BJCP British Journal of Clinical Pharmacology

DOI:10.1111/j.1365-2125.2009.03612.x

Pharmacokinetic and pharmacodynamic analysis of enteric-coated mycophenolate sodium: limited sampling strategies and clinical outcome in renal transplant patients

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Keywords
EC-MPS, IMPDH, limited sampling strategy, renal transplantation, therapeutic drug monitoring

Received
16 October 2009

Accepted
17 November 2009

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Therapeutic drug monitoring of mycophenolate mofetil is a promising tool for reducing acute rejection episodes after renal transplantation.
- Limited sampling algorithms of mycophenolic acid in mycophenolate mofetil-treated renal transplant patients have been established.
- Recently published study results indicate that the intensity of early drug exposure might determine the risk of acute rejection episodes.

WHAT THIS STUDY ADDS

- This study provides pharmacokinetic and pharmacodynamic data of mycophenolic acid in enteric-coated mycophenolate sodium-treated renal transplant patients.
- Limited sampling algorithms are evaluated and a practical sampling strategy with five sampling time points within the first 4 h after oral drug intake is provided in renal transplant patients with combined immunosuppression consisting of enteric-coated mycophenolate sodium and ciclosporin A.
- The association between pharmacokinetic and pharmacodynamic parameters and the risk of adverse events are evaluated.
- Both pharmacokinetic and pharmacodynamic parameters contribute to optimized individual immunosuppression.

AIMS

Pharmacokinetic (PK) and pharmacodynamic (PD) monitoring strategies and clinical outcome were evaluated in enteric-coated mycophenolate sodium (EC-MPS)-treated renal allograft recipients.

METHODS

PK [mycophenolic acid (MPA)] and PD [inosine monophosphate dehydrogenase (IMPDH) activity] data were analysed in 66 EC-MPS and ciclosporin A (CsA)-treated renal allograft recipients. Adverse events were considered in a follow-up period of 12 weeks.

RESULTS

Analyses confirmed a limited sampling strategy (LSS) consisting of PK and PD data at predose, 1, 2, 3 and 4 h after oral intake as an appropriate sampling method (MPA $r^2 = 0.812$; IMPDH $r^2 = 0.833$). MPA AUC_{0-12} of patients with early biopsy-proven acute rejection was significantly lower compared with patients without a rejection (median MPA AUC_{0-12} 28 μg*h ml^{-1} (7–45) *vs.* 40 μg*h ml^{-1} (16–130), $P < 0.01$), MPA AUC_{0-12} of patients with recurrent infections was significantly higher compared with patients without infections (median MPA AUC_{0-12} 65 μg*h ml^{-1} (range 37–130) *vs.* 37 μg*h ml^{-1} (range 7–120), $P < 0.005$). Low 12-h IMPDH enzyme activity curve (AEC_{0-12}) was associated with an increased frequency of gastrointestinal side-effects (median IMPDH AEC_{0-12} 43 nmol*h mg^{-1} protein h^{-1} [range 12–67) *vs.* 75 nmol*h mg^{-1} protein h^{-1} (range 15–371), $P < 0.01$].

CONCLUSIONS

Despite highly variable absorption data, an appropriate LSS might be estimated by MPA AUC_{0-4} and IMPDH AEC_{0-4} in renal transplant patients treated with EC-MPS and CsA. Regarding adverse events, the suggested MPA-target AUC_{0-12} from 30 to 60 μg*h ml^{-1} seems to be appropriate in renal allograft recipients.

Introduction

Mycophenolic acid (MPA) is a potent immunosuppressant that in combination with calcineurin inhibitors and steroids decreases the frequency of acute rejection episodes and chronic allograft failure in renal transplant patients [1–4]. However, data on pharmacokinetics (PK) and pharmacodynamics (PD) of MPA are still incomplete, especially for the enteric-coated mycophenolate sodium (EC-MPS) formulation. MPA is generally administered in a fixed dose, with a standard dose of 1000 mg mycophenolate mofetil (MMF) or the equal dose of 720 mg EC-MPS twice daily. Reduction of the standard dose results in fewer side-effects, although increases the risk of acute rejection. It has been shown that the area under the concentration–time curves (AUCs) vary widely in patients following the same MPA dosage [5]. Therapeutic drug monitoring (TDM) might be useful for optimizing individual immunosuppressive therapy and improving allograft function [6–10]. In the French APOMYGRE study, titration of MMF dose according to plasma MPA concentrations resulted in an increased MPA exposure and a reduced incidence of acute rejection episodes without an increased risk of infections or haematological disorders [11]. In addition, the fixed-dose concentration-controlled (FDCC) trial and earlier studies showed that in the early post-transplant period most patients did not reach the intended target AUC_{0-12} of 30–60 μg*h ml^{-1} [12, 13]. A panel consensus report monitoring MPA stated that evaluation of MPA AUC_{0-12} might be valuable for establishing adequate MPA concentrations early after transplant surgery and providing a basis for flexibility for the practitioner to reduce MMF dose to avoid adverse reactions [14]. However, PK and PD data on EC-MPS are sparse and no formal target AUC has been defined, although most practitioners consider both formulations as equivalent.

In addition to the confounding discussion about TDM in mycophenolic therapy, there is another debate about the best PK monitoring strategy [15–17]. Few data regarding PK and PD analysis of plasma MPA concentration of EC-MPS are available. Consistent with the enteric-coating of the formulation, EC-MPS provides delayed release of MPA in the small intestine instead of the stomach; maximum plasma concentrations occur later compared with MMF [18]. EC-MPS is anticipated to have high interindividual variability in absorption because of a varying release of EC-MPS from the stomach to the small intestine. This observed variation in PK profiles makes TDM for EC-MPS even more challenging. Only one study has evaluated whether a limited sampling strategy (LSS) is appropriate in EC-MPS-treated renal transplant patients, and did not find a suitable algorithm [19].

MPA is a selective, reversible, noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme of lymphocyte proliferation. IMPDH activity is proposed as a potential PD parameter for optimizing MPA therapy [20]. In this study, PK and PD data of this new formulation EC-MPS are presented in renal allograft recipients. This is the first study aiming to verify the current MPA-target AUC from 30 to 60 μg*h ml^{-1} in EC-MPS-treated patients. In addition, a LSS is evaluated for introduction in daily clinical practice.

Methods

Patients

Renal transplant patients from the Department of Nephrology, University of Heidelberg, Germany, who met the following inclusion criteria, were eligible to take part in the present PK and PD analysis: adequate renal transplant function [glomerular filtration rate (GFR) $\geq$ 20 ml min^{-1}], treatment with at least 720 mg EC-MPS daily (from 360 to 1440 mg twice daily), ciclosporin A (CsA) therapy, aged 18–70 years, 8–56 days after transplantation and written informed consent. All patients were on triple immunosuppressive treatment consisting of EC-MPS (Myfortic; Novartis Pharma GmbH, Nuremberg, Germany), CsA (Sandimmun Optoral; Novartis Pharma GmbH, Nuremberg, Germany) and methylprednisolone and all patients had an induction therapy with interleukin-2 antibody (Basiliximab, Simulect; Novartis Pharma GmbH) on days 0 and 4 after transplantation. Exclusion criteria were suspected noncompliance, a history of surgery of the stomach or small intestine, co-medication with an immunosuppressive agent other than CsA, EC-MPS and methylprednisolone and co-medication with cholestyramine, magnesium- or aluminium-containing antacids and rifampicin. The study was approved by the local ethics committee and conducted in accordance with the Helsinki Declaration.

Study design

Blood samples were collected for routine analysis and to determine trough (C_0) plasma CsA and MPA concentrations. Subsequently, each patient was given the morning dose of CsA and EC-MPS and underwent evaluation for PK and PD profiles. Blood samples were taken at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 h after the morning dose of EC-MPS. The blood samples were immediately placed at 4°C after drawing and transferred directly for measurement of MPA concentration and IMPDH activity.

The follow-up period was 12 weeks after transplantation. Clinical data and information about the adverse events, e.g. acute rejection episodes, recurrent infections and gastrointestinal side-effects, were obtained during monthly visits to the outpatient clinic. GFR was calculated by the modification of diet in renal disease (MDRD) formula [21]. Renal allograft biopsies were performed depending on clinical decision. Biopsies were classified in accordance with the BANFF 2005 classification [22]. Early antigen of cytomegalovirus (CMV pp65) was assessed routinely. CMV prophylaxis with valganciclovir was given to

patients with CMV IgG+ donors and all patients had a *Pneumocystis jirovecii* prophylaxis with cotrimoxazole.

Pharmacokinetic assessment

All CsA blood levels were measured using the enzyme-multiplied immunoassay technique (EMIT; Dimension XL, Dade Behring, Marburg, Germany). The within-run and between-run precision of this assay was 8% and 10%, respectively. The limit of detection was <25 μg l^{-1}.

MPA concentrations were measured using the EMIT. The within- and between-run precision of this assay was <9% and <11%, respectively. The limit of detection was <0.5 μg ml^{-1}.

Noncompartmental PK parameters were derived from each individual plasma MPA concentration–time profile using WinNonlin Professional (v.5.2) software (Pharsight Corporation, Montreal, Canada). The AUC for the 12-h MPA exposure (AUC_{0-12}) was calculated by the linear trapezoidal rule and 12-h MPA exposure was estimated by abbreviated sampling strategies and calculated by the developed equations (AUC_{0-3}, AUC_{0-4}). C_{max} and C_{min} were defined as the maximum and minimum daytime MPA concentration after dosing with MPA within the dosing interval. T_{max} was defined as the time to reach the maximum daytime MPA concentration.

Pharmacodynamic assessment

PD analysis was performed by assessing IMPDH activity, as published previously [20, 23, 24]. Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll–Paque gradient centrifugation, and lysed PBMC were incubated with inosine 5′-monophosphate. The xanthosine 5′-monophosphate produced was determined by reversed-phase high-performance liquid chromatography using UV detection (Jasco Corporation, Tokyo, Japan). This method showed intra-assay variation coefficients of <15% across a concentration range of 10–150 nmol mg^{-1} protein h^{-1}, and a lower quantification limit of 10 nmol mg^{-1} protein h^{-1}. The within- and between-run precision of this assay was 11% and 18%, respectively.

The PD parameters derived from each enzyme activity–time profile were calculated using WinNonlin Professional (v.5.2) software. The area under the 12-h IMPDH enzyme activity curve (AEC_{0-12}) was calculated by the linear trapezoidal rule and 12-h IMPDH enzyme activity was estimated by abbreviated sampling strategies and calculated by the developed equations (AEC_{0-3}, AEC_{0-4}). A_{max} and A_{min} were defined as the maximum and minimum daytime enzyme activity after dosing with MPA within the dosing interval. Maximum inhibition of IMPDH activity (I_{max}) was calculated with the following formula $(1 - A_{min}/A_{max}) \times 100$. T_{min} was defined as the time to reach the minimum daytime enzyme activity.

Statistical analysis

All statistical analyses were performed using SPSS for Windows, Version 11.5 (SPSS Inc., Chicago, IL, USA). Data for continuous variables are expressed as median and ranges. Categorical variables are expressed as absolute numbers and percentages. PK and PD parameters were not normally distributed in the study population and the following nonparametric statistical tests were performed: the Mann–Whitney *U*-test (for comparison of sample medians) and the nonparametric Spearman rank correlation coefficient (for correlation between variables). Multivariate logistic regression analyses were calculated to determine the influence of several variables. Correlations and differences between groups were considered significant with an α error of 0.05 and a β error of 0.2 (power of 80%) in a two-sided statistical test.

LSS were established by the following procedure. MPA concentration and IMPDH activity at each sampling time were correlated by linear regression analysis with the total measured MPA AUC_{0-12} and IMPDH AEC_{0-12} in all patients. Multiple stepwise linear regression analysis was performed to give improved correlations with total measured MPA AUC_{0-12} and IMPDH AEC_{0-12}. Equations were in the form of MPA $AUC_{0-12} = K + K_0 \times C_0 + K_1 \times C_1 \ldots K_n \times C_n$, where K, K_0, K_n are fitted constants associated with each timed MPA concentration, C_0, $C_1 \ldots$ C_n are MPA concentrations at 0, $1 \ldots n^{th}$ h post dose. Prediction bias of these LSS-derived estimates was assessed by calculating the percentage of prediction error (PE%) from the formula PE% = 100% × (LSS AUC − total measured AUC)/total measured AUC. Corresponding analyses were performed on IMPDH data.

Results

Patient demographics

A total of 66 full 12-h MPA profiles (AUC_{0-12}) in renal transplant patients [32 male, 34 female; median age 41 years (19–68); median time after transplantation 14 days (10–56)] on a broad range of EC-MPS therapy and stable renal allograft function [median estimated GFR 46 ml min^{-1} (30–76)] were obtained. Daily EC-MPS dosage was 1440 mg in 57 patients, 2880 mg in eight patients and 720 mg in one patient. Median methylprednisolone dosage was 20 mg (12–20) once daily. Median CsA dosage was 150 mg (100–300) twice daily with a median CsA C_0 level of 178 μg l^{-1} (156–281). Patient characteristics are shown in Table 1.

Pharmacokinetic and pharmacodynamic analysis

An overview of the PK and PD data is given in Table 2 demonstrating a broad range of MPA exposure in the patient population. MPA AUC_{0-12} correlated poorly with MPA C_0 concentration ($r^2 = 0.239$; $P < 0.01$) and MPA C_{12} concentration ($r^2 = 0.361$; $P < 0.01$). Individual MPA C_0 and MPA C_{12} differed significantly ($P < 0.05$).

IMPDH AEC_{0-12} correlated with IMPDH A_0 ($r^2 = 0.412$; $P < 0.001$) and with IMPDH A_{12} ($r^2 = 0.450$, $P < 0.05$). Individual IMPDH A_0 and IMPDH A_{12} were not significantly different ($P = 0.07$).

Table 1

Demographic data of 66 renal allograft recipients

Patient characteristic	
Male gender (%)*	48.5 (32/66)
Age (years)†	41 (19–68)
Body mass index (kg m^{-2})†	27 (17–38)
Time after transplantation (days)†	14 (10–56)
Living donation (%)*	42.4 (28/66)
First transplantation (%)*	95.5 (63/66)
Second transplantation (%)*	4.5 (3/66)
Cold ischaemia time (h)†	9.6 (2–24)
HLA mismatches†	3 (0–6)
Panel reactive antibodies > 25%*	0 (0/66)
eGFR (ml min^{-1})†	46 (30–76)
Methylprednisolone dosage (mg day^{-1})†	20 (12–20)
Ciclosporin A dosage (mg day^{-1})†	150 (100–300)
Ciclosporin C_0 level (µg l^{-1})†	178 (156–281)
Mycophenolate sodium dosage (mg day^{-1})†	1440 (720–2880)
Mycophenolate sodium dosage per body weight (mg kg^{-1})†	20 (10–40)

*Number and percentages. †Median and ranges are shown. C_0, predose concentration; eGFR, estimated glomerular filtration rate; HLA, human leucocyte antigen.

MPA $AUC_{0–12}$ and IMPDH $AEC_{0–12}$ did not correlate significantly (Figure 1). The overall PD response is demonstrated by plotting IMPDH activity against MPA plasma concentration (Figure 2). The median MPA concentrations and median IMPDH activities observed over the 12-h sampling period are shown in Figure 3.

Assessment of an abbreviated area under the concentration–time curve

Each MPA concentration at a single time point correlated poorly with $AUC_{0–12}$. MPA $AUC_{0–12}$ correlated best with MPA C_1 ($r^2 = 0.321$, $P < 0.001$), MPA C_3 ($r^2 = 0.320$, $P < 0.001$), MPA C_8 ($r^2 = 0.335$, $P < 0.001$) and MPA C_{12} ($r^2 = 0.424$, $P < 0.001$). Using stepwise linear regression analysis, the best sampling algorithm with a maximum of three sampling time points included plasma MPA concentration at 1, 3 and 8 h after oral EC-MPS intake (C_1, C_3, C_8; $r^2 = 0.836$, $P < 0.001$). Sampling points and corresponding equations are given in Table 3. Two highly predictive formulae with sampling points within the first 4 h were selected: MPA $AUC_{0–3}$ including C_0, C_1 and C_3 and MPA $AUC_{0–4}$ including C_0, C_1, C_2, C_3 and C_4. There was a significant correlation between estimated abbreviated MPA $AUC_{0–3}$ as well as MPA $AUC_{0–4}$ and $AUC_{0–12}$ ($r^2 = 0.702$ and $r^2 = 0.812$). Inclusion of $C_{0.5}$ and $C_{1.5}$ did not rigorously improve accuracy of the LSS. Median PE% were −1% (−73–54) with MPA $AUC_{0–3}$ and −8% (−45–47) with MPA $AUC_{0–4}$.

Assessment of an abbreviated area under the enzyme activity curve

Correlation between IMPDH activity at a single time point and $AEC_{0–12}$ ranged between $r^2 = 0.239$ and $r^2 = 0.689$. The best correlations between $AEC_{0–12}$ and IMPDH activity at single time points were found at 6 h (C_6, $r^2 = 0.684$, $P < 0.001$) and 8 h (C_8, $r^2 = 0.689$, $P < 0.001$). Corresponding to the abbreviated PK sampling strategies, an abbreviated sampling model including A_0 to A_4 to predict $AEC_{0–12}$ was evaluated (Table 4). As shown in Table 3, there was an acceptable correlation between estimated abbreviated IMPDH AEC and $AEC_{0–12}$ using IMPDH $AEC_{0–3}$ including A_0, A_1 and A_3 and IMPDH $AEC_{0–4}$ including A_0, A_1, A_2, A_3 and A_4 ($r^2 = 0.819$ and $r^2 = 0.833$). Median PE% were 8% (−46–139) with IMPDH $AEC_{0–3}$ and 1% (−43–83) with IMPDH $AEC_{0–4}$.

Clinical data – univariate analysis

Patient and graft survival was 100% in the follow-up period of 12 weeks after transplantation. Median MDRD GFR was 52 ml/min (28–96) and neither MPA $AUC_{0–12}$ nor MPA $AUC_{0–3}$ or MPA $AUC_{0–4}$ determined renal allograft function assessed by MDRD GFR at week 12. In the follow-up period, 13 biopsy proven acute rejection episodes were documented (nine borderline rejection, three Banff IA and one Banff IB). MPA $AUC_{0–12}$ and MPA $AUC_{0–4}$ of patients with early acute rejection episodes were significantly lower compared with patients without acute rejection episodes (MPA $AUC_{0–12}$: median 28 µg*h ml^{-1} (7–45) *vs.* 40 µg*h ml^{-1} (16–130), $P < 0.01$; MPA $AUC_{0–4}$: median 32 µg*h ml^{-1} (17–45) *vs.* 40 µg*h ml^{-1} (20–121), $P < 0.05$; MPA $AUC_{0–3}$: median 35 µg*h ml^{-1} (22–56) *vs.* 38 µg*h ml^{-1} (23–125), n.s.). Infections were noted in nine of 66 (13.6%) patients and gastrointestinal side-effects in six of 66 (9.1%). MPA $AUC_{0–12}$, MPA $AUC_{0–4}$ and MPA $AUC_{0–3}$ of patients with infections were significantly higher compared with patients without infections (MPA $AUC_{0–12}$: median 65 µg*h ml^{-1} (37–130) *vs.* 37 µg*h ml^{-1} (7–120), $P < 0.005$; MPA $AUC_{0–4}$: median 62 µg*h ml^{-1} (31–121) *vs.* 36 µg*h ml^{-1} (17–115), $P < 0.05$; MPA $AUC_{0–3}$: median 53 µg*h ml^{-1} (27–125) *vs.* 35 µg*h ml^{-1} (22–115), $P < 0.05$). Neither MPA $AUC_{0–12}$ nor MPA $AUC_{0–4}$ or MPA $AUC_{0–3}$ affected gastrointestinal side-effects (MPA $AUC_{0–12}$: median 51 µg*h ml^{-1} (29–76) *vs.* 38 µg*h ml^{-1} (7–130), n.s.; MPA $AUC_{0–4}$: median 39 µg*h ml^{-1} (30–68) *vs.* 36 µg*h ml^{-1} (17–121), n.s.; MPA $AUC_{0–3}$: median 36 µg*h ml^{-1} (22–125) *vs.* 34 µg*h ml^{-1} (25–56), n.s.). The evaluated LSS MPA $AUC_{0–4}$ confirmed significant MPA $AUC_{0–12}$ differences between patients with and without acute rejections or infections. Association between MPA $AUC_{0–4}$ and clinical outcome is given in Figure 4. Nevertheless, it was impossible to detect patients at risk of acute rejection, infection or gastrointestinal side-effects with the LSS MPA $AUC_{0–3}$.

Neither IMPDH $AEC_{0–12}$ nor IMPDH $AEC_{0–4}$ or IMPDH $AEC_{0–3}$ determined renal allograft function assessed by MDRD GFR at week 12. There was no significant difference between IMPDH $AEC_{0–12}$, IMPDH $AEC_{0–4}$ and IMPDH $AEC_{0–3}$ of patients with and without acute rejections [IMPDH $AEC_{0–12}$: median 64 nmol*h mg^{-1} protein h^{-1} (28–110) *vs.* 74 nmol*h mg^{-1} protein h^{-1} (12–371), NS; IMPDH $AEC_{0–4}$: median 64 nmol*h mg^{-1} protein h^{-1} (40–97) *vs.* 73 nmol*h mg^{-1} protein h^{-1} (30–315), NS; IMPDH $AEC_{0–3}$:

Table 2

Pharmacokinetic and pharmacodynamic parameters of enteric-coated mycophenolate sodium (EC-MPS) in 66 renal transplant patients (median and range)

	All patients $n = 66$	EC-MPS 1440 mg day^{-1} $n = 57$	EC-MPS 2880 mg day^{-1} $n = 8$	Significance P
MPA AUC_{0-12} (μg*h ml^{-1})	38 (7–130)	38 (7–130)	40 (20–65)	NS
MPA C_0 (μg ml^{-1})	1.8 (0.5–23)	1.8 (0.5–23)	1.8 (0.6–16)	NS
MPA C_{12} (μg ml^{-1})	1.5 (0.3–19.4)	1.5 (0.3–19.4)	1.5 (0.5–9.9)	NS
MPA C_{min} (μg ml^{-1})	1.0 (0.5–3.8)	0.9 (0.5–3.8)	1.2 (0.5–1.5)	NS
MPA C_{max} (μg ml^{-1})	11.6 (2.2–45.7)	12 (2.2–45.7)	11.8 (4.7–30.8)	NS
MPA T_{max} (h)	3.0 (0.5–10)	3.0 (0.5–10)	3.0 (2.0–6.0)	NS
Pharmacodynamic data				
IMPDH AEC_{0-12} (nmol*h mg^{-1} protein h^{-1})	74 (12–371)	79 (12–371)	64 (15–110)	<0.05
IMPDH A_0 (nmol mg^{-1} protein h^{-1})	8.4 (1.0–28.5)	8.5 (1.0–28.5)	5.6 (1.1–13.3)	NS
IMPDH A_{12} (nmol mg^{-1} protein h^{-1})	8.0 (0.5–33.5)	8.6 (0.5–33.5)	4.9 (0.7–15.4)	NS
IMPDH A_{max} (nmol mg^{-1} protein h^{-1})	15 (3–48)	15 (5–48)	11 (3–19)	<0.05
IMPDH A_{min} (nmol mg^{-1} protein h^{-1})	1.7 (0–20.7)	1.7 (0–20.7)	1.0 (0–2.3)	<0.05
IMPDH T_{min} (h)	3 (0.5–10)	3 (0.5–10)	3 (2–5)	NS
IMPDH I_{max} (%)	87 (57–100)	87 (57–100)	93 (71–100)	NS

Fifty-seven patients were on 1440 mg day^{-1} and eight patients on 2880 mg day^{-1}. One patient was on EC-MPS 720 mg day^{-1} (data not shown). Significance P is given for difference between the patient groups with EC-MPS 1440 mg day^{-1} and with EC-MPS 2880 mg day^{-1} (Mann–Whitney U-test). A, enzyme activity; AEC, area under the enzyme activity curve; AUC, area under the concentration–time curve; C, concentration; EC-MPS, enteric-coated mycophenolate sodium; I, inhibition; IMPDH, inosine monophosphate dehydrogenase; MPA, mycophenolic acid; T, time.

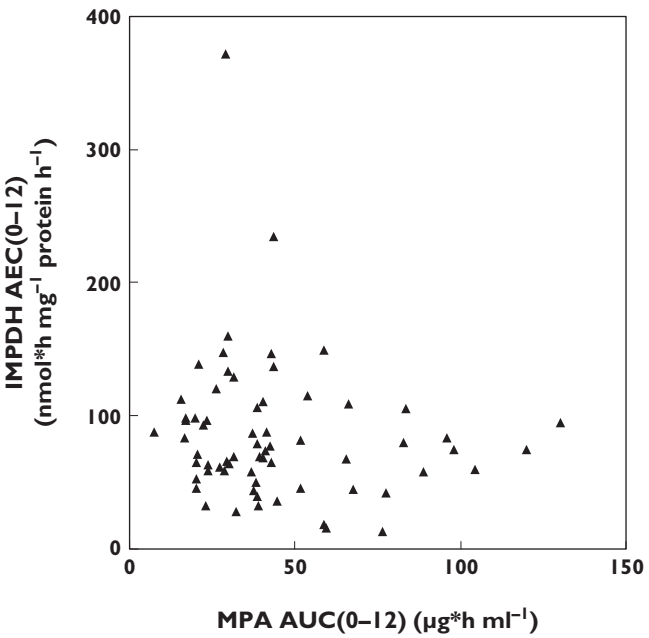

Figure 1

Association of MPA AUC_{0-12} and IMPDH AEC_{0-12} is demonstrated. AUC, area under the concentration–time curve; AEC, area under the enzyme activity curve; IMPDH, inosine monophosphate dehydrogenase; MPA, mycophenolic acid

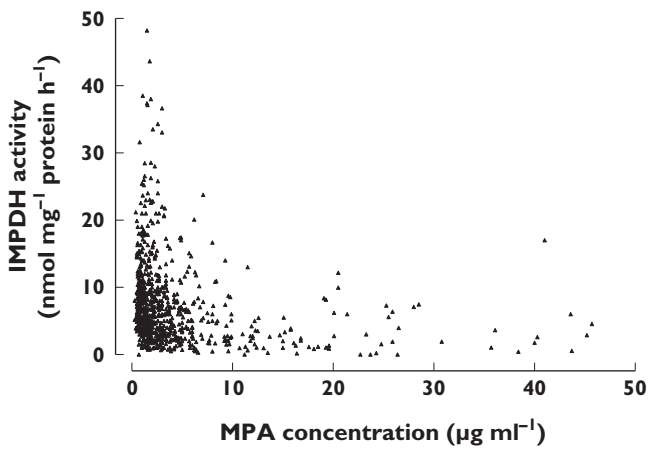

Figure 2

Assessment of pharmacodynamic response by plotting inosine monophosphate dehydrogenase (IMPDH) activity against plasma mycophenolic acid (MPA) concentration

median 61 nmol*h mg^{-1} protein h^{-1} (44–100) *vs.* 78 nmol*h mg^{-1} protein h^{-1} (36–321), NS]. Two patients with an acute rejection episode despite a MPA AUC_{0-12} > 38 μg*h ml^{-1} showed an IMPDH AEC_{0-12} > 100 nmol*h mg^{-1} protein h^{-1}. IMPDH AEC_{0-12}, IMPDH AEC_{0-4} and IMPDH AEC_{0-3} of patients with and without infections did not differ significantly [IMPDH AEC_{0-12}: median 68 nmol*h mg^{-1} protein h^{-1} (12–94) *vs.* 74 nmol*h mg^{-1} protein h^{-1} (15–371), NS; IMPDH AEC_{0-4}: median 71 nmol*h mg^{-1} protein h^{-1} (35–124) *vs.* 72 nmol*h mg^{-1} protein h^{-1} (30–315), NS; IMPDH AEC_{0-3}: median 72 nmol*h mg^{-1} protein h^{-1} (41–117) *vs.* 76 nmol*h mg^{-1} protein h^{-1} (36–321), NS]. IMPDH AEC_{0-12} and IMPDH AEC_{0-4} of patients with gastrointestinal side-effects were significantly lower compared with patients without gastrointestinal symptoms [IMPDH AEC_{0-12}: median 43 nmol*h mg^{-1} protein h^{-1} (12–67) *vs.* 75 nmol*h mg^{-1} protein h^{-1} (15–371), $P < 0.01$; IMPDH AEC_{0-4}: median 55 nmol*h mg^{-1} protein h^{-1} (35–73) *vs.* 75 nmol*h mg^{-1} protein h^{-1} (30–315), $P < 0.05$; IMPDH

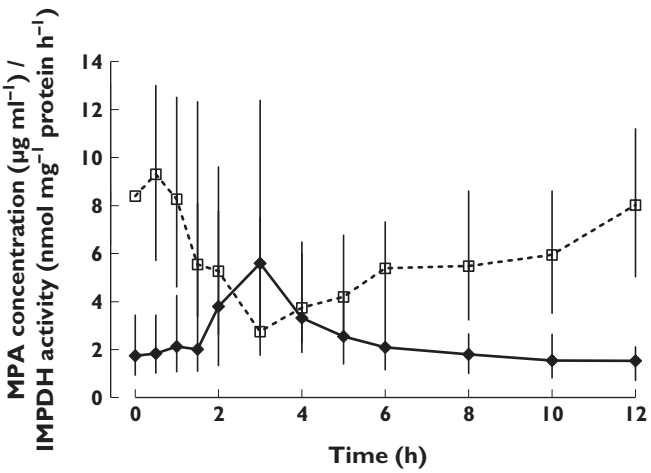

Figure 3

Median time profiles of plasma mycophenolic acid (MPA) concentration and inosine monophosphate dehydrogenase (IMPDH) activity in peripheral mononuclear cells of 66 renal transplant patients on enteric-coated mycophenolate sodium (EC-MPS) therapy are presented (median, 25 and 75 percentile; solid line, MPA concentration; dashed line, IMPDH activity). MPA concentration [µg ml^{-1}] (—◆—); IMPDH activity [nmol/mg protein/h] (-⊟-)

AEC_{0-3}: median 59 nmol*h mg^{-1} protein h^{-1} (41–78) *vs.* 76 nmol*h mg^{-1} protein h^{-1} (36–321), NS].

The evaluated LSS IMPDH AEC_{0-4} confirmed an increased risk of low IMPDH AEC_{0-12} concerning gastrointestinal side-effects. Association of IMPDH AEC_{0-4} and clinical outcome is given in Figure 5. Nevertheless, it was impossible to detect patients at risk of acute rejection, infection or gastrointestinal side-effects with the LSS IMPDH AEC_{0-3}.

Clinical data – multivariate analysis

Multiple regression analyses were performed including the following variables: MPA dosage (mg kg^{-1}), MPA C_{max}, MPA AUC_{0-12}, IMPDH A_{min}, IMPDH AEC_{0-12} and IMPDH I_{max}. MPA AUC_{0-12}, IMPDH I_{max} and IMPDH A_{min} determined the risk of an acute rejection episode. MPA AUC_{0-12} predicted the risk of recurrent infections and IMPDH AEC_{0-12} independently revealed the risk of gastrointestinal side-effects. Data of multivariate logistic regression analyses are given in Table 5.

Discussion

EC-MPS as an enteric-coated formulation of MPA has approved equal clinical efficacy and safety compared with MMF in *de novo* renal transplant patients [25]. However, some PK and PD data differ significantly in both drug formulations [18, 26]. This is the first study assessing PK and PD parameters of EC-MPS in *de novo* renal transplant patients with stable allograft function. Results are similar to published studies [18, 25]. In an individual patient's IMPDH activity, the pharmacological targets of EC-MPS are inversely related to plasma MPA concentration. Evaluation of an appropriate limited PK and PD sampling strategy suggested formulae consisting of five blood samples from 0 to 4 h after oral EC-MPS intake as appropriate tools for estimating MPA 12-h exposure in patients treated with CsA. Clinical associations of MPA exposure were revealed with respect to acute rejection, infection and gastrointestinal side-effects.

Steady-state PK data of MPA (C_{min}, C_{max}, T_{max} and AUC) after oral intake of EC-MPS were consistent with previous results [18, 25]. Consistent with the enteric-coated formulation and delayed release, EC-MPS demonstrated a postponed MPA peak level at 3 h (0.5–10) after oral intake. This study confirmed that predose MPA levels are highly variable in EC-MPS-treated renal transplant patients and differ significantly from MPA C_{12}. In addition, MPA AUC_{0-12} correlated poorly with MPA C_0 and MPA C_{12}. It is of note that MPA exposure varied widely despite corresponding daily MPA dosages and even body-weight-normalized MPA dosages did not determine MPA exposure. These findings support the request for individual drug dosing.

TDM is one of the main aspects of optimizing and personalizing immunosuppressive therapy. As mentioned above, the use of MPA C_0 for monitoring of MPA in EC-MPS treatment is not appropriate because of an absent correlation between MPA C_0 and MPA AUC_{0-12}. Individual MPA exposure should be assessed by MPA AUC_{0-12} profiles. However, these strategies are time consuming, costly and inconvenient for the patients, and therefore impractical in daily clinical work. In the APOMYGRE study, the new dosage was calculated by the Bayesian formula and the results of the LSS correlated significantly with AUC_{0-12} [11]. FDCC investigators used a three-point abbreviated sampling strategy including C_0, $C_{0.5}$ and C_2 [12]. However, limited data on TDM in EC-MPS-treated patients are available. Budde *et al.* [18] and Cattaneo *et al.* [27] demonstrated delayed MPA peak concentration compared with MMF. MPA 12-h exposure was comparable in 18 stable renal transplant patients on MMF therapy converted to EC-MPS [18]. Until now, only de Winter *et al.* [19] have evaluated most recently a LSS for TDM of EC-MPS in renal transplant recipients. However, the authors analysed only the estimation of MPA AUC_{0-12} with LSS for EC-MPS drawn within 2 or 3 h post dose in the maintenance period after transplantation. TDM using an easy abbreviated sampling strategy is challenging with respect to the observed variation in PK profiles of EC-MPS [26].

In this study, stepwise logistic analysis revealed a five-point sampling strategy including blood samples at predose, 1, 2, 3 and 4 h post-EC-MPS intake as a promising tool for adequate and convenient assessment of drug exposure in renal allograft recipients treated with CsA. Such a LSS might be appropriately performed in outpatient clinics. Additional sampling time points at 0.5 and

Table 3

Multiple stepwise linear regression analysis was made to identify a limited sampling strategy to estimate a 12-h exposure of mycophenolate acid in enteric-coated mycophenolate sodium (EC-MPS)-treated renal transplant patients ($n = 66$)

Sampling time (h)	Equation for estimation of $AUC_{0–12}$	Coefficient of determination (r^2)
Best limited sampling strategies with regard to number of sampling points		
1, 3	$21.2 + 2.13\ C_1 + 1.55\ C_3$	0.671
1, 3, 8	$12.7 + 2.05\ C_1 + 1.51\ C_3 + 3.99\ C_8$	0.836
1, 2, 3, 8	$8.36 + 1.61\ C_1 + 0.75\ C_2 + 1.63\ C_3 + 4.13\ C_8$	0.886
1, 2, 3, 4, 8	$3.31 + 1.56\ C_1 + 0.89\ C_2 + 1.07\ C_3 + 1.95\ C_4 + 4.09\ C_8$	0.950
Best limited sampling strategies within the first 4 h ($r^2 > 0.7$)		
0, 1, 3	$19.7 + 1.22\ C_0 + 1.93\ C_1 + 1.39\ C_3$	0.702
1, 3, 4	$16.5 + 2.16\ C_1 + 1.06\ C_3 + 1.92\ C_4$	0.738
1, 2, 3, 4	$12.4 + 1.70\ C_1 + 0.78\ C_2 + 0.99\ C_3 + 2.15\ C_4$	0.792
0, 1, 2, 3, 4	$11.5 + 0.99\ C_0 + 1.56\ C_1 + 0.74\ C_2 + 0.87\ C_3 + 2.09\ C_4$	0.812
0, 0.5, 1, 1.5, 2, 3, 4	$2.56 + 0.57\ C_0 + 0.87\ C_{0.5} + 0.48\ C_1 + 0.61\ C_{1.5} + 0.55\ C_2 + 0.99\ C_3 + 2.08\ C_4$	0.842
Best limited sampling strategies within the first 6 h ($r^2 > 0.7$)		
1, 3, 6	$11.5 + 1.79\ C_1 + 1.59\ C_3 + 4.76\ C_6$	0.797
1, 2, 3, 6	$5.72 + 1.17\ C_1 + 0.92\ C_2 + 1.52\ C_3 + 5.70\ C_6$	0.867
1, 2, 3, 4, 6	$3.21 + 1.21\ C_1 + 0.97\ C_2 + 1.14\ C_3 + 1.50\ C_4 + 4.99\ C_6$	0.904

Table 4

Limited sampling algorithms to estimate a 12-h inhibition of inosine monophosphate dehydrogenase activity in enteric-coated mycophenolate sodium (EC-MPS)-treated renal transplant patients ($n = 66$). Algorithms were calculated by linear stepwise regression analysis

Sampling time (h)	Equation for estimation of $AEC_{0–12}$	Coefficient of determination (r^2)
1, 3	$30.8 + 3.03\ A_1 + 4.27\ A_3$	0.791
0, 1, 3	$24.2 + 1.81\ A_0 + 2.26\ A_1 + 4.0\ A_3$	0.819
0, 1, 2, 3, 4	$19.9 + 1.53\ A_0 + 1.78\ A_1 + 1.02\ A_2 + 2.81\ A_3 + 1.61\ A_4$	0.833

1.5 h after oral intake did not improve accuracy when a LSS of 4 h was applied. It is of note that in this study CsA was the concomitant immunosuppressive agent in all patients. CsA is known to inhibit the multidrug resistance 2 transporters followed by reduction of enterohepatic MPA recycling [28]. A relevant second MPA peak level is not expected in combined EC-MPS and CsA treatment and therefore the establishment of an abbreviated sampling strategy might be possible [29]. The alternative widely used calcineurin inhibitor tacrolimus presents a recirculation issue of MPA affecting the 12-h MPA profiles [30, 31]. This is likely to influence possible LSS and equations in MPA- and tacrolimus-treated patients. However, in this study no tacrolimus-treated patients were evaluated.

Randomized, concentration-controlled trials in renal transplant patients on MMF, CsA and corticosteroids provide the basis of the currently recommended therapeutic range of MPA $AUC_{0–12}$ of 30 to 60 μg*h ml^{-1} to achieve efficacy and avoid toxicity in the early post-transplantation period [6, 16, 32]. Most patients treated with the ester prodrug MMF did not reach the intended target MPA $AUC_{0–12}$ of 30 to 60 μg*h ml^{-1} in the early post-transplant period and TDM might be especially useful in this early time period [12, 13, 32, 33]. In the present study, median MPA $AUC_{0–12}$ was 38 μg*h ml^{-1}, suggesting that a considerable number of the patients exhibited a MPA exposure below MPA $AUC_{0–12}$ of 30 μg*h ml^{-1}. Even patients with increased EC-MPS dosages did not reach sufficient drug exposure. These results are comparable with published data that reveal a low EC-MPS exposure in the first weeks after renal transplantation with a time-dependent PK of MPA despite standard dosing. It is reported that the mean $AUC_{0–12}$ of MPA in the early post-transplantation period is approximately 30–50% lower for the same dose than in the late post-transplantation period [34]. This is especially important because clinical data confirmed an association between MPA exposure and efficacy. The AUC values <30 μg*h ml^{-1} were associated with an increased risk for development of acute rejection, whereas no further reduction in acute rejection was observed in patients with AUC values > 60 μg*h ml^{-1} in MMF-treated renal allograft recipients [32]. However, clinical experience indicates that escalation of MMF dosages is limited by gastrointestinal intolerance.

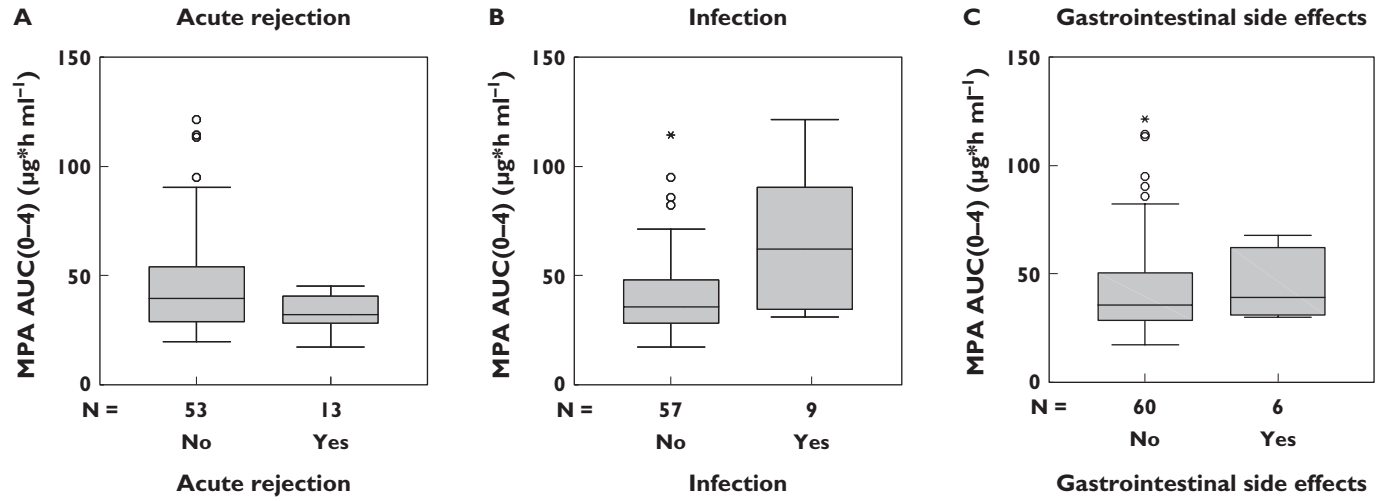

Figure 4

Box and whisker plot shows the median, interquartile range, outliers (°) and extreme values (*) of the evaluated abbreviated sampling strategy MPA AUC_{0-4} in patients (a) with and without acute rejection episode ($P < 0.05$), (b) with and without recurrent infections ($P < 0.05$), and with and without gastrointestinal side-effects (NS). AUC, area under the concentration–time curve; MPA, mycophenolic acid

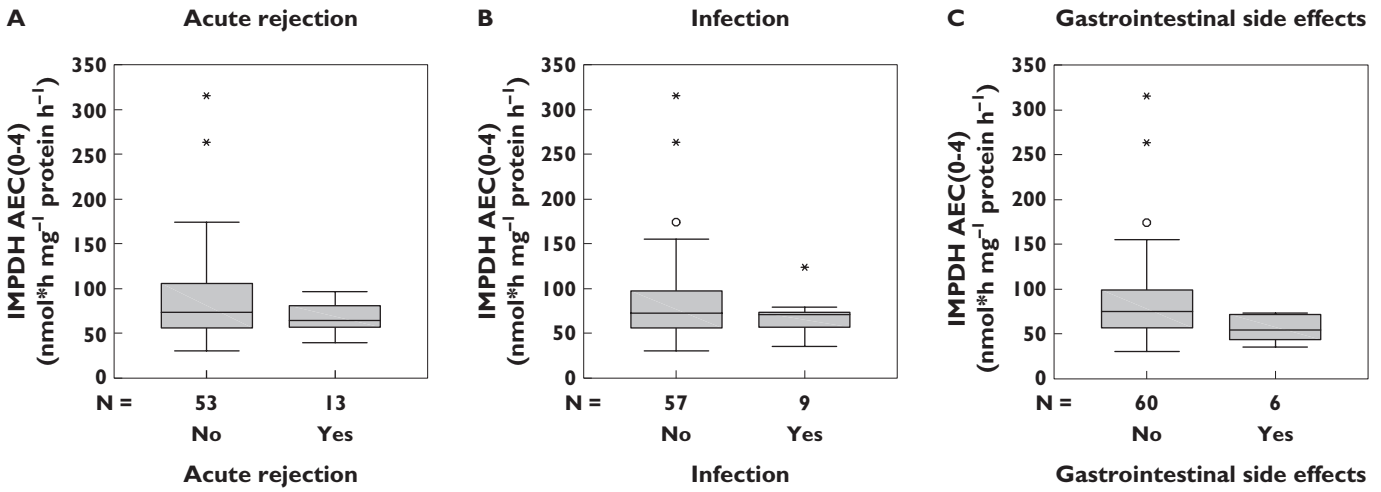

Figure 5

Box and whisker plot shows the median, interquartile range, outliers (°) and extreme values (*) of the evaluated abbreviated sampling strategy IMPDH AEC_{0-4} in patients (a) with and without acute rejection episode (NS), (b) with and without recurrent infections (NS), and (c) with and without gastrointestinal side-effects ($P < 0.05$). AEC, area under the enzyme activity curve; IMPDH, inosine monophosphate dehydrogenase

Several clinical trials have been conducted to investigate whether MPA TDM and concentration-controlled MMF dosing is feasible in clinical practice and whether it is superior to using a fixed MMF dose [33, 35]. Recently, three prospective randomized multicentre studies – the French APOMYGRE study, the FDCC trial and the OPTICEPT trial – have confirmed a clinical benefit of TDM in patients treated with the ester prodrug MMF [9, 10, 36]. In the APOMYGRE trial, the incidence of treatment failure (acute rejection, graft loss, death, discontinuation of MMF) was significantly lower in the concentration-controlled group [11]. In particular, the incidence of acute rejection was significantly lower during the first year (12.3% *vs.* 30.7%) and there was no difference in the incidence of adverse events. In the FDCC trial, immunosuppression consisted of MMF in combination with CsA or tacrolimus [12]. Despite of a similar MPA exposure in patients on fixed-dose and concentration-controlled MPA regimens, in all patients a low MPA exposure at day 3 after transplantation was followed by an increased risk of biopsy-proven acute rejections within the first year after transplantation. In the recently published OPTICEPT trial, MMF dose adjustments were performed based on the MPA trough levels and MPA trough concentrations $\geq$ 1.6 µg ml^{-1} were associated

Table 5

Results of multivariate logistic regression analyses concerning the association between pharmacokinetic and pharmacodynamic data and adverse events are given. Regression coefficient β, significance value *P* and 95% confidence interval are shown

	Coefficient β	*P*-value	Exp [b] (95% CI)
Acute rejection (*R* = 0.50, SE = 0.36, *P* < 0.005)			
MPA $AUC_{0–12}$	–0.49	0.02	0.90 (0.83, 0.98)
MPA C_{max}	0.32	0.13	1.12 (0.98, 1.27)
IMPDH $AEC_{0–12}$	–0.10	0.42	1.02 (0.98, 1.06)
IMPDH A_{min}	–0.43	0.01	0.23 (0.05, 1.0)
IMPDH I_{max}	–0.50	0.005	0.78 (0.65, 0.95)
MPA dosage*	–0.01	0.94	1.03 (0.92, 1.15)
Infections (*R* = 0.44, SE = 0.32, *P* < 0.05)			
MPA $AUC_{0–12}$	0.43	0.05	1.04 (1.00, 1.09)
MPA C_{max}	–0.04	0.87	1.00 (0.88, 1.13)
IMPDH $AEC_{0–12}$	–0.10	0.41	0.99 (0.96, 1.02)
IMPDH A_{min}	–0.05	0.76	0.83 (0.31, 2.23)
IMPDH I_{max}	–0.06	0.76	0.98 (0.84, 1.14)
MPA dosage*	0.12	0.32	1.05 (0.94, 1.18)
Gastrointestinal side effects (*R* = 0.36, SE = 0.28, *P* = 0.05)			
MPA $AUC_{0–12}$	0.11	0.62	0.99 (0.93, 1.06)
MPA C_{max}	–0.07	0.76	1.05 (0.89, 1.24)
IMPDH $AEC_{0–12}$	–0.30	0.02	0.95 (0.90, 1.00)
IMPDH A_{min}	–0.06	0.73	0.87 (0.55, 1.39)
IMPDH I_{max}	–0.07	0.70	0.94 (0.81, 1.10)
MPA dosage*	0.13	0.30	1.05 (0.92, 1.19)

*MPA dosage given in mg kg^{-1}. *A*, activity; AUC, area under the concentration–time curve; *C*, concentration; CI, confidence interval; *I*, inhibition; IMPDH, inosine monophosphate dehydrogenase; MPA, mycophenolic acid; *R*, multiple correlation coefficient; SE, standard error of estimation.

with a longer time to first biopsy-proven acute rejection episode in tacrolimus-treated patients from all three groups [36]. Despite a relatively clear relationship between MPA exposure and clinical efficacy, these prospective randomized multicentre studies have shown that it might be difficult to establish a similarly strong association between the PK parameters of MPA and drug-related adverse events [37]. Our present study has confirmed a relationship between MPA exposure in EC-MPS- and CsA-treated renal transplant patients and acute rejection episodes. In contrast to the mentioned multicentre studies, the present study also suggested a relationship between recurrent infections and MPA exposure.

In this study, all patients diagnosed with biopsy-proven acute rejections displayed a MPA $AUC_{0–12}$ < 45 μg*h ml^{-1} and the median MPA $AUC_{0–12}$ was approximately 30 μg*h ml^{-1} – demonstrating for the first time a exposure response relationship for EC-MPS-treated patients, similar to MMF-treated allograft recipients. In contrast, patients with high MPA exposure were at an increased risk of adverse events. MPA $AUC_{0–12}$ of nearly all patients with recurrent infections was >45 μg ml^{-1} with a median MPA $AUC_{0–12}$ of 65 μg*h ml^{-1}. Only three patients with recurrent infections had a MPA $AUC_{0–12}$ between 39 and 42 μg*h ml^{-1}. PK monitoring of plasma MPA concentrations in renal transplant patients on EC-MPS therapy might be a useful tool for achieving sufficient drug exposure and optimizing individual immunosuppressive treatment. Results of this study support the established MPA $AUC_{0–12}$ target level of 30–60 μg*h ml^{-1} in patients on EC-MPS and CsA immunosuppression.

A PD monitoring strategy by IMPDH activity in lymphocytes as the biological target of MPA was proposed recently in renal transplant patients and in patients with IgA nephritis treated with MMF [23, 38–40]. Assessment of IMPDH activity serves as a surrogate marker of MPD-induced immunosuppression and, in comparison with conventional TDM, might better predict drug efficacy and toxicity. IMPDH activity exhibited high interindividual variability in patients with low intraindividual variability [23]. After oral administration of MMF, close temporal associations between MPA plasma levels and IMPDH inhibition have been reported [18, 40]. This study confirms that individual MPA levels and IMPDH activities run inversely in a daytime observation in EC-MPS treatment. However, the correlation between MPA trough levels and predose IMPDH activity is weak. In most patients, the highest inhibition of IMPDH activity was observed within the first 4 h after oral EC-MPS intake. This is in accordance with earlier studies, which demonstrated the lowest IMPDH activity within the first 3 h in renal transplant patients treated with MMF and CsA [18]. Therefore, a LSS including at least the first 4 h might cover the highly variable absorption period.

There are only limited data on a clinical benefit of IMPDH monitoring in MPA-treated patients. In earlier studies, it has been suggested that assessment of individual IMPDH activity provides clinical benefits. A significant association between IMPDH activity before transplantation and the necessity to reduce MMF dose after transplantation because of MPA-induced complications has been shown. Conversely, dose reduction due to side-effects in patients with low pre-transplant IMPDH activity was followed by an increased risk of acute rejection [23]. This study showed that the incidence of infections was increased in patients with low IMPDH activity despite adequate MPA exposure. In addition, independent of MPA exposure, patients with low IMPDH activity were at an increased risk of gastrointestinal side-effects. These results confirmed an augmented risk of side-effects in patients on EC-MPS treatment with low IMPDH activity assessed by IMPDH $AEC_{0–12}$. Multivariate regression analysis proved that both PK and PD monitoring contribute to an optimized individual immunosuppression. These preliminary data are promising and indicate that IMPDH activity might be a useful surrogate marker of MPA-induced immunosuppression.

There are some study limitations to be considered. First, EC-MPS is combined with CsA, which is known to interfere with the enterohepatic recirculation of MPA. In this study,

LSS of EC-MPS are only evaluated in combination with the calcineurin inhibitor CsA in renal allograft recipients. The study population size was relatively small because of the single-centre design. Prognostic impact of PK and PD EC-MPS monitoring should be prospectively assessed in further clinical trials with long-term follow-up.

Conclusions

This study has confirmed a high interindividual variability of PK and PD data in EC-MPS-treated renal transplant patients. On the basis of the presented results, it is possible to use a limited sampling algorithm for practical monitoring of MPA in renal transplant patients on EC-MPS and CsA therapy. Including PK and practical aspects, a LSS with blood samples drawn within the first 4 h after intake of EC-MPS is recommended. Both PK and PD parameters determine the risk of adverse events. Further data are necessary to confirm clinical benefits of drug monitoring by PK and PD data in EC-MPS-treated patients. In particular, the optimal time point of MPA monitoring has to be evaluated with respect to short- and long-term clinical benefits.

Competing interests

None to declare.

The authors thank Friederike Hug and Sabine Bönisch-Schmidt (Department of Nephrology, University Hospital Heidelberg) for excellent technical assistance.

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