International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

INFLUENCE OF MULTIDRUG RESISTANCE PROTEIN-2 GENETIC POLYMORPHISM ON THE PHARMACOKINETICS OF MYCOPHENOLATE MOFETIL IN THAI KIDNEY TRANSPLANT PATIENTS

Wanarat Anusornsangiam [1], Duangchit Panomvana [1*], Kearkiat Praditpornsilpa [2], Amnart Chaiprasert[3], Surat Thungpanit[4]

[1]Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
[2]Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
[3]Department of Medicine, Phramongkutklao Hospital, Bangkok 10400, Thailand
[4]Department of Medicine, Police General Hospital, Bangkok 10400, Thailand

***Corresponding author e-mail:** Duangchit.p@chula.ac.th

ABSTRACT

The objective of this study was to investigate the influence of multidrug resistance protein (MRP), *MRP-2 -24C>T* polymorphism on the pharmacokinetics of mycophenolate mofetil in 118 Thai kidney transplant patients. The patients with *MRP-2 -24C>T* variant had a predicted area under the concentration-time curve ($AUC_{0\text{-}12\ hr}$) of mycophenolic acid (MPA) significantly lower than the patients with wild-type gene (5.04 versus 5.92 mg x h/L/kg/mg dose, respectively, *p*-value = 0.008). In addition, the oral clearance of MPA in the patients with *MRP-2 -24C>T* variant was significantly higher than that of MPA in the patients with wild-type gene (0.15 versus 0.12 L/h/kg, respectively, *p*-value = 0.025). Therefore, this single nucleotide polymorphism (SNP) reduced MPA exposure and might lead to inferior immunosuppressive effect and eventually loss of clinical outcomes of immunosuppressive drug in Thai kidney transplant patients.

Keywords: Mycophenolate mofetil; Genetic polymorphism; MRP-2; Pharmacokinetics; Kidney transplant patients

INTRODUCTION

Mycophenolate mofetil (MMF), an ester prodrug of mycophenolic acid (MPA), is an immunosuppressive agent used after solid organ transplantation. In kidney transplant patients, MMF is widely used along with calcineurin inhibitors (cyclosporine and tacrolimus) or the proliferation signal inhibitors (sirolimus and everolimus) and corticosteroid at a fixed oral dose (2 g/day) without routinely monitoring plasma levels of MPA.[1,2] Following oral administration, MMF is rapidly and totally converted to MPA. Uridine diphosphate-glucuronosyltransferases (UGTs) metabolize MPA via glucuronidation in the gastrointestinal tract, liver and kidney to be an inactive 7-O-glucuronide MPA (MPAG) and a lesser pharmacological active acyl glucuronide (AcMPAG). Most MPA metabolites are excreted via kidney. However, MPAG is excreted into the bile mediated by canalicular transporter, multidrug resistance-associated protein-2 (MRP-2), and is then converted to MPA via intestinal microflora β-glucuronidase resulting in reabsorption of MPA into the systemic circulation. From the previous study, enterohepatic recirculation of MPA was presumed to account for secondary peak that could occur in the plasma profile of MPA.[3,4]

There has been increasing of interests in the impact of gene polymorphisms of drug metabolizing enzymes and transporters. It was found in the

previous study that single nucleotide polymorphisms (SNPs) in genes encoding for UGTs and drug transporters might cause the MPA pharmacokinetic variability.[5-9] MRP-2 is an adenosine triphosphate (ATP)-dependent efflux transporter encoded by the *MRP-2* gene. MRP-2 is responsible for the biliary excretion of MPAG.

SNPs leading to altered MRP-2 activity might influence this process and therefore affect MPA exposure.[10,11] The most extensive study was a SNP that involved a C to T transition at position -24 on promoter region.[12,13] Neasens et al [14] reported that *MRP-2 -24C>T* polymorphism was associated with oral clearance of MPA; however, there was not significantly different in the trough level. Moreover, there were conflicts in the oral clearance of MPA between the patient with the CC genotype and those with the T allele in the study of Miura et al [15] data regarding the functional significance of this SNP. Hence, the objective of this study was to compare pharmacokinetic parameters of MMF in Thai patients with different *MRP-2 -24C>T* genotypes.

MATERIALS AND METHODS

Study design: This study was designed as a prospective analytical study. The study protocol was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (10 ethical committees), Royal Thai Army Medical Department (14 ethical committees) and Police General Hospital (9 ethical committees). Demographic data and laboratory blood test data were collected from electronic patient database and patient interviews during April 2012 to September 2012.

Patients: Outpatients aged 18 years or over who were receiving MMF dose twice daily for at least one week and who attended the Post-kidney transplantation Clinic at King Chulalongkorn Memorial Hospital, Phramongkutklao Hospital and Police General Hospital, were approached to participate in this study. Patients agreed to be in the study and gave their consents by signing in the informed consent form. Patients with multiple organ transplantations, hepatic disease or taken concomitant drugs that might have drug interaction with MMF such as antacids, cholestyramine, metronidazole, rifampicin were excluded from the study.

Blood sampling: Blood samples were collected from the patients before dosing (i.e. pre-dose), 30 minutes and 2 hr after administration of MMF morning dose. At each blood collection time point, 5 mL of whole blood was collected in the tube containing ethylenediamine tetraacetic acid (EDTA) as an anti-coagulant. Plasma samples were obtained by centrifugation of blood samples at 3000g for 10 minutes at temperature of 20^{o}C. At each collection time point, plasma sample was transferred into a 1.5 mL of microcentrifuge tube for MPA concentration analysis and the buffy coat was collected into a 1.5 mL of microcentrifuge tube for genomic DNA extraction. All samples were kept at -20^{o}C until analysis.

Genotyping: Genomic DNA was extracted from 200 µL of buffy coat sample using a QIAamp DNA blood mini kit (Qiagen, Germany), according to the manufacturer's protocol. The subjects were genotyped for *MRP-2* SNP (*MRP-2 -24C>T*, rs717620) by using a real-time polymerase chain reaction (PCR) method (ABI 7500 Real-Time PCR System; Applied Biosystem, USA) with specific probes (FAM-TaqMan BHQplus probe and CAL Fluor Orange 560-TaqMan BHQplus probe, Biosearch Technologies, Canada) and primers (Biosearch Technologies, Canada).

Measurement of MPA concentrations: MPA concentrations in plasma samples were analyzed using a validated high performance liquid chromatography (HPLC) with UV detector method modified from the method of Elbarbry et al [16] and Patel et al.[17] Briefly, plasma samples were prepared using protein precipitation method (0.1 mol/L cold phosphoric acid in acetonitrile). Chromatographic analysis of MPA and internal standard (carboxy butoxy ether of mycophenolic acid) was achieved with Zorbax Eclipse XDB-C18 (4.6 x 150 mm, particle size 5 µ, Agilent Technologies, USA) protected by a guard column Zorbax Eclipse XDB-C18 (4.6 x 12.5 mm, particle size 5 µ, Agilent Technologies, USA).

The chromatographic separation was performed at ambient temperature with gradient elution. The mobile phase components were methanol and 0.15% phosphoric acid in a ratio of 45:55% vol/vol at the first 4 minutes followed by a ratio of 64:36% vol/vol at 4.5-12 minutes and a ratio of 45:55% vol/vol at 12.5-16 minutes. The flow rate was set and remained at 1 mL/min throughout the 16-minute run. MPA and the internal standard were detected at a UV wavelength of 215 nm. The method was selective and reproducible in the range of 0.25 to 60 µg/mL. For intra-day and inter-day variations of accuracy and precision of this method, the coefficient of variation for the accuracy was less than 8.0% while that for the

precision measured at each quality control sample was less than 12.0%.

MPA area under the concentration-time curve $(AUC)_{0\text{-}12\ hr}$ estimation: Three time points (pre-dose, 30 minutes and 120 minutes after oral morning MMF dose) of blood collection were chosen according to equation that analyzed from the previous study of Tunwongsa [18] where full MPA pharmacokinetic data (series of blood collection samples at 0, 0.25, 0.5, 1, 2, 3, 4, 6 and 12 hours) of 20 Thai post-kidney transplant patients taking cyclosporine and MMF were determined. Limited sampling strategy (LSS) was developed and validated using the two-group method. Pharmacokinetic profiles from 11 subjects were randomly assigned as the index group to develop LSS. The profiles from the remaining 9 subjects were then used to validate the developed LSS. MPA $AUC_{0\text{-}12\ hr}$ of each patient was predicted using the limited sampling strategy equation below (coefficient of determination, r^2 = 0.868; bias = 1.42%; precision = 9.70%): MPA $AUC_{0\text{-}12\ hr}$= 17.808 + 5.56*C_0 + 0.548*$C_{0.5}$ + 2.126*C_2

Statistical Analysis: All data were analyzed using SPSS for Windows version 17.0 (SPSS Co., Ltd., Bangkok, Thailand). Distribution of continuous data was tested for normal distribution with the Kolmogorov-Smirnov test. A two-tailed alpha of less than 0.05 was considered statistically significant. Allele and genotype frequency were measured as percentages and analyzed using *Chi*-squared test. Pharmacokinetic parameters among patients with different *MRP-2* genotypes were compared using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test.

RESULTS

Patient demographics: Patient demographic data were summarized in Table 1. Times after post-transplantation ranged from 3 months to 228 months having a median of 54 months. The number of patients receiving cyclosporine as their immunosuppressive regimen was similar to that of patients receiving tacrolimus (46.60% versus 47.50%, respectively). Most patients (48.30%) received 1000 mg MMF daily dose (mean daily dose of 1211.86±339.10 mg with daily dose range of 500 to 2000 mg).

Genotyping study: *MRP-2 -24C>T* allele frequencies and distribution of genotypes were shown in Table 2. The CC, CT, and TT genotypes were found to be 61.86%, 30.51%, and 7.63%, respectively in Hardy-Weinberg equilibrium. Table 3 showed some selected characteristics of patients with different *MRP-2* genotypes. No significant difference in these characteristics was found among patients with different *MRP-2* genotypes.

MPA and MPAG concentrations: Mean concentrations of MPA and MPAG at pre-dose (C_0), 30 minutes ($C_{0.5}$) and 120 minutes (C_2) after morning MMF dose of patients with different *MRP-2 -24C>T* genotypes were presented in Table 4. No ne of the differences in MPA and MPAG concentrations at any time point were noted in the three *MRP-2 -24C>T* genotypes.

Impact of genetic polymorphism on pharmacokinetic parameters of MPA and its metabolite: The results in Table 5 indicated that patients with homozygous *MRP-2 -24C>T* variant genotype had a predicted MPA $AUC_{0\text{-}12\ hr}$ statistically lower when compared to that of the patients with wild-type gene and patients with heterozygous variant genotype. Clearance of drug (CL/F) in the patients with homozygous or heterozygous variant genotype was more likely to be higher than that in the patients with wild-type; however, the level of difference was not statistically significant. When combined the values obtained from the patients with heterozygous and homozygous variant genotypes, there were statistical differences of predicted MPA $AUC_{0\text{-}12\ hr}$ between patients with wild-type gene and patients with variant gene (*p*-value = 0.008). In addition, CL/F in the patients with *MRP-2* variant gene was statistically higher than that in the patients with wild-type gene (*p*-value = 0.025).

DISCUSSION

The prevalence of *MRP-2 -24C>T* among the 118 patients who participated in this study was 61.86%, 30.51%, and 7.63% for CC, CT and TT genotypes, respectively. In this study, this genotype frequenc y was found to be similar to that previously reported in Asian kidney transplant patients[15,19] and those measured in Caucasian populations.[14,20]

MPAG is extensively produced in the liver, partly excreted into the bile, and substantially hydrolyzed to MPA in the small intestine, leading to MPA reabsorption, which is estimated to the contribution of 10-60% of the total MPA exposure. In the previous study, it was found that the excretion of MPAG into the bile occurred through membrane drug-efflux transporter (MRP-2).[3,4] The results of this study demonstrated the association of the *MRP-2* polymorphism with MPA pharmacokinetics. The patients with *MRP-2 -24C>T* variant had a predicted

MPA $AUC_{0\text{-}12\ hr}$ significantly lower than that of the patients with wild-type gene. This could be implied that the patients with *MRP-2 -24C>T* variant genotype had lower activity for exporting MPAG into the bile resulting in decreasing enterohepatic recirculation of MPA. Meanwhile, the clearance of MPA in the patients with *MRP-2 -24C>T* variant was significantly higher than that of the patients with wild-type gene. The decrease in enterohepatic recirculation of MPA caused more MPAG excretion.

The impact of the *MRP-2 -24C>T* polymorphism might partly affect interindividual variation in MPA pharmacokinetics. Moreover, *MRP-2 -24C>T* SNP might cause inferior outcomes of the immunosuppressive drug. Our results demonstrated in the same direction as Zhou et al [21] who found that there was a strong trend towards decreased exposure to the anticancer drug, irinotecan, in those carrying this SNP. The results were also consistent with a study of Lloberas et al.[20] They determined the relationship between SNPs in *MRP-2* gene and MPA pharmacokinetics in 66 kidney transplant patients. At the end of 3 months, the patients with carriers of the *-24C>T* SNP had a significantly lower MPA $AUC_{0\text{-}12\ hr}$ compared with the patients with wild-type (48.12±4.90 versus 68.73±6.78 mg x h/L, *p*-value = 0.023).

The findings of this study; however, were differed from the previous study performed by Naesens et al [14] who reported that *MRP-2 -24C>T* variant had been associated with an increase in expression and activity resulting in enhanced enterohepatic recirculation and a lower oral clearance of MPA. They investigated the impact of *MRP-2* polymorphism on MPA exposure parameters in 95 Caucasian kidney transplant patients who treated with tacrolimus. They reported no difference in pharmacokinetic parameters (dose-normalised either $AUC_{0\text{-}12\ hr}$ or CL/F) at the day 7 after the kidney transplantation between non-carriers (n = 54) and carriers (n = 41) of the MRP-2 *-24C>T* SNP. On the day 42, 90 and 360 after the transplantation, dose-normalised MPA $AUC_{0\text{-}12\ hr}$ were consistently higher in the carriers of the *MRP-2 -24C>T* SNP (17.0%, 18.3% and 23.0%, respectively) compared to the non-carrier. These differences reached statistical significance only at six weeks after the transplantation (*p*-value = 0.008). The study by Miura et al [15] reported that on the day 28 after the kidney transplantation, there was no significant difference in the dose-normalised $AUC_{0\text{-}12\ hr}$, either trough level or oral clearance of MPA between the patients with wild-type and those with the T allele.

This discrepancy m ight be explained by several reasons. For instances, the period after the transplantation of the previous two studies differ ed greatly from the present study. The discrepancy between the MPA pharmacokinetics at an early period after the transplantation and those at more than three months later after the transplantation could be accounted for clinical factors interfering with MPA or MPAG disposition, such as hypoalbuminuria, anemia, delayed graft function, and corticosteroid doses. The limitation of this study was that the observation was performed on only one SNP of gene encoding the transporter proteins while the influence of other SNPs of genes especially those encoding the drug metabolizing enzyme UGTs has not been analyzed and evaluated. Further study should include the investigation of other genes encoding the transporter proteins and the interaction with those SNPs of genes encoding drug metabolizing enzymes which may have influence on the inter -individual variation in MPA pharmacokinetics.

CONCLUSION

The allele frequencies and genotype frequencies of *MRP-2 -24C>T* variant gene found in this study were similar to those previously reported for other Asian population. The presence *of MRP-2 -24C>T* variant caused significant variation in pharmacokinetic parameters of MPA. The predicted MPA $AUC_{0\text{-}12\ hr}$ was lower and the clearance of MPA was higher in the patients carrying variant genotype than those values found in the patients with wild-type genotype. This study demonstrated that the *MRP-2 -24C>T* SNP might be the useful clinical factor for individualising MPA therapy.

ACKNOWLEDGEMENT

We are grateful to Graduate School and Faculty of Pharmaceutical Sciences, Chulalongkorn University for supporting the research grant.

Table 1: Patient demographics

Data	Number of patients n (%)
Mean age (years old)	45.72±11.96
Sex	
Male/Female	86 (72.90)/32 (29.10)
Cause for transplantation	
- Unknown	56 (47.50)
- Chronic glomerulonephritis	24 (20.30)
- Hypertension	10 (8.50)
- IgA nephropathy	10 (8.50)
- Diabetic nephropathy	6 (5.10)
- Autosomal dominant polycystic kidney disease	4 (3.40)
- Focal segmental glomerulosclerosis	3 (2.50)
- Others	5 (4.20)
Type of donor graft	
Deceased/Living	67 (56.80)/51 (43.20)
Combined immunosuppressant drug	
- Cyclosporine	55 (46.60)
- Tacrolimus	56 (47.50)
- Sirolimus	6 (5.10)
- Everolimus	1 (0.80)
Body weight (kg), mean±S.D.	63.86±13.50
Serum creatinine (mg/dL), mean±S.D.	1.53±0.66
eGFR (mL/min/1.73m^2)*, mean±S.D.	57.29±21.40

** eGFR = estimated glomerular filtration rate was calculated using Modification of Diet in Renal Disease study equation*

Table 2: Allele frequency and genotype frequency of *MRP-2* SNP

Gene	SNP	Allele frequency		Genotype frequency (number of patients, %)			
		Wild-type	Variant	Wild-type *CC*	Heterozygous variant *CT*	Homozygous variant *TT*	*p*-value*
MRP-2 (rs717620)	*-24C>T*	0.77	0.23	73 (61.86)	36 (30.51)	9 (7.63)	0.141

** Chi-squared test*

Table 3: Characteristics of patients with different *MRP-2 -24C>T* genotypes

Patient's characteristic	*CC* (n = 73)	*CT* (n = 36)	*TT* (n = 9)	*p*-value*
Age (years old), mean±S.D.	45.67±11.92	45.58±12.53	46.67±11.96	0.970
MMF daily dose (mg), median	1000	1000	1500	0.133
Serum creatinine (mg/dL), median	1.40	1.36	1.47	0.714
eGFR (mL/min/1.73m^2), median	55.00	61.40	43.60	0.376

** Kruskal-Wallis test*

Table 4: Concentrations of MPA and MPAG among patients with different *MRP-2 -24C>T* genotypes

Concentrations	*CC* (n = 73)	*CT* (n = 36)	*TT* (n = 9)	*p*-value*
MPA (µg/mL) Mean±S.D.				
C_0	2.76±2.37	2.30±1.78	2.40±1.04	0.465
$C_{0.5}$	16.25±14.42	14.08±11.40	21.94±17.89	0.439
C_2	6.20±3.77	6.56±3.61	7.20±3.88	0.533
MPAG (µg/mL) Mean±S.D.				
C_0	50.60±26.96	44.92±24.61	56.15±29.44	0.334
$C_{0.5}$	57.17±30.13	52.25±28.92	63.60±26.59	0.448
C_2	80.53±36.69	78.36±48.07	89.39±31.02	0.360

** Kruskal-Wallis test*

Table 5: Comparison of pharmacokinetic parameters among patients with different *MRP- -24C>T* genotypes

Pharmacokinetic parameters	*CC* (n = 73)	*CT* (n = 36)	*TT* (n = 9)	*p*-value*	*CC* (n = 73)	*CT* and *TT* (n = 45)	*p*-value*
Predicted MPA $AUC_{0\text{-}12\ hr}$ (mg x h/L/kg/mg dose)	5.92	5.06	4.66	**0.013**	5.92	5.04	**0.008**
CL/F (L/h/kg)	0.12	0.15	0.15	0.052	0.12	0.15	**0.025**
MPAG:MPA ratio at C_0	19.00	20.11	24.69	0.841	19.00	20.22	0.705
MPAG:MPA ratio at $C_{0.5}$	4.01	4.09	2.66	0.940	4.01	3.89	1.000
MPAG:MPA ratio at C_2	14.51	12.52	13.49	0.638	14.51	12.99	0.448

CL/F was computed by MMF dose/$AUC_{0\text{-}12\ hr}$ * *Median test*

REFERENCES

1. Golahayan D, Pascual M, Vogt B. Ther Clin Risk Manag, 2009; 5(4): 341-51.
2. Dalal P, Grafals M, Chhabra D, Gallon L. Ther Clin Risk Manag, 2009; 5(1): 139-49.
3. Staatz CE, Tett SE. Clin Pharmacokinet, 2007; 46(1): 13-58.
4. Jeong H, Kaplan B. Clin J Am Soc Nephrol, 2007; 2(1): 184-91.
5. Jonge H, Kuypers DRJ. Transplant Rev, 2008; 22(1): 6-20.
6. Thervet E, Anglicheau D, Legendre C, Beaune P. Ther Drug Monit, 2008; 30(2): 143-50.
7. Betonico GN, Abbud-Filho M, Goloni-Bertollo EM, Panarino-Dertelli E. J Nephrol, 2008; 21(4): 503-9.
8. Dupuis R, Yuen A, Innocenti F. Clin Chim Acta, 2012; 413(7-8): 1318-25.
9. Barraclough KA, Lee KJ, Staatz CE. Pharmacogenomics, 2010; 11(3): 369-90.
10. Felipe CR, Sandes TV, Sampaio M, Park SI, Silva HT, Pestana JOM. Transplant Proc, 2009; 41(5): 1441-55.
11. Shuker N, Bouamar R, Weimar W, van Schaik RHN, van Gelder T, Hesselink DA. Clin Chim Acta, 2012; 413(17-18): 1326-37.
12. Suzuki H, Sugiyama Y. Adv Drug Deliv Rev, 2002; 54(10): 1311-31.
13. Ito S, Ieiri I, Tanabe M, Suzuki A, Higuchi S, Otsubo K. Pharmacogenetics, 2001; 11(2): 175-84.
14. Naesens M, Kuypers DRJ, Verbeke K, Vanrenterghem Y. Transplantation, 2006; 82(8): 1074-84.
15. Miura M, Satoh S, Inoue K, Kagaya H, Saito M, Inoue T, Suzuki T, Habuchi T. Eur J Clin Pharmacol, 2007; 63(12): 1161-9.
16. Elbarbry FA, Shoker AS. J Chromatogr B, 2007; 859(2): 276-81.
17. Patel CG, Akhlaghi F. Ther Drug Monit, 2006; 28(1): 116-22.
18. Tunwongsa K. Relationship between gastrointestinal adverse event and plasma concentrations of mycophenolate mofetil various sampling times in Thai renal transplanted patients [Master's Thesis]. Faculty of Pharmaceutical Sciences: Chulalongkorn University; 2002.
19. Geng F, Jiao Z, Dao YJ, Qiu XY, Ding JJ, Shi XJ, Li ZD, Zhong MK. Clin Chim Acta, 2012; 413(7-8): 683-90.
20. Lloberas N, Torras J, Cruzado JM, Andreti F, Oppenheimer F, Sanchez-Plumed J, Gentil MA, Brunet M, Ekberg H, Grinyo JM. Nephrol Dial Transplant, 2011; 26(11): 3784-93.
21. Zhou Q, Sparreboom A, Tan EH, Cheung YB, Lee A, Poon D, Lee EJD, Chowbay B. Br J Clin Pharmacol, 2005; 59(4): 415-24.