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Full length Research Paper

Integration of genetic and clinical data to analyze pharmacokinetic profile of mycophenolic acid in a population of Chinese patients with glomerular disease

Qian Xiang[1], #Feng Yu[2], Ji-cheng LV[2], Xia Zhao[1], Jing Han[1], Ying Zhou[1], Si-qian Du[1], Ming-hui Zhao[2], Hong Zhang[2], *Yi-min Cui[1] and *Hai-yan Wang[2]
 # Co-First Author

[1]Department of Pharmacy, Base for Clinical Trial, Peking University First Hospital, Beijing 100034 PR. China
[2]Department of Medicine, Renal Division, Peking University First Hospital, Institute of Nephrology, Peking University, Key laboratory of Renal Disease, Ministry of Health of China, Peking University First Hospital, Beijing 100034 PR. China

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We measured the free and plasma concentrations of total Mycophenolic acid (MPA) after single and multiple oral doses of Mycophenolate mofetil (MMF) in 24 glomerular disease patients with different renal function. Clinical characteristics and genetic polymorphisms of UGTs, MRP2, MDR1 and OATP8 were further investigated. After a single oral dose, those patients carrying the MDR1 3435CC allele had a 40.81% higher mean AUC_{0-24} of total MPA compared to MDR1 3435T carriers, and the difference was more significant in patients with an estimated glomerular filtration rate (eGFR) less than 60mL/min/1.73m^2. After repeat doses, MDR1 C3435T genotype in coordination with the MRP2C-24T allele elevated the total MPA level, and serum albumin level were positively correlated with free MPA exposure. Furthermore, there were strong negative correlations between eGFR and AUC_{6-12}/AUC_{0-12} for both free and total MPA. In conclusion, these factors should be evaluated to keep drug safety and guide proper therapy of MMF in patient with glomerular diseases.

Keywords: Mycophenolate mofetil, pharmacokinetics, genetic polymorphism, renal function, glomerular disease

INTRODUCTION

Mycophenolate mofetil (MMF) is an inhibitor of inosine monophosphate dehydrogenase, a key enzyme in the de novo pathway of purine synthesis, which is particularly important for the proliferation of both T and B lymphocytes.

The ester prod rug MMF is rapidly converted in vivo to the main active ingredient- mycophenolic acid (MPA). MPA is then metabolized to the phenolic glucuronide, which is mostly eliminated in the urine, with small amounts present in feces.

Besides the successful application of MMF in organ transplantation, it is evidence from randomize control study to use MMF in lupus nephritis for induction therapy (Ginzler et al., 2005) (Chan et al., 2000) and clinical observational study for the treatment of a common autoimmune glomerular disease, IgA nephropathy (Tang et al., 2010). But, unfortunatively significant inter-individual variable adverse events, such as infections, including deadly pneumocystis pneumonia (Tang et al., 2010), gastrointestinal intolerance, hematologic abnormability have been observed during the treatment of these diseases with MMF (de Winter and van Gelder, 2008). The altered pharmacokinetics of MPA is associated with these effects and several factors, especially renal function, have been reported to affect MPA exposure, but the results of these studies are inconsistent (Kaplan et al., 1998; Naito et al., 2006; Neumann et al., 2003; van Hest et al., 2006). There still lacked study of the pharmacokinetics of MPA in glomerular disease at different stages of renal function.

Furthermore, to investigate the underlying mechanism

*Corresponding Author E-mail: bdyyyljd@126.com

of above phenomenon between-patient variability, it is important to understand the potential genetic background of which candidates are genetic polymorphisms in genes encoding enzymes or transporters involved in MPA pharmacokinetics (Baldelli et al., 2007; Levesque et al., 2008; Lévesque et al., 2007). The role of genetic variation in MPA pharmacokinetics has been characterized in transplant recipients and healthy subjects. However, the genetic variation of MPA on pharmacokinetics has not been evaluated in Chinese population, neither analyzed combining with clinical characteristics, such as renal function, in patients with glomerular disease.

MPA is primarily metabolized by uridine diphosphate–glucuronosyltransferases (UGTs) to the inactive 7-O-glucuronide (MPAG) metabolite and a minor acyl glucuronide (AcMPAG) metabolite. MPA is a substrate of UGT1A7, 1A8, 1A9, and 2B7 (Lévesque et al., 2007). In addition to UGT isoenzymes, membrane transporters play a critical role in MPA deposition in the human body (Naesens et al., 2006). P-glycoprotein (P-gp, which is encoded by the MDR1 gene) and multidrug resistance-associated protein 2 (MRP2) are two membrane adenosine triphosphate -dependent transporters that mediate the excretory transport of various toxins and xenobiotics (Laouari et al., 2001; Maeda and Sugiyama et al., 2008). Many studies demonstrate that the MRP2 C-24T promoter variant effects MPA pharmacokinetics in renal transplant recipients and healthy subjects (Naesens et al., 2006). P-gp co-localizes and shares common substrates with MRP2 (Laouari et al., 2001). More recently, a mouse model demonstrated that the MPA plasma levels and cerebral concentrations were elevated in Mdr1a/1b-/- mice compared to wild-type mice (Wang et al., 2008). In addition, organic anion-transporting polypeptides 8 (OATP8), encoded by the SLCO1B3 genes, have also been reported to be involved in MPA pharmacokinetics (Miura et al., 2007; Tsujimoto et al., 2006).

The present study was designed with three objectives which might influence the pharmacokinetic of MMF in patient with glumerular nephropathy. Firstly, we investigated the clinical factors, especially glomerular filtriation rate (GFR). Secondly, we assessed the impact of genetic polymorphisms in UGTs, MDR1, MRP2, and OATP8 on MPA pharmacokinetics. Finally, we examined the associations between genetic polymorphisms and clinical factors affecting MPA pharmacokinetic parameters.

METHODS

Patients

Twenty-four Chinese patients diagnosed as lupus nephritis or IgA nephropathy between June 2007 and July 2008, with complete clinical and pathological data, from Peking University First Hospital, were recruited into this study. Additional inclusion criteria included an age of at least 18 years and an estimated glomerular filtration rate (eGFR; calculated with the Modification of Diet in Renal Disease (MDRD) formula (Levey et al., 2006)) ranging from 15 to 120 mL/min/1.73 m^2. The patients were further divided into four groups according their glomerular filtriation rate: group 1 with eGFR ranging from 90 to 120 ml/min (e.g. chronic kidney disease stage 1); group 2 with eGFR ranging from 60 to 90 ml/min (e.g. chronic kidney disease stage 2); group 3 with eGFR ranging from 30 to 60 ml/min (e.g. chronic kidney disease stage 3); group 4 with eGFR ranging from 15 to 30 ml/min (e.g. chronic kidney disease stage 4).

If a patient meets any one of all the following criteria, he/ she should be excluded from the study: (1) patients allergic to MMF, MPA or any other ingredients in the drug; (2) a positive result of urine pregnancy test; (3) patients in child-bearing period who cannot maintain effective contraception before, during and 6 weeks after MMF treatment phase; (4) patients with active peptic ulcer or severe diseases of digestive system; (5) patients with genetic deficiency in hypoxanthine-guanine phophoribosyl transferase, such as Lesch-Nyhan syndrome or Kelley-Seegmiller syndrome. None had received MMF or a calcineurin inhibitor before the study. Patients who took drugs that interfered with the absorption, disposition, metabolism or elimination of MMF were also excluded from this study.

Study design

The research was in compliance of the Declaration of Helsinki. The study protocol was approved by the Ethical Review Board of Peking University First Hospital (No.ZB-0701). All subjects gave their written informed consent prior to study participation.

Subjects were given a single 1 g oral dose of MMF on Day 1 of the study. From Day 2 to Day 7, the subjects were given a repeat dose of 1 g MMF twice daily. On Day 1 and Day 7, all of the patients fasted overnight and were not allowed to eat until 2 h after dosing. Blood samples were collected immediately prior to drug administration and at 0.5, 1, 2, 4, 6, 8, and 12 h after drug administration. Since the enterohepatic influence on the course of the AUC-profiles is unknown in the non-transplant use of the drug and to exclude a possible interference of a late secondary MPA peak with the initial peak of the next dosing after 12 h, we decided to evaluate PK profiles over 24 h after first dose (Naito et al., 2006). On Day 1, blood samples were also collected at 16 and 24 h after drug administration. Blood samples were collected in heparinized tubes (Vacutainer; Becton Dickinson; Franklin Lakes, NJ, USA) and centrifuged within 30 min. Plasma

was obtained and stored at -20℃ until assayed.

Assays of total mycophenolic acid (tMPA) and free mycophenolic acid (fMPA)

Solid-phase extraction of total plasma MPA from acidified samples was achieved based on the method of Ya Zhong et al. (Zhong et al., 2006) Free MPA was isolated from plasma by ultra filtration, and the disposable centrifuge cartridges consisted of a sample reservoir containing 10,000 Da MW cutoff membrane (Millipore, MA, USA) were used, and the tube was centrifuged for 40 min at 10,000g and 4 ℃ (Nowak and Shaw et al., 1995). Naproxen (100 mg/L in methanol for working solution, National institute for the control of pharmaceutical and biological products, Beijing; PR China) was used as internal standard.

Transfer 100 µl of patient plasma samples into 1.5 ml tubes and add 200 µl methanol containing 150 mg/L of naproxen to each tube. The prepared solution was vortexed for 30 s. The mixture was then centrifuged at 10,000×g , 40 min, 4℃, as well as fMPA samples. A 10µl aliquot of the upper clear supernatant was injected into the high performance liquid chromatography (HPLC) system for quantitation. Plasma total and fMPA concentrations were analyzed using HPLC as described previously (Zhong et al., 2006) (Srivatsan et al., 2004). Chromatography was performed using an Agilent 1100 series system. The separation was carried out with An Agilent Eclipse XDB-C8 column (150 mm × 4.6 mm, 5µm; Agilent, Ireland), a flow rate of 0.8mL/min was used. The column temperature was kept at 30℃. With the HPLC system was equilibrated with the mobile phase consisting of acetonitrile-32mM glycine buffer, pH 9.2(20:80, v/v), at a flow rate of 0.8 ml/min (Shen et al., 2005). The MPA samples were detected using a fluorescence detector at excitation and emission wavelengths were set at 325 nm and 435 nm, respectively. The validated assay is linear in the range of 0.05–40.0 mg/L for tMPA, 0.005–1.00 mg/L for unbound MPA. Samples above the linear range were diluted and reanalyzed. Inter- and intraday coefficients of variation (CV%) for MPA were less than 7.81%.

Pharmacokinetic analysis

Win Nonlin Professional Edition, Version 3.1 (Pharsight Corp., Mountain View, CA, USA) was used for Pharmacokinetic analysis and simulations. The peak plasma concentration (Cmax) and the time to reach Cmax (Tmax) were directly estimated from the observed plasma concentration–time data. The area under the plasma concentration–time curve from time 0–t h (AUC 0-t) was calculated using the linear trapezoidal rule.

Genetic analysis

DNA was extracted from peripheral whole blood samples obtained from each subject using a DNA Purification kit (Wizard, Promega, USA). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the UGT1A9*3, UGT1A8*2, UGT1A8*3, UGT2B7*2, MDR1 C1236T, MDR1 C3435T and MRP2 C-24T alleles as previously described (Naesens et al., 2006) (Tsujimoto et al., 2006) (Zhong et al., 2006) (Wu et al., 2007). Considering the reported important effect of UGT1A9 T-275A and C-2152T, we also investigated these genetic polymorphisms by PCR-RFLP (Kuypers et al., 2005), though the frequency of the UGT1A9 T-275A and C-2152T alleles is low in the Chinese population (Innocenti et al., 2005). The MDR1 G2677T/A alleles were evaluated by PCR-sequencing, and the primers were in accordance with the study by Pawlik et al. (Pawlik et al., 2005). All methods were verified by sequencing and all sequences were compared with the reference sequence in GenBank by BLAST (http://blast.ncbi.nlm.nih.gov/) to assess genetic variations.

Statistical analysis

Statistical software SPSS 13.0 (SPSS, Chicago, IL, USA) was employed for statistical analysis. Allele and genotype frequencies for the variant SNPs were assessed for deviation from Hardy-Weinberg equilibrium using the χ^2 test. The Mann–Whitney U-test was used to evaluate the significance of differences in pharmacokinetic parameters between the two genotypic groups. Data among three or more different genotypic groups were compared using the Kruskal–Wallis H-test. Univariate regression analysis was used to evaluate variables associated with plasma drug exposure. Those variables were age, sex, weight, hemoglobin levels, serum albumin concentration, glomerular filtration rate, alanine aminotransferase, white blood cell count, and red blood cell count at the time of pharmacokinetic assessment, doses of prednisone, as well as MDR1 C3435T/MRP2 C-24T genotye. Secondly, multiple regression analyses of the factors potentially associated with MPA pharmacokinetic parameters were performed considering MPA AUCs and $AUC_{6\text{-}12}/AUC_{0\text{-}12}$ as the dependent variables. Genotypes of UGT1A8, UGT2B7, MDR1, MRP2, as well as age, weight, eGFR, serum albumin levels, and prednisone levels were considered as independent variables. Correlations between continuous variables were examined using Pearson's correlation analysis and expressed with Pearson's correlation coefficients (r). Statistical significance was considered as $P< 0.05$.

Table 1. Baseline demographics, clinical characteristics, and laboratory parameters of glomerular diseases patients.

Parameter	Total	Group 1	Group 2	Group 3	Group 4
Patients (n)	24	6	7	9	2
Gender (male/female)	9/15	0/6	3/4	5/4	1/1
Age (years)	39.12 ± 11.66	35.67±10.19	37.00±9.59	41.22±12.01	51.50±24.75
Body weight (kg)	62.83 ± 19.53	59.50±24.29	69.57±18.53	61.11±20.05	57.00±8.49
Height (cm)	163.45 ± 8.54	163.67±9.11	162.86±8.86	164.44±9.75	160.50±0.71
BMI (kg/m^2)	24.74 ± 4.37	24.81±5.08	25.99±5.25	24.31±3.65	22.14±3.49
Serum albumin (g/L)	41.56 ± 4.56	38.72±5.13	43.01±2.66	41.63±4.95	44.70±4.95
Serum creatinine (μmol/L)	120.83 ± 60.03	68.33±7.34	96.00±11.45	136.67±21.91	294.00±22.63
Glomerular filtration rate ($mL/min/1.73\ m^2$)	66.81 ± 27.22	101.86±9.46	73.94±10.79	48.80±7.43	17.77±2.85
Serum urea (mmol/L)	9.69 ± 6.26	5.72±1.55	7.56±1.63	9.96±1.18	27.88±8.63
Red blood cell count (10^{12}/L)	4.27 ± 0.61	4.20±0.31	4.56±0.71	4.34±0.51	3.19±0.01
White blood cell count (10^9/L)	7.81 ± 3.43	6.09±2.30	7.19±2.04	8.82±4.53	10.54±3.14
Hemoglobin (g/L)	131.79 ± 20.18	127.17±8.47	138.00±26.12	137.56±16.96	98.00±14.14

RESULTS

Patients' demographics

The subjects consisted of 12 lupus nephritis patients and 12 IgA-nephropathy patients. The participant's baseline demographics and clinical characteristics were summarized in Table 1.

Pharmacokinetics of free and total MPA in 4 groups with different renal function

After first dose, the pharmacokinetics parameters had no significant differences among the four groups with different eGFR. After eleven repeat dosing, the MPA concentration should have reached to a steady-state. (Armstrong et al., 2005) A significant inverse correlations between fMPA $AUC_{6\text{-}12}$ and eGFR values, tMPA $AUC_{6\text{-}12}$ and eGFR values were found (r=-0.552, *P*=0.005 and r=-0.426, *P*=0.038, respectively). Meanwhile, fMPA and tMPA $AUC_{6\text{-}12}/AUC_{0\text{-}12}$ (an estimate of enterohepatic recycling) significantly correlated with eGFR values (r=-0.519, *P*=0.009 and r=-0.651, *P*=0.001, repectively) (Table 2).

Impact of the UGT1A9, UGT1A8 and UGT2B7 polymorphisms on MPA pharmacokinetics parameters

In the present study, all subjects were identified as UGT1A9 -275TT/-2152CC, and no UGT1A9*3 (T98C) or UGT1A8*3 (C277Y) mutations were found. The allelic frequencies of other SNPs were in Hardy-Weinberg equilibrium (*P*>0.05). The allelic frequencies observed in this study were consistent with frequencies reported in Asians (Kagaya et al., 2007). The twenty-four patients in this study had the following genotypes: *1/*1 (4), *1/*2 (14) and *2/ *2 (6) for UGT1A8; *1/*1 (13), *1/*2 (9) and *2/ *2 (2) for UGT2B7, respectively.

Compared with non-carriers, individuals carrying UGT1A8*2 or UGT2B7*2 variations had similar tMPA pharmacokinetic parameters. However, after the first dose, individuals carrying the UGT1A8*2/*2 mutation had a 151.2% and 89.2% higher fMPA $AUC_{0\text{-}6}$ than UGT1A8*1/*2 and UGT1A8*1/*1 carriers (0.94±0.60 vs. 0.37±0.11 and 0.50±0.18, *P*=0.029). In contrast, this significant difference in fMPA were not observed after multiple doses (0.92±0.89 vs. 0.89±0.87 and 0.34±0.13, *P*=0.369). After multiple doses, individuals carrying UGT2B7*2/*2 had a higher $AUC_{0\text{-}12}$ of fMPA than UGT2B7*1/*1 carriers (2.98±0.275 vs. 0.988±0.724, *P* = 0.027).

Impact of MDR1, MRP2 and OATP8 polymorphisms on MPA pharmacokinetics parameters

The influence of MDR1 C3435T allelic variations on pharmacokinetics profiles after a single dose of MPA was summarized in Table 3. The pharmacokinetics parameters of fMPA were not influenced by the MDR1 C3435T genotype. However, patients carrying the MDR1 3435CC allele had a 40.81% higher mean $AUC_{0\text{-}24}$ of tMPA after a single oral dose of MMF compared to the mean value in the MDR1 3435 T carriers (*P*=0.007). After multiple doses, in spite of no significant difference being found in the MDR1 C3435T genotype, the mean values of tMPA $AUC_{0\text{-}12}$ were 37.9% higher in individuals carrying

Table 2. Pharmacokinetic parameters in 4 groups with different renal function.

Parameters	Group 1		Group 2		Group 3		Group 4	
	Single	Repeat	Single	Repeat	Single	Repeat	Single	Repeat
Patients (n)	**6**		**7**		**9**		**2**	
Free MPA								
C_{max} (mg/l/g MMF)	0.47±0.23	0.90±1.18	0.32±0.16	1.09±1.73	0.99±1.34	1.49±1.20	0.20±0.01	0.70±0.82
AUC_{0-6} (mg*h/l/g MMF)	0.51±0.24	0.66±0.85	0.43±0.16	0.87±1.04	0.70±0.56	0.97±0.69	0.27±0.04	0.32±0.18
AUC_{6-12} (mg*h/l/g MMF)	0.17±0.26	0.10±0.05	0.19±0.25	0.19±0.14	0.22±0.23	0.45±0.52	0.14±0.05	1.31±1.70
AUC_{0-12} (mg*h/l/g MMF)	0.67±0.49	0.76±0.89	0.62±0.32	1.06±1.07	0.91±0.58	1.41±0.86	0.40±0.01	1.63±1.87
AUC_{0-24} (mg*h/l/g MMF)	0.91±0.79	NA.	0.84±0.50	NA.	1.14±0.65	NA.	0.67±0.16	NA.
AUC_{6-12}/ AUC_{0-12} (%)	18.13±11.66	19.98±10.86	24.24±18.24	25.27±12.38	25.47±20.37	31.55±19.67	33.97±11.29	60.99±33.81
Total MPA								
C_{max} (mg/l/g MMF)	26.91±8.17	40.32±21.25	27.77±9.01	31.74±10.54	40.17±22.83	39.78±21.29	16.91±7.08	21.44±11.40
AUC_{0-6} (mg*h/l/g MMF)	34.79±8.88	31.83±18.62	35.83±8.06	27.18±7.16	36.24±13.93	28.74±12.55	20.85±12.00	22.58±12.17
AUC_{6-12} (mg*h/l/g MMF) [a]	5.09±3.13	8.74±6.53	8.09±3.40	10.84±5.39	6.79±2.19	12.97±5.41	4.32±0.01	13.93±12.43
AUC_{0-12} (mg*h/l/g MMF)	39.88±10.74	40.56±24.57	43.92±10.11	38.02±11.58	43.03±15.11	41.72±16.94	25.16±11.99	36.51±24.59
AUC_{0-24} (mg*h/l/g MMF)	48.90±13.51	NA.	54.96±12.01	NA.	54.99±20.28	NA.	40.06±23.63	NA.
AUC_{6-12}/ AUC_{0-12} (%)[a]	12.17±5.78	20.31±4.96	18.31±4.96	27.22±7.63	16.61±4.26	31.37±6.12	19.34±9.25	34.52±10.79

Notes: [a], indicates a $P<0.05$ among the four groups after repeat dose.

the MDR1 3435CC allele compared to the MDR1 3435T carriers (47.73±20.96 vs. 35.23±13.11, P=0.161). Furthermore, we found that the difference in the tMPA exposure among patients with the MDR1 C3435T genotype was more significantly prominent in patients with eGFR less than 60 mL/min/1.73 m^2 compared with those higher than 60 mL/min/1.73 m^2 (Figure 1).

For single analysis of correlation between MRP2 C-24T and MPA pharmacokinetics, no significance was found after the first dose or multiple doses. After multiple doses, combined analysis of MDR1 C3435T and MRP2 C-24T revealed that subjects harboring both MDR1 3435CC and MRP2 -24CT alleles had extremely higher tMPA AUC_{0-6}, tMPA AUC_{0-12} and fMPA AUC_{6-12}/AUC_{0-12} values than that in other MDR1 C3435T/MRP2 C-24T carriers (P=0.031, P=0.040 and P=0.031, respectively). (Details in Table 4).

The two patients with OATP8 334TT/699GG allele had a lower mean AUC_{6-12} value of fMPA compared to OATP8 334TG/699GA (n = 5) and 334GG/699AA (n = 17) carriers after multiple doses of MPA (0.055±0.007 vs. 0.328±0.280 and 0.402±0.667, respectively). However, no significant differences were observed among the three groups (P = 0.069), likely due to the limited number of OATP8 334TT/699GG carriers in the present study.

Univariate and multivariate analysis for the factors affecting MPA pharmacokinetics

To further define the multiple factors that may contribute to inter-individual variability in MPA pharmacokinetic parameters, we performed univariate linear regression analyses of patients' demographic and biometric characteristics alongside the pharmacokinetic studies. Except for the UGT1A8*2 and MDR1 C3435T genotype, no additional factors were found to influence free or tMPA exposure after the first oral dose. After multiple doses, eGFR values, serum albumin concentrations, dosage of corticorsteroid (prednisolone), age, sex, weight, MRP2 C-24T/MDR1 C3435T and UGT2B7*2 genotype

Table 3. Distribution of MPA pharmacokinetic parameters according to MDR1 polymorphism in patients with glomerular diseases after the first oral dose of MMF.

	MDR1 C3435T		P-value
	CC	CT T	
N	9	15	
Free MPA			
AUC_{0-6} (mg*h/l/g MMF)	0.58 ± 0.45	0.51±0.35	0.404
AUC_{6-12} (mg*h/l/g MMF)	0.12 ± 0.08	0.23±0.27	0.402
AUC_{0-12} (mg*h/l/g MMF)	0.70 ± 0.44	0.74±0.51	0.788
AUC_{0-24} (mg*h/l/g MMF)	0.92 ± 0.51	0.98±0.68	0.765
AUC_{6-12}/ AUC_{0-12} (%)	19.74 ± 12.19	26.53±19.09	0.531
Total MPA			
AUC_{0-6} (mg*h/l/g MMF)	41.78 ± 10.19	30.10±9.62	0.011
AUC_{6-12} (mg*h/l/g MMF)	7.23 ± 3.82	6.12±2.28	0.655
AUC_{0-12} (mg*h/l/g MMF)	49.01 ± 12.32	36.22±10.79	0.013
AUC_{0-24} (mg*h/l/g MMF)	63.76 ± 15.52	45.28±12.71	0.007
AUC_{6-12}/ AUC_{0-12} (%)	14.36 ± 5.33	17.34±5.46	0.180

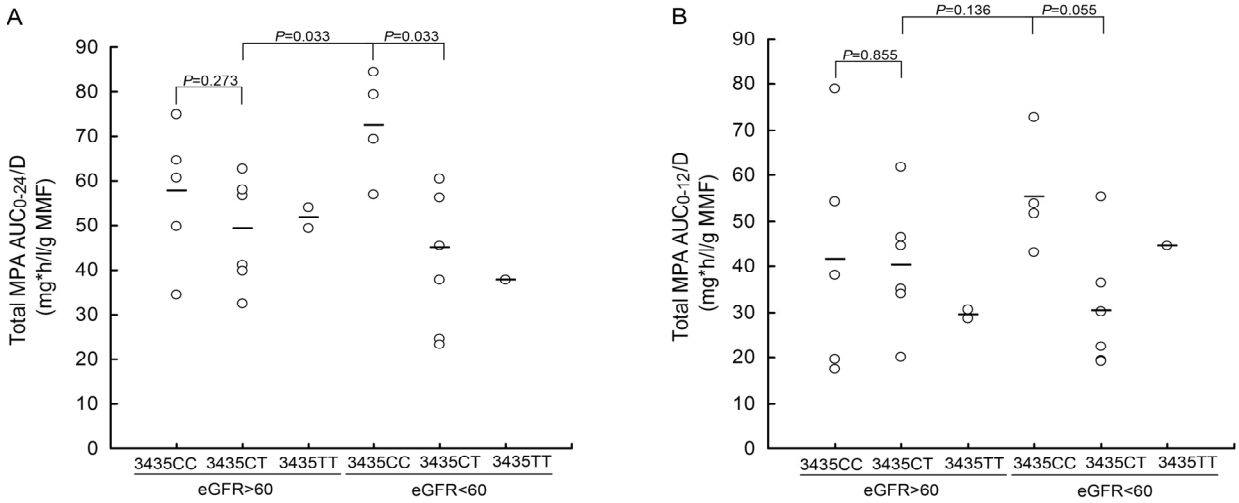

Figure 1. Comparisons of the AUCs of total MPA among different renal function (eGFR) /MDR1 C3435T genotype groups. A, Single dose of MMF and B, multiple doses of MMF. Patients were classified into two main groups according to eGFR, as 60 mL/min/1.73 m^2 < eGFR < 120 mL/min/1.73 m^2 and as 15 mL/min/1.73 m^2 < eGFR< 60 mL/min/1.73 m^2.

Table 4. Pharmacokinetic parameters of MPA in the MDR1 C3435T and MRP2 C-24T genotype groups after multiple doses of total MMF.

	MDR1 3435CC		MDR1 3435T carriers	
	MRP2 -24CT	MRP2 -24CC	MRP2 -24CT T	MRP2 -24CC
N	3	6	5	10
AUC_{0-6} (mg*h/l/g MMF)	44.72 ± 16.54 [a]	29.48 ± 13.77	28.37 ± 13.46	23.22 ± 5.82 [b]
AUC_{6-12} (mg*h/l/g MMF)	16.76 ± 5.53	11.37 ± 7.53	10.41 ± 6.17	10.23 ± 5.31
AUC_{0-12} (mg*h/l/g MMF)	61.48 ± 15.15 [a]	40.86 ± 21.00	38.77 ± 19.08	33.45 ± 9.76 [b]
AUC_{6-12}/ AUC_{0-12} (%)	28.33 ± 12.06	25.55 ± 7.08	26.67 ± 6.41	29.21 ± 8.63

Notes: a, subjects carring MDR1 3435CC/MRP2 -24CT (n=3) had higher values than that in the other MDR1 C3435T/MRP2 C-24T carriers (n=21), P< 0.05;
[b], indicates a P<0.05 when comparing MDR1 3435CC/MRP2 -24 CT carriers.

Table 5. Univariate linear regression analysis of potential predictors affecting MPA pharmacokinetic parameters after multiple doses of MMF.

		Free MPA				Total MPA			
		AUC_{0-6}	AUC_{6-12}	AUC_{0-12}	AUC_{6-12}/ AUC_{0-12} (%)	AUC_{0-6}	AUC_{6-12}	AUC_{0-12}	AUC_{6-12}/ AUC_{0-12} (%)
eGFR	r	0.044	-0.552	-0.288	-0.519	0.022	-0.426	-0.135	-0.651
(mL/min/1.73 m^2)	p	0.840	0.005	0.172	0.009	0.918	0.038	0.529	0.001
Serum albumin	r	0.455	0.489	0.658	0.206	0.120	0.287	0.189	0.366
(g/L)	p	0.025	0.015	<0.001	0.335	0.576	0.175	0.376	0.079
MDR1 C3435T/	r	0.256	-0.254	0.061	-0.356	-0.493	-0.283	-0.459	0.121
MRP2 C-24T[a]	p	0.226	0.230	0.776	0.088	0.014	0.180	0.024	0.574
Age (years)	r	-0.029	0.562	0.304	0.435	-0.069	0.162	0.007	0.174
	p	0.891	0.004	0.148	0.034	0.749	0.451	0.974	0.415
Body weight (kg)	r	0.110	0.035	0.110	0.069	-0.463	-0.360	-0.464	-0.104
	p	0.609	0.870	0.609	0.750	0.023	0.084	0.022	0.627
Gender	r	-0.352	-0.029	-0.306	0.082	0.465	0.277	0.437	-0.148
(male=0/female=1)	p	0.091	0.892	0.147	0.702	0.022	0.189	0.033	0.491

Notes: a, subjects were divided into four groups by MDR1 C3435T/MRP2 C-24T genotype, MDR1 3435CC/MRP2 -24CT=0, MDR1 3435CC/MRP2 -24CC=1, MDR1 3435CT T/MRP2 -24CT T=2, MDR1 3435CT T/MRP2 -24CC=3

were significantly correlated with MPA pharmacokinetics parameters (details in Table 5).

Multivariate analysis was performed considering eGFR values, serum albumin concentrations, prednisone dose levels, age, sex, weight, MRP2 C-24T/MDR1 C3435T genotype, UGT1A8*2 genotype, and UGT2B7*2 genotype together to clarify the main effect on MPA pharmacokinetic parameters after multiple doses. The AUC_{0-12} of fMPA was associated with serum albumin levels, and there were strong negative correlation between eGFR and AUC_{6-12}/AUC_{0-12} for both free and tMPA. Considering sex was significantly correlated with weight (r=0.455, *P*= 0.024) and MRP2 C-24T/MDR1 C3435T genotype (r=0.460, *P*=0.026), we also did multivariate analysis without sex in order to exclude the interaction among the variables. Then, we found that the above significant differences did not change, while the tMPA AUC_{0-6} and AUC_{0-12} were associated with MDR1 C3435T/MRP2 C-24T genotype (Table 6).

Pharmacokinetics of free and total MPA in lupus nephritis and IgA nephropathy

There were no significant differences of the pharmacokinetic indices between lupus nephritis group and IgA nephropathy group, except for fMPA AUC0-12 after multiple doses (r = 0.627, p = 0.001). As LN vs. IgA, after first dose the AUC0-24 for fMPA and tMPA were 0.89±0.45 vs. 1.02±0.76 and 53.18±20.90 vs. 51.25±10.79 respectively, and after multiple doses the AUC0-12 for fMPA and tMPA were 0.56±0.42 vs. 1.77±1.03 and 38.89±19.35 vs. 40.94±15.54 respectively. We supposed that the difference in fMPA was due to the 11 of 13 patients who received prednisone was in lupus nephritis group. Only 2 patients in IgA nephropathy group (n = 12) were taking prednisone.

DISCUSSION

The present study provided the first insight into the role that clinical characteristics and genetic variants of P-gp, MRP2, OATP8, and UGT enzymes effect on MPA metabolism in mild-to-severe renal insufficiency patients with IgA nephropathy and lupus nephritis, which consisted of the most common primary and secondary glomerular demonstrated that SNPs of genes encoding P-gp and MRP2 determined the tMPA exposure in the patients, while fMPA concentrations were related to the serum albumin level. Furthermore, MPA enterohepatic recycling was associated with renal function.

MPA metabolites are excreted into the urine. Therefore, several studies have examined the correlation between renal function and MPA pharmacokinetics. Most studies showed that renal function markedly altered the pharmacokinetics of MPA or MPAG, although the results were paradoxical. Some studies have shown an association between renal impairment and a higher exposure of tMPA (Naito et al., 2006) (Neumann et al., 2003), others showed that MPA clearance increased and diseases in China (Liu et al., 2004). Our study the tMPA AUC_{0-12} decreased in patients with poor renal function (van Hest et al., 2006).

Table 6. Multivariate linear regression analysis of potential predictors affecting MPA pharmacokinetic parameters after multiple doses of MMF.

Parameters	Variables	Model r	Partial r	*P* value
fMPA AUC_{0-12} (mg*h/L/g MMF)	Serum albumin (g/L)	0.766	0.585	0.017
tMPA AUC_{0-6} (mg*h/L/g MMF)[a,b]	MDR1C3435T/MRP2C-24T	0.681	-0.550	0.022
tMPA AUC_{0-12} (mg*h/L/g MMF)[a,b]	MDR1C3435T/MRP2C-24T	0.679	-0.540	0.025
fMPA AUC_{6-12}/ AUC_{0-12} (%)	eGFR (mL/min/1.73 m^2)	0.738	-0.521	0.039
tMPA AUC_{6-12}/ AUC_{0-12} (%)	eGFR (mL/min/1.73 m^2)	0.844	-0.749	0.001

Notes: The following variables were introduced in the model: serum albumin levels, eGFR values, prednisone dose levels, age, sex, weight, the MRP2 C-24T/MDR1 C3435T genotype, UGT2B7*2 genotype and UGT1A8*2 genotype.
a, Sex was not included in the multivariable model.
b, subjects were divided into four groups by MDR1 C3435T/MRP2 C-24T genotype, MDR1 3435CC/MRP2 -24CT=0, MDR1 3435CC/MRP2 -24CC=1, MDR1 3435CT T/MRP2 -24CT T=2, MDR1 3435CT T/MRP2 -24CC=3

Our study investigated the effect of renal function on MPA pharmacokinetics in first and repeated dose, and found that after first dose there was no significant differences between renal function (as eGFR value) and pharmacokinetics parameters. After repeated doses, the AUC_{6-12} for both tMPA and fMPA significantly increased as GFR decreased. In addition, a strong negative correlation between eGFR and $AUC_{6–12}/AUC_{0–12}$ (%) for both fMPA and tMPA was observed. According to the previous studies, the clearance of MPAG decreased with the GFR declining (van Hest et al., 2006), we hypothesize that under steady-state condition more MPAG is available for biliary excretion as GFR declining, and more MPAG will undergo deglucuronidation to MPA in the gut, which is subsequently reabsorbed mainly after 6 hours from MMF administration (Naesens et al., 2007). On the other hand, more increased MPAG in serum will combine completely of albumin with MPA, thus induced higher concentration of fMPA. Thereby, our results indicated that renal impairment indirectly determined the amount of MPA absorption through enter hepatic recirculation resulted in increased fMPA and tMPA AUC_{6-12} values and then elevated trend of fMPA AUC_{0-12}. However, the influence of renal impairment in MPA exposure (AUC_{0-12}) is indirect and limited. Therefore, we further investigated other factors which may affect on MPA pharmacokinetics.

The present study provides evidence of the influence of MDR1 genetic variation on MPA pharmacokinetic variability and suggests that patients with the MDR1 3435 CC allele may be exposed to higher levels of tMPA. To our knowledge, this significant difference has not been previously reported. Transplant patients are usually treated with a combination of MMF and a calcineurin inhibitor (cyclosporine or tacrolimus), which are substrates and inhibitors of P-gp and may alter the function or expression of P-gp (Yu et al., 2008). This combined treatment likely eliminates the difference in MPA pharmacokinetics between the different genotypes (Johnson et al., 2008).On the other hand, the significant difference has not been reported in healthy subjects who are not required to take any other medication. Thus, we hypothesized that the difference among the MDR1 C3435T genotype may correlate with impaired renal function. Further combined analysis of the MDR1 C3435T genotype and renal function confirmed a correlation between this genotype and renal function. The effect of the MDR1 C3435T allele was more significant in patients with worse renal function than in patients with better renal function Therefore, according to our data, if a study involved more MDR1 3435 CC carriers with poor renal function and more MDR1 3435 T carriers with better renal function, they would conclude that MPA exposure increased in patients with lower eGFR. Conversely, the mean MPA exposure for MDR1 3435CC/eGFR> 60 mL/min/1.73 m^2 patients was higher than MDR1 3435T/eGFR< 60 mL/min/1.73 m^2 patients.

The mechanism for why the tMPA exposure is elevated in patients with MDR1 3435CC allele and is more significant in patients with poor renal function remains to be elucidated. Our data do not support an impact of MDR1 3435CC on enterohepatic recirculation after a single or repeated doses of MPA in patients with renal impairment, as reflected by unchanged AUC_{6-12}/AUC_{0-12} values for tMPA. In the present study, tMPA AUC_{0-6} significantly elevated in patients with the MDR1 3435CC allele, while the AUC_{6-12} value slightly increased. Therefore, increased exposure to MPA seems to occur mainly in absorption, probably because of degraded P-gp function or expression level in the intestine by MDR1 3435CC alleles.

MPA is a drug with low-intermediate hepatic extraction and a high degree of plasma protein binding, such that total hepatic clearance would be impacted by both plasma protein binding and hepatic intrinsic clearance (CLint). In

this regard it is of interest that, as indicated above, there were no differences in free MPA exposure (Table 3). One would anticipate that genotypic variants impacting on hepatic clearance of MPA would result in changes in the CLint parameter, leading to changes in the free MPA level of exposure. This suggests that the observed differences in total MPA are most likely the result of variation in MDR1/P-glycoprotein expression at the intestinal mucosa impacting the extent of systemic absorption.

Studies using a chronic renal failure (CRF) rat model showed that the expression level of P-gp in intestine, liver and kidneys may be altered under CRF conditions (Laouari et al., 2001; Naud et al., 2008; Naud et al., 2007). In intestine of CRF rats, the level of P-gp was significantly lower compared to control animals (Naud et al., 2007). According to the present study, the alteration in P-gp expression for CRF patients may be different in MDR1 3435CC and 3435T carriers. Thus, further studies are required to clearly assess the influence of the uremic toxins on P-gp and the roles that MDR1 genetic polymorphisms play in vitro and in vivo. In the present study, we also detected the presence of MDR1 C1236T and MDR1 G2677T/A alleles. We analyzed the correlations between genotypes/haplotypes and pharmacokinetics; however, no significant differences were observed. In addition, we cannot exclude the possibility that other unknown SNPs of MDR1 were affected.

After 11 multiple dosings when the MPA concentration is suppose to have reached a steady-state (which requires at least eight doses of MMF (Armstrong et al., 2005), the tMPA exposure was still elevated in MDR1 3435 CC carriers. However, the difference in tMPA exposure did not reach statistical significance. Under steady-state conditions, it can be explained that the importance of P-gp on the MPA bioavailability decreased due to the influence of other factors, such as renal function, UGT enzymes, or other transports.

According to previous studies, other transporters (such as MRP2) may play a role in MPA disposition (Naesens et al., 2006). An investigation of the functional significance of the variation in the 5'-UTR of MRP2 suggested that the C-24T variation was associated with 18.7% reduced activity in human hepatoblastoma HepG2 cells (Haenisch et al., 2007). However, for single analysis of correlation between MRP2 C-24T and MPA pharmacokinetics, no significance was observed after the first dose or under steady-state conditions. Further analysis showed that MDR1 C3435T had a concomitance effect with MRP2 C-24T on the absorption and enterohepatic recycling of free and tMPA after multiple doses. The AUCs of tMPA were greater in MDR1 3435CC/MRP2 -24CT carriers than in the other MDR1 C3435T/ MRP2 C-24T genotypes. These data imply that impaired function or reduced expression of both MDR1 and MRP2 might result in significant increase in absorption, but reduce biliary and renal excretion, thus, increase systemic exposure to drug and/or probably to its metabolites. Drug-transporter interactions must be considered in patients receiving MMF.

In the end, multivariate analysis confirmed the effect of renal function on AUC6–12/AUC0–12 for both fMPA and tMPA, and serum albmin level on fMPA exposure.

In conclusion, the present study described the influence of multi-factors on MMF pharmacokinetics in patients with different renal function. We should totally evaluate the above multiple factors affecting MMF pharmacokinetics in patients with different renal function, keep drug safety and guide proper therapy in clinical practice. An important finding of this study was that the MDR1 3435CC allele increased the tMPA exposure in patients, an effect independent of the UGT1A8, UGT1A9, and UGT2B7 genetic variations, and in co-ordination with the MRP2 -24T allele, to elevate the tMPA level after multiple doses of MMF. Moreover, the effect of the MDR1 C3435T allele was likely to be related to renal function. This clearly warrants additional studies with complete and detailed genetic profiling of MDR1 and MRP2 genes and their association with serum albumin level and renal function in inter-individual variation of MPA pharmacokinetics.

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Conflict of interest

The authors declare that they have no conflict of interest.

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