Published in final edited form as:

Ther Drug Monit. 2014 December ; 36(6): 716–723. doi:10.1097/FTD.0000000000000081.

The Effects of Unbound Mycophenolic Acid (MPA) on Inosine Monophosphate Dehydrogenase (IMPDH) Inhibition in Pediatric Kidney Transplant Patients

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Abstract

Background—Mycophenolic acid (MPA) is a key immunosuppressive drug that acts through inhibition of inosine monophosphate dehydrogenase (IMPDH). MPA is commonly measured as part of therapeutic drug monitoring as the total concentration in plasma. However, it has been postulated that the free (unbound) fraction of MPA (fMPA) is responsible for the immunosuppressive effects. In this study, a sensitive low volume high performance liquid chromatography (HPLC) assay was developed to measure fMPA concentrations in order to explore the relationship between fMPA and IMPDH activity.

Methods—To obtain fMPA concentrations, plasma samples were filtrated using Centrifree® Ultrafiltration Devices. The ultrafiltrate was analyzed by HPLC using a Kinetex® C18 column (2.6 μ m, 3.0 $\times$ 75 mm). fMPA concentrations were compared to total MPA concentrations available in 28 pediatric kidney transplant patients at 3 consecutive occasions post-transplantation. The relationship between fMPA and IMPDH activity was analyzed using an $E_{\max}$ -model.

Results—The HPLC-assay using 25 μ L of the ultrafiltrates was validated over a range from 2.5 to 1000 μ g/L with good accuracy, precision and reproducibility. Total and free MPA concentrations were well correlated ($R^2 = 0.85$, $P < 0.0001$) although large intra-and inter-individual variability in the bound MPA fractions was observed. The overall relationship between fMPA concentrations and IMPDH inhibition using the $E_{\max}$ -model was comparable to that of total MPA as previously

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Part of this work has been presented as on oral presentation at the 13th international congress of the International Association of Therapeutic Drug Monitoring and Clinical Pharmacology (IATDMCT) 2013 in Salt Lake City.

Conflict of Interest

All authors declare no conflict of interest.

reported. The model estimated EC₅₀ (164.5 µg/L) is in good agreement with reported *in vitro* EC₅₀ values.

Conclusions—This study provides a simple HPLC method for the measurement of fMPA and a pharmacologically reasonable EC₅₀-estimate. The good correlation between total and free MPA concentrations suggests that routine measurement of fMPA to characterize mycophenolate PK/PD does not seem warranted although the large variability in the bound fractions of MPA warrants further study.

Keywords

mycophenolic acid; inosine monophosphate dehydrogenase; pediatric kidney transplant; free concentration; PK/PD

Introduction

Mycophenolic acid (MPA) is a key immunosuppressive drug which is administered as the pro-drug mycophenolate mofetil (CellCept, MMF). The *in vivo* conversion of MMF into its active moiety MPA is catalyzed by esterases and almost complete before reaching the systemic circulation.(1) In blood, 99.9% of MPA is distributed into plasma and the fraction of MPA which is bound to plasma proteins, predominantly human serum albumin, is 97% under normal physiology.(2)(3) Total MPA exposure as characterized by the area under concentration-time curve (AUC) has been associated with clinical outcome.(4)(5) In a pivotal randomized double-blind clinical trial, investigators showed that a higher area under the curve (AUC) value of total (bound and unbound) MPA (tMPA) was associated with a reduced risk of acute graft rejection in adult renal transplant patients. An AUC of 15 µg·h/mL was associated with effective treatment in half of the adult kidney transplant patients.(6) In pediatric kidney transplant patients, a tMPA AUC_{0-12h} of less than 33.8 mg·L/h in the initial post-transplant period was associated with risk of acute rejection.(7) A recent consensus report recommends a tMPA AUC_{0-12h} range of 30–60 mg·L/h as the therapeutic target in both adult and pediatric renal transplant patients.(8)

MPA acts through reversible and noncompetitive inhibition of Inosine Monophosphate Dehydrogenase (IMPDH).(9) Two IMPDH isoforms have been identified; isoform type I which is present in most human cells and isoform type II which is predominantly expressed in human B and T lymphocytes. MPA predominantly inhibits isoform type II, resulting in an effective drug for immunosuppressive combination with calcineurin inhibition.(10)(11) Notwithstanding the fact that MPA can act through several other mechanisms to prevent graft rejection as well, IMPDH inhibition can be used as a biomarker of immunosuppressive effect of MPA in lymphocytes. IMPDH inhibition is well correlated with MPA concentration with IMPDH activity being reduced with increasing MPA levels.(12)

It has been postulated that the pharmacological effect of MPA is best described by the free (unbound) MPA (fMPA) concentration.(2) However, there is large inter-individual variability in fMPA concentrations due to various (patho-)physiological factors. To date, no studies have been performed to investigate the relationship between fMPA and IMPDH inhibition in pediatric kidney transplant patients. As renal impairment is associated with

lower serum albumin levels and with albumin concentrations fluctuating, especially early post-transplant, it may be clinically relevant to measure fMPA concentrations to predict immunosuppressive efficacy.(13)(14)(15) Only a few high performance liquid chromatography (HPLC) methods for the quantification of fMPA have been published. All reported methods are based on relatively large filtrate volumes and have relatively high lower limits of quantification (LLOQ) in the range of 4–10 µg/L.(13)(16)(17) Since drawing small blood volumes is preferred in pediatric patients, a sensitive method with a smaller filtrate and injection volume is desirable for this population. For instance much lower LLOQ values have been reported with liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) (i.e., 0.5 µg/L), but MS methodology has some disadvantages in terms of equipment and operational cost.(18)

In this study, a sensitive and simple HPLC assay was developed and validated requiring only a small volume of ultrafiltrate. A more sensitive method with a low volume requirement is beneficial especially given the restrictions in the amount of blood that can be safely drawn for study purposes in pediatric patients. The developed assay was used for the analysis of fMPA concentrations of samples from a PK/PD study in pediatric patients, with the purpose of exploring the relationship between fMPA and IMPDH inhibition.(12)

Materials and Methods

Clinical Samples

tMPA concentrations and IMPDH activity measurements over time were obtained from a cohort of 28 pediatric kidney transplant patients on MMF as recently described.(12) Patient demographic characteristics are summarized in Table 1. Ultrafiltrate samples for the measurement of fMPA were prepared using a portion of the tMPA PK plasma sample at the same sampling time (see Sample preparation) and were stored at –80°C.(12) This procedure was intended to simultaneously study the PK of tMPA and fMPA and their inhibitory effects on IMPDH. The prospective study was a multicenter study with approval by the institutional review boards of all participating institutions.(12) Written consent was obtained from every parent and patient assent when applicable (12–18 y). All patients were on MMF, tacrolimus, corticosteroids and received induction therapy. Pharmacokinetic samples were collected predose and at 20, 40 minutes, 1, 1.5, 2, 3, 4, 6 and 9 hours postdose at 3 consecutive occasions: early posttransplant (day 1–3), at hospital discharge (day 5–9) and at stable treatment (after 3 or more months). A sparse sampling schedule was offered for patients with blood volume or study visit time restrictions.(12)

Chemicals and reagents

Phosphate buffered saline (PBS) 10x solution pH 7.4 and monopotassium phosphate were obtained from Fisher Scientific (Pittsburgh, PA). Acetonitrile and methanol were obtained from Tedia (Fairfield, OH). MPA and the internal standard (IS), the carboxybutoxy ether of mycophenolic acid, were generous gifts of La Roche Bioscience (Palo Alto, CA). All aqueous solutions were made using Millipore water and methanol.

Sample preparation

Blood samples were collected in EDTA tubes and centrifuged immediately; retrieved plasma samples were stored in a refrigerator (4°C). At the end of the study day plasma samples were stored in a -80°C freezer (within 24h). After centrifuge, plasma was divided into two portions: one for the analysis of tMPA and one for ultrafiltration for the analysis of fMPA concentrations. For fMPA, an aliquot plasma was filtrated according to the method of Nowak and Shaw (1998) (30 min at 2000 ×g) using Centrifree® Ultrafiltration Devices (Millipore, Billerica, MA) yielding approximately 50–100 µL filtrate.(2)(12)(19) The filter was first rinsed with methanol:water (50%/50% vol/vol) to eliminate potential interfering substances which elute around the same retention time as MPA and the used IS.(13)(17) The filtration procedure and tMPA analysis, as previously described, were performed within 30 days of blood sampling. All samples, including the ultrafiltrates, were stored at -80°C until analysis.(12)

Measurement of free (unbound) MPA

25 µL of the ultrafiltrate was mixed with 10 µL of IS (875 µg/L) into 1.5 mL Eppendorf tubes (Fisher Scientific, Pittsburgh, PA) for analysis. The HPLC system consisted of a G1354A quaternary pump, a thermostatted autosampler, a degasser and a diode array detector set at 215 nm (Agilent Technologies 1100 series, Santa Clara, CA). The column used was a Kinetex® 2.6 µm C₁₈ column (3.0 × 75 mm, Phenomenex, Torrance, CA) at a flow rate of 0.75 ml/min. Peaks were manually integrated using ChemStation for LC 3D Systems (Agilent Technologies, Santa Clara, CA). A standard flow cell (Agilent Technologies, Santa Clara, CA) was used. The isocratic mobile phase consisted of 29% acetonitrile and 71% 0.02M KH₂PO₄ pH=3.0. The samples were transferred into a micro-volume polypropylene vial insert (National Scientific, Rockwood, TN) and 30 µL was injected into the HPLC system.

Assay validation

Solutions for validation were made in-house in PBS.(13)(16)(18) Blank human plasma was obtained from Hoxworth Blood Center (Cincinnati, OH) and was used to test for interfering compounds in the biological matrix after filtration. Validation was performed according to the FDA guidelines for bioanalytical method validation.(20) The limit of detection (LOD) was determined at the lowest concentration where a MPA peak could still be visibly detected against background noise. The LLOQ was defined as the concentration with a precision of at least 20% and an accuracy of 80–120% (n=5). Quality control (QC) samples were prepared at the following concentrations: 7.5 µg/L, 250 µg/L and 750 µg/L. Intra-day precision was determined by analyzing each QC sample five times on the same day. Inter-day precision was determined by analyzing the QC samples in duplicate on five different days. The calibration curve was set using the following concentrations: 2.5, 5, 10, 50, 100, 500 and 1000 µg/L (n=5) and was used to examine linearity over the working range. The curve was considered to be linear when R² 0.995. Besides the requirement of linearity, an additional requirement for the variation of the 5 measurements of each concentration was applied. The variation of the 5 measurements of each concentration of the calibration curve should not exceed 15% and for a concentration of 2.5 µg/L (LLOQ) 20%. Finally, accuracy

was investigated by comparing the nominal concentration to the average measured concentrations in the QC samples (n=5) which should fall within 85 to 115% of the actual MPA concentration.

Pharmacokinetic Analysis

Non-compartmental analysis in Phoenix (WinNonlin 6, version 6.2.1, Pharsight, Certara, Mountain View, CA) was used for the estimation of fMPA pharmacokinetic parameters. To obtain the fMPA AUC_{0-12h}, the predose concentration of fMPA was substituted for the concentration at 12 hours post-dose. In one patient whose predose sample was not available, the fMPA concentration at 9 hours post-dose was used for both the concentration predose and 12 hours postdose.

Pharmacokinetic and Pharmacodynamic Analysis

IMPDH activity measurements were obtained from the previous study.(12) In brief, the lysate of mononuclear cells (MNC) isolated from whole blood was incubated with inosine 5'-monophosphate (IMP) and the product xanthosine 5'-monophosphate (XMP) and adenosine 5'-monophosphate (AMP) were measured by HPLC. Enzyme activity was expressed as produced XMP (nmol) per time unit (h) per mg protein (nmol/h/mg protein). An E_{max}-model was used to describe the relationship between the fMPA concentration and IMPDH activity using the following equation: $\text{IMPDH activity} = E_0 - ((E_0 - E_{\text{max}}) \cdot C / (\text{fEC}_{50} + C))$, where E_{max} is the maximum IMPDH inhibition, E₀ is the baseline activity with no MPA inhibition, and fEC₅₀ is the free MPA concentration (C) when 50% of maximum IMPDH inhibition is reached. GraphPad Prism 4 for Windows, version 4.03 (GraphPad Software, La Jolla, CA), was used for the nonlinear E_{max}-model data analysis.

Statistical Analysis

The Pearson correlation test was used to assess the relationship between free and total MPA in all samples where both fMPA (>2.5 µg/L) and tMPA was quantifiable.(23) The D'Agostino and Pearson omnibus normality test was used to test normality for average MPA binding percentages on each occasion. When normality was confirmed, a one-way ANOVA was used to compare the means of the three occasions. The Bartlett's test was used to compare the variances. In addition, a paired student's t-test was performed to compare the binding percentages within the patients between the different occasions. Subsequently, the Wilcoxon signed-rank test was used to compare the fMPA AUC_{0-12h} among the three different occasions. In order to gain insight into the effects of low albumin levels (<32 g/L), a two-tailed t-test was performed comparing the ratio of fMPA-AUC_{0-12h} and tMPA-AUC_{0-12h}. The cut-off value of 32 g/L was based on the observation by Atcheson et al. (2004), who reported that therapeutic drug monitoring of fMPA in patients with albumin levels <32 g/L is useful, while in patients with a higher albumin level therapeutic drug monitoring of tMPA would suffice.(24) A P-value of 0.05 was considered statistically significant.

Results

Development and validation of an HPLC method for fMPA measurement

The HPLC method for the measurement of fMPA was validated as described in the method section. Typical retention times were between 6.2 and 6.6 minutes for MPA and between 7.2 and 7.5 minutes for the IS. No interfering signal was observed in blank plasma samples. The LOD was 2.0 µg/L. The LLOQ was 2.5 µg/L with a coefficient of variation (CV) of 11% and an accuracy of 16%. The intra-day precision for the high, medium and low QC samples was 3% (n=5), 3% (n=5) and 4% (n=5), respectively. The inter-day precision for the high, medium and low QC samples were 5% (n=10), 5% (n=10) and 6% (n=10), respectively. Adequate linearity over the range 2.5–1000 µg/L was observed with the following equation: $y=0.0045764x - 0.00408$ ($R^2=0.996$; weighting factor=1/X). The variations of the concentrations of the calibration curve did not exceed 15% (2–7%). Accuracy for the high, medium and low QC samples were 98% (n=5), 94% (n=5) and 85% (n=5), respectively.

Free mycophenolic acid measurements in clinical samples

625 fMPA concentrations in clinical study samples were measured with the validated assay. fMPA concentrations were well correlated with tMPA concentrations previously measured (n=602, $R^2 = 0.85$, $P<0.0001$, the Pearson's product-moment correlation). The fMPA concentration-time profiles of all patients are shown in figure 1. Large inter-individual variation in fMPA was observed at all three occasions; early posttransplant (n=27), at hospital discharge (n=25) and at stable treatment (n=17). Consistent with previous observations in tMPA concentrations, the values for C_{max} and fMPA AUC_{0-12h} increased over time. The fMPA AUC_{0-12h} on stable treatment was significantly higher than that at hospital discharge ($P<0.05$, the Wilcoxon signed-rank test) (figure 2, table II).

The fraction of MPA bound to serum proteins is shown in figure 3 for every individual patient on each occasion. The mean fraction bound early posttransplant was 88.2% (range: 80.1–99.5%), at hospital discharge 89.1% (range: 82.2–99.1%) and at stable treatment 89.7% (range: 85.6–91.9%). Thus, large intra- and inter patient variability in the free fraction of MPA was observed. Although the mean MPA fractions bound appeared similar among the three occasions, the variance was significantly different (Bartlett's test; figure 3), indicating that the fraction of bound MPA stabilized over time. Patients who had low albumin levels (<32 g/L) had a significantly higher ($P<0.02$) fMPA-AUC_{0-12h} to tMPA-AUC_{0-12h} ratio than patients with normal albumin levels.

Relationship between fMPA-PK and IMPDH activity

Mean fMPA PK-time profiles at the three occasions are displayed in figure 4 together with mean IMPDH activity-time profiles observed previously. An increase in fMPA concentration resulted in a decrease of IMPDH activity on all three occasions. This relationship between IMPDH inhibition and fMPA concentration was mathematically described by using the E_{max} -model (figure 5). Parameter estimates for E_0 and fEC_{50} were 5.04 nmol/h/mg protein (SE=0.47; 95% confidence interval=4.12–5.97) and 164.5 µg/L (SE=40; 95% confidence interval=87–242), respectively. The R^2 values of this analysis

were similar when using tMPA or fMPA, and were estimated as 0.27 and 0.20, respectively. However no significant between-occasion difference in patients was observed.

Discussion

A sensitive low volume HPLC-method for the determination of fMPA was successfully developed and validated. The LLOQ of 2.5 µg/L was well below the reported LLOQ values of 10 µg/L (13)(23), 5 µg/L (LOD)(17) and 4 µg/L (16) in previous publications. Although lower LLOQ values were reported with an LC-MS/MS method (0.5 µg/L)(18), the developed HPLC-diode array system was considered well suited for its purpose in terms of accuracy, precision, linearity and reproducibility. The assay has advantages in terms of equipment and operation costs compared with an LC-MS/MS method. In addition, time required for sample preparation (2–3 minutes) and actual runtime of 10 minutes for fMPA detection were both considered relatively short and sufficient, even though an LC-MS/MS method has a shorter runtime of only 4 minutes with comparable sample preparation time as the developed HPLC method.(18)

The purpose of this study was to provide a more complete picture on the PK and PD of free MPA since the free fraction of MPA has been proposed to best predict the immunosuppressive effect.(2) Consistent with tMPA concentration profiles, significant intra-and inter-individual variability in the concentration-time profiles of fMPA was observed in our cohort of pediatric kidney transplant patients. The overall trends in the pharmacokinetic parameters of fMPA ($C_{\max}$, $T_{\max}$, AUC_{0-12h} and CL/F) were compatible to those observed for tMPA. This observation was supported by a good correlation between fMPA and tMPA ($R^2 = 0.85$). The pharmacokinetic properties in patients changed over time after kidney transplantation, including MPA protein binding. This indicates the importance of therapeutic drug monitoring of tMPA and/or fMPA on a regular basis in order to maintain adequate MPA exposure to ensure long-term graft survival.(12)

Reported binding percentages of MPA to human serum albumin have been in the range of 97% to 99% in different populations.(2)(23)(25)(26) Binding percentages in pediatric kidney transplant patients have not been reported. In this study, large intra-and inter-patient variability in binding percentages was observed early posttransplant and at hospital discharge. The binding profile became more stable over time with binding values in the range of 85.6 to 91.9% on stable treatment. These binding percentages were significantly lower than reported values in other patient populations. There are several possible reasons to explain this. First, excessive albumin loss through the kidney is common in kidney transplant patients.(27) According to a study in adult kidney transplant patients, 50% of patients (n=608) experienced albuminuria one year after transplantation.(28) Consequentially, fewer albumins available for binding would result in higher free concentrations. Secondly, MPAG and endogenous compounds, and co-medications could compete for binding sites leading to lower binding.(29–31) Chronic renal insufficiency has been associated with higher free fractions of MPA in adult renal transplant patients.(29) This in turn could result in MPAG accumulation leading to MPAG competing for albumin binding sites.(29) Third, the binding of mainly acidic drugs (like MPA) to albumin is decreased in chronic kidney failure due to the accumulation of endogenous compounds

which compete for the same binding sites. In addition, a structural change in albumin's tertiary structure (e.g. via carbamylation) during chronic kidney failure leads to decreased binding of drug including MPA.(30) As for drug-drug protein binding interactions with other immunosuppressants, tacrolimus, cyclosporine A and prednisone did not have any impact on the protein binding of MPA.(31) Another potential reason to consider is surgical stress and reported lower albumin production.(32) In our patient population, the effect of albumin concentration on MPA exposure was analyzed. The higher ratio of fMPA-AUC_{0-12h} and tMPA-AUC_{0-12h} in patients with low albumin is indicative of a higher free fraction. This requires further exploration in future studies.

An important clinical question is whether therapeutic drug monitoring of fMPA has advantages over current practice of monitoring of tMPA. In this study, the fEC₅₀-value of 164.5 µg/L was estimated by using the E_{max}-model, and is in accordance with reported *in vitro* values of IMPDH II inhibition (ranging from 3.5 to 480.5 µg/L, including a value of 32.0 µg/L recently determined by Dunkern et al.)(33). The fEC₅₀-estimate is much lower than the EC₅₀-value obtained from the analysis with total MPA which was 0.97 mg/mL.(12) Since only the free concentration is considered to exhibit the pharmacological effect, this value cannot be derived from the tMPA concentration measurements. Thus, the fMPA concentration provided an *in vivo* pharmacological insight into IMPDH inhibition, as the fEC₅₀-value was comparable to the reported *in vitro* IMPDH inhibition parameters. However, there was a good correlation between fMPA and tMPA and the coefficient of determination ($R^2 = 0.85$) was even higher than the R^2 of 0.77, reported by Reine et al. (2012) for measurements of fMPA and tMPA.(23) Furthermore, the overall relationship of fMPA versus IMPDH inhibition in terms of goodness of fit with the E_{max}-model was similar to that of tMPA ($R^2=0.21$ and 0.27 for fMPA and tMPA, respectively). Practically, the use of Centrifree® Ultrafiltration Devices is more time-consuming and generates additional cost with no faster and qualitatively comparable alternative. These facts do not indicate any clear advantage of the routine measurement of fMPA over the current standard practice of tMPA monitoring.

There are some limitations to this study. One is related to the stability of MPA in ultrafiltrate samples. The ultrafiltrates were prepared during the study period from February 2006 to December 2008 as part of the prospective study described by Fukuda et al. (2011) from a portion of plasma sample immediately after sampling.(12) However, there are no data regarding long term stability of MPA samples. The stability of fMPA was previously investigated by Streit et al. (2004) who reported good stability for up to six months and after three freeze and thaw cycles which was within 15% of baseline across all measurements. (18) Samples in current study were all filtrated before storage and anticipated to be more stable than samples stored as plasma due to the absence of serum proteins. However, as fMPA stability has not been confirmed in the samples, results should be interpreted with some caution.

Another topic of note is the expression of IMPDH activity. In this study, IMPDH activity was expressed as xanthosine monophosphate (XMP) per protein concentration in line with previously reported results. Glander et al. (2009) recommended the use of AMP-normalized XMP as a ratio of XMP to AMP instead of XMP per protein concentration. They describe a

relatively low variability in the ratio of XMP to AMP due to elimination of the effect of a possible contamination of the samples with extracellular proteins in the case of protein normalization.(34) When using the ratio of XMP to AMP in our analysis some samples showed higher variability with the ratio in comparison to normalization to protein. Therefore, XMP per protein concentration was used to express IMPDH activity, which seemed to work well for both tMPA and fMPA data.

Conclusion

This study describes a sensitive low volume HPLC method for the measurement of fMPA. A pharmacologically reasonable fEC₅₀-value for IMPDH inhibition was estimated. The observed good correlation between total and free MPA concentrations suggests that routine measurement of fMPA to better predict the level of immunosuppression does not seem warranted although the large variability in levels of MPA protein binding warrants further study.

Acknowledgments

Source of Funding:

This work was supported by NIH grants 5U10HD037249 (TF, AAV) and 5K24HD050387 (AAV) and an investigator initiated research grant from Roche Laboratories (AAV). We thank colleagues, Hasan Jafri, MD, George McCracken, MD, and Mouin Seikaly (UT Southwestern, Dallas, TX) and staff in the Utah and UT Southwestern NICHD Pediatric Pharmacology Research Unit network for their support. We gratefully acknowledge the financial support from the Dr. Saal van Zwaneberg Stichting, the Groninger Universiteitsfonds and the KNMP Stipendiafonds, to Thomas Smits to stay and study at the Cincinnati Children's Hospital Medical Center.

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Figure 1.

Concentration time profiles of free mycophenolic acid (fMPA) in pediatric kidney transplant patients: (A) early posttransplant (n=27), (B) at hospital discharge (n=25) and (C) at stable treatment (n=17). The dotted lines early posttransplant show the individual concentration-time profile of two patients on intravenous (IV) treatment (stop time of infusion, after 2hr, is set as 0hr in graph), all lines are profiles after oral administration.

Figure 2.

Boxplot of the free mycophenolic acid (fMPA) area under the curve from 0 to 12h (AUC_{0-12h}) for the three different occasions; (1) early posttransplant, (2) at hospital discharge and (3) at stable treatment. The line in the middle of the boxes is the 50th percentile; the entire box covers AUC_{0-12h} values between the 25th and the 75th percentiles. The bars display the entire range of AUC_{0-12h} values.

Figure 3.

The fraction of mycophenolic acid (MPA) bound to serum proteins at different occasions (A) early posttransplant (n=28), (B) at hospital discharge (n=26) and (C) at stable treatment (n=17). X-axis shows individual patient ID. This fraction is calculated according to the following formula: $((t\text{MPA}-f\text{MPA})/t\text{MPA}) \cdot 100\%$. The number of a patient is the same on every occasion. Error bars show the 95% confidence intervals of the measurements of all the samples from one patient on one day each occasion (n=4–10). One patient (#4) had a negative percentage for the fraction bound, as fMPA concentration was higher than tMPA concentration due to an unknown reason. For this reason, this patient was excluded for further analysis of the bound fraction one the first occasion.

Figure 4.

Mean ($\pm$ SEM) free mycophenolic acid (fMPA) concentration (■) and inosine monophosphate dehydrogenase (IMPDH) activity (▲) at 3 different occasions; (A) early posttransplant, (B) at hospital discharge and (C) at stable treatment. A total of 25 PK-profiles of fMPA early posttransplant, 25 at hospital discharge and 17 at stable treatment were used. Besides, average IMPDH PD-time profiles were based on 19 profiles early posttransplant, 19 at hospital discharge and 13 at stable treatment. The following measurements of MPA concentration and IMPDH activity were excluded from this analysis since there were limited number of blood samples at this time point to take an average; MPA at 0.5h (n=12) IMPDH activity at 20 min (n=2) and 3h (n=2) early posttransplant and at 20 min (n=2) at hospital discharge.

Figure 5.

Scatter plot of the inosine monophosphate dehydrogenase (IMPDH) activity versus the concentration of free mycophenolic acid (fMPA). The solid line represents the best fitting curve in the $E_{\max}$ -model with 274 pairs where both IMPDH-activity and fMPA concentration were available.

Table I

Patient demographics (n=28).

	Mean (Median)	Range
Age at transplantation, years	12.5 (14.5)	2.1–20.2
Gender (female/male)	11/17	
Race (African-American/Caucasian)	4/24	
Etnicity (hispanic/non-hispanic)	2/26	
Donor type (deceased/living)	11/17	
Weight, kg	44.8 (41.3)	10.3–106.4
Height, cm	140.5 (147.8)	81.2–175.8
Body Surface Area (BSA), m ²	1.30 (1.34)	0.49–2.21
Days after transplant of PK/PD profile		
Early posttransplant (n=27)	2.44 (2)	1–5
Prior to hospital discharge (n=25)	7.04 (6)	3–17
Stable period (n=17)	185 (143)	81–611
Creatinine clearance, mL/min per 1.73 m²		
Early posttransplant (n=27)	102.7 (87.6)	14.4–269.5
Prior to hospital discharge (n=25)	116.0 (110.8)	20.5–228.3
Stable period (n=17)	100.3 (93.7)	62.5–223.3
Albumin concentration, g/L		
Early posttransplant (n=19)	31 (29)	22–52
Prior to hospital discharge (n=25)	35 (35)	21–47
Stable period (n=18)	43 (43)	31–56

- 1) Body Surface Area (BSA) is calculated using the Mosteller formula.(21)
- 2) The creatinine clearance was determined in accordance with the equation of Schwartz.(22) A few patients had a creatinine clearance of < 30 mL/min per 1.73 m²: 4 patients early posttransplant and 1 patient at hospital discharge.
- 3) One patient used cyclosporine at the first two occasions instead of MPA, and was therefore excluded for these occasions.
- 4) Another patient with an age of 20.2 years at the time of transplantation was included, because of an impaired physical and mental development. All other patients were 18 years or younger at the moment of transplantation.
- 5) After the first occasion two patients left the study and after the second occasion nine patients ended their participation for different reasons (e.g. rejection or personal reasons)
- 6) A substantial number of patients early posttransplant had albumin levels < 32 g/L (13 out of 18 patients or 72%) Several patients continued to have albumin levels < 32 g/L: 6 patients out of 25 (24%) at hospital discharge and one patient at stable treatment.

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Table II

Descriptive pharmacokinetic parameters of free mycophenolic acid (fMPA)

	Early posttransplant N = 25		At hospital discharge N = 25		At stable treatment N = 17	
	Mean	Range	Mean	Range	Mean	Range
MMF Dose (mg)	513	200–1000	543	200–1000	588	100–1500
MMF Dose/BSA (mg/m ²)	420	167–602	436	230–602	422	169–689
fMPA C _{max} (µg/L)	618	52–1695	881	77–2215	1041	269–2784
fMPA T _{max} (h)	2.2	0.0–9.1	1.8	0.4–8.8	1.6	0.3–6.0
fMPA AUC _{0–12h} (h·µg/L)	2400	167–5764	2550	170–5889	3460	1035–8321
fMPA CL/F (L/h)	383	73–2989	282	72–1473	214	60–966
fMPA AUC _{0–12h} (h·mg/L)*	21.0	4.0–48.1	25.2	10.2–44.5	41.6	11.8–91.8

C_{max}, highest concentration observed in an individual PK-profile, T_{max}, timepoint where the highest concentration is observed, AUC_{0–12h}, the Area Under the Curve calculated using the linear trapezoidal method, CL/F, estimation of the body clearance, calculated as dose/AUC_{0–12h}

* Previously published by Fukuda et al. (2011)(12). Data from early posttransplant in two patients on i.v. from were excluded in the current analysis.