Variability in the pharmacokinetics of mycophenolic acid

Implications for therapeutic drug monitoring

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Preface

Mycophenolate mofetil (MMF) is an immunosuppressive drug used to prevent rejection following solid organ transplantation. MMF was introduced in 1995 with a recommended fixed-dose regimen of 1 g twice daily. Nowadays, dose individualization using therapeutic drug monitoring (TDM) of the area under the concentration-time curve from 0 to 12 hours postdose (AUC_{0-12}) of the active compound, mycophenolic acid (MPA), is advocated to optimize the treatment. The recommended target range for the MPA AUC_{0-12} in renal transplant recipients is 30-60 mg*h/L.[1] A practical and suitable manner of determining the MPA AUC_{0-12} are abbreviated AUC measurements, in which the AUC_{0-12} is estimated by a limited sampling strategy. In renal transplant recipients, it has been shown that limited sampling strategies estimate MPA AUC_{0-12} with sufficient accuracy and precision.[2] The aim of this thesis was to further explain the differences in the pharmacokinetics of MMF seen between renal transplant recipients, investigate the validity of these results in other populations, or when different formulations are used, and to describe the effects of these results on individualization of the MMF treatment. In Chapter 1 of this thesis, an overview of the pharmacokinetics of MPA in renal transplant recipients and the added value of TDM are discussed.

MPA is a highly protein bounded drug, which binds reversibly to albumin. The free fraction is thought to be responsible for the immunosuppressive effect of MPA. Cyclosporine comedication, low plasma albumin level, and impaired renal function are associated with a decrease in total MPA AUC, but the unbound concentration is hardly affected.[3] The effect of these covariates on total and unbound MPA concentrations is clarified in Chapter 2.1. The effect of MMF dose on the pharmacokinetics of MPA is evaluated in Chapter 2.2. In Chapter 2.3 the differences in the pharmacokinetics of MPA between adult and pediatric renal transplant recipients are examined.

Besides solid organ transplantation, MMF is increasingly used to prevent graft-versus-host disease following hematopoietic stem cell transplantation and for treatment of autoimmune diseases. TDM may be a valuable tool to optimize MMF therapy in these patients as well. The differences in the pharmacokinetics of MPA between renal transplant recipients and hematopoietic stem cell transplant patients and patients with autoimmune diseases and the consequences of these differences for TDM are reported in Chapter 3.

To prevent gastrointestinal adverse events, which are frequently seen during MMF treatment, enteric-coated mycophenolate sodium (EC-MPS) was developed. EC-MPS and MMF are both prodrugs of MPA, and showed similar efficacy and safety profiles. In Chapter 4, the pharmacokinetic profile of MPA is compared following administration of EC-MPS and MMF.

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Chapter 1

INTRODUCTION

Therapeutic drug monitoring of mycophenolic acid: does it improve patient outcome?

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Abstract

Treatment with the immunosuppressive agent mycophenolate mofetil (MMF) decreases the risk of rejection after renal transplantation and improves graft survival compared with azathioprine. The exposure to the active metabolite mycophenolic acid (MPA) is correlated to the risk of developing acute rejection. The interpatient variability in exposure of MPA is wide relative to the proposed therapeutic window of the MPA AUC_{0-12} (30-60 mg*h/L). The pharmacokinetics of MPA are influenced by patient characteristics such as gender, serum albumin concentration, renal function, comedication and pharmacogenetic factors. Therapeutic drug monitoring is likely to reduce interpatient variability. Limited sampling strategies are used to predict the full AUC_{0-12}. Three prospective randomized studies compared concentration-controlled MMF therapy to a fixed-dose regimen. Outcomes of these studies showed conflicting results and longer follow up is needed to further clarify the role of therapeutic drug monitoring in increasing the therapeutic potential of MMF.

Introduction

Mycophenolate mofetil (MMF) is an immunosuppressive agent used in renal, heart and liver transplant patients. MMF was introduced in 1995 for the prevention of acute rejection at a fixed-dose regimen of 1 g p.o. b.i.d. in renal allograft recipients.[1] A pooled efficacy analysis found a significant decrease in the rate of rejection in renal transplant recipients from 40.8% in placebo or azathioprine treatment to 19.8 and 16.5% for the groups treated with MMF 2 g and 3 g, respectively.[2] The immunosuppressive therapy after renal transplantation has shifted in the past years from azathioprine, cyclosporine A (CsA) and corticosteroids to a combination of MMF, tacrolimus and corticosteroids. At the same time the posttransplant rates of rejection further decreased from ~40% to 20% in 1995-2001.[3]

After oral administration, the prodrug MMF is rapidly and completely absorbed and hydrolysed to the active compound mycophenolic acid (MPA). MPA is mainly glucuronidated to the inactive 7-O-mycophenolic acid glucuronide (MPAG). This metabolite is excreted in the urine and in bile. Besides MPAG, two other metabolites of MPA are formed, 7-O-glucoside and the active acyl glucuronide (AcMPAG), both in smaller amounts.[4] The enterohepatic recirculation of MPAG causes a secondary plasma peak of MPA.[1, 5] According to a Japanese study the contribution of enterohepatic recirculation to MPA exposure is higher at night time compared to daytime.[6]

MPA is a selective inhibitor of inosine monophosphate dehydrogenase (IMPDH). This enzyme is an important factor in the *de novo* synthesis of guanine nucleotides. MPA inhibits T- and B-lymphocyte proliferation by inhibition of IMPDH activity, which ultimately results in its immunosuppressive effect. The free fraction of the highly protein bound MPA (97%) is thought to be responsible for this effect.[7]

Recently, therapeutic drug monitoring (TDM) is a frequently discussed topic that may further improve MMF therapy. This review focuses on the need for dose individualisation in MMF therapy and subsequently on the possible methods for TDM. The results of three prospective randomized trials comparing MMF treatment with and without dose individualisation are discussed to interpret the efficacy of TDM. The data presented in this paper are only related to the MMF formulation and not to the enteric-coated mycophenolate sodium (EC-MPS). The latter has different and more variable pharmacokinetics, and the algorithms to predict MPA exposure developed for MMF cannot be used for EC-MPS.

The need for therapeutic drug monitoring

It has been suggested that a number of criteria should be met for a drug to be considered a suitable candidate for TDM. These criteria include a clear relationship between drug concentration and effect, a small therapeutic index and considerable interpatient pharmacokinetic

variability. Furthermore, the pharmacological response of the drug should be difficult to assess or to distinguish from adverse events.[8]

In clinical studies with MMF therapy, no correlation was observed between dose and graft rejection, whereas the pharmacokinetic parameters of MPA did show a relationship with efficacy.[9] The correlation between pharmacokinetic parameters and effect in renal transplant recipients has been established in a number of clinical trials.[9-11] Rejecters had lower MPA area under the concentration-time curve (AUC_{0-12}) and predose levels (C_0) compared to non-rejecters.[12] These parameters poorly correlate (r=0.1) with the given MMF dose.[13] A randomized concentration-controlled trial (RCCT) by Hale et al followed renal transplant patients on a combined therapy of MMF, CsA and prednisone for 6 months. A total of 150 patients were randomized to three AUC target groups: low (16.1 mg*h/L), intermediate (30.3 mg*h/L) and high (60.6 mg*h/L). The risk of biopsy-proven acute rejection was increased in the low target group. A statistical significant relationship was found between the incidence of biopsy-proven acute rejection and MPA AUC ($p<0.001$) and MPA C_0 ($p=0.01$), in which AUC showed the best correlation.[9] Similar correlations have been demonstrated in pediatric renal transplant patients by Weber et al The risk for acute rejection in the first 3 weeks posttransplantation was increased in children with MPA AUC_{0-12} values <33.8 mg*h/L ($p=0.005$) or predose levels <1.2 mg/L ($p<0.001$).[10] A Canadian group stressed the importance of early adequate MPA exposure based on a study with 94 CsA- and MMF treated renal transplant recipients. In these patients the MPA AUC_{0-12} (estimated with limited sampling) at day 3 was strongly associated with increased risk of acute rejection.[11] The discussed studies all included patients who were cotreated with CsA.[9-11] A statistically significant relationship was, however, not seen in renal transplant recipients with a therapy based on concurrent therapy with tacrolimus.[14] Kuypers et al followed 100 renal transplant patients for 12 months and found a trend towards a higher incidence of acute rejection in recipients who did not reach both a target tacrolimus AUC_{0-12} of 150 mg*h/L and an MPA AUC_{0-12} of 45 mg*h/L ($p=0.07$).[15] This difference between CsA and tacrolimus could be caused by the decrease in MPA AUC due to the inhibition of the enterohepatic recirculation of MPA by CsA as explained later. In patients cotreated with tacrolimus, higher MPA exposure was found compared to CsA cotreatment[16-19], whereby more patients reach the therapeutic window. The amount of patients needed to establish a significant correlation between MPA exposure and effect is high due to the low incidence of acute rejection when patients have an MPA exposure in the therapeutic window.

The most frequently seen side effects in MMF therapy are gastrointestinal symptoms, haematological disorders and infections.[20] The correlation between pharmacokinetic variables and adverse events is more difficult to establish than the correlation with efficacy. In part, this is due to the lower incidence of some of these adverse events, but it can also be difficult to distinguish MMF related adverse events from adverse events caused by other factors, like concurrently used (immunosuppressive) drugs. Some large studies were not able to find a relationship between MPA pharmacokinetic parameters and toxicity.[11, 21] In the RCCT study (n=150), no significant correlation was seen between adverse events and MPA C_0, MPA C_{max} or

MPA AUC_{0-12}, whereas MMF dose was significantly related to the occurrence of adverse events, which were mostly of gastrointestinal origin in this trial.[21] Analyses by Kiberd et al (n=94) for predictors of toxicity were negative for MPA exposure (AUC_{0-12}, C_0 and C_2 (concentration at t=2h)). Toxicity was defined here as the need to reduce or discontinue the dose of MMF for clinical symptoms or for abnormal laboratory values.[11] Nevertheless, other smaller studies did find a correlation between MPA exposure and adverse events. A significant relationship between adverse events, especially leukopenia and anemia, and MPA exposure (AUC_{0-12}, C_0 and C_{max}) was seen in renal transplant recipients cotreated with CsA or tacrolimus.[15, 22-24] The non-protein bound (free) concentration of MPA (fMPA) was not determined in any of these trials. In studies in which fMPA was monitored, patients who experienced infections or haematological events, including leukopenia, had a significantly higher fMPA AUC.[10, 25] It is unclear at present why fMPA does not have a clear concentration-effect relationship with efficacy.

A therapeutic window for MPA AUC_{0-12} of 30-60 mg*h/L has been established based on the aforementioned clinical studies.[26] These limits are a reasonable target for renal transplant recipients when MMF is coprescribed with CsA and when the MPA concentrations are determined by high-performance liquid chromatography (HPLC).[26] This therapeutic window for MPA AUC_{0-12} is comparable with predose levels in the target range of 1-3.5 mg/L, or 1.7-4.0 mg/L when MMF is combined with tacrolimus.[26-27] However, MPA pharmacokinetics exhibit large inter- and intrapatient variability, in both AUC_{0-12} and predose levels.[1] The MPA AUC_{0-12} in renal transplant recipients after administration of MMF 1 g is in the range of ~10-100 mg*h/L[13-14, 28], whereas a therapeutic window of 30-60 mg*h/L is recommended.[26] MPA clearance following other kinds of transplantation (liver, heart and bone marrow) also shows a 10-fold variability.[27] Several factors, such as comedication and time after transplantation, influence the MPA exposure. A simulation model showed only 13% of the patients receiving MMF 1 g b.i.d. had an MPA AUC of 30-60 mg*h/L 1 week after transplantation, which increased in 4-5 months to 67% of the patients.[29] Intrapatient variability in MPA exposure is relatively low compared to interpatient variability.[28] Pharmacokinetic monitoring is expected to increase the effect of MMF treatment because it would increase the number of patients on target due to the large interpatient variability and small intrapatient variability.

MMF exerts its action through inhibition of IMPDH. The activity of IMPDH displays high interpatient variability. This variability was seen in healthy volunteers and pre- and posttransplantation in renal transplant recipients.[30-32] An inverse relationship between plasma MPA concentration and IMPDH activity within the MMF dose interval was demonstrated.[30, 33-34] Glander et al found associations between low pretransplant IMPDH activity and the need for dose reduction due to adverse events ($p<0.004$) and between high pretransplant IMPDH activity and rejection ($p<0.01$).[31] This means that patients with low pretransplant IMPDH activity need less MMF to get the same immunosuppressive effect. These findings suggest that pharmacodynamic monitoring of IMPDH activity may be suitable to individualise MMF therapy. However, more research on this topic is needed.[35]

Factors influencing MPA exposure

Demographic factors that influence MPA pharmacokinetics

Gender

A number of studies observed a significant relationship between MPA pharmacokinetic parameters and gender.[36-38] Morissette et al found gender-related differences in the MPAG:MPA ratio. The average MPAG:MPA ratio was significantly increased in men compared to women.[36] Borrows et al found increased MPA predose levels in female renal transplant recipients compared to males (p=0.002).[37] The relationship between patient factors and pharmacokinetic parameters has been studied by developing a population pharmacokinetic model for MPA following oral administration of MMF. In the final model it appears that males have an 11% higher MPA clearance than females.[38] Other studies found no effect of gender on the pharmacokinetics of MPA.[39-41] In one study, the mean AUC in females was higher than in males, but this difference was not significant (female:male ratio = 1.094 ;90% confidence interval [CI]: 0.975-1.228).[39] In a population pharmacokinetic meta-analysis containing 13,346 MPA concentration-time data points from 468 renal transplant patients, no significant relationship was found between gender and MPA exposure.[40] Overall, the studies give conflicting results. Some small studies suggest that MPA metabolism is reduced in women, but a meta-analysis found no correlation between gender and MPA pharmacokinetics. A possible effect has been suggested to result from a competitive inhibition of uridine 5'-diphosphate glucuronosyl transferase (UGT) enzymes by estrogen.[36]

Race

African-American (AA) renal transplant patients have been recognized to be at higher risk for early acute rejection episodes.[42] AA renal transplant recipients have significantly less benefit of MMF treatment considering risk of acute rejection compared with Caucasians.[43] The benefit of MMF compared to azathioprine on long-term outcomes is equivalent for both ethnicities.[44] To produce comparable benefit:risk ratios in AA renal allograft recipients as in non-AA renal transplant patients, higher MMF doses may be required. In AA renal transplant patients a higher MMF dose is needed to produce a significant benefit in acute rejection compared with Caucasians (1.5 g b.i.d. and 1 g b.i.d., respectively).[42] This difference in clinical outcome between AA and Caucasian patients cannot be explained by a difference in MPA exposure, as no significant differences in the pharmacokinetics of MPA were found between AA and Caucasian stable renal transplant recipients. The exposure to both MPA and MPAG (defined by AUC_{0-12}, C_{max} or C_0) was comparable between the two ethnic populations.[39, 45] The variability in MPA predose levels was also found to be unaffected by ethnicity in comparison with other races (white, Indo-Asian, Afro-Caribbean).[37] These results indicate that the racial differences in renal graft survival are not caused by pharmacokinetic differences. The explanation for the requirement of higher MMF doses in AA transplant patients must be sought elsewhere. The increased risk of rejection in AA renal transplant recipients is probably caused by heightened immune responsiveness.[45-46] This stresses the importance of adequate

immunosuppressive drug exposure with target differentiation depending on race to achieve better outcome.[45-46]

In contrast to AA patients, in Chinese patients with standard dose MMF, higher MPA AUC levels were reached.[47] In addition, the algorithms used to predict full AUC on the basis of reduced sampling strategies appear to be different in this population. This stresses the limited applicability of reduced sampling techniques.

Bodyweight

A population pharmacokinetic analysis of Le Guellec et al (n=60) found that bodyweight was positively correlated with oral MMF clearance. Considering bodyweight in the pharmacokinetic model reduced the unexplained variability in clearance from 34.8 to 28.2%. The magnitude of this reduction in variability, however, is not sufficiently strong to recommend dosing on a per kilogram basis.[48] In the study of Staatz et al (n=117) a trend towards increased MPA clearance with higher bodyweight was found. Inclusion of patient weight into the model resulted in 1.3% absolute reduction in interpatient variability.[49] Other large trials of van Hest et al (n=468), Kuypers et al (n=100) and Borrows et al (n=117) did not show a correlation between bodyweight and MPA pharmacokinetics.[37, 40-41] These results suggest that, even if bodyweight does influence MPA pharmacokinetics, the amount of effect will not reach clinical relevance.

Pathophysiological factors influencing MPA pharmacokinetics

Diabetes

In a retrospective analysis of the RCCT data the influence of diabetes on MPA pharmacokinetics was analysed. The results did not show significant differences in MPA exposure (AUC_{0-12}, clearance and C_{max}) between diabetic (n=7) and nondiabetic (n=129) renal transplant patients. On day 11 after transplantation, there was significant difference in MPA T_{max} between 6 diabetics and 125 non-diabetics (1.59 hours for diabetic versus 0.67 hours for nondiabetic patients, p=0.04).[50] A population pharmacokinetic meta-analyse of 468 renal transplant patients confirmed an increased T_{max} in patients with diabetes (p=0.045).[40] The increased T_{max} is likely to be caused by slower absorption as a consequence of gastroparesis.[40, 50] This difference does not effect the overall MPA exposure for patients with diabetes, thus the effect on T_{max} has no clinical relevance. Other studies also found no difference in MPA exposure between diabetics and non-diabatics after renal transplantation.[39, 49]

Protein binding of MPA

MPA is a highly protein bound drug (±97%) and binds reversibly to serum albumin. As is the case for many other drugs, the free fraction is thought to be responsible for the immunosuppressive effect of MPA.[1] Besides, increased exposure to fMPA causes an elevated risk of certain MMF related side effects.[10, 25] The clearance of MPA is also correlated with the free fraction.[25] The MPA free fraction depends on the serum albumin concentration and renal function of the

patient. An increased albumin concentration is correlated with a decrease in free fraction and fMPA $AUC_{0\text{-}12}$.[5, 7, 25] fMPA levels are significantly elevated in patients with an impaired renal function.[45, 51] The protein binding of MPA is reduced in renal impairment due to an effect of uraemia and the increase of MPAG, which has been shown *in vitro* to compete with MPA for binding sites on albumin.[45] In addition, in a paediatric patient population, renal impairment and decreased albumin levels were associated with increased free fraction and increased fMPA AUC.[52] In addition to MPAG, salicylate can displace MPA from albumin binding sites in high concentrations.[7] A decrease in fMPA levels leads to a reduction of MPA clearance and increase in total MPA levels.[5, 53] The influence of albumin concentration on MPA clearance and exposure was established in two population pharmacokinetic models.[38, 49]

Influence of renal dysfunction on pharmacokinetics of MPA

Increased serum creatinine and decreased glomerular filtration rate are associated with decreased MPA predose levels.[37] Renal dysfunction leads to an increase in fMPA exposure due to elevated MPAG levels.[7, 45, 51] In a population pharmacokinetic model, reduced creatinine clearance correlated significantly with increased MPA clearance.[38] The creatinine clearance accounts for 19% of the intrapatient variability of MPA clearance.[54] In general, renal dysfunction leads to decreased MPA concentrations, increased MPAG concentrations and increased free MPA fractions.

Influence of comedication on MPA pharmacokinetics

Immunosuppressive agents

Coadministration of other immunosuppressive agents, especially calcineurin inhibitors (CNIs), can influence MPA exposure. The CNIs CsA and tacrolimus are used in combination with MMF in renal transplant recipients. Several studies compared MPA pharmacokinetics when given with either CsA or tacrolimus. Significantly increased MPA clearance[16-17] and decreased MPA predose levels[18-19] were found in case of CsA coadministration compared to tacrolimus coadministration. Exposure to the metabolite MPAG was significantly higher in renal transplant recipients treated with MMF in combination with CsA.[16, 55] Discontinuation of CsA in the immunosuppressive therapy leads to increased MPA predose levels[56-57], suggesting that the differences depend on an effect of CsA on the pharmacokinetics of MPA. The effect can be explained by reduced enterohepatic recirculation of MPAG in case of CsA cotreatment due to inhibition of the multi-drug resistance-associated protein 2 (MRP2) enzyme.[18, 58-59] MRP2 is responsible for the excretion of MPAG in bile.[60-61] In addition, in the population pharmacokinetic analysis of Cremers et al, supportive clinical evidence for the inhibitory effect of CsA on the enterohepatic recirculation of MPA was provided. This study showed that total MPAG clearance was lower in CsA cotreated patients than in tacrolimus cotreated patients, despite a similar renal function in both groups.[59] Besides this effect of CsA on the enterohepatic recirculation of MPAG, tacrolimus may have a contribution to the differences in MPA pharmacokinetics. When MMF was administered in combination with tacrolimus, the MPA clearance decreased compared with no CNI.[16] Tacrolimus may increase

MPA levels due to inhibition of UGT, which is responsible for the formation of MPA to the inactive metabolite MPAG[17], but this effect is too small to be clinically relevant.

The combination of MMF and sirolimus can be used as a CNI-free regime. In sirolimus-cotreated patients, elevated MPA exposure[62-63] and decreased exposure to its metabolites[62] were found compared to cotreatment with CsA. The exposure to MPA, fMPA and MPAG in sirolimus-cotreated patients compared to tacrolimus-cotreated patients was not significantly different.[64] Coadministration of corticosteroids may increase MPAG levels, possibly due to induction of UGT activity. This was shown in a study by Cattaneo et al, in which steroid discontinuation was correlated with an increase in MPA AUC_{0-12} and a decrease in MPA clearance and MPAG predose levels.[65] Other studies did not find an association between MPA clearance and steroid therapy.[40, 48]

Non-immunosuppressive agents

Even though Morii et al found a significant decrease in MPA exposure when renal transplant recipients were cotreated with ferrous sulphate[66], this drug interaction could not be established in other studies.[37, 67] The influence of the presence of ferrous sulphate on the physicochemical properties of MMF and MPA could not show the formation of an MMFiron complex.[68] Coadministration of antacids decreases the bioavailability of MMF due to chelation.[5] Aciclovir cotreatment had no effect on MPA levels, but resulted in increased MPAG and acyclovir AUCs by 10.6 and 21.9%, respectively. Ganciclovir may also compete with MPAG for tubular secretion in patients with increased plasma concentrations as seen in renal impairment.[53] Treatment with oral antibiotics with activity against the Gram-negative anaerobes in the gut, which contain most of the glucuronidase activity, reduce enterohepatic cycling and, hence, MPA levels.[5, 37] Colestyramine decreases MPA and MPAG AUC_{0-12} by 39 and 34%, respectively, by interfering with the enterohepatic recirculation of MPA.[5] Pieper et al found a significant reduction by a mean of 25% of the MPA AUC after a single dose of sevelamer ($p>0.05$).[69] A case-report presented a drug interaction between MMF and rifampin, in which rifampin caused a twofold higher MPA clearance, probably as a result of induction of UGT or MRP2.[70]

The impact of pharmacogenetic variation on MPA pharmacokinetics

Several single nucleotide polymorphisms (SNPs) have been identified in genes of enzymes involved in MPA pharmacokinetics. The pharmacogenetic research is focused on the enzymes UGT, MRP2 (both pharmacokinetic effects) and the IMPDH types I and II (pharmacodynamic effect).[14]

MPA is metabolised to MPAG by the UGT enzyme family. The most important UGT isoforms for the glucuronidation to MPAG and AcMPAG are UGT1A9 and -2B7, respectively.[71-72] The variability in the expression of UGT1A9 differs by a factor of 17 in human liver microsomes. The glucuronidation of MPA in these microsomes was significantly correlated with UGT1A9

protein levels. In livers with the *T275A* and *C2152T* SNPs (incidence: 5/38), MPA glucuronidation was significantly higher compared with wild-type livers.[73] In renal transplant recipients cotreated with tacrolimus, carriers of the *T275A* and/or *C2152T* SNP (11% of the patients) had significantly decreased MPA AUC_{0-12} (50.5 vs. 63.0 mg*h/L; $p<0.001$) compared with non-carriers.[74-75] No significant correlation was found for the UGT1A9*3 polymorphism in renal transplant patients. Mutation of the UGT2B7 gene (*G840A*) is associated with a significantly higher AcMPAG:MPA ratio (2- and 2.6-fold higher ratios in heterozygous and homozygous mutated patients, respectively; $p<0.05$) due to an increased production of AcMPAG.[76] In a Japanese renal transplant population the *C802T* SNP was not associated with a difference in MPA exposure, whereas AcMPAG concentrations were not measured.[6]

MRP2 is responsible for the biliary excretion of MPAG. CsA reduces the enterohepatic recirculation of MPA through inhibition of MRP2.[60-61] Van Agteren et al determined the impact of MRP2 gene polymorphism on MPA exposure in renal transplant patients. Heterozygosity for the *C3972T* SNP was found in 117/259 and homozygosity in 28/259 of the patients. Carriers of *C3972T* polymorphism in the MRP2 gene have little but significantly decreased MPA AUC_{0-12} (59.0 vs. 64.7 mg*h/L; $p=0.045$) when tacrolimus is co-administrated.[77] Naesens et al found that the *C24T* and *C3972T* polymorphisms are associated with higher MPA concentrations in a subgroup of patients with liver dysfunction.[78]

MPA exerts its immunosuppressive activity by inhibition of IMPDH. The MPA concentration has an inverse correlation with IMPDH activity within the MMF dose interval[30, 33-34], even though the baseline variability of IMPDH activity in humans is very wide. High pretransplant IMPDH activity was associated with acute rejection, which suggests the need for dose individualisation based on IMPDH activity.[31] The IMPDH enzyme consists in two isoforms, type I and type II. MPA has a five-fold higher inhibitory affinity for IMPDH-II than IMPDH-I.[79] Therefore, pharmacogenetic analysis of IMPDH-II may contribute more to individualisation of MMF therapy.[80] SNPs of both isoforms have been identified, but no associations between these polymorphisms and the incidence of acute rejection were detected.[81-83] However, Grinyo et al found a significantly increased acute rejection in carriers of the *T3757C* polymorphism of IMPDH-II (odds ratio: 2.99; 95% CI: 1.27-6.99; $p=0.012$).[84]

Time-dependent pharmacokinetics of MPA

MPA clearance of renal transplant patients decreases over time for at least 30%.[9, 14, 41, 85] In addition, paediatric renal transplant patients exhibit a two-fold increase in total MPA AUC_{0-12} over time.[85] Shaw et al showed that the increase in MPA AUC_{0-12} over time was due to a decrease in MPA clearance, and only occurred in patients with impaired renal function.[45] When renal function improves over time posttransplant, the MPAG clearance increases and MPA protein binding recovers as a result of less displacement by MPAG.[7, 45] Other time-varying factors, such as increasing albumin levels and the gradual tapering of CsA dose and target levels in the same period, may play a role.[86] Van Hest et al described the time-dependent

clearance of MPA in a population pharmacokinetic meta-analysis. In the first 6 months after renal transplantation the MPA clearance decreased from 34 to 20 L/h. Simultaneous changes in creatinine clearance from 19 to 71 mL/min, albumin level from 35 to 40 g/L, haemoglobin from 97 to 120 g/L and CsA predose level from 225 to 100 ng/mL were responsible for this decrease in MPA clearance and could explain 19, 12, 4 and 3% of the intrapatient variability, respectively.[54]

Therapeutic drug monitoring of MPA

Methods of dose individualisation

As mentioned before, TDM may be used as a tool to optimize MMF treatment in renal transplant recipients given the large interpatient variability and the established concentration-effect relationship. The variability in MPA exposure is wide compared to the therapeutic window[13, 26-28] and is influenced by many factors. The clinical outcome correlates significantly with MPA concentration, but not with dose.[10-11, 21] MPA AUC_{0-12} has a better correlation with the risk of rejection than predose levels.[9, 21, 87] However, AUC_{0-12} measurements are more difficult to perform for practical reasons.[26, 53] A suitable alternative may be abbreviated AUC measurements, in which the AUC_{0-12} is determined by limited sampling regimes.

A priori methods

In *a priori* methods no concentration data are used; only patient characteristics, such as gender or bodyweight, known to influence the concentration profile are used. These methods are simple to implement and partly compensate for interindividual pharmacokinetic variability, but their predictive performance is limited.[88] The influence of patient factors may be used to predict the optimal dose, which until now was never done for MMF, except for paediatric patients in which dose is based on body surface area.

A posteriori methods

A posteriori methods (adaptive control methods) require concentration data and sometimes patient characteristics known to partly explain the interindividual pharmacokinetic variability.[88] *A posteriori* methods can be used to estimate pharmacokinetic parameters such as clearance and exposure. The methods can be divided in multiple regression analysis (MRA) and maximum *a posteriori* Bayesian analysis methods.[89]

Multiple regression analysis

These models intend to estimate exposure using a small number of blood samples collected at specific time points. MRA is a limited sampling strategy in which the relationship between AUC and the various timed concentrations is determined and described by the function in Equation 1:

$$AUC = A_0 + A_1C_{t1} + A_2C_{t2} + \ldots A_iC_{ti} \quad \text{(Eq. 1)}$$

Where C_{t1}, C_{t2},…C_{ti} are concentrations obtained at times t_1, t_2…t_i and A_0, A_1, A_2…A_i are constant values associated with each timed concentration.[88-89] Several MRA limited sampling strategies using 3-4 samples during a 2 to 3-h period have been developed to predict full MPA AUC values, with correlation coefficients ≤ 0.95.[50, 90-92] Advantages of the MRA limited sampling method are the easy development and use. Deviations of the time of sample drawing or the presence of extreme patient conditions (e.g. very poor renal function) hinder the accurate application of the method.[89]

Maximum a posteriori Bayesian estimation

Bayesian analysis can be performed using a pharmacokinetic population model and a few concentration data collected at several time points after drug administration. The method uses *a priori* population pharmacokinetic parameters and concentration-time data of the individual for estimation of individual parameters.[88-89] Advantages of Bayesian estimation compared with MRA are the flexible sampling times, the estimation of several pharmacokinetic parameters, the improved adaptability to patients with unusual pharmacokinetics and better possibilities to check the modelling efficiency visually.[88-89, 93] To perform Bayesian analysis a computer with specialized software programs is required due to the complicated calculations and algorithms that are used.[89] Le Guellec et al[48] developed a Bayesian method for MPA to estimate $AUC_{0\text{-}12}$ in renal transplant recipients. The method was validated by comparison of predicted $AUC_{0\text{-}12}$ versus observed MPA AUC by trapezoidal method (correlation coefficient 0.946).[48] The Bayesian estimators of MPA exposure at different posttransplantation periods, designed by Prémaud et al, have been used for MPA dose adaptation in a fixed-dose versus concentration-controlled study[93], in which the model has been used to adjust MMF dose in the concentration-controlled arm of the study. In the other arm, the MMF dose was adjusted only on clinical signs or symptoms. Results of this study show that the MPA AUC of patients in the concentration-controlled group reached the target value of 40 mg*h/L earlier after transplantation compared with the control group.[94] These results indicate that maximum *a posteriori* Bayesian estimation is a suitable tool for application of TDM in MMF therapy.

Effect of therapeutic drug monitoring

Three prospective, randomized, multi-centre studies have been started to determine the contribution of TDM to outcome in MMF treated renal transplant patients: the fixed-dose versus concentration-controlled (FDCC) study; the Apomygre study; and the OptiCept study.[95-97] The risk of acute rejection and toxicity between a concentration-controlled regime and a fixed MMF dosing regimen are compared.[14] In the FDCC study, MPA exposure is estimated with abbreviated AUCs. Patients (n=901) cotreated with a CNI and corticosteroids were compared between a concentration-controlled and a fixed-dose group. The results of this study showed no difference in biopsy-proven acute rejection (p=0.96). In addition, no difference was found in MPA exposure between the concentration-controlled and the fixed-

dose patients.[96] The Apomygre study compared fixed-dose with concentration-controlled MMF treatment in patients cotreated with basiliximab, CsA and steroids (n=130). Dose adjustments were calculated with the Bayesian estimation approach. Final results showed significantly higher AUCs in concentration-controlled patients at 2 weeks and 3 months post-transplantation ($p<0.0001$) as well as less rejection ($p=0.01$) in the concentration-controlled group.[97] Finally, the results of the Opticept study (n=720), which adjusted MMF doses based on MPA predose level measurement, showed no significant differences in acute rejection between the concentration-controlled group and the fixed-dose group.[95]

Conclusion therapeutic drug monitoring

The therapeutic window of the exposure to MPA is small compared with the pharmacokinetic variability at a fixed MMF dose. MPA exposure measured by AUC_{0-12} gives the best prediction of the risk of rejection, but measurements are impractical. Determining of MPA AUC by limited sampling strategies seems to be the most suitable parameter for TDM. A good prediction of full AUC by limited sampling can be achieved with MRA or maximum *a posteriori* Bayesian estimation. The advantages of the latter over MRA are that sampling time is more flexible and that patient factors can be taken into account. However, for CsA, Bayesian estimation was also shown to be of added benefit, but has not been widely adapted for this drug. Reasons for small-scale use in daily practice may be related to limited expertise in Bayesian estimation, or to poor communication between clinicians and pharmacists/clinical pharmacologists. Measurement of MPA AUC values and dose adjustment after 1 week and after 1 or 2 months posttransplantation in the majority of the patients are likely to be sufficient to obtain target exposure in most patients.[28] The first measurement can be done while the patient is still admitted to the hospital, and aims to identify gross over- or underexposure to MPA. Following dose adjustment a second mini-AUC (0, 30, 120 min) can be done somewhere around week 4, to check if the patient is still within the therapeutic range. The limited number of samples proposed will prevent a huge increase in the cost of patient care. Formal cost-effectiveness studies have not been performed yet. More frequent monitoring seems necessary if significant changes in patient condition or comedication occur.

Expert Opinion

TDM is expected to be beneficial in MMF therapy considering the variability in MPA exposure and relationship between exposure and the risk of rejection. The Apomygre study[97] indicated a beneficial effect of TDM on outcome in MMF treatment in renal transplant patients. The concentration-controlled patients reached higher MPA AUCs and experienced less rejection compared with the fixed-dose patients without an increased rate of infectious complications.[97] In the larger FDCC study[96], however, no benefit of TDM on the risk of rejection was observed, probably because the performed dose adjustments were not able to sufficiently

separate MPA exposure in the concentration-controlled and fixed-dose groups. The difference in outcome between the two studies may also be caused by the use of MRA instead of Bayesian estimation to predict full AUC after limited sampling.

If we take a step back, and consider the outcomes of the three prospective trials, then the overall impression is one of disappointment. Where based on the experience obtained in the 1990s, a clear added value for TDM was expected, we are faced with conflicting results. Those who have been in favor for TDM for this drug will find sufficient positive results to maintain their faith in monitoring. In contrast, the non-believers will interpret the results as negative, and also they will not change their position on the value of TDM. Most likely, it will require more studies, in more homogeneous populations, and with dedicated clinicians, to get the final answer.

Pharmacodynamic monitoring of IMPDH activity for MMF dose individualisation might be a promising approach because this biomarker also has a relationship to the clinical effect.[31] The practical difficulty of the IMPDH assays (labor intensive, expensive, technically challenging) compared with the methods for the determination of MPA plasma concentrations are, however, a serious drawback for implementation of this approach. A clear parallel can be drawn to the experience with the CNIs, where monitoring calcineurin activity has only been occasionally studied.

Besides renal transplantation, MMF is also increasingly used in other solid organ transplantation, stem cell transplantation and autoimmune diseases. The influence of the described patient characteristics on MPA pharmacokinetics and the need for TDM may also apply to these indications. Further research is needed to optimize the treatment with MMF and elucidate the need and ways of dose individualisation in all these indications. Investigators are encouraged in these areas to initiate observational studies first, to obtain more knowledge regarding concentration-effect relationships and optimized exposure for these indications. More insights from these other indications could potentially strengthen the position of TDM in the area of renal transplantation. There is also a need to further specify optimized MPA exposure in situations with reduced or discontinued CNI treatment, where most likely MPA target concentrations would be different.

In conclusion, the question raised in the title cannot yet be answered definitively. There are still reasons to believe it will, but so far the studies do not all show a consistent contribution.

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Chapter 2

PHARMACOKINETICS OF MYCOPHENOLATE MOFETIL IN RENAL TRANSPLANT RECIPIENTS

Chapter 2.1

Pharmacokinetic role of protein binding of mycophenolic acid and its glucuronide metabolite in renal transplant recipients

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Abstract

Mycophenolic acid (MPA), the active compound of mycophenolate mofetil (MMF), is used to prevent graft rejection in renal transplant recipients. MPA is glucuronidated to the metabolite MPAG, which exhibits enterohepatic recirculation (EHC). MPA binds for 97% and MPAG binds for 82% to plasma proteins. Low plasma albumin concentrations, impaired renal function and coadministration of cyclosporine have been reported to be associated with increased clearance of MPA. The aim of the study was to develop a population pharmacokinetic model describing the relationship between MMF dose and total MPA (tMPA), unbound MPA (fMPA), total MPAG (tMPAG) and unbound MPAG (fMPAG). In this model the correlation between pharmacokinetic parameters and renal function, plasma albumin concentrations and cotreatment with cyclosporine was quantified. tMPA, fMPA, tMPAG and fMPAG concentration-time profiles of renal transplant recipients cotreated with cyclosporine (n=48) and tacrolimus (n=45) were analyzed using NONMEM. A 2- and 1-compartment model were used to describe the pharmacokinetics of fMPA and fMPAG. The central compartments of fMPA and fMPAG were connected with an albumin compartment allowing competitive binding (bMPA and bMPAG). tMPA and tMPAG were modeled as the sum of the bound and unbound concentrations. EHC was modeled by transport of fMPAG to a separate gallbladder compartment. This transport was decreased in case of cyclosporine cotreatment ($p<0.001$). In the model, clearance of fMPAG decreased when creatinine clearance (CrCL) was reduced ($p<0.001$), and albumin concentration was correlated with the maximum number of binding sites available for MPA and MPAG ($p<0.001$). In patients with impaired renal function cotreated with cyclosporine the model adequately described that increasing fMPAG concentrations decreased tMPA AUC due to displacement of MPA from its binding sites. The accumulated MPAG could also be reconverted to MPA by the EHC, which caused increased tMPA AUC in patients cotreated with tacrolimus. Changes in CrCL had hardly any effect on fMPA exposure. A decrease in plasma albumin concentration from 0.6 to 0.4 mmol/L resulted in ca. 38% reduction of tMPA AUC, whereas no reduction in fMPA AUC was seen. In conclusion, a pharmacokinetic model has been developed which describes the relationship between dose and both total and free MPA exposure. The model adequately describes the influence of renal function, plasma albumin and cyclosporine co-medication on MPA exposure. Changes in protein binding due to altered renal function or plasma albumin concentrations influence tMPA exposure, whereas fMPA exposure is hardly affected.

Introduction

Mycophenolate mofetil (MMF) is currently the most prescribed immunosuppressive agent in renal transplant recipients to prevent graft rejection.[1] After oral administration the prodrug MMF is rapidly hydrolyzed to the active agent mycophenolic acid (MPA). The majority of MPA is metabolized to the inactive 7-O-mycophenolic acid glucuronide (MPAG), which exhibits enterohepatic recirculation (EHC). MPA is a selective, reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH). IMPDH has an important role in the *de novo* purine synthesis in T and B lymphocytes.[2] Inhibition of this pathway causes immunosuppression, contributing to the prevention of graft rejection. Although introduced as a fixed-dose drug, debate has emerged with respect to the potential contribution of therapeutic drug monitoring (TDM) of MPA area under the total plasma concentration time curve (AUC_{0-12}). [3-6] The target range for total MPA (tMPA) AUC_{0-12} in renal transplant recipients cotreated with cyclosporine is 30-60 mg*h/L.[7]

MPA is a highly protein bound drug, with a bound fraction of approximately 97%, which binds reversibly to serum albumin.[8-9] The free fraction is thought to be responsible for the immunosuppressive effect of MPA.[8-9] The main metabolite MPAG is approximately 82% protein bound.[9] Low plasma albumin concentrations and impaired renal function are associated with an increased clearance of tMPA and with a decreased AUC_{0-12} of tMPA.[10] The effect of impaired renal function on unbound MPA (fMPA) plasma concentrations is however not clear: some studies have reported an increase in fMPA AUC_{0-12} [11-15], whereas in other studies unbound exposure was unchanged.[16-17]

Coadministration of the calcineurin inhibitor cyclosporine influences MPA pharmacokinetics. In renal transplant recipients, significantly increased tMPA clearance and decreased tMPA AUC_{0-12} occurs with cyclosporine coadministration compared with tacrolimus coadministration.[18-19] This effect can be explained by reduced EHC of MPAG in case of cyclosporine cotreatment due to inhibition of the multi-drug resistance-associated protein 2 (MRP2) enzyme.[20]

Decreased tMPA exposure correlates with a higher risk for acute rejection[21-23], whereas an increase in unbound MPA exposure may produce hematological toxicity and infections.[23-24] On the basis of these data it is unclear whether a decreased total MPA AUC_{0-12}, caused by impaired renal function or low albumin concentrations, should be corrected with an increase in MMF dose, as the patient subsequently may be at risk for adverse events.

The aim of this study was to develop a population pharmacokinetic model, which describes the relationship between MMF dose and both total and unbound plasma concentrations of MPA and MPAG. Using this model, it will be investigated how renal function, plasma albumin and cotreatment with cyclosporine influence the pharmacokinetics of tMPA and fMPA. The developed model may provide insight in the necessity to adjust the MMF dose in situations of impaired renal function and low albumin concentrations.

Methods

Patients

Pharmacokinetic data from two previously performed studies were used for the current analysis. In the first trial, a randomized concentration-controlled trial (RCCT)[25], de novo renal transplant recipients were divided into three MPA AUC target groups. All patients in this study were cotreated with cyclosporine and corticosteroids as concomitant immunosuppressive therapy. In this study, tMPA, fMPA and total MPAG (tMPAG) concentrations were measured at day 3, 7, 11, 21, 28, 56, 84, 112 and 140 after transplantation. On days 3, 7 and 11 post-transplantation, sample times were predose and 0.33, 0.66, 1.25, 2, 6, 8 and 12 hours after oral intake of MMF. On the remaining occasions, sample times were predose and 0.33, 0.66, 1.25 and 2 hours postdose. Concentrations were measured using a validated high-performance liquid chromatography (HPLC) method.[26] MMF dose was adjusted based on these measurements. From a subset of 88 patients fMPA concentrations were measured on one or two of the nine occasions, which were nominally day 11 and/or day 140. These fMPA concentrations were analyzed using a validated ultrafiltration procedure.[27]

In the second trial, the IMPDH-activity study[28], de novo renal transplant recipients started with 1000 mg MMF twice daily, combined with tacrolimus and corticosteroids. MMF dose was adjusted based on clinical evaluations. In the IMPDH-activity study tMPA, fMPA, tMPAG and unbound MPAG (fMPAG) concentrations were measured at day 6, 21, 49 and 140 after transplantation. On day 6, samples were taken predose and 0.5, 1, 2, 6 and 12 hours postdose. On the remaining occasions, sample times were predose and 0.5 and 2 hours after oral intake of MMF. Concentrations were measured using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.[29] For the determination of unbound plasma concentrations of MPA and MPAG, plasma was ultrafiltrated first. A detailed description of these studies was published previously.[25, 28]

Pharmacokinetic analysis

Basic model

The MMF dose and analysed MPA and MPAG concentrations were converted to molar equivalents by dividing them by their molecular weight (MMF 433.5 g/mol; MPA 320.3 g/mol; MPAG 496.5 g/mol). The data of both studies were simultaneously fitted using the nonlinear mixed-effects modeling software program NONMEM (version VI, level 2.0; Globomax LLC, Ellicott City, MD, USA). The first-order (FO) method was used throughout to fit the logarithmically transformed concentration-time data, because of the high computational intensity of the first order conditional estimate method.

The minimum value of objective function (OFV) was used as a criterion for model selection. If the difference between two nested models was larger than the critical value from a

chi-squared distribution with degrees of freedom equal to the difference in the number of estimated parameters, the models were significantly different from each other. A decrease in OFV>10.83 showed a significant improvement of a nested model with one degree of freedom of p<0.001. Model adequacy was further evaluated by using various residual plots ("goodness-of-fit" plots) and values of random effects variances. To assess the graphical goodness of fit, extensive plotting was available via Xpose[30], a purpose built set of subroutines in S-plus (version 6.1; Insightful Corp, Seattle, WA, USA).

A compartmental pharmacokinetic model was developed for fMPA and fMPAG. Several structural models were tested. Models with 1 or 2 compartments were evaluated, as well as models with and without absorption lag-time (T_{LAG}). Furthermore, it was evaluated whether absorption was best described as a zero-order or first-order process. Pharmacokinetic parameters were estimated in terms of central and peripheral volume of distribution (V), clearance (CL), and intercompartmental clearance (Q). Because bioavailability (F) could not be quantified, V, CL and Q values correspond to the ratios V/F, CL/F, and Q/F. Addition of interpatient variability (IPV), described using an exponential error model, was evaluated for each pharmacokinetic parameter. The covariance between values for IPV was estimated using a variance-covariance matrix. Residual variability between observed and predicted MPA plasma concentrations was described using an additional error model for logarithmically transformed data.

Protein bound MPA (bMPA) and bound MPAG (bMPAG) were described by addition of a protein binding compartment, containing a maximum number of binding sites (B_{max}) at which MPA and MPAG could bind (Fig. 1). In this model MPA and MPAG were allowed to bind competitively with the protein binding sites, and replace each other from these binding sites as described by

$$\frac{dA_1}{dt} = - k_{12} \bullet A_1 \qquad \text{(Eq. 1)}$$

$$\frac{dA_2}{dt} = k_{12} \bullet A_1 - k_{25} \bullet A_2 - k_{23} \bullet A_2 + k_{32} \bullet A_3 - k_{24} \bullet A_2 \bullet \left(B_{MAX} - A_4 - A_6\right) + k_{24} \bullet A_4 + k_{72} \bullet A_7 \qquad \text{(Eq. 2)}$$

$$\frac{dA_3}{dt} = k_{23} \bullet A_2 - k_{32} \bullet A_3 \qquad \text{(Eq. 3)}$$

$$\frac{dA_4}{dt} = k_{24} \bullet A_2 \bullet \left(B_{MAX} - A_4 - A_6\right) - k_{42} \bullet A_4 \qquad \text{(Eq. 4)}$$

$$\frac{dA_5}{dt} = k_{25} \bullet A_2 - k_{56} \bullet A_5 \bullet \left(B_{MAX} - A_4 - A_6\right) + k_{65} \bullet A_6 - k_{50} \bullet A_5 - k_{57} \bullet A_5 \qquad \text{(Eq. 5)}$$

$$\frac{dA_6}{dt} = k_{56} \bullet A_5 \bullet \left(B_{MAX} - A_4 - A_6\right) - k_{65} \bullet A_6 \qquad \text{(Eq. 6)}$$

$$\frac{dA_7}{dt} = k_{57} \bullet A_5 - k_{72} \bullet A_7 \qquad \text{(Eq. 7)}$$

where A_n represents the amount of a substance in the n^{th} compartment and k_{nm} represents the rate constant for transport between compartment n and compartment m. Values for the dissociation constant (K_D) can be calculated from this differential equations by dividing k_{42} by k_{24} for MPA and k_{65} by k_{56} for MPAG. The concentrations of tMPA and tMPAG were modeled as the sum of the unbound and bound concentrations.

Furthermore, to describe the EHC of the drug a gallbladder compartment was added, which is responsible for the reconversion of fMPAG into fMPA (Fig.1). It was evaluated whether transport of fMPAG to the gallbladder was best described as a zero-order or first-order process. The gallbladder emptied into the central compartment of fMPA at a certain time point postdose.[31]

Covariate model

Finally, to explain IPV, relationships were investigated between pharmacokinetic parameters and patient characteristics known to influence MPA pharmacokinetics. Covariates assessed were renal function, plasma albumin concentration, and cyclosporine comedication. Renal function was tested by calculation of the creatinine clearance (CrCL) according to Cockcroft and Gault.[32] Continuous covariates, such as albumin concentration (Alb) were modeled by using an exponential model, as shown in equation 8.

$$\theta_i = \theta_{pop} \bullet (Alb / 0.5)^{\theta_{alb}} \qquad \text{(Eq. 8)}$$

where θ_i represents the parameter for the i^{th} individual, θ_{pop} is the population value with Alb=0.5 mmol/L, and θ_{alb} is an exponent determining the shape of the correlation. Categorical variables, such as cyclosporine comedication, were modeled proportionally as shown in equation 9.

$$\theta_i = \theta_{pop} \bullet \theta_{CsA}^{CsA} \qquad \text{(Eq. 9)}$$

where CsA=1 for patients cotreated with cyclosporine and CsA=0 for patients not cotreated with cyclosporine, and θ_{CsA} represents the fractional change of the parameter in patients cotreated with cyclosporine.

Model validation

The final model was validated by a visual predictive check.[33] Data sets (n=50) were simulated from the original data set using the final model. Per time point, the dose-corrected median simulated concentrations plus 95-percentile intervals were compared graphically with the observed concentrations for fMPA, tMPA, fMPAG, and tMPAG separately. The data set was analyzed separately for different categories of the covariates included in the final model.

Simulation study

The final model was used to examine the impact of changes in covariates on the pharmacokinetics of MPA and MPAG. Simulations were performed to demonstrate how the disposition of MPA and MPAG was affected by clinically relevant changes in renal function, plasma albumin

concentration and cyclosporine comedication. Simulations were performed with the final model for 50 patients treated with 1 g MMF twice daily and cyclosporine, and for 50 patients treated with 1 g MMF twice daily and tacrolimus. CrCL was initially set on 50 mL/min, and was decreased to 30 and 10 mL/min. The initial value for albumin concentration was 0.5 mmol/L, which was varied to 0.4 and 0.6 mmol/L. Changes in concentration-time profiles, free fraction and AUC_{0-12} values of fMPA, tMPA, fMPAG and tMPAG were assessed.

Results

Patients

The data set contained 489 tMPA, 489 fMPA, 488 tMPAG, and 210 fMPAG plasma concentrations obtained from 75 patients cotreated with cyclosporine or tacrolimus. Each patient participated in one or two pharmacokinetic assessments at different time points after transplantation. In total, 93 concentration-time profiles were used for the analysis. Patient characteristics are described in Table I.

Table I: Patient characteristics

Cotreatment	Cyclosporine (n=48 profiles)	Tacrolimus (n=45 profiles)
Gender (male/female)*	30/17	18/10
Age (years)*	51 (21-70)	53 (19-76)
Weight (kg)*	67 (42-99)	78 (44-113)
Number of patients with DGF *	4	11
Number of diabetic patients pretransplantation*	2	2
Time after transplantation (days)	11 (7-155)	11 (4-115)
Plasma albumin (mmol/L)	0.51 (0.38-0.61)	0.51 (0.35-0.68)
Creatinine clearance (mL/min)#	44 (8-107)	45 (8-154)
Heamoglobine (mmol Fe/L)	9.6 (6.9-13.0)	8.0 (4.2-13.0)
ASAT (U/L)	15 (6-50)	19 (6-236)
ALAT (U/L)	20 (7-155)	27 (7-534)
MMF dose (mg bid)	1350 (400-2200)	1000 (500-1500)
CNI daily dose (mg)	512.5 (250-1125)	8 (1-20)
CNI predose concentration (µg/L)	267.5 (21.0-619.1)	10.0 (1.5-30.0)

Parameters are presented as median (range) of all profiles, for patients treated with the calcineurin inhibitors (CNI) cyclosporine and tacrolimus. DGF, delayed graft function; ASAT, aspartate aminotransferase; ALAT, alanine transaminase; MMF, mycophenolate mofetil.

* parameters for all patients, of which some participated in two pharmacokinetic assessments

calculated with Cockcroft&Gault-formula

Pharmacokinetic analysis

Compartmental model with protein binding

The final model is shown in Figure 1, corresponding typical population pharmacokinetic estimates are summarized in Table II. The standard errors of the estimated parameters could not be obtained due to rounding errors of the final run. The pharmacokinetics of fMPA were

described with a two-compartment model, containing a central- and peripheral volume of distribution. fMPA from the central compartment bound to the protein binding sites. The number of binding sites (B_{MAX}) was limited, which resulted in a saturable binding process. The pharmacokinetics of fMPAG were modeled with one, central compartment. From this compartment, fMPAG bound to the same binding sites as fMPA, which resulted in competitive binding. Due to this competition MPA and MPAG were able to displace each other from the protein binding sites. Inclusion of the competitive binding between MPA and MPAG significantly improved the model compared to the model without competition (ΔOFV=- 37). The protein binding and unbinding rate constants of fMPA (k_{24} and k_{42}) and fMPAG (k_{56} and k_{65}) provide information on the affinity of both substances to the protein binding sites, which is higher for fMPA (K_D=1100 µmol) than for fMPAG (K_D=7000 µmol). The interpatient variability (IPV) in protein binding was described for B_{MAX} using an exponential error model.

Enterohepatic recirculation

The EHC was modeled by using a gallbladder compartment (Fig. 1, compartment 7). Transport of fMPAG from the central compartment to the gallbladder compartment was characterized using the first order rate constant k_{57}. Emptying of the gallbladder into the central compartment of fMPA occurred at a certain time point (T_{GB}) with rate constant k_{72} and duration D_{GB}. Unfortunately, insufficient data were collected between 4 and 10 hours postdose, the period at which the gallbladder is expected to empty. As a result, the parameters describing the

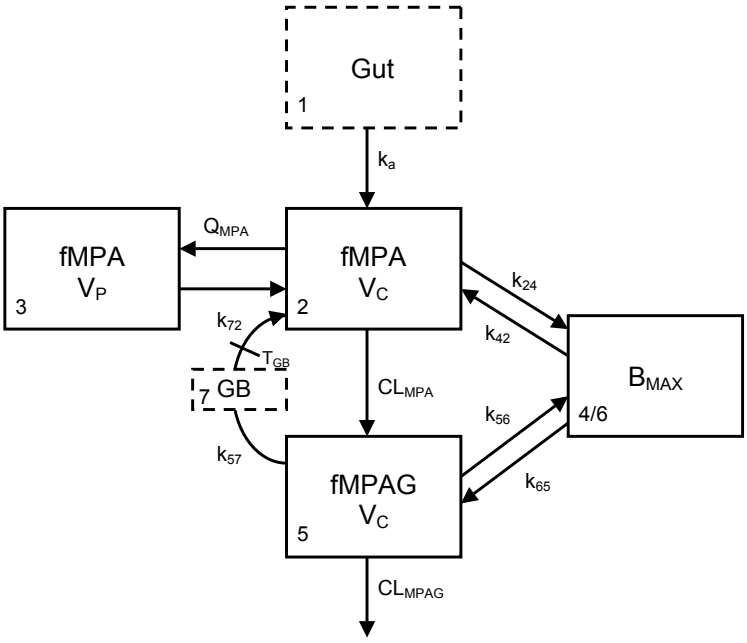

Figure 1: Graphical representation of the final model. MPA and MPAG bind competitively to the protein binding sites. fMPA is cleared to fMPAG by first-order elimination. fMPAG is eliminates by a first-order process (CL_{MPAG}), or undergoes enterohepatic recirculation via the gallbladder compartment.

Table II: Parameter estimates of the pharmacokinetic model

Parameter	Value	IPV (%)
T_{LAG} (h)	0.231	161
k_a (h^{-1})	4.00 *	
V_c fMPA (L)	189	116
CL fMPA (L/h)	747	97
V_p fMPA (L)	34300	
Q fMPA (L/h)	2010	
k_{24} ($h^{-1}\mu mol^{-1}$)	0.153	
B_{MAX} (µmol)	35100	48
k_{42} (h^{-1})	169	
V_c fMPAG (L)	8.56	
k_{56} ($h^{-1}\mu mol^{-1}$)	0.0133	
k_{65} (h^{-1})	93.1	
CL fMPAG (L/h)	4.75	106
T_{GB} (h)	7.90	141 *
D_{GB} (h)	1.00 *	
k_{72} (h^{-1})	10.0 *	
k_{57} (h^{-1})	0.0796	71 *
Residual variability		
Additive error tMPA (mmol/L)	0.52	
Additive error fMPA (mmol/L)	0.993	
Additive error tMPAG (mmol/L)	0.186	
Additive error fMPAG (mmol/L)	0.551	
Covariate effects		
CrCL on CL fMPAG	1.36	
Albumin on B_{MAX}	1.39	
CsA on k_{57}	0.002	

IPV, interpatientvariability; T_{LAG}, lag-time; k_a, first order absorption rate constant; V_c, volume of distribution of the central compartment; CL, clearance; V_p, volume of distribution of the peripheral compartment; Q, intercompartmental clearance; k_{nm}, rate constant between compartment n and m; B_{MAX}, maximum number of protein binding sites; T_{GB}, time after oral dose of gallbladder emptying; D_{GB}, duration of gallbladder emptying; CrCL, creatinine clearance; MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide; t, total concentration; f, unbound concentration; CsA, cyclosporine.

* These parameters were fixed in the final model.

gallbladder emptying, D_{GB} and k_{72}, were fixed at 1 h and 10 h^{-1}. IPV was described with an additional error model for T_{GB} and with an exponential error model for k_{57}. However, the IPV of k_{57} had to be fixed at 71%, to prevent variability on EHC to take on extreme values. This value was based on the variance in EHC from 10-60% as described in literature.[9] In comparison with a model without EHC (k_{57}=0 h^{-1}), addition of the EHC significantly improved the model (ΔOFV=-43).

Covariate analysis

Decreased plasma albumin concentrations are known to be associated with reduced protein binding of MPA.[34] Inclusion of plasma albumin concentration as covariate of B_{MAX} (equation 3) significantly improved the model (ΔOFV=-26). The IPV of fMPA CL, fMPAG CL and B_{MAX} decreased slightly with 1, 6 and 1%, respectively. A decrease in albumin from 0.6 to 0.4 mmol/L

resulted in a decrease in the number of binding sites from 45200 to 25700 µmol, as is graphically shown in Figure 2A.

Impaired renal function reduced renal clearance of MPAG.[35] In the present study, a significant correlation was observed between CrCL and fMPAG CL. Introduction of CrCL improved the goodness of the fit (ΔOFV=-48). The IPV of fMPA CL, fMPAG CL and B_{MAX} decreased with 25, 57 and 17%, respectively. A decrease in CrCL from 45 to 25 mL/min resulted in a decrease from 4.75 to 2.14 L/h in clearance of fMPAG (Fig. 2B). No correlation was seen between CrCL and fMPA CL.

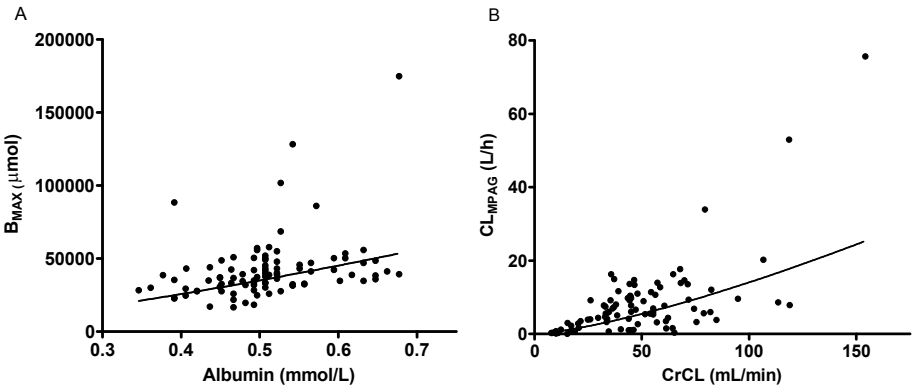

Figure 2: Correlation of pharmacokinetic parameters versus covariate effects for (a) the number of protein binding sites (B_{MAX}) and plasma albumin level, and (b) fMPAG clearance and creatinine clearance (CrCL). The line represents the estimated correlation between the pharmacokinetic parameter and the covariate.

Cyclosporine decreases the EHC of MPAG by inhibition of MRP2.[20] In the present study, implementation of cyclosporine as covariate on k_{57} significantly improved the model (ΔOFV=-17). In patients cotreated with cyclosporine k_{57} is very small with a value of 0.000159 h^{-1} compared to 0.0796 h^{-1} in patients cotreated with tacrolimus. Inclusion of cyclosporine as a covariate on fMPA clearance did not significantly improve the model further.

Evaluation of the final model

The goodness-of-fit plots (Fig. 3) of the final model were evaluated for tMPA, fMPA, tMPAG and fMPAG separately. The scatter plots of predicted and individually predicted versus observed concentrations showed no structural bias, except for a small underprediction of the maximum concentration of tMPA and fMPA. The weighted residuals exhibited a homogeneous distribution over the whole sampling period (data not shown).

Figure 4 shows the results of the visual predictive check for the final model. The results are presented separately for patients cotreated with cyclosporine and tacrolimus. Good agreement between the simulated and observed concentrations was apparent at all sampling time points. However, there seemed to be a small underprediction of the maximum tMPA and fMPA concentration. The visual predictive check was performed separately for patients

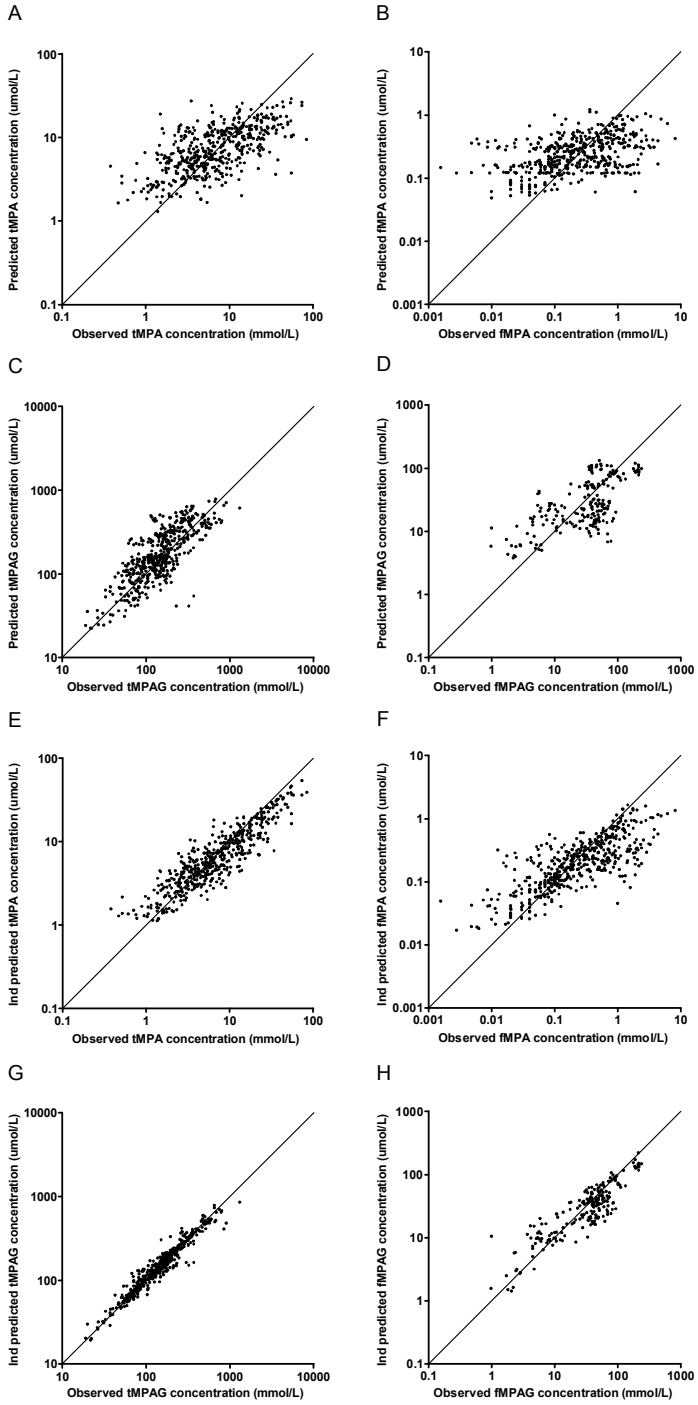

Figure 3: Goodness-of-fit plots of observed versus population predicted concentrations of (a) tMPA, (b) fMPA, (c) tMPAG and (d) fMPAG, and observed versus individually predicted concentrations of (e) tMPA, (f) fMPA, (g) tMPAG and (h) fMPAG. In the plots the line of identity is presented.

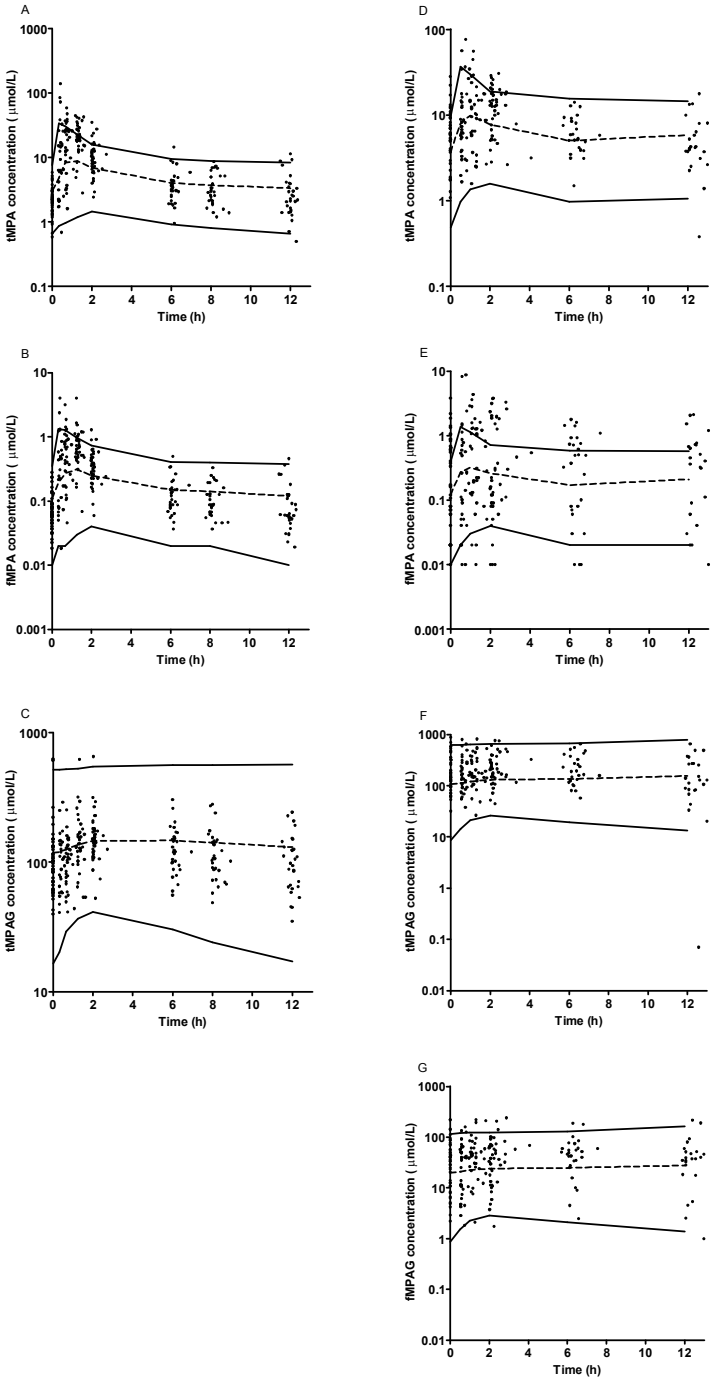

Figure 4: Visual predictive check of the comparison of median (dashed line) with 95-percentile interval (solid lines) of 50 simulated data sets and the observed concentrations (dots). Concentrations are corrected to an MMF dose of 1 g. Concentrations of (a) tMPA, (b) fMPA and (c) tMPAG for patients cotreated with CsA, and (d) tMPA, (e) fMPA, (f) tMPAG and (g) fMPAG for patients cotreated with tacrolimus.

with CrCL<30 mL/min, CrCL 30-50 mL/min and CrCL>50 mL/min as well as for patients with albumin concentrations <0.5 mmol/L and >0.5 mmol/L. The results of these visual predictive checks on these selections gave similar results (data not shown).

Simulations

The pharmacokinetic profiles of tMPA, fMPA, tMPAG and fMPAG were simulated for 50 renal transplant recipients receiving 1 gram MMF twice daily using the final model. Covariate effects were set at typical values: plasma albumin concentration 0.5 mmol/L, CrCL 50 mL/min and comedication was cyclosporine or tacrolimus. Subsequently, the effect of a change in plasma albumin concentration or CrCL was evaluated.

A decrease in plasma albumin concentrations from 0.6 to 0.4 mmol/L, resulted in decreased tMPA concentrations, whereas the effect on fMPA, tMPAG and fMPAG concentrations was small (Fig.5). The free fraction of both, MPA and MPAG, almost doubled when albumin concentrations decreased from 0.6 to 0.4 mmol/L (Fig.6). This effect was larger for patients cotreated with cyclosporine (MPA: 2.4 to 5.3%, MPAG: 13.7 to 26.1%) than for patients cotreated with tacrolimus (MPA: 2.7 to 4.1%, MPAG: 14.9 to 21.3%). Furthermore, tMPA AUC_{0-12} values were decreased in patients with low albumin concentrations (Fig.7). A decrease in albumin concentrations

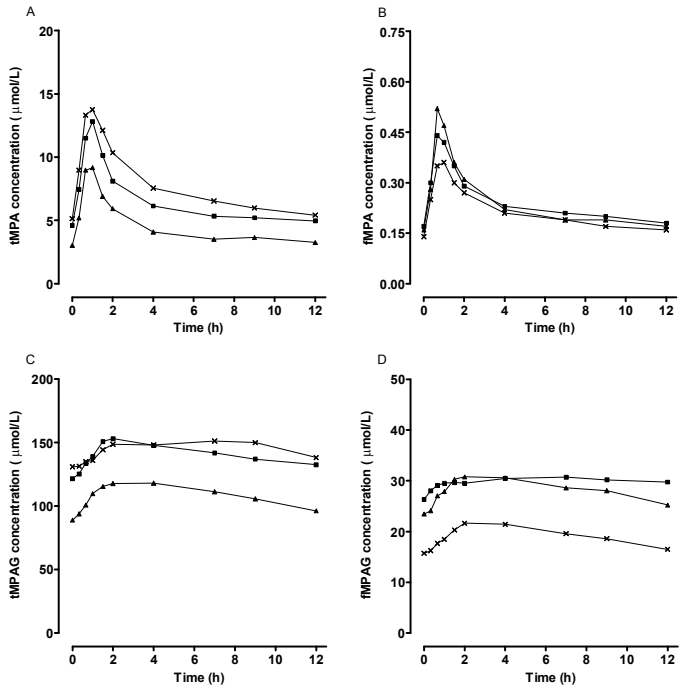

Figure 5: Influence of albumin levels on pharmacokinetic profiles. Median concentration-time profiles of (a) tMPA, (b) fMPA, (c) tMPAG and (d) fMPAG in patients cotreated with CsA and concentration-time profiles of (e) tMPA, (f) fMPA, (g) tMPAG and (h) fMPAG in patients cotreated with tacrolimus. Concentration-time profiles were simulated for 50 patients with albumin levels of (▲) 0.4 mmol/L, (■) 0.5 mmol/L and (x) 0.6 mmol/L.

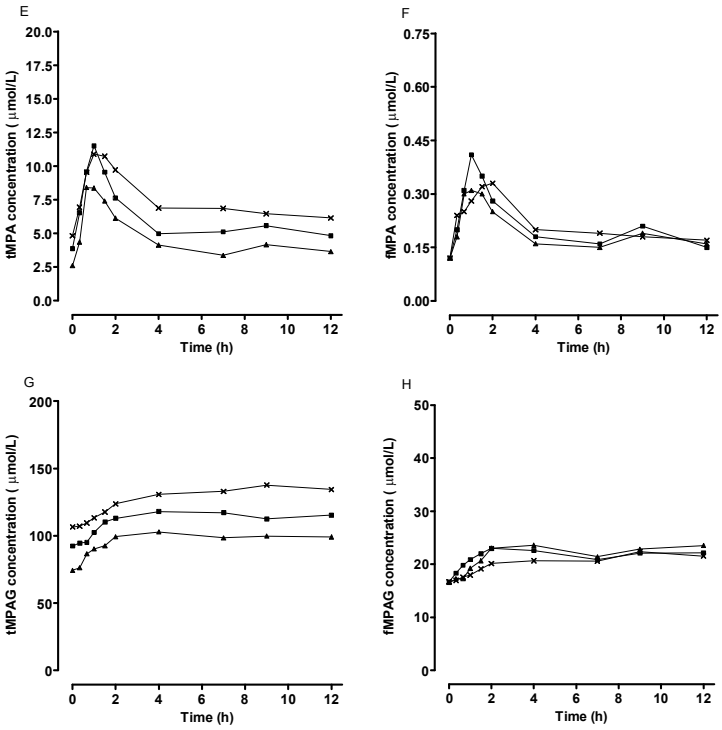

Figure 5. continued

from 0.6 to 0.4 mmol/L, resulted in a decrease in mean tMPA AUC from 30.1 to 17.7 mg*h/L in patients cotreated with cyclosporine and from 31.1 to 20.4 mg*h/L in patients cotreated with tacrolimus. Consequently, a decrease in albumin concentrations may cause a major underexposure to tMPA, as the lower limit of the therapeutic window of tMPA AUC is 30 mg*h/L. The exposure to fMPA, tMPAG and fMPAG remained however stable, as indicated by Figure 7B-D.

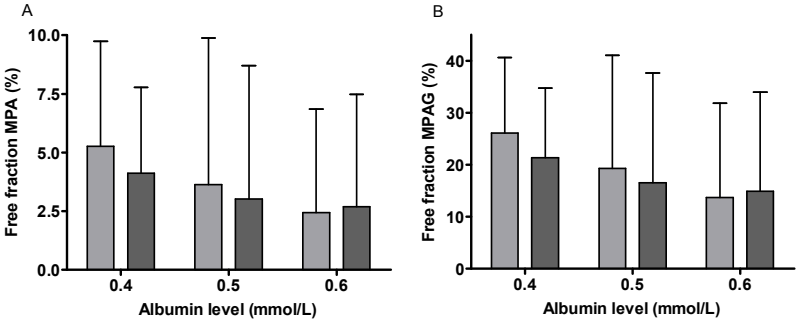

Figure 6: Influence of albumin levels on the free fraction of (a) MPA and (b) MPAG. Free fractions are presented as median and 95-percentile range of 50 simulated patients cotreated with CsA (light) or tacrolimus (dark).

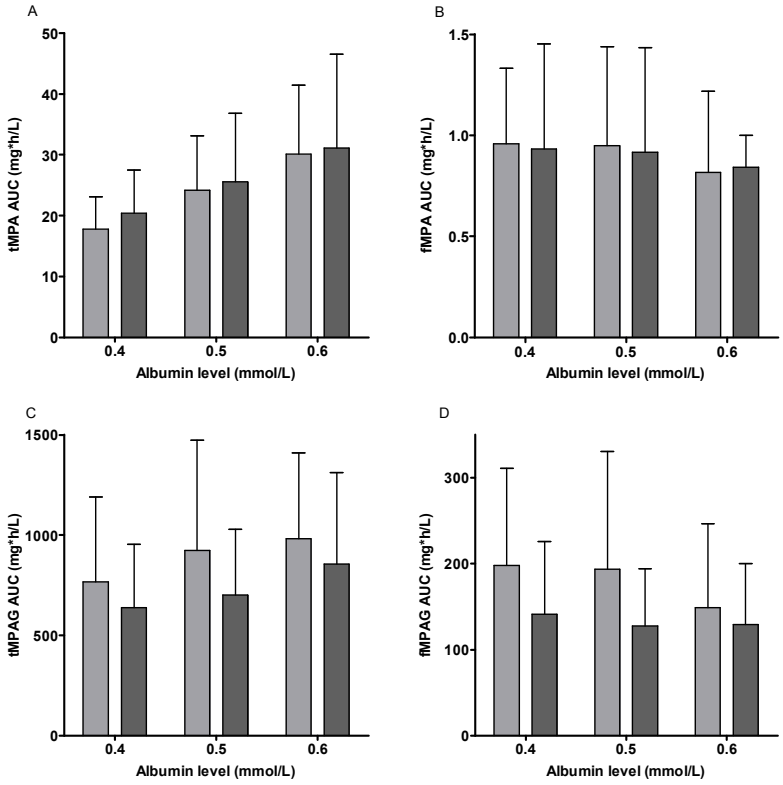

Figure 7: Influence of albumin levels on exposure of (a) tMPA, (b) fMPA, (c) tMPAG and (d) fMPAG for patients cotreated with CsA (light) or tacrolimus (dark). AUCs are presented as mean and 95% confidence interval of 50 simulated patients.

A decrease in renal function, characterized by a change in CrCL from 50 to 10 mL/min, had a large impact on the MPAG concentrations (Fig.8). Both, tMPAG and fMPAG concentrations increased, especially in patients cotreated with cyclosporine. The changes in the concentration-time profiles of tMPA and fMPA were small. A slight difference was seen at the end of the curve, where the tacrolimus cotreated patients showed a higher EHC with impaired renal function (Fig.8 E+F). This was caused by increased concentrations of fMPAG. The latter undergoes EHC and is subsequently converted to MPA. tMPAG and fMPAG AUC values were higher in patients with impaired renal function (Fig.9). Corresponding AUC values for tMPAG increased from 831 to 3794 mg*h/L for patients cotreated with cyclosporine and from 723 to 2647 mg*h/L in patients with tacrolimus as comedication. A decrease in CrCL from 50 to 10 mL/min resulted in increased tMPA AUC in patients treated with tacrolimus (25.1 to 31.6 mg*h/L). The opposite effect was seen in patients treated with cyclosporine, in which tMPA AUC values decreased from 23.9 to 21.5 mg*h/L. Furthermore, fMPA AUC values remained stable when renal function decreased, except for a small increase in fMPA AUC in patients with CrCL of 10 mL/min and tacrolimus as comedication. The free fraction of both MPA and MPAG increased in patients treated with cyclosporine (Fig.10). In tacrolimus treated patients no difference in free fraction was seen.

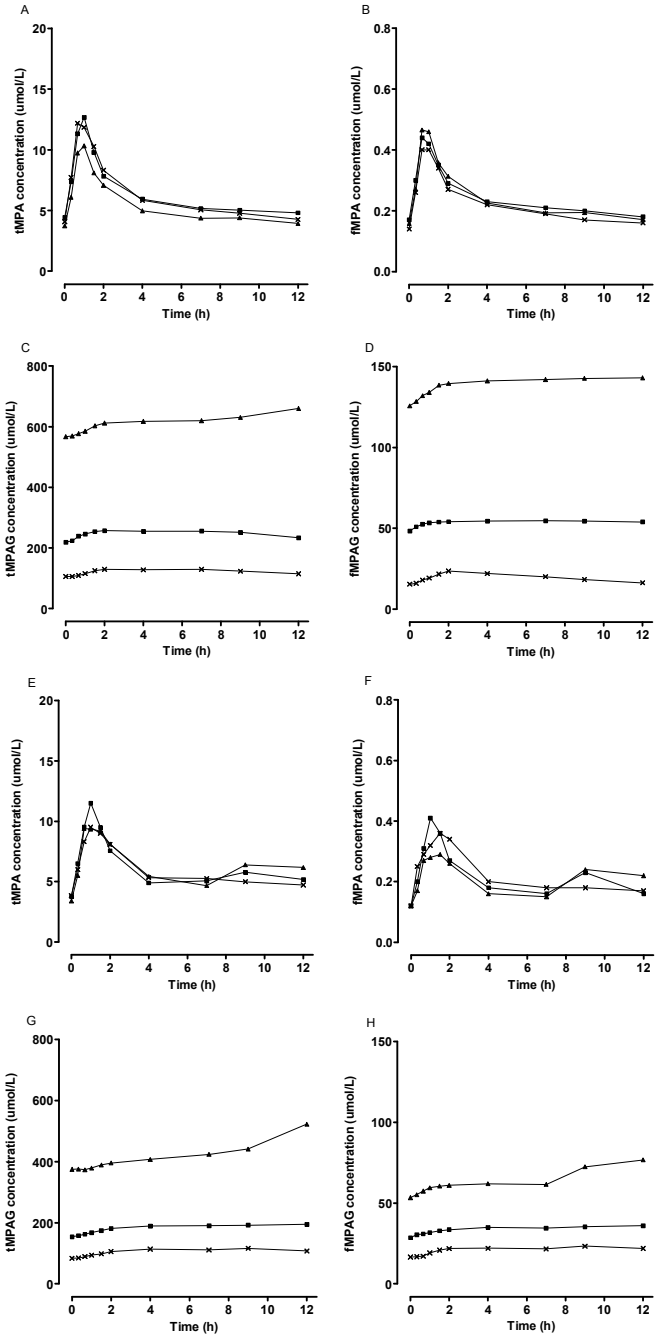

Figure 8: Influence of creatinine clearance (CrCL) on pharmacokinetic profiles. Median concentration-time profiles of (a) tMPA, (b) fMPA, (c) tMPAG, and (d) fMPAG in patients cotreated with CsA and concentration-time profiles of (e) tMPA, (f) fMPA, (g) tMPAG, and (h) fMPAG in patients cotreated with tacrolimus. Concentration-time profiles were simulated for 50 patients with CrCL of (▲) 10 mL/min, (■) 30 mL/min and (x) 50 mL/min.

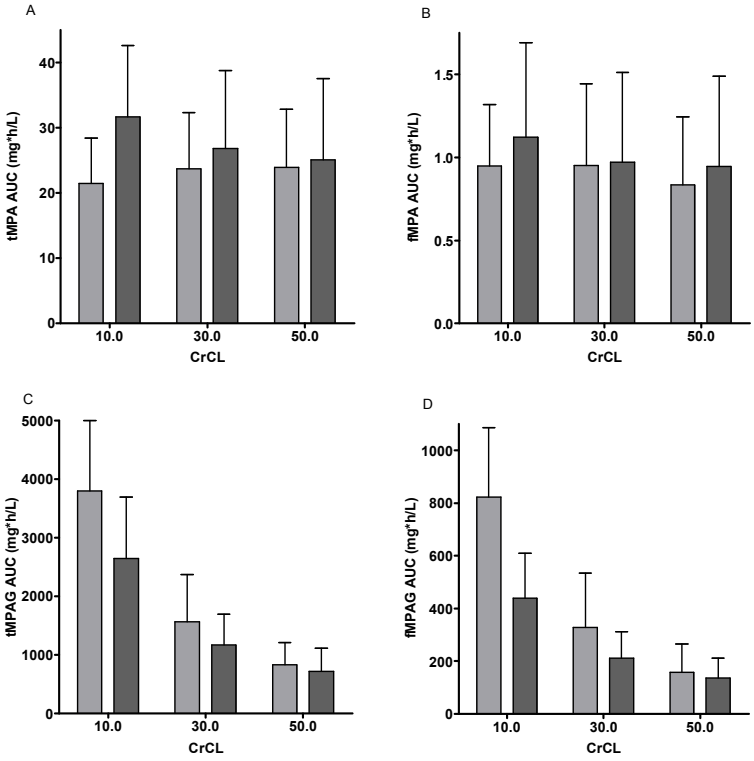

Figure 9: Influence of creatinine clearance (CrCL) on exposure of (a) tMPA, (b) fMPA, (c) tMPAG and (d) fMPAG for patients cotreated with CsA (light) or tacrolimus (dark). AUCs are presented as mean and 95% confidence interval of 50 simulated patients.

Discussion

A population pharmacokinetic model has been developed describing the protein binding of MPA and the main metabolite MPAG for renal transplant recipients receiving MMF and cyclosporine or tacrolimus (Fig.1). The model provided a semi-mechanistic explanation for increase of tMPA AUC and decrease in MPA free fraction with increasing plasma albumin concentrations, renal function and tacrolimus comedication. Simulations with the final model can be used to provide information about the effect of changes in albumin concentration or renal function on tMPA and fMPA exposure.

Van Hest et al[36] have previously published an empirical model describing the protein binding of MPA. The current model is superior compared to the model of van Hest et al due to a more mechanism-based character of the model. Our model described the protein binding process with a mass balance, based on physiological processes. Mechanism-based models have much better properties for extrapolation and prediction than empirical models.[37]

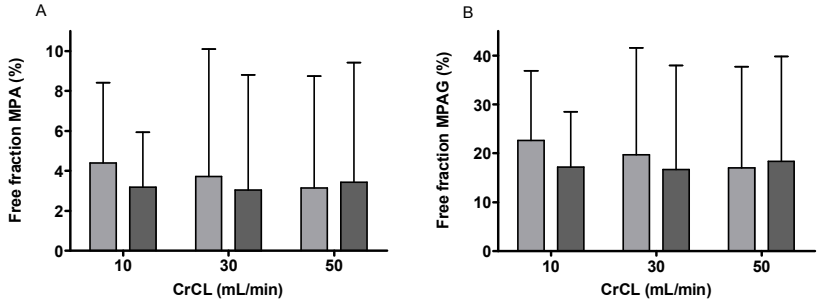

Figure 10: Influence of creatinine clearance (CrCL) on the free fraction of (a) MPA and (b) MPAG. Free fractions are presented as median and 95-percentile range of 50 simulated patients cotreated with CsA (light) or tacrolimus (dark).

In the current model, a competitive protein binding process was incorporated, which described the interaction between MPA and MPAG. Increasing MPAG concentrations were associated with increased free fractions of MPA and decreased tMPA exposure. The model did not only describe the protein binding of MPA, but also of the main metabolite MPAG. This was possible due to availability of fMPAG concentration-time profiles, which were not available in the study of van Hest et al[36] This addition allowed the protein binding process to be competitive between MPA and MPAG.

Due to the complexity of the model, runtimes were very long (55 h for the final run). The final run did not minimize successfully, due to rounding errors. As a result, no standard errors of the parameter estimates were obtained. Other methods to obtain information about the accuracy of the estimated parameters, like the bootstrap resampling method and the jackknife, could not be used because of the high computational intensity. However, the visual predictive check revealed a good agreement between the simulated and observed concentrations at all sampling time points, which indicates that the estimations of the pharmacokinetic parameters are reasonable.

The parameter B_{MAX} is defined as the maximum number of binding sites, to which MPA and MPAG could bind. In theory, if the volume of plasma is roughly 3L and albumin (median: 510 µmol/L) has at least one binding site, then the total number of binding sites in plasma should be ≥510 µmol/L*3L=1530 µmol. In the model, B_{MAX} was estimated to be 35100 µmol, which is indeed larger than 1530 µmol. B_{MAX} may be larger due to the fact that other proteins are also able to bind MPA or that each albumin molecule binds more than one MPA molecule. Albumin is not confined to plasma, but is continuously filtered into interstitial fluid, and then returned to plasma via the thoracic duct. Albumin-bound drugs may therefore be present in plasma, which contains 40% of albumin in the body, and in interstitial fluid, which contains the remaining 60%.[38] The latter may increase the available number of binding sites as well.

In the model, the maximum number of binding sites available for MPA and MPAG was restricted. The number of binding sites was correlated with the plasma albumin concentra-

tion (Fig.2A). A decrease in plasma albumin concentrations resulted in less binding sites, causing an increase in the free fraction of MPA and MPAG (Fig.6), which has previously been reported.[27, 34] Due to this increase in the free fraction, relatively more fMPA is available for clearance, resulting in a decreased tMPA AUC (Fig.7). However, the fMPA AUC was unaffected. The phenomenon that free concentrations are independent of protein binding is characteristic for drugs with a low extraction ratio.[39] The theoretical hepatic extraction ratio of MPA is low (0.20).[40] The simulations showed that changes in albumin concentrations cause clinically relevant changes in tMPA AUC. A decrease in albumin concentration from 0.6 to 0.4 mmol/L, resulted in a decrease in tMPA AUC from 30.1 to 17.7 mg*h/L in patients treated with cyclosporine and from 31.1 to 20.4 mg*h/L in patients treated with tacrolimus. This implies that the tMPA AUC could drop below the minimal effective value of 30 mg*h/L and that the MMF dose should be increased. This correlation between tMPA AUC and albumin concentrations is previously seen.[34, 41] In contrast, the fMPA AUC is almost unaffected by the change in albumin concentration. A decrease in albumin concentration from 0.6 to 0.4 mmol/L resulted in an increase in fMPA AUC from 0.82 to 0.96 mg*h/L for patients cotreated with cyclosporine and from 0.84 to 0.93 mg*h/L in patients cotreated with tacrolimus.

The simulations showed that changes in CrCL have major effects on both tMPAG and fMPAG exposure, but the effects on tMPA and fMPA exposure are smaller. The CNI used in patients with impaired renal function leads to a differential effect on tMPA and fMPA exposure. In case of cyclosporine, tMPA AUC decreased and fMPA AUC remains the same. While in patients cotreated with tacrolimus, an increased exposure to tMPA and a small increase in fMPA AUC was seen when CrCL decreased to 10 mL/min. These opposing effects can be explained as follows: In patients cotreated with tacrolimus, impaired renal function leads to accumulation of MPAG (Fig.2B). Accumulating MPAG concentrations result in increased transport of MPAG to the gallbladder, leading to increased recirculation of MPAG to MPA. Because of the extra recirculation, MPAG does not accumulate to an extent where it can displace MPA from its protein binding sites. The result is increased tMPA and fMPA (Fig.9) due to extra recirculation and no change in unbound fraction of MPA. In patients cotreated with cyclosporine, the accumulated MPAG following impaired renal function can not be compensated for by increased recirculation because cyclosporine minimizes EHC due to inhibition of MRP2.[20] As a result MPAG displaces MPA from its protein binding sites, leading to an increased unbound fraction of MPA (Fig.10).[11, 35] The increased fMPA exposure is immediately compensated for by an increase in MPA glucuronidation as MPA is a drug with a low extraction ratio.[40] The result is decreased tMPA exposure, unchanged fMPA exposure and an increased MPA unbound fraction. The simulated effects of CrCL on exposure to tMPA and fMPA in patients treated with cyclosporine or tacrolimus are in accordance with previously published results.[10, 31, 41-42]

In vitro analysis showed that fMPA is the pharmacologically active compound, which is responsible for inhibition of IMPDH.[27] Patients with elevated fMPA exposure have an increased risk for leucopenia and infections.[23-24] However, although a relationship between fMPA exposure and the risk for acute rejection should be expected, it has not been demonstrated

yet. On the other hand, a correlation between tMPA exposure and the risk for acute rejection has been reported.[6, 21-22] Clearly, more information is needed about the relationship between fMPA exposure and the risk for acute rejection and side effects to interpret the clinical effect of changes in protein binding of MPA. In general, fMPA is thought to be responsible for the immunosuppressive effect[8-9, 27] and changes in fMPA are supposed to be clinically relevant. Alterations in both albumin concentrations and renal function have little effect on fMPA AUC and have thereby little clinical relevance. However, special attention is necessary in patients with impaired renal function cotreated with tacrolimus as the increased fMPAG can cause elevated exposure to both tMPA and fMPA.

In conclusion, this model describes the protein binding of both, MPA and its main metabolite MPAG, and the relationship with albumin concentrations, renal function and cyclosporine. When albumin concentrations decrease, tMPA exposure decreases, but fMPA exposure remains unaffected. The increase in MPAG due to impaired renal function is followed by a decrease in tMPA in patients cotreated with cyclosporine and by an increase in tMPA in patients cotreated with tacrolimus. Again, fMPA exposure is hardly affected by the changes in renal function. Changes in protein binding, caused by alterations in albumin concentrations or renal function, will not or hardly influence the exposure of a patient to the probably active agent fMPA. Therefore, changes in protein binding have little clinical relevance if fMPA is indeed the biologically active fraction.

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Chapter 2.2

Nonlinear relationship between mycophenolate mofetil dose and mycophenolic exposure: implications for therapeutic drug monitoring

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Abstract

Mycophenolate mofetil (MMF) is an immunosuppressive drug used in renal transplant patients. Upon oral administration it is hydrolyzed to the active agent mycophenolic acid (MPA). In renal transplant recipients, MMF therapy is optimal when the AUC of MPA is 30-60 mg*h/L. When MMF doses are adjusted, a linear relationship between dose and MPA exposure is assumed. In this study, the linearity of MMF pharmacokinetics was investigated. MPA concentration-time profiles from renal transplant recipients cotreated with cyclosporine (n=140) or tacrolimus (n=101) were analyzed retrospectively using nonlinear mixed-effects modeling. In the developed population pharmacokinetic model MPA clearance and the central volume of distribution were correlated with cyclosporine co-administration and time posttransplantation. The pharmacokinetics of MPA were not linear. Bioavailability decreased with increasing MMF doses. Compared to an MMF dose of 1000 mg (=100%), relative bioavailibility was 123, 111, 94 and 90% in patients receiving MMF doses of 250, 500, 1500 and 2000 mg in combination with cyclosporine ($p<0.001$); respective values in tacrolimus cotreated patients were 176, 133, 85 and 76% ($p<0.001$). Due to the decreasing relative bioavailability, MPA exposure will increase less than proportional with increasing MMF doses. In conclusion, MMF exhibits nonlinear pharmacokinetics. This should be taken into account when performing therapeutic drug monitoring.

Introduction

Mycophenolate mofetil (MMF) is an immunosuppressive drug used in renal transplant patients. Upon oral administration it is hydrolyzed to the active agent mycophenolic acid (MPA). In 2007, Le Meur et al published the results of the APOMYGRE study, that showed that therapeutic drug monitoring (TDM) of MPA reduces the risk of treatment failure and acute rejection in renal allograft recipients without an increase in adverse events.[1] Also others have investigated the value of performing TDM for MPA in renal transplant patients.[2] When the MMF dose is adjusted most physicians assume a linear relationship between dose and MPA exposure, i.e. linear pharmacokinetics. So far, linear pharmacokinetics have, however, not been assessed. This may be caused by the fact that MPA exerts complex pharmacokinetic properties.[3] Furthermore, factors complicating the assessment of linear pharmacokinetics are the changes in MPA clearance in the first three months after transplantation, the influence of co-medication and the within-patient variability.[4-5]

In an early dose-ranging study in cyclosporine treated patients Sollinger et al compared a 1000 mg MMF bid dose with a 1500 mg MMF bid dose, and found that with the 50% higher dose the area under the MPA concentration versus time curve (AUC) was also about 50% higher (12.3 ± 5.8 mg*h/L vs 19.5 ± 13.9 mg*h/L).[6] These results are in accordance with a linear relationship between MMF dose and MPA AUC. In the randomized concentration-controlled trial (RCCT), a total of 154 cyclosporine cotreated adult recipients of a deceased kidney graft were randomly allocated to receive MMF treatment targeted at three predefined MPA AUC values. During the first 6 months after transplantation, plasma samples for nine AUCs were collected.[7] At day 3 posttransplantation the median assigned daily doses of MMF were 0.90, 1.90 and 3.40 grams and corresponding MPA AUC values were 13.9, 24.6, and 39.1 mg*h/L. In this study, the increase in AUC is less than proportional, which supports nonlinear pharmacokinetics for MMF. With a convex relationship between dose and exposure, increasement of the MMF dose may produce a less than expected increase in MPA exposure. Alternatively, the decrease of MPA AUC may be overestimated when the MMF dose is decreased. Clearly, this may have significant implications for TDM of MPA.

In this study, a population pharmacokinetic model was developed in which the effect of calcineurin inhibitor cotreatment on MPA disposition and the time-dependency of the pharmacokinetics was quantified. The developed population model was used to evaluate the relationship between MMF dose and the pharmacokinetic parameters of MPA.

Methods

Patients

MPA plasma concentration-time profiles obtained from renal transplant recipients treated with MMF and cyclosporine (n=140) or MMF and tacrolimus (n=101) were combined and analysed simultaneously. The data were obtained from the RCCT and the IMPDH-activity study, which have been published earlier.[7-8] In the RCCT study[7], de novo renal transplant recipients were divided into three MPA AUC target groups. All patients in this study received cyclosporine and corticosteroids as concomitant immunosuppressive therapy. In this study plasma MPA concentrations were measured at day 3, 7, 11, 21, 28, 56, 84, 112 and 140 after transplantation. On days 3, 7 and 11 posttransplantation, sample times were predose and 0.33, 0.66, 1.25, 2, 6, 8 and 12 hours after oral intake of MMF. On the remaining occasions, sample times were predose and 0.33, 0.66, 1.25 and 2 hours postdose. MPA concentrations were measured using high-performance liquid chromatography (HPLC).[9] The MMF dose was adjusted on basis of the measured MPA concentrations. In the IMPDH-activity study[10], de novo renal transplant recipients started with 1000 mg MMF twice daily, combined with tacrolimus and corticosteroids. The MMF dose was adjusted based on clinical evaluations. In the IMPDH-activity study MPA plasma concentrations were measured at day 6, 21, 49 and 140 after transplantation. On day 6, samples were taken predose and 0.5, 1, 2, 6 and 12 hours postdose. On the remaining occasions, sample times were predose and 0.5 and 2 hours after oral intake of MMF. Concentrations were measured using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.[8]

Pharmacokinetic analysis

Due to the heterogenous nature of the data the population pharmacokinetic analysis was performed by using nonlinear mixed-effects modeling (NONMEM). By application of this technique typical pharmacokinetic parameters and their relationship with covariates can be estimated (fixed effects) as well as the inter- and intrapatient variability (random effects).

Basic model

All data were analyzed simultaneously using NONMEM (Verion VI, level 1.0; GloboMax LLC, Ellicott City, MD). Since MPA plasma concentrations were modelled, MMF doses were converted to the equivalent MPA content by multiplying the MMF dose by 0.739. Data were logarithmically transformed, and the first-order estimation method was used throughout the entire model-building process. A two-compartment model with lag-time (T_{LAG}) and first-order absorption and elimination was fitted to the MPA concentrations, as reported earlier.[5, 11] Pharmacokinetic parameters were estimated in terms of volume of distribution of the central compartment (V_c), clearance (CL), volume of distribution of the peripheral compartment (V_p) and intercompartmental clearance (Q). Because no intravenous data were available, absolute bioavailability (F) cannot be estimated. As a result V_c, CL, V_p and Q correspond to the ratios V_c/F, CL/F, V_p/F and Q/F, respectively. At some point in the analysis bioavailability was compared

for the different doses (see below). In this case the relative bioavailability (F_{rel}) of an MMF dose of 1000 mg was arbitrarily set at 100%. Interpatient variability (IPV) and interoccasion variability (IOV) of the pharmacokinetic parameters were modelled using an exponential error model. The covariance between values for IPV was estimated using a variance-covariance matrix. Residual variability between observed and predicted MPA plasma concentrations was described using an additional error model.

The population model was built stepwise. A specific assumption was tested at each step. The main decision criterion was the likelihood ratio test. In NONMEM modeling, the minimum value of objective function (OFV) can be used as a criterion for model selection. If the difference in OFV between two nested models is larger than the critical value from a chi-squared distribution with degrees of freedom equal to the difference in the number of estimated parameters, the models are significantly different from each other. A decrease in the OFV>10.8 shows a significant improvement of a nested model with one degree of freedom of $p<0.001$. Model adequacy was further evaluated by using various residual plots ("goodness-of-fit" plots) and values of random effects variances. To analyze the graphical goodness of fit, extensive plotting was available through the use of Xpose,[12] a purpose built set of subroutines in S-plus (version 6.1; Insightful Corp. Seattle, WA).

Covariate model

To explain IPV and IOV, relationships were investigated between pharmacokinetic parameters and patient characteristics. The correlation between the use of calcineurin inhibitors and the pharmacokinetic parameters was tested using equation 1.

$$CL = \theta_{pop} \bullet \theta_{CsA}{}^{CsA} \qquad \text{(Eq. 1)}$$

where θ_{pop} is the typical clearance in patients using tacrolimus (exponent CsA=0) and θ_{CsA} is the fractional change in MPA clearance in patients cotreated with cyclosporine (exponent CsA=1).

The time-dependent changes of MPA pharmacokinetics were modelled as described in equation 2.

$$CL = \theta_{pop}(1 + \theta_{\Delta} * e^{-\theta_{rate} * time}) \qquad \text{(Eq. 2)}$$

where θ_{Δ} is the relative change of MPA CL at day 3 compared to its stabilized value at six months posttransplantation and θ_{rate} is a first order rate constant describing the decrease of CL posttransplantation from day 0 to day 180.

The relationship between the MMF dose and the pharmacokinetic parameters of MPA was tested as follows (equation 3).

$$F_{rel} = \theta_{pop} * (dose / 1000)^{\theta_{dose}} \quad \text{(Eq. 3)}$$

in which θ_{pop} is the F_{rel} in individuals which received 1000 mg MMF twice daily, which was arbitrarily set at the value of 1 and θ_{dose} is an exponent determining the shape of the relationship. The final model was developed by forward inclusion and backward elimination.[13] Covariates were introduced in univariate analyses. When inclusion of a covariate caused a decrease in OFV>3.8 ($p<0.05$), the covariate was considered to be statistically significant. Subsequently, a multivariate analysis with backward elimination was done to obtain the final model. All covariates selected after the univariate analyses were included in an intermediate model. If the elimination of a covariate caused an increase in OFV>10.8 ($p<0.001$), then the covariate remained in the model and was considered to be significant.

Model validation

As an internal validation method, a bootstrap resampling method[14] was applied, using the Wings for NONMEM software (Dr N. Holford, version 612, March 2007, Auckland, New Zealand). Two hundred bootstrap data sets were generated by sampling randomly from the original data set with replacement. Parameters were estimated for each of the replicate data sets using the final model. The validity of the model was evaluated by comparing the median values and 95-percentile range of the bootstrap replicates with the observed concentrations in the original data set.

Results

Patients

The population pharmacokinetic model was developed using 7739 MPA samples originating from 1402 concentration-time profiles obtained from 241 renal transplant recipients. The pharmacokinetic profiles of patients cotreated with cyclosporine (n=140) are presented in Figure 1A, and pharmacokinetic profiles of patients cotreated with tacrolimus (n=101) in Figure 1B. Sampling occasions varied from day 3 to day 168 after renal transplantation. MMF doses ranged from 250 to 2200 mg twice daily. Patient characteristics are presented in Table I.

Pharmacokinetic analysis

Basic model

The concentration-time data of all patients were fitted simultaneously to several pharmacokinetic models. A two-compartment model with first-order elimination adequately described the data. The delayed absorption was characterized by a T_{LAG} and a first-order absorption rate constant (k_a). In the basic model, F_{rel} was fixed on 100%. Introduction of IPV for T_{LAG}, k_a, V_c, CL and F_{rel} significantly improved the fit of the model for each parameter ($p<0.001$). IOV could be estimated for k_a, V_c, CL and F_{rel}, each improved the model even further ($p<0.001$).

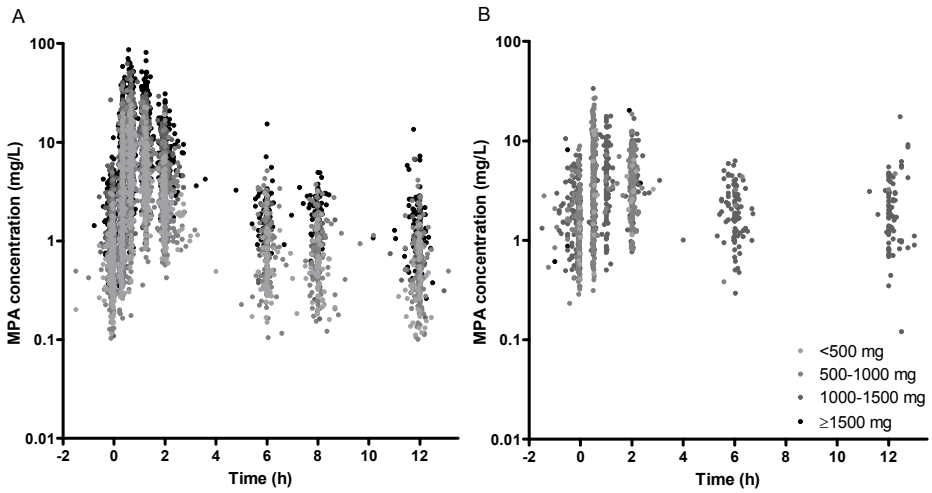

Figure 1: Concentration-time profiles of patients co-treated with cyclosporine (a) and tacrolimus (b).

Table I: Patient characteristics

Cotreatment	Cyclosporine	Tacrolimus
Gender (m/f)	88/52	73/28
Age (years)	50 (19-70)	53 (19-76)
Height (cm)	170 (150-190)	175 (150-195)
Body weight (kg)	69 (37-104)	80 (44-145)
Time posttransplantation (days)	26 (1-152)	23 (3-168)
Creatinine (μmol/L)	141 (53-1238)	145 (61-1190)
Albumin (g/L)	36 (20-53)	39 (10-50)
ASAT (U/L)	14 (2-289)	21 (8-236)
ALAT (U/L)	14 (1-653)	24 (1-534)
PK-day	26 (4-152)	23 (3-168)
MMF dose twice daily (mg)	1150 (250-2200)	750 (250-1500)
CNI daily dose (mg)	450 (125-2100)	8 (1-20)

Parameters are presented as median (range), separated for the cotreatment of the calcineurin inhibitors (CNI), cyclosporine and tacrolimus. ASAT, aspartate aminotransferase; ALAT, alanine transaminase; PK-day, number of days between the transplantation and the pharmacokinetic assessment; MMF, mycophenolate mofetil.

Covariate model

Equation 1 was used to evaluate possible differences in the pharmacokinetic parameters of MPA between patients receiving either cyclosporine or tacrolimus. Significant differences were observed for CL and V_c. CL was significantly higher in patients receiving cyclosporine whereas V_c was significantly lower ($p<0.001$). Introduction of the differences in CL and V_c between patients cotreated with cyclosporine or tacrolimus improved the fit of the model to the data; the OFV was reduced by 151.7 points ($p<0.001$). Interpatient and -occasion variability was explained. IPV decreased from 44 to 38% for MPA CL and from 124 to 105% for V_c. The respective reductions for IOV were from 9.3 to 7.3% and from 57 to 52%.

Subsequently, the change of the pharmacokinetic parameters in time was evaluated using equation 2. A significant decrease in CL and V_c was seen in time during the first six months after transplantation, which was different between patients cotreated with cyclosporine and tacrolimus (ΔOFV=-530.8, p<0.001). MPA CL decreased from 35.3 to 18.6 L/h in patients cotreated with cyclosporine and from 26.9 to 13.6 L/h in patients cotreated with tacrolimus (Fig. 2A). V_c decreased from 104 to 49 L in patients cotreated with cyclosporine and from 297 to 54 L in patients cotreated with tacrolimus in the first six months after transplantation (Fig. 2B). Interpatient and -occasion variability was explained. IPV decreased from 38 to 31% for MPA CL and from 105 to 71% for V_c. The respective reductions for IOV were from 7.3 to 3.3% and from 52 to 50%.

Finally, the correlation between MMF dose and the different pharmacokinetic parameters was evaluated in univariate analyses. Inclusion of a relationship with CL, V_p and F_{rel} resulted in a significant improvement of the fit of the model; corresponding reductions in OFV were

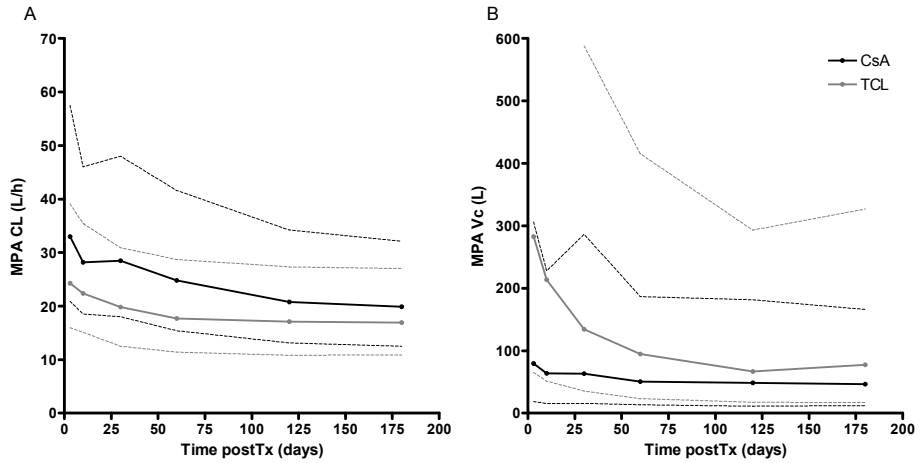

Figure 2: Post hoc changes in time after transplantation of MPA clearance (CL) and volume of distribution of the central compartment (V_c). The median and 90-percentile range are presented for patients co-treated with cyclosporine (black) and tacrolimus (grey).

19.8, 17.6 and 39.0 points (p<0.001). All these relationships were included in the intermediate model. After the backward elimination procedure, only the relationship between MMF dose and F_{rel} remained in the population model; relationships with CL and V_c could be eliminated without significantly worsening the fit of the model. Figure 3 shows the correlation between MMF dose and F_{rel}. The values estimated for the pharmacokinetic parameters and the precision of the estimates are presented in Table II. Compared to 1000 mg MMF twice daily, median F_{rel} (and 95% confidence interval) was 123% (109-139%), 111% (104-118%), 94% (91-98%), and 90% (85-96%) in patients receiving MMF doses of 250, 500, 1500 and 2000 mg twice daily respectively in combination with cyclosporine (p<0.001); corresponding values for patients receiving tacrolimus were 176% (134-233%), 133% (116-153%), 85% (78-92%) and 76% (65-87%) (p<0.001). IPV and IOV of relative bioavailability were 41 and 24%, respectively.

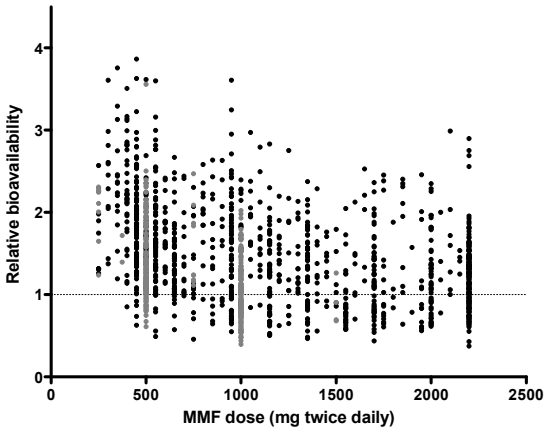

Figure 3: Correlation between mycophenolate mofetil (MMF) dose and relative bioavailability (F_{rel}). Patients cotreated with cyclosporine are represented in black, and patients cotreated with tacrolimus in grey.

Model validation

The goodness-of-fit plots of the final model (Fig. 4) show no structural bias, except for a small underprediction of the maximum MPA concentration. The original data set was used to generate 200 bootstrap data sets, which were fitted with the final model. The median estimates and 95-percentile range resulting from the bootstrap procedure are very similar to the population estimates of the final model, see Table II. This demonstrates that the estimates for the fixed and random effects in the final model are accurate and that the model is stable.

Post hoc analysis

The exponents in equation 3 were negative and significantly different from zero with a value of -0.15 for cyclosporine and -0.41 for tacrolimus. This indicates that bioavailbility decreases when doses increase. As a result, MMF does not exhibit linear pharmacokinetics. The correlation between MMF dose ("administered MMF dose") and MMF dose multiplied by F_{rel} ("exposed MMF dose") was investigated using simulations of the final model (Fig.5). A patient receiving 500 mg MMF cotreated with tacrolimus shows an MPA exposure corresponding to 665 mg "exposed MMF dose". To double the obtained MPA AUC with this dose, the "exposed MMF dose" needs to be doubled to 1330 mg. The corresponding "administered MMF dose", as deduced from Figure 5, is 1620 mg. In this case, to double the MPA exposure, the administered dose needs to be increased to 3.2 times its original value.

Discussion

A population pharmacokinetic model for MPA was developed in renal transplant recipients, who were followed for six months after transplantation. Differences were seen in MPA CL and V_c between patients cotreated with either cyclosporine or tacrolimus ($p<0.001$). The pharmacokinetics of MPA were time dependent with decreasing CL and V_c following transplantation.

Table II: Pharmacokinetic parameter estimations

Parameter	Final model (CV in %)	Bootstrap (95-percentile range)
T_{LAG} (h)	0.21 (2)	0.21 (0.15-0.26)
k_a (h^{-1})	3.9 (10)	3.8 (2.5-5.0)
V_c (L)	68 (14)	70 (52-98)
CL (L/h)	17.0 (9)	16.8 (13.9-20.2)
V_p (L)	229 (9)	244 (202-326)
Q (L/h)	38.4 (7)	38 (32-53)
Residual error	0.45 (2)	0.44 (0.42-0.47)
Calcineurine inhibitor effect		
θ_{CsA} of CL	1.13 (11)	1.16 (0.93-1.40)
θ_{CsA} of V_c	0.72 (18)	0.73 (0.50-0.97)
Time dependency		
CsA: θ_{Δ} of CL	0.87 (11)	0.90 (0.74-1.14)
CsA: θ_{rate} of CL (day^{-1})	0.019 (19)	0.020 (0.013-0.031)
TCL: θ_{Δ} of CL	0.50 (36)	0.55 (0.26-1.00)
TCL: θ_{rate} of CL (day^{-1})	0.040 (35)	0.038 (0.003-0.085)
CsA: θ_{Δ} of V_c	1.13 (27)	1.25 (0.58-2.15)
θ_{rate} of V_c (day^{-1})	0.045 (22)	0.047 (0.026-0.072)
TCL: θ_{Δ} of V_c	3.2 (27)	3.2 (1.8-5.4)
Relationship MMF dose Frel		
CsA: θ_{dose} of F	-0.15 (30)	-0.15 (-0.24- -0.06)
TCL: θ_{dose} of F	-0.41 (25)	-0.41 (-0.62- -0.19)
Interpatient variability		
T_{LAG} (%)	7.4 (207)	17 (4-155)
k_a(%)	125 (18)	140 (102-199)
V_c(%)	71 (29)	70 (41-108)
CL(%)	31 (24)	34 (23-49)
F_{rel}(%)	41 (17)	44 (34-59)
Interoccasion variability		
k_a(%)	117 (10)	120 (106-134)
V_c(%)	50 (21)	48 (19-70)
CL(%)	3.8 (297)	5.3 (0.1-12)
F_{rel}(%)	24 (12)	24 (20-26)

Values of estimated parameters with coefficient of variation (CV). IPV, interpatient variability; IOV, interoccasion variability; OFV, minimum value of objective function; T_{LAG}, lag-time; k_a, absorption rate constant; V_c, volume of distribution of the central compartment; CL, clearance; V_p, volume of distribution of the peripheral compartment; Q, intercompartmental clearance; CsA, cyclosporine; TCL, tacrolimus; F_{rel}, relative bioavailability; θ_{CsA}, fractional change in patients cotreated with CsA; θ_{Δ}, relative change in time posttransplantation; θ_{rate}, first order rate constant describing the relative change of a parameter posttransplantation; θ_{dose}, exponent determining the shape of the relationship between a parameter and the MMF dose. For example: MPA clearance in cyclosporine cotreated patients: $CL=\theta_{pop}*\theta_{CsA}^{CsA}=17.0*1.13=19.2$ L/h; MPA clearance at 30 days posttransplantation in tacrolimus cotreated patients: $CL=\theta_{pop}(1+\theta_{\Delta}*e^{-\theta rate*time})=17.0*(1+0.50*e^{-0.040*30})=19.6$ L/h; Relative bioavailability of 1.5 g MMF in patients cotreated with tacrolimus: $F_{rel}=\theta_{pop}*(dose/1000)^{\theta dose}=1*(1500/1000)^{-0.41}=0.85$.

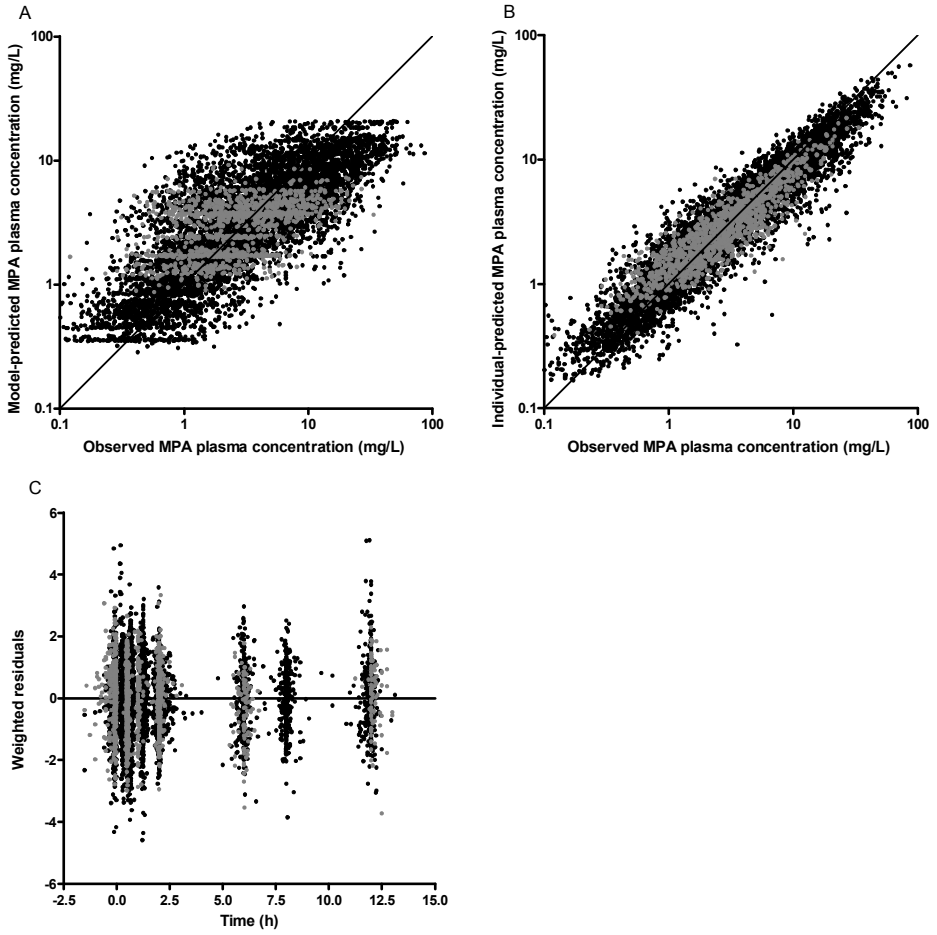

Figure 4: Goodness-of-fit plots of the final model. Model-predicted mycophenolic acid (MPA) concentration versus observed MPA concentration (a), individual-predicted MPA concentration versus observed concentration (b), and weighted residuals versus time (c). The solid line in (a) and (b) is the line of identity. The solid line in (c) is the line for x=0. Patients cotreated with cyclosporine are represented in black, and patients cotreated with tacrolimus in grey.

Finally, in the developed model a relationship between MMF dose and relative bioavailability F_{rel} was observed. Due to the decrease of F_{rel} with rising MMF doses, an increase of the MMF dose will produce an increase in MPA AUC that is less than proportional.

The basic model and estimated pharmacokinetic parameters of the present model were comparable with those of previously published models for MPA in renal transplant recipients.[15-17] In a recent study a value of 16 L/h was reported for MPA CL for patients more than 6 months after transplantation cotreated with cyclosporine,[16] which is comparable to the values found in this study (19.2 L/h). Shum et al, reported MPA CL to be 27.1 L/h in the first month after

transplantation in patients cotreated with cyclosporine, which corresponds to the value of 28.7 L/h found at one month posttransplantation of the present study.[17]

The pharmacokinetics of MPA are dependent on the calcineurin inhibitor that is co-administered. V_c was 49 L when patients were cotreated with cyclosporine and 68 L in patients cotreated with tacrolimus. MPA CL was increased in patients cotreated with cyclosporine (19.2 L/h) compared to tacrolimus cotreated patients (17.0 L/h). The latter can be explained by the interaction of cyclosporine with the enterohepatic recirculation of the drug. The enterohepatic recirculation is decreased by inhibition of the multidrug resistance-associated protein 2 (MRP2) transporter by cyclosporine. MRP2 is responsible for the excretion of MPAG in bile, which can be reabsorbed in the gut as MPA.[18] The intact enterohepatic recirculation contributes to MPA exposure, and explains the reduced clearance, in tacrolimus cotreated patients.

Time-dependent changes in MPA pharmacokinetics cause at least a 30 to 50% increase in MPA AUC during the first weeks posttransplantation.[19] In this study, the structural change in V_c and CL was described with a formula, which was previously reported by van Hest et al[4] V_c decreased from 104 L at day 0 to 49 L at day 180 following transplantation in cyclosporine cotreated patients and from 286 to 68 L in tacrolimus cotreated patients. This decrease in V_c might be explained by the increase in renal function and albumin levels in time posttransplantation.[5, 15] MPA CL decreased from 35.9 to 19.2 L/h for cyclosporine and from 25.5 to 17.0 L/h for tacrolimus cotreated patients. This is caused by a combination of improving renal function, increasing albumin levels, increasing hemoglobin levels, and decreasing cyclosporine predose target concentrations during the first six months posttransplantation.[4]

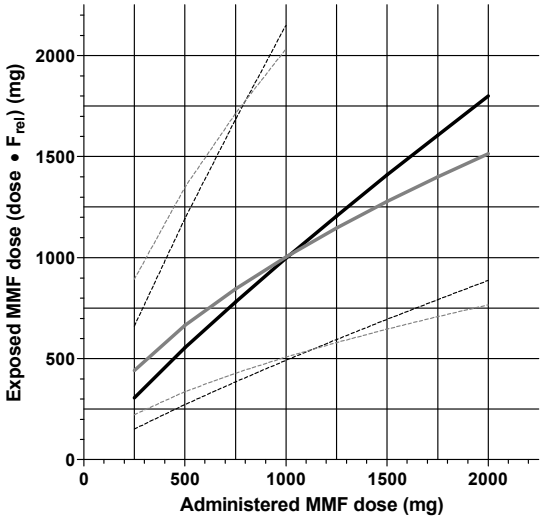

Figure 5: Conversion from administered mycophenolate mofetil (MMF) dose to the exposed MMF dose, corrected for relative bioavailability (F_{rel}). The median and 90-percentile range are presented for patients cotreated with cyclosporine (black) and tacrolimus (grey).

The population pharmacokinetic analysis demonstrated that bioavailability was not constant over the studied dose range of MMF. Bioavailability decreased significantly with rising MMF doses. As a result, MMF exhibits no linear pharmacokinetics. For example, to double the MPA AUC of a patient treated with tacrolimus and 500 mg MMF twice daily, the MMF dose needs to be increased to 1620 mg, which is 3.2 times its original value (Fig. 5). The decrease in bioavailability with higher doses may be caused by a saturable absorption process of MPA from the gut. Hereby, a limited amount of MPA can be absorbed when high doses are administered. Another possible explanation might be saturation of the enterohepatic circulation, which is responsible for the reabsorption of MPAG in the gut as MPA. At higher doses relativity less MPAG is recirculated and more will be excreted by the kidney producing less exposure to MPA. Consequently, the effect of this mechanism will be less in patients treated with cyclosporine which may explain the slightly different relationship between MMF dose and F_{rel} in cyclosporine cotreated patients compared to tacrolimus cotreated patients.

Figure 5 may be used to calculate the MMF dose needed for a certain change in MPA AUC in an individual patient. Separate correlations between "admininstered MMF dose" and "exposed MMF dose" are presented for patients cotreated with cyclosporine and tacrolimus. When a patient treated with 1000 mg MMF twice daily has a MPA AUC of 20 mg*h/L the patient is underexposed according to the presumed therapeutic window of MPA AUC of 30-60 mg*h/L. Following linear pharmacokinetics, doubling the MMF dose would result in an adequate exposure of 40 mg*h/L. However, due to the lower bioavailability of this higher dose, the "exposed MMF dose" of 2000 mg will be 1520 mg in tacrolimus cotreated patients and 1800 mg in cyclosporine cotreated patients. This increase in MMF dose will on average result in an AUC of 30 mg*h/L for the tacrolimus cotreated patient and 36 mg*h/L for the cyclosporine cotreated patient. Clearly, decreasing bioavailability may produce underexposure to MPA when the MMF dose is increased and this may have consequences for the efficacy of MMF therapy.

The bioavailability of MPA was improved through ester derivatization to MMF.[20] MMF absorption has been reported to be almost completely. In healthy subjects, bioavailability after single-dose oral administration of 1.5 g MMF was 85.7% for the MPA AUC_{0-24} and 93.3% for the MPA $AUC_{0-\infty}$ in comparison with the intravenous formulation.[21] However, MMF bioavailability seems to be decreased to 48.5% in liver transplant patients treated with 1 g oral MMF twice daily.[22] Also in hematopoietic stem cell transplant recipients treated with 1 g MMF in combination with cyclosporine, a decreased and highly variable bioavailability was seen (F=72.3%, range 20.5-172%).[23] In combination with our findings, it would seem that changes in bioavailability might contribute to the variability seen in patients treated with MMF. This should be taken into account when performing therapeutic drug monitoring.

In conclusion, the population pharmacokinetic analysis of MPA demonstrated that bioavailability of MMF is not constant when doses are varied. An Increase of the MMF dose will produce a less than proportional increase of the MPA exposure. This should be taken into

account when performing TDM. A correction of the MMF dose for the differences in bioavailabilty, as presented in Figure 5, can be used to calculate the dose needed to get the average patient on target. When higher MPA exposure is needed the MMF dose must generally be increased more than proportional to produce the desired MPA AUC.

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Chapter 2.3

Development of a Bayesian estimator for monitoring mycophenolate mofetil therapy in pediatric renal transplant recipients

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Abstract

Mycophenolate mofetil (MMF) is an important immunosuppressive agent used in adult and pediatric renal transplant recipients. The efficacy of the MMF therapy is correlated to the area under the concentration time curve (AUC) of its active metabolite, mycophenolic acid (MPA). The aim of this study was to develop a limited sampling strategy based on a population pharmacokinetic model, which describes the AUC_{0-12} of MPA in pediatric renal transplant recipients. MPA concentration versus time profiles (n=79) obtained from 52 pediatric renal transplant recipients (median age 14.4 years, range 4.5-18.9 years) were available. A population pharmacokinetic model was developed using nonlinear mixed-effects modeling. The final model was used to develop a Bayesian estimator, which could estimate the AUC_{0-12} based on limited concentration-time samples. The data were best described using a 2-compartment model with a lag-time, first-order absorption and first-order elimination, with allometric scaling of the pharmacokinetic parameters. The enterohepatic recirculation was described with a separate gallbladder compartment. A decrease in albumin concentration was significantly correlated with an increase in MPA clearance. The MPA clearance of pediatric renal transplant recipients (8.8 L/h/70 kg) was relatively low compared to adults. Using the final model and MPA concentration samples at 0, 0.5 and 2 hours postdose the Bayesian estimator could estimate MPA AUC_{0-12}. Validation of the estimator produced a bias of -5.8 mg*h/L (95% CI: -12.6/1.0 mg*h/L) and a precision of 15.4 mg*h/L (9.5/19.5 mg*h/L). The accuracy of the Bayesian estimator is not good enough for use in clinical practice. In conclusion, a population pharmacokinetic model was developed including an EHC. The MPA clearance of pediatric renal transplant recipients is low compared to adults. Furthermore, a Bayesian estimator was developed to estimate the MPA AUC_{0-12} in pediatric renal transplant recipients. Unfortunately, the model could not explain enough variability in the pharmacokinetics of MPA to accurately estimate the MPA AUC based on a limited sampling strategy.

Introduction

Increasingly, mycophenolate mofetil (MMF) has replaced azathioprine as immunosuppressive agent in patients undergoing kidney transplantation.[1] In comparison with azathioprine, the use of MMF significantly reduced the risk of acute rejection.[2] After absorption, MMF is converted into the active compound mycophenolic acid (MPA), which inhibits purine synthesis by reversible inhibition of inosine monophosphate dehydrogenase (IMPDH). In particular IMPDH type II is inhibited, that is expressed predominantly in activated lymphocytes. In this manner, especially proliferation of lymphocytes is impeded.[3] MMF is rapidly absorbed, followed by a maximum plasma concentration of MPA an hour after intake.[4] The majority of MPA is metabolized to the inactive 7-O-mycophenolic acid glucuronide (MPAG), which undergoes enterohepatic recirculation (EHC),[5] resulting in a smaller second MPA plasma peak 6-12 hours after MMF intake.[4] Patients should not be underexposed to MPA, because of the increasing risk of acute rejection.[6-7] On the other hand, overexposure is associated with more severe side-effects like bone marrow depression and gastro-intestinal complaints.[8] The recommended exposure to MPA is between 30 and 60 mg*h/L.[9-10] Individualizing MMF dosing based on drug exposure significantly improves patient outcome after renal transplantation.[11]

Intra- and inter-individual differences in the pharmacokinetics of MPA have been observed.[12-13] Variability in pharmacokinetics in adults is mostly dependent on albumin concentration, renal function, hemoglobin levels and co-medication with cyclosporine.[14] The few published articles concerning children suggest that in children pharmacokinetics depend on age, serum total protein levels, co-therapy with cyclosporine and graft function, although results vary.[4, 9, 15-16]

The most reliable assessment of MPA exposure is obtained through the repetitive measurement of 12-hour MPA area under the concentration-time curves (AUC_{0-12}).[17] Determination of MPA-AUC_{0-12} requires 8 – 10 blood samples and is cumbersome. This in contrast to a limited sampling strategy, which is patient friendly, especially in children, and requires less costs and time. Therefore, the development of a practical limited sampling strategy that can predict the AUC is desirable. For adult renal transplant recipients several limited sampling strategies are available,[18-20] whereas limited research has been performed in children. Development changes in children are associated with changes in absorption, distribution and metabolism, making extrapolation of the results of studies in adults undesirable.[21] Two studies developed a limited sampling strategy for pediatric renal transplant recipients using multiple linear regression analysis.[22-23] A maximum a posteriori Bayesian estimator (MAP-BE), a limited sampling strategy based on a population pharmacokinetic model, will however be preferred over an algorithm due to its flexibility in sampling times and possibility to include the effect of covariates on the pharmacokinetics of MPA. Payen et al developed a MAP-BE to estimate MPA AUC in pediatric and adolescent renal transplant recipients.[24] The authors constructed a population pharmacokinetic model and Bayesian estimator using concentration-time samples obtained at 1 and 4 hours postdose to estimate the MPA AUC_{0-12}. The model was however

not able to describe the EHC of MPA, due to the fact that most of the included patients were cotreated with cyclosporine, which inhibits the EHC of MPA.[25] The aim of this study was to develop a new population pharmacokinetic model taking the EHC into account. This model was used to develop a Bayesian estimator to estimate the MPA AUC_{0-12} for pediatric renal transplant recipients, cotreated with either cyclosporine or tacrolimus.

Patients and methods

Patients

Concentration-time profiles, obtained from 52 pediatric renal transplant recipients treated with MMF were analyzed retrospectively. The data were obtained from the Wilhelmina Children's hospital, Utrecht and Erasmus MC - Sophia Children's hospital, Rotterdam, in the Netherlands. Pharmacokinetic, demographic and laboratory data were collected from patient medical records and therapeutic drug monitoring databases. Patients were included if they were ≤18 years old, received a kidney transplant and were at least one month post-transplantation. Patients who underwent a multi-organ transplantation were excluded. MPA pharmacokinetic profiles were obtained during daytime. In the Wilhelmina Children's hospital, 23 patients were included. For each patient, 7 blood samples were collected at steady-state at 0, 0.5, 1, 2, 4, 6, and 12 hours after MMF dose administration. The blood samples were analysed using a validated HPLC method. In the Erasmus MC – Sophia Children's hospital, 29 patients were included. Abbreviated AUCs were collected at this center, with samples taken at 0, 0.17, 0.5, 1.5, 2, and 4 hours postdose. In the chronologically first 27 pharmacokinetic profiles assessed, concentrations of MPA in plasma were measured using enzyme multiplied immunoassay technique assay (EMIT MPA Assay System; Dade Behring, San Jose, CA, USA). For the following 18 profiles, MPA concentrations were analysed using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.[26]

Pharmacokinetic modeling

Basic model

All data were analyzed simultaneously using the nonlinear mixed-effects modeling software program (NONMEM Verion VI, level 2.0; GloboMax LLC, Ellicott City, MD, USA). Because NONMEM estimated pharmacokinetic parameters for MPA, MMF doses were converted to the equivalent MPA content by multiplying the MMF dose by 0.739. Data were logarithmically transformed, and the first-order estimation method was used throughout the entire model-building process.

In the first step of the population analysis, a compartmental pharmacokinetic model was developed. Several structural models were tested. Models with one or two compartments were evaluated, as well as models with and without lag time. Furthermore, it was evaluated

whether absorption was best described as a zero- or first-order process. Finally, addition of the EHC to describe the second peak in the concentration-time profile was evaluated.[17] Pharmacokinetic parameters were estimated in terms of central and peripheral volume of distribution (V), clearance (CL) and intercompartmental clearance (Q). Because bioavailability (F) could not be quantified, V, CL and Q values correspond to the ratios V/F, CL/F and Q/F, respectively.

Interpatient variability (IPV) and interoccasion variability (IOV) for all pharmacokinetic parameters was modeled using an exponential (equation 1) or additive (equation 2) error model.

$$CL_{ij} = \theta_{pop} * exp(\eta_i + \kappa_j) \quad \text{(Eq. 1)}$$

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

where CL_{ij} represents the MPA clearance of the i^{th} individual on the j^{th} occasion, θ_{pop} represents the population value for MPA clearance, η represents the interindividual random effect with mean 0 and variance ω^2, and κ represents the interoccasion random effect with mean 0 and variance π^2. The covariance between values for IPV was estimated using a variance-covariance matrix. Residual variability between observed (C_{obs}) and predicted (C_{pred}) MPA plasma concentrations was described using an additional error model for logarithmic transformed data (equation 3).

$$\ln C_{obs} = \ln C_{pred} + \varepsilon_i \quad \text{(Eq. 3)}$$

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

The population model was built stepwise. A specific assumption was tested at each step. The main decision criterion was the likelihood ratio test. In NONMEM modeling, the minimum value of objective function (OFV) can be used as a criterion for model selection. If the difference in OFV between two nested models is larger than the critical value from a chi-squared distribution with degrees of freedom equal to the difference in the number of estimated parameters, the models are significantly different from each other. A decrease in the OFV>10.83 shows a significant improvement of a nested model with one degree of freedom of $p<0.001$. Model adequacy was further evaluated by using various residual plots ("goodness-of-fit" plots) and values of random effects variances. To analyze the graphical goodness of fit, extensive plotting was available through the use of Xpose,[27] a purpose built set of subroutines in S-plus (version 6.1; Insightful Corp. Seattle, WA).

Covariate model

To explain IPV and IOV, relationships were investigated between pharmacokinetic parameters and patient characteristics. For missing covariates the median of this parameter was

used for this analysis, except for height, which was estimated based on the patients' age and body weight. To account for variability in pharmacokinetic parameters due to the varying size of the individual children, the parameter values were standardized to a body weight of 70 kg using allometric scaling (equation 4).[28-29]

$$CL_i = \theta_{pop}\left(W_i / 70\right)^{PWR} \quad \text{(Eq.4)}$$

where CL_i represents the MPA clearance of the i^{th} individual, θ_{pop} represents the population value for MPA clearance representative for a person of 70 kg, W_i is the weight of the i^{th} individual and PWR is an exponent determining the shape of the correlation. The value for PWR was fixed at literature values of 0.75 for clearance and 1 for the volumes of distribution.[30] This standardization allows comparison of pediatric parameter estimates with those reported for adults. It was evaluated if estimation of the exponent PWR improved the goodness of the fit.[31]

Other covariates tested were patient age, gender, body height, time posttransplantation, hospital of inclusion, concurrent use of cyclosporine, cyclosporine daily dose/kg, serum creatinine concentration, glomerular filtration rate estimated according to the Schwartz formula, modified to current and local practice (eGFR),[32-33] serum albumin concentration, urea concentration, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT). The effect of the differences between the analytical methods used to determine the MPA concentration was tested as covariate of the residual error. First, all potential covariates were added separately to the basic model for univariate analysis. Continuous covariates were modeled exponentially (equation 5).

$$CL_i = \theta_{pop} * (eGFR / 60)^{\theta_{eGFR}} \quad \text{(Eq. 5)}$$

in which θ_{pop} is the MPA CL in individuals with the median eGFR of the population (60 mL/min) and θ_{eGFR} is an exponent determining the shape of the relationship. Categorical variables were modeled proportionally (equation 6).

$$CL_i = \theta_{pop} * \theta^{CsA} \quad \text{(Eq. 6)}$$

in which θ_{pop} is the MPA CL in individuals without cyclosporine (CsA=0) and θ is the fractional change of CL due to concurrent use of cyclosporine (CsA=1). Whether a variable had a significant effect was determined with the likelihood ratio test. When inclusion of a covariate caused a decrease in OFV>3.8 ($p<0.05$), the covariate was considered to be statistically significant. Second, a multivariate analysis with backward elimination was done to construct the final model. All covariates selected during the first stage were included in an intermediate model. Covariates were excluded separately from the intermediate model. If the elimination of a covariate caused an increase in OFV>10.8 ($p<0.001$), the covariate remained in the model.

Model validation

As an internal validation method, a bootstrap resampling method was applied,[34] using the Wings for NONMEM software (Dr N. Holford, version 612, March 2007, Auckland, New Zealand). Two hundred bootstrap data sets were generated by sampling randomly from the original data set with replacement. Parameters were estimated for each of the replicate data sets using the final model. The validity of the model was evaluated by comparing the median values and 95-percentiles of the bootstrap replicates with the estimates of the original data set. The final model was further validated by the application of a visual predictive check.[35] One hundred data sets were simulated from the original data set using the final model. Per time point the median simulated concentrations plus 90-percentile intervals were compared graphically with the observed MPA concentrations.

Bayesian estimator

The final model was used to develop a MAP-BE for MMF in pediatric renal transplant recipients. The MAP-BE was developed using the post hoc option of NONMEM. The best limited sampling strategy was selected based on a combination of 3 sampling times within 4 hours postdose. The individual parameters and AUC_{0-12} of the patients were estimated using the population pharmacokinetic model. Subsequently, the predictive performance of the developed MAP-BE was evaluated. All pharmacokinetic profiles containing concentration-time samples at 0, 0.5, 1, 2, 4, 6 and 12 hours postdose (n=20) were included in the validation data set of this analysis. The validation data set was randomly split into 4 data sets, each containing 5 pharmacokinetic profiles. Each of these data sets was used as a validation data set in 4 separated runs. All remaining profiles were included in the index data set, which was used to obtain population parameter values of the final model. These values and the sampling times included in the limited sampling strategy were used to estimate the AUC_{0-12} of the 5 profiles in that part of the validation data set. Finally, the bias or mean prediction error (equation 7) and precision or root mean squared prediction error (equation 8) were calculated.

$$MPE = \frac{\sum_{i=1}^{N}(pe_i)}{N} \qquad \text{(Eq. 7)}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(pe_i^{\,2})}{N}} \qquad \text{(Eq.8)}$$

where N represents the number of pairs of estimated and measured AUC, and pe_i is the difference between the estimated and the measured AUC. Reference AUC_{0-12} values were calculated using the linear trapezoidal rule and all available concentration-time data.

Results

Patients

The data set contained 488 MPA plasma concentration-time samples obtained from 52 pediatric renal transplant recipients. Each patient participated in 1 to 4 pharmacokinetic assessments at different times posttransplantation. The median time posttransplantation was 23.6 months (range, 1.6-127.5 months). The median age at the time of the pharmacokinetic assessment was 14.4 years (range, 4-18 years). The patient characteristics are presented in Table I. In 30 assessments patients received prednisolone and MMF without cotreatment with a calcineurin inhibitor, in 23 assessments in combination with tacrolimus, and in 26 assessments in combination with cyclosporine. Some data were not available on the day of the pharmacokinetic assessment for all patients. From the 79 pharmacokinetic profiles, information about height, serum albumin concentration, urea concentration, ALAT and ASAT was missing for 3, 4, 3, 8 and 7 profiles, respectively. Figure 1 shows all observed concentration-time profiles. In some patients, a second peak was seen at approximately 6 hours postdose.

Pharmacokinetic analysis

The concentration-time data of all patients were fitted simultaneously to several pharmacokinetic models. The data were best described using a 2-compartment model with a lag-time

Table I: Patient characteristics

Characteristics	Median (range)
Gender (m/f)	31/48
Age (years)	14.4 (4.5-18.9)
Height (cm)	147 (99-185)
Body weight (kg)	43.8 (15.5-87.6)
Serum albumin concentration (g/L)	42.0 (23.7-52.0)
Serum urea (mmol/L)	8.2 (3.8-30.2)
Serum creatinine (µmol/L)	105 (44-237)
eGFR (mL/min/1.73m2)	59.5 (25.6-121.7)
ALAT (U/L)	15 (<6-149)
ASAT (U/L)	22 (<12-108)
Time posttransplantation (days)	707 (47-3824)
Immunosuppresive drugs	
Mycophenolate mofetil dose (mg/kg/day)	27.8 (5.7-59.5)
Tacrolimus dose (mg/kg/day), n=23	0.12 (0.03-0.22)
Cyclosporine dose (mg/kg/day), n=26	4.7 (1.9-8.8)
No calcineurin inhibitor, n=30	
Prednisolone (mg/kg/day)	0.15 (0-0.93)
Other concomitant drug	
Antacida	n=12
Antibiotics	n=17

Data are presented as median (range) from 79 pharmacokinetic profiles obtained from 52 pediatric renal transplant recipients. Information with respect to height and biochemical parameters were not available for all patients.

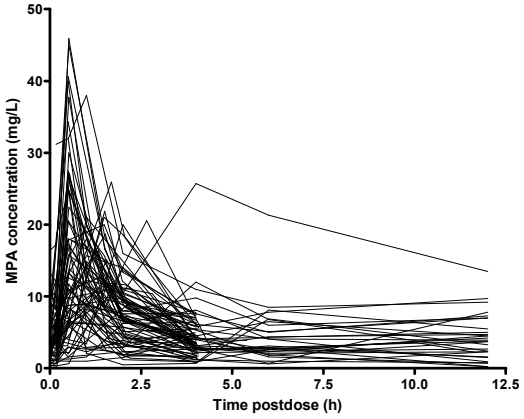

Figure 1: Mycophenolic acid (MPA) concentration-time curves of 52 pediatric renal transplant recipients (n=79).

(T_{lag}), first-order absorption and first-order elimination. The EHC was described with an extra gallbladder compartment, which was filled continuously from the central compartment with rate k_{24} (Fig. 2). Emptying of the gallbladder compartment into the gastrointestinal compartment occurred at a certain time point postdose (T_{GB}), with rate k_{41} and duration D_{GB}. Afterwards, MPA was reabsorbed into the central compartment. Because insufficient data were collected around the EHC peak, k_{41} and D_{GB} were fixed at literature values of 10 h^{-1} and 0.5 h to produce a fast and complete emptying of the gallbladder.[17] Introduction of IPV for T_{lag}, the central volume of distribution (V_C), CL, Q, k_{24}, and T_{GB} significantly improved the fit of

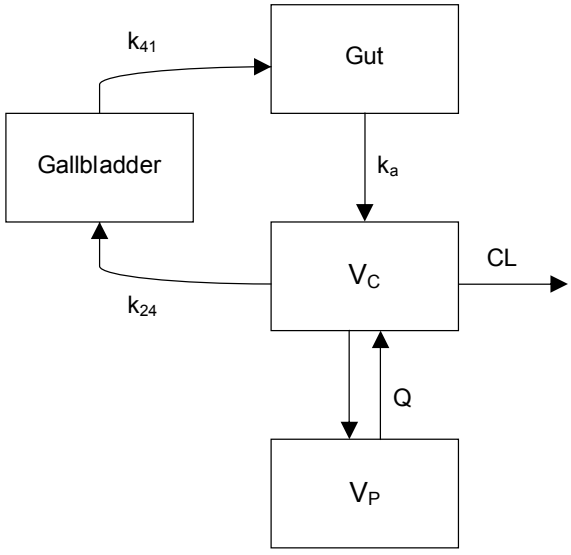

Figure 2: Population pharmacokinetic model, used to describe MPA concentration-time profiles from pediatric renal transplant recipients. V_C, volume of distribution of the central compartment; V_P, volume of distribution of the central compartment; k_a, absorption rate constant; k_{nm}, rate constant from compartment n to compartment m; CL, clearance; Q, intercompartmental clearance.

the model. The IPV was best described with an exponential error model for T_{lag}, V_C, CL, and Q; corresponding values were 26, 101, 44 and 97%, respectively. The exponential IPV of k_{24} was fixed at a value of 141% and the additional IPV of T_{GB} was fixed at a value of 2 h. Introduction of IOV for CL (61%) further improved the fit of the model.

To account for variability in pharmacokinetic parameters due to the varying size of the individual children, the parameter values were standardized to a body weight of 70 kg using allometric scaling. Introduction of allometric scaling with fixed values for PWR significantly improved the fit of the model ($p<0.001$). Estimation of these values resulted in a PWR of 0.60 for clearance, and a PWR of 0.99 for the volumes of distribution. The estimated values for PWR did not result in a significant improvement of the fit compared to the fixed values of 0.75 for clearance and 1 for the volumes of distribution, and were for this reason fixed at these values in the final model.

The univariate analysis of the covariates produced an intermediate model with the following correlations: gender, height, time posttransplantation, hospital of inclusion, cyclosporine daily dose/kg, serum creatinine concentration, serum albumin concentration, ALAT, and ASAT for V_C; gender, height, time posttransplantation, cyclosporine daily dose/kg, serum creatinine concentration, eGFR, serum albumin concentration, and urea concentration for CL; age, gender, time posttransplantation, concurrent use of cyclosporine, serum creatinine concentration, eGFR, serum albumin concentration, and ALAT for Q; and age, gender, height, time posttransplantation, concurrent use of cyclosporine, cyclosporine daily dose/kg, serum creatinine concentration, eGFR, serum albumin concentration, and ALAT for k_{24} ($p<0.05$). During the backward elimination, only albumin concentration for CL resulted in a significant increase in OFV when excluded from the intermediate model. Figure 3 shows the correlation between albumin concentration and MPA CL.

Table II shows the parameter estimations of the final model. The MPA CL was estimated to be 8.8 L/h/70 kg. This results in a median MPA CL of 5.2 L/h for the pediatric renal transplant recipients included in this study. Another part of MPA was recirculated by the EHC. The part of MPA transported to the gallbladder was 12% (95-percentile range: 2-36%). The median posthoc time of gallbladder emptying was 5.7 h after MMF administration.

The goodness-of-fit plots of the final model are shown in Figure 4. The scatter plots of predicted and individually predicted versus observed concentrations show no structural bias, except for a small underestimation of the maximum concentration of MPA. The weighted residuals were homogeneously distributed over the sampling time period. The estimated parameters of the final run are compared to the results of 200 bootstrap replicates (Table II). The median estimates resulting from the bootstrap procedure are similar to the population estimates of the final model. This demonstrates that the estimation of the parameters of the final run is accurate and that the model is stable. Figure 5 represents the results of the visual predictive check. This validation method showed a good agreement between the simulated

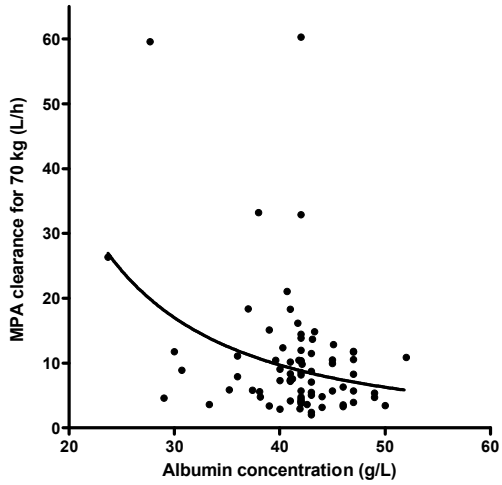

Figure 3: Correlation between serum albumin concentration and mycophenolic acid (MPA) clearance as estimated by the pharmacokinetic model for a child of 70 kg. The dots represent the individual patients, and the solid line is the correlation as estimated by the final model.

Table II: Parameter estimates of the population pharmacokinetic model

Parameter	Final model	Bootstrap analysis		
		median	90-percentile range	
T_{LAG} (h)	0.144	0.148	0.117	0.234
k_a (h^{-1})	3.68	4.19	2.38	10.7
$V_C=\theta_1*(Wt/70)$				
θ_1 (L/70 kg)	64.1	73	48.3	108
$CL=\theta_2*(Wt/70)^{0.75}*(Alb/42)^{Alb\sim CL}$				
θ_2 (L/h/70 kg)	8.82	10.2	5.9	12.1
Alb~CL	-1.95	-1.49	-2.98	0.218
$V_p=\theta_3*(Wt/70)$				
θ_3 (L/70 kg)	400	224	108	635
$Q=\theta_4*(Wt/70)$				
θ_4 (L/h/70 kg)	29.6	25.5	15.7	34.4
k_{24} (h^{-1})	0.004	0.00847	0.00129	0.0406
T_{GB} (h)	4.74	5	4.04	10.4
D_{GB} (h)*	0.5	0.5	-	-
k_{41} (h^{-1})*	10	10	-	-
Residual error	0.478	0.458	0.397	0.501
Variability				
IPV T_{LAG} (%)	26	27	6	220
IPV V_C (%)	101	107	64	147
IPV CL (%)	44	40	15	78
IPV Q (%)	97	158	65	466
IPV k_{24} (%)*	141	141	-	-
IPV T_{GB} (%)*	2	2	-	-
IOV CL (%)	61	50	30	86

Estimations for the pharmacokinetic parameters of the final model and the bootstrap procedure. T_{LAG}, lag-time; k_a, absorption rate constant; V_C, volume of distribution of the central compartment; CL, clearance; V_p, volume of distribution of the peripheral compartment; Q, intercompartmental clearance; k_{nm}, rate constant from compartment n to compartment m; T_{GB}, time of opening the gallbladder compartment; D_{GB}, duration of gallbladder opening; IPV, interpatient variability; IOV, interoccasion variability. *These values needed to be fixed in the final model.

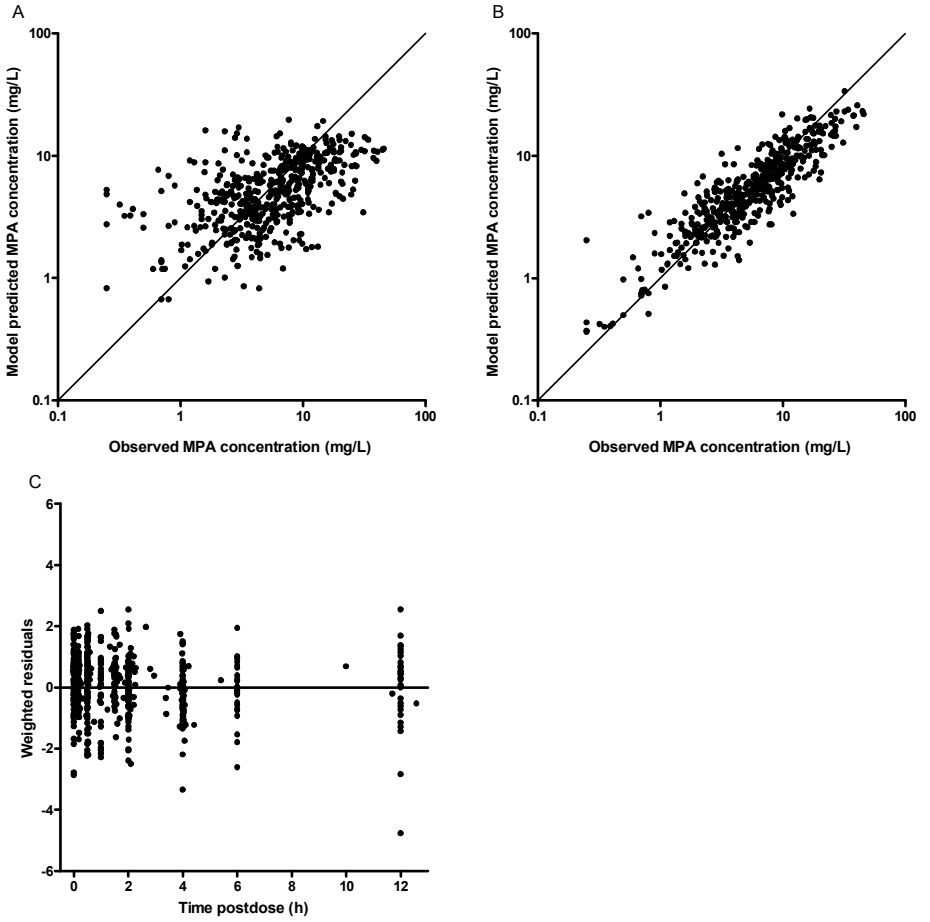

Figure 4: Goodness-of-fit plots of the final model. Plots of observed MPA concentration versus model predicted (a) and Bayesian predicted (b) and weighted residuals versus time (c). The solid line in (a) and (b) is the line of identity.

and observed concentrations at all sampling time points, except for a small underprediction of the maximum concentration of MPA at approximately 1 hour postdose.

Bayesian estimator

The final model and the total data set were used to develop a MAP-BE to predict the MPA $AUC_{0\text{-}12}$ on the basis of a limited number of samples. On the basis of bias and precision, the selected optimal sampling schedule consisted concentrations obtained at 0, 0.5 and 2 hours postdose. To validate the MAP-BE, the parameters of the final model were estimated again for each index data set containing all the pharmacokinetic profiles, except for the 5 profiles in the validation data set. The results of the estimations were comparable to the results of the parameter estimations of the final model. The developed MAP-BE adequately estimated

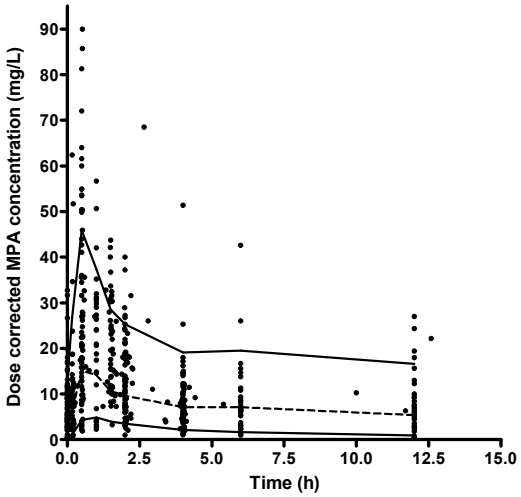

Figure 5: Visual predictive check. Comparison of median (dashed line) and 90-percentile range (solid line) of 100 simulated data sets and the observed concentrations (dots).

the MPA $AUC_{0\text{-}12}$ in the four validation data sets. When compared with $AUC_{0\text{-}12}$ assessed by the trapezoidal rule, bias was -5.8 mg*h/L (95%CI: -12.6/1.0 mg*h/L) and precision was 15.4 mg*h/L (95%CI: 9.5/19.5 mg*h/L). The Bland-Altman plot in Figure 6 shows no structural bias for the estimated MPA $AUC_{0\text{-}12}$ using the developed MAP-BE. In the validation data set, 4 pharmacokinetic profiles had an observed AUC<30 mg*h/L. The estimated AUC of all these profiles was also <30 mg*h/L. The observed AUC>60 mg*h/L were estimated to be >60 mg*h/L in 7 of the 12 profiles. The validation results of the three Bayesian estimators producing the best estimation of the $AUC_{0\text{-}12}$ are presented in Table III. The added value of including an extra sampling time at 6 h postdose was evaluated. This resulted in a bias of -6.6 mg*h/L (95%CI: -11.6/-1.6 mg*h/L) and a precision of 12.3 mg*h/L (95%CI: 6.3/16.1 mg*h/L).

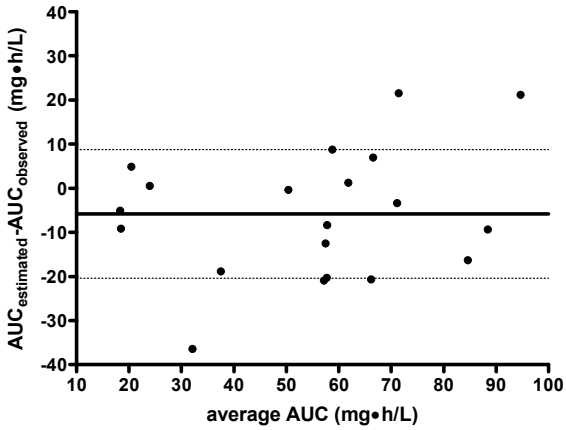

Figure 6: Bland-Altman plot for the agreement between measured mycophenolic acid (MPA) AUC and MPA AUC estimated using the developed Bayesian estimator. The line represents the mean bias, dashed lines are plus and minus two times the standard deviation of the mean.

Table III: Predictive performance of the developed Bayesian estimators

Sampling times	Bias	Precision
0, 1, 2 h	-5.3 (-12.0/1.4)	15.0 (7.8/19.7)
0, 1, 4 h	-6.2 (-12.6/0.3)	14.8 (5.4/20.2)
0, 0.5, 2 h	-5.8 (-12.6/1.0)	15.4 (9.5/19.5)
0, 0.5, 2, 6 h	-6.6 (-11.6/-1.6)	12.3 (6.3/16.1)

Data are presented as mean (and 95% confidence interval).

Discussion

In this study, a population pharmacokinetic model was developed for pediatric renal transplant recipients treated with MMF. In this model, the MPA concentration-time profiles were described using a two-compartment model with time-lagged, first order absorption and first order elimination. The EHC of the drug was included in the model using a gallbladder compartment. A Bayesian estimator was developed based on the population pharmacokinetic model and concentration-time samples obtained at 0, 0.5 and 2 hours after MMF dose administration.

Payen et al [24] have previously described a population pharmacokinetic model and developed a Bayesian estimator to estimate the MPA AUC_{0-12} in pediatric renal transplant recipients. The differences with the current model are that we were able to describe the EHC of MPA in the population pharmacokinetic model, due to inclusion of a higher number of patients cotreated with tacrolimus or without a calcineurin inhibitor. Furthermore, we applied allometric scaling to the pharmacokinetic parameters, which allows comparison of the estimated parameters with values found for adult renal transplant recipients.[28]

Several population pharmacokinetic models have been described for adult renal transplant recipients.[14, 20, 36-39] The estimated values for MPA CL in adult renal transplant recipients in the maintenance period after transplantation are 16-23 L/h. Compared to these studies, the children in the current study have a relatively low MPA CL of 8.8 L/h/70 kg. The covariate analysis showed a clear correlation between the MPA CL and albumin concentration, which is also seen in adult renal transplant recipients.[14, 38, 40] A decrease in plasma albumin concentrations from 45 to 35 g/L resulted in an increase in MPA CL from 7.7 to 12.6 L/h/70 kg. The correlation between MPA CL and albumin concentration can be explained through MPA protein binding. When albumin levels increase, MPA protein binding increases, resulting in a decrease in the free MPA fraction and consequently less MPA available to be cleared.[39] Correlations between MPA CL and renal function or EHC and cyclosporine cotreatment, as reported in some studies in adult renal transplant recipients, were not seen in this group of patients.[14, 39-40] The lacking correlation with renal function might be caused by the fact that patients included in this study needed to be at least one month posttransplantation, which resulted in a group of patients with a relatively good renal function. Van Hest et al[14] showed this correlation for adult renal transplant recipients cotreated with cyclosporine with a creatinine clearance <25 mL/min. Furthermore, the correlation between renal function and MPA CL seems to be different for patients treated with or without

cyclosporine.[39] Our data set contained a combination of patients treated with and without cyclosporine. Concerning the cyclosporine effect, only one third of patients in this study were cotreated with cyclosporine. Furthermore, of these patients, the number of pharmacokinetic profiles including samples later than 6 hours was limited (n=6). And in these few profiles, not much samples were taken at the time of EHC (6-12 hours postdose). To describe the effect of cyclosporine on the EHC of MPA clearly, frequent sampling between 6 and 12 hours postdose would be necessary in patients cotreated with and without cyclosporine.

The final model was used to develop and validate a Bayesian estimator to estimate MPA AUC_{0-12} in pediatric renal transplant recipients. The best MAP-BE was based on MPA concentrations at 0, 0.5 and 2 hours after oral MMF administration. The validation of this Bayesian estimator resulted in a bias not significantly different from zero and a precision of 15.4 mg*h/L. Given a therapeutic window from 30-60 mg*h/L, these results were not considered to be acceptable.[41] The Bayesian estimator developed by Payen et al, which is mainly based on cyclosporine cotreated patients, was more accurate (precision: 6.0 mg*h/L).[24] The better precision of this study could be explained by the decrease in variability in the second part of the concentration-time profile, due to inhibition of the EHC by cyclosporine.[25, 42] In the current study, we were not able to explain the high variability seen in the EHC with covariates. As a result, the Bayesian estimator can not estimate this part of the pharmacokinetic profile accurate enough for use in clinical practise. Addition of 6 h postdose as an extra sampling time resulted in a small improvement of the precision of the MAP-BE (12.3 mg*h/L). Further research is needed to obtain more information about the pharmacokinetics of MPA in pediatric renal transplant recipients, especially for the children cotreated with tacrolimus. More pharmacokinetic profiles should be obtained to develop a Bayesian estimator that can accurately estimate the MPA AUC_{0-12}. In these profiles more sampling times between 6 and 12 hours postdose should be obtained.

MPA concentrations in this study were determined using EMIT, HPLC or LC-MS/MS. In the population pharmacokinetic model, no significant difference in MPA levels caused by these different analytical methods was observed. A comparison between the two methods showed that EMIT overestimated MPA levels and AUC, with variations depending on the posttransplantation time.[43] This overestimation of the MPA concentration is highest in the early phase posttransplantation, due to elevated AcMPAG levels as a consequence of impaired renal function, which could explain the fact that this overestimation was not seen in the current study.[43]

In conclusion, a population pharmacokinetic model was developed to describe the pharmacokinetics of MPA in pediatric renal transplant recipients. A significant correlation was seen between albumin concentration and MPA CL. Compared to adult renal transplant recipients, the MPA CL seen in these children was relatively low (8.8 L/h/70 kg). The final model was used to develop a Bayesian estimator, which can estimate the MPA AUC_{0-12} using concentration-time samples obtained at 0, 0.5 and 2 hours after MMF administration. Unfortunately, this Bayesian estimator is not accurate enough to be used to estimate the MPA AUC_{0-12} in clinical practise.

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Chapter 3

PHARMACOKINETICS OF MYCOPHENOLATE MOFETIL IN OTHER POPULATIONS

Chapter 3.1

Therapeutic drug monitoring for mycophenolic acid in patients with autoimmune diseases

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Introduction

Mycophenolate mofetil (MMF, CellCept®) has become the most frequently used immunosuppressive drug in kidney transplant recipients.[1] Since its approval for the prevention of acute rejection after kidney transplantation in 1995 in the USA and in 1996 in Europe, the use of azathioprine has been rapidly diminishing, giving way to the use of MMF. A second formulation of mycophenolic acid (MPA), the active metabolite of MMF, has become available as enteric-coated mycophenolate sodium (EC-MPS, Myfortic®). Randomized clinical trials have shown that EC-MPS 720 mg b.i.d. is therapeutically equivalent to MMF 1000 mg b.i.d. with a comparable safety profile.[2-3] These equimolar doses of EC-MPS and MMF produce equivalent MPA exposure. The delayed-release formulation, EC-MPS, exhibits more variable predose MPA concentrations and more variable peak concentrations.[4]

Because of the favourable experience with MMF in transplant recipients, combining good efficacy with relatively few side effects, its use has also been tried in patients with autoimmune diseases.[5] Following case reports and case series of the successful use of MMF, controlled trials have been started.[6] Increasing evidence suggests that MMF can be used not only for the prevention of rejection in solid organ transplant recipients, but also for the treatment of several immunologically mediated (renal) diseases.[7]

Lupus nephritis

Of the diseases for which MMF may be a first-line drug, systemic lupus erythematosus or lupus nephritis is the most promising.[8] A recent meta-analysis of randomized controlled trials, showed that MMF not only had higher efficacy in inducing remission in severe lupus nephritis, but also caused fewer side effects compared to pulsed cyclophosphamide.[9] Also for maintenance therapy in lupus nephritis MMF seems to be a good alternative to azathioprine.[9] The upcoming publication of the results of a large phase III clinical trial (Aspreva Lupus Management Study, ALMS) should provide us with more comparative data on the efficacy and safety of MMF as induction and maintenance therapy in lupus nephritis.[10]

ANCA-associated vasculitis

In 2007, Stassen et al reported on the use of MMF for induction of remission in 32 patients with active anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis.[11] The patients in this study could not be treated with cyclophosphamide, for varying reasons. Complete remission was obtained in 25 (78%) patients, and partial remission in 6 (19%) patients. Only one patient did not respond. Also, other groups found high percentages of responders on MMF therapy in ANCA-associated vasculitis.[12-13]

Optimal dosing

Initially, in patients with lupus nephritis MMF doses of up to 2 g daily were used. Subsequent dosing regimens started with 1 g MMF/day and titrated on a weekly basis up to a maximum of 3 g/day.[14] A similar dose escalation is used in the ALMS trial. It is questionable whether standard dose therapy is the best way to treat a patient, given the large inter-individual variability in pharmacokinetics.[15] In renal transplant patients monitoring of MPA exposure to optimize MMF treatment is a heavily debated topic.[16-19] Several studies have shown a correlation between MPA exposure and efficacy.[20] This is remarkable, as most renal transplant patients are being treated with three or sometimes four immunosuppressive drugs in the first months after transplantation. Apparently exposure to only one (MPA) of these three or four drugs is so important that it affects the incidence of acute rejection in these patients. Recently, a French study showed a reduced incidence of acute rejection in concentration-controlled MMF treated renal transplant recipients compared to treatment with a fixed dose regimen.[21]

Since patients with autoimmune disease are regularly treated with only one or two immunosuppressive drugs, an adequate MPA exposure may be even more important compared to renal transplant recipients receiving multiple immunosuppressive drugs. Also, in patients treated for autoimmune diseases MPA has highly variable pharmacokinetics, and dose is a poor predictor for MPA exposure.[22] Factors affecting the between-patient variability of MPA have been extensively investigated in transplant patients, and are likely the same for lupus patients, as they are drug related.[23-24] Patients with a poor renal function (creatinine clearance <25 mL/min), and patients with low albumin (<32 g/L) are known to have lower MPA exposure. This is explained by the fact that the clearance of MPA depends on its non-protein bound fraction.[25] Hypoalbuminaemia, as well as renal insufficiency, results in a higher free fraction of MPA, and this higher free fraction results in a higher MPA clearance. Other factors influencing MPA pharmacokinetics are listed in Table I. If in patients with autoimmune disease MPA exposure can be shown to correlate with either efficacy or toxicity, then therapeutic drug monitoring could contribute to optimize patient care.

Table I: Factors influencing MPA pharmacokinetics.

Factor	Mechanism
Creatinine clearance (<25 mL/min)	Increased MPA clearance (through higher free fraction)
Plasma albumin concentration (<32 g/L)	Increased MPA clearance (through higher free fraction)
Sex (male gender)	Increased MPA clearance (more glucuronidation activity)
Aluminium/magnesium containing antacids	Lower MPA bioavailability
Sevelamer co-therapy	Lower MPA bioavailability
Glucocorticoid co-therapy	Mechanism unknown, possibly lower MPA bioavailability
Cyclosporine co-therapy	Lower MPA exposure due to interruption of enterohepatic circulation
Colestyramine co-therapy	Lower MPA exposure due to interruption of enterohepatic circulation
Rifampicin co-therapy	Mechanism unknown, possibly due to faster glucuronidation of MPA
Antibiotic co-therapy	Lower MPA exposure due to interruption of enterohepatic recirculation
Polymorphisms in metabolizing enzymes	Increased MPA clearance (more glucuronidation activity)

Correlating MPA exposure to clinical outcome

Neumann et al report on the value of measuring MPA plasma concentrations in patients with autoimmune diseases.[26] The study consisted of two parts. In the first part of the study the correlation between 12-h trough MPA concentrations and full area under the concentration-time curve (AUC) of MPA was investigated. Despite a rather weak correlation between trough and AUC the authors decided to longitudinally monitor a cohort of patients in the second part of the study, collecting serial trough values, which were linked to the occurrence of adverse events and to disease recurrence. Optimal efficacy, i.e. prevention of recurrence to active disease, was associated with higher MPA trough concentrations (> 3.0 mg/L). A remarkable finding is the observation that in this study adverse events were clustered in patients with a high MPA exposure. This is in contrast with studies in renal transplant patients, in whom tolerability was poorly correlated with MPA concentrations. The authors define the upper threshold of the therapeutic window based on toxicity. In renal transplant patients, the upper threshold of the therapeutic window is not based on increased toxicity, but merely on a lack of further improvement of efficacy above a certain exposure.

Unanswered questions

Neumann et al are careful in the interpretation of their data. They acknowledge that this is an exploratory study. The therapeutic window of MPA concentrations between 3.5 and 4.5 mg/L may serve as a starting point for prospective studies, but is subject to change. Prospective studies should be adequately powered to deal with other patient or disease characteristics influencing the propensity to relapse. Although homogeneous patient populations would be preferred for establishing correlations between drug exposure and clinical outcome, in reality patients with lupus nephritis form rather heterogeneous populations, and we want to get a better idea of optimal target levels in patients with a lower or higher risk of relapse. In the study by Neumann et al patients were not suffering from the more severe stages of autoimmune diseases. Obviously, this may have consequences for optimal target concentrations.

For routine clinical practice, trough concentrations are more practical compared to obtaining a full AUC. Given the poor correlation between trough and AUC, for prospective trials it would be better to use a more robust measurement of MPA exposure than troughs only. As an alternative to the latter abbreviated sampling strategies may be used to accurately estimate AUC. Such abbreviated sampling strategies have been developed for transplant patients[27], but those are not necessarily valid for patients with autoimmune disease, given the differences in MPA pharmacokinetics between the two patient populations. Indeed, initial studies showed that for lupus patients specifically developed sampling strategies should be used.[28]

What can we do now?

The data presented should not be considered strong evidence in favour of MPA monitoring. Nor should their predictions of a therapeutic window be looked upon as an established guidance for routine clinical practice. We need more pharmacokinetic/pharmacodynamic analyses to decide on the value of therapeutic drug monitoring for MPA in this patient population.

For current patient care, however, even at this moment measurement of MPA plasma concentrations can be of some help. In patients with lupus nephritis in whom MMF is used as induction therapy one would expect to see a clinical response within a period of 1 month in most patients. If, after 1 month of therapy, in non-responders MPA (trough) plasma concentrations are found to be low (say <2 mg/L), then a dose increase may have favourable effects on the likelihood of reaching remission. However, if in the same patient MPA trough is >4.0 mg/L already, then a further dose increase would not seem to be a good idea, as it may cause toxicity without additional benefit in efficacy. In such patients switching to another agent may be the preferred way to go.

Conclusion

MMF is an effective immunosuppressive drug, which is increasingly used in the remission induction and maintenance therapy of lupus nephritis. Therapeutic drug monitoring may be beneficial considering the between-patient variability in MPA exposure and the first indications of a correlation between exposure and efficacy/safety in patients with autoimmune diseases. Prospective pharmacokinetic/pharmacodynamic studies are needed to elucidate the true value of dose individualization for this indication and to identify the subsets of patients that can benefit from monitoring.

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Chapter 3.2

Limited sampling strategies for therapeutic drug monitoring of mycophenolate mofetil therapy in patients with autoimmune disease

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Abstract

Mycophenolate mofetil (MMF) is increasingly used for the treatment of autoimmune diseases (AID). In renal transplant recipients, it has been demonstrated that adjustment of the MMF dose according to the area under the plasma concentration versus time curve (AUC) of mycophenolic acid (MPA), the active moiety of MMF, improves clinical outcome. The aim of this study was to develop a maximum a posteriori Bayesian estimator (MAP-BE) to estimate MPA AUC_{0-12} in patients with AID using a limited number of samples. The predictive performance of the MAP-BE was compared with a multiple linear regression method. Full MPA concentration versus time curves were available from 38 patients with AID treated with MMF. Nonlinear mixed-effects modeling was used to develop a population pharmacokinetic model. Patients were divided in an index and a validation data set. The pharmacokinetic model derived from the index data set was used to develop several MAP-BEs. The Bayesian estimators were used to predict AUC_{0-12} in the validation data set on the basis of a limited number of blood samples. The bias and precision of these predictions were compared with those of limited sampling strategies developed with multiple linear regression. The absorption of MPA was described with 2 first-order processes with a short and a long lag time and a subsequent first-order elimination. The 2-compartment model accounted for the enterohepatic recirculation of MPA as well. Using 1-4 samples, MPA AUC_{0-12} was adequately estimated by the MAP-BE. Bias (-5.5%) was not significantly different from zero, and precision was below 27%. The predictive performance of the multiple linear regression method was comparable. In conclusion, MAP-BEs were developed for the estimation of MPA AUC_{0-12} in patients with AID. The predictive performance was good and comparable to those of the multiple linear regression method. Due to its flexibility with respect to sample times, the MAP-BE may be preferred over the multiple linear regression method.

Introduction

Mycophenolate mofetil (MMF) is now the most prescribed drug for renal transplant recipients.[1] After oral administration, the prodrug MMF is rapidly hydrolyzed to the active agent mycophenolic acid (MPA). The majority of MPA is metabolized to the inactive 7-O-mycophenolic acid glucuronide (MPAG), which undergoes enterohepatic recirculation (EHC). MPA inhibits the immune system due to a selective reversible inhibition of inosine monophosphate dehydrogenase.[2] Although introduced as a fixed-dose drug, debate has emerged with respect to the potential contribution of monitoring MPA plasma concentrations as a basis for MMF dose adjustment [therapeutic drug monitoring (TDM)].[3-4] The between-patient variability in MPA pharmacokinetics[5] and the observation that the risk for acute rejection increases with lower MPA plasma concentrations suggest that a strategy of TDM for MMF therapy could improve outcome.[6-7] Evidence for the added value of TDM of MPA comes from a recent randomized study in adult renal transplant recipients, where a significantly lower incidence of biopsy-proven acute rejection was found in the TDM-based dosing group compared with a fixed-dose group.[8]

The most reliable assessment of MPA exposure is obtained through the repetitive measurement of 12-hour MPA area under the concentration-time curves (AUC_{0-12}). Unfortunately, routine application of the full MPA AUC_{0-12} regimen, consisting of 8-10 samples drawn over a 12-hour time interval, is impractical from a clinical point of view. Predose assessment of MPA is a commonly used alternative to MPA AUC_{0-12}. However, correlations between predose concentrations and MPA AUC_{0-12} are relatively weak (r^2=0.4-0.5),[9] and there is greater within-patient variability for predose concentrations than for AUC_{0-12}. Abbreviated AUC strategies have been proposed to represent the best of both worlds, yielding greater accuracy than predose measurements, although being less cumbersome than a 12-hour sampling strategy. For MPA, limited sampling strategies (LSSs) using a predose sample and 2 postdose samples have shown good agreement between the estimated and measured AUC, with correlation coefficients (r^2) ranging from 0.84 to 0.95.[9-11]

Following anecdotal reports describing benefits of MMF in patients with systemic lupus erythematosus (SLE) and lupus nephritis, small studies and finally large, randomized, controlled trials have established the use of MMF in these patients, particularly those with lupus nephritis.[12] MMF use in other autoimmune disease (AID), like antineutrophil cytoplasmic antibody-associated systemic vasculitis, has only been evaluated in smaller studies and in very few randomized controlled trials.[13] Many studies currently are ongoing with this immunosuppressive agent.[14-18] The publication of the Phase III Aspreva Lupus Management Study, comparing induction therapy with MMF or cyclophosphamide in combination with corticosteroids for 24 weeks for lupus nephritis, and maintenance treatment with MMF or azathioprine thereafter is expected in 2009.[19]

Interindividual variability of MMF pharmacokinetic parameters in patients with AID was found to be as high as in renal transplant patients.[20] Whereas in renal transplant patients, treatment regimens often consist of 3 or 4 immunosuppressive drugs, AIDs are typically treated with 1 or 2 drugs concomitantly. This would imply that the success of treatment would be even more dependent on the MPA concentrations reached, and thus, TDM in patients with AID may be a valuable tool to optimize individual immunosuppressive therapy.[21] Recently, a correlation is seen between MPA through levels and recurrence of active disease in patients with AID.[22] To facilitate prospective trials, we decided to develop and validate a maximum a posteriori Bayesian estimator (MAP-BE) for MMF treatment in patients with AID and compare the predictive performance of this MAP-BE with an LSS based on linear regression for estimating MPA exposure.

Methods

Patients

Concentration-time samples from 38 patients (20 males and 18 females; age (mean ± SD) 52.4 ± 18.3 years) with AID treated with MMF who participated in a pharmacokinetic study were analyzed retrospectively. This study was based on a set of patients of whom the relationship between MPA concentrations and clinical outcome is already published.[22] This article describes the development of LSSs for the estimation of MPA AUC_{0-12} in patients with AID, using the same data set. See Neumann et al[22] for a detailed description of the methods of this pharmacokinetic study. The clinical study was institutional review board approved. For the post hoc analysis of the data as described in this article, no additional institutional review board approval was requested as all data were anonimized. Briefly, 26 patients with antineutrophil cytoplasmic antibody-associated systemic vasculitis and 12 patients suffering from SLE were enrolled in the pharmacokinetic study. The patients received MMF (1 g twice daily) for at least 10 weeks before the study. More than half of the patients received a low dose of steroids (2.5-7.5 mg prednisolon) as comedication. Three patients (8%) used cyclosporine as concomitant immunosuppressive drug. After a 12-hour overnight fast, a 1 g dose of MMF was administered orally. Blood samples were collected before drug intake and then 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, 14, and 24 hours thereafter. During the 24-hour collection period, no additional MMF dose was given. Concentrations of MPA in plasma were determined using enzyme multiplied immunoassay technique assay (EMIT MPA Assay System, Dade Behring, San Jose, CA). The main characteristics of the patients are listed in Table I.

Pharmacokinetic modeling

Basic model

All data were analyzed simultaneously using the nonlinear mixed-effects modeling software program (NONMEM Verion VI, level 1.0; GloboMax LLC, Ellicott City, MD). Because NONMEM

Table I: Patient characteristics.

	Index data set (n=19)	Validation data set (n=19)	p-value
Sex (m/f)	11/8	7/12	0.53
Age (years)	50.0±8.5 (20-82)	54.5±7.7 (25-79)	0.91
Weight (kg)	73.4±6.8 (45-111)	71.5±7.7 (48-106)	0.51
Serum albumin (g/L)	43.3±1.7 (34.0-49.8)	45.6±2.3 (35.9-54.6)	0.30
Creatinine clearance (mL/min)	62.3±12.5 (32.8-108)	70.5±14.7 (16-136)	0.61
Proteinuria (g/24h)	0.69±0.35 (0-2.5)	0.59±0.4 (0-3.7)	0.61
Hemoglobin (g/dL)	12.5±0.5 (10-14.7)	12.3±0.7 (8.9-14.1)	0.22
Primary disease	13 AASV 6 SLE	13 AASV 6 SLE	

For the index and validation group the patient characteristics are expressed as mean ±95% confidence interval (range). AASV, antineutrophil cytoplasmic antibody-associated vasculitis; SLE, systemic lupus erythematosus.

estimated pharmacokinetic parameters for MPA, MMF doses were converted to the equivalent MPA content by multiplying the MMF dose by 0.739. Data were logarithmically transformed, and the first-order estimation method was used throughout the entire model-building process because the first-order conditional estimate method did not minimize successfully.

In the first step of the population analysis, a compartmental pharmacokinetic model was developed. Several structural models were tested. Models with 1 or 2 compartments were evaluated, as well as models with and without lag time. Furthermore, it was evaluated whether absorption was best described as a zero- or first-order process with single- or double-peak absorption profiles. Finally, several methods were evaluated to describe the EHC peak.[23-24] Pharmacokinetic parameters were estimated in terms of central and peripheral volume of distribution (V), clearance (CL) and intercompartmental clearance (Q). Because bioavailability (F) could not be quantified, V, CL and Q values correspond to the ratios V/F, CL/F and Q/F, respectively. Interpatient variability (IPV) for all pharmacokinetic parameters was modeled using an exponential (equation 1a) or additive (equation 1b) error model.

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

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

where CL_i represents the MPA clearance of the i^{th} individual, θ_{pop} represents the population value for MPA clearance, and η represents the interindividual random effect with mean 0 and variance ω^2. The covariance between values for IPV was estimated using a variance-covariance matrix. Residual variability between observed (C_{obs}) and predicted (C_{pred}) MPA plasma concentrations was described using an additional error model for logarithmic transformed data (equation 2).

$$ln\,C_{obs} = ln\,C_{pred} + \varepsilon_i \quad \text{(Eq. 2)}$$

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

The population model was built stepwise. A specific assumption was tested at each step. The main decision criterion was the likelihood ratio test. In NONMEM modeling, the minimum value of objective function (OFV) can be used as a criterion for model selection. If the difference in OFV between 2 nested models is larger than the critical value from a chi-squared distribution with degrees of freedom equal to the difference in the number of estimated parameters, the models are significantly different from each other. A decrease in the OFV>10.83 shows a significant improvement of a nested model with 1 *df* of p<0.001. Model adequacy was further evaluated by using various residual plots ("goodness-of-fit" plots) and values of random effects variances. To analyze the graphical goodness of fit, extensive plotting was available through the use of Xpose,[25] a purpose built set of subroutines in S-plus (version 6.1; Insightful Corp. Seattle, WA).

Covariate model

To explain IPV, relationships were investigated between pharmacokinetic parameters and patient characteristics. Covariates tested were patient age, sex, weight, kind of disease, concurrent use of cyclosporine, serum creatinine concentration, creatinine clearance (CrCL), proteinuria, hemoglobin, serum albumin concentration, lymphocyte, and leukocyte count. First, all potential covariates were added separately to the basic model for univariate analysis. Continuous covariates were modeled exponentially (equation 3).

$$CL_i = \theta_{pop} * (CrCL / 66)^{\theta_{CrCL}} \quad \text{(Eq. 3)}$$

in which θ_{pop} is the MPA CL in individuals with the median CrCL of the population (66 mL/min) and θ_{CrCL} is an exponent determining the shape of the relationship. Categorical variables were modeled proportionally (equation 4).

$$CL_i = \theta_{pop} * \theta^{CsA} \quad \text{(Eq. 4)}$$

in which θ_{pop} is the MPA CL in individuals without cyclosporine (CsA=0) and θ_{CsA} is the fractional change of CL due to concurrent use of cyclosporine. Whether a variable had a significant effect was determined with the likelihood ratio test. When inclusion of a covariate causes a decrease in OFV>3.84 (p<0.05), the covariate was considered to be statistically significant.

Second, a multivariate analysis with backward elimination was done to obtain the final model. All covariates selected during the first stage were included in an intermediate model. Covariates were excluded separately from the intermediate model. If the elimination of a covariate caused an increase in OFV>10.83 (p<0.001), then the covariate remained in the model.

Model validation

As an internal validation method, a bootstrap resampling method[26] was applied, using the Wings for NONMEM software (Dr N. Holford, version 612, March 2007, Auckland, New Zealand). Two hundred bootstrap data sets were generated by sampling randomly from the original

data set with replacement. Parameters were estimated for each of the replicate data sets using the final model. The validity of the model was evaluated by comparing the median values and 95-percentiles of the bootstrap replicates with the estimates of the original data set.

The final model was further validated by the application of a visual predictive check.[27] One hundred data sets were simulated from the original data set using the final model. Per time point, the median simulated concentrations plus 95-percentile intervals were compared graphically with the observed MPA concentrations.

Limited sampling strategies

Design of MAP-BEs

The patient data set was split into 2 data sets: an index data set (n=19) and a validation data set (n=19). The index data set was used to obtain population parameter values of the final model. MAP-BEs were developed using the post hoc option of NONMEM. The best LSSs were selected on the basis of a combination of 1, 2 or 3 sampling times within 4 hours post dose. The individual parameters and $AUC_{0\text{-}12}$ of the patients were estimated using the population model. Subsequently, the predictive performance of the developed MAP-BEs was evaluated with the validation data set by calculating bias or mean prediction error (equation 5) and precision or root mean squared prediction error (equation 6).

$$MPE = \frac{\sum_{i=1}^{N}(pe_i)}{N} \quad \text{(Eq.5)}$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}(pe_i^{\,2})}{N}} \quad \text{(Eq.6)}$$

where N represents the number of pairs of estimated and measured AUC, and pe_i is the difference between the estimated and the measured AUC. Reference $AUC_{0\text{-}12}$ values were calculated using the linear trapezoidal rule and all available data concentrations. To assess the agreement between the calculated $AUC_{0\text{-}12}$ and the reference $AUC_{0\text{-}12}$, the r^2 was calculated.

LSSs developed with multiple linear regression

A second method, multiple linear regression, was used to develop an LSS as well. The index data set was used to develop the LSSs. MPA concentrations at each sampling time point were correlated with the reference $AUC_{0\text{-}12}$ using linear regression analysis. Those sampling time points that showed the best correlation were combined to create LSSs. The correlation was calculated in a multiple stepwise linear regression analysis, with the reference $AUC_{0\text{-}12}$ as independent variable and the MPA concentrations at the different sample time points as explanatory variables. The validation data set was used to determine bias, precision, and r^2 of the LSSs. These results were compared with the MAP-BEs.

Results

Patients

The data set contained 492 MPA plasma concentrations obtained from 38 patients with AID. Each patient participated in 1 pharmacokinetic assessment of a 24-hour AUC. Figure 1 shows the observed MPA plasma concentration-time profiles for all patients. In some patients, a second and third peak was seen at approximately 6 and 10 hours after administration. Mean AUC_{0-12} was 66.0 mg·h/L, with a range between 27.6 and 129.4 mg·h/L.

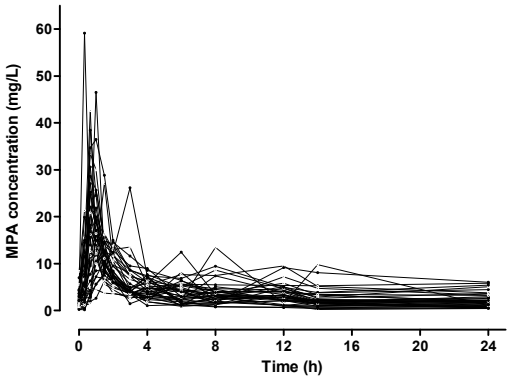

Figure 1: MPA concentration-time profiles. Pharmacokinetic curves obtained in 38 patients with AIDs receiving 1 g MMF.

Pharmacokinetic modeling

The concentration-time data of all patients were fitted simultaneously to several pharmacokinetic models. Figure 2 shows the final basic model. A 2-compartment model with a central (V_C) and a peripheral volume of distribution (V_P) and first-order elimination adequately described the data. To describe the absorption phase, a double-peak model with a short lag time ($T_{LAG,short}$) and a longer lag time ($T_{LAG,long}$) followed by first-order absorption was used. The fraction of the dose transported through the short (P) and long absorption compartment (1-P) was estimated. The EHC was modeled with an extra gallbladder compartment, which was filled continuously from the central compartment with rate k_{46}. Emptying of the gallbladder into the gastrointestinal compartment occurred at 2 time points (T_{GB1} and T_{GB2}) with rate k_{63} and duration D_{GB}, followed by reabsorption of MPA into the central compartment. Because insufficient data were collected around the EHC process, several parameters need to be fixed based on prior information to make the model numerically identifiable. The part of MPA clearance that was recirculated into the gallbladder (EHCP), calculated with equation 7, was fixed at 37%.

$$EHCP = \frac{k_{46}}{k_{46} + CL / V_C} x100\% \qquad \text{(Eq. 7)}$$

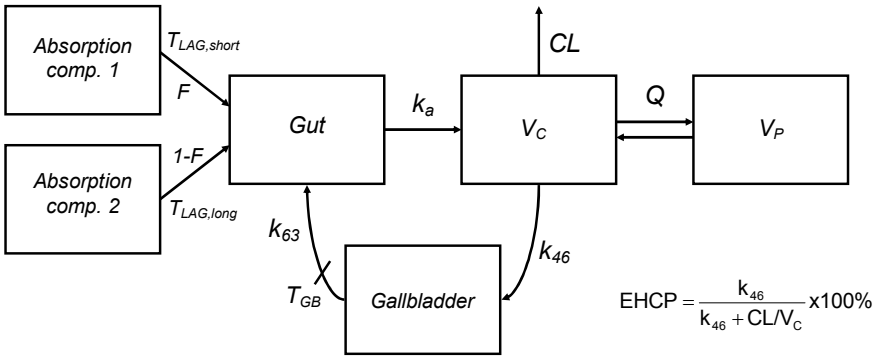

Figure 2: Population pharmacokinetic model. Model used to describe MPA concentration-time profiles in patients with AIDs. P, part of dose transported trough the absorption compartment with short T_{LAG}; $T_{LAG,short}$, short lag time; $T_{LAG,long}$, long lag time; k_a, rate of absorption; V_C, volume of distribution of the central compartment; CL, clearance; V_P, volume of distribution of the peripheral compartment; Q, intercompartmental clearance; k_{46}, filling rate of gallbladder; T_{GB}, time of gallbladder compartment opening; EHCP, part of MPA recycled in the body; and k_{63}, rate of gallbladder emptying.

The best values for the parameters T_{GB1}, T_{GB2}, D_{GB} and k_{63} were determined after fixing these parameters at several values. The best values for these parameters resulted in a bolus (D_{GB}=0.1 hours) gallbladder emptying at 6 and 10 hours after MMF administration. The optimal rate of emptying was 1 per hour.

Introduction of IPV for k_a, V_C, and CL as an exponential error model significantly improved the fit of the model; corresponding values were 186, 54, and 41%. Introduction of IPV of $T_{LAG,long}$, T_{GB1} and EHCP further improved the fit of the model. IPV for these parameters however had to be fixed at certain values. IPV of EHCP was described exponentially and fixed at a value of 35%, which limited the EHCP of 95% of the patients between 10% and 61%.[28-29] For $T_{LAG,long}$, IPV was fixed at 32% with an exponential error model. With this restriction, it was possible to discriminate between delayed absorption (T_{LAG}<1hour) and EHC. The latter occurred usually after at least 4 hours. The longest individual absorption lag time estimated was 0.83 hours. The first EHC peak in the individual pharmacokinetic profiles varied between approximately 4 and 8 hours after administration. To get 95% of all first EHC peaks within this range, the IPV of T_{GB1} was fixed at an additive error value of 2 hours, resulting in a range of 2.3-7.9 hours. The second EHC peak (T_{GB2}) was fixed at a value of four hours after T_{GB1}. Typical population estimates for the pharmacokinetic parameters for this basic model are summarized in Table II.

The screening of the relationship between patient factors and pharmacokinetic parameters produced an intermediate model with the following correlations: age, creatinine concentration and leukocyte count for k_a; age, creatinine concentration, and CrCL for CL; and sex, kind of disease, creatinine concentration, and CrCL for EHCP ($p<0.05$). During the backward elimination, only CrCL for CL resulted in a significant increase in OFV when excluded from the intermediate model ($\Delta OFV=14.1$, $p<0.001$). Figure 3 shows the correlation between CrCL and MPA CL. The inclusion of this covariate in the final model partly explained IPV (Table II). The

Table II: Parameter estimates of the pharmacokinetic models.

	Basic model OFV=-116.9		Final model OFV=-130.8		Bootstrap		Index data set	
Parameter	Value	CV (%)	Value	CV (%)	Mean	CV (%)	Value	CV (%)
F_{fast}	0.718	10	0.713	9	0.704	20	0.675	13
$T_{LAG,short}$ (h)	0.288	5	0.287	5	0.278	14	0.304	3
$T_{LAG,long}$ (h)	0.643	3	0.645	3	0.624	17	0.663	1
k_a (h^{-1})	6.19	25	6.2	22	8.87	123	8.87	36
V_C (L)	51.8	11	52.4	17	50.7	16	50.0	16
CL (L/h)	7.92	8	8.27	5	7.34	19	8.61	8
V_P (L)	279	41	262	5	440	79	205	39
Q (L/h)	15.9	16	16.2	22	17.8	15	18.1	24
T_{GB1} (h)*	6	-	6	-	6	-	6	-
T_{GB2} (h)*	T_{GB1}+4	-	T_{GB1}+4	-	T_{GB1}+4	-	T_{GB1}+4	-
D_{GB} (h)*	0.1	-	0.1	-	0.1	-	0.1	-
EHCP*	0.37	-	0.37	-	0.37	-	0.37	-
k_{63} (h^{-1})*	1	-	1	-	1	-	1	-
ERR	0.415	6	0.414	6	0.503	37	0.409	9
CrCL on CL	-	-	0.42	26	0.408	7	0.454	35
IPV								
$T_{LAG,long}$ (%)*	32	-	32	-	32	-	32	-
k_a (%)	186	32	182	40	141	98	200	55
V_C (%)	53.8	47	53	48	78	33	72	64
CL (%)	40.5	31	34	41	64.3	49	34	51
T_{GB1} (h)*§	2	-	2	-	2	-	2	-
EHCP (%)*	35	-	35	-	35	-	35	-

Estimations and their coefficient of variation (CV) for the pharmacokinetic parameters of the basic model, the final model, the index data set with the final model and the bootstrap procedure are described in this table. OFV, value of objective function; F_{fast}, part of dose ending up in the fast absorption compartment; $T_{LAG,short}$, lag time short absorption; $T_{LAG,long}$, lag time long absorption; k_a, rate of absorption; V_C, volume of distribution of the central compartment; CL, clearance; V_P, volume of distribution of the peripheral compartment; Q, intercompartmental clearance; T_{GBn}, time of n^{th} opening gall bladder compartment; D_{GB}, duration of gall bladder opening; EHCP, part of MPA recycled in the body; k_{63}, rate of gall bladder emptying; ERR, residual random error; CrCL, creatinine clearance; IPV, interpatient variability; *, the parameter is fixed at this value; §, additional variability.

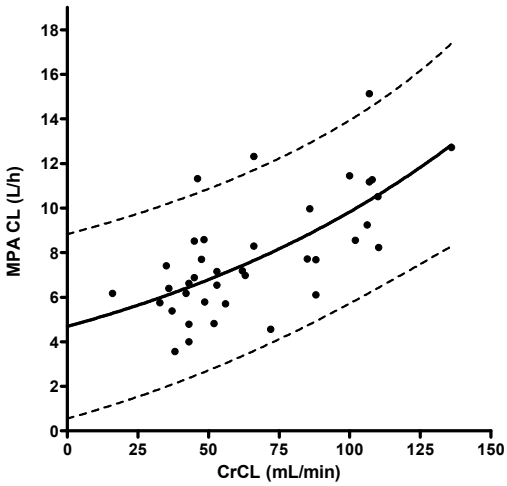

Figure 3: Correlation between creatinine clearance (CrCL) and MPA clearance (CL). Plot of the mean (solid line) influence of CrCL on MPA CL and the 95% confidence interval of the predicted correlation (dotted lines).

estimate for IPV for CL decreased from 40.5% to 34.0%. Figure 4 illustrates the goodness-of-fit plots of the final model. The scatter plots of predicted and individually predicted versus observed concentrations show no structural bias, except for a small underprediction of the maximum MPA concentration. The weighted residuals were homogeneously distributed over the sampling time period.

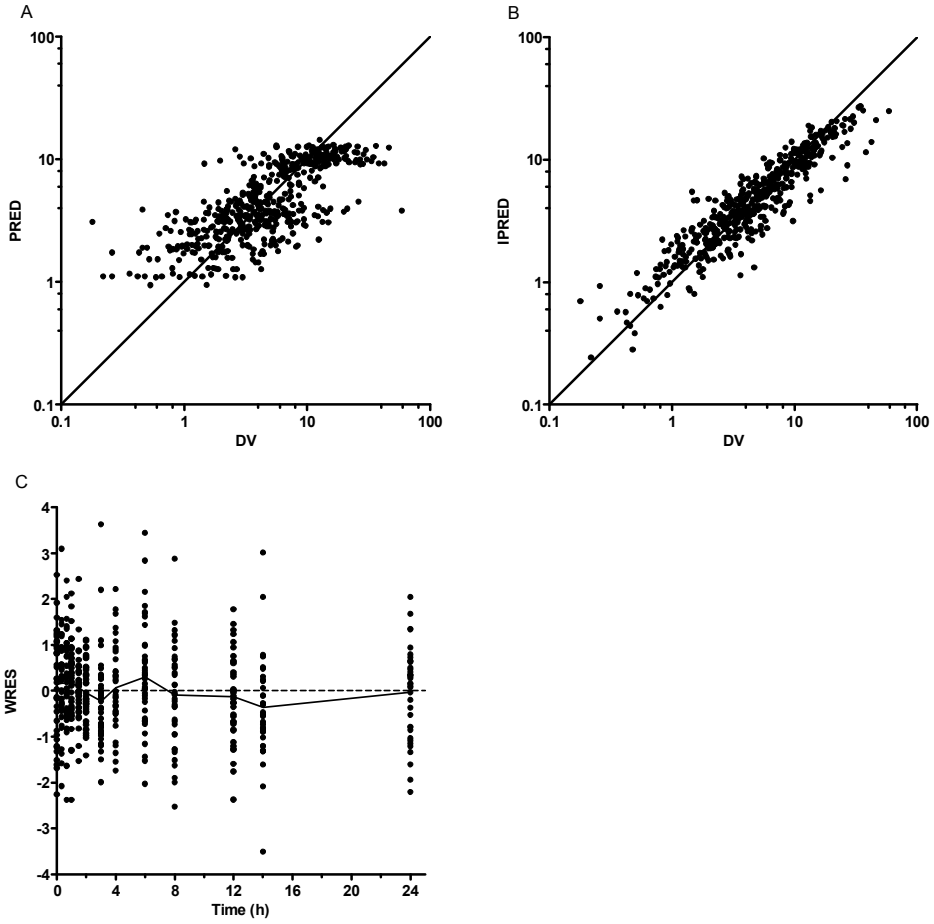

Figure 4: Goodness-of-fit plots of the final model. Plots of observed MPA concentration (DV) versus model predicted (PRED) (a) and Bayesian predicted (IPRED) (b) and weighted residuals (WRES) versus time (c). The solid line in (a) and (b) is the line of identity. The solid line in (c) is the average of the WRES.

The results of 200 bootstrap replicates are given in Table II. The mean estimates resulting from the bootstrap procedure are very similar to the population estimates of the final model. This demonstrated that the estimates for the fixed and random effects in the final model are accurate and that the model is stable.[26] The visual predictive check (Fig .5) revealed a good agreement between the simulated and observed concentrations at all sampling time points.

However, there seemed to be a small underprediction of the maximum MPA concentration at approximately 1 hour after MMF administration.

Limited sampling strategies

The parameters of the final model were estimated again in the index data set, which produced comparable results (Table II). The patient characteristics of the index and validation data set were similar (Table I). The population pharmacokinetic parameters obtained in the index group were used to develop MAP-BE to predict MPA AUC_{0-12} on the basis of a limited number of samples. On basis of predictive performance (bias and precision), the following sampling times between 0 and 4 hours post dose were selected for the optimal sampling schedules: 1 sample: 0 hour; 2 samples: 0+1 hour; 3 samples: 0+1+3 hours (Table III). The developed MAP-BEs adequately estimated AUC_{0-12} in the validation data set. When compared with AUC_{0-12} assessed by the trapezoidal rule, bias was not significantly different from zero and precision was below 27%. An extra sample at 6 hours post dose was included in the LSS to provide more information about the extent of EHC. This extra sample improved the precision of the LSS from 23.0% to 18.9% (Table III). The predictive performance for an individual patient is illustrated in Figure 6.

In the second LSS, multiple linear regression was used to select MPA plasma concentration sampling times to predict AUC_{0-12}. In the index data set, MPA concentrations at each sample time point until 4 hours after oral intake of MMF were correlated with the MPA AUC_{0-12} as assessed by the trapezoidal rule. The correlation was best for the predose sample (r^2=0.68).

Table III: Predictive performance of the LSSs in the validation data set.

Sampling times LSS	Algorithm	MPE (%)	RMSE (%)	r^2
MAP-BE t=0		-5.3 (-18.3,7.7)	26.8 (13.1,35.6)	0.38
MAP-BE t=0+1		-5.6 (-17.6,6.4)	24.9 (11.9,32.4)	0.46
MAP-BE t=0+1+3		-5.5 (-16.6,5.6)	23.0 (12.6,30.0)	0.53
MAP-BE t=0+1+3+6		-5.5 (-14.4,3.5)	18.9 (11.3,24.2)	0.66
MLR t=0	$AUC=38.3+11.7xC_0$	3.4 (-9.8,16.6)	26.8 (1.9,37.9)	0.48
MLR t=0+0.67	$AUC=30.8+10.1xC_0+0.7xC_{0.67}$	4.8 (-7.4,17.0)	25.1 (11.5,33.5)	0.53
MLR t=0+1+3	$AUC=17.5+7.1xC_0+1.0xC_1+2.6xC_3$	0.8 (-10.4,12.0)	22.6 (13.5,29.0)	0.61
MLR t=0+1+3+6	$AUC=12.3+4.7xC_0+1.2xC_1+2.7xC_3+1.8xC_6$	-0.4 (-9.0,8.1)	17.3 (9.0,22.7)	0.70

Bias (MPE) and precision (RMSE) are represented as means and 95% confidence interval. MAP-BE, maximum a posteriori Bayesian estimator; MLR, multilinear regression; r^2, correlation coefficient.

The multiple stepwise regression analysis revealed that the best prediction of the measured AUC_{0-12} was derived by the combination of the time points t=0+0.67 hours post dose for a 2-sample LSS and t=0+1+3 hours post dose for a 3-sample LSS (Table III). Inclusion of the fourth sample (t=6 hours) improved the predictive performance of the LSS. To test the agreement between the measured MPA AUC_{0-12} and the predicted AUC_{0-12}, the developed LSSs were applied to the validation data set. The validation of the strategies yielded a precision below 27% and a bias not significantly different from zero.

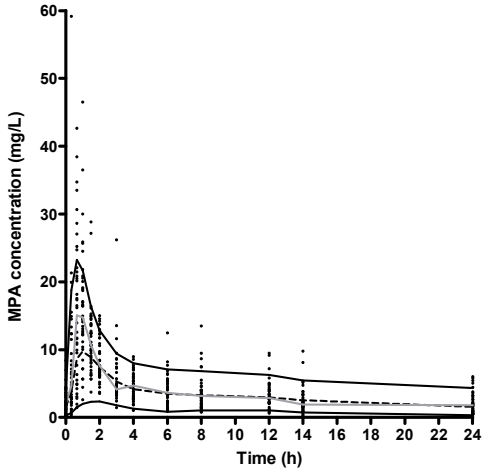

Figure 5: Visual predictive check. Comparison of median (dashed line) and 95-percentile interval (solid black lines) of 100 simulated data sets and observed MPA concentrations (dots). The grey line represents the median observed concentration-time curve.

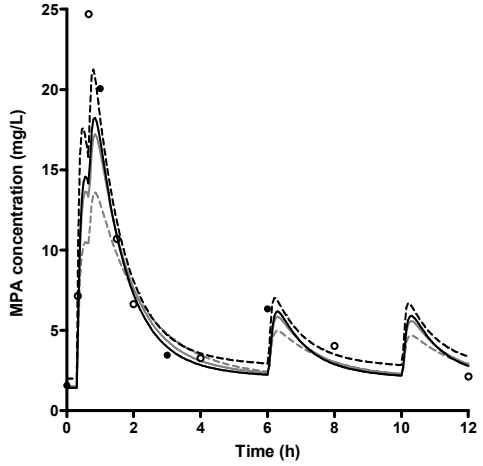

Figure 6: MAP-BE of the MPA concentration-time profile. The curves represent MAP Bayesian-predicted MPA concentration-time profile of a patient using 1 (t=0, dashed grey line), 2 (t=0+1, solid grey line), 3 (t=0+1+3, solid black line) or 4 samples (t=0+1+3+6, dashed black line). The filled dots represent the concentration-time points implemented in at least one of the LSSs, and the open dots represent the other observed MPA samples.

Discussion

In this study, a population pharmacokinetic model was developed for MPA in patients with AID (Fig. 2). In the model, the MPA concentration-time profiles were described using a double-peak absorption model, a central and peripheral compartment and a gallbladder compartment for EHC. LSSs were developed on basis of 1-3 samples during the first 4 hours after ingestion using MAP-BE and multiple linear regression. Both methods gave similar bias and precision. By increasing the number of sample times, the precision of the predicted

AUC_{0-12} increased. Sampling at 0, 1 and 3 hours after oral MMF administration resulted in the best predictive performance for both methods (Table III). We would like to stress that the enteric coating of the other currently available MPA formulation (enteric-coated mycophenolate sodium) results in importantly different pharmacokinetics and that the developed LSSs only apply to the MMF formulation.[30-31]

Little is known about the pharmacokinetics of MPA in patients with AID. Comparison with pharmacokinetics in renal transplant recipients may be complicated. Most renal transplant patients are cotreated with a calcineurin inhibitor, like cyclosporine or tacrolimus. Cotreatment with cyclosporine results in decreased EHC, due to inhibition of multidrug resistance-associated protein-2 (MRP2).[32] Furthermore, a decrease in MPA clearance over time is seen in renal transplant recipients, due to changes in renal function and albumin levels.[33-34] The results of the population pharmacokinetic model of the current study may be compared with studies describing MPA pharmacokinetics for patients in a stable posttransplantation phase receiving a cyclosporine-free regimen and including an EHC. MPA clearance in patients with AID (8.3 L/h) was slightly lower compared with renal transplant recipients cotreated with tacrolimus[24] (11.9 L/h) and healthy volunteers[35] (10.2 L/h).

In renal transplant recipients cotreated with cyclosporine, van Hest et al reported a negative correlation between CrCL and MPA CL.[36] The authors stated that MPAG CL was reduced by an impaired renal function. The increased MPA CL could be caused by displacement of MPA from albumin due to accumulation of the metabolite MPAG.[36] The covariate analysis of the current study also identified a relationship between CrCL and MPA CL. However, the correlation is positive instead of negative, MPA CL decreased with impaired renal function (Fig. 3). One reason for this different correlation is the fact that patients in this study did not use cyclosporine, which is known to inhibit EHC. Furthermore, this might be caused by an increased EHC. Decreased renal clearance of MPAG will cause accumulation of this metabolite, resulting in an increased availability of MPAG for biliary excretion. As a result, more MPAG will undergo deglucuronidation to MPA in the gut, which is reabsorbed. This explanation is supported by previous studies[37-38] in which a similar correlation was found in patients cotreated with tacrolimus.

The final model was used to develop and validate a MAP-BE to estimate AUC_{0-12} on the basis of 3 sampling times within 4 hours post dose. The best LSS was based on MPA concentrations at predose, 1 and 3 hours after oral intake of MMF. Given a 10-fold IPV, and a therapeutic window from 30 to 60 mg·h/L in renal transplant patients[39], a precision of ≤25% and a bias not significantly different from zero were considered to be acceptable. The mean bias (and 95% confidence interval) of the developed LSS was -5.5% (-16.6 to 5.6%) and mean precision 23.0%, which indicates that the developed LSS has an acceptable predictive performance. Inclusion of a fourth sample during EHC (t=6 hours) resulted in a further improvement of precision (18.9% versus 23.0%) but impairs practical application. Several MAP-BEs have been developed in renal transplant patients treated with MMF and cyclosporine.[40-41] Correlation coefficients (r^2>0.85) and precision (<13%) for these models are better than the values in the

present study. The mean bias of a previously published MAP-BE for MPA in renal transplant patients (7.7%)[40] is comparable with the bias found in the current study (-5.5%). The better correlation coefficient and precision could be explained by the decrease in variability in the second part of the concentration-time profile, due to the inhibition of EHC by cyclosporine.[32]

For the multiple linear regression method, AUC_{0-12} was best predicted when 3 samples were taken at predose and 1 and 3 hours post dose. The mean bias was less compared with the MAP-BE (0.8% versus -5.5%), but both were not significantly different from zero. The precision was comparable between both methods (22.6% versus 23.0%). Inclusion of an extra sampling time during EHC (t=6 hours) again improved the predictive performance of the LSS slightly (precision: 17.3% versus 22.6%). Pawinski et al[9] developed a LSS for MPA with multiple linear regression in renal transplant recipients cotreated with tacrolimus with a correlation coefficient of r^2=0.86, which is higher compared with the current study. This might be explained by less variability in the pharmacokinetic profile in that group of patients. Furthermore, an extension of the study using more patients may increase the correlation coefficient.

MPA concentrations in this study are determined using EMIT. A comparison of liquid chromatography-tandem mass spectrometry and EMIT showed that EMIT overestimated MPA levels and AUC, with variations depending on the posttransplantation period and sampling time.[42] This overestimation could largely be explained by the cross-reactivity of the anti-MPA antibody with the acylglucuronide metabolite of MPA.[43] Consequently, the LSSs developed using concentrations measured with this method cannot be used to estimate AUC_{0-12} using MPA concentrations determined with other analytical methods.

In a previous study, Zahr et al[44] developed a MAP-BE using an empiric pharmacokinetic model for MMF in 20 patients with SLE. In this study, a 1-compartment model with first-order elimination convoluted with a triple γ distribution was used to fit the MPA concentration-time data. In the current study, a more mechanistic approach was used. A gallbladder compartment was connected to a 2-compartment model (Fig 2). This extra compartment makes true circulation of MPA possible. Zahr et al[44] estimated AUC_{0-12} with samples taken at 0.67, 2 and 3 hours post dose. However, predicted performance was not assessed in an independent group of patients. As a result, a comparison between the empiric method of Zahr et al and our method is not possible.

In conclusion, MAP-BEs were developed for the estimation of MPA AUC_{0-12} in patient with AID taking MMF. The predictive performance of the MAP-BEs was good and comparable to those of the multiple linear regression method. Due to its flexibility with respect to sample times, the MAP-BE may be preferred over the multiple linear regression method. Optimal sampling times are predose and 1 and 3 hours after MMF administration. These results can be used for designing prospective trials, with either an observational or an interventional approach, in patients with AID, to further study the pharmacokinetic behavior of MPA and/or to study the added value of TDM for MPA in these patients.

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Chapter 3.3

Pharmacokinetics of mycophenolate mofetil in hematopoietic 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 hematopoietic stem cell transplantation (HSCTx). Few pharmacokinetic data are available about the use of MMF for this indication. This case series aimed at analyzing the pharmacokinetics of MMF in a population of HSCTx recipients representative for everyday practice. From 15 HSCTx 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/F), apparent half-life, and total MPA area under the curve for hours 0 to 12 (AUC_{0-12}, normalized to 1000 mg MMF) were, respectively, 56 L/h (range: 29-98 L/h), 2.3 hours (range: 0.8-5.7 hours), and 18.0 mg*h/L (range: 10-35 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} (normalized to 1000 mg MMF) was 224 µg*h/L (range: 56-411 µg*h/L). Because of high CL/F, total MPA exposure in HSCTx recipients is low and apparent half-life is short in comparison with reference values from renal transplantation. Exposure may be improved in HSCTx 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 rapid absorption from the gut, with maximum MPA peak concentrations generally occurring within 1 hour after oral MMF administration.[3] MPA is primarily metabolized 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 accumulates 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 in comparison with 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 hematopoietic stem cell transplantation (HSCTx) as well as to promote engraftment in nonmyeloablative HSCTx.[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 HSCTx recipients are largely based on pharmacokinetic data from renal transplant studies, as such data are scarce in HSCTx. This means that the starting MMF dose generally is 15 mg/kg twice daily orally, which in most HSCTx patients results in the standard MMF dose recommended in renal transplantation (1000 mg twice daily). Preliminary pharmacokinetic data from HSCTx recipients after standard MMF dosing showed low MPA trough levels in comparison with data from renal transplantation.[10,16-18] Confirmation of low MPA exposure, assessed by predose levels and area under the curve for hours 0 to 12 (AUC_{0-12}), was provided by 3 studies in HSCTx patients treated with nonmyeloablative conditioning.[12,19,20] Standard twice daily oral MMF dosing also seemed to result in low MPA exposure in four HSCTx patients after myeloablative conditioning.[21] These results suggest that in HSCTx the pharmacokinetics of MMF are different than in renal transplantation. This pilot study aimed at describing the pharmacokinetics of MPA, MPAG, AcMPAG, and free MPA in a case series of allogeneic HSCTx recipients representative for every day practice after application of an MMF dosing regimen, based on renal transplantation 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 HSCTx 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 intravenously (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 underwent the same conditioning regimens as patients with a related donor. In addition, they were given rabbit antithymocyte globulin (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 undergoing 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 by 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 of 1.0 to 3.5 mg/L has been adopted.[22]

Infection prophylaxis consisted of ciprofloxacin, 500 mg orally twice daily, and fluconazole, 200 mg orally once daily, starting on the first day of the conditioning regimen, until the granulocyte count was above 0.5x10^9/L. All patients received valacyclovir, 500 mg three times daily, from the first day of the conditioning regimen until 1 year posttransplantation, except patients at risk for cytomegalovirus (CMV) infection, who were given valganciclovir, 450 mg once daily. Patients at risk for CMV were defined as those 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 polymerase chain reaction (PCR) above 500 replicates (CMV-PCR was routinely performed every week). Every patient received co-trimoxazole, 480 mg once daily, for prevention of *Pneumocystis Carinii* infection when administration of ciprofloxacin and fluconazole was stopped, until 1 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 because of 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.

Bioanalytics

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. Twentyfive microliters of internal standard solution (tolmetin, 120 mg/L, in methanol) and 75 µL of acetonitrile were 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 32,000 *g*. The supernatant was placed in a new polypropylene tube and centrifuged for 10 minutes at 32,000 *g*. Twenty microliters 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), 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). 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 on the basis of a 4-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 to103% and for MPAG in the range of 95 to108%.

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 required 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 analyzed with WinNonlin version 4.1 (Pharsight Corporation, Mountain View, CA, 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_z) 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-normalized 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), clearance and V_z values correspond to the apparent oral values of this parameters (the ratio of CL/F and V_z/F respectively). 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). Apparent half-life ($t_{½}$) of MPA was calculated on the basis of estimates for CL/F and V_z/F.[27]

Statistics

Statistical tests were performed with the software package SPSS 10.1 for Windows (SPSS, Chicago, IL, USA). Pharmacokinetic data and patient characteristics are presented as median and range, because 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 HSCTx 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] Data from this patient

are not included in the group analyses. Patient characteristics are summarized in Table I. Ten patients received their transplant from a related donor. Eleven patients recieved a myeloablative conditioning regimen (Table I). 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.

Table I: Patient characteristics at the time of pharmacokinetic assessment.

Characteristic	No of patients or median value (range)
Female/Male*	6/9
Age (years)	32 (17-58)
Diagnosis	
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 < 0.5x10^9/L)	12
Body weight (kg)	70 (45-89)
Creatinine clearance (mL/min)	132 (46-265)
Albumin concentration (g/L)	33 (26-37)
MMF starting dose¶ (mg)	1000 (500-1500)
MMF daily dose (mg) at time of pharmacokinetic assessment*	3000 (1500-4000)
Cyclosporine predose level (ng/mL) at time of pharmacokinetic assessment	356 (198-562)

In total, concentration-time curves were drawn from 15 hematopoietic 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 leukemia; MDS-RAEBt, myelodysplastic syndrome-refractory anemia with excess of blasts in transformation; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's lymphoma; ATG, antithymocyte globulin; MMF, mycophenolate mofetil; TBI, total body irradiation.

¶Starting MMF dose was 15 mg/kg orally twice daily, rounded up or down to the nearest 250 mg MMF increment. Thereafter, MMF doses were adjusted to keep total MPA trough levels >1 mg/L.

*Mycophenolate mofetil was always given twice daily orally.

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 HSCTx of pharmacokinetic assessment was 8 days (range: 4-26 days). From 1 patient, 2 concentration-time profiles were taken, on days 7 and 15 after

HSCTx. 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.

At the time of pharmacokinetic assessment, acute GVHD was reported in 6 patients: 5 patients had grade 1 skin acute GVHD, for which they received triamcinolon ointment 0.1% topically twice daily, and 1 patient had grade 3 acute GVHD which was treated with i.v. prednisolone 90 mg twice daily. One patient experienced graft rejection, and the remaining 14 patients all reached complete donor chimerism within 3 months after transplantation. Reported side effects were mainly mucositis, in 9 patients, and infections, in 7 patients.

Pharmacokinetics of MPA and its metabolites

The results of the pharmacokinetic analysis for total MPA, MPAG, AcMPAG, and free MPA are presented in Table II and Figures 1 to 3. Figure 1 represents the concentration-time curves,

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

Component	Parameter	Median (range) (n=16*)
Total MPA		
	$AUC_{0\text{-}12}$ (mg*h/L)	25 (7.6-35)
	Dose normalized $AUC_{0\text{-}12}$ (mg*h/L)	18 (10-35)
	CL/F (L/h)	56 (29-98)
	V_z/F (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		
	$AUC_{0\text{-}12}$ (mg*h/L)	643 (219-1859)
	Dose normalized $AUC_{0\text{-}12}$ (mg*h/L)	430 (146-1638)
	C_{max} (mg/L)	88 (30-260)
	T_{max} (h)	2.5 (1.0-6.0)
AcMPAG		
	$AUC_{0\text{-}12}$ (mg*h/L)	3.5 (0.72-8.5)
	Dose normalized $AUC_{0\text{-}12}$ (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		
	$AUC_{0\text{-}12}$ (µg*h/L)	275 (62-616)
	Dose normalized $AUC_{0\text{-}12}$ (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 MPA (%)		0.96 (0.54-3.1)

*In total, concentration-time curves were drawn from 15 hematopoietic stem cell transplant patients (excluding the patient presented as a case report). From 1 patient 2 concentration-time curves were drawn. Values are not dose normalized, unless otherwise stated. Dose normalized $AUC_{0\text{-}12}$ values are normalized to 1000 mg mycophenolate mofetil.

MPA, mycophenolic acid; MPAG, glucuronide metabolite of MPA; AcMPAG, acyl glucuronide metabolite of MPA; AUC, area under the concentration-time curve; C_{max}, maximum concentration; T_{max}, time of maximum concentration after oral administration of mycophenolate mofetil; $t_{½}$, apparent half-life; CL/F, apparent oral clearance; V_z, volume of distribution.

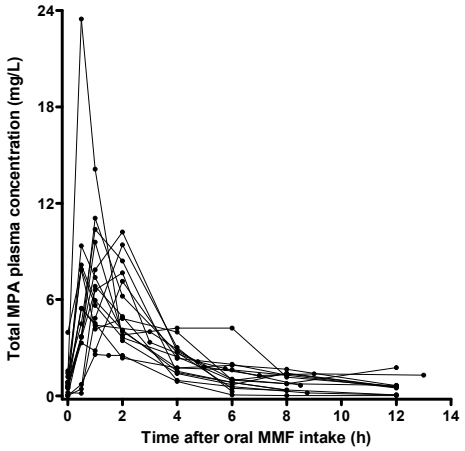

Figure 1: Individual concentration-time profiles of total mycophenolic acid (MPA) in 15 hematopoietic stem cell transplant recipients (excluding the patient presented as a case report). Concentration-time data are normarlized to 1000 mg MMF. Two profiles are from 1 patient.

normalized to 1000 mg MMF, of every patient and shows that total MPA is rapidly absorbed, reaching peak concentrations within 2 hours after oral administration. There was no clear explanation for the highest observed dose normalized maximum total MPA concentration of 23 mg/L. Dose normalized 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: only 4 concentration-time curves (25%) exhibited an increase in the total MPA concentration beyond 6 hours after MMF intake. The largest observed increase was 0.73 mg/L. The free MPA concentration-time curves, normalized to 1000 mg MMF, (Fig. 2) show that in 7 cases (44%), a modest increase in the free MPA concentration, occurred with a maximum increase of 18

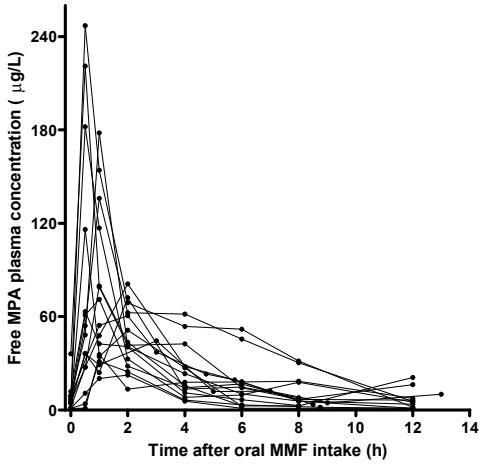

Figure 2: Individual concentration-time profiles of free mycophenolic acid (MPA) in 15 hematopoietic stem cell transplant recipients (excluding the patient presented as a case report). Concentration-time data are normalized to 1000 mg MMF. Two profiles are from 1 patient.

µg/L. The average curves of total MPA, MPAG and AcMPAG, normalized to 1000 mg MMF (Fig. 3), also show a minor contribution of EHC to drug exposure.

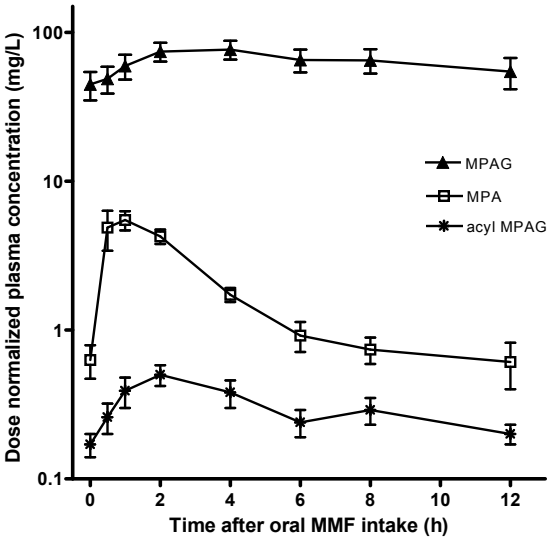

Figure 3: Mean dose normalized concentration-time curves with standard errors of total mycophenolic acid (MPA), MPA glucuronide metabolite (MPAG), and MPA acyl glucuronide metabolite (AcMPAG). Data are from 15 patients and 16 concentration-time profiles (excluding the patient presented as a case report).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

Median MPA CL/F was 56 L/h with a 3.4-fold between-patient variability (Table II). Median total MPA AUC_{0-12} normalized to 1000 mg MMF was 18 mg*h/L (mean dose normalized total MPA AUC_{0-12} was 20 mg*h/L) and median total MPA $t_{½}$ was 2.3 hours (Table II). Median total MPA predose level was 0.63 mg/L (Table II), 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 MPA CL/F (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 CL/F and serum albumin levels (r_s=-0.02, p=0.93), between CL/F and CsA dose (r_s=-0.24, p=0.36) or between CL/F and CsA predose level (r_s=-0.37, p=0.16).

Median MPAG AUC_{0-12} and AcMPAG AUC_{0-12}, both normalized to 1000 mg MMF, were 430 mg*h/L and 3.2 mg*h/L, respectively (Table II). 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 normalized free MPA AUC_{0-12} was 224 µg*h/L and median free fraction was 0.96% (Table II).

Because of 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.

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 characterize 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 HSCTx. Peripheral stem cells from an HLA-matched unrelated donor were transplanted after non-myeloablatiave conditioning with fludarabine, TBI and ATG. Complete donor chimerism was reached within 3 months after transplantation. At the time of pharmacokinetic assessment, chronic extensive GVHD and multiple infections were present. GVHD was treated with oral prednisolone, 75 mg twice daily. 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 (MPA CL/F=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 already below 0.5 mg/L at 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 1 week later a second pharmacokinetic assessment was done after oral intake of MMF. Total MPA AUC_{0-12} was 1.8 mg*h/L (MPA CL/F= 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 HSCTx.[10-12,15,18-20,29,30] In this study of the pharmacokinetics of MPA and its metabolites in a case series of HSCTx recipients, a high median MPA CL/F of 56 L/h, a low median dose-normalized total MPA AUC_{0-12} of 18 mg*h/L, and a remarkably short median MPA $t_{½}$ of 2.3 h were observed. Table III shows that the findings for total MPA AUC_{0-12} are in agreement with results from other studies in HSCTx recipients.[12,19,21] Table III also presents total MPA AUC_{0-12} data from renal transplant recipients for comparison.[31-33] It appears that with the same MMF dose, MPA exposure is almost 50% lower in HSCTx patients than in renal transplant recipients. The values in HSCTx recipients would be considered subtherapeutic 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]

Table III: Comparison of the results for mean dose-normalized total MPA AUC_{0-12} with literature data from hematopoietic stem cell transplant recipients and with literature data from renal transplant recipients.

Study	Mean dose-normalized MPA AUC_{0-12}	Reference
Present study:		
Van Hest et al (n=15)	20*	-
Studies in HSCTx recipients:		
Giaccone et al (n=34, day 7 after transplantation)	23#	19
Giaccone et al (n=34, day 21 after transplantation)	24#	19
Maris et al (n=31, day 7 after transplantation)	20¶#	12
Nash et al (n=4, day 21 after transplantation)	12¶#	21
Studies in renal transplant recipients:		
Weber et al (n=10, day 21 after transplantation)	31*	31
Shaw LM et al (n=20, day 7 after transplantation)	34*	32
Johnson et al (n=10, day 5 after transplantation)	36*	33

All data were obtained after oral administration of mycophenolate mofetil (MMF) in patients who were concurrently treated with cyclosporine.

*total MPA AUC_{0-12} data are based on 1000 mg MMF.

#total MPA AUC_{0-12} data are based on a MMF dose of 15 mg/kg.

¶these values concern medians. Mean values were not reported in the study by Maris et al[12] and Nash et al[21]

Median dose-normalized 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 HSCTx, which found free MPA AUC_{0-12} to be 211 and 251 µg*h/L on day 7 and day 21, respectively.[19]

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, which frequently occur with MMF therapy in renal transplant recipients.[6] There are no suitable data available from the renal transplant literature for comparison, 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 HSCTx patients.

The present unselected population of 15 HSCTx 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 HSCTx. Eight concentration-time curves were drawn within the first week after HSCTx, 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 HSCTx recipients and, importantly, the predominant finding of low MPA exposure occurred in nearly all patients. This indicates that the high MPA CL/F in patients undergoing HSCTx is a very explicit effect. The clinical consequence is that higher MMF doses or more

frequent dosing is necessary to attain higher total and free MPA exposure. The theory that higher exposure can also improve clinical outcome is strengthened by the recent finding that low free MPA exposure was associated with an increased risk for GVHD.[20] In addition, MMF administration 3 times a day has indeed been found to optimize total MPA exposure in patient undergoing HSCTx,[19,21,39] which subsequently was predictive for a higher degree of donor T-cell chimerism in patients undergoing nonmyeloablative conditioning and unrelated donor HSCTx.[19]

Because of the small sample size and the heterogeneity of the study population, the causes of the high MPA CL/F cannot be determined with the present data. The following observations, however, may have played a role, but they are speculative and warrant further research. First, 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] Few concentration-time profiles from this study, however, showed secondary MPA peak concentrations (Fig. 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 MPA CL/F, however, could not be found. Further factors adding to the reduced contribution of EHC may be 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.

A second factor may be low bioavailability. Unfortunately, no intravenous data were available in this study to assess bioavailability, but low bioavailability seems a plausible factor, 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 two studies, which showed a median bioavailability of 63 and 72% in HSCTx recipients with high between-patient variability, evidenced by observed bioavailabilities as low as 13%.[21,41] This median value is lower than values from renal transplantation.[42] 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 HSCTx recipients in previous studies.[17,21,39]

Finally, low albumin levels may contribute to the high MPA CL/F in HSCTx recipients. From renal transplantation it is known that low serum albumin levels lead to high CL/F, presumably through an increased MPA free fraction.[32,43] Although this analysis could not identify a relationship between serum albumin levels and CL/F, 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 MPA CL/F observed in this patient. A previous study in patients treated with nonmyeloablative conditioning and unrelated donor HSCTx did find a positive correlation between total MPA AUC and serum albumin levels.[19]

In conclusion, exposure to MPA after twice daily oral MMF administration in a case series of stem cell transplant recipients representative for daily practice is low in comparison with reference values from renal transplantation, as a result of high MPA CL/F. More frequent MMF dosing is likely to optimize 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 can be formulated.

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Chapter 3.4

Explaining the pronounced differences in clearance of mycophenolic acid between renal transplant recipients, hematopoietic stem cell transplant recipients and patients with autoimmune disease

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Abstract

For more than a decade mycophenolate mofetil (MMF) has been used as an immunosuppressive drug in solid organ transplant recipients to prevent graft rejection. After oral administration the prodrug MMF is rapidly hydrolyzed to the active metabolite mycophenolic acid (MPA). MMF is currently increasingly being used in hematopoietic stem cell transplantation (HSCTx) and autoimmune diseases (AID). The pharmacokinetics of MPA are markedly different in these patients. In comparison with renal transplant recipients (RTx), MPA clearance is increased in HSCTx patients and decreased in AID. The aim of this study was to characterise MPA clearance in RTx, HSCTx and AID patients and to test whether the differences in clearance can be explained by clinical chemical parameters. MPA concentration-time profiles from 19 RTx patients cotreated with cyclosporine, 17 RTx patients cotreated with tacrolimus, 38 HSCTx patients cotreated with cyclosporine and 38 patients with AID were analysed retrospectively with nonlinear mixed effects modeling (NONMEM, FOCE). The following covariates were tested: indication for MMF treatment, gender, age, weight, plasma albumin, cyclosporine cotreatment, dose and predose blood level, creatinine clearance, and haemoglobin. Pharmacokinetics of MPA were described by a 2-compartment model with time-lagged first order absorption. MPA clearance was correlated in univariate analysis with plasma albumin, cyclosporine dose and predose blood level, creatinine clearance, heamoglobin and indication for MMF treatment (RTx, HSCTx or AID) ($p<0.05$). All significant covariates were combined in an intermediate multivariate model, followed by backward elimination. Indication for MMF treatment could be removed from the intermediate model without compromising the fit. The correlation between clearance and cyclosporine predose levels and plasma albumin remained significant in the final model ($p<0.001$) and could explain the difference in clearance between the different indications for MMF treatment. Median clearance was 29.8, 47.5, and 10.6 L/h in RTx, HSCTx and AID patients, respectively. In conclusion, plasma albumin concentrations and cyclosporine predose levels explain the difference in MPA clearance between RTx, HSCTx and AID patients.

Introduction

After oral administration the prodrug mycophenolate mofetil (MMF) is rapidly hydrolyzed to the active agent mycophenolic acid (MPA). The majority of MPA is metabolized to the inactive 7-O-mycophenolic acid glucuronide (MPAG), which exhibits enterohepatic recirculation (EHC). MPA is a selective, reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH). IMPDH has an important role in the *de novo* purine synthesis in T and B lymphocytes.[1] Inhibition of this pathway causes immunosuppression, which is contributing to the prevention of graft rejection. Although introduced as a fixed-dose drug, debate has emerged with respect to the potential contribution of therapeutic drug monitoring (TDM) of MPA area under the concentration-time curve (AUC_{0-12}).[2-6]

Previous studies in renal transplant recipients (RTx) showed that MPA exposure is significantly correlated to renal function and plasma albumin level.[7-9] Low plasma albumin levels and accumulation of MPAG due to impaired renal function decrease the binding of MPA to albumin. The subsequent increase of unbound MPA produces an increase in MPA clearance, resulting in decreased MPA exposure. Furthermore, cyclosporine comedication is correlated with increased MPA clearance.[7-9] Cyclosporine inhibits the EHC of MPA, due to inhibition of the multidrug resistance-associated protein 2 (MRP2) enzyme.[10]

MMF is now the most prescribed immunosuppressive agent in RTx recipients to prevent graft rejection.[11] It has been shown to be more effective on acute rejection rates and on long-term outcomes than azathioprine.[12-14] The success of MMF within solid organ transplantation has triggered the increasing application of MMF in other diseases. MMF was demonstrated to be effective in the prevention and treatment of graft-versus-host disease (GVHD) after hematopoietic stem cell transplantation (HSCTx)[15-16] and to be beneficial for patients with autoimmune diseases (AID), like systemic lupus erythematosus, lupus nephritis and antineutrophil cytoplasmic antibody-associated systemic vasculitis.[17-18] Nowadays, MMF is increasingly used after HSCTx and in patients with AID.

Most pharmacokinetic studies of MMF are performed in RTx recipients. However, the pharmacokinetics are different in HSCTx patients and patients with AID. In comparison with RTx recipients, MPA clearance is increased in HSCTx patients and decreased in patients with AID.[19-20] The reason for these differences is not clear. The aim of this study was to characterise MPA clearance in RTx, HSCTx and AID patients and to test whether the differences in clearance can be explained by clinical chemical parameters.

Methods

Patients

MPA concentration-time profiles from 36 RTx patients, 38 HSCTx patients and 38 patients with AID treated with MMF were combined and analyzed simultaneously. The data were obtained from five different clinical trials, which are presented in Table I.[19, 21-24] One of these studies has not been published yet. All of the studies used were approved by the institutional review board. Patient characteristics are presented in Table II. The patient characteristics were compared with an ANOVA test using SPSS version 11.5.0 software for Windows (SPSS Inc., Chicago, IL, USA).

Table I: Studies included in the data set

Study	Patients	CNI	Number of patients	Sampling times	Time postTx (days)[b]
Van Gelder et al[21]	RTx	CsA	19	t=0, 0.33, 0.67, 1.25, 2, 6, 8, 12 h	11 (9-15)
Sombogaard et al[23, 24]	RTx	TCL	17	t=0, 0.5, 1, 2, 6, 12 h	6 (3-8)
van Hest et al[19]	HSCTx	CsA	18	t=0, 0.5, 1, 2, 4, 6, 8, 12 h	10 (4-139)
Sombogaard et al[a]	HSCTx	CsA	20	t=0, 2 h	14 (6-39)
Neumann et al[22]	AID	none	38	t=0, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 12, 14, 24 h	n.a.

a Unpublished data

b Time after transplantation presented as median (and range)

RTx, renal transplantation; HSCTx, hematopoietic stem cell transplantation; AID, autoimmune disease; CNI, calcineurin inhibitor; CsA, cyclosporine; TCL, tacrolimus; n.a., not applicable.

Table II: Patient characteristics

Group	HSCTx	RTx	AID	p-value
Number of patients	38	36	38	
Gender (% male)	68	75	47	0.035
Age (y)	43 (17-65)	50 (19-75)	55 (20-82)	0.061
Weight (kg)	77 (45-105)	79 (44-112)	72 (45-111)	0.294
Haemoglobin (mmol Fe/L)	5.7 (4.3-8.8)	6.0 (4.3-8.1)	7.7 (5.5-9.1)	<0.001
Creatinine clearance (mL/min)	91 (16-265)	48 (10-154)	55 (16-136)	<0.001
Plasma albumin (g/L)	35 (15-42)	34 (25-40)	44 (34-55)	<0.001
MMF dose (mg)	1000 (500-2000)	1000 (400-2200)	1000 (1000-1000)	0.038
Use of cyclosporine (%)	100	53	0	<0.001
Cyclosporine predose level (mg/mL)	356 (198-722)	267 (21-619)	n.a.	<0.001

Data are presented as median (and range). Significant differences were tested with ANOVA.

MMF, mycophenolate mofetil; RTx, renal transplantation; HSCTx, hematopoietic stem cell transplantation; AID, autoimmune disease; n.a., not applicable.

Pharmacokinetic analysis

The pharmacokinetic data of the RTx, HSCTx, and AID patients were pooled and simultaneously fitted using the nonlinear mixed-effects modeling software program (NONMEM, version VI, level 1.0, Globomax LLD, Ellicot City, MD, USA). Because NONMEM estimated pharmacokinetic parameters for MPA, MMF doses were converted to the equivalent MPA content by mul-

tiplying the MMF dose by 0.739. Data were logarithmically transformed, and the first-order conditional estimate (FOCE) method was used throughout the entire model-building process.

The population model was built stepwise. A specific assumption was tested at each step. The main decision criterion was the likelihood ratio test. In NONMEM modeling, the minimum value of objective function (OFV) can be used as criterion for model selection. If the difference in OFV between two nested models is larger than the critical value from a chi-squared distribution with degrees of freedom equal to the difference in the number of estimated parameters, the models are significantly different from each other. A decrease in the OFV>10.83 shows a significant improvement of a nested model with one degree of freedom of $p<0.001$. Model adequacy was further evaluated by using various residual plots ("goodness-of-fit" plots) and values of random-effects variances. To analyze the graphical goodness-of-fit, extensive plotting was available through the use of Xpose,[25] a purpose built set of subroutines in S-plus (version 6.1; Insightful Corp. Seattle, WA, USA).

In the first step of the population analysis, a two-compartment model with time lagged first-order absorption and first-order elimination was used, as described for MPA in the literature.[7, 20] Typical values for the pharmacokinetic parameters lag-time (T_{LAG}), absorption rate constant (k_a), central volume of distribution (V_C), clearance (CL), peripheral volume of distribution (V_P), and intercompartmental clearance (Q) were estimated. Since bioavailability (F) could not be quantified, V_C, CL, V_P, and Q values correspond to the ratios of V_C/F, CL/F, V_P/F, and Q/F, respectively. Improvements of the model by inclusion of interpatient variability (IPV) was tested for all pharmacokinetic parameters. IPV was modeled using an exponential error model (equation 1).

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

where CL_i represents the MPA clearance of the i^{th} individual, θ_{pop} represents the population value for MPA clearance, and η_i is the interindividual random effect with mean 0 and variance ω^2. The covariance between values for IPV was estimated using a variance-covariance matrix. Residual variability between observed (C_{obs}) and predicted (C_{pred}) MPA plasma concentrations was described using an additional error model for logarithmically transformed data (equation 2).

$$ln\, C_{obs} = ln\, C_{pred} + \varepsilon_i \qquad \text{(Eq. 2)}$$

where ε_i represents the residual random error with mean 0 and variance σ^2. In the elimination phase, a first value under the lower limit of quantification (LLOQ) concentration was included in the data set at a value of half the LLOQ; subsequent sub-LLOQ observations were deleted.

In the second part of the modeling procedure, the influence of covariates on MPA clearance was evaluated. Covariates tested were indication for MMF treatment (RTx, HSCTx, or AID), gender, age, weight, haemoglobin, creatinine clearance, plasma albumin, cyclosporine comedication (yes/no), cyclosporine dose, and cyclosporine predose level. The influence

of the indication for MMF treatment was evaluated by estimating a separate value for MPA clearance for RTx patients, HSCTx patients and patients with AID. Continuous covariates were modeled exponentially (equation 3).

$$CL_i = \theta_{pop}(alb/40)^{\theta_{alb}} \quad \text{(Eq. 3)}$$

in which θ_{pop} is the MPA clearance in individuals with albumin levels (alb) of 40 g/L and θ_{alb} is an exponent determining the shape of the relationship. In case of a continuous variable, which can become zero a constant value of one is added to this parameter to prevent mathematical problems. Categorical variables were modelled proportionally (equation 4).

$$CL_i = \theta_{pop} \bullet \theta_{CsA}^{CsA} \quad \text{(Eq. 4)}$$

where θ_{pop} is the MPA clearance in individuals who did not use cyclosporine (CsA=0) and θ_{CsA} is the fractional change in clearance due to concurrent use of cyclosporine (CsA=1). Covariates were introduced in univariate analyses. When inclusion of a covariate caused a decrease in OFV>3.84 ($p<0.05$), the covariate was considered to be statistically significant. Subsequently, a multivariate analysis with backward elimination was done to obtain the final model.[26] All covariates selected after the univariate analyses were included in an intermediate model. If the elimination of a covariate caused an increase in OFV>10.83 ($p<0.001$), then the covariate remained in the model and was considered to be significant.

Model validation

As an internal validation method, a bootstrap resampling method[27] was applied, using the Wings for NONMEM software (Dr N. Holford, version 612, March 2007, Auckland, New Zealand). Two hundred bootstrap data sets were generated by sampling randomly from the original data set with replacement. Parameters were estimated for each of the replicate data sets using the final model. The validity of the model was evaluated by comparing the median values and 95-percentiles of the bootstrap replicates with the estimates of the original data set. Furthermore, the final model was validated by the application of a visual predictive check.[28] Data sets (n=100) were simulated from the original data set using the final model. Per time point, the dose-corrected median simulated concentration plus 95-percentile intervals were compared graphically with the observed concentrations.

Results

Patients

From the RTx recipients 19 patients were cotreated with cyclosporine, and 17 patients were cotreated with tacrolimus. Pharmacokinetic assessments in this group were obtained shortly

after transplantation (Table I). In total, 245 MPA concentration-time samples were obtained from 36 RTx recipients. All 38 HSCTx patients were treated with MMF and cyclosporine. During the pharmacokinetic assessments 206 concentration-time samples were drawn in these patients. The patients suffering from AID were not cotreated with a calcineurin inhibitor. The pharmacokinetic data in this group consisted of 492 concentration-time samples obtained from 38 patients with AID. The concentration time profiles of all patients are presented in Figure 1. From the patient characteristics (Table II), significant differences in gender, hemoglobin levels, creatinine clearance, plasma albumin, MMF dose and cyclosporine comedication were seen between the three indications ($p<0.05$).

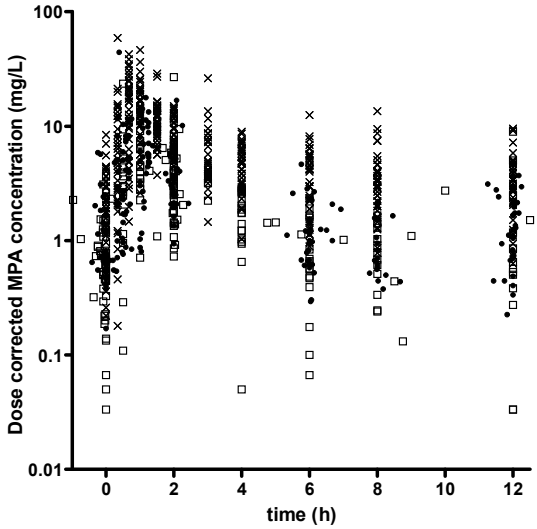

Figure 1: Dose corrected concentration-time data. Mycophenolic acid (MPA) concentration versus time for hematopoietic stem cell transplant patients (□), renal transplant recipients (●) and patients with autoimmune diseases (x), dose-corrected towards 1 g MMF.

Pharmacokinetic analysis

The concentration-time data of all patients were fitted simultaneously to develop a pharmacokinetic model. A two-compartment model with time-lagged first-order absorption and first-order elimination was used to describe the data. The values for the pharmacokinetic parameters T_{LAG}, k_a, V_C, CL, V_P, and Q and their coefficient of variation (CV) are presented as model 1 in Table III, in which k_a was fixed at a value of 4. IPV was estimated for V_C and CL using an exponential error model.

The difference in CL between RTx, HSCTx and AID patients was evaluated through estimation of a separate value for MPA CL for each indication (model 2, Table III). Introduction of this covariate reduced the OFV by 88.3 points compared to the basic model, and resulted in a decrease in IPV for CL from 76 to 44%. Further univariate analysis of the relationship between patient factors and MPA CL produced an intermediate model with the covariates indication for MMF treatment,

albumin level, cyclosporine comedication, cyclosporine predose level, haemoglobin level, and creatinine clearance ($p<0.05$). During the backward elimination procedure it appeared that cyclosporine comedication, haemoglobin level, creatinine clearance and indication for MMF treatment did not result in a significant increase of OFV when excluded from the intermediate model. These correlations were therefore not incorporated in the final model. Albumin level and cyclosporine predose level caused a significant increase of OFV when eliminated from the model ($p<0.001$, model 3, Table III). A decrease in albumin level from 40 to 30 g/L resulted in an increase in MPA CL of 11% (Fig. 2A). MPA CL was 50% reduced in patients cotreated with tacrolimus or without a calcineurin inhibitor compared to patients cotreated with cyclosporine with a predose level of 140 mg/L (Fig. 2B). The inclusion of these covariates in the final model resulted in a decrease in IPV for CL from 76 to 52% when compared with the basic model.

The goodness-of-fit plots of the final model (Fig. 3) show no structural bias. The original data set was used to generate 200 bootstrap data sets, which were fitted with the final model. The median estimates and 95-percentile range resulting from the bootstrap procedure were very similar to the population estimates of the final model (Table III). This demonstrates that the estimates for the fixed and random effects in the final model were accurate and that

Table III: Pharmacokinetic parameters

	Model 1	Model 2	Model 3	Bootstrap final model	
	(CV in %)	(CV in %)	(CV in %)	median	95-percentile range
OFV	362.8	274.5	273.4		
T_{LAG} (h)	0.294 (3)	0.296 (3)	0.295 (3)	0.296	(0.262-0.308)
k_a (h^{-1}) *	4	4	4	4	-
V_C (L)	90.5 (12)	79.6 (13)	86.5 (13)	85.3	(61.3-109)
CL (L/h)	25.1 (7)	-	32.0 (7)	31.4	(27.2-35.5)
CL HSCTx (L/h)	-	41.5 (7)	-	-	-
CL RTx (L/h)	-	28.9 (7)	-	-	-
CL AID (L/h)	-	13.1 (7)	-	-	-
V_P (L)	257 (28)	203 (26)	205 (24)	219	(140-404)
Q (L/h)	24.2 (8)	25.9 (8)	25.0 (8)	25.7	(21.4-31.9)
CsA C_0 ~ CL	-	-	0.14 (10)	0.14	(0.11-0.17)
Alb ~ CL	-	-	-0.37 (57)	-0.42	(-1.10--0.01)
residual error	0.59 (5)	0.59 (5)	0.59 (5)	0.59	(0.52-0.66)
IPV Vc (%)	113 (20)	117 (20)	119 (20)	114	(83-136)
IPV CL (%)	76 (12)	44 (19)	52 (16)	51	(42-61)

*k_a was fixed at 4 h^{-1}. OFV, minimum value of objective function; T_{LAG}, lag-time; k_a, absorption rate constant; V_C, central volume of distribution; CL, clearance; RTx, renal transplantation; HSCTx, hematopoietic stem cell transplantation; AID, autoimmune disease; V_P, peripheral volume of distribution; Q, intercompartmental clearance; CsA C_0, cyclosporine predose level; Alb, albumin level; IPV, Interpatient variability; CV, coefficient of variation.

the model is stable. Figure 4 shows the results of the visual predictive check, separated for indication for MMF treatment and comedication. The visual predictive check revealed a good agreement between the simulated and observed concentrations at all sampling time points. The η-shrinkage of the model was acceptable, 12.5% for the IPV of V_C and 6.5% for the IPV of CL. The ε-shrinkage was 8.6%.[29]

Individual post hoc estimations of MPA CL for the different indications for MMF treatment estimated with the final model are presented in Figure 5. Median (and range) of MPA CL was 47.5 L/h (16.5-133.0 L/h) in HSCTx patients, 29.8 L/h (11.2-63.7 L/h) in RTx patients, and 10.6 L/h (5.4-30.8 L/h) in patients with AID. In RTx patients median clearance was higher in patients cotreated with cyclosporine (33.1 L/h) compared with tacrolimus cotreated patients (25.9 L/h).

Discussion

In this study, a population pharmacokinetic model was developed for MPA in RTx recipients, HSCTx patients and patients with AID. Differences in MPA CL were described between the three patient groups. We demonstrate that the pronounced differences in MPA CL are explained by the differences in albumin levels and cyclosporine predose concentrations. Median MPA CL was 29.8, 47.5, and 10.6 L/h in RTx, HSCTx and AID patients, respectively. MPA CL was lowest in the AID patients, in whom albumin levels were normal, and who were free from co-treatment with CsA. The higher MPA CL in RTx patients is explained by the fact that they did have lower albumin levels, and part of them CsA co-treatment. HSCTx patients had low albumin levels and were all cotreated with high doses of CsA, resulting in the highest MPA CL of the three patient populations.

In the model, a correlation was seen between MPA CL and albumin levels (Fig. 2a). A decrease in plasma albumin levels from 40 to 25 g/L resulted in an elevation of MPA CL from 32.0 to 38.0 L/h in patients with a cyclosporine predose level of 140 mg/L. A similar effect was previously reported in RTx patients.[7, 9] The relation between MPA CL and albumin levels can be explained through MPA protein binding. When albumin levels increase, MPA protein binding increases, resulting in a smaller free MPA fraction and consequently less MPA available to be cleared.[30]

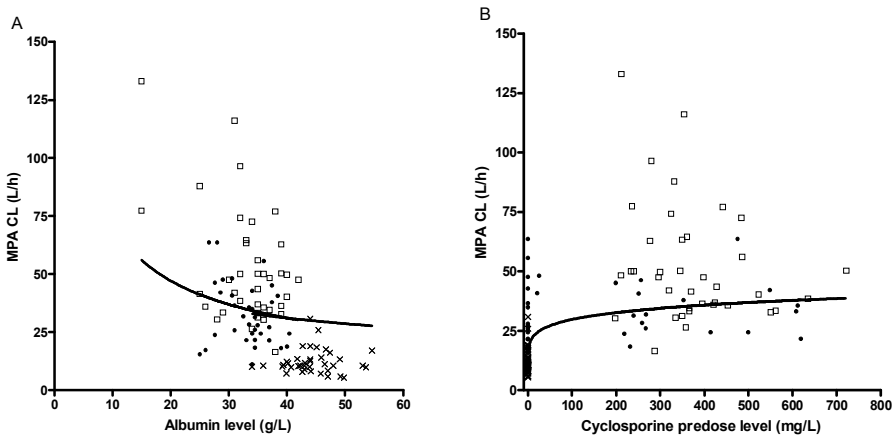

Figure 2: Correlation between MPA CL and (a) albumin levels and (b) cyclosporine predose levels for hematopoietic stem cell transplant patients (□), renal transplant recipients (●) and patients with autoimmune diseases (X). The line respresents the correlation fitted with the model.

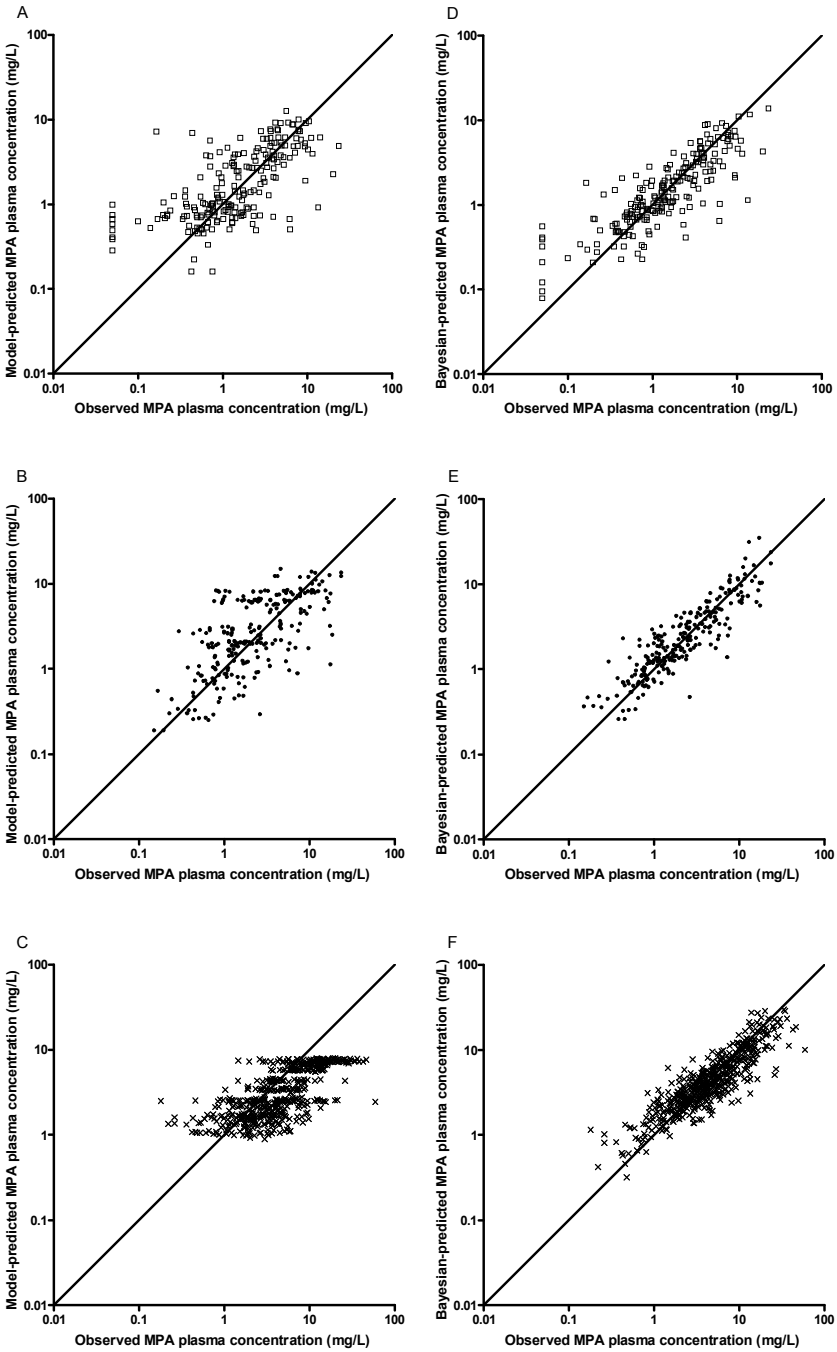

Figure 3: Goodness-of-fit plots for the final model. (a-c) Model-predicted mycophenolic acid (MPA) concentrations versus observed MPA concentration, (d-f) individual-predicted MPA concentration versus observed concentration and (g-i) weighted residuals versus time for hematopoietic stem cell transplant patients (□), renal transplant recipients (●) and patients with autoimmune diseases (X). The solid line in (a-f) represents the line of identity. The solid line in (g-i) is the line for y=0.

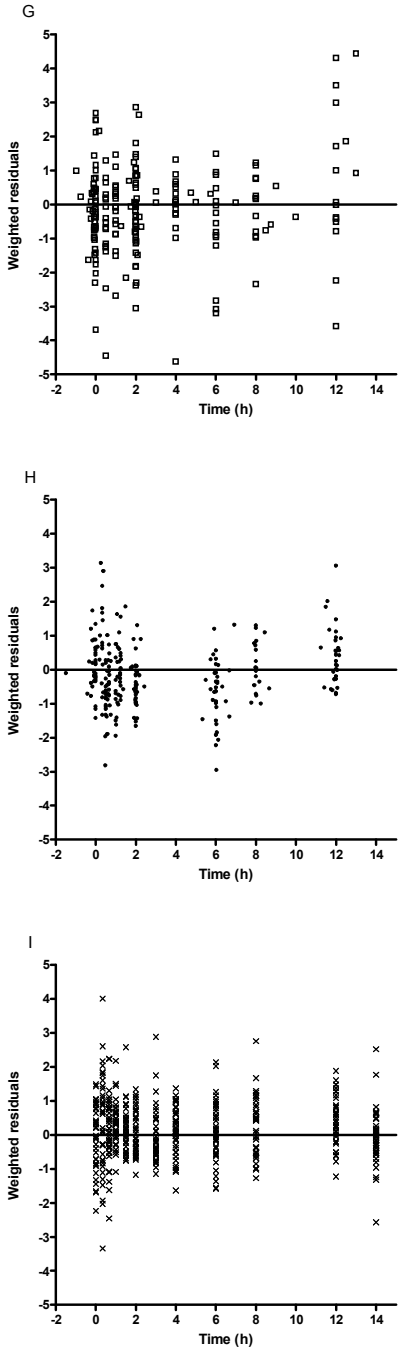

Figure 3. Continued

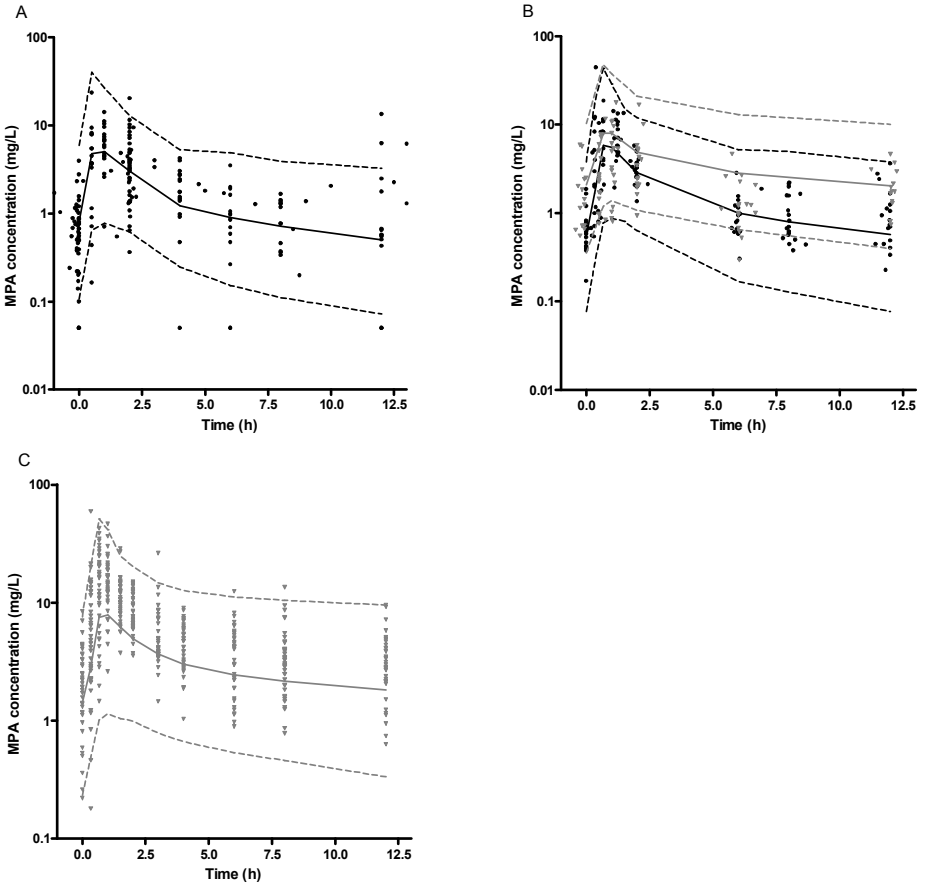

Figure 4: Visual predictive check. Comparison of the median (solid line) and 95-percentile range (dotted line) of 100 simulated data sets and the observed concentration-time points (dots) for (a) hematopoietic stem cell transplant patients, (b) renal transplant recipients and (c) patients with autoimmune diseases. The data are presented separately for patients cotreated with cyclosporine (black circle) or no cyclosporine (grey triangle) and are dose-corrected towards 1 g MMF.

The influence of cyclosporine predose levels on MPA CL can be explained by the EHC of MPA. In case of cotreatment with cyclosporine, the EHC is decreased by cyclosporine-induced inhibition of MRP2. MRP2 has been reported to be responsible for the excretion of MPAG in bile.[10] A similar effect of cyclosporine on MPA CL has been reported previously.[9] In this study, MPA CL decreased from 38.2 to 32.0 L/h when cyclosporine predose levels decreased from 500 to 140 mg/L. The MPA CL in tacrolimus cotreated patients was 16.1 L/h.

In RTx recipients a median value for MPA CL of 29.8 L/h was estimated, 25.9 L/h in tacrolimus cotreated patients and 33.1 L/h in cyclosporine cotreated patients. The pharmacokinetic assessment of MPA in these patients was obtained shortly after transplantation, between day 3 and day 15 posttransplantation. These results are comparable to the previously reported value for MPA CL of 32 L/h in RTx patients cotreated with cyclosporine in the first

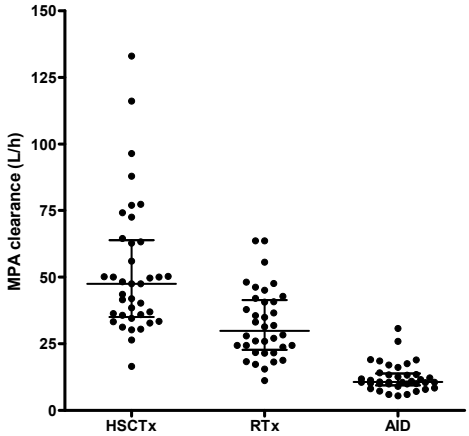

Figure 5: Differences in mycophenolic acid (MPA) clearance between hematopoietic stem cell transplant patients (HSCTx), renal transplant recipients (RTx) and patients with autoimmune diseases (AID). Data are presented as median and interquartile range.

week posttransplantation.[31] The MPA CL was increased to 47.5 L/h in HSCTx patients, due to cyclosporine comedication with high predose levels (median: 356 mg/mL) and low plasma albumin levels (median: 35 g/L). MPA CL was previously determined in a clinical trial to be 56 L/h for HSCTx patients.[19] In patients suffering from AID, the MPA CL was as low as 10.6 L/h. This value is also comparable to literature values of similar patients (8.3 L/h).[20] Higher plasma albumin levels (median: 44 g/L) and a therapy without cyclosporine could explain this low MPA CL compared with HSCTx and RTx patients.

The differences in MPA CL will have consequences for the exposure to MPA of the patients. HSCTx patients will have lower MPA AUC values than RTx patients after administration of an equal MMF dose. Under the same circumstances, MPA AUC will be relatively increased in patients with AID. To achieve similar exposure to MPA as in RTx recipients, the MMF dose needs to be increased in HSCTx patients and decreased in patients with AID.

In conclusion, a population pharmacokinetic model was developed to describe the pharmacokinetic differences in MPA clearance between RTx, HSCTx and AID patients. Median MPA clearance was 29.8, 47.5, and 10.6 L/h in RTx, HSCTx and AID patients, respectively. Plasma albumin levels and cyclosporine predose concentrations could explain the differences in MPA clearance between the three indications for MMF treatment.

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Chapter 4

MYCOPHENOLATE MOFETIL VERSUS ENTERIC-COATED MYCOPHENOLATE SODIUM

Chapter 4.1

Population pharmacokinetics of mycophenolic acid: a comparison between enteric-coated mycophenolate sodium and mycophenolate mofetil in renal transplant recipients

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Abstract

Objective: The pharmacokinetics of mycophenolic acid (MPA) were compared in renal transplant patients receiving either mycophenolate mofetil (MMF) or enteric-coated mycophenolate sodium (EC-MPS).

Methods: MPA concentration-time profiles were included from EC-MPS (n=208) and MMF treated (n=184) patients, 4-257 months after renal transplantation. Population pharmacokinetic analysis was performed using nonlinear mixed-effects modeling (NONMEM). A two-compartment model with first-order absorption and elimination was used to describe the data.

Results: No differences were detected in MPA clearance, intercompartmental clearance, or the central or peripheral volume of distribution. Respective values and interpatient variability (IPV) were; 16 L/h (39%), 22 L/h (78%), 40 L (100%) and 518 L (490%). EC-MPS was absorbed more slowly than MMF with respective absorption rate constant values of 3.0 h^{-1} and 4.1 h^{-1} ($p<0.001$, IPV 187%). A mixture model was used for the change-point parameter lag-time (T_{lag}) in order to describe interpatient variability in this parameter adequately for EC-MPS. Following the morning dose of EC-MPS the T_{lag} values were 0.95, 1.88 and 4.83 h for 51, 32 and 17% of the population (IPV 8%), respectively. The morning T_{lag} following EC-MPS was significantly different from both the T_{lag} following MMF administration (0.30 h, $p<0.001$ (IPV 11%)) and the T_{lag} following the evening dose of EC-MPS (9.04 h, $p<0.001$, IPV 40%). *Post hoc* analysis showed that T_{lag} was longer and more variable following EC-MPS administration (morning median 2.0 h (0.9-5.5 h), evening median 8.9 h (5.4-12.3 h)) than following MMF administration (median 0.30 h (0.26-0.34 h), $p<0.001$). The morning MPA predose concentrations were higher and more variable than following MMF administration, with respective values of 2.6 mg/L (0.4-24.4 mg/L) and 1.6 mg/L (0.2-7.6 mg/L). The correlation between predose concentrations and the area under the plasma concentration-time curve (AUC) was lower in EC-MPS treated patients ($r^2=0.02$) than in MMF treated patients ($r^2=0.48$).

Conclusion: Absorption of MPA was delayed and also slower following EC-MPS administration than following MMF administration. Furthermore, the T_{lag} varied more in EC-MPS treated patients. MPA predose concentrations were poorly correlated with the MPA AUC in both MMF and EC-MPS treated patients.

Background

Mycophenolate mofetil (MMF) is an immunosuppressive agent used in renal transplant recipients to prevent graft rejection. Three clinical trials in renal transplant patients have demonstrated that MMF is more efficacious as an immunosuppressant than azathioprine or placebo.[1-3] Following oral administration, the prodrug MMF is rapidly hydrolyzed to the active agent mycophenolic acid (MPA). The majority of MPA is metabolized to the inactive 7-O-mycophenolic acid glucuronide (MPAG), which exhibits enterohepatic recirculation (EHC). The minority of MPA is metabolized to the presumably active acyl-glucuronide (AcMPAG). MPA is a selective, reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH). IMPDH has an important role in the *de novo* purine synthesis in T and B lymphocytes.[4] Inhibition of this pathway causes immunosuppression, contributing to the prevention of graft rejection.

Gastrointestinal adverse events are frequently observed in renal transplant recipients treated with MMF. To abrogate these gastrointestinal adverse events and improve the clinical outcome, enteric-coated mycophenolate sodium (EC-MPS) was developed. In two clinical trials, EC-MPS 720 mg twice daily and MMF 1000 mg twice daily showed similar efficacy and safety profiles.[5, 6] MPA exposure, reflected by the area under the plasma concentration-time curve (AUC), is similar during administration of EC-MPS 720 mg and MMF 1000 mg, but differences in the pharmacokinetic profile have been reported.[7-9] During EC-MPS therapy, MPA predose plasma concentrations (C_0) are higher, MPA peak concentrations are lower and the time to reach the peak concentration is longer and varies more than with MMF therapy.[7-9]

In MMF treated renal transplant recipients, it has been demonstrated that biopsy-proven acute rejection is significantly correlated with both the MPA AUC ($p<0.001$) and MPA C_0 values ($p=0.01$), whereas no correlation between the efficacy and dose is present.[10] Moreover, the MPA AUC is a better predictor of the risk of rejection than MPA C_0 values.[10] The target range for the MPA AUC in renal transplant recipients cotreated with cyclosporine is 30-60 mg*h/L.[11] This AUC range is more or less associated with C_0 values of 1-3.5 mg/L.[11] Recently, the Apomygre (Adaptation de Posologie du MMF en Greffe Rénale) study[12] showed that therapeutic drug monitoring (TDM), using a limited sampling strategy to determine the MPA AUC, can reduce the risk of treatment failure and acute rejection in the first year after renal transplantation. The rates of biopsy-proven rejection were 7.7% in the concentration-controlled group and 24.6% in the group that received fixed doses of MMF ($p=0.01$).

The aim of this study was to compare the pharmacokinetics of MPA following administration of EC-MPS and MMF. A population pharmacokinetic analysis was performed using concentration-time data obtained from seven clinical studies with EC-MPS and/or MMF. In comparison with the "classic" two-stage pharmacokinetic analysis, the population pharmacokinetic approach has the advantage that data sets originating from several clinical studies with different sampling schedules can be combined easily. As a result, typical pharmacoki-

netic parameters and their corresponding interpatient variability (IPV) can be estimated in large, unbalanced and heterogeneous populations.

Patients and methods

Studies

MPA plasma concentration-time profiles obtained from 259 renal transplant recipients receiving maintenance therapy with either EC-MPS (n=208 profiles) or MMF (n=184 profiles) 4-257 months after renal transplantation were combined and analyzed simultaneously. The data were obtained from seven different clinical trials;[8-10, 13-16] and two unpublished studies (Table I). All concentration-time data were provided by the respective principal investigators, all of whom are coauthors of this study. The immunosuppressive regimens, number of subjects, number of pharmacokinetic assessments, time after renal transplantation and sampling times after oral administration of the drugs are described in Table I. MPA pharmacokinetic profiles were obtained during daytime. Information with respect to sex, bodyweight, age, serum albumin, serum creatinine and creatinine clearance was not available for all patients. Further details of the studies have been reported elsewhere.[8-10, 13-16] Most of the EC-MPS treated patients were Caucasians in a stable phase after renal transplantation. A comparable group of patients receiving MMF was created by combining data from Caucasian renal trans-

Table I: Studies included in the data set

Study [reference]	Immunosuppressive drug regimen (no. of PK profiles)	No. of subjects	No. of PK curves	Mean time after transplantation in days (range)	Sampling interval (hr)	Sampling times
Hilbrands et al[unpublished]	EC-MPS+Cyclosporine (n=7)	7	7	2095 (1289-3803)	0-12	12
Neumann et al[unpublished]	EC-MPS (n=1) EC-MPS+Cyclosporine (n=6) EC-MPS+TCL (n=3) EC-MPS+EVL (n=1)	11	11		0-12	11
Budde et al[8, 9, 42, 43]	EC-MPS+Cyclosporine (n=43) EC-MPS+Cyclosporine+EVL (n=12) EC-MPS+TCL (n=42) EC-MPS+TCL+EVL (n=2) EC-MPS+EVL (n=9)	47	108	1899 (381-7722)	0-12	10
Cattaneo et al.[15]	EC-MPS+Cyclosporine (n=42)	10	42	525 (180-982)	0-12	12
Tedesco-Silva et al.[13]	EC-MPS+Cyclosporine (n=40) MMF+Cyclosporine (n=40)	40	80	657 (SD 329)	0-12	13
Van Gelder et al[10, 16],a	MMF+Cyclosporine (n=99)	99	99	140 (127-151)	0-2	5
Pescovitz et al[14],b	MMF+Cyclosporine (n=45)	45	45	1183 (235-3795)	0-12	10

[a] From this study only data from protocol day 140 was included. [b] From this study only Caucasian renal transplant patients were included in the data set, since most of the EC-MPS treated patients were Caucasians. EC-MPS, enteric-coated mycophenolate sodium; MMF, mycophenolate mofetil; Cyclosporine, cyclosporine; TCL, tacrolimus; EVL, everolimus.

plant patients from a study by Pescovitz et al[14] and data from patients in the randomized concentration-controlled trial.[10, 16] Concerning the latter study, only data from protocol day 140 were included in this analysis.

Pharmacokinetic analysis

The pharmacokinetic data of the patients treated with EC-MPS and/or MMF were pooled. Data from all patients were simultaneously fitted using the nonlinear mixed-effects modeling software program (NONMEM Version VI, level 1.0; GloboMax LLC, Ellicott City, MD, USA). By using NONMEM, typical pharmacokinetic parameters and their interpatient variability (IPV) can be estimated for the patient population. In the present study, the first-order method was used, since the first-order conditional estimate method with interaction did not minimize successfully. Since pharmacokinetic parameters for MPA were estimated, all MMF and EC-MPS doses were converted to the equivalent MPA content by multiplying the dose by 0.739 for MMF and by 0.936 for EC-MPS.

In the first step of the population analysis, a compartmental pharmacokinetic model was developed. Typical values for pharmacokinetic parameters (lag-time (T_{lag}), absorption rate constant (k_a), volume of distribution of the compartments (V), clearance (CL) and intercompartimental clearance (Q)) were estimated. Since bioavailability (F) could not be quantified, CL, Q and V values of MPA corresponded to the ratios CL/F, Q/F and V/F, respectively. IPV for each pharmacokinetic parameter was modeled using an exponential error model (equation 1):

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

where CL_i represents the MPA clearance of the i^{th} individual, θ_{pop} represents the population value for MPA clearance, and η represents the interindividual random effect with a mean of zero and variance of ω^2. The covariance between values for IPV was estimated using a variance-covariance matrix. The concentration data were logarithmically transformed. Residual variability between observed (lnC_{obs}) and predicted (lnC_{pred}) MPA plasma concentrations was described using an additional error model (equation 2).

$$lnC_{obs} = lnC_{pred} + \varepsilon_i \quad \text{(Eq. 2)}$$

where ε represents the residual random error with a mean of zero and variance of σ^2.

In NONMEM, estimation of a change-point parameter, such as the T_{lag}, is difficult, especially when this parameter exhibits large IPV. This estimation problem may be overcome by using a mixture model for the T_{lag}.[17] In this approach, all subjects are directed to a number of subgroups with different typical values for the T_{lag}. Estimation difficulties were further reduced by constraining T_{lag} with a logit transformation.[18]

In the second part of the modeling procedure, possible differences in pharmacokinetic parameters between MMF and EC-MPS administration were evaluated. Differences were investigated using equation 3.

$$CL_i = \theta_{pop} * \theta^{drug} \quad \text{(Eq. 3)}$$

where drug=0 for MMF treated patients and drug=1 for EC-MPS treated patients, and θ represents the fractional change in MPA clearance in EC-MPS treated patients.

The population model was built stepwise. A specific assumption was tested at each step (e.g. a two-compartment versus a three-compartment model). The main decision criterion was the likelihood ratio test (see Statistical analysis section). Model adequacy was further evaluated by using various residual plots ("goodness-of-fit" plots) and values of random-effects variances. To analyse the graphical goodness-of-fit, extensive plotting was available through the use of Xpose,[19] a purpose built set of subroutines in S-plus version 6.1 software (Insightful Corporation, Seattle, WA, USA).

Bayesian *post hoc* analysis of the developed population model was used to obtain individual estimations of the parameter T_{lag} and the predose concentration following ingestion of EC-MPS and MMF. The T_{lag} values, predose concentrations and the correlation between *post hoc* predose concentrations and MPA exposure were compared between both groups.

Validation

Two procedures were used to validate the final model. As an internal validation method, a bootstrap resampling method was applied.[20] This method has been implemented in the software package Wings for NONMEM version 612 (Dr N. Holford, March 2007, Auckland, New Zealand). 1000 bootstrap data sets were generated by sampling randomly from the original data set with replacement. Parameters were estimated for each of the replicate data sets using the final model developed earlier. The validity of the model was evaluated by comparing the median values and 95-percentiles (2.5^{th}-97.5^{th} percentiles) of the bootstrap replicates with the estimates of the original data set. The final model was further validated by application of a visual predictive check.[21] 100 data sets were simulated from the original data set using the final model. Per time point, the average simulated concentrations plus 95% confidence intervals were compared graphically with the observed average MPA concentrations.

Statistical analysis

In NONMEM modeling, the minimum objective function value (OFV) can be used as a criterion for model selection. If the difference in the OFV between two nested models is larger than the critical value from a chi-squared (X^2) distribution with degrees of freedom equal to the difference in the number of estimated parameters, the models are significantly different

from each other. A decrease in the OFV>10.83 shows a significant improvement of a nested model with one degree of freedom of p<0.001.

Post hoc values of pharmacokinetic parameters were compared using various statistical tests. Depending on the results of the Kolmogorov-Smirnov test, data were analysed with an unpaired student's T-test or a Mann-Whitney U-test. More than two parameters were compared with an ANOVA or the Kruskal-Wallis test was used. Categorical data were compared using a X^2-test. Correlation coefficients were determined with a Pearson correlation test. The statistical analyses were performed using SPSS 11.5.0 software for Windows (SPSS Inc., Chicago, IL, USA). Differences between parameters of p≤0.05 were considered significant.

Results

Data description

The data set contained 3764 MPA plasma concentrations obtained from 259 renal transplant recipients. In total, 208 concentration-time profiles were available from patients treated with EC-MPS and 184 from patients treated with MMF. Each patient participated in at least one (median 1, range 1-6) pharmacokinetic assessment of either a 2-hour or a 12-hour AUC at different time points after transplantation. A median of 10 (range 4-15) concentration samples was obtained per AUC. The patient characteristics of both groups are described in Table II.

Table II: Patient characteristics.

Characteristics	EC-MPS	n	MMF	n	p-value
MPA dose (mg)[a]	674 (337-1348)	208	739 (185-1626)	184	<0.001
Sex (m/f)	117/50	167	89/55	144	0.125
Bodyweight (kg)	76 (40-124)	167	73 (44-108)	144	0.007
Age (years)	45 (21-79)	167	51 (19-74)	144	0.031
Serum creatinine (umol/L)	156 (71-413)	168	130 (79-280)	143	<0.001
Creatinine clearance (mL/min)	63.0 (27.4-153.4)	166	58.8 (24.9-122.5)	143	0.069
Serum albumin (g/L)	41.3 (27.5-48.1)	167	39.0 (32.0-53.0)	143	0.019
Cyclosporine predose (mg/L)	110 (25-700)	109	178 (53-522)	136	<0.001
Number CsA comedication[b]	150 (72%)	208	184 (100%)	184	0.048
Number TCL comedication[b]	47 (23%)	208	0 (0%)	184	<0.001
Number EVL comedication[b]	24 (12%)	208	0 (0%)	184	<0.001

Data are presented as median (range). Information with respect to sex, bodyweight, age, serum albumin, serum creatinine and creatinine clearance was not available for all patients.

[a]The amount of the drug was converted to equivalent MPA doses

[b]Described as the number of patients cotreated with the drug (percentage of total patients)

EC-MPS, enteric-coated mycophenolate sodium; MMF, mycophenolate mofetil; CsA, cyclosporine; TCL, tacrolimus; EVL, everolimus.

Pharmacokinetic analysis

Figures 1A and 1B show the observed MPA plasma concentration-time points for EC-MPS and MMF treated patients. Some typical concentration-time profiles are shown in Figure 2. For both

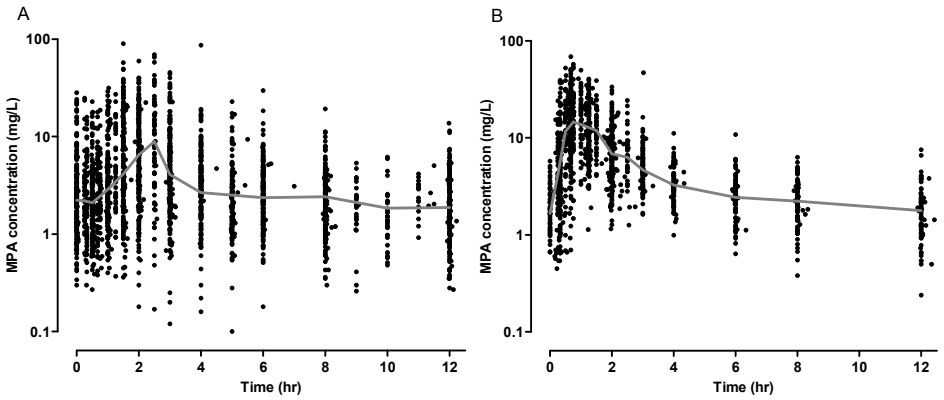

Figure 1: Observed concentration-time data. Mycophenolic acid (MPA) concentration versus time for (a) all patients treated with enteric-coated mycophenolate sodium and (b) all patients treated with mycophenolate mofetil.

formulations, absorption was delayed following ingestion of the drug. Furthermore, in 18 (8.7%) of the profiles of patients taking EC-MPS, MPA predose concentrations were higher than the maximal MPA concentration in the subsequent 12-hour observation period (Fig. 2B). In all patients receiving MMF, maximal MPA concentrations were higher than predose concentrations.

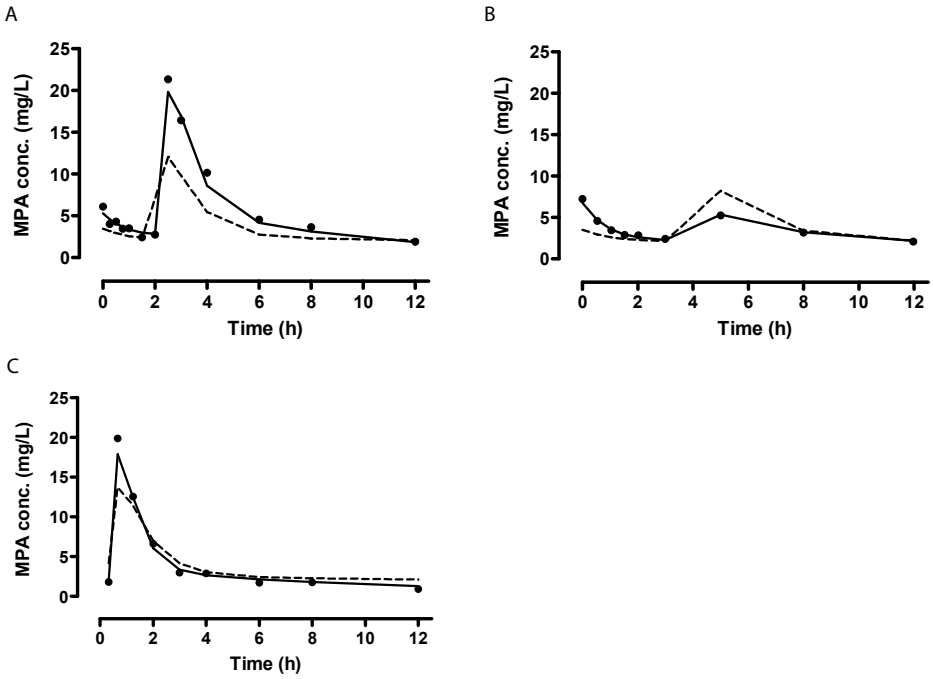

Figure 2: Observed and predicted characteristic concentration-time profiles. Plots of observed (dots), model-predicted (dashed line), and Bayesian-predicted (solid line) mycophenolic acid (MPA) concentration versus time from two patients treated with enteric-coated mycophenolate sodium (a) and (b) and one patient treated with mycophenolate mofetil (c). Note that in (b), the concentration at time zero was higher than in the following 12-h observation period.

The concentration-time data of all patients were fitted simultaneously to several pharmacokinetic models. A two-compartment model with first-order elimination adequately described the data. The delayed absorption was characterised by a lag-time and a first-order absorption process (model 1, Table III). The latter described absorption better than zero-order absorption, Weibull-absorption[22, 23] and transit-compartments,[24, 25] as judged by the OFV and goodness-of-fit plots. Minimization of model 1 was successful in NONMEM; However, standard errors were not obtained.

Table III: Parameter estimates of the pharmacokinetic models.

	Model				Bootstrap		
Parameter	1	2	3	4	Median	95-percentile range	
OFV	1328	682	186	68	-	-	-
T_{lag} EC-MPS and MMF (h)	0.21	-	-	-	-	-	-
T_{lag} EC-MPS morning 1 (h)	-	0.97	0.97	0.95	0.96	0.62	0.99
T_{lag} EC-MPS morning 2 (h)	-	-	1.88	1.88	1.94	1.39	2.46
T_{lag} EC-MPS morning 3 (h)	-	-	4.87	4.83	4.95	3.95	5.98
T_{lag} EC-MPS evening (h)	-	7.03	8.46	9.04	8.52	2.92	9.29
T_{lag} MMF (h)	-	0.22	0.30	0.30	0.24	0.18	0.31
k_a EC-MPS and MMF (h^{-1})	1.6	2.4	4.1	-			
k_a EC-MPS (h^{-1})	-	-	-	3.0	3.9	2.8	5.4
k_a MMF (h^{-1})	-	-	-	4.1	4.1	2.7	5.6
$V_{central}$ (L)	62	75	43	40	45	37	58
CL (L/h)	16.2	17.0	16.0	16.0	16.1	14.9	17.2
$V_{peripheral}$ (L)	229	430	557	518	394	190	290775
Q (L/h)	71	32	21	22	22	19	28
POP with T_{lag} EC-MPS 1	-	-	0.50	0.51	0.51	0.29	0.67
POP with T_{lag} EC-MPS 2	-	-	0.32	0.32	0.32	0.16	0.53
POP with T_{lag} EC-MPS 3	-	-	0.18	0.17	0.17	0.23	0.12
Residual error	0.51	0.45	0.41	0.39	0.39	0.36	0.43
Interpatient variability (%)							
T_{lag} EC-MPS and MMF	0.1	-	-	-	-	-	-
T_{lag} EC-MPS morning	-	1.1	2.9	8.0	1.0	0	14
T_{lag} EC-MPS evening	-	155	57	40	59	35	6000
T_{lag} MMF	-	39	33	11	12	1.0	34
k_a	15000	310	175	187	200	158	360
$V_{central}$	15100	210	96	100	114	81	172
CL	40	37	38	39	38	33	43
$V_{peripheral}$	3500	410	500	490	390	171	293500
Q	1010	105	70	78	71	56	96

OFV, minimum value of the objective function; EC-MPS, enteric-coated mycophenolate sodium; MMF, mycophenolate mofetil; T_{lag}, lag-time; k_a, absorption rate; $V_{central}$, volume of distribution of the central compartment; CL, clearance; $V_{peripheral}$, volume of distribution of the peripheral compartment; Q, intercompartmental clearance; POP, part of the population.

Equation 3 was used to evaluate possible differences in pharmacokinetic parameters between EC-MPS and MMF treated patients. No significant differences were observed for the volume of distribution of the central compartment (V1/F), CL/F, the volume of distribution

of the peripheral compartment (V2/F) or Q/F. The T_{lag} was approximately 5-fold longer for EC-MPS than for MMF; respective values were 0.98 and 0.21 h. Incorporation of the difference in the T_{lag} in model 1 reduced the OFV by 380 points ($p<0.001$). Despite the improved fit of the model, goodness-of-fit plots indicated that predose concentrations were underestimated following ingestion of EC-MPS. The inclusion of a separate T_{lag} for the morning and evening dose of EC-MPS significantly improved the model further (model 2, Table III, ΔOFV = -266, $p<0.001$). It should be noted, however, that MPA concentrations were only assessed during the daytime. As a result, the T_{lag} for the evening doses was estimated on the basis of concentration-time points observed before absorption of the morning dose started. No differences were detected in the T_{lag} for the morning- and evening doses of MMF. In model 2, the T_{lag} following morning administration of EC-MPS was typically estimated to be 0.97 hours with IPV of 1.1%. Goodness-of-fit plots, however, indicated that these values were underestimated. This underestimation in NONMEM was due to the difficulty in estimating the change-point parameter T_{lag}, which exhibited considerable IPV. Subsequently, a mixture model was introduced for the T_{lag} of the morning dose of EC-MPS. With an optimum number of three subgroups, a significant decrease in the OFV was obtained when compared with the previous model (model 3, Table III, ΔOFV = -496, $p<0.001$), and also the goodness-of-fit plots showed an improved correlation between predicted and observed concentrations. The introduction of a mixture model on the T_{lag} of the EC-MPS evening dose or MMF did not result in any further improvement of the model. Introduction of a different k_a for MMF and EC-MPS further improved model 3 (model 4, Table III, ΔOFV = -118, $p<0.001$). The k_a was decreased following EC-MPS compared with MMF.

Finally, the model was extended with a bile compartment to describe EHC of MPA.[26] This did not, however, improve the fit of the model to the data. Clearance was not found to be dependent on the use of cyclosporine.

For the final model (model 4, Table III), Figure 3 depicts the correlations between the observed MPA concentrations, model-predicted MPA concentrations and individual-predicted MPA concentrations, as well as the distribution of the weighted residuals during the dose interval. Data in the model-predicted versus observed (Fig. 3A) and individual-predicted versus observed (Fig. 3B) plots are symmetrically distributed around the line of identity, indicating the goodness of the fit. No trends were observed in the weighted residuals versus time plot (Fig. 3C). The concentration-time curves of the three typical patients were well described by the individual-predicted values (Fig. 2).

Validation

The original data set was used to generate 1000 bootstrap data sets, which were fitted with the final model. The estimated pharmacokinetic parameters from the bootstrap analysis compared favourably with the estimations of the final model, indicating the validity of the final model (Table III). The visual predictive check (Fig. 4) revealed an acceptable agreement

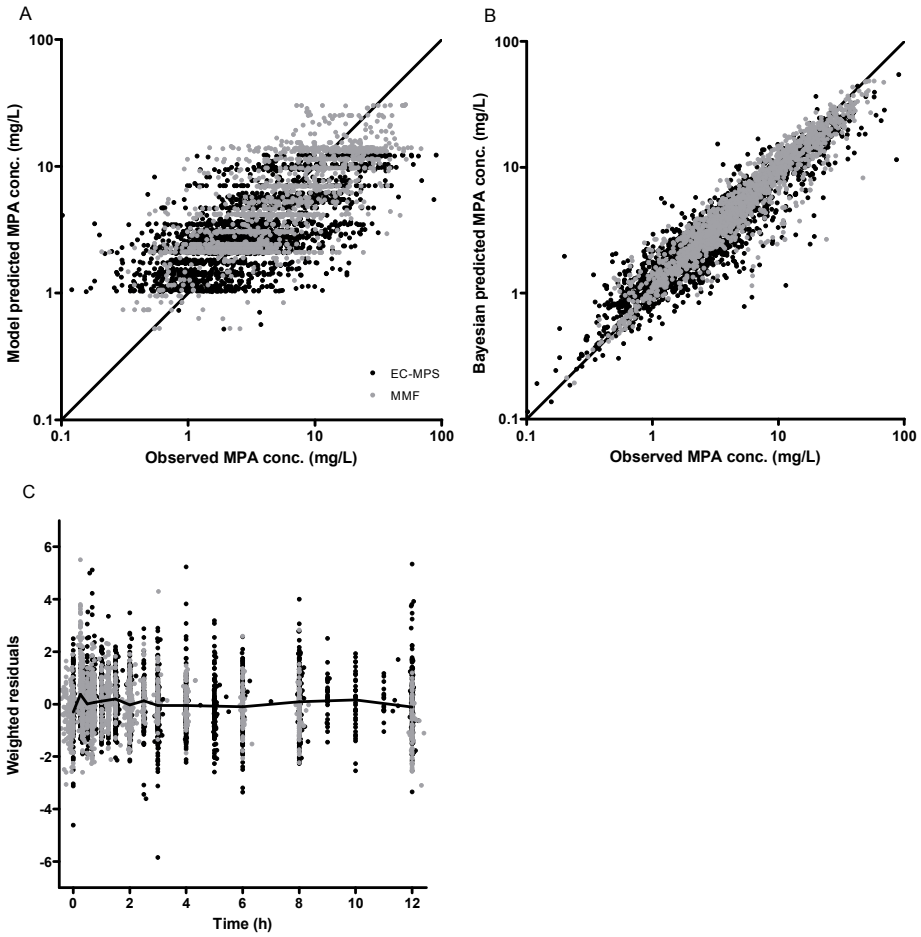

Figure 3: Goodness-of-fit plots for the final model (model 4). Model predicted mycophenolic acid (MPA) concentration versus observed MPA concentration (a), individual predicted MPA concentration versus observed concentration (b) and weighted residuals versus time (c). The solid line in (a) and (b) is the line of identity. The solid line in (c) is the average of the weighted residuals.

between the 95% confidence interval band of 100 simulated data sets and the average observed concentration. However, there appeared to be an under-prediction of the maximum concentration for EC-MPS and a small overprediction of the MPA concentration at 6 hours for both drugs.

Post hoc analysis

Individual Bayesian estimations of the T_{lag} and predose concentrations were compared for the various subgroups. Following EC-MPS ingestion, the T_{lag} was longer than following MMF ingestion (Fig. 5). The absorption of MPA following the EC-MPS evening dose was remarkably delayed. As a result, the T_{lag} between the EC-MPS morning dose, the EC-MPS evening dose

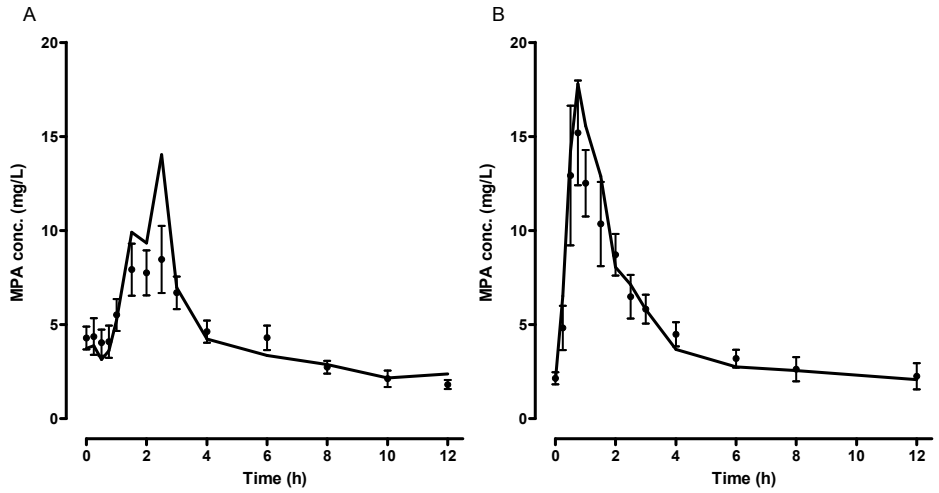

Figure 4: Visual predictive check. Comparison of 100 simulated data sets (mean ± 95% confidence interval) with the average observed concentrations (solid line) for enteric-coated mycophenolate sodium (a) and mycophenolate mofetil (b). The 100 data sets were simulated on basis of the final model.

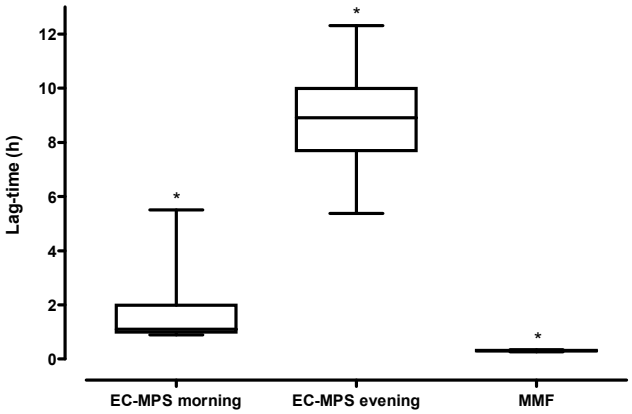

Figure 5: Delay in mycophenolic acid absorption. Box plot of Bayesian-predicted lag-time (t_{lag}) following ingestion of the morning and evening doses of enteric-coated mycophenolate sodium (EC-MPS) and ingestion of mycophenolate mofetil (MMF; there was no difference between morning and evening doses). The box represents the median and the 25th and 75th percentiles of the data. The whiskers represent the range. * indicates a significant difference from the other groups ($p<0.001$).

and the MMF dose was significantly different ($p<0.001$): median values and range were 2.0 h (0.9-5.5 h), 8.9 h (5.4-12.3 h) and 0.30 h (0.26-0.34 h), respectively.

Subsequently, the *post hoc* MPA predose concentrations of EC-MPS and MMF treated patients were compared (Fig. 6). The median MPA morning predose concentrations (C_0) (and range) were 2.6 mg/L (0.4-24.4 mg/L) following EC-MPS ingestion and 1.6 mg/L (0.2-7.6 mg/L) following MMF ingestion. The evening predose concentrations (C_{12}) were 1.6 mg/L (0.1-4.8 mg/L) for EC-MPS treated patients and 2.1 mg/L (0.2-6.2 mg/L) for MMF treated patients. C_0

levels of EC-MPS treated patients were almost 2-fold higher than EC-MPS C_{12} concentrations and MMF C_0 values ($p<0.05$).

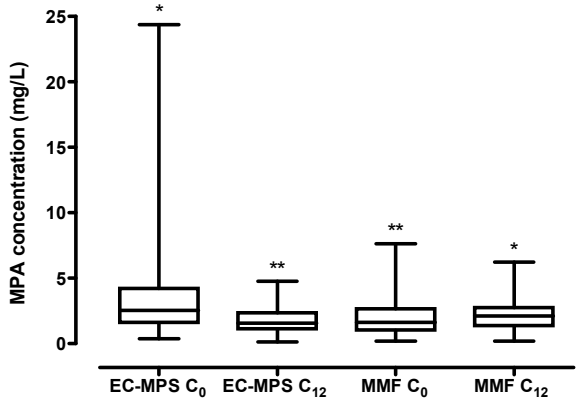

Figure 6: Predose levels of mycophenolic acid (MPA). Box plot of Bayesian-predicted MPA predose concentrations in the morning (C_0) and evening (C_{12}) for patients using enteric-coated mycophenolate sodium (EC-MPS) and mycophenolate mofetil (MMF). The box represents the median and the 25th and 75th percentiles of the data. The whiskers represent the range.
*incicates a significant difference from the predose concentration of all other groups ($p<0.05$).
**indicates a significant difference from the EC-MPS C_0 and the MMF C_{12} ($p<0.05$).

Figure 7 shows the correlation between MPA predose concentrations and the MPA AUC. The correlation coefficient was higher in MMF treated patients ($r^2=0.48$, $p<0.001$) than in EC-MPS treated patients ($r^2=0.02$, $p<0.001$).

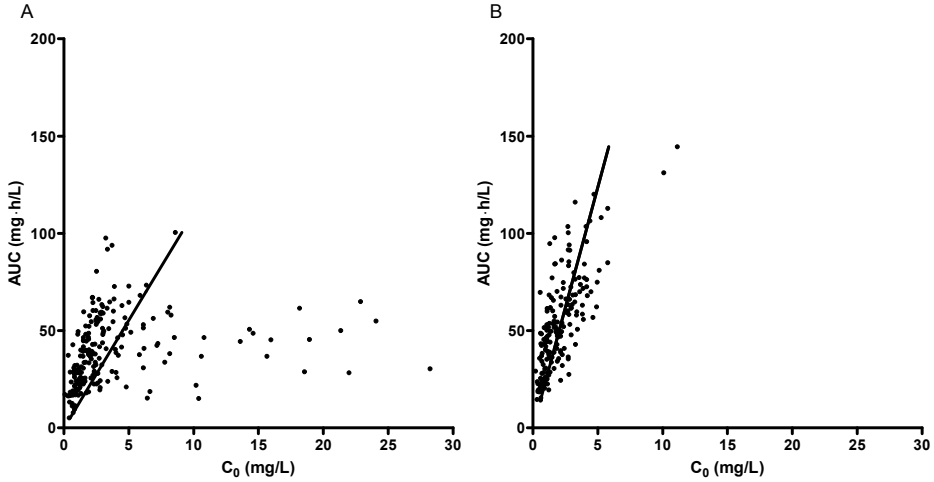

Figure 7: Correlation of the area under the plasma-concentration curve (AUC) and mycophenolic acid (MPA) predose concentration (C_0). Correlation between the MPA AUC and the MPA C_0 following administration of enteric-coated mycophenolate sodium (r^2: 0.02, $p<0.001$) (a) and mycophenolate mofetil (r^2: 0.48, $p<0.001$) (b). The line represents the trendline.

Discussion

This is the first study in which a population pharmacokinetic model was developed for MPA in patients receiving either MMF or EC-MPS. Application of nonlinear mixed-effects modeling allowed the combination of heterogeneous data sets and assessment of pharmacokinetic parameters in a large population of 259 renal transplant patients. In the present study, clinically relevant differences in absorption pharmacokinetics were observed between the two formulations. Differences in the pharmacokinetic profile of MPA between both drugs have been described earlier, but only with a limited number of patients (n≤40).[7-9, 15]

The final model and pharmacokinetic parameters were comparable with previously published models for MPA in patients using MMF.[27-29] In other population pharmacokinetic studies in renal transplant patients taking MMF, absorption of MPA was characterized by a double absorption-phase model[30] or zero-order absorption.[31] In the present study, implementation of these absorption characteristics models did not improve the fit of the model to the data, as indicated by the minimum objective function and the goodness-of-fit plots. Weibull absorption or transit compartiment absorption did not improve the model either.[22-25]

The absorption of MPA was more delayed and variable in EC-MPS treated patients than in MMF treated patients. Furthermore, the absorption of MPA was slower following EC-MPS ingestion. These differences resulted in higher MPA predose concentrations in EC-MPS treated patients.

In earlier studies,[7-9, 15] higher MPA predose concentrations and lower and delayed maximal concentrations were reported for EC-MPS. The present analysis demonstrated that this is explained by a longer T_{lag} and slower absorption following ingestion of EC-MPS. Clearly, MPA absorption is delayed in patients using EC-MPS due to the enteric coating of the drug. Moreover, the formulation of the drug produces greater interpatient variability in the T_{lag} for EC-MPS than for MMF (Fig. 5).

Interestingly, the T_{lag} was estimated to be longer for the evening dose than for the morning dose (Fig. 5). As a result, in a considerable number of patients, the predose concentrations were comparable with the maximal concentration observed following the morning dose (Fig. 2B). The longer T_{lag} for the evening dose is likely to be the result of the circadian effect of gastric emptying. Gastric emptying has been reported to be delayed by 53.6% in the evening.[32] Two studies[33, 34] in Japanese renal transplant recipients treated with MMF showed a delayed MPA absorption at night compared with the daytime.

The rate of absorption of MPA was lower in EC-MPS treated patients than in MMF treated patients (3.0 versus 4.1 h^{-1}). This difference was independent of the observed differences in the T_{lag}. Nevertheless, the absorption of MPA was fast in both formulations, with absorption

half-lives of 0.23 h for EC-MPS and 0.17 h for MMF. As a result, the observed differences in the rate of absorption will not be clinically relevant.

An alternative explanation for the high predose concentration could be the presence of EHC following the evening dose of EC-MPS. An attempt was made to include EHC in the model, but the data set did not contain information allowing accurate description of the recirculation process. In case of cotreatment with cyclosporine, EHC may be decreased by cyclosporine-reduced inhibition of the multidrug resistance-associated protein 2 (MRP2) enzyme. MRP2 has been reported to be responsible for the excretion of MPAG in bile.[35] It has been indicated that elevated MPAG concentrations increase clearance of MPA through interaction at the protein-binding site.[36, 37] The possible effect of cyclosporine cotreatment on MPA clearance was tested in the population model. However, no significant influence of cyclosporine on MPA clearance was detected.

In this study, EC-MPS and MMF treated patients exhibited different demographic and pathofysiological characteristics. In a previous study, the relationship between patient characteristics and pharmacokinetic parameters was extensively studied.[28] In this study exposure to MPA was reported to be significantly influenced by renal function, albumin and haemoglobin levels and cyclosporine predose concentrations. In the present study albumin levels were different in both groups. However, this small difference (41.3 vs 39.0 g/L) was not considered to be of clinical relevance. Cotreatment with cyclosporine was tested as a covariate of the final model but showed no significant influence.

No deviations were present in the plot of weighted residuals versus time (Fig. 3C). However, in the visual predictive check, a small overestimation was observed at 6 hours, which was probably caused by the presence of EHC in some patients. It should be noted that in the present study, samples were only collected following the morning dose. A better insight into possible diurnal variation of absorption or the possible contribution of EHC should be obtained by taking samples after the evening dose as well.

A mixture model was needed to describe the variability in the T_{lag}. NONMEM software handles the T_{lag} as a change-point parameter, which causes an abrupt increase in the absorption rate at a certain time point. This nonphysical approach makes it difficult for NONMEM to estimate the exact change-point and especially the variability of the parameter. A mixture model makes it possible to estimate the amount of variability by dividing the population into subgroups. A transit absorption model (with serial compartments) may also be used to avoid the estimation problem of the T_{lag}.[24, 25] However, in the present study implementation of this model did not produce realistic estimations. Through implementation of the mixture model for the T_{lag} following the morning dose of EC-MPS in the model, variability in this parameter could adequately be described. Furthermore, it allowed the estimation of individual T_{lag} values and predose concentrations by Bayesian *post hoc* analysis. Morning predose concentrations were 2.6 mg/L for EC-MPS and 1.6 mg/L for MMF (Fig. 6). The higher MPA predose concentrations

in patients taking EC-MPS can be explained by the delayed absorption, especially following the evening dose, and its corresponding IPV. As a result, MPA morning predose concentrations ranged from 0.2 to 7.6 mg/L for MMF and from 0.4 to 24.4 mg/L for EC-MPS. Budde et al[38] previously reported similar results; MPA predose concentrations were 31.1% higher with EC-MPS (2.40 mg/L) than with MMF (1.83 mg/L) with increased interpatient (123 versus 76%) and intrapatient variability (64 versus 42%).

The increased IPV in the T_{lag}, rate of absorption and predose concentrations has important implications for the application of TDM in patients using EC-MPS. Greater variability explains the poor relationship between MPA predose concentrations and the AUC in EC-MPS treated patients (r^2: 0.02 versus 0.48). Budde et al[38] also compared this correlation between EC-MPS and MMF and reported comparable results (r^2: 0.16 versus 0.45). Because AUC measurements are more difficult to perform in clinical practice, limited sampling strategies or *a posteriori* Bayesian algorithms are alternatives to accomplish reliable estimations of the MPA AUC in MMF treated renal transplant recipients.[39, 40] The relevance of TDM with such Bayesian algorithms was recently demonstrated by Le Meur and colleagues.[12, 41] Because of the unpredictability of the absorption time, development of a practically applicable limited sampling strategy for EC-MPS will be impracticable. A complete AUC from time zero to 12 hours is likely to be necessary to obtain reliable estimates of MPA exposure in patients treated with EC-MPS. This will limit the feasibility of TDM for this drug.

Conclusion

A population pharmacokinetic model has been developed in which the pharmacokinetics of MPA following oral administration of both EC-MPS and MMF were compared. Following ingestion of EC-MPS, absorption of MPA into the central circulation was slower and more delayed in comparison with MMF. Moreover, the IPV of the T_{lag} was greater in EC-MPS treated patients. As a consequence, MPA predose concentrations reflect MPA exposure even worse in patients using EC-MPS than in patients using MMF. It is expected that clinically feasible limited sampling strategies will not reliably reflect MPA exposure in patients taking EC-MPS, because of the unpredictable absorption profile of EC-MPS.

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Chapter 4.2

Limited sampling strategies drawn within 3 hours postdose poorly predict mycophenolic acid area-under-the-curve after enteric-coated mycophenolate sodium

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Abstract

Previous studies predicted that limited sampling strategies (LSS) for estimation of mycophenolic acid (MPA) area-under-the-curve (AUC_{0-12}) after ingestion of enteric-coated mycophenolate sodium (EC-MPS) using a clinically feasible sampling scheme may have poor predictive performance. Failure of LSS was thought to be due to the slow absorption of MPA causing late and variable times of maximum MPA concentration and variable predose concentrations. The aim of this study was to formally test the performance of LSS by developing and validating LSS for estimation of MPA AUC_{0-12} after EC-MPS administration. Pharmacokinetic data from 109 renal transplant recipients collected during the maintenance period after transplantation were analysed retrospectively. LSS were developed separately for renal transplant patients who concurrently used cyclosporine (n=79) and for patients not concurrently treated with cyclosporine (n=30). Data were split into an index and a validation data set. For clinical feasibility reasons, a LSS could consist of a maximum of 3 sampling time points with the latest sample drawn 2 hours after drug administration. LSS with the latest sample drawn 3 hours after drug administration or even later were also tested. The validation of the developed LSS showed that MPA AUC_{0-12} for patients concurrently treated with cyclosporine was best estimated by AUC_{0-12} [mg*h/L] = 36.536 + 1.642x$C_{0.5}$ + 0.569x$C_{1.5}$ + 0.905xC_2 (r^2=0.33, bias=-1.0 mg*h/L, precision=24 mg*h/L), whereas AUC_{0-12} [mg*h/L] = 19.801 + 1.827x$C_{0.5}$ + 1.111xC_1 + 1.429xC_2 was the best AUC_{0-12} estimator for patients not cotreated with cyclosporine (r^2=0.31, bias=0.4 mg*h/L, precision=14.5 mg*h/L). Both LSS showed poor precision and overestimation of AUC_{0-12} values below the therapeutic window and underestimation of AUC_{0-12} values above the therapeutic window of MPA. Using C_3 as latest sampling time point improved the fit slightly, but not satisfactory, with r^2 still <0.40 and precision still >14.0 mg*h/L. Estimation of MPA AUC_{0-12} with LSS for EC-MPS drawn within 2 or 3 hours postdose in renal transplant recipients in the maintenance period is likely to result in biased and imprecise results.

Introduction

Mycophenolic acid (MPA) is the active immunosuppressive molecule of the prodrugs mycophenolate mofetil (MMF) and of enteric-coated mycophenolate sodium (EC-MPS).[1,2] MPA is widely used to prevent acute rejection after kidney, liver and heart transplantation.[3,4] The pharmacokinetics of MPA are characterized by extensive binding to plasma albumin (unbound fraction of less than 3%) and metabolism to the pharmacologically inactive phenolic mycophenolic acid glucuronide metabolite (MPAG) in liver, kidney, and intestinal mucosa.[5-7] MPAG can undergo enterohepatic recirculation, leading to reabsorption of MPA from the gut and multiple peak concentrations in the concentration-time profile of MPA.[5] Finally, MPAG is eliminated through the kidneys.

It has been advocated that for optimal efficacy, a MPA area-under-the-curve (AUC_{0-12}) greater than 30 mg*h/L is necessary.[8,9] This target is not reached with standard doses MMF or EC-MPS in all patients but, nevertheless, therapeutic drug monitoring (TDM) is not routinely practiced for MPA in most centers. The added value of TDM has been tested in 2 prospective randomized trials. The first study showed a significant reduction in the incidence of biopsy-proven acute rejection in renal transplant patients in whom the MMF dose was based on MPA concentration measurements compared with patients receiving standard doses of MMF.[10] The second study compared the same 2 MMF dosing regimens, but could not find a significant difference in both biopsy-proven acute rejection and in MPA exposure.[9] The unexpected results of the second study were in part explained by nonadherence to the required dose increases after the first MPA concentration measurements. Another explanation might be the difference in the limited sampling strategy (LSS) used between the studies. Both studies used a LSS to estimate MPA AUC_{0-12}.[9-12] However, the Bayesian approach used by Le Meur et al[10] allowed a more individualized estimation. For MMF, it has been shown that LSS can estimate MPA AUC_{0-12} with sufficient accuracy and precision, using clinically practical sampling schemes consisting of 3 concentration-time samples drawn between predose and 2 hours postdose.[13-15] For EC-MPS, performance of LSS using samples only drawn within 2 or 3 hours postdose, which are considered clinically feasible, have not been published yet. From a theoretical point of view, estimation of MPA AUC_{0-12} with LSS for EC-MPS using sampling schemes similar as LSS for MMF may have less predictive performance. The reason is that EC-MPS releases MPA more slowly and more variable than MMF, due to the enteric coating, which causes the time of maximum MPA concentration (usually between 2 and 6 hours, or sometimes even later, for EC-MPS compared to 1 hour for MMF) to fall outside the sampling scheme.[16-19] Besides, higher and more variable MPA predose concentrations compared with MMF have been observed for EC-MPS.[20]

The aim of this study was to develop and validate LSS based on samples drawn within 2 or 3 hours postdose for estimation of MPA AUC_{0-12} after EC-MPS administration and to investigate whether estimation of MPA AUC_{0-12} with this LSS can be done accurately and precisely.

Patients and methods

Patients

Pharmacokinetic data were collected from 109 renal transplant recipients during the maintenance period after transplantation (Table I). The data were obtained from 3 different clinical trials, all of which had received institutional review board approval, number of subjects, number of pharmacokinetic assessments, sampling times after oral administration of the drugs and the analytical method used to determine MPA concentrations are described in Table I. MPA concentration-time profiles (Fig. 1) were obtained during daytime. In the study of de Winter et al,[25] plasma samples were taken predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours after oral EC-MPS administration. In 2 patients, the sample at 2.5 hours postdose and in 1 patient, the sample at 12 hours postdose was not available. Budde et al,[18,22] measured MPA concentrations predose, 0.5, 1, 1.5, 2, 3, 5, 8, and 12 hours postdose, and no data were missing in this study. In the second study of Budde et al[21,22] samples were taken predose, 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 hours postdose. MPA concentrations were missing at t=3 hours postdose in 1 patients and at t=12 hours postdose in another patient. The study of Tedesco-Silva et al[24] sampled the patients predose, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 hours postdose. In this study, none of the MPA concentrations were missing.

Table I: Studies included in the data set

Study reference	Immunosuppressive drug regimen (no. of pharmacokinetic profiles)	No. of subjects	No. of pharmacokinetic curves	Sampling interval (h)	No. of sampling times	Analytical method
de Winter et al[25]	EC-MPS+CsA (n=7)	7	7	0-12	12	HPLC
Budde et al[18,22]	EC-MPS+CsA (n=36) EC-MPS+CsA+EVL (n=12)	32	48	0-12	10	EMIT
Budde et al[21,22]; Arns et al[23]	EC-MPS+TCL (n=42) EC-MPS+TCL+EVL (n=2) EC-MPS+EVL (n=9)	30	53	0-12	9	HPLC
Tedesco-Silva et al[24]	EC-MPS+CsA (n=40)	40	40	0-12	13	HPLC

Mean values (and range) are presented. EC-MPS, enteric-coated mycophenolate sodium; MMF, mycophenolate mofetil; CsA, cyclosporine; TCL, tacrolimus; EVL, everolimus, HPLC, high performance liquid chromatography; EMIT, enzyme multiplied immunoassay technique.

Limited sampling strategies

LSS were developed separately for renal transplant patients who concurrently used cyclosporine (n=79) and for patients not concurrently treated with cyclosporine (n=30). Data were randomly split into two data sets. The first was an index data set, which was used to develop the LSS. It contained 48 randomly selected concentration-time profiles from renal transplant recipients concurrently treated with cyclosporine and 27 profiles from randomly selected patients who did not receive cyclosporine. MPA concentrations at each sampling time point were correlated with the measured MPA AUC_{0-12} using linear regression analysis. Those MPA

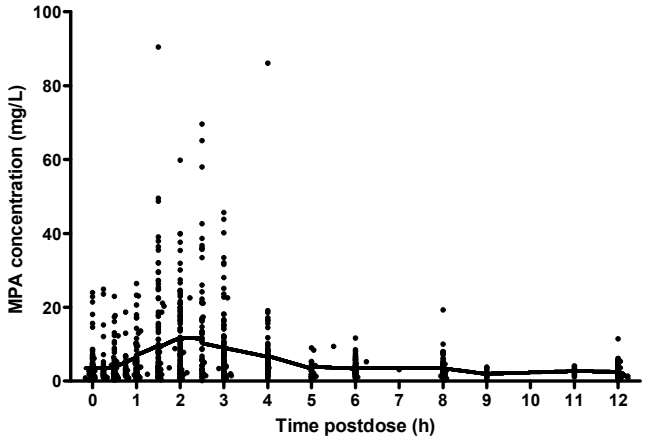

Figure 1: Observed mycophenolic acid (MPA) concentration versus time data for all patients treated with enteric-coated mycophenolate sodium (EC-MPS). The line respresents the trendline of the data.

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 reasons of clinical applicability, a LSS could consist of a maximum of 3 sampling time points with the latest sample being collected maximally 2 hours after administration of EC-MPS. Because it is known that the maximum concentration of MPA after EC-MPS administration often occurs beyond 2 hours, it was tested whether LSS with the latest time point being 3 hours after EC-MPS intake, and LSS with the latest time point beyond 3 hours, would result in improved predictive performance.

The second data set was a validation data set, which included the remaining 47 concentration-time profiles from renal transplant recipients concurrently treated with cyclosporine and the remaining 26 profiles from patients not receiving cyclosporine. The patients in this data set had no significant differences in patient characteristics compared to the index data set (Table II). The validation data set was used to correlate the AUC_{0-12} estimated with the LSS with the measured MPA AUC_{0-12}, calculated with the linear trapezoidal rule, thus verifying the performance of the developed strategy. These results were used to compare the predictive performance of the LSS for EC-MPS with the performance of validated LSS in renal transplant recipients after MMF administration for which a measure of bias or precision was available in literature.

To assess the agreement between the measured and the estimated MPA AUC_{0-12}, 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_{0-12} with the measured AUC_{0-12} were obtained using formulas 1 and 2, respectively.[26]

$$MPE(mg \cdot h/L) = \frac{\sum_{i=1}^{N}(pe_i)}{N} \quad \text{(Eq. 1)}$$

$$RMSE(mg \cdot h/L) = \sqrt{\frac{\sum_{i=1}^{N}(pe_i^2)}{N}} \quad \text{(Eq. 2)}$$

N represents the number of pairs of estimated and measured $AUC_{0\text{-}12}$ and pe_i is the difference between the estimated and the measured $AUC_{0\text{-}12}$. Bias and precision were visualized by plotting the average $AUC_{0\text{-}12}$ resulting from the abbreviated and the full profile as described by Bland and Altman.[27]

Statistics

Statistical tests were performed with the software package SPSS 15.0 for Windows (SPSS Inc. Chicago, IL, USA). Pharmacokinetic data are expressed as median and range, because not all data were normally distributed. Bias and precision of the comparison between measured and estimated MPA $AUC_{0\text{-}12}$ are expressed as mean and their 95% confidence intervals. The Mann-Whitney *U* test was used to test for statistical differences. A p-value of 0.05 was considered statistically significant.

Results

Pharmacokinetic data from 109 renal transplant patients were included in the analysis. The demographics and pharmacokinetic parameters of the population are presented in Table II. The index data set with patients concurrently treated with cyclosporine contained 48 concentration-time profiles from 38 patients. The index data set with patients not concur-

Table II: Mycophenolic acid pharmacokinetic parameters

	Cyclosporine		Non cyclosporine	
Data set	Index	Validation	Index	Validation
n	48	47	27	26
EC-MPS dose (mg)	990±375	1019±363	880±321	969±334
AUC (mg*h/L)	61.8 (5.7-145)	59.2 (14.7-178.9)	38.7 (8.8-86.9)	40.3 (14.2-78.6)
C_{max} (mg/L)	19.0 (1.3-90.4)	22.7 (2.1-86.1)	11.5 (1.6-31.7)	12.0 (2.0-23.0)
T_{max} (h)	2.0 (0-12)	2.5 (0-8)	1.5 (0-12)	2.0 (0-12)
Predose level (mg/L)	2.6 (0.4-21.4)	2.1 (0.09-24.0)	1.49 (0.57-7.22)	1.64 (0.34-15.7)
Age (years)	45 (16-67)	50 (19-68)	42 (16-67)	43 (19-68)
Weight (kg)	78 (46-109)	79 (49-115)	72 (46-124)	73 (49-121)
Gender (% male)	73%	72%	78%	62%
Time posttransplantation (days)	1023 (292-6155)	800 (197-7694)	1361 (242-6155)	1740 (197-7722)

Data are presented as median (and range), except for the enteric-coated mycophenolate sodium (EC-MPS) dose, which is presented as mean±standard deviation. n, number of pharmacokinetic profiles; AUC, area-under-the-curve; C_{max}, maximum concentration; T_{max}, time of C_{max}

rently treated with cyclosporine contained 27 profiles from 19 patients. The validation data sets consisted of 47 profiles from 42 patients cotreated with cyclosporine and 26 profiles from 21 patients who did not receive cyclosporine. Pharmacokinetic parameters of patients in the index data set were not statistically significantly different from pharmacokinetic parameters of patients in the validation data set (Table II). MPA concentrations at each sample time point in the index data set, until 2 hours after oral intake of EC-MPS (C_0, $C_{0.5}$, C_1, $C_{1.5}$, C_2), were correlated with the measured MPA AUC_{0-12} in a linear regression analysis. The strongest correlation coefficients (r^2) for a single time point with MPA AUC_{0-12} was C_2 in the data set with patients cotreated with cyclosporine (r^2=0.30, p<0.0001) and C_1 in the data set with noncyclosporine users (r^2=0.46, p<0.0001), whereas predose levels showed weak correlations (r^2=0.084, p=0.045 in the data set with cyclosporine users and r^2=0.091, p=0.127 in the data set with noncyclosporine users). The multiple stepwise regression analysis revealed that the best estimate of the measured MPA AUC_{0-12} in patients concurrently treated with cyclosporine was derived by the combination of the sampling time points at 0.5, 1.5, and 2 hours after oral intake of EC-MPS. The correlation coefficient was 0.42, mean bias was 0.0 mg*h/L and precision was 21.7 mg*h/L (Table III). The accompanying algorithm is described by formula 3.

$$AUC_{0-12}\,(mg*h/L) = 36.536 + 1.642xC_{0.5} + 0.569xC_{1.5} + 0.905xC_2 \qquad \text{(Eq. 3)}$$

The best LSS for patients not cotreated with cyclosporine consisted of the sampling time points 0.5, 1, and 2 hours after EC-MPS administration and had a r^2 of 0.69, a bias of 0.0 mg*h/L, and a precision of 9.4 mg*h/L (Table III, formula 4).

$$AUC_{0-12}\,(mg*h/L) = 19.801 + 1.827xC_{0.5} + 1.111xC_1 + 1.429xC_2 \qquad \text{(Eq. 4)}$$

Incorporation of C_3 as latest acceptable time point (Table III, formula 5) did not significantly improve the predictive performance of the LSS in terms of precision for patients concurrently treated with cyclosporine (14.6 mg*h/L versus 21.7 mg*h/L; p=0.998). Likewise, no improvement in predictive performance was observed when C_3 was incorporated in a LSS for patients not treated with cyclosporine (Table III, formula 6).

$$AUC_{0-12}\,(mg*h/L) = 20.646 + 1.836xC_{0.5} + 1.023xC_2 + 2.017xC_3 \qquad \text{(Eq. 5)}$$

$$AUC_{0-12}\,(mg*h/L) = 23.771 + 1.211xC_{0.5} + 1.274xC_1 + 1.334xC_3 \qquad \text{(Eq. 6)}$$

To test the agreement between the measured MPA AUC_{0-12} and the estimated AUC_{0-12}, the 4 developed LSS were applied to the validation data sets. The validation of the 4 LSS resulted in nonsignificant biases but markedly worse r^2 values (all < 0.4) and values for precision (all >14 mg*h/L; Table III). The performance of the LSS was further characterised by a percentage of 53.2, 66.0, 59.6 and 46.8% of estimated AUCs that fell within 75-125% of the measured MPA AUC_{0-12} for formulas 3, 4, 5, and 6, respectively. Although the LSS had a nonsignificant bias, the Bland-Altman plots in Figure 2 show that all LSS overestimated AUC_{0-12} when the measured

Table III: Predictive performance of limited sampling strategies

	Index data set			Validation data set			
Samples	r^2	Bias (mg*h/L)	Precision (mg*h/L)	r^2	Bias (mg*h/L)	Precision (mg*h/L)	Within 75-125% compared to full AUC (%)
CsA							
T=0.5/1.5/2	0.42	0.0 (-6.4/6.4)	21.7 (15.0/26.8)	0.33	-1.0 (-8.1/6.1)	24.0 (3.2/33.7)	53.2
T=0.5/2/3	0.74	0.0 (-4.3/4.3)	14.6 (11.4/17.2)	0.37	-0.8 (-8.0/6.4)	24.3 (0/35.5)	59.6
T=0.5/2/4[a]	0.72	0.0 (-4.7/4.7)	11.1 (5.6/14.7)	0.64	-4.2 (-10.0/1.5)	14.0 (8.2/18.1)	66.6
T=2/3/8[b]	0.74	0.0 (-5.0/5.0)	10.4 (6.5/13.3)	0.75	-11.1 (-24.9/2.7)	29.2 (16.3/38.0)	77.7
Non CsA							
T=0.5/1/2	0.69	0.0 (-3.8/3.8)	9.4 (1.8/13.1)	0.31	0.4 (-5.6/6.4)	14.5 (9.3/18.4)	66.0
T=0.5/1/3	0.62	0.0 (-4.2/4.2)	10.4 (5.2/13.7)	0.36	-3.3 (-9.0/2.5)	14.3 (10.5/17.2)	46.8
T=1/1.5/5[c]	0.78	1.3 (-3.9/6.4)	12.7 (7.5/16.3)	0.41	-3.5 (-9.1/2.2)	13.7 (8.8/17.2)	37.5

Data are presented as mean (and 95% confidence interval). Sampling times >3h postdose were not available for all patients. The number of concentration time profiles included in the index and validation data set of these limited sampling strategies were [a]25 and 24; [b]20 and 18; [c]26 and 24. CsA, cyclosporine; r^2, correlation coefficient.

Table IV: Bias (in mg*h/L) of the LSS, separately for three AUC ranges

AUC_{0-12} range (mg*h/L)	LSS_{0-2} CsA	LSS_{0-3} CsA	LSS_{0-2} non CsA	LSS_{0-3} non CsA
<30	20.6 (16.2/25.0)*	6.8 (0.7/12.9)*	11.2 (4.7/17.7)*	11.1 (5.8-16.3)*
30-60	6.2 (0.5/12.0)*	6.0 (1.0/11.0)*	0.7 (-5.5/6.9)	-7.6 (-13.3/-2.0)*
>60	-13.0 (-25.1/-0.9)*	-8.2 (-22.3/6.0)	-19.8 (-35.3/-4.3)*	-18.6 (-31.4/-5.8)*

Data are presented as mean (and 95% confidence interval). *Bias is significantly different from zero. CsA,cyclosporine; AUC, area-under-the-curve; LSS, limited sampling strategy.

MPA AUC_{0-12} is <30 mg*h/L, whereas they underestimated AUC_{0-12} when the measured AUC_{0-12} is >60 mg*h/L. The specific bias for each range is presented in Table IV.

The difference between the measured AUC_{0-12} and the estimated AUC_{0-12} could be caused by a bias in the calculation of the measured AUC_{0-12} due to missing concentration-time points in some profiles or the differences in the analytical methods used. Both possible explanations were evaluated. However, application of the LSS to only those pharmacokinetic profiles in which one of the sampling time points was missing showed no extra bias compared with those in which the measured AUC was based on complete sampling profiles. Also, no differences in predictive performance of the LSS were seen between the different studies included, suggesting that there was no effect of the analytical method used in the participating centers.

Table III and Figure 2 show that LSS including C_3 did not perform better than LSS including C_2 as latest sampling time point. LSS which included samples drawn at time points beyond 3 hours also did not improve predictive performance (Table III), although this analysis was hindered by the fact that C_4, C_5, C_6, and C_8 samples were only available for a part of the study population.

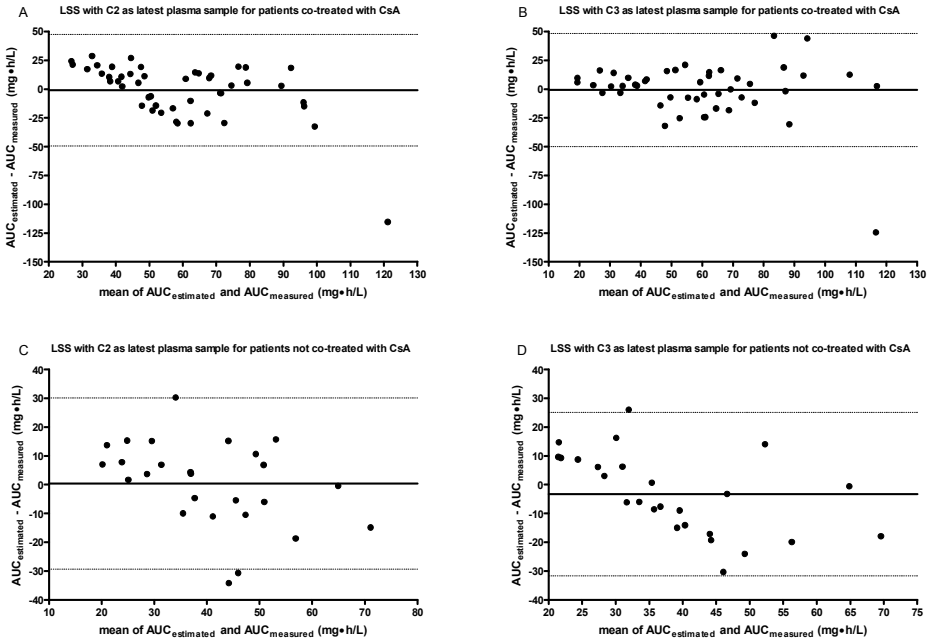

Figure 2: Bland-Altman plots for the agreement between measured MPA AUC_{0-12} and estimated MPA AUC_{0-12} for the four developed and validated limited sampling strategies. The line represents the mean bias, dotted lines are plus and minus two times the standard deviation of the mean. CsA, cyclosporine; MPA, mycophenolic acid; AUC_{0-12}, area-under-the-curve for a 12 hour dosing interval.

The predictive performance of the LSS for EC-MPS from the validation data set were compared with validated LSS for MMF described in literature (Table V). Both, r^2 and precision, were worse in LSS for EC-MPS compared with LSS for MMF.

Table V: Comparison between limited sampling strategies for EC-MPS and MMF

Reference	Sampling times (h)	r^2	Precision (mg*h/L)	Immunosuppressive regimen
Pawinski et al[13]	0, 0.5, 2	0.86	-	MMF+TCL
Weber et al[15]	0, 0.5, 2	0.76	-	MMF+CsA in peadiatrics
Figurski et al[30]	0, 0.66, 2	0.86	4.1	MMF+CsA
Van Hest et al[31]	0, 0.67, 2	0.67	8.1	MMF+CsA
Current study	0.5, 1.5, 2	0.33	24.0	EC-MPS+CsA
Current study	0.5, 2, 3	0.37	24.3	EC-MPS+CsA
Current study	0.5, 1, 2	0.31	14.5	EC-MPS+TCL
Current study	0.5, 1, 3	0.36	14.3	EC-MPS+TCL

Limited sampling strategies are validated with an independent validation data set.

MMF, mycophenolate mofetil; TCL, tacrolimus; CsA, cyclosporine; EC-MPS, enteric-coated mycophenolate sodium.

Discussion

For TDM of MPA, current consensus articles recommend estimation of MPA AUC_{0-12} with a LSS, eventually with a Bayesian estimator.[9,10] LSS are recommended because measuring full AUC_{0-12} is impractical, whereas MPA predose concentrations are only weakly correlated with

AUC_{0-12}.[28] Limited sampling during 2 to 3 hours after MMF administration can lead to acceptable estimation of MPA AUC_{0-12}, and different algorithms have been published, although many do not report a measure of bias or precision based on a validation data set.[28] These LSS cannot be used for EC-MPS because EC-MPS delivers MPA in a different and more variable way than MMF during absorption from the gut.[18,29] LSS for estimation of MPA AUC_{0-12} drawn within 3 hours after EC-MPS administration have not been published yet, and several articles suggested that it would be challenging to obtain a reliable estimation of MPA exposure after EC-MPS intake.[18,29]

The results of the validation of the 4 LSS for estimation of MPA AUC_{0-12} after EC-MPS administration, using only samples obtained within 3 hours postdose, show that the LSS do not perform sufficiently well to be used in the clinical setting. First, the LSS are insufficiently precise, with deviations from true AUC values of >14 mg*h/L. The large imprecision in the LSS for EC-MPS could well lead to unjustified dose adjustments based on MPA AUC_{0-12} estimates that may be 14 mg*h/L too high or too low. Second, although the LSS for estimation of MPA AUC_{0-12} on average do not have a significant bias, the Bland-Altman plots in Figure 2 show that a bias is certainly present, with an overestimation in case of AUC_{0-12}<30 mg*h/L and an underestimation if AUC_{0-12}>60 mg*h/L. The clinical consequence could be that EC-MPS dose increases may be initiated too late for true AUC_{0-12} values <30 mg*h/L and that dose decreases may not be carried out for true AUC_{0-12} values >60 mg*h/L. Third, correlation coefficients in the index data set of the LSS within 3 hours postdose for renal transplant recipients treated with EC-MPS were lower ($r^2 \leq 0.74$) than comparable LSS for estimation of AUC_{0-12} under MMF therapy ($r^2 \geq 0.75$).[13,29-31] These correlation coefficients decreased below $r^2<0.4$ during validation of the LSS (Table III). Correlation coefficients and precision of the LSS for estimation of AUC_{0-12} in EC-MPS treated patients cotreated with cyclosporine ($r^2<0.4$, precision≥24 mg*h/L) were worse than those of a comparable and validated LSS for MMF (Table V).[31,32]

The explanation for the poor predictive performance of the LSS for estimation of MPA AUC_{0-12} after EC-MPS administration probably lies in the highly variable absorption of the drug. Imprecision could be the result of high variability in the time to MPA maximum concentration and in MPA predose concentrations, which has been reported after EC-MPS compared with MMF administration.[17] A recent study reported coefficient of variation values for MPA predose concentrations of 42% for MMF and 82% for EC-MPS, whereas values for time to MPA maximum concentration ranged between 0.5 and 2 hours for MMF and between 0 and 6 hours for EC-MPS.[18] Underestimation of high AUC_{0-12} values could be due to a time to MPA maximum concentration of more than 2 hours after EC-MPS intake. Consequently, the maximum concentration is not captured in the limited sampling scheme. Extension of the LSS from C_2 as latest sampling time point to C_3 did not result in an improvement of the predictive performance (Table III). Most likely, later time points are necessary for improvement, but when these time points were included in the LSS, no evident improvement was observed in predictive performance (Table III). Because the studies included in the present analysis used different sampling schemes, C_4, C_5, C_6 and C_8 samples were not available for all patients.

The lower number of available concentration-time profiles may have contributed to the poor predictive performance of LSS with a sample drawn beyond 3 hours after EC-MPS intake. Future studies need to show whether incorporation of sampling time points beyond 3 hours will result in better predictive performance. However, if samples more than 3 hours postdose result in improved predictive performance, this will be at the cost of reduced practical utility.

Overestimation of low AUC_{0-12} values was observed during validation of the LSS. This may be due to the fact that EC-MPS concentration-time profiles have been observed in this study with a time to MPA maximum concentration within an hour after drug ingestion (Table II).[33] The presented LSS are not capable of adjusting for a high concentration early in the limited sampling scheme, leading to overestimation of AUC_{0-12}. Besides, there were some profiles with very low peak concentrations.

Data included in this study were obtained during the maintenance period after transplantation. Because the pharmacokinetics of MPA change over time after transplantation, with a decrease in MPA clearance due to recovering renal function, plasma albumin concentration, and tapering of immunosuppression,[34] separate LSS will be necessary for AUC_{0-12} estimation in the initial period. However, it is unlikely that LSS for estimation of MPA AUC_{0-12} during EC-MPS treatment in the initial period after transplantation will perform better than the LSS presented here, because pharmacokinetic variability is usually higher in the initial period than in the maintenance period after renal transplantation.

Part of the poor predictive performance of the LSS may be overcome with the use of a Bayesian estimator for EC-MPS. Such an approach may reduce bias because it can compensate for complex absorption profiles with very early or very late high concentrations. Nevertheless, the high unexplained variability during the absorption phase makes it unlikely that precision will improve much.[17]

In conclusion, estimation of MPA AUC_{0-12} with LSS drawn within 2 or 3 hours after EC-MPS administration in patients in the maintenance period after renal transplantation is likely to result in biased and imprecise results, so cannot be recommended for use in routine clinical practice.

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Chapter 5

GENERAL DISCUSSION

Introduction

Mycophenolate mofetil (MMF) is an immunosuppressive drug used to prevent rejection following solid organ transplantation. After oral administration, the prodrug MMF is rapidly hydrolyzed to the active agent mycophenolic acid (MPA). The majority of MPA is glucuronidated to the inactive 7-O-mycophenolic acid glucuronide (MPAG). MPAG undergoes enterohepatic recirculation (EHC), which causes a secondary peak of MPA in the concentration-time profile.[1-2] MPA is a selective, reversible inhibitor of inosine monophosphate dehydrogenase, which has an important role in the proliferation of T- and B-lymphocytes. Inhibition of this pathway causes immunosuppression, contributing to the prevention of graft rejection.[3]

MMF was introduced in 1995 with a recommended fixed-dose regimen of 1 g twice daily. Nowadays, dose individualization using therapeutic drug monitoring (TDM) is thought to further optimize MMF treatment.[4-6] The interpatient variability in MPA exposure is wide compared with the therapeutic window and is influenced by many factors.[7] Coadministration of cyclosporine, low plasma albumin levels and impaired renal function are associated with increased MPA clearance and decreased MPA area under the concentration-time curve (AUC).[8] The incidence of biopsy-proven acute rejection is correlated with the MPA AUC and the MPA predose level, of which AUC showed the best correlation.[9] The recommended target range for the MPA AUC in renal transplant recipients is 30-60 mg*h/L.[5] However, AUC measurements are more complicated to perform for practical reasons.[10] A suitable alternative are abbreviated AUC measurements, in which the AUC is determined by limited sampling strategies (LSS). For TDM in routine practice LSS are the preferred method.

Protein binding

MPA is a highly protein bound drug (~97%). As is the case for many other drugs, the free fraction is thought to be responsible for the immunosuppressive effect of MPA. High unbound MPA (fMPA) exposure is correlated with an elevated risk of MMF related side effects.[11] However, no correlation between fMPA exposure and efficacy is found, as is the case for total MPA (tMPA) AUC. Furthermore, analytical methods to measure fMPA concentrations are more complex than tMPA assays, and no therapeutic window has been determined for fMPA. Consequently, TDM for MMF therapy is mostly performed by monitoring tMPA plasma concentrations.

Low plasma albumin levels and impaired renal function are associated with an increased clearance of tMPA and consequently a decreased tMPA AUC.[8] The effect of these covariates on fMPA exposure in renal transplant recipients is clarified in Chapter 2.1. When albumin levels decrease, tMPA exposure decreases, but fMPA exposure remains unaffected. This is caused by an increase in the MPA free fraction. Impaired renal function causes an increase in MPAG concentrations, which is followed by a decrease in tMPA exposure in patients cotreated with cyclosporine. This

can be explained by displacement of MPA from its binding sites. In patients cotreated with tacrolimus, impaired renal function is followed by an increase in tMPA exposure. Accumulating MPAG concentrations result in increased transport of MPAG to the gallbladder, leading to increased recirculation of MPAG to MPA. fMPA exposure is hardly affected by the changes in renal function. As a result, changes in protein binding, caused by alterations in albumin levels or renal function, will not or hardly influence the exposure to fMPA. These findings should be taken into account when performing TDM. At present, adjustment of the dose due to changes in albumin level or renal function is not recommended. Some authors argue that a dose change is not needed as the relatively low tMPA AUC is accompanied by a higher unbound fraction, resulting in unchanged immunosuppressive potency. On the other hand, the correlations between tMPA exposure and acute rejection are stronger than those between fMPA exposure and acute rejection, and no therapeutic window is defined for the fMPA AUC. These reasons would vote for implementing a dose change based upon the lower tMPA AUC in patients with poor renal function or hypoalbumenia. More research is needed to define a therapeutic window for fMPA AUC and explore the possible advantages of its use in these patients.

Initial mycophenolate mofetil dose

Early exposure to adequate MPA levels is important. An MPA AUC <30 mg*h/L at day 3 posttransplantation is associated with an increased risk of acute rejection.[12] However, due to a relatively high MPA clearance in the immediate phase posttransplantation many patients treated with 1 g MMF twice daily do not reach this target range in the first weeks posttransplantation. The number of patients with an AUC <30 mg*h/L is higher in cyclosporine cotreated patients than in tacrolimus cotreated patients, due to inhibition of the EHC by cyclosporine. In a large randomized trial by van Gelder et al[13] patients treated with 1.98 g MMF per day and cyclosporine had a mean MPA AUC of 33.1 mg*h/L at day 3 posttransplantation, whereas patients treated with 1.99 g MMF and tacrolimus had a mean MPA AUC of 46.1 mg*h/L. Higher initial MMF doses could be given during the early critical period posttransplantation, to increase the number of patients reaching an AUC >30 mg*h/L. Two randomized, controlled trials compared an intensified dosing strategy with standard therapy in the early phase posttransplantation.[14-15] The first study compared a 5-day 3 g daily MMF loading dose with standard 2 g MMF daily dosing in renal transplant recipients cotreated with tacrolimus. The increased MPA AUC levels achieved, resulted in a trend towards fewer treated acute rejections at 6 months posttransplantation (p=0.055).[15] In the other study, the percentage of renal transplant recipients cotreated with cyclosporine with an MPA AUC >30 mg*h/L on day 3 posttransplantation increased from 40.7 to 81.8% after receiving 2.88 g instead of 1.44 g enteric-coated mycophenolate sodium (EC-MPS) per day. The results of these studies show that an intensified dosing strategy in the early phase posttransplantation results in an increased number of patients reaching an AUC >30 mg*h/L, which will presumably result in an decreased risk for acute rejection, especially in patients cotreated with cyclosporine.

Therapeutic drug monitoring

In renal transplant recipients treated with MMF, limited sampling strategies (LSS) have shown good agreement between the estimated and measured MPA AUC.[16-18] In these patients, different LSS are needed for adults and for pediatrics, and for patients cotreated with cyclosporine or patients cotreated with tacrolimus, because of differences in MPA pharmacokinetics. In cyclosporine cotreated patients MPA clearance is increased due to inhibition of the EHC of MPAG in these patients.[19] Clearance has an important role in the estimation of the MPA AUC using LSS. The benefit of TDM by using a LSS was shown by Le Meur et al[4] A significant reduction in the incidence of biopsy-proven acute rejection was seen in renal transplant recipients in whom MMF dose was based on AUC values estimated with a LSS compared with patients receiving fixed doses of MMF.

Besides solid organ transplantation, MMF is increasingly used following hematopoietic stem cell transplantation and autoimmune diseases. Interindividual variability of the pharmacokinetics of MPA in these patients was found to be as high as in renal transplant recipients.[20-21] TDM may be a valuable tool to optimize MMF therapy in these patients as well. However, LSS developed for renal transplant recipients are not necessarily valid for patients with other indications, due to the differences in MPA pharmacokinetics between the populations.

Hematopoietic stem cell transplantation

The success of MMF in renal transplant recipients has triggered the increasing use of MMF in the prophylaxis and treatment of acute and chronic graft-versus-host disease (GVHD) after hematopoietic stem cell transplantation.[22-23] Initially, MMF dose and dose interval applied in hematopoietic stem cell transplant patients were largely based on pharmacokinetic data from renal transplant studies. However, MPA pharmacokinetics differ between these patients (Chapter 3.3). In hematopoietic stem cell transplant patients, exposure to tMPA and fMPA is low compared with renal transplant recipients treated with a similar dose of MMF.

The delivery of optimal immunosuppressive therapy is critical in balancing the beneficial graft versus tumor effects with the adverse effects on engraftment and GVHD. So, an optimal MPA exposure seems to be very important in these patients. As in renal transplant recipients, a correlation between pharmacokinetic parameters and effect was found in hematopoietic stem cell transplant patients. Total MPA predose concentrations ≥1mg/L were associated with an increase in engraftment ($p<0.01$), and decreased severity of acute GVHD ($p=0.02$).[21, 24] Jacobson et al[21] also observed that a low fMPA AUC (<300µg*h/L) was associated with a higher risk for acute GVHD≥II. Giaccone et al[25] found a correlation between steady-state concentrations <2.5mg/L and increased graft rejection ($p<0.01$), without differences in acute GVHD. This predose concentration and steady-state concentration, which is consistent with an AUC_{0-12} <30mg*h/L, are similar to the therapeutic window recommended for renal transplant recipients. However, due

to high cyclosporine predose concentrations and low albumin levels MPA clearance is very high in hematopoietic stem cell transplant patients (Chapter 3.4). Higher doses are needed to achieve these optimal concentrations in hematopoietic stem cell transplant patients compared with renal transplant recipients. Dose adjustments based on MPA concentrations are important to titrate the dose of MMF and to get the patient on target. Consequently, LSS developed in renal transplant recipients could not be used to determine the MPA AUC in hematopoietic stem cell transplant patients. Estimations of exposure would probably be higher than the true values for AUC due to overestimation of the end of the pharmacokinetic profile, as a consequence of the lower MPA clearance in renal transplant recipeints compared to hematopoietic stem cell transplant recipients. Ng et al[26] and Saint-Marcoux et al[27] developed a LSS to estimate the MPA AUC_{0-12} in hematopoietic stem cell transplant recipients. These LSS can be used to perform TDM and optimize the MMF treatment in these patients.

Autoimmune diseases

MMF has also been shown to be effective in patients with autoimmune diseases, such as systemic lupus erythematosus (SLE) and antineutrophil cytoplasmic antibody-associated systemic vasculitis (AASV).[28-29] Recently, oral MMF and intravenous cylcophosphamide were compared as induction treatment for active lupus nephritis in a multinational, randomized, controlled trial.[30] This study showed no significant differences between both treatments with regard to response rate and adverse events. The low number of immunosuppressive drugs used simultaneously with MMF in these patients implies that optimal dosing of MMF could be even more important than in renal transplant recipients to increase the effect of the therapy. Neumann et al[31] found a correlation between MPA predose concentrations and recurrence of active disease and adverse events in patients suffering from SLE or AASV. The best results for maintenance or remission of the underlying disease and prevention of adverse events were observed for MPA predose concentrations between 3.5 and 4.5 mg/L. However, the number of patients with autoimmune diseases in which the pharmacokinetics of MPA is studied is limited. Further research is needed to examine the optimal therapeutic window for MPA in these patients. The therapeutic window defined by Neumann et al[31] can serve as a starting point for this research. Furthermore, these pharmacokinetic studies could better use more robust measurements of MPA exposure than predose concentrations alone (Chapter 3.1). Preferably, LSS should be used to estimate MPA AUC. However, the LSS developed for renal transplant recipients can not be used in patients with autoimmune diseases, due to differences in MPA pharmacokinetics. MPA clearance is decreased in patients with autoimmune diseases compared with renal transplant recipients, due to high albumin levels and the absence of cyclosporine cotreatment (Chapter 3.4). In Chapter 3.2, a LSS was developed to accurately estimate MPA AUC in patients with autoimmune diseases using sampling times at 0, 1, and 3 hours postdose. This LSS can be used to further explore the pharmacokinetic behavior of MPA and study the added value of TDM in patients with autoimmune diseases

Dose adjustments

After determination of the MPA AUC, the MMF dose needs to be adjusted to get the AUC of the patient in the therapeutic window. When adjusting the MMF dose, the MPA exposure is assumed to have a linear relationship with the MMF dose. In Chapter 2.2, we investigated this relationship in renal transplant recipients, and came to the conclusion that the pharmacokinetics of MMF were not linear. Due to a decrease in bioavailability, MPA exposure will increase less than proportional with increasing MMF doses. When higher MPA exposure is needed, the MMF dose needs to be increased more than proportional to produce the desired MPA AUC. This effect is larger in tacrolimus cotreated patients than in cyclosporine cotreated patients. The nomogram, presented in Chapter 2.2, can be used to calculate the dose adjustment needed to get the average patient cotreated with cyclosporine or tacrolimus on target.

Due to elevated MPA clearance, high daily doses are needed to achieve the target concentrations in hematopoietic stem cell transplant patients. However, increasing the MMF dose seems to result in a less than proportional increase in MPA exposure, especially in tacrolimus cotreated patients.[32] For this reason, increasing the dose frequency is preferred, because this will result in higher daily exposure after administration of the same daily dose. Patients with autoimmune diseases are generally treated with lower MMF doses, for which the decrease in bioavailability will be limited.

Enteric-coated mycophenolate sodium

MMF and EC-MPS are both prodrugs of the active compound MPA. EC-MPS was developed to abrogate the frequently observed gastrointestinal adverse events and improve the clinical outcome. Unfortunately, EC-MPS and MMF showed similar efficacy and safety profiles.[33-34] In Chapter 4.1, the pharmacokinetics of MPA after administration of EC-MPS and MMF were compared. Following EC-MPS administration, absorption of MPA was more delayed than following MMF administration as a result of the enteric-coating. Consequently, the lag-time varied much more in EC-MPS treated patients, resulting in unpredictable pharmacokinetic profiles. In 8.7% of the pharmacokinetic profiles of EC-MPS, MPA predose concentrations were higher than the maximal MPA concentration in the subsequent 12-hour observation period. As a result, estimation of the MPA AUC using a LSS is very difficult, and results in biased and imprecise results (Chapter 4.2). As a consequence, a full AUC_{0-12} needs to be obtained to perform reliable therapeutic drug monitoring for EC-MPS, which is very unpractical. Therefore, TDM of EC-MPS cannot be recommended for use in routine clinical practice. In a setting where TDM is not performed the formulations of MPA can be assumed to be therapeutically equivalent. However, due to the practical problems in the implementation of TDM for EC-MPS, MMF is the first choice treatment when MPA treatment is monitored.

Conclusion

Changes in protein binding, caused by alterations in albumin levels or renal function, will not or hardly influence the exposure to fMPA in renal transplant recipients. However, due to the stronger correletion between tMPA AUC and acute rejection than between fMPA AUC and acute rejection, dose adjustments are based on tMPA AUC. To achieve adequate MPA AUC values in the early phase posttransplantation intensified dosing strategies can be used. Afterwards, the MMF therapy can be optimized by TDM. LSS are a suitable tool to estimate the MPA AUC for TDM of MMF in renal transplant recipients. Due to differences in MPA clearance, specifically validated LSS are needed to estimate the MPA AUC in hematopoietic stem cell transplant patients and patients with autoimmune diseases. Following AUC determination, dose adjustments are needed to get the MPA AUC of the patient on target. The nomogram, presented in Chapter 2.2, accounts for the nonlinear relationship between MMF dose and MPA exposure. This nomogram can support the physician to convert TDM data to a desired AUC in order to optimize the MMF treatment. In patients treated with EC-MPS, a LSS will produce biased and imprecise results, due to the unpredictable absorption profile. In these patients a LSS is not recommended for use in clinical practice.

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SUMMARY

Mycophenolate mofetil (MMF) is an immunosuppressive drug used in solid organ transplant recipients, hematopoietic stem cell transplant patients and patients suffering from autoimmune diseases. After oral administration, the prodrug MMF is absorbed and hydrolyzed to the active compound mycophenolic acid (MPA). The main metabolite of MPA, 7-O-mycophenolic acid glucuronide (MPAG), undergoes enterohepatic recirculation (EHC). Due to the existence of a concentration-effect relationship and the large interpatient variability, therapeutic drug monitoring (TDM) may be instrumental in the individualization of MMF treatment.

Chapter 1 extensively reviews the literature to present a clear overview of the need for TDM of MMF in renal transplant recipients. Based on studies that show a correlation between MPA exposure and effect, a therapeutic window for the MPA AUC from 30-60 mg*h/L has been recommended. Furthermore, the pharmacokinetics of MPA are influenced by covariates such as serum albumin levels, renal function and comedication. TDM is likely to reduce the interpatient variability and improve the results of MMF treatment. Limited sampling strategies (LSS) can be used to accurately estimate the MPA AUC using sampling schemes that can be implemented in routine practice. Three prospective randomized studies tried to show the added value of TDM by comparing concentration-controlled MMF therapy with a fixed-dose regimen in renal transplant recipients. However, the results of these studies showed conflicting results.

In Chapter 2.1, a mechanism-based pharmacokinetic model was developed to describe the relationship between covariates and both total and unbound plasma concentrations of MPA and MPAG in renal transplant recipients. In the model, unbound MPA and unbound MPAG competed for a limited number of binding sites. The EHC was described by transport of unbound MPAG to the gallbladder compartment, which empties at certain times in the central compartment of unbound MPA. This transport was decreased in case of cyclosporine cotreatment. Furthermore, clearance of unbound MPAG decreased with impaired renal function. The increasing MPAG concentrations were able to displace MPA from its binding sites, resulting in faster clearance and a decreased total MPA exposure in cyclosporine cotreated patients. However, in tacrolimus cotreated patients the accumulated MPAG could also be reconverted to MPA by the EHC, which caused the total MPA AUC to increase. Changes in renal function had hardly any effect on unbound MPA exposure. Subsequently, the albumin concentration was correlated with the number of binding sites available for MPA and MPAG. A decrease in albumin caused an elevated free fraction, which resulted in an decrease of the total MPA AUC, whereas almost no effect on unbound MPA was seen. In conclusion, changes in protein binding due to altered renal function or plasma albumin levels influence total MPA exposure, whereas unbound MPA exposure is hardly affected.

When MMF doses are adjusted based on an AUC value, a linear relationship between MMF dose and MPA exposure is assumed. In Chapter 2.2 the relationship between MMF dose and the pharmacokinetics of MPA was evaluated. A population pharmacokinetic model was developed in which the already known effects of cyclosporine cotreatment and time

posttransplantation were correlated with MPA clearance and the volume of distribution of the central compartment. A relationship between MMF dose and bioavailability was seen. Compared to an MMF dose of 1000 mg, relative bioavailability was 123, 111, 94, and 90% in renal transplant recipients receiving doses of 250, 500, 1500, and 2000 mg, respectively in combination with cyclosporine; corresponding values for patients receiving tacrolimus were 176, 133, 85, and 76%. As a result, MMF does not exhibit linear pharmacokinetics. The correlation between MMF dose and MPA exposure can be calculated with the nomogram presented in Chapter 2.2. Due to decreased bioavailability, MPA exposure will increase less than proportional with increasing MMF doses. The lack of this linear relationship between MMF dose and MPA exposure may partly explain why a proportion of patients does not reach the therapeutic window of MPA exposure following dose adjustments based on TDM.

The pharmacokinetics of MPA are different between children and adults. In Chapter 2.3, a population pharmacokinetic model is developed to describe the MPA concentration-time profiles obtained from 52 pediatric renal transplant recipients. The data were best described using a 2-compartment model with a lag-time, first-order absorption and first-order elimination. An extra compartment was used to describe the EHC. To account for variability in pharmacokinetic parameters due to the varying size of the individual children, the parameters were standardized to a body weight of 70 kg using allometric scaling. A decrease in albumin level was correlated with an increase in MPA clearance ($p<0.001$). The MPA clearance of pediatric renal transplant recipients (8.8 L/h/70 kg) was relatively low compared to adults. The final model was used to develop a Bayesian estimator to estimate the MPA AUC in these patients.

MMF is increasingly used in patients with autoimmune diseases. In Chapter 3.1, the use of MMF for this indication is reviewed. MMF showed to be very effective in inducing remission and as maintenance therapy in patients suffering from systemic lupus erythematosus (SLE). TDM may be very useful in patients with autoimmune diseases to optimize the MMF treatment. A recent pilot study showed that MPA predose concentrations are correlated with both, disease recurrence and adverse events in patients with autoimmune diseases. A therapeutic window for MPA predose concentrations between 3.5-4.5 mg/L was proposed. However, more research is needed to decide on the value of TDM in these patients. In these studies the correlation between pharmacokinetic parameters and effect can best be determined using a LSS to estimate the AUC. For this purpose, in Chapter 3.2 the pharmacokinetics of MPA in patients with autoimmune diseases are described and a LSS is developed to estimate the MPA AUC. In 38 patients with autoimmune diseases, treated with 1 g MMF twice daily, an average MPA AUC of 66.0 mg*h/L was seen. The data were best described with a 2-compartment model with lag-time, first-order absorption, first-order elimination and an EHC. MPA clearance in patients with autoimmune diseases (8.3 L/h) was low compared to renal transplant recipients. This clearance was further decreased in patients with impaired renal function. LSS were developed with Bayesian estimation and linear regression using 1-4 samples. Optimal sampling times are predose, and 1 and 3 hours postdose. The predictive performance of the

LSS developed with both methods resulted in good accuracy and precision. However, due to its flexibility with respect to sampling times, the Bayesian estimator may be preferred over the multiple regression method.

Furthermore, MMF is also increasingly used in the prophylaxis and treatment of graft-versus-host disease after hematopoietic stem cell transplantation. In Chapter 3.3, the pharmacokinetics of MMF in 15 hematopoietic stem cell transplant patients were described. Although the MMF daily dose was relatively high (median 3000 mg/day), the exposure to total MPA was relatively low (median 25 mg*h/L). Median apparent oral MPA clearance in these patients was 56 L/h (range: 29-98 L/h), which is high compared to reference values from renal transplant recipients. Exposure to MPA can be improved by higher or more frequent MMF dosing.

In comparison with renal transplant recipients, MPA clearance is increased in hematopoietic stem cell transplant recipients and decreased in patients with autoimmune diseases. In Chapter 3.4, the differences in MPA clearance between these three patient groups was characterized and explained by clinical chemical parameters. To this extent, MPA concentration-time curves from renal transplant recipients, hematopoietic stem cell transplant patients and patients with autoimmune diseases were combined and analyzed retrospectively with nonlinear mixed-effects modeling. The pharmacokinetics of MPA were described by a 2-compartment model with time-lagged first order absorption. MPA clearance was correlated with several covariates. Plasma albumin level and cyclosporine predose concentration significantly improved the fit of the model and could explain the difference in clearance between the different indications for MMF treatment. Median clearance was 29.8, 47.5, and 10.6 L/h in renal transplant recipients, hematopoietic stem cell transplant patients and patients with autoimmune diseases, respectively.

To abrogate gastrointestinal adverse events seen in MMF treated patients, enteric-coated mycophenolate sodium (EC-MPS) was developed. MMF and EC-MPS showed similar efficacy and safety, but the pharmacokinetic profile of MPA is different. In Chapter 4.1, the pharmacokinetics of MPA following MMF and EC-MPS were compared. To this extent, concentration-time data from seven clinical studies with EC-MPS and/or MMF were combined and analyzed simultaneously. No differences were detected in MPA clearance, intercompartmental clearance, or the central or peripheral volume of distribution. Differences were seen in the absorption profile. EC-MPS was absorbed more slowly than MMF with respective absorption rate constant values of 3.0 and 4.1 h^{-1}. The morning lag-time following EC-MPS (2.0 h) was significantly different from both the lag-time following MMF administration (0.30 h) and the lag-time following the evening dose of EC-MPS (8.9 h). The morning MPA predose concentrations after EC-MPS administration were higher and more variable than following MMF administration, with respective median (and range) of 2.6 mg/L (0.4-24.4 mg/L) and 1.6 mg/L (0.2-7.6 mg/L). As a consequence, MPA predose concentrations reflect MPA exposure even worse in patients treated with EC-MPS than in patients treated with MMF. In Chapter 4.2, a LSS for EC-MPS was developed for patients who were concurrently using cyclosporine

and patients not concurrently treated with cyclosporine. For clinical feasibility reasons, a LSS could consist of a maximum of 3 sampling time points with the latest sample drawn 3 hours postdose. The LSS developed to estimate MPA AUC following EC-MPS administration resulted in biased and imprecise results. The LSS showed poor precision (>14.0 mg*h/L) and overestimation of AUC values below the therapeutic window and underestimation of AUC values above the therapeutic window. The LSS did not reliable estimate MPA exposure due to the unpredictable absorption profile of EC-MPS.

In conclusion, LSS are a suitable tool to estimate the MPA AUC for TDM of MMF. Due to differences in MPA clearance, specifically validated LSS are needed to estimate the MPA AUC in renal transplant recipients, hematopoietic stem cell transplant patients and patients with autoimmune diseases. Following AUC determination, dose adjustments are needed to get the MPA AUC of the patient on target. When higher MPA exposure is needed, the MMF dose needs to be increased more than proportional to produce the desired MPA AUC.

APPENDICES

NEDERLANDSE SAMENVATTING

Inleiding

Na een orgaantransplantatie kan het immuunsysteem van de patiënt het getransplanteerde orgaan als lichaamsvreemd herkennen. Hierdoor zal het immuunsysteem proberen het orgaan te vernietigen. Deze reactie wordt een afstotingsreactie genoemd. Om deze afstotingsreactie te voorkomen wordt het immuunsysteem van een transplantatie patiënt onderdrukt met geneesmiddelen. Meestal wordt hiervoor het geneesmiddel mycofenolaat mofetil (MMF) gebruikt in combinatie met ciclosporine of tacrolimus. In dit proefschrift wordt vooral gekeken naar MMF. Dit is een geneesmiddel dat het immuunsysteem onderdrukt door de vermenigvuldiging van witte bloedcellen te remmen. Deze witte bloedcellen spelen een belangrijke rol in de afstotingsreactie. De actieve component van MMF is mycofenolzuur (MPA). In het lichaam wordt MPA afgebroken, waarbij mycofenolzuur glucuronide (MPAG) het belangrijkste afbraakproduct is. Dit MPAG wordt via de urine uitgescheiden. Een deel van het gevormde MPAG kan echter via de galblaas naar de darmen worden getransporteerd. Hier wordt het weer omgezet in MPA, wat wederom wordt opgenomen in het bloed. Dit fenomeen wordt een enterohepatische kringloop genoemd. Als in een grafiek de concentratie van MPA in het bloed uitgezet wordt tegen de tijd na inname (concentratie-tijd curve), zullen er door deze kringloop meerdere pieken van de MPA concentratie te zien zijn.

Bij de introductie van MMF werd een dosering van twee maal per dag 1 gram geadviseerd voor iedereen. Door MPA concentraties in het bloed te meten, kan de blootstelling aan het geneesmiddel worden berekend als het oppervlak onder de concentratie-tijd curve (AUC). De MPA AUC is dus een maat voor de blootstelling van de patiënt aan de actieve vorm van het geneesmiddel MMF. Na inname van de geadviseerde dosering MMF verschilt de MPA AUC tussen patiënten van 10 tot 100 mg*uur/l. Uit onderzoek is gebleken dat bij patiënten met een MPA AUC tussen de 30 en 60 mg*uur/l het risico op afstoting en bijwerkingen minimaal is. Om dit te bereiken heeft een deel van de patiënten een hogere dosering nodig dan twee maal per dag 1 gram, terwijl een ander deel van de patiënten juist een lagere dosering nodig heeft om op een goede MPA AUC uit te komen.

De MMF therapie kan per patiënt worden geïndividualiseerd door oorzaken te vinden, die vooraf kunnen voorspellen of een patiënt een relatief hoge of lage MPA AUC zal krijgen. Voor niertransplantatiepatiënten is bekend dat behandeling met ciclosporine, de nierfunctie van de patiënt en de concentratie van het lichaamseigen eiwit albumine in het bloed een belangrijke invloed hebben op de MPA AUC. Zo zal een patiënt met een lage albumine concentratie en een slechte nierfunctie een relatief hoge dosering nodig hebben om een goede MPA AUC te bereiken. Daarnaast kan de MMF therapie per patiënt geïndividualiseerd worden door de MPA AUC te bepalen. De dosering wordt verhoogd als een patiënt een te lage MPA AUC heeft, of verlaagd indien de MPA AUC van de patiënt te hoog is. De MPA AUC kan worden bepaald door gedurende de periode tussen twee doseringen op verschillende tijdstippen de concentratie van MPA in het bloed te meten. Vervolgens worden deze meetpunten door een lijn met elkaar verbonden en wordt de oppervlakte onder deze lijn berekend. Dit is echter een zeer

tijdrovende methode, aangezien de tijd tussen twee doseringen meestal 12 uur bedraagt. In de praktijk wordt bij niertransplantatiepatiënten de MPA AUC vaak bepaald door 2 tot 3 maal in de eerste 2 tot 4 uur na inname van het geneesmiddel de MPA concentratie in het bloed te meten. Met behulp van deze concentraties en een specifiek computer model waarmee het verloop van de concentraties over de tijd kan worden beschreven, kan vervolgens de MPA AUC goed worden geschat.

Bij niertransplantatiepatiënten is uit eerder onderzoek bekend dat de albumine concentratie, nierfunctie van de patiënt en behandeling met ciclosporine invloed hebben op de MPA AUC van een patiënt. Het doel van dit proefschrift was om deze effecten en hun mechanisme te beschrijven met een model. Ook is onderzocht of de resultaten voor het individualiseren van de MMF therapie bij niertransplantatiepatiënten ook gelden in andere patiëntgroepen of bij gebruik van een andere toedieningsvorm van MPA. Dit maakt het mogelijk ook bij deze patiënten de dosering te optimaliseren om het effect van het geneesmiddel te verbeteren.

MMF BIJ NIERTRANSPLANTATIEPATIËNTEN

MPA is voor een groot deel gebonden aan eiwitten die in het bloed aanwezig zijn, waarvan albumine de belangrijkste is. Het kleine deel van de totale hoeveelheid MPA dat niet gebonden is aan eiwitten (~3%) is waarschijnlijk verantwoordelijk voor het effect van het geneesmiddel. Dit ongebonden deel is tevens het deel dat beschikbaar is voor de omzetting in MPAG. Voor niertransplantatiepatiënten is bekend dat de MPA AUC beïnvloed wordt door verschillende patiëntkarakteristieken, zoals de albumine concentratie in het bloed en de nierfunctie van de patiënt. In hoofdstuk 2.1 is een model over de eiwitbinding gemaakt dat de effecten van verschillende patiëntkarakteristieken op zowel de totale als de ongebonden MPA concentraties laat zien. Hierbij is de invloed van drie patiëntkarakteristieken beschreven: behandeling met ciclosporine, de albumine concentratie in het bloed en de nierfunctie van de patiënt. Behandeling met het geneesmiddel ciclosporine remt de enterohepatische kringloop van het geneesmiddel. Hierdoor wordt er meer MPA afgebroken en neemt de heropname af, waardoor de totale MPA AUC afneemt. Een daling van de albumine concentratie in het bloed, leidt tot een daling van het percentage gebonden MPA. Hierdoor is er meer ongebonden MPA beschikbaar voor afbraak en zal MPA sneller worden omgezet naar MPAG. Het gevolg hiervan is een afname in de MPA AUC. Tenslotte leidt een slechte nierfunctie tot een sterke verhoging van de MPAG concentraties, doordat MPAG minder snel door de nieren wordt uitgescheiden. Bij patiënten die niet met ciclosporine behandeld worden, heeft dit een toename van de omzetting van MPAG naar MPA tot gevolg, waardoor de MPA AUC stijgt. Indien patiënten wel behandeld worden met ciclosporine, daalt de MPA AUC juist als gevolg van de slechte nierfunctie. Deze veranderingen hebben vooral effect op de totale MPA AUC, terwijl de AUC van het ongebonden MPA nauwelijks verandert. Omdat de totale MPA AUC een beter beeld geeft van het risico op afstoting, wordt de MMF dosering aangepast aan de hand van de resultaten van de totale MPA AUC.

Na het bepalen van de MPA AUC wordt indien nodig de MMF dosering aangepast. Het doel hiervan is het bereiken van een MPA AUC tussen de 30 en 60 mg*uur/l. Bij het aanpassen van de dosering wordt uitgegaan van een lineaire relatie tussen MMF dosering en MPA AUC. Dit betekent dat bij een verdubbeling van de dosering, de MPA AUC ook zal verdubbelen. In hoofdstuk 2.2 van dit proefschrift is echter gebleken dat deze relatie niet lineair is. Ondanks correctie voor de bekende effecten van patiëntkarakteristieken op de MPA AUC, is er een correlatie gevonden tussen het percentage MPA dat opgenomen wordt en de dosering. Bij een verdubbeling van de dosering, zal de AUC hierdoor minder dan tweemaal zo hoog zijn. Dit effect is groter bij patiënten die niet worden behandeld met ciclosporine.

Een model om de MPA AUC te schatten voor volwassen patiënten die een niertransplantatie hebben ondergaan kan niet voor kinderen worden gebruikt. In hoofdstuk 2.3 is een model gemaakt om de MPA concentraties te beschrijven bij kinderen die een niertransplantatie hebben ondergaan. Hieruit blijkt dat kinderen MPA relatief langzaam afbreken. De snelheid waarmee dit gebeurd, is afhankelijk van het gewicht van het kind en de albumine concentratie in het bloed van het kind. Grote kinderen krijgen dan ook een hogere MMF dosering dan kleine kinderen. De verschillen in de MPA AUC tussen kinderen met een gelijk gewicht en een vergelijkbare albumine concentratie zijn echter nog vrij groot. Het meten van de MPA AUC en het aanpassen van de dosering aan de hand van de resultaten lijkt dan ook noodzakelijk om de MMF therapie bij deze kinderen te optimaliseren.

MMF BIJ ANDERE PATIËNTGROEPEN

Naast het voorkomen van afstoting na orgaantransplantaties, wordt MMF steeds meer gebruikt bij andere patiëntgroepen. In hoofdstuk 3.1 is beschreven dat MMF effectief is gebleken bij patiënten met specifieke auto-immuunziekten. Bij deze patiënten wordt een lichaamseigen weefsel door het immuunsysteem als vreemd herkend en aangevallen. Door behandeling met MMF wordt deze reactie van het immuunsysteem geremd. Het aanpassen van de dosering aan de hand van gemeten concentraties zou bij deze patiënten een goede aanvulling kunnen zijn op de huidige MMF therapie. Een model voor het schatten van de MPA AUC bij niertransplantatiepatiënten kan niet zondermeer gebruikt worden voor andere patiëntgroepen omdat de MPA concentraties verschillen. Dit verschil wordt voornamelijk veroorzaakt door de snelheid waarmee MPA wordt afgebroken. Met als gevolg dat voor iedere patiëntgroep een eigen model ontworpen dient te worden, waarmee met behulp van enkele concentraties een goede schatting van de MPA AUC gemaakt kan worden. In hoofdstuk 3.2 wordt een model gepresenteerd voor patiënten met auto-immuunziekten. De afbraak van MPA is relatief langzaam bij deze patiëntgroep vergeleken met niertransplantatiepatiënten. Met dit model en de gemeten MPA concentraties op 0, 1 en 3 uur na MMF inname kan de MPA AUC voor patiënten met auto-immuunziekten goed worden geschat.

Bij een stamceltransplantatie, waarbij voorafgaand aan de transplantatie het zieke immuunsysteem van de patiënt met chemotherapie wordt vernietigd, worden stamcellen van het gezonde afweersysteem van een donor gegeven. Deze stamcellen vormen het nieuwe immuunsysteem van de patiënt. Ze kunnen het lichaam van de patiënt als vreemd herkennen, waardoor een afstotingsreactie gericht tegen het lichaam van de patiënt zou kunnen ontstaan. Deze reactie richt zich vooral op de huid, darmen en lever van de patiënt en wordt graft-versus-host disease genoemd. MMF wordt veel gebruikt bij stamceltransplantatiepatiënten om graft-versus-host disease te voorkomen. In hoofdstuk 3.3 is de MPA concentratie bij stamceltransplantatiepatiënten gemeten en is het verloop hiervan in de tijd beschreven. Ondanks een relatief hoge MMF dosering bij deze patiënten werd er een lage MPA AUC gemeten. Dit is het gevolg van een relatief snelle afbraak van MPA in deze patiëntgroep. Bij stamceltransplantatiepatiënten zal dan ook een hogere dosering nodig zijn om een AUC tussen de 30 en 60 mg*uur/l te bereiken.

In hoofdstuk 3.4 is geprobeerd de verschillen in de afbraaksnelheid van MPA bij niertransplantatiepatiënten, stamceltransplantatiepatiënten en patiënten met auto-immuunziekten te verklaren. Er is één model gemaakt waarmee de MPA concentraties van alle drie de patiëntgroepen beschreven konden worden. De hoeveelheid bloed die gezuiverd wordt van MPA was hierbij 29,8 l/uur voor niertransplantatiepatiënten, 47,5 l/uur voor stamceltransplantatiepatiënten en 10,6 l/uur voor patiënten met auto-immuunziekten. De verschillen in de afbraaksnelheid van MPA tussen deze patiëntgroepen konden volledig worden verklaard door het gemeten verschil bij de patiënten in albumine en ciclosporine concentraties. In vergelijking met niertransplantatiepatiënten hebben stamceltransplantatiepatiënten een lage albumine concentratie in het bloed en hoge doseringen ciclosporine, waardoor ze MPA relatief snel afbreken. Bij patiënten met auto-immuunziekten worden juist hoge albumine concentraties gemeten. Daarnaast worden deze patiënten meestal niet behandeld met ciclosporine, waardoor MPA bij deze patiënten relatief langzaam wordt afgebroken. De MMF dosering die nodig is om een goede MPA AUC te bereiken zal hierdoor relatief hoog zijn voor stamceltransplantatiepatiënten, en relatief laag voor patiënten met auto-immuunziekten.

EC-MPS BIJ NIERTRANSPLANTATIEPATIËNTEN

Een ander geneesmiddel met dezelfde werkzame component, MPA, is maagsap-resistent mycofenolaat natrium (EC-MPS). MMF en EC-MPS hebben een vergelijkbare effectiviteit en veiligheid. De concentratie-tijd curve van beide geneesmiddelen is echter verschillend. In hoofdstuk 4.1 is een model ontwikkeld om de verschillen in de concentratie-tijd curve tussen deze twee geneesmiddelen te beschrijven. De opname van MPA uit het geneesmiddel EC-MPS begint later en is trager in vergelijking met MMF. Ook vertoont de opname veel grotere verschillen tussen patiënten en verschillen tussen de ochtend en avond dosering. Door de onvoorspelbaarheid van de opname van MPA in het bloed, is het moeilijk een schatting te maken van de MPA AUC aan de hand van een beperkt aantal gemeten concentraties vlak

na inname van EC-MPS. In hoofdstuk 4.2 is geprobeerd een dergelijk model te ontwikkelen, maar de resultaten van de voorspelde AUC waarden waren onjuist en onnauwkeurig. Het is hierdoor lastig om de EC-MPS dosering te individualiseren aan de hand van gemeten concentraties.

Conclusie

Na inname van MMF verschillen de in het bloed gemeten MPA concentraties tussen patiënten. Om de behandeling te optimaliseren wordt aanbevolen de MPA AUC te bepalen, en de dosering aan te passen aan de hand van de resultaten. Hierbij wordt gestreefd naar een MPA AUC tussen de 30 en 60 mg*uur/l. De MPA AUC na inname van MMF kan goed geschat worden met behulp van een model en een beperkt aantal gemeten concentraties. Door verschillen in de afbraaksnelheid van MPA zijn er echter verschillende modellen nodig voor niertransplantatiepatiënten, stamceltransplantatiepatiënten en patiënten met auto-immuunziekten. Na het bepalen van de MPA AUC dient de dosering te worden aangepast om de AUC in het best werkzame gebied van 30 tot 60 mg*uur/l te krijgen. Als een hogere MPA AUC gewenst is, zal de MMF dosering echter meer dan proportioneel verhoogd moeten worden om de juiste AUC te bereiken. Naast MMF kan ook EC-MPS gebruikt worden als toedieningsvorm voor MPA. Door de grote variabiliteit in de opname van MPA is het bepalen van de MPA AUC bij patiënten die behandeld worden met EC-MPS echter lastiger.

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Brenda

CURRICULUM VITAE

Brenda de Winter werd geboren op 25 mei 1982 te Rotterdam. In 2000 behaalde zij het VWO diploma aan het Sint Laurenscollege te Rotterdam, waarna ze met de studie farmacie startte aan de Universiteit Utrecht. Tijdens haar studie heeft ze haar onderzoeksproject gericht op de farmacokinetiek van mycofenolaat mofetil in stamceltransplantatiepatiënten uitgevoerd in de apotheek van het Erasmus MC (Hoofdtuk 3.3). In 2005 behaalde ze haar doctoraal en in 2006 sloot zij haar studie af met het apothekersexamen. In september van datzelfde jaar begon ze als onderzoeker in dienst van het Erasmus MC. Dit onderzoek stond onder supervisie van Prof.dr. A.G. Vulto, dr. R.A.A. Mathôt en dr. T. van Gelder en heeft geresulteerd in dit proefschrift. Vanaf december 2009 is ze als postdoc werkzaam aan de Universiteit van Limoges.

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de Winter BC, Bakker C, Cransberg K, Knops NB, van Gelder T, Bartelink IH, Mathot RA. Development of a Bayesian estimator for monitoring mycophenolate mofetil therapy in pediatric renal transplant recipients. *Submitted*.

PHD PORTFOLIO

Name PhD student:	Brenda de Winter
Erasmus MC department:	Hospital Pharmacy
PhD period:	2006-2009
Promotor:	Prof.dr. Arnold G. Vulto
Supervisors:	Dr. Teun van Gelder and Dr. Ron A.A. Mathot

	Year	Workload (hours)
Courses		
European Transplant Fellow Workshop ESOT, Barcelona, Spain	2006	24
Introductory Biostatistics for Researchers, Utrecht University, Utrecht, the Netherlands	2006	64
Binary and Categorical Data Modeling, Analysis & Simulation using NONMEM, Copenhagen, Denmark	2007	16
Hesperis course of ESOT, St. Gallen, Switzerland and Leiden, the Netherlands	2007-2008	80
English Biomedical Writing and Communication, Erasmus MC, Rotterdam, the Netherlands	2008	112
Multiple and Logistic Regression Analysis, Utrecht University, Utrecht, the Netherlands	2008	15
Conferences		
Annual congress Dutch Society of Clinical Pharmacology and Biopharmacy, Lunteren, the Netherlands	2005	8
Spring meeting of Dutch Society of Clinical Pharmacology and Biopharmacy, Utrecht, the Netherlands	2007	6
Annual meeting of the American Society of Clinical Pharmacology and Therapeutics, Orlando, USA	2008	40
Annual congress Dutch Transplantation Society, Zeewolde, the Netherlands	2008	13
Annual congress Dutch Society of Clinical Pharmacology and Biopharmacy, Lunteren, the Netherlands	2008	8
Dutch Hospital Pharmacy day, Rotterdam, the Netherlands	2008	8
Meeting for Dutch PhD students and postdocs in Nephrology, Rotterdam, the Netherlands	2008	8

	Year	Workload (hours)
Annual congress Population Approach Group Europe, St Petersburg, Russia	2009	24
International Congress of Therapeutic Drug Monitoring & Clinical Toxicology, Montreal, Canada	2009	40

Oral Presentations

Annual congress of Dutch Transplantation Society, Zeewolde, the Netherlands — 2008

- *Population pharmacokinetics of MPA: a comparison between EC-MPS and MMF in renal transplant recipients*

Annual congress Dutch Society of Clinical Pharmacology and Biopharmacy, Lunteren, the Netherlands — 2008

- *Limited sampling strategies for mycophenolic acid in patients with autoimmune disease*

International Congress of Therapeutic Drug Monitoring & Clinical Toxicology, Montreal, Canada — 2009

- *Dose proportionality of mycophenolate mofetil in renal transplant recipients*
- *Explaining the differences in clearance of mycophenolic acid between renal transplant recipients, hematopoietic stem cell transplant recipients and patients with autoimmune disease*
- *Limited sampling strategies drawn within three hours postdose for estimation of mycophenolic acid area-under-the-curve after enteric coated mycophenolate sodium are unsuitable for use in clinical practice*
- *Limited sampling strategies for mycophenolic acid in patients with autoimmune disease*

Poster presentations

Annual congress Dutch Society of Clinical Pharmacology and Biopharmacy, Zeewolde, the Netherlands — 2005

- *Overestimation of mycophenolic acid concentration for EMIT compared to HPLC in mycophenolate sodium (Myfortic®) treated kidney transplant patients*

	Year	Workload (hours)
Annual meeting of the American Society of Clinical Pharmacology and Therapeutics, Orlando, USA	2008	
· *Population pharmacokinetics of MPA: a comparison between EC-MPS and MMF in renal transplant recipients*		
Annual congress Dutch Society of Clinical Pharmacology and Biopharmacy, Zeewolde, the Netherlands	2008	
· *Population pharmacokinetics of mycophenolic acid: a comparison between enteric-coated mycophenolate sodium and mycophenolate mofetil in renal transplant patients*		
Dutch Hospital Pharmacy day, Rotterdam, the Netherlands	2008	
· *Population pharmacokinetics of mycophenolic acid: a comparison between enteric-coated mycophenolate sodium and mycophenolate mofetil in renal transplant patients*		
· *Limited sampling strategies for mycophenolic acid in patients with autoimmune disease*		
Annual congress Population Approach Group Europe, St Petersburg, Russia	2009	
· *Mechanism-based pharmacokinetic modeling of protein binding of mycophenolic acid and its glucuronide metabolite in renal transplant recipients*		

Teaching

Education in pharmacy, 5th/6th year medical students, Erasmus MC, Rotterdam, the Netherlands	2006-2009	100