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No relevant pharmacokinetic interaction between pantoprazole and mycophenolate in renal transplant patients: a randomized crossover study

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Pantoprazole is a frequently prescribed proton pump inhibitor (PPI) in renal transplant patients as gastrointestinal side effects, such as heartburn, are common under immunosuppressive therapy with mycophenolate.
- PPIs influence the bioavailability of drugs by raising gastric pH, which may lead to different dissolution rates or changes in the solubility of drugs.

WHAT THIS STUDY ADDS

- This crossover study analysed the effect of pantoprazole on MMF and EC-MPS pharmacokinetics in renal transplant patients under maintenance immunosuppressive therapy.
- For pantoprazole intake, bioequivalence was not established for either MMF or EC-MPS.
- Further analysis showed no impact of pantoprazole on MPAG pharmacokinetics or MPA pharmacodynamics.

AIMS

Mycophenolic acid (MPA) suppresses lymphocyte proliferation through inosine monophosphate dehydrogenase (IMPDH) inhibition. Two formulations have been approved: mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS). Pantoprazole (PAN) inhibits gastric acid secretion, which may alter MPA exposure. Data from healthy volunteers suggest a significant drug–drug interaction (DDA) between pantoprazole and MPA. In transplant patients, a decreased MPA area under the concentration–time curve (AUC) may lead to higher IMPDH activity, which may lead to higher acute rejection risk. Therefore this DDA was evaluated in renal transplant patients under maintenance immunosuppressive therapy.

METHODS

In this single-centre, open, randomized, four-sequence, four-treatment crossover study, the influence of PAN 40 mg on MPA pharmacokinetics such as (dose-adjusted) $AUC_{0-12\,h}$ (dAUC) was analysed in 20 renal transplant patients (>6 months post-transplantation) receiving MMF (1–2 g day^{-1}) and EC-MPS in combination with ciclosporin. The major metabolite MPA glucuronide (MPAG) and the IMPDH activity were also examined.

RESULTS

MMF + PAN intake led to a lowest mean dAUC for MPA of 41.46 ng h ml^{-1} mg^{-1} [95% confidence interval (CI) 32.38, 50.54], while MPA exposure was highest for EC-MPS + PAN [dAUC: 46.30 ng h ml^{-1} mg^{-1} (95% CI 37.11, 55.49)]. Differences in dAUC and dose-adjusted maximum concentration (dCmax) were not significant. Only for MMF [dAUC: 41.46 ng h ml^{-1} mg^{-1} (95% CI 32.38, 50.54)] and EC-MPS [dAUC: 43.39 ng h ml^{-1} mg^{-1} (95% CI 33.44, 53.34)] bioequivalence was established for dAUC [geometric mean ratio: 101.25% (90% CI 84.60, 121.17)]. Simultaneous EC-MPS + PAN intake led to an earlier time to Cmax (tmax) [median: 2.0 h (min–max: 0.5–10.0)] than EC-MPS intake alone [3 h (1.5–12.0); $P = 0.037$]. Tmax was not affected for MMF [1.0 h (0.5–5.0)] ± pantoprazole [1.0 h (0.5–6.0), $P = 0.928$]. No impact on MPAG pharmacokinetics or IMPDH activity was found.

CONCLUSION

Pantoprazole influences EC-MPS and MMF pharmacokinetics but as it had no impact on MPA pharmacodynamics, the immunosuppressive effect of the drug was not impaired.

Introduction

Mycophenolic acid (MPA) is commonly used for immunosuppressive combination therapy after renal transplantation [1]. It is a reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the *de novo* synthesis of guanosine nucleotides. IMPDH activity reflects the immunosuppressive response of MPA and the determination of its activity is an appropriate pharmacodynamic monitoring tool [2]. Primarily, MPA is metabolized to MPA glucuronide (MPAG), which is present in plasma in 20–100-fold higher concentrations than MPA. Enterohepatic recirculation of MPA/MPAG is responsible for 10–60% of MPA exposure and contributes to the high pharmacokinetic variability of the drug [3].

There are two different MPA formulations on the market: the prodrug mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS). After MMF intake, the prodrug is rapidly released in the stomach at low pH and hydrolysed to active MPA, while the EC-MPS tablet dissolves in the small intestine at a higher pH owing to its delayed-release formulation [3].

Pantoprazole (PAN), a proton pump inhibitor (PPI), inhibits gastric acid secretion and is used frequently as concomitant medication for the treatment or prophylaxis of gastrointestinal disorders in renal transplant patients [4]. PPIs are known to modify the release of other drugs by increasing gastric pH [5]. Higher pH values might interfere with the dissolution of the tablet and the hydrolysis of MMF to MPA, and thereby potentially lead to lower bioavailability. This hypothesis is supported by a crossover study in healthy volunteers in which lower MPA exposure [area under the concentration–time curve (AUC)] and lower maximum concentrations (Cmax) were observed for MMF with concomitant PPI intake. The best solubility of the MMF tablet is below a pH value of 4 [6]. By contrast, for the EC-MPS tablet, fast dissolution was found to be achieved at pH $>$5.5 [7]. MPA absorption seems not to be affected by PPIs in healthy volunteers or in heart transplant patients [7–9].

In order to examine the drug–drug interaction of PPIs with the currently available MPA formulations thoroughly, we performed an open-label, randomized, four-sequence, four-period crossover study in stable renal allograft recipients on ciclosporin and determined a complete 12 h MPA and MPAG profile. Additionally, we analysed IMPDH activity in order to determine fully the effect of PPIs on the pharmacodynamic response of MPA.

Methods

The main objective of the present study was to evaluate a potential interaction of PAN on the bioavailability of MPA after MMF or EC-MPS intake in stable renal allograft recipients. Additional pharmacokinetic data of the main metabolite MPAG and the biomarker IMPDH activity were evaluated. The protocol was approved by the ethics committee of the federal state of Berlin and was conducted in accordance with the Helsinki Declaration and the Federal Institute of Drugs and Medical Devices (BfarM, Bonn, Germany; Eudra-CT number: 2010-021275-92; ClinicalTrials.gov Id: NCT01801280). All enrolled patients gave written informed consent.

Inclusion/exclusion criteria

Key inclusion criteria included adult (≥18 years) stable renal allograft recipients, who were ≥ 6 months post-transplant and receiving ciclosporin with or without glucocorticoids. PPIs, H_2-antagonists or similar medication interfering with MPA absorption had to be discontinued one month before the start of the study.

Exclusion criteria included patients with acute rejection within 1 month before the start of the study, with low renal function [glomerular filtration rate (GFR) $<$30 ml min^{-1} estimated by Cockcroft Gault (eGFR-CG)], on clopidogrel therapy, who were HIV, hepatitis C or hepatitis B antigen positive, or who had gastrointestinal disorders which could affect MPA absorption.

Study design

Each patient was randomized to one of four different sequences according to the (computerized) Latin square design and simultaneously took MMF (Roche, Basel, Switzerland), EC-MPS (Novartis, Nuremberg, Germany) with/out PAN 40mg once-in-the-morning (o.m.) (Nycomed, Konstanz, Germany) (Figure 1).

MPA doses ranged between 500 mg and 1000 mg MMF twice daily (360–720 mg EC-MPS). Target trough levels for ciclosporin (Novartis, Germany) were 75–115 ng ml^{-1}. Study medication had to be taken for 10–14 days for each period. At each visit, a full 12 h pharmacokinetic and pharmacodynamic profile was determined for MPA and MPAG, and for IMPDH activity. Lithium–heparin blood samples were collected before study medication intake and 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h and 12 h after drug administration. On the next day, a new treatment period started. Routine safety parameters, including vital signs, clinical chemistry, haematology and gastrointestinal side effects, were determined at baseline and at every visit.

Measurement of pharmacokinetic data for MPA and MPAG

Plasma for MPA and MPAG analysis was separated and stored at –80°C. An isocratic high-pressure liquid chromatography (HPLC) assay was used for simultaneous analysis of MPA (LGC Standards, Wesel, Germany) and MPAG (TRC Inc., Toronto, Canada) with hexobarbitone (LGC standards) as internal standard, respectively.

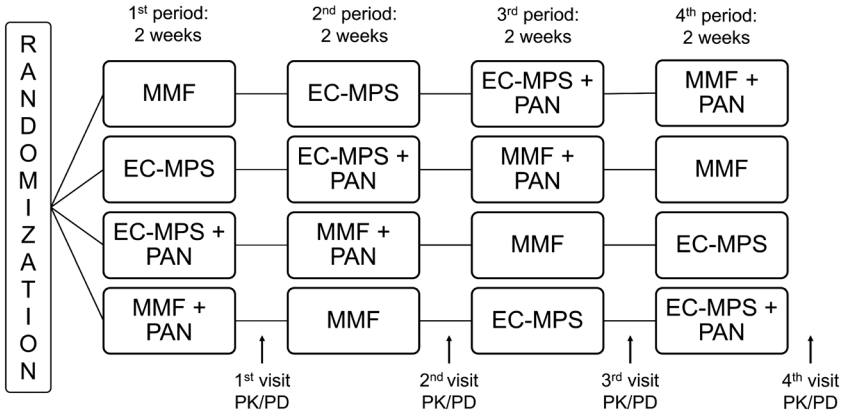

Figure 1

Study design. The study design of this four-sequence, four-period, four-treatment crossover study is shown. It was intended to include five patients into each sequence but in fact five patients were included into sequence D and four patients into sequences A, B and C, respectively. Every treatment period lasted 2 weeks. EC-MPS, enteric-coated mycophenolate sodium; MMF, mycophenolate mofetil; PAN, pantoprazole; PD, pharmacodynamics; PK, pharmacokinetics

A RP C18 column (Phenomenex Luna 5 μm C18(2) 100 A (150 × 4,6 mm), Aschaffenburg, Germany) was used as a stationary phase and was kept at 55°C. The mobile phase consisted of acetonitrile and 50 mmol KH_2PO_4 (30:70; v/v) at a pH of 2.4; the flow rate was 1.5 ml min^{-1}.

The pre-analytical procedure of collecting lithium–heparin plasma included protein precipitation with acetonitrile. Linearity was assessed between 0.50 μg ml^{-1} and 40.00 μg ml^{-1} for MPA and 5.00 μg ml^{-1} to 350.00 μg ml^{-1} for MPAG. The accuracy of calibration standards for MPA ranged from 88.82% to 111.52% [lower limit of quantification (LLOQ): 99.18–114.13%] and for MPAG from 88.12% to 111.92% (LLOQ: 84.65–103.37%). We either prepared quality control (QC) samples by dilution from stock solutions or used external standards from ChromSystems [Mycophenolic Acid/Glucuronide Plasma Controls (lyoph.)] which were placed at the beginning and the end of each run. Intraday variation for QC samples of MPA ranged from 0.03% to 6.50% and 0.10% to 3.84% for MPAG. The relative standard deviation of the inter-day variation of QC samples for MPA ranged from 0.08% to 7.40%, and for MPAG from 3.69% to 8.63%; for calibration standards for MPA, it ranged from 1.55% to 9.14%, and for MPAG from 1.52% to 6.58%.

Measurement of IMPDH activity

IMPDH activity was measured by an established method by Glander, using an isocratic ion-pair HPLC method [10]. Enzyme activity was calculated by normalizing the produced xanthine 5′ monophosphate (XMP) to the internal standard adenosine 5′-monophosphate (AMP). Two control samples were used at the beginning and the end of each run. The within-run and between-run precision was <10.09% and <9.00%, respectively.

Pharmacokinetic and pharmacodynamic parameters

The primary endpoint of the study was the bioavailability (defined as $AUC_{0-12\,h}$) of MPA after MMF and EC-MPS application alone or in combination with PAN. Calculation of pharmacokinetic and pharmacodynamic parameters for MPA and MPAG was performed using WinNonlin (Version 6.3; Pharsight Corporation, Mountain View, CA, USA) using noncompartmental analysis.

Calculated pharmacokinetic parameters for MPA were: $AUC_{0-12\,h}$ by the linear-up/log-down trapezoidal rule; Cmax and time to Cmax (tmax). For MPAG, AUC, Cmax and tmax were determined.

For IMPDH, the following pharmacodynamic parameters were calculated: maximum activity (Amax), minimum activity (Amin), activity at baseline (A0), area under the enzyme activity curve (AEC), maximum inhibition (Imax; i.e. Amin/Amax). Data were pooled for each treatment and an inhibitory maximal efficacy (Emax) model was used for calculating the half-maximal inhibitory concentration (IC50): $E = E0 - (Imax \times C)/(C + IC50)$, where E0 is the estimated baseline effect.

Statistical analysis and sample size

A possible interaction of PAN with the bioavailability of MPA and Cmax was analysed using the linear mixed effect model (LMEM); the sequence and medication were used as the fixed factor and patient nested within sequence was used as a random effect. Natural logarithmized pharmacokinetic data (such as AUC and Cmax) and the corresponding dose-adjusted data (such as dose-adjusted Cmax (dCmax) and dose-adjusted AUC (dAUC)) were analysed by WinNonLin, respectively. MPA pharmacokinetic parameters were dose adjusted to equimolar doses of MPA. Amax, Amin, A0, AEC and Imax for

IMPDH activity were analysed using LMEM. For MPAG, GFR was added as an additional covariate in the model.

For statistical analysis, MMF was set as the reference treatment for the other three treatment options and these were analysed together using LMEM. When EC-MPS was set as the reference treatment, it was only compared with EC-MPS + PAN. To evaluate comparability, geometric mean ratios (GMRs) were calculated for the differences between the reference treatment and the other treatment groups, including 90% confidence intervals (CI). Comparability was accepted if the point estimates and the 90% CI remained within the 0.80–1.25 interval. Tmax values were not transformed or dose adjusted, and were described as medians (minimum – maximum). Statistical analysis was performed using the exact Wilcoxon signed-rank test. Demographic parameters were analysed using the analysis of variance/Friedman test and descriptive methods. Safety and pharmacokinetic/pharmacodynamic data were presented as proportions, with mean ± 95% CI of the mean or median (minimum – maximum).

The total sample size was estimated to be a maximum of 24 for the 4 × 4 crossover study. This sample size would be sufficient to obtain 80% power to detect a 25% decrease in the bioavailability of MPA after PAN intake, with a coefficient of variation (CV) of 20%. In order to correct this grossly estimated sample size, a formal power calculation was proposed in the protocol after completion of 16 evaluable pharmacokinetic profiles. Contrary to the assumptions, we observed a CV of >40% in the MPA AUC and a difference in the bioavailability of MPA ± PAN of about 11% after $n = 17$ evaluable pharmacokinetic profiles. According to the prespecified power calculation, a sample size of 39 patients would be necessary to obtain 80% power for the detection of differences in MPA bioavailability after PAN administration. As the study could enrol no more than 24 patients, no further patients were enrolled in the study and final analysis was performed as specified in the protocol.

Results

Demographics

In total, 20 patients were enrolled into the study at the Charité Hospital from January 2012 to March 2013. Two of them (10%) withdrew consent and discontinued the study (Table 1). One patient was excluded from analysis owing to noncompliance. In summary, 68 complete pharmacokinetic/pharmacodynamic profiles were available for analysis. Twelve out of the remaining 17 patients received standard dosing, three received 1500 mg MMF/1080 mg EC-MPS and two received 1000 mg MMF/720 mg EC-MPS.

Gastrointestinal side effects occurred in all treatment periods. Three patients (15%) experienced heartburn and one patient abdominal discomfort during MMF treatment. During EC-MPS treatment, three side effects were reported (diarrhoea, flatulance and lack of appetite). Only one side effect occurred during MMF + PAN treatment (diarrhoea) and two during EC-MPS + PAN treatment (nausea and diarrhoea).

Table 1

Demographic characteristics

	No. of patients (%)
Patients included	20 (100%)
Patients completing the study	18 (90%)
Patients eligible for analysis	17 (85%)
Male	12 (60%)
Caucasian	20 (100%)
Patients receiving standard dosing (2 × 1 g MMF)	12 (60%)
Patients receiving low-dose glucocorticoids*	6 (30%)
Primary kidney disease	
Glomerulonephritis	11 (55%)
Vasculitis	2 (10%)
ADPKD	2 (10%)
Other†	5 (25%)
	Mean (SD)
Age at transplantation (years)	44 (16)
Time after tx until study entry (years)	6.5 (5.5)
Estimated glomerular filtration rate – Cockcroft Gault baseline† (ml min^{-1})	54.1 (17.1)
Albumin baseline* (g l^{-1})	44.2 (16.4)
Ciclosporin levels at baseline* (μg l^{-1})	91.2 (29.0)
Methylprednisolone dose ($n = 6$) (mg)	3.1 (1.0)

*No significant changes were found between the other treatment periods (data not shown). †Other primary kidney disease: hereditary (one patient) or interstitial nephritis (two patients), renal cell carcinoma (one patient), nephrocalcinosis (one patient). Abbreviations are as follows: ADPKD, autosomal dominant polycystic kidney disease; MMF,mycophenolate mofetil; tx, transplantation.

Pharmacokinetics of MPA

For all treatment options in reference to MMF, no significant changes in dCmax or dAUC were found (Table 2). After MMF + PAN intake, dAUC was decreased by up to 10.86% in comparison with MMF alone. Comparing the MPA exposure of EC-MPS + PAN and MMF alone, we found an increase of 11.51%, and for MMF in comparison with EC-MPS of 0.98%. dCmax was decreased by 22.28% for MMF + PAN *vs.* MMF intake alone, and by 7.50% comparing EC-MPS and MMF. For EC-MPS + PAN intake in comparison with MMF, dCmax increased by 10.24% (Figure 2).

dAUC was bioequivalent for MMF and EC-MPS. For MMF + PAN, the geometric mean ratio was lowest and exceeded the lower 90% CI compared with MMF alone (Figure 3). The highest values were observed for dAUC and dCmax after EC-MPS + PAN intake. Significant differences in tmax were found for MMF *vs.* EC-MPS ($P = 0.003$) and MMF *vs.* EC-MPS + PAN ($P = 0.028$).

Table 2

Main pharmacokinetic parameters of mycophenolic acid (MPA) at steady state ($n = 17$)

Treatment	Mean	95% CI	*P*-value	Geometric mean ratio (GMR) (%)	90% CI for GMR
$AUC_{0-12\ h}$ (μg h ml^{-1})					
MMF	55.70	42.09, 69.32			
MMF + pantoprazole	49.80	37.45, 62.15	0.304	89.47	74.76, 107.07
EC-MPS	53.64	39.76, 67.53	0.460	92.33	77.15, 110.50
EC-MPS + pantoprazole	57.20	43.52, 80.88	0.779	103.07	86.13, 123.35
$dAUC_{0-12\ h}$ (ng h ml^{-1} mg^{-1})					
MMF	41.46	32.38, 50.54			
MMF + pantoprazole	37.79	28.48, 47.10	0.304	89.47	74.76, 107.07
EC-MPS	43.39	33.44, 53.34	0.908	101.25	84.60, 121.17
EC-MPS + pantoprazole	46.30	37.11, 55.49	0.259	113.03	94.44, 135.27
Cmax (μg ml^{-1})					
MMF	19.49	14.32, 24.67			
MMF + pantoprazole	15.93	11.59, 20.27	0.228	78.88	56.93, 109.29
EC-MPS	20.34	13.25, 27.42	0.331	82.63	59.64, 114.49
EC-MPS + pantoprazole	22.16	15.54, 28.78	0.942	101.44	73.22, 140.55
dCmax (ng ml^{-1} mg^{-1})					
MMF	14.61	11.10, 18.12			
MMF + pantoprazole	11.92	8.79, 15.05	0.228	78.88	56.93, 109.29
EC-MPS	16.05	10.79, 21.32	0.615	90.61	65.40, 125.55
EC-MPS + pantoprazole	17.44	12.86, 22.02	0.586	111.24	80.29, 154.12
tmax (h)	**Median**	**Minimum – Maximum**	***P*-value**		
MMF*	1.0	0.5, 5.0			
MMF + pantoprazole*	1.0	0.5, 6.0	0.928		
EC-MPS*	3.0	1.5, 12.0	0.003		
EC-MPS + pantoprazole*	2.0	0.5, 10.0	0.028		

Analysis was done for non-dose-adjusted (AUC, Cmax, tmax) and dose-adjusted pharmacokinetic data (dAUC, dCmax). Pharmacokinetic parameters were dose adjusted to 1 g equimolar doses of MPA. Linear mixed-effect model was performed with all four treatment options in reference to MMF in one statistical model. For bioequivalence analysis, MMF was set as the reference as well. *Tmax values shown as median (minimum – maximum). The Wilcoxon signed-rank test was performed in reference to MMF. Abbreviations are as follows: AUC, area under the concentration-time curve; dAUC, dose-adjusted AUC; CI, confidence interval; Cmax, maximum concentration; dCmax, dose-adjusted Cmax; EC-MPS, enteric coated mycophenolate sodium; MMF, mycophenolate mofetil; tmax, time to maximum concentration.

Co-administration of EC-MPS and PAN resulted in earlier tmax compared to EC-MPS alone ($P = 0.037$). The ratio for dAUC EC-MPS + PAN in reference to EC-MPS was 111.63% (90% CI 92.94, 134.08%) and for dCmax 122.76% (90% CI 91.26, 165.13%). Difference in dAUC was 9.56%, and in dCmax 15.79%. No significant impact of PAN were found for either dCmax ($P = 0.244$) or dAUC ($P = 0.310$).

In addition, the analysis of additional pharmacokinetic parameters revealed no significant impact of PAN on other MPA pharmacokinetics (Table S1).

Pharmacokinetics of MPAG

AUC values for MPAG were comparable between treatment groups. PAN intake did not affect the concentration–time profile of MPAG and had no significant effect on AUC, Cmax or tmax. As expected, eGFR-CG had a strong influence on the pharmacokinetics of MPAG (Table 3).

IMPDH activity

EC-MPS (AEC CV = 39.16%) had a larger pharmacodynamic variability than MMF (AEC for MMF: CV = 29.37%). Overall, PAN had no significant effect on MPA pharmacodynamic factors such as AEC, Amin, A0 or Imax (Table 4). Only Amax displayed a trend towards lower values during administration of MMF + PAN than MMF alone ($P = 0.060$). The IC50 for MMF alone was 5.02 μg ml^{-1}, for MMF + PAN was 3.48 μg ml^{-1}, for EC-MPS was 6.00 μg ml^{-1} and for EC-MPS + PAN was 4.06 μg ml^{-1} (Figure 4).

Discussion

This was the first randomized, crossover study in stable renal transplant patients under immunosuppressive maintenance therapy that analysed the influence of PAN on MPA pharmacokinetics and pharmacodynamics under steady-state conditions. PAN is a widely used PPI against gastrointestinal side effects in renal transplant patients [4]. In healthy volunteers, PPIs were found to have a significant drug–drug-interaction with MMF, but not with EC-MPS [7, 8]. In the present study, bioequivalence was not reached for PAN intake, regardless of the

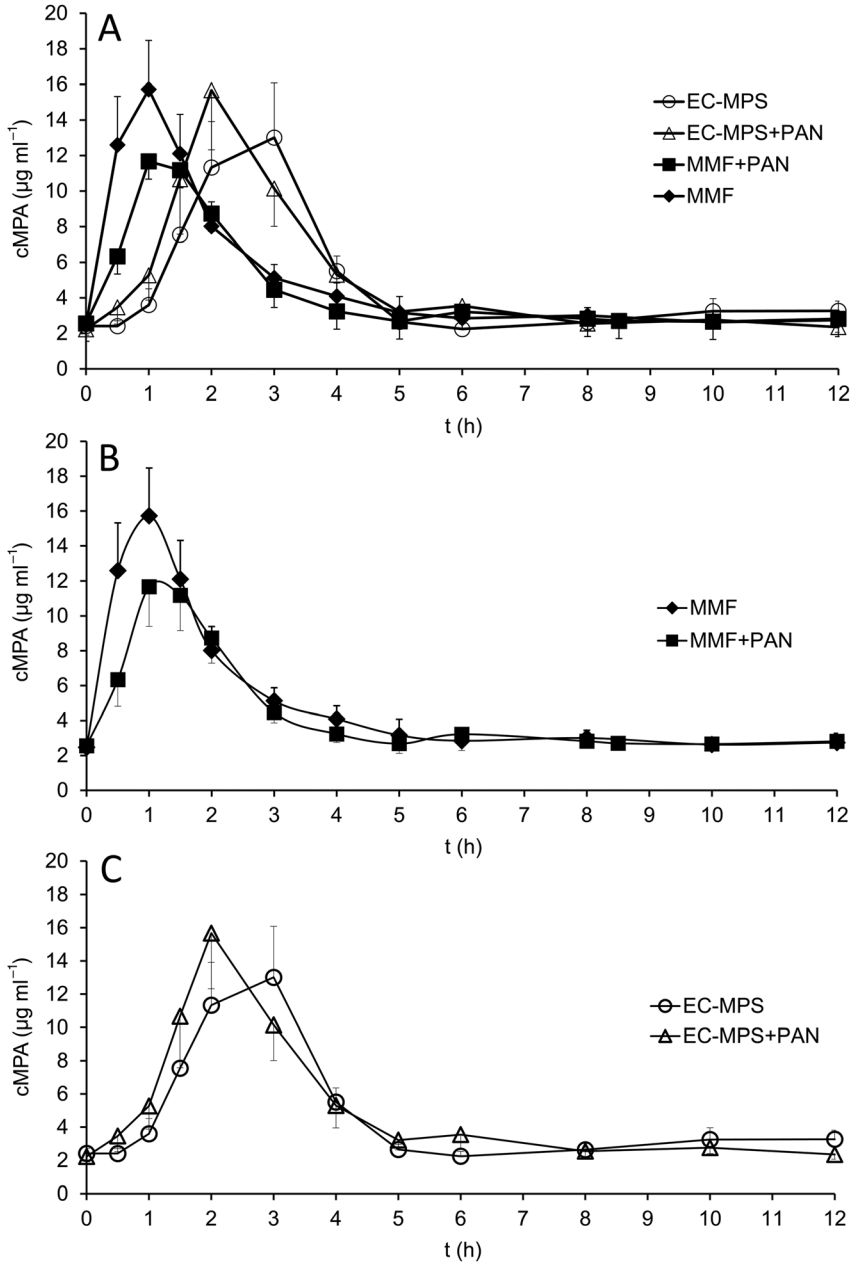

Figure 2

Concentration–time profiles of different treatment options for mycophenolic acid (MPA) ($n = 17$). Mycophenolate mofetil with and without pantoprazole (MMF ± PAN), enteric-coated mycophenolate sodium with and without PAN (EC-MPS ± PAN) (A); MMF ± PAN (B); EC-MPS ± PAN (C). No significant differences were found for PAN intake with either MMF or EC-MPS for (dose-adjusted) maximum concentration (Cmax) and (dose-adjusted) area under the curve (P = NS). Time to Cmax (tmax) was delayed for EC-MPS in comparison with MMF ($P = 0.003$). Tmax occurred significantly earlier after PAN 40 mg intake together with EC-MPS than for EC-MPS intake alone ($P = 0.037$). For MMF ± PAN, tmax was not affected (P = NS). Symbols show mean MPA plasma concentrations, and error bars the standard error at each time point ($n = 17$). cMPA, plasma concentration of MPA; NS, not significant

formulation. Intake of PAN with MMF was associated with a nonsignificant decrease in dCmax (by 22.3%) and MPA exposure (by 11.5%). By contrast, we found a nonsignificant increase in dCmax (by 15.8%) and dAUC (by 9.6%) and an earlier tmax after EC-MPS intake with PAN compared with EC-MPS alone ($P = 0.037$). Given the high variability of MPA pharmacokinetics in renal transplant patients, these trends did not reach statistical significance. In line with these observations, we did not observe a significant influence on MPAG pharmacokinetics or IMPDH activity after simultaneous intake of PAN with either MMF or EC-MPS, further excluding any clinically meaningful influence of PAN on MPA pharmacokinetics and pharmacodynamics.

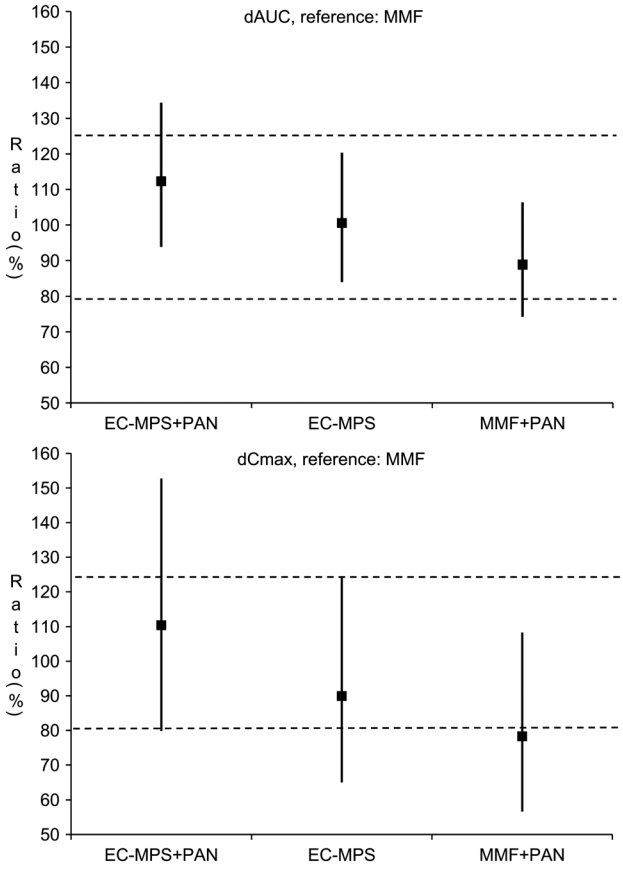

Figure 3

Bioequivalence results for dose-adjusted maximum concentration (dCmax) and area under the curve (dAUC) in reference to mycophenolate mofetil (MMF) for mycophenolic acid (MPA) ($n = 17$). The point estimates and 90% confidence intervals of log-transformed, dose-adjusted area under the curve (A) and Cmax (B) MPA are shown for all treatment options in reference to MMF. The dotted lines are the upper and lower limit for bioequivalence (80–125%). Comparing MMF and enteric-coated mycophenolate sodium (EC-MPS), bioequivalence was established for dAUC but not for dCmax

Table 3

Pharmacokinetic parameters of mycophenolic acid glucuronide (MPAG) under steady-state conditions ($n = 17$)

Treatment	Mean	95%CI of mean	*P*-value
$AUC_{0-12\ h}$ (μg h ml^{-1})			
MMF	1510.78	1140.21, 1881.35	
MMF + pantoprazole	1544.72	1133.11, 1956.33	0.989
EC-MPS	1497.69	1191.65, 1803.73	0.979
EC-MPS + pantoprazole	1578.42	1191.65, 1803.73	0.478
eGFR-CG (ml min^{-1})*	51.96	48.16, 55.75	0.001
Cmax (μg ml^{-1})			
MMF	179.20	140.26, 218.14	
MMF + pantoprazole	168.79	121.91, 215.67	0.212
EC-MPS	167.16	136.52, 197.80	0.367
EC-MPS + pantoprazole	169.58	135.33, 203.84	0.432
eGFR-CG (ml min^{-1})*	51.96	48.16, 55.75	0.006
tmax (h)			
MMF†	2.0	1.5, 8.0	
MMF + pantoprazole†	2.0	0.5, 8.0	0.723
EC-MPS†	4.0	0.0, 6.0	0.730
EC-MPS + pantoprazole†	3.0	1.0, 8.0	0.362

Significance testing was performed for all treatment options in reference to MMF in one model by the linear mixed-effect model. *GFR was calculated for all treatment options. †Tmax is shown as median (minimum – maximum). The Wilcoxon signed-rank test was performed in reference to MMF. Abbreviations are as follows: AUC, area under the concentration-time curve; CI, confidence interval; Cmax, maximum concentration; dCmax, dose-adjusted Cmax; EC-MPS, enteric coated mycophenolate sodium; eGFR-CG, glomerular filtration rate estimated by Cockcroft Gault; MMF, mycophenolate mofetil; tmax, time to maximum concentration.

Gastric acid suppression by PPIs depends on which of these agents is used, the dose administered, dosing frequency and treatment duration [11]. *In vivo*, average gastric pH rises to values >5 in patients with a duodenal ulcer after 14 days of PAN 40 mg intake each morning [12]. For complete and fast dissolution of a MMF tablet, a pH <4 is needed; at higher pH values, the dissolution rate decreases [7]. In addition, solubility of MMF is lower at higher pH [6]. This could result in a lower amount of MPA, which is available for absorption. In contrast to MMF, EC-MPS dissolves normally at higher pH values in the small intestine and changes to gastric pH should have only a minor impact on MPA absorption.

We found an earlier tmax after EC-MPS intake with PAN than with EC-MPS alone. Earlier *in vitro* tablet dissolution has been observed at a pH of 5 for EC-MPS [7]. This effect could lead to earlier dissolution of an EC-MPS tablet under PAN treatment, leading to a decreased lag time until Cmax and therefore to an earlier tmax than after intake of an EC-MPS tablet alone.

It has been shown that the pharmacodynamic effect of PPIs such as PAN is dose dependent [13]. In this study MPA was administered twice a day and PAN once daily, whereas pharmacokinetic parameters were measured only on the morning after simultaneous intake of both drugs. Several studies have shown that pH values are lower in the evening when PAN 40 mg is administered once in the morning, both in healthy volunteers and in patients with a duodenal ulcer [14, 13]. MPA absorption was probably less affected in the evening, which might also contribute to the minor differences in AUC and Cmax between MPA and MPA ± PPI observed in this study.

Application of PAN 40 mg twice daily leads to a more evenly distributed gastric pH >5 [12], which could additionally interact with the dissolution of an MPA tablet after simultaneous intake in the evening, leading to a greater decrease in MPA AUC after MMF intake.

The present study showed a minor drug–drug interaction of both MPA formulations with PAN. After MMF +PAN intake, MPA Cmax and exposure were slightly reduced, while tmax decreased and exposure was slightly higher for EC-MPS with PAN. As a result, strict

Table 4

Pharmacodynamic parameters of mycophenolic acid (MPA) under steady-state conditions ($n = 17$)

Treatment	Mean	95% CI	*P*-value
AEC (μmol h s^{-1} mol^{-1} AMP)			
MMF	859.37	724.24, 981.89	
MMF + pantoprazole	815.91	704.82, 928-38	0.126
EC-MPS	917.00	740.21, 1113.44	0.204
EC-MPS + pantoprazole	885.52	752.82, 997.17	0.209
Amin (μmol s^{-1} mol^{-1} AMP)			
MMF	19.58	13.97, 25.19	
MMF + pantoprazole	21.09	17.49, 29.14	0.464
EC-MPS	26.64	13.86, 38.46	0.591
EC-MPS + pantoprazole	20.94	10.42, 30.99	0.668
Amax (μmol s^{-1} mol^{-1} AMP)			
MMF	119.35	102.37, 135.57	
MMF + pantoprazole	106.19	91.45, 124.10	0.060
EC-MPS	129.23	100.71, 158.80	0.217
EC-MPS + pantoprazole	129.37	112.24, 142.26	0.278
A_0 (μmol s^{-1} mol^{-1} AMP)			
MMF	77.97	61.91, 93.29	
MMF + Pantoprazole	76.18	59.43, 92.92	0.850
EC-MPS	72.91	56.00, 89.82	0.530
EC-MPS + pantoprazole	74.53	58.28, 90.78	0.729
Imax (%)			
MMF	83.56	79.54, 87.59	
MMF + pantoprazole	79.25	75.46, 83.04	0.446
EC-MPS	80.20	73.16, 87.24	0.443
EC-MPS + pantoprazole	83.32	76.17, 90.47	0.718

Significance testing was performed for all treatment options in reference to MMF in one model by the linear mixed-effect model. Abbreviations are as follows: AEC, area under the enzyme activity curve; Amin, minimum activity; AMP, adenosine 5′-monophosphate; Amax, maximum activity; A_0, baseline activity; CI, confidence interval; EC-MPS, enteric coated mycophenolate sodium; Imax, maximum inhibition; MMF, mycophenolate mofetil.

bioequivalence criteria are not fulfilled when PAN is added to one of the MPA formulations. Similarly to previous studies in healthy volunteers or heart transplant patients [7–9, 15], differences in dCmax and dAUC values did not reach statistical significance with EC-MPS. Other studies found a significant decrease in MPA exposure and/or Cmax values after concomitant therapy with PAN and MMF. However, some of these studies [16–19] used abbreviated MPA kinetics, which has some weaknesses. Others employed an immunoassay in which the acyl glucuronide of MPAG (AcMPAG) concentrations potentially interact with MPA plasma levels, whereas we used a specific HPLC method for MPA and MPAG determination. Finally, an influence on MPA kinetics could depend on the PPI used for treatment. Miura *et al*. found a significant influence of lansoprazole on Cmax but not on MPA exposure. For rabeprazole, no significant decrease in AUC or Cmax was found [20]. In a single-dose crossover study in healthy volunteers, Rupprecht *et al*. observed a significant decrease in MPA AUC and Cmax [8]. The study was limited by the fact that the MPAG AUC was low and not in steady-state conditions after a single administration of MPA. Therefore, enterohepatic recirculation of MPAG could not have influenced MPA exposure. In the present study, all renal allograft recipients received the same MPA dose for at least one month before the start of the study. In addition, MPA was administered during the clinical study for two weeks, twice daily, simultaneously with PAN 40 mg each morning in order to maintain stable steady-state conditions.

Both MPA formulations were well tolerated in all treatment groups. The pharmacokinetic parameters for MMF *vs*. EC-MPS alone were comparable and reached strict bioequivalence criteria for the dose-adjusted MPA AUC. This finding is in line with previous studies demonstrating bioequivalence between both formulations [21–23]. Bioequivalence criteria were not fulfilled for dCmax owing to high variability. As expected, tmax was significantly delayed for EC-MPS *vs*. MMF ($P = 0.003$).

In order to put the effects of PAN into clinical perspective, we also determined the pharmacokinetics of MPAG, the main metabolite of MPA. Any clinically relevant change in MPA absorption should be reflected by a concomitant change in MPAG, provided that additional medication with ciclosporin or glucocorticoids, and other variables (liver function, renal function and albumin levels) remain stable [24]. In our cohort, all of these parameters, which could have influenced MPA pharmacokinetics, were stable during all four treatment periods. GFR but not pantoprazole had a significant impact on MPAG pharmacokinetics.

To assess the pharmacodynamic consequences of the potential drug–drug interaction between PAN and MPA, we also determined IMPDH activity. The target enzyme activity measurement reflects a better MPA-induced immunosuppressant effect [1, 2]. In patients with autoimmune disease, a significant decrease in MPA pharmacokinetics and in IMPDH activity was found. In this study IMPDH activity was normalized to cellular protein content [19]; however, we used an internal standard for normalization, which better reflects cellular IMPDH activity [10]. Our results clearly demonstrated no impact of PAN on the pharmacodynamic response. This confirms the results of a previous study in heart transplant patients, in which no significant changes in IMPDH activity were found after EC-MPS intake [9]. For all treatment options, IC50 for MPA remained below 6 μg ml^{-1}, which is comparable with the results of other studies in renal transplant patients [21, 25]. The lowest mean dCmax of 11.92 μg ml^{-1} was found for MMF + PAN, which would exhibit only a marginal effect on further enzyme inhibition. Therefore, a decrease in dCmax caused by PAN does not lead to a relevant impact on the immunosuppressive effect of MPA.

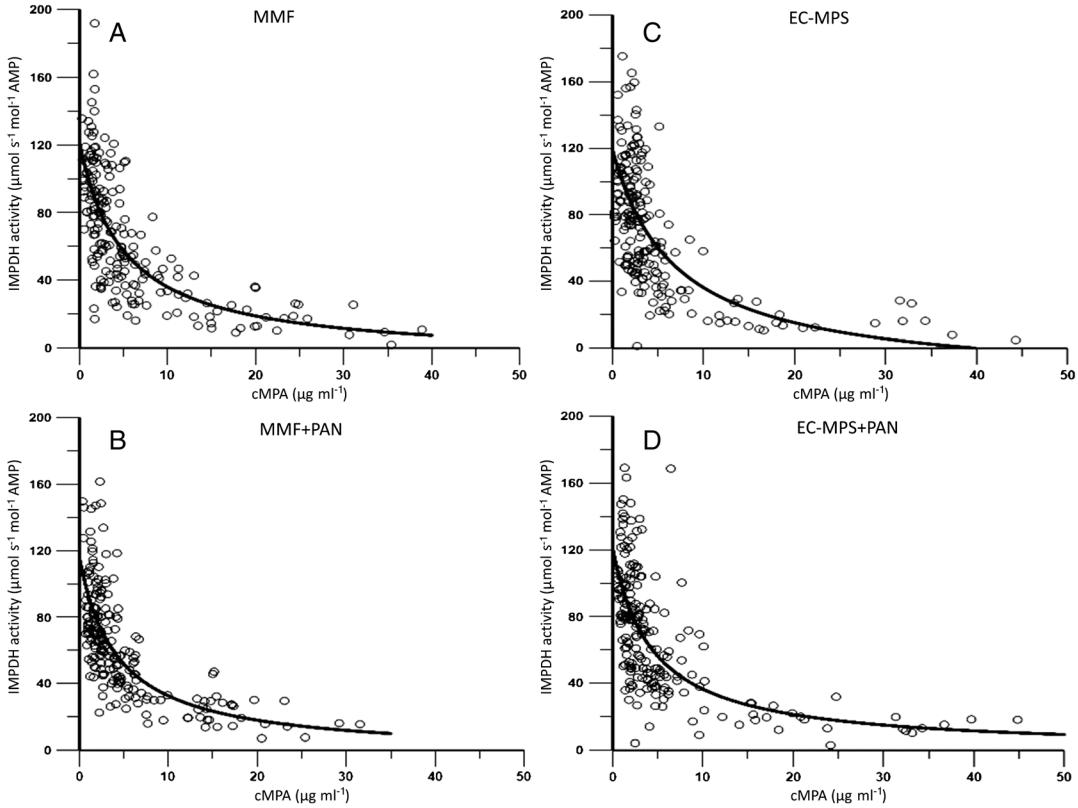

Figure 4

Pharmacokinetic/pharmacodynamic (PK/PD) relationship for mycophenolic acid (MPA) for all treatment options ($n = 17$). Inosine monophosphate dehydrogenase (IMPDH) activity is plotted against MPA plasma concentrations. The circles show the observed values, and the solid line represents the predicted values. The inhibitory Emax model was used to calculate the half-maximal inhibitory concentration (IC50) for each treatment. The median IC50 for mycophenolate mofetil (MMF) alone (A) was 5.02 µg ml^{-1}; for MMF + pantoprazole (PAN) (B) was 3.48 µg ml^{-1}; for enteric-coated mycophenolate sodium (EC-MPS) alone was 6.00 µg ml^{-1} (C); and for EC-MPS + PAN was 4.06 µg ml^{-1} (D). AMP, adenosine 5′-monophosphate; cMPA, plasma concentration of MPA; Emax, maximal effect

Dose-adjusted AUC values for MPA for all treatment options remained within the therapeutic window (30–60 µg*h ml^{-1}) [24, 26]. It is known that underexposure is associated with a higher rejection risk [24]. Recently published data showed no influence of PPIs after MMF intake on acute rejection risk in non-black patients (similar to our study, in which only Caucasian people participated) within one year after undergoing a renal transplant [27]. In another study conducted in Chinese transplant patients, no significant increase in acute rejections or delay in graft function was observed for patients receiving omeprazole with MMF or EC-MPS, although significantly higher pharmacokinetic values were found for EC-MPS [15]. Therefore, we conclude that the attenuated MPA peak concentration under MMF + PAN treatment might be of lesser concern in stable renal transplant patients. However, in the present crossover study, the main objective was to analyse a possible pharmacokinetic interaction between PAN and MPA. We did not focus on the clinical outcome due to a change in MPA pharmacokinetics. Therefore, a long-term effect of PAN or other PPIs on MPA exposure in relation to the administered formulation or dose should be evaluated in further studies, focusing more on clinical aspects such as acute rejections or side effects.

There were some limitations to the present study. MPA is known for its high pharmacokinetic variability [24], which was observed during all treatment periods. According to our power analysis, the study would have needed additional 26 patients to show a significant difference, given the small treatment effect and high variability. We chose to terminate the study according to protocol because such a small treatment effect would be of little clinical relevance. Although the difference in treatment effect was small, we were able to show that MMF or EC-MPS intake in combination with PAN does not meet strict bioequivalence criteria for combination with PAN in ciclosporin-treated renal allograft recipients.

However, the pharmacodynamic analysis and stable MPAG pharmacokinetics argue against a clinically relevant and meaningful drug–drug interaction as the immunosuppressive effect of MPA was not affected. As this rigorous crossover study was conducted in patients who had been transplanted at least 6 months before study enrolment under maintenance immunosuppressive therapy, additional prospective analysis is needed

to assess a clinical meaningful impact of PAN on MPA pharmacokinetics in the early post-transplant period.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: OR has received honoraria from Novartis and Roche in the past 3 years. KB has consultancy agreements with Bristol-Myers Squibb, Effimune, Hexal, Novartis, Pfizer, Chiesi and Veloxis over the last few years; and has received research grants for clinical studies, speaker's fees, honoraria, travel expenses and payment for development of educational presentations from Astellas, Aicuris, BmT GmbH, Bristol-Myers Squibb, Chiesi, Fresenius, Hexal, Novartis, Otsuka, Pfizer, Roche, Siemens and Veloxis in the last three years.

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Contributors

OR performed the data analysis and wrote the manuscript. She was involved in the study management and measurement of the study samples. PG was involved in designing the study and writing the study protocol. She took part in the study management and in reviewing the manuscript. She was in overall charge of the bio-analytical process. PH and MM were involved in the measurement of study samples and bio-analytical supervision. SB, DK, FH, ES, ES, MD and HHN were responsible for patient recruitment, dose administration and blood collection. KB was the principal investigator. He was involved in designing the study and writing the study protocol, contributed to the study management and data analysis, and was involved in writing the manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1

Additional pharmacokinetic parameters of mycophenolate. ($n = 17$)