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Mycophenolic Acid Metabolites Acyl-Glucuronide and Glucoside Affect the Occurrence of Infectious Complications and Bone Marrow Dysfunction in Liver Transplant Recipients

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Background: Mycophenolic acid (MPA) prodrugs are anti-proliferative immunosuppressive agents commonly used after organ transplantation. Although they are generally well tolerated by patients, adverse effects may occur. It is postulated that MPA metabolites could also contribute to these adverse effects.

Material/Methods: The objective of this study was the assessment of concentrations of total MPA and its metabolites, phenyl glucuronide (MPAG), acyl glucuronide (AcMPAG) and glucoside (GluMPA), using liquid chromatography combined with mass spectrometry (LC/MS/MS) in two groups: kidney transplant recipients and liver transplant patients. Associations of MPA and its metabolites with adverse effects were analyzed.

Results: The study group consisted of 211 recipients of liver or kidney transplants who received immunosuppressive therapy, including MPA prodrugs. Multivariant analysis showed a positive influence of MPA on gastroenterotoxicity in kidney transplant recipients. In liver patients, gastroenterotoxicity was associated with lower MPAG concentrations. A positive influence of AcMPAG on bacterial infections in liver transplant patients was observed. In liver transplant recipients, a positive influence of MPA and a negative influence of GluMPA levels on the PLT count were revealed. MPA and its metabolites did not influence the hemoglobin levels in both groups. There were no significant relationships among MPA, its metabolites and WBC counts.

Conclusions: In kidney transplant recipients, total MPA trough concentration is associated with gastroenterotoxicity and its monitoring could have important role in management of gastrointestinal complications. The quantification of AcMPAG in liver recipients receiving MPA may be helpful in avoiding bacterial infections. GluMPA seems to have a toxic effect on thrombopoiesis.

MeSH Keywords: Kidney Transplantation • Liver Transplantation • Mycophenolic Acid

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Background

Mycophenolic acid (MPA) is widely used for the treatment of patients undergoing solid organ transplantation as part of a multiple drug regimen, usually with concomitant cyclosporine or tacrolimus and corticosteroids to prevent graft rejection. MPA blocks the conversion of inosine monophosphate by inosine monophosphate dehydrogenase (IMPDH), a key enzyme in the *de novo* purine biosynthetic pathway [1]. Although mycophenolic acid pro-drugs, including mycophenolate mofetil (MMF) and mycophenolate sodium (MPS), are generally well-tolerated in patients, such adverse effects as: infections, leucopoenia, anemia, and gastrointestinal problems may occur, necessitating dose reduction or discontinuation and thereby potentially jeopardizing patient and graft outcomes. Dose reduction and discontinuation of MPA therapy have been associated with an increased risk of acute rejection episodes and kidney graft loss [2].

In contrast to other immunosuppressants, mycophenolate mofetil and mycophenolate sodium preparations were introduced for clinical use without a recommendation of therapeutic drug monitoring (TDM). Clinical experience has shown that it is possible and, in some cases, necessary to use various methods of TDM, such as the assessment of the area under the concentration-time curve (AUC) and minimum plasma drug concentration (through concentration). These evaluations have shown high inter- and intra-individual variability in the MPA exposure parameters. Although several MPA metabolites have been identified, their assessment is not used routinely. The major MPA metabolite is phenolic glucuronide (MPAG), and other minor metabolites include 7-O-glucoside (GluMPA) and acyl-glucuronide (AcMPAG) [3,4]. Whereas MPAG is inactive, AcMPAG is capable of inhibiting human IMPDH *in vitro* and has been considered toxic [4,5]. There is scarce information regarding GluMPA levels and actions in solid organ transplant (SOT) patients.

The aim of the study was to assess the levels of the total MPA and of its three metabolites, MPAG, AcMPAG, and GluMPA, using liquid chromatography combined with tandem mass spectrometry (LC/MS/MS) in SOT recipients. We hypothesized that metabolites of MPA could affect the occurrence of the adverse effects of the drug.

Material and Methods

This study involved the participation of 211 solid organ transplant recipients. All of the patients under the care of our transplant center were eligible for inclusion. All consecutive outpatients who gave their written informed consent to participate in the study were included. All blood samples were taken during routine blood tests on outpatient visits to the Transplant and Nephrology Clinic between November 2011 and July 2012. Blood was taken just before the administration of the morning dose of MPA, between 8.30 and 9.00 am (trough concentration) and after fasting for at least 8 hours. Blood was collected in EDTA tubes and placed at +4°C, then centrifuged to obtain plasma. Plasma was stored at –80°C until the time of determination of MPA and its metabolites concentrations by LC/MS/MS method. Blood sampling was accompanied by the collection of relevant laboratory and clinical data.

Renal function was assessed with the use of estimated glomerular filtration rate (eGFR) by Modification of Diet in Renal Disease (MDRD) [6]. MPA dosage: To standardize the data on daily mycophenolate mofetil and mycophenolate sodium dosage (MPA_{cd}), mycophenolate sodium doses were converted to equivalent mycophenolate mofetil doses (MPA_{cd}=MPS [mg/day] ×1.3889). Gastroenterotoxicity in patients was assessed based on patients' medical history. Symptoms, including diarrhea, abdominal pain, and vomiting, especially recurrent vomiting, were identified. We excluded other causes of these problems, such as CMV infection or other infections on the basis of fever incidence; elevated C-reactive protein levels; or positive CMV DNA PCR. According to the clinical symptoms, the patients were divided into two groups: those with gastroenterotoxicity (recurrent or few episodes of diarrhea, abdominal pain or vomiting, not related to infection, with the resolution of the symptoms following MPA dose reduction) and those without gastroenterotoxicity (no typical symptoms or symptoms related to infection). The incidence of infections was based on the presence of suggestive clinical characteristics and was confirmed by microbiologic studies of representative biological samples and agreeable results from additional tests, including elevated CRP, procalcitonin, and white blood cell counts. CMV infection was diagnosed on the basis of typical clinical symptoms and positive CMV DNA PCR results. Anemia was diagnosed if the blood hemoglobin was <120 g/L (women) or <130 g/L (men).

Chemicals. The chemicals used included the following: LC-MS grade – methanol, 25% ammonium hydroxide and formic acid (J.T. Baker), and analytical grade - ammonium acetate (POCh, Gliwice, Poland). Ultra-pure water was obtained from a water purification system (Mili-Q, Millipore, Milford, MA, USA). MPA, deuterated MPA (MPA-d3), AcMPAG, MPAG, and GluMPA (Toronto Research Chemicals Inc., North York, Canada) were a gift from Roche Poland. Stock solutions were prepared in methanol and stored at –20°C. As an internal standard for MPA and all metabolites, MPA-d3 was applied.

Sample preparation. MPA and all metabolites were quantified in the blood plasma. The whole blood samples were centrifuged (10 min at 1000 RCF) to obtain the plasma. Sample preparation was performed as follows: 100 µL of plasma was

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Table 1. Monitored transitions, cone voltages, collision energies, and retention times of the analyzed compounds.

Immunosuppressive agent	MRM transition	Cone voltage	Collision energy	Retention time [min]
Mycophenolic acid (MPA)	338.16>207.10 (qt)	10	15	1.81
	338.16>275.19	10	15	
MPA glucoside	500.21>303.02 (qt)	15	20	1.50
	500.21>275.10	15	25	
	500.21>207.12	15	30	
MPA acyl glucuronide	514.19>207.12 (qt)	15	35	1.64
	514.19>303.02	15	20	
	514.19>321.10	15	10	
MPA phenyl glucuronide	514.19>321.10 (qt)	15	10	1.33
	514.19>303.02	15	20	
	514.19>207.12	15	35	
MPA-d3	341.22>210.04	10	25	1.80

qt – quantification transition; MPA – mycophenolic acid; MPA-d3 – deuterated mycophenolic acid; MRM – multiple-reaction monitoring (MRM) mode.

transferred into a 1.5-mL silanized conical test tube (Sigma Aldrich), and then 250 µL of methanol (with MPA-d3) was added for protein precipitation and analyte extraction. After the mixture was vortexed (1 min) and centrifuged (2 min at 18626 RCF), the entire supernatant was transferred to the vial and analyzed by LC/MS/MS.

Instrumentation. The instrumentation consisted of a Waters Acquity Ultra Performance Liquid Chromatograph coupled with a Waters TQ-S triple-quadruple mass spectrometer. For the instrument control and data acquisition, MassLynx software was used. LC/MS/MS analysis was performed in the positive electrospray ionization mode (ESI). The mass spectrometer was operated in a multiple-reaction monitoring (MRM) mode. The concentration of each analyte was calculated per MPA-d3.

Analyses

For chromatographic separation, we applied the UPLC BEH Phenyl column (50×2.1 mm, 1.7 µm, Waters), thermostated at 45°C. Mobile phase A consisted of 300 µL of formic acid and 900 µL of 25% NH_4OH in 1000 mL of water, and mobile phase B consisted exclusively of methanol. The flow rate of the mobile phase was set at 0.5 mL/min, and the injection volume was 10 µL for the analysis of MPA, GluMPA and AcMPAG and 2 µL for the analysis of MPAG. The gradient scheme was 3% B initially, followed by an increase to 90% B at 2.0 min. At 2.3 min, the mobile phase reverted to the initial conditions (3% B). The total analysis time was 3 min, including re-equilibration time. For all of the analyzed compounds, the mass spectrometer optimized settings were as follows: capillary voltage=2.5 kV, desolvation temperature=200°C, desolvation gas flow=800 L/h, cone gas flow=150 L/h, nebulizer gas pressure=7.0 bar, source temperature=150°C. The MRM transitions, cone voltages, collision energies and retention times used in the described methods are presented in Table 1. The first MRM transition of each compound served as a quantitative transition; the second, as a confirmation transition. To define the relationship between the concentration and detector responses of analytes, 6-level calibrators were prepared for each MPA metabolite, as well as for the parent compound. The concentrations of the calibrators covered entire ranges of the expected (determined empirically based on several patients' samples prior to the validation process) concentrations in the patients' samples (1–7 µg/ml for MPA, 0.01–1 µg/ml for MPA glucoside, 0.5–5 µg/ml for AcMPAG and 10–200 µg/ml for MPAG). The mean R2 coefficients of the calibration curves for all compounds from 7 sample batches were not lower than 0.97. The imprecision level of the method was assessed using 120 in-house control samples and was determined by measuring 4 sets of 10 samples at three concentration levels within the ranges of expected concentration in the patient. Imprecision values for all compounds were determined at the following four concentration levels, expressed as a coefficient of variation (CV): <6% for MPA, <12.3% for GluMPA, <11.4% for AcMPAG and up to 26% for MPAG. The mean recovery for all of the analytes was as follows: 93.9% for MPA, 88.3% for GluMPA, 92.2% for AcMPAG and 93% for MPAG.

Statistics

The analyzed database comprised 249 medical records for 211 patients. The data were weighted according to the number of

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Table 2. Demographic, clinical, and laboratory characteristics of the study groups: kidney (n=162) and liver transplant recipients (n=49).

Characteristic	Ktx (n=162)		Ltx (n=49)	
Age [years]	47.95	(12.1)	50.83	(12.73)
Sex (Female)	67	(41.36%)	23	(46.94%)
BMI [kg/m^2]	25.49	(3.99)	25.75	(3.98)
eGFR MDRD [ml/min/1.73 m^2]	44.9	(18.18)	59.14	(24/36)
Hb [g/dL]	13.16	(1.97)	13.02	(2.14)
WBC [G/L]	7.81	(2.63)	6.45	(2.39)
PLT [G/L]	223	(72–449)	177	(9–705)
AlAT [U/L]	24	(8–294)	41	(8–185)
MPA [µg/mL]	2.28	(0.31–19.82)	1.06	(0.14–5.4)
GluMPA [µg/mL]	0.07	(0.004–0.96)	0.03	(0.0–0.24)
AcMPAG [µg/mL]	0.77	(0.15–7.14)	0.45	(0.14–2.84)
MPAG [µg/mL]	70.3	(0.66–409.71)	31.44	(1.43–168.77)
MPA corrected dose [mg/day]	1500	(500–2500)	1000	(500–2000)
Time from TX [months]	58	(1–294)	38	(1–154)
GS+Tac+MPA	60	(37.04%)	21	(42.86%)
GS+CsA+MPA	55	(33.95%)	4	(8.16%)
Tac+MPA	18	(11.11%)	12	(24.49%)
CsA+MPA	13	(8.03%)	8	(16.33%)
GS+MPA	11	(6.79%)	3	(6.12%)
GS+SIR+MPA	3	(1.85%)		–
MPA	1	(0.62%)	1	(2.04%)
EVE+MPA	1	(0.62%)		–
Gastroenterotoxicity	27	(16.67%)	8	(16.33%)
Infectious complications	73	(45.06%)	17	(34.7%)
Bacterial infections	56	(34.57%)	11	(22.45%)
Viral infections	31	(19.14%)	8	(16.33%)

BMI – body mass index; TX – type of transplantation; Ktx – kidney transplantation; Ltx – liver transplantation; eGFR MDRD – estimated glomerular filtration rate calculated by Modification of Diet in Renal Disease equation; AlAT – alanine aminotransferase; MPA – mycophenolic acid; GluMPA – MPA glucoside; AcMPAG – MPA acyl-glucuronide; MPAG – MPA phenolic glucuronide; GS – glucocorticosteroids; CsA – cyclosporine; Tac – tacrolimus; SIR – sirolimus; EVE – everolimus; CMV – cytomegalovirus; WBC – white blood cell count; RBC – red blood cell count; Hb – hemoglobin; PLT – blood platelet count.

records for each patient, such that each unit observation represents one patient. The analyses were corrected by using the Bonferroni correction. Normality was estimated with the Kolmogorov-Smirnov test. Unless specified otherwise, continuous data are described as means ±SD for a normal distribution, or as medians and ranges for data with any non-normal distribution. The differences between the normally distributed variables were assessed with Student's t test; in other cases, the Mann-Whitney U test was applied. Correlations between the parameters were calculated with the Spearman correlation coefficient for all non-normally distributed values. Then, we used the multivariate analysis methods and a number of regression models were constructed to control for the effects of patients' sex, age, eGFR level and transplant organ on the

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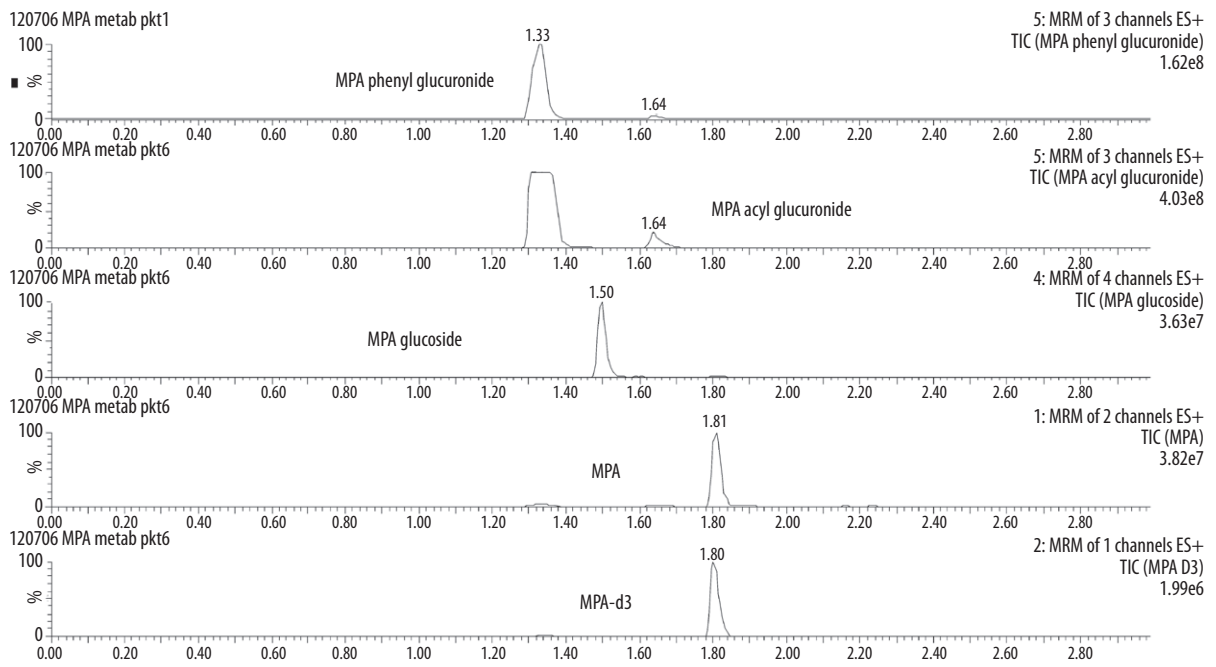

Figure 1. Chromatograms of mycophenolic acid and its derivatives: acyl glucuronide, glucoside, and phenyl glucuronide.

concentrations of MPA or its metabolites. Other medical factors, including hemoglobin, red blood cell count (RBC), platelet count (PLT), white blood cell count (WBC), infection and gastroenterotoxicity, were also analyzed. In the latter two cases, in which the dependent variables were binary, logistic regression models were applied; linear regression was used in all other cases. A p-value of <0.05 was considered to be significant. All statistical analyses were performed using IBM SPSS Statistics software version 19.0 for Windows.

Results

Patients' demographics and concentrations of MPA, MPAG, AcMPAG and GluMPA are shown in Table 2. Chromatograms for all compounds in this method are presented in Figure 1.

In the kidney transplant recipients (Ktx) in the group with gastroenterotoxicity (GT) MPA concentrations were higher in comparison with the group without GT (Table 3). There were no significant differences in the MPAG, AcMPAG or GluMPA concentrations between the group with GT and the group with no gastroenterotoxicity in Ktx group (Table 3). Multivariant analysis using logistic regression using gastroenterotoxicity as a dependent variable confirmed that in kidney recipients MPA concentrations were associated with GT, independent of the patient's sex, age, eGFR, MPA corrected dosage, and metabolite concentrations (Table 4). In liver transplant recipients (Ltx) in the group with GT, there were significantly MPAG concentrations in comparison with the group with no GT (Table 3). However, there was no difference between those groups in MPA concentrations. Logistic regression in liver patients confirmed negative influence of MPAG concentrations on gastrointestinal adverse effects (Table 4).

Ktx patients with bacterial infections or all types of infections had higher MPAG concentrations in comparison with the group without infections (Table 3). However, logistic regression, including the patients' sex, age, eGFR level, MPA dosage and metabolite concentrations as controlled variables, revealed no significant relationships among MPA, MPAG, AcMPAG and GluMPA and infectious complications (all types of infections, bacterial and viral infections) in this population (Table 4). In liver transplant recipients there was no difference between group with and without infections in MPA or metabolite concentrations (Table 3). However, multivariant analysis showed the positive influence of AcMPAG on bacterial infections in liver transplant patients (Table 4).

In kidney transplant recipients MPAG and GluMPA was significantly negatively correlated with hemoglobin (Table 5). Based on the linear regression, MPA or its metabolites did not influence hemoglobin concentration in both Ktx and Ltx patients (Table 4).

Univariate analysis and linear regression using PLT count as a dependent variable revealed no significant relationships among MPA, MPAG, AcMPAG and GluMPAG and PLT counts in

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Table 3. Comparison of concentrations of MPA and its metabolites in patients with and without adverse effects in the 2 groups: kidney and liver transplant recipients.

		Ktx (n=162)			Ltx (n=49)		
		Yes	No	p	Yes	No	p
GT	MPA	3.4 (0.99–12.47)	2.19 (0.31–19.82)	0.003*	1.23 (0.14–3.06)	1.02 (0.15–5.41)	0.99
	GluMPA	0.07 (0.01–0.96)	0.07 (0.004–0.77)	0.12	0.02 (0.0–0.06)	0.04 (0.003–0.24)	0.15
	AcMPAG	1.05 (0.41–2.41)	0.72 (0.15–7.14)	0.13	(0.36–0.14)	0.48 (0.14–2.84)	0.13
	MPAG	77.83 (24.89–158.29)	66.86 (0.66–409.71)	0.39	21.74 (1.43–31.44)	39.14 (2.38–168.77)	0.016*
Viral infections	MPA	2.28 (0.31–19.82)	2.42 (0.73–16.71)	0.61	1.12 (0.18–3.44)	1.06 (0.14–5.41)	0.99
	GluMPA	0.06 (0.004–0.77)	0.08 (0.01–0.96)	0.33	0.04 (0.003–0.08)	0.02 (0.0–0.24)	0.59
	AcMPAG	0.77 (0.15–7.14)	0.94 (0.22–3.95)	0.48	0.55 (0.18–1.2)	0.44 (0.14–2.84)	0.41
	MPAG	69.0 (0.66–409.71)	83.58 (17.0–222.24)	0.4	33.55 (3.45–47.23)	31.44 (1.43–168.77)	0.56
Bacterial infections	MPA	2.28 (0.85–7.85)	2.27 (0.31–19.82)	0.64	1.05 (0.15–5.41)	1.1 (0.14–4.36)	0.56
	GluMPA	0.07 (0.01–0.96)	0.07 (0.004–0.77)	0.23	0.04 (0.003–0.1)	0.03 (0.0–0.24)	0.9
	AcMPAG	0.91 (0.17–3.04)	0.72 (0.15–7.14)	0.12	0.61 (0.14–2.84)	0.44 (0.14–2.79)	0.45
	MPAG	85.48 (1.0–409.71)	60.86 (0.66–314.31)	0.01*	27.87 (3.45–132.5)	31.52 (1.43–168.77)	0.91
All infections	MPA	2.28 (0.73–16.71)	2.3 (0.31–19.82)	0.87	1.05 (0.15–5.41)	1.01 (0.14–4.36)	0.73
	GluMPA	0.07 (0.01–0.96)	0.06 (0.004–0.77)	0.13	0.04 (0.003–0.1)	0.03 (0.0–0.24)	0.98
	AcMPAG	0.9 (0.17–3.95)	0.72 (0.15–7.14)	0.08	0.46 (0.14–2.84)	0.44 (0.14–2.79)	0.59
	MPAG	83.58 (1.0–409.71)	57.93 (0.66–314.31)	0.017*	29.36 (3.45–132.92)	31.52 (1.43–168.77)	0.78

GT – gastroenterotoxicity; Ktx: kidney transplantation; Ltx: liver transplantation; MPA: mycophenolic acid; GluMPA: MPA glucoside; AcMPAG: MPA acyl-glucuronide; MPAG: MPA phenolic glucuronide; * p<0.05.

Ktx group (Tables 4, 5). MPAG concentrations were significantly positively correlated with PLT count in Ltx group (Table 5). In the group of liver transplant recipients, we observed a statistically significant positive influence of MPA and a negative influence of GluMPA levels on PLT count after adjusting for patient sex, age, eGFR, AlAT, MPAG, and MPA corrected dosage (Table 4). In kidney and liver transplant recipients there were no associations between MPA and its metabolites and WBC count both in univariate and multivariate analyses (Tables 4, 5).

Discussion

Here, we have presented an analysis of levels of MPA and its three metabolites in group of MPA-treated transplant recipients. We have used our novel LC-MS/MS method allowing for the complete separation of MPA and its derivatives. We tried to link MPA and its metabolites to side effects in two different groups: kidney and liver graft recipients.

Our analysis revealed higher concentrations of MPA in kidney transplant recipients with gastroenterotoxicity. The relationship

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Table 4. Relationship between MPA and its metabolites and their therapeutic complications in the 2 groups: kidney and liver transplant recipients (multivariate analyses results).

Independent variable	Dependent variable	Ktx (n=162)		Ltx (n=49)	
		B	p	B	p
MPA	Gastroenterotoxicity	0.260	0.04*	8.130	0.07
MPAG		–0.005	0.421	–0.330	0.04*
AcMPAG		–0.626	0.126	–0.738	0.605
GluMPA		0.967	0.625	–57.113	0.147
MPA	Viral infections	0.160	0.060	–0.012	0.976
MPAG		0.000	0.957	–0.054	0.096
AcMPAG		–0.031	0.909	–0.591	0.584
GluMPA		2.158	0.269	3.986	0.846
MPA	Bacterial infections	–0.224	0.093	–0.531	0.370
MPAG		0.004	0.322	0.023	0.158
AcMPAG		0.156	0.469	2.040	0.05*
GluMPA		2.229	0.254	7.063	0.705
MPA	All types of infections	–0.02	0.76	–0.01	0.97
MPAG		0.004	0.28	0.003	0.88
AcMPAG		0.19	0.38	0.83	0.27
GluMPA		1.6	0.38	–0.81	0.96
		β	p	β	p
MPA	Hemoglobin	–0.081	0.350	–0.063	0.804
MPAG		–0.134	0.115	–0.453	0.075
AcMPAG		0.129	0.108	–0.193	0.356
GluMPA		0.071	0.398	–0.072	0.801
MPA	PLT	0.098	0.343	0.650	0.004*
MPAG		0.068	0.499	–0.001	0.997
AcMPAG		–0.110	0.244	0.330	0.087
GluMPA		0.009	0.928	–0.520	0.042*
MPA	WBC	–0.055	0.522	–0.083	0.638
MPAG		0.047	0.644	–0.104	0.696
AcMPAG		–0.078	0.419	0.128	0.551
GluMPA		0.164	0.101	0.092	0.750

Ktx – kidney transplantation; Ltx – liver transplantation; MPA – mycophenolic acid; GluMPA – MPA glucoside; AcMPAG – MPA acyl-glucuronide; MPAG – MPA phenolic glucuronide; WBC – white blood cell count; PLT – platelet count; * p<0.05.

between high MPA trough concentrations and adverse drug effects has been reported by several investigators. In a study of 22 kidney transplant recipients, the MPA trough concentration was significantly higher in patients with episodes of diarrhea, infection or hematological adverse effects than in those without such events; these findings are in agreement with the results of the our study [7]. However, other studies have revealed contradictory results in this respect. There was no association between the incidence of GT symptoms and thrombocytopenia and the total and free MPA pharmacokinetic parameters in the group of pediatric kidney transplant recipients [8]. In a prospective, randomized, double-blind, multicenter and controlled study of 150 renal transplant recipients, a dose-dependent increase in the adverse effects of kidney recipients was observed in the first 6 months following transplantation. However, no relationship between MPA trough concentrations or AUCs and adverse effects was detected [9]. In a retrospective study, 4 of 27 kidney transplant recipients with

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Table 5. Correlations of MPA and its metabolites concentrations with hemoglobin concentration, blood platelets count, and white blood cells count in the 2 groups: kidney and liver transplant recipients.

		Ktx (n=162)		Ltx (n=49)	
		r	p	r	p
Hb	MPA	–0.001	0.99	0.21	0.16
	GluMPA	–0.19	0.015*	0.09	0.54
	AcMPAG	–0.02	0.8	0.03	0.86
	MPAG	–0.28	0.0003*	–0.09	0.55
Plt	MPA	0.05	0.54	0.24	0.09
	GluMPA	0.06	0.43	0.25	0.09
	AcMPAG	–0.02	0.77	0.15	0.3
	MPAG	0.07	0.4	0.32	0.02*
WBC	MPA	–0.01	0.92	0.09	0.54
	GluMPA	0.05	0.57	0.06	0.67
	AcMPAG	0.02	0.8	0.05	0.72
	MPAG	0.11	0.15	0.004	0.98

Ktx – kidney transplantation; Ltx – liver transplantation; MPA – mycophenolic acid; GluMPA – MPA glucoside; AcMPAG – MPA acyl-glucuronide; MPAG – MPA phenolic glucuronide; Plt – blood platalets; Hb – hemoglobin; WBC – white blood cells; * p<0.05.

gastrointestinal adverse effects had significantly lower MPA AUC values. It was suggested that, in patients with gastrointestinal toxicity, drug absorption is decreased, leading to further local irritation [10].

Some authors have postulated the involvement of AcMPAG in causing gastrointestinal disturbances among patients treated with mycophenolate mofetil or sodium [11]. We did not observe such associations in kidney recipients. Other studies were also unable to confirm the relationship between MPA metabolites and GT: Grinyo et al. did not find correlations between MPA, AcMPAG exposure or maximal concentration (C max) of AcMPAG and the occurrence of gastrointestinal symptoms after 7 days and after 1 month in their pharmacokinetic study of 82 renal transplant recipients [12]. Heller et al. were not able to find any relationship between AcMPAG concentrations and the incidence of diarrhea in renal transplant recipients [13].

Little is known about MPA metabolites and their associations with gastrointestinal problems in liver transplant recipients. We have found a negative influence of MPAG levels on gastroenterotoxicity in liver transplant recipients. The clinical significance of this finding remains unclear. It cannot be excluded that lower MPAG levels are the consequence of decreased enterohepatic circulation in the course of diarrhea. Interestingly, MPA concentrations were not related to gastrointestinal symptoms in this group. In a study of 67 liver transplant patients the occurrence of diarrhea was not related to pharmacokinetics of MPA and its metabolites [14].

There are few reports available on the impact of the concentrations of MPA and its metabolites on the incidence of infectious complications in SOT patients. It has been proposed that AcMPAG may contribute to side effects of the MPA formulations, which include myelotoxicity, infections and gastroenterotoxicity [15]. Those toxic effects may be mediated by interleukin-6 and tumor necrosis factor alpha induced by AcMPAG in human mononuclear leukocytes [11]. Interestingly, it has been demonstrated that some of the effects of AcMPAG are independent of the major mechanism of MPA action, which is associated with inosine monophosphate dehydrogenase inhibition [16]. It has been speculated that AcMPAG could exert an antiproliferative effect and inhibit proliferation of human mononuclear leukocytes via a mechanism independent of guanosine triphosphate (GTP) depletion [15]. Other studies have shown contradictory results regarding the influence of MPA levels on infectious complications. The mean MPA through concentrations were higher in 13 patients with adverse effects, mainly infectious, compared with those without adverse effects in a retrospective study of 30 renal transplant recipients [17]. On the other hand, in a retrospective study of 21 renal transplant patients over the first 28 days following transplantation, no differences in the drug dosage or MPA AUC were detected between recipients with and without viral infections [18]. Some authors reported relationship between high free MPA-AUC and MPA-Cmax levels and infections [8]. The authors did not observe such a relationship when the total MPA pharmacokinetic parameters were analyzed.

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In our study in kidney transplant recipients neither MPA nor its metabolites influenced bacterial or viral infections' occurrence. However, in liver transplant recipients unfavorable influence of AcMPAG on bacterial infections' incidence was observed. We have not found any reports concerning relationship among MPA, its metabolites and infection episodes in Ltx patients. This finding seems to be interesting and suggests the need of further research on this topic.

In multivariate analyses we did not confirmed negative correlations of GluMPA and MPAG concentrations and hemoglobin levels in Ktx patients. However, other authors observed a negative influence of MPA, MPAG and AcMPAG on erythropoiesis. In a prospective study of 100 renal transplant patients, those with anemia and leucopenia had significantly higher MPA AUC12 than those with normal RBC and WBC [19]. A prospective multicenter study of 33 kidney transplant patients demonstrated a relationship of high MPAG and AcMPAG levels with leucopenia and anemia [20]. In the group of 106 renal transplant recipients, the MPAG pharmacokinetic parameters correlated negatively with hemoglobin and hematocrit [21]. The authors of this paper concluded that MPAG might be a predicting factor for the side effects of mycophenolates. Ting et al. reported that anemia, leucopenia, infection and rejection occurred in lung and heart transplant recipients with higher AcMPAG AUC [22]. Inconclusive results of the research to date suggest that also in this field there is a need of analyses of larger patients' groups. In liver transplant recipients we did not observe the influence of MPA metabolites on hemoglobin levels. We did not also find any reports on this issue in the literature. We hope to obtain more conclusive results from analyses of larger group, which is currently recruited by our team.

In kidney transplant recipients we found no associations between MPA or its metabolites and thrombocytopenia. It is consistent with observations made by other investigators. Weber et al. did not observe any associations between free or total MPA pharmacokinetic parameters and thrombocytopenia in kidney transplant recipients [8]. In liver transplant patients GluMPA was associated with thrombocytopenia, suggesting a disadvantageous effect of GluMPA on thrombocytopoiesis in the bone marrow. Additionally, higher MPA levels were associated with higher Plt count, which was confirmed by multivariate analyses. The significance of this finding remains elusive and needs further investigation.

The relationship between MPA levels and gastrointestinal symptoms, observed in kidney transplant patients, was not confirmed in the liver transplant group, possibly because the number of patients in this group was too small. The fact that the association between MPA metabolites and other adverse effects occurred only in liver transplant recipients is interesting. We hypothesized that it might be caused by lower eGFR values in the Ktx group in comparison with liver transplant recipients. It could result in increased accumulation of MPA metabolites and cause attenuation of interplay between them and the analyzed complications.

The present study has some limitations, including its observational nature and limitation to a single center. The lack of exclusion criteria reflects the natural characteristics of this patient population after solid organ transplantation. The heterogeneity of the group is also left open to being influenced by confounding factors, such as various lengths of time elapsed since transplantation, 2 different MPA prodrugs, and different immunosuppression regimens. It has been proved that there are some differences between MMF and MPS preparations in terms of maximal concentration and AUC profiles, but their impact on IMPDH activity is not well defined [23,24]. In pediatric liver transplant patients there was no statistically significant difference between formulations of MPA in the gene expression of IMPDH 2, in the AUC(0-12h), or in C max, but peak concentration occurred later with MPS [25]. Parallel analysis of the concentrations of MPA and its metabolites in patients after Ktx and Ltx allowed a unique comparison of differences in their impact on adverse reactions occurrence between kidney and liver transplant recipients.

Conclusions

In conclusion, there are differences in the relationship between concentrations of MPA or its metabolites and adverse effects in kidney graft recipients and liver graft recipients. Those differences only partially could be explained. Precise measurements of the total MPA trough concentrations seem to be important in kidney recipients to monitor adverse gastrointestinal effects. On the other hand, the quantification of AcMPAG concentrations in liver transplant recipients receiving immunosuppressive therapy with MPA may be helpful in avoiding bacterial infections. Additionally, GluMPA, of unknown significance so far, seems to have a toxic effect on thrombopoiesis in this group. The assessment of the GluMPA concentrations could help to prevent thrombocytopenia. Further studies are needed to verify if monitoring of MPA and its metabolites would be beneficial for the long-term management of patients receiving mycophenolate formulations. The quantification of MPA metabolites could then be an important part of therapeutic monitoring and may be helpful in establishing safer immunosuppressive therapies.

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