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Received for publication: 31.8.10; Accepted in revised form: 15.2.11

Nephrol Dial Transplant (2011) 26: 3784–3793
doi: 10.1093/ndt/gfr130
Advance Access publication 22 March 2011

Influence of MRP2 on MPA pharmacokinetics in renal transplant recipients–results of the Pharmacogenomic Substudy within the Symphony Study

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Abstract

Background. The aim of this study was to determine the relationship between single-nucleotide polymorphisms (SNPs) in MRP2 genes and mycophenolic acid (MPA) pharmacokinetics in renal transplant recipients of the Symphony Pharmacogenomic substudy.

Methods. Sixty-six renal transplant recipients of eight Spanish centres were randomized into four branches of immunosuppressive regimen: low dose of cyclosporine, standard dose of cyclosporine, tacrolimus and sirolimus, all in addition to mycophenolate mofetil and steroids. Fifty-five patients were genotyped for SNPs in MRP2, C24T and C3972T. Pharmacokinetic sampling was done before MPA administration and up to 12 h post-dose at Day 7, 1 month and 3 months post-transplant. Relationships of area under the curve (AUC) of MPA and MPAG plasma

sampling with the presence of MRP2 SNPs and with the immunosuppressive regimens were studied.

Results. At steady-state conditions, MPA-reduced exposure was observed in C24T variant allele in MRP2 (CC: 68.73 ± 6.78; *T: 48.12 ± 4.90, P = 0.023); no significant differences linked to C3972T SNP were observed. Taking into account groups of treatment, lower MPA AUC in variant allele of C24T was only found under macrolides treatment with statistically significant differences at Month 3 (Tac and SRL, CC: 86.52 ± 10.98 versus *T: 41.99 ± 4.82, P = 0.001; CsA, CC: 52.31 ± 5.30 versus *T: 54.24 ± 8.30, P = 0.772); for C3972T, the same tendency was found but differences at steady state did not reach statistical significance.

Conclusions. Renal transplant recipients T carriers of C24T MRP2 with macrolides treatment were associated with reduced MPA AUC in steady-state conditions. Patients treated with cyclosporine lost the effect of this polymorphism.

Keywords: Immunosuppressors; MRP2; mycophenolic acid; pharmacokinetics; single-nucleotide polymorphism

Introduction

Mycophenolate mofetil (MMF) is a pro-drug that is rapidly and almost completely absorbed from the gut where it is de-esterified to form active mycophenolic acid (MPA). MPA is primarily metabolized by uridine diphosphate-glucuronosyltransferase (UGT) 1A to the inactive form 7-*O*-MPA-glucuronide (MPAG) [1, 2] and by UGT2B7 to the pharmacologically active form acyl-glucuronide of MPA (AcMPAG) in the liver, gastrointestinal tract and kidney [2]. Later MPAG is excreted into the bile by multidrug resistance-associated protein 2 (MRP2; ABCC2 or cMOAT, canalicular multispecific organic anion transporter) [3, 4]. In the gut, MPAG transforms into MPA by bacterial deconjugation, which is absorbed in the colon. Because of this enterohepatic circulation, the initial MPA plasma concentration peaks at 1 h and is followed by a second increase in the MPA plasma concentration, occurring 6–12 h after oral administration. Preliminary studies in renal transplant recipients demonstrated that high free MPA AUC but not total MPA correlates with an increased risk for MPA-related haematological toxicity [5]. Because free MPA concentrations determine MPA's immunosuppressive action, factors that alter protein binding can affect the pharmacodynamic effect of the drug. These factors include hypoalbuminaemia or renal insufficiency which occurs in the early post-transplantation period. The accumulation of MPAG in patients with impaired renal function significantly reduces MPA binding to the albumin, increasing the MPA-free fraction [6]. In human subjects, any interference with enterohepatic circulation reduces MPA AUC by 35–40%. Finally, the majority of the absorbed MMF is eliminated by the kidneys as MPAG, mainly via tubular secretion [7, 8].

There is a growing interest in the impact of gene polymorphisms of drug-metabolizing enzymes and transporters. Some authors have suggested that MPA and/or MPAG pharmacokinetic variability could be caused by the single-nucleotide polymorphisms (SNPs) in genes encoding for UGTs and drug transporters including MRP2 and P-glycoprotein (Pgp) [9–11].

MRP2 is a member of the superfamily of ATP-binding cassette (ABC) transporters. This protein is expressed at the apical (canalicular) membrane of hepatocytes, the luminal membrane of proximal renal tubular cells and also in epithelial cells of the intestine, the placenta, and the blood-brain barrier [12–14]. MRP2 is considered to be the main transporter involved in MPAG excretion, both in the liver and in the proximal renal tubule. Several SNPs in the gene encoding this transporter have been described [14]. These SNPs may partly explain the large interindividual variation in MPA pharmacokinetics. C24T SNP in the MRP2 5′-UTR region occurs with a relatively high allelic frequency (18%) [14, 15] and the presence of this SNP has been related to a significantly higher dose-corrected MPA trough levels in stable renal allograft recipients. C24T polymorphisms have been furthermore associated with oral clearance of MPA [16].

Furthermore, strong interactions have been observed between MPA and other co-administered drugs such as cyclosporine and rifampin [3, 10]. Cyclosporine is an inhibitor of a variety of drug transporters [17–19] and may exert this effect through its interaction with drug transport and metabolic enzymes.

The present study evaluated the impact of MRP2 polymorphisms on MPA exposure parameters in *de novo* renal transplant recipients of the Symphony substudy [20] to assess the influence of these SNPs in the large interindividual variation in MPA and MPAG pharmacokinetics.

Materials and methods

This pharmacogenomic study is within the framework of the Symphony trial and it was based in the results of the pharmacokinetic substudy [20]. The Symphony substudy was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization—Good Clinical Practice Guidelines and with local ethical committee or institutional review board approval at each centre. All patients provided written informed consent before inclusion into the study.

Table 1. Sample description at baseline ($n = 55$)

	CsA[a] ($n = 30$)	Tac[b] ($n = 13$)	SRL[c] ($n = 23$)	P-value
Age, mean (SD)[d]	48.18 (10.18)	52.36 (13.43)	47.41 (12.03)	P = 0.444
Sex male (%)[e]	65.5	72.7	41.2	P = 0.165
Creatinine (mg/dL), mean (SD)[d]	8.52 (2.81)	8.17 (2.58)	6.89 (2.93)	P = 0.289
Albumin (g/dL), mean (SD)[d]	4.25 (0.45)	4.01 (0.55)	4.18 (0.80)	P = 0.633

[a]Standard immunosuppression with normal and lose dose of cyclosporine, MMF and corticosteroids (CS).
[b]Low dose of tacrolimus with daclizumab induction, MMF and CS.
[c]Low dose of sirolimus with daclizumab induction, MMF and CS.
[d]Kruskal–Wallis test.
[e]Chi-squared test. SD, standard deviation

Subjects

Sixty-six renal transplant recipients of eight Spanish centres took part in the Pharmacokinetic Symphony substudy. Patients were originally randomized into four branches of immunosuppressive regimen, all of them consisting of daclizumab induction, MMF and corticosteroids potentiated by either low dose of cyclosporine (CsA) or standard dose of CsA, tacrolimus (Tac) or sirolimus (SRL). During the follow-up, we did not observe statistical significance among CsA low and standard AUCs (nanograms per millilitre per hour). At Day 7 (low CsA 3347.64 ± 1173.90, standard CsA 5638.81 ± 3744.89, P = 0.094), at Month 1 (low CsA 3681.16 ± 1579.99, standard CsA 4356.40 ± 1306.00, P = 0.397) and at Month 3 (low CsA 3464.97 ± 742.72, standard CsA 3541.05 ± 1634.57, P = 0.829), the post-transplant values tended to converge. For this reason, low and standard dose of cyclosporine were summed into one group (CsA) for the statistical analyses, giving three treatment groups according to immunosuppressive regimen: low and standard dose of cyclosporine (CsA; $n = 30$), tacrolimus (Tac; $n = 13$) and sirolimus (SRL; $n = 23$).

Pharmacokinetic analysis

The $AUC_{0–12}$ of MPA and its metabolites between treatment groups were compared at each time along the follow-up. Pharmacokinetic data were collected on Day 7 and on Months 1 and 3 post-transplant. For this purpose, at each visit, 11 blood samples were collected: before the first MMF administration of the day [pre-dose (Time 0)] and up to 12 h post-dose (at 20, 40, 75 min and 2, 3, 4, 6, 8, 10 and 12 h post-dose). Pharmacokinetic analysis of MPA was carried out with a standard noncompartmental model using WinNonlin. All AUC results were dose corrected at 2 g/day to obtain the right correlation in the pharmacokinetic dates.

Table 2. Immunosuppressive regimens and pharmacokinetics of MPA and MPAG

	Drugs	*n*	Mean	SD	SE	P[a]
AUC MPA[b] Day 7	Tac[c], SRL[d]	17	55.188	19.961	4.841	0.009
	CsA[e]	22	40.251	17.275	3.683	
AUC MPA Month 1	Tac, SRL	22	70.367	25.862	5.514	0.001
	CsA	21	46.047	16.030	3.498	
AUC MPA Month 3	Tac, SRL	25	70.490	34.156	6.831	0.023
	CsA	22	52.946	19.831	4.228	
AUC MPAG[f] Day 7	Tac, SRL	16	1073.669	675.086	168.771	0.029
	CsA	22	1609.767	691.632	147.456	
AUC MPAG Month 1	Tac, SRL	23	813.721	327.465	68.281	0.064
	CsA	20	1244.194	879.209	196.597	
AUC MPAG Month 3	Tac, SRL	25	851.744	583.386	116.677	0.204
	CsA	23	959.674	456.653	95.219	
AUC-free MPA Day 7	Tac, SRL	17	1.392	0.414	0.100	0.123
	CsA	21	1.237	0.538	0.117	
AUC-free MPA Month 1	Tac, SRL	23	1.568	0.992	0.207	0.284
	CsA	20	1.391	1.002	0.224	
AUC-free MPA Month 3	Tac, SRL	20	1.677	0.947	0.212	0.299
	CsA	19	1.311	0.427	0.098	
AUC-free MPA/total MPA Day 7	Tac, SRL	17	0.027	0.009	0.002	0.163
	CsA	21	0.035	0.018	0.004	
AUC-free MPA/total MPA Month 1	Tac, SRL	22	0.024	0.016	0.003	0.100
	CsA	19	0.026	0.008	0.002	
AUC-free MPA/total MPA Month 3	Tac, SRL	20	0.023	0.006	0.001	0.563
	CsA	19	0.026	0.010	0.002	

[a]Mann–Whitney's *U*-test.
[b]Area under curve of mycophenolic acid.
[c]Low dose of tacrolimus with daclizumab induction, MMF and corticosteroids (CS).
[d]Low dose of sirolimus with daclizumab induction, MMF and CS.
[e]Standard immunosuppression with normal and lose dose of cyclosporine, MMF and CS.
[f]Area under curve of 7-*O*-MPA-glucuronide. SD, standard deviation.

Table 3. Ratio of MPA $AUC_{0–12}$: MPAG $AUC_{0–12}$ among four treatment groups of adult renal allograft patients receiving MMF 2 g/day[a]

	Standard-dose cyclosporine	Low-dose cyclosporine	Low-dose tacrolimus	Low-dose sirolimus
Day 7	$n = 13$	$n = 17$	$n = 13$	$n = 21$
Median	0.0156	0.0319	0.0588	0.0641
(range)	(0.004–0.070)	(0.016–0.152), P*= 0.036	(0.016–0.124), P*= 0.025/P† = 0.241	(0.007–0.181), P*=0.001/P† = 0.017
Month 1	$n = 12$	$n = 17$	$n = 15$	$n = 22$
Median	0.0368	0.0477	0.0758	0.1024 (0.033–0.305),
(range)	(0.015–0.075)	(0.028–0.206), P* = 0.002	(0.051–0.241), P* = 0.001/P† = 0.041	P* = 0.001/P† = 0.032
Month 3	$n = 12$)	$n = 18$	$n = 16$	$n = 19$
Median	0.0458	0.0622	0.0919	0.1187 (0.035–0.499),
(range)	(0.027–0.137)	(0.018–0.277), P* = 0.314	(0.049–0.286), P* = 0.003/P† = 0.075	P* = 0.011/P† = 0.191

[a]AUC0–12, = area under the concentration–time curve from 0 to 12 h; MPA, = mycophenolic acid; MPAG, = 7-O-MPA-glucuronide. * versus standard-dose cyclosporine; †versus low-dose cyclosporine (between-group comparisons conducted with Mann-–Whitney tests). AUC0–12 values are dose- normalized.

Table 4. MRP2 polymorphisms (C3972T and C24T) and pharmacokinetics of MPA and MPAG

	n	Mean	SD	SE	P[a]
MRP2: C3972T					
AUC MPA[b] Day 7					
CC	13	45.135	22.667	6.287	0.279
*T	24	49.410	17.870	3.648	
AUC MPA Month 1					
CC	14	54.687	24.991	6.679	0.650
*T	22	55.051	17.450	3.720	
AUC MPA Month 3					
CC	15	56.707	20.351	5.254	0.978
*T	25	57.543	22.430	4.486	
AUC MPAG[c] Day 7					
CC	13	1357.988	764.331	211.987	0.805
*T	23	1390.404	717.466	149.602	
AUC MPAG Month 1					
CC	14	927.254	402.657	107.614	0.626
*T	22	866.846	461.066	98.300	
AUC MPAG Month 3					
CC	15	1035.241	565.290	145.957	0.176
*T	26	800.375	519.300	101.843	
AUC-free MPA Day 7					
CC	13	1.153	0.497	0.138	0.068
*T	23	1.412	0.477	0.099	
AUC-free MPA Month 1					
CC	13	1.204	0.436	0.121	0.420
*T	23	1.516	0.969	0.202	
AUC-free MPA Month 3					
CC	12	1.471	0.661	0.191	0.728
*T	23	1.373	0.593	0.124	
AUC-free MPA/total MPA Day 7					
CC	13	0.028	0.011	0.003	0.657
*T	23	0.031	0.013	0.003	
AUC-free MPA/total MPA Month 1					
CC	12	0.024	0.009	0.003	0.773
*T	22	0.024	0.007	0.002	
AUC-free MPA/total MPA Month 3					
CC	12	0.027	0.011	0.003	0.667
*T	23	0.023	0.006	0.001	
MRP2: C24T					
AUC MPA Day 7					
CC	23	46.586	21.502	4.484	0.247
*T	14	50.079	16.140	4.314	
AUC MPA Month 1					
CC	23	60.322	29.422	6.135	0.730
*T	14	52.944	17.853	4.771	
AUC MPA Month 3					
CC	25	68.731	33.911	6.782	0.023
*T	16	48.121	19.588	4.897	
AUC MPAG Day 7					
CC	23	1353.447	681.406	142.083	0.729
*T	13	1423.373	820.989	227.701	
AUC MPAG Month 1					
CC	23	911.511	383.598	79.986	0.616
*T	14	867.387	509.576	136.190	
AUC MPAG Month 3					
CC,	26	978.774	559.249	109.678	0.195
*T	16	744.980	476.087	119.022	
AUC-free MPA Day 7					
CC	23	1.184	0.449	0.094	0.008
*T	13	1.556	0.495	0.137	
AUC-free MPA Month 1					
CC	22	1.274	0.547	0.117	0.370
*T	15	1.621	1.089	0.281	
AUC-free MPA Month 3					
CC	23	1.664	0.887	0.185	0.118
*T	13	1.183	0.417	0.116	
AUC-free MPA/total MPA Day 7					
CC	23	0.029	0.013	0.003	0.172
*T	13	0.033	0.011	0.003	

Continued

Table 4. *Continued*

	n	Mean	SD	SE	P[a]
AUC-free MPA/total MPA Month 1					
CC	21	0.022	0.008	0.002	0.130
*T	14	0.026	0.007	0.002	
AUC-free MPA/total MPA Month 3					
CC	23	0.026	0.009	0.002	0.587
*T	13	0.023	0.006	0.002	

[a]Mann–Whitney's *U*-test.
[b]Area under curve of mycophenolic acid.
[c]Area under curve of 7-*O*-MPA-glucuronide. SD, standard deviation.

For the analysis of pharmacokinetic interactions between drugs, C_{max} and $AUC_{0–12}$ values for MPA and its metabolites, cyclosporine, tacrolimus and sirolimus were normalized by the dosage of the medication taken prior to blood sampling.

Quantification of MPA and MPAG concentration

Plasma concentrations of MPA and MPAG were measured by high-performance liquid chromatography as previously reported [20]. Free MPA levels were determined using the Centrifree Micropartition® system (Amicon) following the methods of Nowak and Shaw [6]. Analysis of cyclosporine, tacrolimus and sirolimus exposures is referred to as the 'third drug' in this manuscript: whole-blood cyclosporine and tacrolimus concentrations were measured using enzyme immunoassay EMIT® methods in a Cobas Mira autoanalyser (Dade-Behring, Palo Alto, CA). SRL levels were determined using high-performance liquid chromatography with tandem mass spectrometry according to the methods described by Grinyo *et al.* [20]. All measurements were performed at the Laboratory of Pharmacology Hospital Clinic, Barcelona, Spain.

Genotyping of MRP2 polymorphisms

Due to the lack of DNA sample, 11 patients had to be withdrawn so genotyping was performed on 55 of the 66 renal transplant recipients. Patients were genotyped for SNPs in MRP2 gene, C24T and C3972T. DNA was extracted from a peripheral whole-blood sample using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Sydney, Australia) and was stored at −80°C until analysis. Genotyping procedures were performed by the MassARRAY™ SNP genotyping system (Sequenom Inc., San Diego, CA), following the manufacturer's instructions. The method involves multiplex polymerase chain reaction and Single base extension assays, designed by the AssayDesigner software (Sequenom Inc.), and followed by mass spectrometry analysis with the Bruker Autoflex MALDI-TOF mass spectrometer (Bruker Daltonics, Billerica, MA). Spectral output was analysed and checked using MassARRAY™ Typer 3.4 software (Sequenom Inc.). The genotyping platform is at the Spanish National Genotyping Centre's facilities at Santiago de Compostela University.

Statistical analysis

Demographic variables (age and sex), baseline characteristics and transplantation-related data as biochemical parameters and immunosuppressant doses were described by frequencies (percentages) and mean (standard deviation). Nonparametric statistics (Kruskal–Wallis, and chi-square test) were applied to study differences in baseline data according to immunosuppressive regimens. Moreover, differences at each time of the follow-up in AUC plasma samplings (MPA and MPAG) with respect to the presence of MRP2 polymorphisms and the immunosuppressive regimens were studied by using nonparametric Kruskal–Wallis and Mann–Whitney's *U*-tests. Finally, mixed-effect models (with analyses of covariance - ANCOVA-) were applied to test the effect of the treatments, polymorphisms (both inter-factors) and follow-up measurements (intra-factor) on AUC plasma samplings with controlled levels of creatinine and albumin.

Results

In total, 66 renal transplant recipients (58.6% males; mean age: 47.9 ± 11.8 years) were included. Descriptive analysis of the sample is shown in Table 1. As it can be seen, demographic variables (sex and age) did not show differences among groups of patients according to treatment. No significant differences either for biochemical parameters (creatinine or albumin) were observed at baseline.

The MMF daily dose range across the branches of immunosuppressive regimen was 1680–1946 mg over the first 3 months of treatment. Cyclosporine mean daily dose on Day 7 was 425 mg (±133 mg) in the standard-dose group and 236 mg (±58 mg) in the low-dose group, which decreased to 222 mg (±81 mg) and 183 mg (±79 mg), respectively, by Month 3. These doses corresponded to median trough levels by Day 7, Months 1 and 3 of 292, 218 and 164 ng/mL for the standard-dose group and 75.5, 109 and 80.5 ng/mL for the low-dose group, respectively. During the first 3 months, the tacrolimus and sirolimus daily dose ranges were 4.3–5.8 and 2.9–3.5 mg, respectively (median trough levels by Day 7, Months 1 and 3: 8.1, 7.7 and 7.1 ng/mL and 4.6, 7.5 and 7.8 ng/mL, respectively). The mean exposure ($AUC_{0–12}$) to cyclosporine, tacrolimus or sirolimus as appropriate during the first 3 months in the high-dose cyclosporine, low-dose cyclosporine, Tac and SRL groups were 4842.3–9230.7, 2796.2–3601.6, 129.3–152.3 and 134.1–160.9 μg/h/mL, respectively.

The general analyses of MPA, MPAG and free MPA pharmacokinetic according to the immunosuppressive regimens are shown in Table 2. MPA levels were statistically significantly lower under cyclosporine treatment at time point Day 7, Months 1 and 3 after transplantation. MPAG levels, on the contrary, were higher under cyclosporine treatment, marginally significant at time point Day 7. The ratio MPA/MPAG exposure also showed the correlation between lower levels of MPA and higher level of MPAG in patients receiving low-dose and standard-dose CsA compared to patients treated with tacrolimus or sirolimus (Table 3). Differences between the standard-dose cyclosporine group and the low-dose tacrolimus and low-dose sirolimus recipients were still observed at Month 3 (all $P < 0.05$ versus standard-dose cyclosporine). There were no significance between-group differences in the ratio of MPA/MPAG between both doses of CsA at Month 3 ($P = 0.314$).

Regarding the relation between MRP2 (C24T and C3972T) polymorphisms and MPA or MPAG pharmacokinetics, *ABCC2* C24T CC, CT and TT genotypes were detected in 30 (62.5%), 16 (33.3%) and 2 (4.16%) recipients, respectively. C3972 CC, CT and TT were found in 29 (60.4%), 16 (33%) and 3 (6.25%). The genotype distribution was in Hardy–Weinberg equilibrium.

Table 5. MRP2 polymorphism (C24T) and pharmacokinetics of MPA and MPAG; differences according to the immunosuppressive regimens

Drug	MRP2 C24T	*n*	Mean	SD	SE	P[a]
Tac[b], SRL[c]	AUC MPA[d] Day 7					
	CC	9	62.836	23.159	7.720	0.266
	*T	7	48.273	11.588	4.380	
	AUC MPA Month 1					
	CC	10	83.464	30.136	9.530	.026
	*T	8	54.669	16.704	5.906	
	AUC MPA Month 3					
	CC	12	86.520	38.038	10.981	0.001
	*T	8	41.999	13.633	4.820	
	AUC MPAG[e] Day 7					
	CC	9	976.069	606.799	202.266	0.346
	*T	6	1280.355	826.750	337.519	
	AUC MPAG Month 1					
	CC	11	770.363	309.819	93.414	0.934
	*T	8	761.776	324.316	114.663	
	AUC MPAG Month 3					
	CC	12	996.324	709.347	204.771	0.217
	*T	8	554.241	275.236	97.311	
	AUC-free MPA day 7					
	CC	9	1.391	0.240	0.080	0.427
	*T	7	1.473	0.566	0.214	
	AUC-free MPA Month 1					
	CC	11	1.471	0.583	0.176	0.509
	*T	8	1.319	0.496	0.175	
	AUC-free MPA Month 3					
	CC	11	2.150	1.006	0.303	0.003
	*T	7	0.979	0.357	0.135	
	AUC-free MPA/total MPA Day 7					
	CC	9	0.024	0.007	0.002	0.153
	*T	7	0.031	0.011	0.004	
	AUC-free MPA/total MPA Month 1					
	CC	10	0.019	0.006	0.002	0.131
	*T	8	0.024	0.007	0.002	
	AUC-free MPA/total MPA Month 3					
	CC	11	0.024	0.006	0.002	0.441
	*T	7	0.022	0.004	0.002	
CsA[f]	AUC MPA Day 7					
	CC	14	36.140	12.332	3.296	0.030
	*T	7	51.886	20.556	7.769	
	AUC MPA Month 1					
	CC	13	42.520	10.786	2.992	0.335
	*T	6	50.645	20.664	8.436	
	AUC MPA Month 3					
	CC	13	52.311	19.107	5.299	0.772
	*T	8	54.243	23.466	8.297	
	AUC MPAG Day 7					
	CC	14	1596.048	630.236	168.438	0.941
	*T	7	1545.960	860.502	325.239	
	AUC MPAG Month 1					
	CC	12	1040.897	410.910	118.620	0.512
	*T	6	1008.202	697.312	284.676	
	AUC MPAG Month 3					
	CC	14	963.731	418.532	111.858	0.838
	*T	8	935.719	571.651	202.109	
	AUC-free MPA Day 7					
	CC	14	1.051	0.507	0.136	0.003
	*T	6	1.652	0.428	0.175	
	AUC-free MPA Month 1					
	CC	11	1.078	0.450	0.136	0.160
	*T	7	1.967	1.490	0.563	
	AUC-free MPA Month 3					
	CC	12	1.218	0.448	0.129	0.160
	*T	6	1.421	0.371	0.152	
	AUC-free MPA/total MPA Day 7					
	CC	14	0.031	0.016	0.004	0.458
	*T	6	0.035	0.013	0.005	
	AUC-free MPA/total MPA Month 1					

Continued

Table 5. *Continued*

Drug	MRP2 C24T	*n*	Mean	SD	SE	P[a]
	CC	11	0.026	0.009	0.003	0.421
	*T	6	0.028	0.007	0.003	
	AUC-free MPA/total MPA Month 3					
	CC	12	0.026	0.011	0.003	1.000
	*T	6	0.024	0.007	0.003	

[a]Mann–Whitney's *U*-test.
[b]Low dose of tacrolimus with daclizumab induction, MMF and corticosteroids (CS).
[c]Low dose of sirolimus with daclizumab induction, MMF and CS.
[d]Area under curve of mycophenolic acid.
[e]Area Under Curve of 7-*O*-MPA-glucuronide.
[f]Standard immunosuppression with normal and lose dose of cyclosporine, MMF and CS. SD, standard deviation.

The pharmacokinetic parameters of MPA, MPAG and free MPA in the *ABCC2* C24T and C3972T genotypes are shown in (Table 4). As it can be seen, at Month 3, MPA exposure was associated with the presence of C24T (carriers of the C24T SNP had significantly lower MPA AUC). Free MPA AUC is statistically higher for C24T SNP carriers at Day 7 but at steady-state conditions, AUC is decreased (although not statistically significant). On the other hand, there were no significant differences in MPAG pharmacokinetic parameters associated to C24T or C3972T polymorphisms.

In Table 5, a separate comparison of pharmacokinetic parameters in the presence or absence of C24T SNP for each treatment (macrolides versus CsA) is presented. The tendency of lower MPA AUC can be seen in T carriers under macrolides treatment, with statistically significant differences at time points Months 1 and 3. On the contrary, MPA AUC was seen to be higher for T carriers under cyclosporine treatment, with a statistically significant difference at Day 7. Free MPA AUC is statistically significantly lower for T carriers under macrolides treatment at Month 3 and statistically significantly higher for T carriers under cyclosporine treatment at Day 7, showing the same tendency at the other time points.

A similar analysis for C3972T is presented in Table 6. In this case, MPA AUC was seen to be lower for T carriers under macrolides treatment, showing statistically significant differences at Month 1. On the contrary, higher values for MPA AUC were found for T carriers under CsA treatment, also showing statistically significant differences at Month 1.

Finally, Table 7 summarizes the main results of the ANCOVA analyses to measure the effects of the interaction of treatments, polymorphisms (inter-factors) and follow-up measurements (intra-factor) with controlled levels of creatinine and albumin. As it can be seen, the results of the ANCOVA analyses support the tendencies highlighted in the analyses described above. However, low percentages of variance in AUC levels were explained by each of the effects or interactions (and its associated error) included within these models (partial η^2 in Table 7).

Discussion

This article provides additional data to the pharmacokinetic substudy of Symphony, including the effects of MRP2 SNPs on the MPA exposure. Pharmacokinetics results supported the current evidence that the differences in MPA exposure between patients receiving MMF plus cyclosporine and MMF plus Tac or SRL are attributable to an interaction with cyclosporine, rather than with tacrolimus or sirolimus.

MRP/*ABCC*2 is a protein expressed at the luminal membrane of proximal renal tubular cells and is a key factor in MPA pharmacokinetic. This study demonstrates an association of the MRP2 (C24T and C3972T) SNPs with MPA pharmacokinetics in renal transplant recipients. Patients with a C24T SNP in MRP2 (T-carriers) showed a marked decrease in the MPA AUC in steady-state conditions. Our results confirm the reported correlation between ABCC2 C24T polymorphisms and the oral clearance of MPA [16, 21].

Naesens *et al.* [16] described the impact of the MRP2 C24T polymorphism on MPA pharmacokinetics. Renal recipients with this SNP were protected from a reduction in MPA exposure associated with mild liver dysfunction and hence prevents early under-immunosuppression and loss of clinical efficacy, suggesting an association between this SNP and lower oral clearance of MPA in steady-state conditions. In our study, results showed that the presence of MRP2 C24T SNPs led to a lower total and free MPA exposure at Month 3. These differences were significant in total MPA in stable renal recipients. MPAG exposure did not show differences with the presence or the absence of the C24T SNP.

In addition, the *ABCC2* C24T polymorphism seems to be associated with enhanced enterohepatic circulation of MPA. Hesselink *et al.* [3] reported that the decrease in the MPA exposure is caused by the inhibition of MRP2 protein. It could be explained either by the effect of CsA on MRP2 inhibition or by a C24T SNP in MRP2 gene [3, 19, 22, 23]. In this way, MRP2 C24T SNP could be associated with a lack of response of the gene promoter for inflammatory repressor mechanisms, thereby preserving MRP2 expression, MRP2 activity and MPA/MPAG enterohepatic recirculation within the normal range [16]. In our study, the pharmacokinetic parameters of MPA were influenced by C24T MRP2 genetic polymorphisms depending on the immunosuppressive treatment. CsA is a known potent competitive inhibitor of MRP2 and a pharmacokinetic interaction between this immunosuppressor and MPA mediated by MRP/*ABCC*2 has been described [3]. The MPA exposure

Table 6. MRP2 polymorphism (C3972T) and pharmacokinetics of MPA and MPAG; differences according to the immunosuppressive regimens

Drug	MRP2 C3972T	*n*	Mean	SD	SE	P[a]
Tac[b], SRL[c]	AUC MPA[d] Day 7					
	CC	5	62.442	27.665	12.372	0.692
	*T	11	53.747	16.156	4.871	
	AUC MPA Month 1					
	CC	5	85.178	6.181	2.764	0.020
	*T	12	57.853	19.169	5.533	
	AUC MPA Month 3					
	CC	6	74.603	12.060	4.923	0.066
	*T	13	55.967	23.627	6.553	
	AUC MPAG[e] Day 7					
	CC	5	668.310	186.527	83.417	0.066
	*T	10	1312.520	758.216	239.769	
	AUC MPAG Month 1					
	CC	6	756.762	361.932	147.758	1.000
	*T	12	747.637	294.320	84.963	
	AUC MPAG Month 3					
	CC	6	953.995	806.862	329.400	0.930
	*T	13	741.262	541.293	150.128	
	AUC-free MPA Day 7					
	CC	5	1.304	0.144	0.064	0.234
	*T	11	1.482	0.471	0.142	
	AUC-free MPA Month 1					
	CC	6	1.429	0.353	0.144	0.708
	*T	12	1.359	0.631	0.182	
	AUC-free MPA Month 3					
	CC	5	1.668	0.813	0.363	0.527
	*T	12	1.480	0.753	0.217	
	AUC-free MPA/total MPA Day 7					
	CC	5	0.024	0.008	0.004	0.396
	*T	11	0.029	0.009	0.003	
	AUC-free MPA/total MPA Month 1					
	CC	5	0.018	0.002	0.001	0.140
	*T	12	0.024	0.007	0.002	
	AUC-free MPA/total MPA Month 3					
	CC	5	0.022	0.008	0.003	0.206
	*T	12	0.024	0.005	0.001	
CsA[f]	AUC MPA Day 7					
	CC	8	34.319	9.789	3.461	0.111
	*T	13	45.739	19.045	5.282	
	AUC MPA Month 1					
	CC	9	37.748	9.588	3.196	0.050
	*T	10	51.690	15.441	4.883	
	AUC MPA Month 3					
	CC	9	44.776	15.280	5.093	0.136
	*T	12	59.250	21.968	6.342	
	AUC MPAG Day 7					
	CC	8	1789.036	655.368	231.708	0.277
	*T	13	1450.315	709.654	196.823	
	AUC MPAG Month 1					
	CC	8	1055.124	404.887	143.149	0.790
	*T	10	1009.898	590.310	186.672	
	AUC MPAG Month 3					
	CC	9	1089.406	379.571	126.524	0.133
	*T	13	859.488	511.124	141.760	
	AUC-free MPA Day 7					
	CC	8	1.059	0.620	0.219	0.054
	*T	12	1.347	0.494	0.143	
	AUC-free MPA Month 1					
	CC	7	1.011	0.426	0.161	0.160
	*T	11	1.687	1.252	0.377	
	AUC-free MPA Month 3					
	CC	7	1.331	0.553	0.209	0.821
	*T	11	1.257	0.348	0.105	
	AUC-free MPA/total MPA Day 7					
	CC	8	0.031	0.013	0.004	0.877
	*T	12	0.033	0.016	0.005	
	AUC-free MPA/total MPA Month 1					
	CC	7	0.028	0.010	0.004	0.435
	*T	10	0.025	0.007	0.002	

Continued

Table 6. *Continued*

Drug	MRP2 C3972T	*n*	Mean	SD	SE	P[a]
	AUC-free MPA/total MPA Month 3					
	CC	7	0.031	0.011	0.004	0.160
	*T	11	0.022	0.007	0.002	

[a]Mann–Whitney's *U*-test.
[b]Low dose of tacrolimus with daclizumab induction, MMF and corticosteroids (CS).
[c]Low dose of sirolimus with daclizumab induction, MMF and CS.
[d]Area under curve of mycophenolic acid.
[e]Area under curve of 7-*O*-MPA-glucuronide.
[f]Standard immunosuppression with normal and lose dose of cyclosporine, MMF and CS. SD, standard deviation.

Table 7. General key findings highlighted by ANCOVA analyses (mixed-effect models)

Covariates: creatinine and albumin levels at baseline
MRP2 C24T
AUC MPA[a]
Significant effect of the interaction of times of follow-up with drugs (macrolides, normal CsA and low CSA). $F_{2,21}$= 3.355; P = 0.054; partial $\eta^2 = 0.242$.
Significant effect of the interaction of MRP2 C24T (T carriers versus non-carriers) with drugs (macrolides, normal CsA and low CSA). $F_{2,21} = 7.280$; P = 0.004; partial $\eta^2 = 0.41$.
AUC MPAG[b]
Significant effect of times of follow-up (Day 7 and at 1 month). $F_{1,14} = 5.107$; P = 0.04; partial $\eta^2 = 0.41$.
Significant effect of drugs (macrolides, normal CsA and low CSA). $F_{2,14} = 10.501$; P = 0.002; partial $\eta^2 = 0.60$.
AUC-free MPA
Significant effect of the interaction of MRP2 C24T (T carriers versus non-carriers) with drugs (macrolides, normal CsA and low CSA). $F_{2,11} = 4.155$; P = 0.045; partial $\eta^2 = 0.43$.
AUC-free/TOTAL MPA
No significant effects found.
MRP2C3972T
AUC MPA
Significant effect of the interaction of times of follow-up (Day 7 and at 1 month) with drugs (macrolides, normal CsA and low CSA). $F_{2,14} = 4.564$; P = 0.030; partial $\eta^2 = 0.39$.
Significant effect of the interaction of MRP2SC39724T (T carriers versus non-carriers) with drugs (macrolides, normal CsA and low CSA). $F_{2,14} = 4.971$; P = 0.023; partial $\eta^2 = 0.41$.
AUC MPAG
Significant effect of the interaction of times of follow-up (between AUC MPAG levels at Day 7 and at 1 month) with creatinine. $F_{1,14} = 6.582$; P = 0.022; partial η^2= 0.32.
Significant effect of the interaction of times of follow-up (between AUC MPAG levels at Day 7 and at 1 month) with MRP2SC39724T (T carriers versus non-carriers) and drugs (macrolides, normal CsA and low CSA). $F_{2,14} = 3.947$; P = 0.044; partial $\eta^2 = 0.36$.
AUC-free MPA:
No significant effects found.
AUC-free/total MPA:
No significant effects found.

[a]Area under curve of mycophenolic acid.
[b]Area under curve of 7-*O*-MPA-glucuronide.

in low-dose CsA group showed higher levels than normal-dose CsA group but the statistical significance disappeared at Month 3. For this reason and due to the small number of patients, the analysis of C24T and C3972T polymorphisms as normal- and low-dose CsA was merged in one group [20].

Our results showed that only renal patients treated with macrolides, both tacrolimus and sirolimus, presented differences between T carriers and non-carriers in total and free MPA exposure in steady-state conditions, suggesting an interference of the biliary excretion of MPAG Naesens *et al*. [24]. When patients were treated with CsA the effect of the SNP in the steady-state conditions was lost, suggesting that the effect of CsA on enterohepatic circulation overcomes depending on the SNPs. Nevertheless, patients with C24T genetic polymorphsim and treated with cyclosporine presented differences on total and free MPA at Day 7 before achieving the stable MPA levels. It suggests a masking effect of cyclosporine against the C24T genetic polymorphism so in this case, in patients treated with CsA, to normalize dose according the C24T SNP is not necessary.

The MRP2 C3972T SNPs are described to protect solid organ transplant recipients from the reduction in MPA exposure associated with renal dysfunction [16]. As C3972T is a silent SNP without effect on amino acid translation, it is unlikely that this SNP influences MRP2 expression or functional activity, and the association between this SNP and MPA pharmacokinetics is most probably mediated via linkage disequilibrium with C24T [25]. In our study, we found differences between patients with SNP in C3972T and patients non-carriers depending on the immunosuppressor treatment. Under macrolides therapy, T carriers showed a lower total MPA exposure at steady-state conditions presenting a similar pattern to C24T SNP. On the contrary, in patients treated with CsA, the effect of the SNP is the opposite. In this way, T carriers would need less dose of MPA to achieve the same MPA exposure as non-carriers only under CsA treatment. This effect is due to the interference of CsA on enterohepatic recirculation by MRP2 inhibition [3].

In conclusion, this study describes the impact of the MRP2 C24T and C3972T polymorphisms on MPA pharmacokinetics depending on the immunosuppressive regimen in steady-state conditions. In consequence, when patients are treated with macrolides, these MRP2 SNPs really should be considered to normalize MPA doses to avoid low drug exposure. Further studies in drug–transporters interaction in patients receiving mycophenolic acid and/or other immunosuppressors might be useful to deep in their pharmacokinetics. Future studies are needed to approach in different mechanisms to illustrate the relation of MRP2 polymorphismes and MPA exposure, which could be explained studying the correlation of MRP2 SNPs with MRP2 expression and activity.

Limitations of the study

This study has a relatively small number of patients due the exhaustive pharmacokinetic study. On the other hand, some of the DNA samples were not processed for logistic problems intrinsic in multi-centre studies. We considered 3 months as enough time of follow-up to achieve steady-state conditions. There were possible influencing factors such as diarrhoea but these data were not collected in the study.

Acknowledgements. Funding for this study was provided by F. Hoffmann La–Roche Ltd. Editorial assistance was provided by Tracy Harrison and Richard Glover from Wolters Kluwer Health with the financial support of F. Hoffmann La–Roche Ltd. We thank Susana Traseira for her initial support to the study and would also like to acknowledge the support from Roche Farma S.A, Spain, for critically reviewing the manuscript. We are sincerely grateful to Anna Caldes and Maria Sarrias for the logistic support in hospital samples recruitment. We are grateful to Helena Colom for her critical comments. We thank all the healthy volunteers who have contributed to the study. This study was supported by Roche Foundation and grants from Instituto de Salud Carlos III (CP06/00067) and Ministerio de Sanidad y Consumo (FIS PI070768).

Transparency declarations.

Authors declare that they have had no involvements that might raise the question of bias in the work reported or in the conclusions, implications or opinions stated.

Conflict of interest statement. None declared.

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Received for publication: 2.6.10; Accepted in revised form: 16.2.11