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Effects of uridine diphosphate glucuronosyltransferase 2B7 and 1A7 pharmacogenomics and patient clinical parameters on steady-state mycophenolic acid pharmacokinetics in glomerulonephritis

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Abstract

Purpose—The role of pharmacogenomics, clinical and demographic parameters in pharmacokinetic predictions was evaluated in patients receiving mycophenolic acid (MPA).

Methods—A cohort study design of patients with glomerulonephritis secondary to lupus nephritis and anti-neutrophil cytoplasmic antibody (ANCA) vasculitis was employed. Forty-six patients with lupus nephritis and ANCA vasculitis who were receiving MPA were recruited from the nephrology clinic. The study assessed the relative single and combined roles of genomic,

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clinical, and demographic characteristics on pharmacokinetic parameters using general linear models. The study focused on polymorphisms in *UGT1A7*, *UGT2B7*, and *ABCB1/MDR1*; all of which have limited data available concerning MPA pharmacokinetics. All patients had pharmacokinetic assessments for MPA and glucuronide metabolites (MPAG, AcMPAG). Genotyping was performed for known variants of UGTs (*UGT1A9*, *UGT1A7*, *UGT2B7*), and multidrug resistance protein (*ABCB1*/*MDR1*), involved in MPA disposition. Analyses included univariate and multivariate linear modeling.

Results—In univariate analyses, *UGT2B7* heterozygosity (coefficient 0.3508; R^2=0.0873) and *UGT1A7* heterozygosity (coefficient 0.3778; R^2=0.0966) predicted increased apparent oral clearance of MPA. *UGT1A7* heterozygosity (coefficient −0.4647; R^2 0.0897) predicted lower MPA trough concentrations. In multivariate assessments, higher urinary protein excretion, lower serum creatinine, and increased weight predicted greater apparent oral clearance of MPA (p< 0.0001). White race and higher serum creatinine predicted higher MPA trough concentrations (p<0.0001). Higher exposure to MPA was predicted by decreased urinary protein excretion and increased serum creatinine.

Conclusions—Clinical and demographic parameters were 2–4 times more important in MPA disposition than genotypes and explained 30–40% of the pharmacokinetic parameters.

Keywords

Mycophenolic acid; UGT2B7; UGT1A7; ABCB1; MDR1; Glomerulonephritis

Introduction

Autoimmune-related kidney diseases such as systemic lupus erythematosus (SLE) and anti-neutrophil cytoplasmic antibody (ANCA) small vessel vasculitis (SVV) are treated with myriad drugs approved for use in the transplant and cancer populations. These treatments commonly include but are not limited to mycophenolate mofetil/sodium, glucocorticoids, and cyclophosphamide. Treatments with these drugs are considered “off-label” with respect to Food and Drug Administration (FDA) labeling. Intrinsic to off-label usage is the uncertainty pertaining to the effects of disease-related clinical covariates on drug disposition (pharmacokinetics). Patients with glomerulonephritis can have reductions in serum albumin and kidney function (glomerular filtration rate [GFR] or estimated creatinine clearance [eClCr]), and elevations in proteinuria, all of which may alter drug disposition. Kidney transplant patients, on the contrary, have primarily reductions in GFR and less commonly alterations in serum albumin and urinary protein excretion. Reductions in serum albumin may increase clearance through metabolism and excretion by increasing the unbound drug. Increases in urinary protein excretion may increase excretion through clearance of the bound drug. Among the various forms of glomerulonephritis, there can be a predilection for patients of certain ages, races, and genders; factors that may result in variable drug disposition. For drugs such as mycophenolic acid (MPA), the active moiety of mycophenolate mofetil and mycophenolate sodium, there is inherently wide inter-individual variability in pharmacokinetics. [1, 2] Hence, the alterations in clinical and/or demographic covariates in the glomerulonephritis population compared with the kidney transplant population could lead to variability in pharmacokinetics above and beyond that which would be predicted from studies employing the latter patients.

There are several reports in transplant and healthy normal populations that suggest altered MPA pharmacokinetics secondary to single nucleotide polymorphisms (SNPs) in the uridine glucuronosyltransferase metabolizing enzymes (UGTs) [3–8]. Polymorphisms in the *UGT1A9* gene and influence on MPA have been most frequently described. The *UGT1A9 T-275A* and *C-2152T* promoter SNPs have been associated with enhanced metabolism of

mycophenolic acid [3, 8]. *UGT1A9* SNPs at nucleotide base positions 8 and 98 have been associated with enhanced exposure to MPA, suggesting a reduction in metabolism. Less well described are the effects of polymorphisms in the *UGT2B7* gene, with one report describing an increase in MPA exposure in patients with the *UGT2B7 C802T* variant [3]. Several limitations exist for these published pharmacogenomic reports. The studies comprised mostly Caucasian and Asian populations and therefore generalizability to patients of other ethnic subpopulations receiving MPA may be limited. Also, there is not always consistency in results between in vitro and in vivo approaches; reduced intrinsic clearance was noted in an in vitro evaluation of the effects of *UGT1A8 *2* and *3*, while in vivo studies showed a lack of effect by *UGT1A8* variants on MPA disposition [3, 5, 7]. In addition to polymorphisms in drug-metabolizing enzymes, it is known that polymorphisms in the *ABCC2* gene, which encodes the multidrug resistance-associated protein MRP2, can influence the disposition of MPA [9, 10]. There are more limited data that suggest that polymorphisms in the multidrug resistance transporter gene *ABCB1* or *MDR1* may also influence the disposition of MPA [11, 12]. These studies support the need to evaluate SNP frequencies within the populations of specific glomerular diseases and within patient demographic subpopulations to understand the contribution of pharmacogenetics as opposed to the effects of demographics or clinical covariates on variability in MPA pharmacokinetics.

In this study, we investigated the ability of genomic, clinical, and demographic patient characteristics to predict the pharmacokinetic outcomes of MPA (bound and unbound) and its phenolic- and acyl-glucuronide metabolites (MPAG and AcMPAG) in patients with glomerulonephritis secondary to SLE and ANCA SVV using linear statistical models. In order to expand on the existing knowledge of MPA and pharmacogenomics, we focused on the less well-described influence of polymorphisms in *UGT2B7*, *UGT1A7*, and *ABCB1* genes, but also sought to characterize the influences of *UGT1A9* genes in glomerulonephritis. We hypothesized that genetic variations in *UGT2B7* and *UGT1A7*, and in *ABCB1* contribute to the disposition of MPA and its glucuronidated metabolites. We also explored the separate and combined contributions of the pharmacogenomic, disease-related, and demographic patient characteristics to the prediction of the disposition of total MPA, unbound MPA, and the glucuronide metabolites MPAG and AcMPAG.

Materials and methods

Research subjects

Patients with biopsy-confirmed SLE or ANCA SVV with kidney manifestations who were receiving maintenance therapy on a stable dose of MPA (Cellcept®; Roche, Nutley, NJ, USA) for at least 2 weeks were evaluated for enrollment. MPA dosing regimens were prescribed by the treating nephrologists and ranged from 250 mg twice daily to 1,500 mg twice daily. The patients participated in a 24-h MPA pharmacokinetics evaluation approved by the Biomedical Institutional Review Board and conducted in the inpatient clinical research center. Specific details of these studies and results from noncompartmental pharmacokinetics for MPA and AcMPAG were previously described [13, 14]. Briefly, blood samples were collected at times 0, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 12, and 24 h and urine was collected from 0–6, 6–12, and 12–24 h. Plasma and urine samples were assayed for MPA and MPAG by high-performance liquid chromatography (HPLC) with ultraviolet detection assay [15]. Plasma and urine standard curves for MPA were linear over the range of 0.2–200 μg/mL and 1–50 μg/mL respectively. Plasma and urine standard curves for MPAG were linear over the range of 1–200 μg/mL and 5–1,500 μg/mL respectively. The AcMPAG metabolite was assayed in plasma and urine by liquid chromatography/ mass spectrometry (LC/MS) developed in the laboratory. Tuning, operation, peak integration, and data analysis were performed in negative ion mode using multiple reaction monitoring (Analyst software

v.1.4.1; Applied Biosystems). Reverse phase separation (gradient elution using 0.1% acetic acid aqueous phase and 100% acetonitrile organic mobile phase) was accomplished using a Zorbax RX-C8 150×2.1 mm column with a 5-μm particle size (Agilent Technologies, Santa Clara, CA, USA). Analysis required a 10-μL injection and a solvent flow of 0.3 mL/min. Total run time, including equilibration, was 15 min per injection. The internal standard was azidothymidine. Plasma and urine standard curves for AcMPAG were linear over the range of 0.01–50 μg/mL and 1–500 μg/mL respectively. Unbound plasma fraction was determined by filtration via a Centrifree® Micropartition device (Millipore, Cork, Ireland) with a filter cut-point of 30,000 Da. Unbound concentrations were calculated as unbound fractions multiplied by total concentrations.

Clinical data were abstracted from medical charts and included serum creatinine (SCr), estimated creatinine clearance (eClCr) by Cockcroft and Gault [16], urinary protein to creatinine ratio (UP:Cr), serum albumin, and steroid dose. Abstracted demographic data included age, weight, race, and gender.

Genotyping assessments

A 5-mL whole blood sample was collected into an EDTA-containing vacutainer and genomic DNA was isolated using a Flexigene Qiagen kit (Qiagen, Valencia, CA, USA). Genotyping was conducted for several published *UGT1A9*, *UGT1A7*, and *UGT2B7* SNPs reported to result in alterations in MPA metabolism (Table 1) [3–5, 7, 8]. Additionally, *ABCB1/ MDR1* polymorphismss were evaluated secondary to published data, suggesting the role of the P-glycoprotein transporter in MPA disposition (Table 1) [11]. Genotyping assessments for *UGT1A7 T622C* (c___287260_10; Applied Biosystems, Foster City, CA, USA) and *MDR1 C1236T* (c___7586662_10; Applied Biosystems) were conducted using commercially available assays. Genotyping for *UGT1A9 C98T*, *UGT1A9 T-275A*, and *MDR1 C3435T* was conducted using custom assays manufactured by Applied Biosystems. Allelic discrimination was assessed for all Applied Biosystems products using 5 μL of TaqMan Universal PCR Master Mix, No AmpErase UNG (×2; Applied Biosystems), 0.25 μL (of 40× assay) or 0.5 μL (of 20× assay), 10–20 ng genomic DNA and a total reaction volume of 10 μL as per the manufacturer's instructions. The reactions were cycled with an initial denaturation of 95°C for 10 min followed by 50 cycles of 92°C for 15 seconds, and then 60°C for 1.5 min on an Applied Biosystems 7900 Taqman PCR instrument. Prior to conducting the allelic discrimination reactions, a subset of samples were sequenced using the primers noted in Table 2 in order for them to serve as positive controls for the former assays. Genotyping for *UGT1A9 G8A*, *UGT1A9 C-2152T*, and *UGT2B7 C802T* was conducted by Polymorphic DNA Technologies (Alameda, CA, USA). All genotyping results were coded as 0 (wildtype/ wildtype), 1 (heterozygote), or 2 (variant/variant).

Statistical analysis strategy and methods

Descriptive statistical methods were applied to the pharmacokinetic, demographic, clinical, and genotype data. Graphical visualization of the data and summary tabulations of frequencies, means, standard deviations, and ranges were evaluated. Each of the pharmacokinetic outcome variables was transformed to natural log (ln scale) prior to use in the statistical computations. The observed genotype frequencies for each defined locus were used in a Chi-squared test procedure for the testing of deviation from the Hardy–Weinberg equilibrium.

Putative relationships between pharmacokinetic outcomes and patient characteristics were explored using descriptive methods (e.g., estimation of Spearman's correlation coefficients), linear models for natural log (ln) scale pharmacokinetic variables, hypothesis testing, and exploratory model-building methods (e.g., stepwise variable selection algorithms, all

possible regressions, etc.). For these analyses a set of pharmacokinetic outcome variables of interest for total MPA, unbound MPA, MPAG and AcMPAG was selected.

The clinical and demographic patient characteristics of interest included serum albumin, UP:Cr, eClCr, weight, age, race, gender, and glucocorticoid dose. Since patients of various races participated in the study, race was coded as Caucasian (1) vs NonCaucasian (2) for analyses. The genotypes of interest focused on the allelic variation at each of the targeted SNP loci: *UGT1A9 G8A*, *C98T*, *C-2152T*, *T-275A*, *UGT2B7 C802T*, *UGT1A7 T622C*, and *ABCB1*/*MDR1 C1236T* and *C3435T*. Genotypes were coded as 0 (wildtype), 1 (heterozygote), or 2 (homozygous variant). In cases of the minimal numbers of the "2" code, assessments included only 0 and 1.

Following descriptive graphical examinations of the relationships between the ln pharmacokinetic outcomes (lnPK) and the various patient characteristic variables, simple univariate models were fitted for each of the lnPK variables conditional on the selected clinical, demographic or genotype variable. Univariate relationships with p values <0.05 were employed in building multivariate models. Next, the combined set of genotype, clinical, and demographic variables was used to fit various multivariable linear models for the lnPK outcomes via the application of variable selection algorithms (e.g., stepwise selection, backward elimination, etc.) For each lnPK variable, a final model was selected based on considerations of the statistical significance of the candidate predictor variables and the overall model R^2.

Auxiliary analyses were also performed to evaluate the plausibility of assumptions made (e.g., analysis of residuals) and to evaluate the sensitivity of the results to reasonable perturbations of the methods used. All statistical computations were performed using SAS System software (Version 9.1; SAS Institute, Cary, NC, USA).

Results

Demographic and clinical data (Table 3) and noncompartmental pharmacokinetic data for MPA, MPAG, and acyl-MPAG (Table 4) were available for 27 SVV patients and 19 SLE patients. $AUC_{6–12}$ /$AUC_{0–12}$ was used as a measure of enterohepatic recycling as demonstrated by others [3, 8]. The racial distribution of these 46 patients was 59% Caucasian, 28% African–American, and 13% Other (Asian [n=3], Native American [n=2], not specified [n=1]). Sixty-seven percent of study participants were female. At the time of the pharmacokinetic analysis, these subjects exhibited a wide range of clinical laboratory parameters: eClCr (18.3 to 185 mL/min), UP:Cr (0.0 to 7.9), and serum albumin (26 to 52 g/L). The frequency data for *UGT* and *ABCB1*/*MDR1* genotypes in the evaluated SVV vasculitis and SLE nephritis patients are provided in Table 5. All SNP frequencies were in Hardy–Weinberg equilibrium. The frequencies for the *UGT1A9* polymorphisms were too low to be able to incorporate them into any planned univariate and multivariate model assessments.

Analyses of univariate models for the lnPK outcomes were performed to evaluate the separate predictive value of genotype, clinical, and demographic patient characteristics. The univariate models with p<0.05 are summarized in Table 6. The coefficient gives an estimate of the direction and magnitude of the changes in the outcomes based on the level of the dependent variable. For those models evaluating only clinical and demographical factors, the fit (R^2) ranged from ~0.10 to ~0.32. Notable contributors (R^2 ~0.20 to 0.32) to MPA trough concentrations, exposure (AUC), and oral clearance were kidney function measures (Scr and eClcr). The eClcr was positively related to unbound MPA oral clearance and negatively predictive for MPA AUC. This appears consistent with the relationship between unbound

drug and glomerular filtration rate on renal clearance, e.g. increased unbound drug, increased losses through renal clearance by filtration. The small value for the coefficient mirrors the fact that usually only 3% of a MPA dose is eliminated by the kidneys [17]. Urinary protein and serum albumin were moderate contributors (R^2~0.13) to MPA and metabolite disposition. Demographic factors (age, race, weight) and glucocorticoid dose were less contributory (R^2 ~0.10) to the disposition of MPA and MPAG. However, age contributed ~20% toward the exposure ($AUC_{0–12}$ and $AUC_{6–12}$) of AcMPAG, e.g., increased age led to increased exposure. Genotype factors were generally less contributory (R^2=0.09) to MPA disposition. Genotypes for *UGT1A7* (*T622C*) and *UGT2B7* (*C802T*) appeared to be predictive of MPA oral clearance, exposure (AUC), and maximal plasma concentration. The *UGT2B7 C802T* heterozygote predicted increased renal clearance of MPA (R^2=0.1974) and AcMPAG metabolite and decreased MPA $AUC_{0–12}$, $AUC_{6–12}$, and increased oral clearance. The homozygous variant genotype for *UGT2B7 C802T* was predictive of increased MPA $AUC_{6–12}$ and decreased renal clearance of MPA (R^2=0.0897). The *UGT1A7* heterozygote was predictive of increased MPA oral clearance and decreased maximal plasma concentration. The homozygous variant for the *UGT1A7* occurred in only one patient, so the contribution of this SNP to MPA disposition was not able to be assessed. The *ABCB1*/*MDR1* SNPs were not found to significantly predict lnPK variables in the univariate assessments.

The clinical, demographic, and genotype variables from univariate models in Table 5 were assessed in multivariate models to predict the combined influence of these parameters on pharmacokinetics (Table 6). The goodness of fit of the models conditional on all variables (clinical, demographic, and genotype) was generally much better than the goodness of fit of the models conditional on clinical, demographic, or genotype variables alone. For MPA, a higher UP:Cr and lower SCr appeared to be predictive of increased oral clearance. One model incorporated weight as a significant variable in predicting oral clearance, improving the fit of the model R^2 from 0.3526 to 0.4397. A lower UP:Cr and higher SCr were predictive of increased $AUC_{0–12}$ (R^2=0.3622) and $AUC_{6–12}$ (R^2=0.4931), which is consistent with the reciprocal relationship between oral clearance and AUC. White race and higher SCr were both predictive of an increased MPA trough plasma concentration (R^2=0.4244). The multivariate model for renal clearance (R^2=0.2763) incorporated both weight and *UGT2B7* genotype. This latter model was the only multivariate assessment that incorporated a genotype variable.

The significant multivariate relationships observed for the MPAG metabolite included AUC and renal clearance. Increased serum creatinine and Caucasian race were predictors of increased $AUC_{0–12}$ (R^2=0.2950) and $AUC_{6–12}$ (R^2=0.3420). Additionally, decreased Scr and female gender were predictors of increased renal clearance (R^2=0.2636). MPAG is primarily eliminated by the kidneys and clearance is inversely related to AUC, so it is predictable that Scr would influence both AUC and renal clearance.

Similar to MPAG, renal function (Scr or eClcr) was also important in predicting the AcMPAG AUC and renal clearance. Increased $AUC_{0–12}$ was predicted by Caucasian race and decreasing eClCr (R^2=0.4542), while $AUC_{6–12}$ was predicted by increased age and Scr (R^2=0.4092). Both decreased serum albumin and Scr were predictive of increased AcMPAG renal clearance (R^2=0.4239).

Discussion

In this study, we sought to characterize the roles of clinical and demographic factors in glomerulonephritis, as well as genomic alterations in selected *UGTs* (*1A9, 1A7*, and *2B7*) and *ABCB1*/*MDR1*, in the pharmacokinetics of MPA and its glucuronide metabolites

MPAG and AcMPAG using linear models. This research was conducted since MPA is often used in an off-label indication for the treatment of autoimmune-mediated glomerulonephritis. When drugs are used off-label in patient populations that are different from where the drug was originally approved, there is a potential for pharmacokinetic alterations that may require dosing changes to enable an appropriate exposure (AUC), optimizing outcomes and minimizing adverse effects. This is particularly relevant for glomerulonephritis where, unlike kidney transplant patients, primarily with decreases in GFR, glomerulonephritis patients can have reductions in GFR in addition to decreases in serum albumin and increases in urinary protein excretion. Previous reports by our research team suggested altered MPA oral clearance in patients with lupus nephritis and ANCA-associated vasculitis [13, 14], as opposed to what was previously reported for kidney transplant recipients [17, 18]. In smaller patient populations employing less sophisticated statistical analyses, we found that nonwhite race [14] (for SVV patients), and decreased serum albumin [13] (for SLE nephritis patients) favored increases in oral clearance. However, we also reported an overall reduction in the metabolic capacity (e.g., metabolic ratio; MPAG AUC/MPA AUC) in the glomerulonephritis patients [13, 14] compared with kidney transplant recipients. Our current study of an expanded population of glomerulonephritis patients found relevant alterations in MPA pharmacokinetics influenced by clinical covariates and pharmacogenomic factors. This study is novel as it describes these former interactions in a glomerulonephritis population and seeks to elucidate the relative contribution of each factor on pharmacokinetics. Additionally, this study evaluated the influence on MPA pharmacokinetics of less well-described polymorphisms in *UGT2B7*, *UGT1A7* and *ABCB1*/*MDR1*. The resultant multivariable models explained 30–50% of the pharmacokinetic outcomes of MPA. Genomic factors alone explain about 10% of the pharmacokinetic outcomes MPA.

Our current cohort of 46 patients with glomerulonephritis represented a spectrum of laboratory abnormalities that would be typical in patients with these disorders, i.e., some patients with mild disease and others with moderate to severe manifestations. The study population was hence broad enough in the clinical manifestations of the glomerular disease to be able to make inferences about the effects of the disease parameters on the pharmacokinetics of MPA and its glucuronide metabolites. Our regression results (Tables 6, 7) suggested a primary importance of kidney function, through either SCr or eClcr, for the prediction of most pharmacokinetic parameters for MPA and its metabolites. This finding is important as it reminds clinicians to be mindful of the effects of kidney disease on the disposition of drugs such as MPA, which are not readily eliminated unchanged by the kidneys. It is consistent with suggestions by others [19], that drug metabolism and transport derangements, among other unknown effects, occur in kidney disease, and these effects can alter the pharmacokinetics of drugs. In addition to kidney function, UP:Cr also contributed toward the prediction of the pharmacokinetics of MPA and its metabolites. An elevated UP:Cr predicted reduced exposure (AUCs) and increased oral clearance for MPA. Two previous publications by our group also highlight the need to be cognizant of the effects of UP:Cr and/or serum albumin on the pharmacokinetics of highly bound drugs, particularly when assessing total drug concentrations [13, 20]. According to multivariate regression data from the present study, an increase in UP:Cr from 0.5 to 3.5 at a stable SCr of 2 mg/dL (176.8 mol/L) would result in a MPA $AUC_{0–12}$ decrease of 25 units (from 76 μg · h/mL to 51 μg · h/mL). Similarly, at a stable UP:Cr of 0.5, an increase in SCr from 2 mg/dL to 5 mg/dL (176.8 mol/L to 442 mol/L) would result in a tripling of the $AUC_{0–12}$ (from 76μg · h/mL to 228 μg · h/mL).

The glomerulonephritis study population reported here included mostly Caucasian and African–American patients (59% and 28% respectively), but relatively few patients of other races to enable ascertainment of a multitude of race-related effects on MPA disposition.

Caucasian race was predictive of higher MPA trough concentrations, and higher exposure (AUC) to the metabolites MPAG and AcMPAG. Our results contrast with data from the kidney transplant literature that have not reported associations between race and MPA disposition [21, 22]. Our multivariate regression results show that at a stable SCr of 2 mg/dL (176.8 mol/L), Caucasian patients have a 2-fold higher Ctr concentration than non-Caucasians. Within Caucasian patients, a doubling of SCr would result in an 8-fold increase in Ctr concentration.

Female subjects were adequately represented in our study (67%), but have historically been under-represented in biomedical research. Our results suggest that female gender may predict a higher renal clearance of the MPAG metabolite. Since SCr was also contributory to increased renal clearance in the linear regression model, our data suggest that either there exists an added effect of female gender on top of the effect of decreased SCr on MPAG renal clearance and/or there is an interaction between decreased SCr and female gender. Since it is generally assumed that female subjects have a lower SCr value for level of kidney function compared with males, the latter explanation may be warranted. However, the MPAG metabolite is a known substrate for the multidrug resistance-associated proteins (MRPs) [23] and this transporter is located in the kidney tubules. Previous animal data (rats) suggest increased liver expression and increased activity of MRP2 in females as compared with males [24, 25], and this differential activity of MRP2 may also explain the gender-related influence on renal clearance of MPAG.

Four SNPs in three genes were evaluated in glomerulonephritis patients to assess their role in the disposition of MPA and its metabolites. The specific SNPs were selected based on their hypothesized, yet limited in vivo data on their influences on human MPA pharmacokinetics [3, 4, 26]. Additionally, previous research has established that UGT1A7, UGT1A9, and UGT2B7 have significant catalytic activities for MPA [1, 27–30]. *UGT2B7 C802T* has been purported to result in 25% increases in total AUC and 48% increases in unbound AUC for MPA, as well as increases in maximal plasma concentrations and urinary AcMPAG. [3, 4, 26]. Our univariate models employing only genotypes showed increased recycling ($AUC_{6–12}$) and decreased renal clearance for MPA in the variant homozygous group. These results seem consistent with the previous publications. Increased oral clearance, decreased $AUC_{0–12}$ and $AUC_{6–12}$, and increased renal clearance were demonstrated in patients exhibiting heterozygosity for *UGT2B7 C802T*. Increased AcMPAG renal clearance was also demonstrated in the *UGT2B7* heterozygous group. An increase in MPA renal clearance may be a reflection of a reduction in the renal clearance of AcMPAG. The finding of a decrease in MPA $AUC_{0–12}$ in the heterozygous group cannot currently be explained, but may be due to the intermediate effect of this genotype and its greater frequency compared with the homozygous variant. In the multivariable models, *UGT2B7* heterozygosity was the only genetic factor remaining, where it predicted increased MPA renal clearance. A recent study has described the expression of *UGT2B7* in the kidney [31], suggesting a greater contribution of the metabolic enzyme toward the renal clearance of MPA through its metabolites. Regarding the *UGT1A7 T622C* SNP, a previous study in Japanese patients failed to detect any MPA pharmacokinetic alterations [6]. A recent review publication by Knights and Miners [30] describes the expression of UGT1 and UGT2 protein in human kidney tissues, with positive expression of UGT1A7 described. A major issue toward determination of specific UGT proteins in tissues is related to the availability of specific antibodies. Widespread use of absolute quantitative LC/MS methodologies should help to confirm the absolute amounts of UGTs, including 1A7 in various human tissues. These data will provide a more definitive assessment regarding the contribution of specific tissues to the metabolism of MPA. In our univariate assessments analyzing only genotype variables, heterozygosity of the UGT1A7 variant contributed toward increased oral clearance and decreased maximal plasma concentration values. While there are currently no

human studies demonstrating the effects of the *ABCB1*/*MDR1* polymorphism on MPA pharmacokinetics, an animal study in *ABCB1*/*MDR1*-deficient mice suggests the possibility of increased MPAG concentration when the activity of this protein is low [11], suggesting decreased export function and resulting in decreased clearance. We failed to detect any effects of *ABCB1*/*MDR1 C1236T* and *C3435T* on the alteration of the systemic pharmacokinetics of MPA, suggesting a minimal to absent role of this transporter on MPA pharmacokinetics.

Although the current study's findings demonstrated moderate effects of clinical and demographic variables and minimal effects of *UGT1A7* and *UGT2B7* genotypes on explaining the disposition of MPA and its metabolites, the overall effects of these former genotypes should not be discounted secondary to limitations in the study. The main limitation surrounds the limited number of patients who contributed to the homozygous variant genotype groups. Employing a larger population of patients, perhaps by attempting to select study patients based on specific genotypes may have enhanced the evaluation of the effects of various genotypes on MPA pharmacokinetics. Regarding *UGT1A7 T622C*, only one patient was classified as a homozygous variant, limiting our ability to fully evaluate the potential impact of this genotype on MPA pharmacokinetics. Our assessments surrounding the *UGT1A7* SNP encompassed the homozygous wildtypes and heterozygotes. Since heterozygotes in drug-metabolizing gene SNPs often have less alteration in function than homozygous variants, the differences in the pharmacokinetic variables between these groups may be more difficult to detect in smaller studies. The numbers were somewhat less limited for *UGT2B7* genotype assessments where a total of 10 patients were included in the homozygous variant group. Similarly, the *ABCB1*/*MDR1* homozygous variants at nucleotide base positions 1236 and 3435 were represented by only 4 and 5 patients respectively, also limiting the ability to evaluate the full role of this covariate in MPA pharmacokinetics. We did not evaluate the influence of genetic variations in additional efflux transporters such as *ABCC2*, since previous studies have evaluated for alterations in MPA disposition. While the patients in this study represented a fairly broad range of laboratory values for UP:Cr and serum albumin, they were primarily representative of patients with mild to moderate forms of glomerulonephritis. It is conceivable that more acute and/or severe forms of glomerulonephritis might have additional alterations in MPA disposition. Lastly, as we have assessed numerous pharmacokinetic variables in our patients, larger studies will be needed to validate the most relevant clinical findings of the current study.

Conclusions

The results from this study demonstrated the potential importance of factoring in clinical and demographic variables when assessing the disposition of drugs such as MPA in patients with glomerulonephritis. In this glomerulonephritis cohort, the predictive value of clinical and demographic covariates, especially kidney function (eClcr and Scr), urinary protein:creatinine ratio, serum albumin, and race were more profound than that of the *UGT1A7*, *UGT2B7*, and *ABCB1/MDR1* genotypes on MPA pharmacokinetics. The former covariates explained 2–4 times more of the variability in the pharmacokinetic variables of MPA than did the genotype covariates. Our data suggest the need for further research and larger pharmacogenomic studies in glomerulonephritis to adequately assess the contributions of genetics- and disease-related perturbations to MPA metabolism and transport.

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Table 1

Single nucleotide polymorphisms (SNPs)

Gene	Location	Sequence with SNP denoted
UGT1A9	G8A	GTTCTCTGATGGCTT[G/A]CACAGGGTGGACCAG
UGT1A9	C98T	TAGTGCCCA[C/T]GGATGGGAG
UGT1A9	T-275A	TTAATAATTCTGCT[T/A]CTAAACTTAACATTGCAG
UGT1A9	C-2152T	CGCTTCCCGGGTT[C/T]AAGTGATTCTCCTGCC
UGT2B7	C802T	GGAATTTTCAGTTTCCT[C/T]ATCCACTCTTACCAAAT
UGT1A7	T622C	AGAGAGTA[T/C]GGAACCAC
ABCB1/MDR1	C1236T	GATCTTGAAGGG[C/T]CTGAACCTGAAGGTGCAG
ABCB1/MDR1	C3435T	GTCACAGGAAGAGAT[C/T]GTGAGGGCAGCAAA

MDR, multidrug resistance; UGT, uridine diphosphate glucuronosyltransferase

Table 2

Sequencing and polymerase chain reaction (PCR) primers

Primer pair	Location	Sequence (5′-3′)
1	*UGT1A9* G8A and C98T	F-CCTGCTCTCAGCTGCAGTTCTCT R-CTTCACTGTGCAATTCAGTGATCTT
2	*UGT1A9* C-2152T	F-GTAGGTCTTTTACATTTCC R-CCTGAAACAGCAAAACCAA
3	*UGT1A9* T-275A	F-TTGCTTAGAGTATGAGTTGCCATCTT R-TTTGTATGTTTTCCAGACAACAGTAGC
4	*UGT2B7* C802T	F-GTAAATATCTGTGTCATC R-GACTATAGAATCATTTCTACTG
5	*UGT1A7* T622C	F-GTGCCCTGCTCCTCTTTCCTAT R-ACGGGTTTGGGATACTCCAAA
6	*ABCB1*/*MDR1* C1236T	F-GAAGAGTGGGCACAAACCAGATA R-CATCCCCTCTGTGGGGTCATA
7	*ABCB1/MDR1* C3435T	F-GAGCCCATCCTGTTTGACTG R-GCATGTATGTTGGCCTCCTT

Table 3

Demographics, clinical and pharmacokinetic data (mean ± standard deviation). Data represent 27 small vessel vasculitis and 19 lupus nephritis prospective patients (total n=46). Data provided as mean (SD); range

Parameter	Data
Age (years)	46.0 (15.0); 22–78
Race (white/black/other)	27/13/6
Gender (male/female)	15/31
Weight (kg)	85.1 (19.7); 47–124
eClCr (mL/min)	93.4 (46.3); 18–185
UP:Cr	0.76 (1.48); 0–7.9
Serum albumin (g/L)	40.9 (50.2); 26–52

Table 4

Pharmacokinetics of mycophenolic acid and metabolites in glomerulonephritis

	MPA $_{total}$(*n*=46)	**MPA $_{free}$(*n*=46)**	**MPAG(*n*=46)**	**AcMPAG(*n*=41)**
C_{max} (μg/mL)[a]	20.9 (17.9)	0.30 (0.39)	63.9 (50.2)	0.91 (1.08)
T_{max} (h)	1.46 (1.48)	N/A	3.02 (2.54)	1.68 (1.65)
C_{tr} (μg/mL)[a]	4.11 (4.46)	0.07 (0.11)	31.7 (27.8)	0.28 (0.63)
AUC $_{0–12}$ (μg · h/mL)[a]	66.3 (43.8)	1.07 (1.57)	498 (433)	3.88 (4.80)
Cl/F (mL/min)	305 (173)	31.7 (28.6)[b]	N/A	N/A
AUC $_{6–12}$ (μg · h/mL)[a]	24.1 (19.9)	N/A	214 (191)	1.53 (2.12)
Cl_R/F (mL/min)	3.74 (4.70)	N/A	46.5 (45.9)	45.9 (53.4)
Free fraction (%)	1.63 (1.49)	N/A	12.3 (6.74)	N/A
T ½ (h)	14.5 (18.7)	N/A	16.9 (26.1)	10.4 (8.26)
AUC $_{6–12}$/AUC $_{0–12}$ %	35.4 (12.2)	N/A	39.7 (41.9)	0.37 (0.14)

AcMPAG, acyl MPAG; AUC $_{0–12}$, area under the plasma concentration time curve from 0–12 h; AUC $_{6–12}$, area under the plasma concentration time curve from 6–12 h; AUC $_{6–12}$/AUC $_{0–12}$, fraction of AUC due to enterohepatic recycling; Cl/F, oral clearance; Cl_R/F, renal clearance; C_{max}, maximum concentration in plasma after a dose; C_{tr}, minimum concentration in plasma after a dose; eClcr, estimated creatinine clearance; MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide; N/A, not applicable; Tmax, time to maximum plasma concentration; T½, half life

[a] Data concerning MPA were dose-normalized

[b] L/min

Table 5

Genotype frequency distributions (frequency [*n*])

		Small vessel vasculitis(*n* = 27)	Lupus nephritis(*n* = 19)
UGT1A9			
G8A	G/G	1.0 (27)	1.0 (19)
	G/A	0 (0)	0 (0)
	A/G	0 (0)	0 (0)
C98T	C/C	0.96 (26)	1.0 (19)
	C/T	0.04 (1)	0 (0)
	T/T	0 (0)	0 (0)
C-2152T	C/C	0.96 (26)	1.0 (19)
	C/T	0.04 (1)	0 (0)
	T/T	0 (0)	0 (0)
T-275A	T/T	0.96 (26)	1.0 (19)
	T/A	0.04 (1)	0 (0)
	A/A	0 (0)	0 (0)
UGT1A7[a]			
T622C	T/T	0.46 (12)	0.47 (9)
	T/C	0.54 (14)	0.47 (9)
	C/C	0 (0)	0.06 (1)
UGT2B7[a]			
C802T	C/C	0.31 (8)	0.47 (9)
	C/T	0.42 (11)	0.37 (7)
	T/T	0.27 (7)	0.16 (3)
ABCB1/MDR1			
C1236T	C/C	0.37 (10)	0.53 (10)
	C/T	0.48 (13)	0.47 (9)
	T/T	0.15 (4)	0 (0)
C3425T	C/C	0.33 (9)	0.37 (7)
	C/T	0.52 (14)	0.58 (11)
	T/T	0.15 (4)	0.05 (1)

ANCA, anti-neutrophil cytoplasmic antibody

[a]Genotyping results missing in one ANCA vasculitis patient

Table 6

Final univariate models for the separate effects of clinical, demographic, and genotype parameters on the prediction of pharmacokinetic outcomes

Dependent variable	[a] Independent variable	Coefficient	Model *p*	Model R^2
Clinical and demographic factors				
Mycophenolic acid				
C_{max}	Albumin	0.4902	0.0280	0.1051
	Steroid Dose	0.0485	0.0261	0.1151
Ctr	Age	0.0191	0.0193	0.1182
	Race	0.4924	0.0478	0.0861
	Albumin	0.4940	0.0384	0.0939
	UP:Cr	−0.2128	0.0099	0.1416
	Scr	0.6891	<0.0001	0.3218
	eClcr	−0.0098	0.0004	0.2527
$AUC_{0–12}$	SCr	0.4261	0.0004	0.2490
	Age	0.0114	0.0494	0.0850
	Albumin	0.3690	0.0272	0.1060
	eClcr	−0.0066	0.0006	0.2372
	UP:Cr	−0.1558	0.0070	0.1537
$AUC_{6–12}$	UP:Cr	−0.2292	0.0010	0.2219
	SCr	0.5980	<0.0001	0.3271
	eClcr	−0.0078	0.0010	0.2193
Cl/F	Age	−0.0127	0.0305	0.1020
	Albumin	−0.3733	0.0287	0.1041
	UP:Cr	0.1595	0.0069	0.1546
	SCr	−0.4250	0.0006	0.2377
	eClcr	0.0072	0.0003	0.2642
	Weight	0.0075	0.0989	0.0607
Cl_R/F	Weight	0.0221	0.0225	0.1152
Cl_{unb}	eClcr	0.0071	0.0326	0.1066
AUC_{unb}	eClcr	−0.0068	0.0397	0.0991
Ctr_{unb}	Scr	0.5738	0.0156	0.1343
	eClcr	−0.0086	0.0276	0.1129
Mycophenolic acid glucuronide				
$AUC_{0–12}$	Age	0.0183	0.0325	0.0998
	Caucasian	0.6100	0.0175	0.1216
	UP:Cr	−0.1779	0.0415	0.0911
	SCr	0.5059	0.0059	0.1596
$AUC_{6–12}$	Age	0.0181	0.0396	0.0928
	Caucasian	0.6143	0.0200	0.1169
	UP:Cr	−0.1834	0.0407	0.0918
	SCr	0.5960	0.0014	0.2100

Dependent variable	[a] Independent variable	Coefficient	Model *p*	Model R^2
	eClcr	−0.0077	0.0108	0.1386
Cl_R/F	Age	−0.0204	0.0402	0.0943
	SCr	−0.6148	0.0030	0.1869
	eClcr	0.0084	0.0181	0.1232
	Female	0.6003	0.0518	0.0851
Acyl-mycophenolic acid glucuronide				
$AUC_{0–12}$	Age	0.0437	0.0009	0.2497
	Caucasian	0.9104	0.0311	0.1137
	Albumin	1.0663	0.0093	0.1611
	UP:Cr	−0.2927	0.0311	0.1137
	eClcr	−0.0182	<0.0001	0.3487
$AUC_{6–12}$	Age	0.0298	0.0067	0.1780
	UP:Cr	−0.2361	0.0282	0.1205
	Scr	0.9490	<0.0001	0.3375
	eClcr	−0.0160	<0.0001	0.4347
Cl_R/F	Age	−0.0320	0.0159	0.1473
	Albumin	−1.0760	0.0054	0.1913
	eClcr	0.0130	0.0045	0.1981
Genotype factors				
Mycophenolic acid				
Cl/F	*UGT1A7* heterozygote	0.3508	0.0462	.0877
	UGT2B7 heterozygote	0.3778	0.0355	0.0966
$AUC_{0–12}$	*UGT2B7* heterozygote	−0.3702	0.0354	0.0967
$AUC_{6–12}$	*UGT2B7* heterozygote	−0.4844	0.0240	0.1105
	UGT2B7 variant/variant	0.5968	0.0185	0.1198
Cmax	*UGT1A7* heterozygote	−0.4647	0.0432	0.0897
Cl_R/F	UGT2B7 heterozygote	1.1748	0.0022	0.1974
	UGT2B7 variant/variant	−0.9237	0.0456	0.0897
Acyl-mycophenolic acid glucuronide				
Cl_R/F	*UGT2B7* heterozygote	0.8323	0.0408	0.1083

Cl_{unb}, unbound oral clearance; $Cmax_{unb}$, unbound maximum concentration in plasma after a dose; Cmax, maximum concentration in plasma after a dose; Ctr, minimum concentration in plasma after a dose; Ctr_{unb}, unbound minimum concentration in plasma after a dose; Scr, serum creatinine

[a]The natural logarithmic transformation was used for all dependent variables except for acyl MPAG MR

Table 7

Final multivariable linear models of the combined effects of genotype, clinical, and demographic parameters on pharmacokinetics

Dependent parameters[a]	Independent parameters (*p* value)	Coefficient	Model *p* value	Model R^2
Mycophenolic acid				
Ctr	Caucasian race (0.008)	0.5384	<0.0001	0.4244
	Scr (<0.0001)	0.7074		
$AUC_{0–12}$	UP:Cr (0.008)	−0.1346	<0.0001	0.3622
	Scr (0.0005)	0.3925		
$AUC_{6–12}$	UP:Cr (0.0005)	−0.1996	<0.0001	0.4931
	Scr (<0.0001)	0.5482		
Cl/F (1)	UP:Cr (0.0084)	0.1384	<0.0001	0.3526
	Scr (0.0008)	−0.3905		
Cl/F (2)	Weight (0.0143)	0.0090	<0.0001	0.4397
	UP:Cr	(0.0053)	0.1387	
	Scr (0.0002)	−0.4153		
Cl_R/F	Weight (0.0382)	0.0185	0.0011	0.2763
	UGT2B7 Het (0.0039)	1.0715		
Mycophenolic acid glucuronide				
$AUC_{0–12}$	Caucasian race (0.0063)	0.6443	0.0005	0.2950
	SCr (0.0022)	0.5280		
$AUC_{6–12}$	Caucasian race (0.0052)	0.6544	0.0001	0.3420
	SCr (0.0004)	0.6182		
Cl_R/F	Female gender (0.0425)	0.5703	0.0016	0.2636
	SCr (0.0027)	−0.6012		
Acyl-mycophenolic acid glucuronide				
$AUC_{0–12}$	Caucasian race (0.0100)	0.8772	<0.0001	0.4542
	eClCr (<0.0001)	−0.0182		
$AUC_{6–12}$	Age (0.0408)	0.0197	<0.0001	0.4092
	SCr (0.0005)	0.8193		
Cl_R/F	Albumin (0.0030)	−0.9947	<0.0001	0.4239
	SCr (<0.0005)	−0.09506		

[a]The natural logarithmic transformation was used for all dependent variables except AcylMPAG $AUC_{0–12}$. Box–Cox transformation was used for AcylMPAG $AUC_{0–12}$ (lambda=0.2)