Disposition of Mycophenolic Acid and Its Glucuronide Metabolites in Subjects with Glomerulonephritis: Implications of Genes and Effects on Kidney Outcomes

Melanie S. Joy, Pharm.D.

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Approved By:

Philip C. Smith, Ph.D.

Dhiren Thakker, Ph.D.

Kim R. Brouwer, Pharm.D., Ph.D.

Ronald J. Falk, M.D.

Joseph K. Ritter, Ph.D.

Abstract

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Under the direction of Philip C. Smith, Ph.D.

Glomerulonephritis is the third most frequent cause of end-stage kidney disease in the U.S. population. Treatments include immunosuppressant agents such as mycophenolate mofetil. The purpose of undertaking the studies included in this dissertation were to assess the pharmacokinetic alterations of mycophenolic acid in glomerulonephritis, to evaluate the role of patient-level demographic data, clinical data, and genomic alterations on pharmacokinetics, and to evaluate determinants of treatment-related outcomes. We evaluated the pharmacokinetics of mycophenolic acid in 45 patients receiving maintenance mycophenolic acid therapy. Pharmacogenomic assessments were conducted in 85 patients to evaluate genotype frequencies of drug metabolizing enzymes (uridine diphosphate glucuronosyltransferases; UGTs) and drug transporters (including multidrug resistance protein; MDR1) and mycophenolic acid disposition and relative risk of autoimmune diseases. mRNA expression patterns and their relationships to genomics were conducted in 45 patients. The pharmacokinetics showed enhanced oral clearance and reduced metabolic ratios in glomerulonephritis patients. Pharmacokinetics were more highly influenced by serum creatinine/creatinine clearance, urinary protein excretion, race, and gender, than single nucleotide polymorphisms in the *UGTs* or

MDR1. The expression of transcript for drug metabolizing genes and transporter genes was variable across SLE and SVV treated versus untreated patients and healthy controls. The drug transporters were expressed in most patients, while the UGTs were expressed in only 50% of patients. Differences in transcript expression by race, treatment, disease, and genotypes were demonstrated. A disease-gene association risk was found in the study; the relative risk of SVV was increased in patients who were heterozygous or homozygous for the *UGT2B7 C802T* polymorphism. Kidney-related outcomes, as assessed by urinary protein to creatinine ratio, were worsened in patients with the *UGT1A7* C622T polymorphism and improved with the *MDR1 C3435T* polymorphism. Composite outcomes (dialysis, death, or transplantation) were increased in patients who had reduced transcript expression for *ABCB1* in peripheral blood leukocytes. The conducted studies demonstrated the highly complex relationships between drug disposition, patient-level clinical and demographic data, and genome-level variability. Numerous opportunities exist to further delineate these relationships in cell-based assays, animal models of glomerulonephritis, and larger translational studies that assess serial measurements of drug exposure and transcript expression.

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Table of Contents

List of Tables

Page

List of Figures

List of Abbreviations

AcMPAG	Acyl-mycophenolic acid glucuronide
Ae	Amount excreted in the urine
ANCA	Antineutrophil cytoplasmic antibodies
ANOVA	Analysis of variance
AUC	Area under the plasma concentration time curve
cDNA	Copy deoxyribonucleic acid
Clcr	Creatinine clearance
Cl/F	Apparent oral clearance
Cl_{NR}/F	Apparent nonrenal clearance
Cl_{R}/F	Apparent renal clearance
Cl_{unb}/F	Apparent unbound oral clearance
Cmax	Maximum concentration in plasma
Ctr	Minimum concentration in plasma
FOCE	First order conditional estimate
HPLC	High pressure liquid chromatography
MDR1	Multidrug resistant protein
MOVF	Minimized objective function
MPA	Mycophenolic acid
MPAG	Mycophenolic acid glucuronide
MR	Metabolic ratio
mRNA	Messenger ribonucleic acid
MRT	Mean residence time

SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SVV	Small vessel vasculitis
Tmax	Time to maximum concentration in plasma
UGT	Uridine diphosphate glucuronosyltransferase
UP:Cr	Urinary protein to creatinine ratio
UV	Ultraviolet

Chapter One

Introduction

Introduction

Autoimmune diseases account for 15% of the ~ 500,000 patients with end-stage renal disease (ESRD) cases in the U.S, just after diabetes mellitus and hypertension. [1] Additionally, while the exact percentages are currently unknown, these diseases afflict some of the ~20 million individuals in the U.S. with chronic kidney disease who are not yet dialysis dependent. [2] Antineutrophil cytoplasmic autoantibody (ANCA) small vessel vasculitis (SVV) and systemic lupus erythematosus (SLE) are two autoimmune diseases that often afflict the kidneys. ANCA SVV typically strikes an older, predominantly Caucasian patient population of roughly equal gender distribution. SLE nephritis, in contrast, affects a predominantly younger, female, African-American population. Although the natural course of ANCA SVV and SLE nephritis heralds poor outcomes, standard therapeutic approaches using the combination of glucocorticoids and cyclophosphamide results in improved, but less than optimal outcomes.

The current treatment approaches for both ANCA SVV and SLE nephritis are based on therapy with a regimen of either cyclophosphamide or mycophenolic acid (MPA) as either the sodium salt or mofetil, with or without glucocorticoids. Data from the University of North Carolina Kidney Center suggest that both cyclophosphamide and MPA are used extensively in both glomerulonephritis populations in North Carolina. In fact, ANCA SVV treatment data from the University of North Carolina showed an 84.7% remission rate in patients treated with combined therapy (cyclophosphamide and glucocorticoids) when compared to a 56% remission rate in patients receiving glucocorticoids alone. [3] However even with combined therapy employing cyclophosphamide plus prednisone, approximately 40% of ANCA SVV patients who initially respond tend to relapse within the first six months. [3] Two recent publications have described the use of MPA for inducing remission of ANCA SVV.[4, 5] In one study, 35 patients with moderate renal involvement who were prescribed mycophenolate mofetil or intravenous cyclophosphamide were followed for treatment related outcomes. [5] Birmingham Vasculitis Disease Activity Scores (BVAS) (mean±SD) were lower in the MPA versus cyclophosphamide

treatment group (0.2±0.89 vs 2.6±1.7, $p < 0.05$) at 6 months. The percentage of patients with complete remission at six months was higher in the MPA vs cyclophosphamide group (77.8% vs 47.1%), and serum ANCA titers were reduced to normal in 41.7% and 16.7% of MPA vs cyclophosphamide groups, respectively. [5] The side effects were similar between treatment groups. [5] Another recent study evaluated remission responses in 32 patients who received MPA (as mofetil) and prednisolone as they were not candidates for cyclophosphamide therapy. [4] This study reported complete remission in 78%, partial remission in 19%, and non-response in 3% of patients. Fifty-two percent of the initial complete responders and 100% of the partial responders relapsed. The median relapse-free survival rate was 16 months. Relapse-free survival rates at 1, 3, and 5 years were reported to be 63%, 38%, and 27%, respectively.[4]

For SLE nephritis patients receiving a regimen of cyclophosphamide and glucocorticoids, a 71% and 50% five-year renal survival rate has been reported in Caucasian and African-American patients, respectively. [6] Mycophenolic acid and cyclophosphamide have shown equal renal outcomes. [7] Hence, therapy with MPA for induction and maintenance of remission has gained favor in SLE nephritis. [7, 8] A recent meta-analysis reported on the use of MPA for induction and maintenance of severe lupus nephritis. [9] A total of 307 patients from four randomized controlled trials were included for assessment of MPA versus cyclophosphamide, and two trials were included for MPA versus azathioprine. In the induction assessment, MPA therapy increased the relative risk for a complete remission rate (Relative Risk 3.10) and decreased the relative risk of infection (Relative Risk 0.65) and leukopenia (Relative Risk 0.66) versus cyclophosphamide. Mycophenolic acid was similar to azathioprine with respect to SLE nephritis prognosis outcomes and side effects (amenorrhea and herpes zoster). [9] These data show consistent results demonstrating the viability of MPA treatment in patients with ANCA SVV and SLE nephritis.

Glomerulonephritis

Treatment Issues

The published data for MPA therapy in SLE nephritis (as compared to cyclophosphamide) have shown at least equivalent renal outcomes (if not improved), and reduced side effects of leukopenia, amenorrhea, and infections. [7-9] The data for MPA therapy in the treatment of ANCA SVV is more sparse than SLE nephritis, but the limited data from generally smaller sized studies has been consistent with the data from SLE nephritis. [4, 5, 10, 11]

Even as MPA is gaining favor in the treatment of SLE nephritis and ANCA SVV, several limitations to treatment regimens currently exist. The primary limitation is that dosage regimens employing MPA are based mainly on regimens used in renal transplantation. Clinicians typically treat patients with a protocol based on targeting a dose of 1 to 1.5 grams twice daily by initiating therapy with 500 mg twice daily and advancing the dose based on maintaining leukocyte counts above 3.0 to 5.0 x 10^9/L and minimizing gastrointestinal side effects. Additionally, there is no goal for MPA exposure (area under the plasma concentration time curve, i.e. AUC) that has been established in patients with glomerulonephritis. Data from the kidney transplant literature suggest that MPA AUC $_{0\text{-}12}$ targets of 30 to 60 mg hr/L are effective for patients receiving triple drug combinations with MPA, corticosteroids, and calcineurin inhibitors. [12] The second limitation is the inability to predict the patient's overall response on outcomes based on measurable data such as pharmacokinetic variables from patients with glomerulonephritis. Few studies have been conducted that have assessed the pharmacokinetics of MPA in glomerulonephritis and none of these have attempted to evaluate the contribution of pharmacokinetics to treatment-related outcomes. [13-16] The third limitation is the absence of data that evaluates initial and long-term kidney outcomes according to phenotype and genotype differences in drug metabolizing enzymes and transporters and/or differences in disease severity, both of which may lead to alterations in pharmacokinetics of MPA in patients with glomerulonephritis. Together, these limitations reduce our knowledge and the ability to prescribe specific dosages and regimens that

may be beneficial in glomerulonephritis patients as a whole, and for individual patients within this disease category. The exploration of possible genotype-phenotype relationships may be necessary to improve outcomes for these glomerulonephritides that exhibit resistant and relapsing characteristics.

Alterations in Drug Disposition

Treatment approaches for glomerulonephritis in general have been borrowed from other disease populations, hence, the disposition of drugs used in treating these diseases have never been rigorously evaluated in patients. Treatment of glomerulonephritis is complicated by several important pharmacokinetic concerns. First, incorrect dosing of prescribed medications may occur due to the unique loss of drug in the urine. Urinary losses are not normally a concern for drugs that are highly and reversibly bound to plasma proteins such as albumin, secondary to the intact glomerular filtration barrier. However, the relative impact of urinary loss of bound drug that may undergo a clearance mechanism has not been established in glomerulonephritis, where there are varying degrees of proteinuria. A second reason for incorrect dosing includes an increase in “unbound” or “free” fraction in the plasma associated with hypoalbuminemia. Increased “unbound” fractions can result in increased elimination through pathways such as glomerular filtration, tubular secretion, and hepatic and extrahepatic metabolism. The contribution of reduced kidney function (e.g. glomerular filtration rate) in the setting of serum albumin abnormalities requires clarification regarding the impact on unbound concentrations. A third concern in proteinuric states is the presence of altered body composition, edema, and increases in the volume of distribution of medications. Chronic proteinuria may alter various independent and dependent pharmacokinetic parameters including C_{max} and C_{ss} (maximal concentration of drug in plasma after a single dose or at steady state, respectively), T_{max} (time to maximal plasma concentration), K_{el} (terminal elimination rate constant), $T_{1/2}$ (elimination half-life), Vd (volume of distribution in central and peripheral body compartments), Cl (clearance), and AUC (area under the plasma concentration time curve).

Pharmacokinetic parameters in patients with proteinuria that differ from those determined in "normal" populations could potentially result in drug under- or over-dosing, and reduced efficacy and/or increased toxicity, especially if the unbound pharmacokinetic values are sufficiently altered. One limitation of the available pharmacokinetic data in patients with kidney disease is the absence of data for varying degrees of proteinuria/albuminemia and concurrent alterations in the glomerular filtration rate. Comprehensive pharmacokinetic assessments in inadequately evaluated diseased populations, e.g. glomerulonephritis, has the potential to result in more appropriate drug-dosage regimens for potentially useful medications and hence, may allow improved efficacy and safety for medications.

Chronic Kidney Disease and Alterations in Drug Disposition

There are several examples of reductions in albumin binding of drugs in glomerulonephritis that leads to increased unbound fractions. [17, 18] The highly protein bound drugs (protein binding ≥ 90%) are most causally implicated. On the contrary, increased alpha-1 acid glycoprotein levels have been documented in chronic kidney disease, potentially leading to enhanced binding of basic drug moieties. [19] More recently, it has been reported that chronic kidney disease is associated with qualitative and quantitative reductions in metabolism via several different pathways. When rat hepatocytes were incubated with serum from patients with severe chronic kidney disease, the levels of CYP450 protein and mRNA were reduced by more than 45% for the 1a2, 2c6, 2c11, 2d1/2c2, 3a2, and 4a1/4a3 isoforms. [20] Hepatic acetylation pathways were also diminished; Nat1 and Nat2 protein and gene expression studies were decreased in a rat model of chronic kidney disease. [21] Studies in patients with chronic kidney disease have revealed reductions in the nonrenal clearance of drugs that are substrates for CYP2D6, 2C8, 2C9, 2C19, 3A4, 2B6, 2E1, N-acetylation, and glucuronidation pathways. [22-31] Drug transporters also have also been suggested to be altered in chronic kidney disease. Protein expression of intestinal drug transporters (P-glycoprotein (Pgp), multidrug resistance proteins (Mrp2, Mrp3)) were reported to be reduced by > 40% in rats with chronic renal failure.[32]

Activities of Pgp and Mrp2 were decreased by 30% and 25%, respectively, in a rat model of chronic kidney disease, suggesting increased bioavailability of certain drugs. [32] In the liver, reductions in organic anion transporting polypeptides (Oatp1, Oatp2, Oatp4), and increases in Mrp2, Mrp3, and Pgp proteins have been described in rat models of chronic kidney disease. [33-35] A conflicting report, however, suggested no change in Mrp2 protein expression, but enhanced mRNA expression. [35] Quantitative changes in kidney transport proteins also have been described in chronic kidney disease including reductions in Oat1 and Oct2, and increases in Pgp and Mrp2. [34, 36, 37] These data support the hypothesis that drug disposition may be altered in patients with chronic kidney disease in general, but there is currently a paucity of data regarding drug disposition in glomerulonephritis.

Mycophenolic Acid

Pharmacokinetic Disposition of Mycophenolic Acid in Renal Transplantation

Mycophenolic acid (as the mofetil, Cellcept®) originally was approved in the mid-1990's for prophylaxis of rejection in renal transplant recipients. Hence, most of the data pertaining to MPA pharmacokinetics has been derived from the renal transplant population. It is thus instrumental to fully understand the pharmacokinetic behavior of MPA in the renal transplant population in order to comprehend the deviations from this behavior that may be observed in populations representing off-label uses, such as glomerulonephrits. Figure 1.1 demonstrates the proposed metabolic scheme for MPA and includes the chemical structures for MPA and its glucuronide metabolites. [38]

As shown in Figure 1.1, the morpholino-ester prodrug of MPA (mycophenolate mofetil) undergoes hydrolysis by esterases (in the stomach, small intestine, blood, and liver) resulting in the absorption of MPA (LogP 3.2) most likely via an active mechanism secondary to the structure having a negative charge at physiologic pH. [39] MPA is presented to the liver where it is glucuronidated by uridine diphosphate glucuronosyltransferase (UGT) enzymes to the phenolic metabolite mycophenolic acid glucuronide (MPAG) and the acylated form of MPAG

(AcMPAG). Glucuronidation can also occur in the small intestine and kidney. [40] As the MPA metabolites have enhanced polarity as compared to MPA itself, they are primarily eliminated in the urine. It is estimated that 93% of a MPA dose is eliminated in the urine; primarily as MPAG (~87%) and secondarily as AcMPAG (1%). [41] The urine is responsible for eliminating only ~3% as unchanged MPA. [41] Mycophenolic acid glucuronide undergoes bliliary excretion from the liver and the excreted metabolite is subjected to de-glucuronidation by β-glucuronidases of microorganisms in the gastrointestinal tract. The de-glucuronidation process results in the formation of MPA and this cycling process is referred to as enterohepatic recycling (further described later). A comprehensive discussion regarding transport of MPA metabolites is provided later in this chapter.

Central to MPA disposition is the wide intra- and inter-patient variability demonstrated in renal transplant recipients. [41, 42] Additionally the time period after transplantation is important for assessment of MPA pharmacokinetics; the early post-transplant phase (up to 3 months) generally has been associated with lower C_{tr}, C_{max}, and AUC_{0-12} values, while the later post-transplant phase (> 3 months) has been associated with higher values for these parameters. [41] There has been a keen interest among transplantation specialists in developing therapeutic drug monitoring tools using Ctr and AUC_{0-12}. However, variability in C_{max} and the presence of enterohepatic recycling complicate the adoption of an abbreviated area under the curve method for assessment of exposure. The current recommendation in renal transplant recipients receiving triple drug therapy with MPA, a calcineurin inhibitor, and glucocorticoids is to maintain an AUC_{0-12} of 30 to 60 mg h/L as measured by HPLC. [12, 43] Recommendations based on EMIT measurement methods which can overestimate MPA concentrations secondary to the presence of AcMPAG, other drug combinations, or other disease indications have not been established. Table 1.1 lists the mean±SD pharmacokinetic variables for MPA (total and unbound) and its

metabolites, MPAG and AcMPAG in adult renal transplant recipients receiving twice daily MPA dosing. [44-49]

Mycophenolic Acid and Enterohepatic Recycling

Mycophenolate mofetil is a prodrug that is de-esterified by plasma and tissue esterases to MPA. MPA is 72% by weight of a dose of mycophenolate mofetil that is available to the liver as MPA. [45] In contrast, MPA is 100% by weight of the dose of mycophenolate sodium (Myfortic®). After metabolism by the UGTs, the MPA glucuronide metabolites that are produced in the liver are, in part, exported across the bile canalicular membrane and expelled into the intestine. The MPAG can either be eliminated into the feces (6% of a dose in humans) or transported across the intestinal epithelial cells into the blood by uptake transporters. [45] However, most MPAG in blood is from the liver and not via uptake from the gut wall after biliary excretion. While intestinal transport has not been established definitely, it is known that OATPs are expressed in the liver and intestine, and MPA pharmacokinetics are altered in the presence of polymorphisms in OATPs. [50-52] β-glucuronidases in the intestine can cleave the sugar moiety of the glucuronide metabolites resulting in the release of MPA in the intestines and subsequent absorption into the systemic circulation where MPA is once again available to the liver for metabolism. In humans, renal elimination is comprised of 3% unchanged MPA and 87-91% of the dose excreted as glucuronides. [41] The renal elimination of acylMPAG in patients has been estimated at 1%, with potential increases possible in renal insufficiency. [45] The proposed disposition for MPA and its glucuronide metabolites with reference to enterohepatic recycling are presented in Figure 1.2.[53]

As noted in Figure 1.2, the MPAG can undergo transport across the hepatic basolateral membrance into blood for clearance via the kidneys or can undergo biliary excretion and subsequent enterohepatic recycling. Hence, as kidney function declines, MPAG plasma concentrations may be elevated, with subsequent shunting of the MPAG through the biliary excretion route. It is plausible that enhanced recycling could then lead to increased MPA exposure through an apparent decrease in metabolic clearance via diminished kidney function.

Although effects of diminished kidney function on UGTs have not been reported, reduced metabolism should be entertained because reductions in the expression and function of phase I enzymes (cytochrome P450s) expression and function have been reported in patients with declining kidney function. [54]

The impact of enterohepatic recycling can be visualized upon review of a plasma concentration-time profile whereby a second MPA plasma concentration peak is demonstrated in the 6-12 hour portion of a 12- hour dosing interval (Figure 1.3). The implication of the second MPA peak is that the total exposure to MPA is enhanced, which can contribute to efficacy and toxicity. Dosage recommendations based on pharmacokinetic assessments that fail to examine the concentration-time profile through 12 hours may under-predict exposure and elevate the risk of toxicities.

Drug Interactions

Several potential drug interactions have been described for MPA. Early reports described small increases in plasma MPAG AUC $_{0\text{-}24}$ with concomitant acyclovir, suggesting either inhibition of secretion or competition for secretion, likely by the multidrug resistance proteins (MRPs). [45] Additionally, a small decrease in ganciclovir renal clearance was reported when it was co-administered with MPA. [55] The most clinically important drug-drug interaction in the renal transplant arena is that of MPA with cyclosporine. Notably, cyclosporine is suggested to inhibit the biliary secretion of MPAG by the MRP2 transporter resulting in reduced enterohepatic recycling and lower exposure to MPA. [56] The accumulation of MPAG in plasma may result in competition with MPA for albumin binding, thus increasing the MPA unbound fraction. Glucocorticoids are known to cause induction of drug metabolizing enzymes including UGTs and also have been purported to reduce the bioavailability of MPA. [57, 58] Other therapies that have been suggested to cause induction of UGTs include oral contraceptives and rifampin. [59, 60] Sevelamer (Renagel®) has been reported to reduce MPA AUC ~25% secondary to modification of protein binding and/or interference with enterohepatic recycling. [45] Metal ions including

calcium and iron have been documented to decrease exposure to MPA secondary to chelation in the gastrointestinal tract. [61, 62] Reduction of intestinal glucuronidases secondary to the antimicrobials norfloxacin and metronidazole can reduce MPA and MPAG exposure (AUCs) by up to 33% and 41%, respectively. [63]

Uridine Diphosphate Glucuronosyltransferases

Uridine Diphosphate Glucuronosyltransferases and Mycophenolic Acid

The UGTs are metabolizing enzymes that are responsible for creating polar metabolites of endogenous substrates (e.g. bilirubin and thyroxine) and xenobiotics through conjugation with uridine diphosphate glucuronic acid (UDPGA). The UGTs primarily metabolize drugs with nucleophilic functional groups including oxygen (carboxylic acids, alcohols, phenols), nitrogen (amines), sulfur (thiols) and activated carbon centers. [64] There are two main human families of UGTs; UGT1A and UGT2B. Isozymes of UGT1A are the result of modifications within Exon 1 of the *UGT1* gene. Isozymes of UGT1 that are involved in MPA metabolism include UGT1A8, UGT1A9, and UGT1A7, while the predominant isozyme of UGT2 is UGT2B7. [41, 65] MPA has relatively lower affinity for UGT1A9 in human liver microsomes leading to a high Km (low affinity), whereas the affinity of MPA for UGT1A7 and UGT2B7 are higher (low Km) as depicted in Table 1.2. [65]

The transplant literature has documented considerable variability in MPA pharmacokinetic parameters and inter-individual differences in UGT activity have been reported to be on the order of 8 to 30-fold. [65, 66] Additionally, the efficiency of UGTs for formation of MPAG and AcMPAG is variable and is tissue dependent: MPAG (kidney > liver > intestine) and AcMPAG (liver > kidney > intestine). [65] The role of one UGT versus another in MPA metabolism may be dependent on the dose and/or overall concentration as well as the specific organ.

UGT2B7 has been suggested to be involved with the formation of AcMPAG. As acyl glucuronides have been associated with idiosyncratic drug reactions, there is interest in evaluating the role of this glucuronide in MPA-associated adverse events. Regarding the UGT

protein, the N-terminal location is responsible for the enzymatic activity within the endoplasmic reticulum of the cell, while the C-terminal portion is thought to be responsible for anchorage to the plasma membrane and binding of the co-substrate uridine diphosphate glucuronic acid (UDPGA). [64] Decreased function of the UGTs could lead to increased exposure to the parent drug (MPA), while increased function could lead to reduced MPA relative to inactive metabolites. The repercussions from the previously described circumstances may be increased efficacy balanced with toxicity from MPA itself versus reduced efficacy and potential toxicity from the acyl metabolite. Glucocorticoids, oral contraceptives, and rifampin are the few published examples of potential UGT modulators; all are purported to be enzyme inducers. [57-60]

Genetic Variations and Uridine Diphosphate Glucuronosyltransferases

There is large variability in the expression and activity of UGTs, and single nucleotide polymorphisms are thought to be at least partially responsible. [66-69] Distinctive racial distributions in the frequency of these identified polymorphisms in *UGT* genes have not been thoroughly evaluated. The presence of the promoter polymorphisms *UGT1A9 T-275A* and *C-2152T* result in significantly lower MPA exposures and less enterohepatic recycling. [70] *UGT1A9*3* carriers (*C98T*) have been reported to have higher MPA and AcMPAG exposure, while the *UGT1A9*2* (*G8A*) and *UGT1A8*2* and *UGT1A8*3* single nucleotide polymorphisms appear to exert little change in pharmacokinetics. [70] The *UGT2B7*2/*2* genotype (*C802T*) has been reported to confer higher unbound and total MPA. [70] Recent reports have associated the *UGT2B7 C802T* single nucleotide polymorphism with prostate cancer; possibly implicating this gene in disease risks. [71] While not directly relevant for MPA, the antineoplastic agent irinotecan (Camptosar®) is metabolized by UGT1A1 to the active metabolite SN-38 and the *UGT1A1*28* polymorphism confers increased neutropenic risks. This pharmacogenetic finding and clinical consequences have been incorporated into the FDA-appoved product literature for irinotecan leading to decreased dosage recommendations in patients who are homozygous variant for the *UGT1A1*28* single nucleotide polymorphism.

The influences of genetic polymorphisms on variations in drug metabolism and outcomes are important to consider in patients with glomerulonephritis receiving MPA. Unlike renal transplant recipients who demonstrate primary alterations in glomerular filtration rate, patients with glomerulonephritis may have variations in pharmacokinetics secondary to low serum albumin, proteinuria, and altered glomerular filtration rate. Since targeted MPA concentration ranges for glomerulonephritis have not been established, it will be necessary to account for multiple aspects of patient variability including single nucleotide polymorphisms, in order to enable better empiric dosing strategies.

Drug Tranporters

Drug Transporters and Mycophenolic Acid Disposition

The polar metabolites of MPA (MPAG and AcMPAG) require active transport for uptake and efflux from cells. Mycophenolic acid has been shown to inhibit human OAT1, while MPA, MPAG and AcMPAG can inhibit human OAT3. [72, 73] This may lead to interactions with other substances (e.g. para-aminohippurate and estrone sulfate) that are substrates for these transporters. For MPAG, OATPs are thought to be the primary transporters involved in cellular uptake, while MRPs have been implicated in its efflux from cells. [74, 75] OATPs are members of the SLCO family, and they can transport a multitude of drugs with various chemical characteristics including statins, digoxin, and methotrexate. MRPs are members of the ABCC family; MRP2 has been suggested to export MPAG. [56, 76] Breast cancer resistance protein (BCRP) is a member of the ABCG family and it has also been implicated in MPAG transport. [77] While we have not previously evaluated OATP, we have identified MRP2 transcript expression in peripheral leukocytes in our laboratory and hence alterations in MRP2 may be important in MPA lymphocyte responses. MRP2 is localized in the bile canalicular membrane, intestinal wall, and apical membrane of the proximal tubule. [56, 78] MRP2 transport of cephalosporins, azidothymidine, statins, and products of phase II metabolism have been described.

Genetic Variations in Uptake and Efflux Transporters Relevant to Mycophenolic Acid

Information concerning the effect of polymorphisms of drug transporting genes on MPA disposition has not been completely elucidated. Homozygosity for the *SLCO1B3 T334G* allele (in the presence of the *ABCC2 C-24T* allele) resulted in lower oral clearance of MPA in a population of Japanese kidney transplant recipients. [52] Naesens et al. reported significantly higher dose-corrected MPA trough levels and more diarrhea in renal allograft recipients who had the *C-24T* variant of *MRP2*. [75] None of the studies to date have sought to evaluate the effects of concomitant polymorphisms in uptake and efflux transporters as well as in *UGT*s.

Rationale and Overview of Proposed Research

The objective of this thesis proposal is to evaluate pharmacokinetic and pharmacogenomic factors that may be associated with altered outcomes to MPA therapy in patients with ANCA SVV and SLE nephritis. The goals of this research are to understand and improve treatment responses to MPA in these patients. The central hypothesis of the thesis is that the metabolism and transport of MPA are different in individual patients with ANCA SVV and SLE nephritis and these differences account for variations in systemic or tissue exposure and thus influence outcomes in these kidney diseases. The specific questions that will be evaluated by this project include: 1) Are there alterations in pharmacokinetic parameters for MPA in lupus nephritis and ANCA vasculitis as compared to the published values from transplant recipients, with reference to glomerular filtration rate, proteinuria, and serum albumin?, 2) Is the olism and exposure to glucuronide metabolites of MPA (phenolic and acyl glucuronide) altered?, 3) What is the degree of transcript expression for metabolizing enzymes and transporters in peripheral blood leukocytes? and 4) Does the presence of variant alleles and/or genotypes associated with altered conversion of MPA to glucuronide metabolites affect pharmacokinetics and disease outcomes? This research will evaluate pharmacokinetics, expression phenotype, and genotype, and will correlate the findings of these studies to determine associations with patient outcomes. In addition, these studies will be the first evaluation of their kind in patients with SLE nephritis

and ANCA SVV, and should generate useful pharmacokinetic profiles for MPA to assist with appropriate dosing. The studies proposed in this thesis research program are *innovative* in that they fill a void in our knowledge of the disposition of highly protein bound drugs in subjects with glomerulonephritis and the role of UGTs in altering the kidney outcomes of MPA-based treatment strategies. The contributions to the treatment of ANCA SVV and SLE nephritis patients will be *significant* because of the expansion of knowledge regarding this common therapy as well as exploration of methods to individualize dosing regimens to improve treatment responses. Clinicians will benefit from this research because it will reduce some of the "guess-work" involved in prescribing appropriate treatment regimens for patients with ANCA SVV and SLE nephritis.

Specific Aims

The specific aims and methods to address the objective of the proposal are cited below.

Aim 1. Evaluate the pharmacokinetic parameters for MPA in subjects with ANCA SVV and SLE nephritis with variable levels of kidney function as reported by glomerular filtration rate, proteinuria, and disease activity. Preliminary data from the University of North Carolina population of patients suggest increased total and renal clearance of MPA in patients with clinically significant levels of proteinuria. Additionally, patients with glomerulonephritis appear to exhibit alterations in pharmacokinetic variables as compared to published data from transplant patients.

a. Perform noncompartmental pharmacokinetic analyses of MPA using plasma and urine collected from 40 subjects; 20 with ANCA SVV and 20 with SLE nephritis. Analyses will include total and unbound plasma concentration data.
b. Develop a compartmental pharmacokinetic model for MPA that incorporates the components of renal elimination and metabolic clearance.
c. Develop a statistical model to evaluate the effects of changes in clinical characteristics (e.g. glomerular filtration rate, proteinuria, serum albumin) on

pharmacokinetic parameters deemed to be of paramount importance in affecting exposure of tissues to MPA, and ultimately renal outcomes.

Aim 2. Evaluate the exposure to MPAG and AcMPAG in subjects with ANCA SVV and SLE nephritis as a function of variable kidney function as reported by glomerular filtration rate and proteinuria.

a. Perform noncompartmental pharmacokinetic analyses of MPAG and AcMPAG using plasma and urine collected from a subset of subjects from Specific Aim #1.
b. Develop a compartmental pharmacokinetic model for MPAG and AcMPAG that incorporates the components of renal elimination and metabolic clearance.
c. Develop a statistical model to evaluate the effects of changes in clinical characteristics (e.g. glomerular filtration rate, proteinuria, serum albumin) on pharmacokinetic parameters deemed to be of paramount importance in effecting exposure of tissues to MPAG and AcMPAG, and kidney outcomes.

Aim 3. Genotype ANCA SVV and SLE nephritis subjects for known single nucleotide polymorphisms in UGTs *1A9, 1A7*, and *2B7* because these have been associated with altered pharmacokinetic parameters (AUC, C_{max}, C_{tr}) for MPA and glucuronides and may explain part of the variability in patient outcomes. Determine mRNA expression patterns for *UGT1A9, UGT1A7, UGT2B7, ABCB1, ABCC2*, and *SLCO1A2* in leukocytes of patients with glomerulonephritis.

a. Genotype subjects with ANCA SVV and SLE nephritis for known single nucleotide polymorphisms in UGTs including *UGT1A9* promoter enhanced activity SNPs (*C-2152T, T-275A*), *1A9*2 (G8A)* and *UGT1A9*3 (T98C)* (both associated with reduced activity), *UGT 1A7*4* (*T622C*) (reduced activity), UGT 2B7*2 (C802T) (associated with enhanced formation of the AcMPAG).

b. Develop a statistical model to evaluate the association between key MPA and glucuronide pharmacokinetic parameters from subjects and the presence of variant alleles in *UGTs.*

c. Evaluate mRNA expression patterns of drug transporters and drug metabolizing enzymes in leukocytes and determine associations with genotype and pharmacokinetic parameters

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Table 1.1 Steady-State Pharmacokinetics of Mycophenolic Acid and Its Phenolic Glucuronide in Adult Kidney Transplant Recipients [44-49]

	C_{max} (mg/L)	T_{max} (hr)	C_{tr} (mg/L)	AUC $_{0-12}$ (mg h/L)
MPA $_{total}$	23.2±11.9	0.9±0.2	1.22±0.42	61.3±28.7
MPA $_{free}$	0.21±0.03	1.37±0.19	0.02±0.005	0.57±0.05
MPAG	111±26.5	3.0±1.2	75.8±40.0	1040±290
AcMPAG	1.95 (0.88-5.35)	1.63(1.25-2.0)	0.33±0.40	32±19

Abbreviations

AUC – area under the plasma concentration time curve

Cmax – maximum concentration in plasma

Ctr – trough concentration in plasma

MPA – mycophenolic acid

MPAG – mycophenolic acid glucuronide

AcMPAG – acyl mycophenolic acid glucuronide

Tmax – time to maximum plasma concentration

Table 1.2[65] Representative Enzyme Kinetic Values for the Conversion of Mycophenolic Acid to Mycophenolic Acid Glucuronide by Human Recombinant Uridine Diphosphate Glucuronosyltransferases as Depitcted by Eadie-Hofstee Plots.

Enzyme	Km (μM)	Vmax (pmol/min/mg)	Vmax/Km
UGT1A9	276	106	0.38
UGT1A7	159	85.2	0.54
UGT2B7	123	39.0	0.32

Abbreviations

Km – plasma concentration at one-half of the maximum rate of metabolism

UGT – uridine glucuronosyltransferase

Vmax – maximum rate of metabolism

Figure Legends

Figure 1.1. Structures of Mycophenolic Acid and Metabolic Pathways.

Figure 1.2. Depiction of the Disposition and Recycling of Mycophenolic Acid (MPA) and Its Glucuronide (MPAG). After MPA glucuronidation to MPAG in the hepatocyte, MPAG either undergoes efflux at the apical hepatocyte membrane resulting in biliary excretion, or undergoes efflux at the basolateral hepatocyte membrane resulting in uptake into the blood. The former pathway is contributory toward enterohepatic recycling, while the later pathway contributes toward renal clearance. Abbreviations: MMF – mycophenolate mofetil; MPA – mycophenolic acid; MPAG – mycophenolic acid glucuronide.

Figure 1.3. Mycophenolic Acid Plasma Concentration Versus Time Curve. This patient was receiving a Cellcept® dose every 12 hours. After the 4 hour time period, a second peak at 6 hours occurs and demonstrates the enterohepatic recycling phase. (Joy MS data)

Figure 1.1 [38] Reprinted with permission.

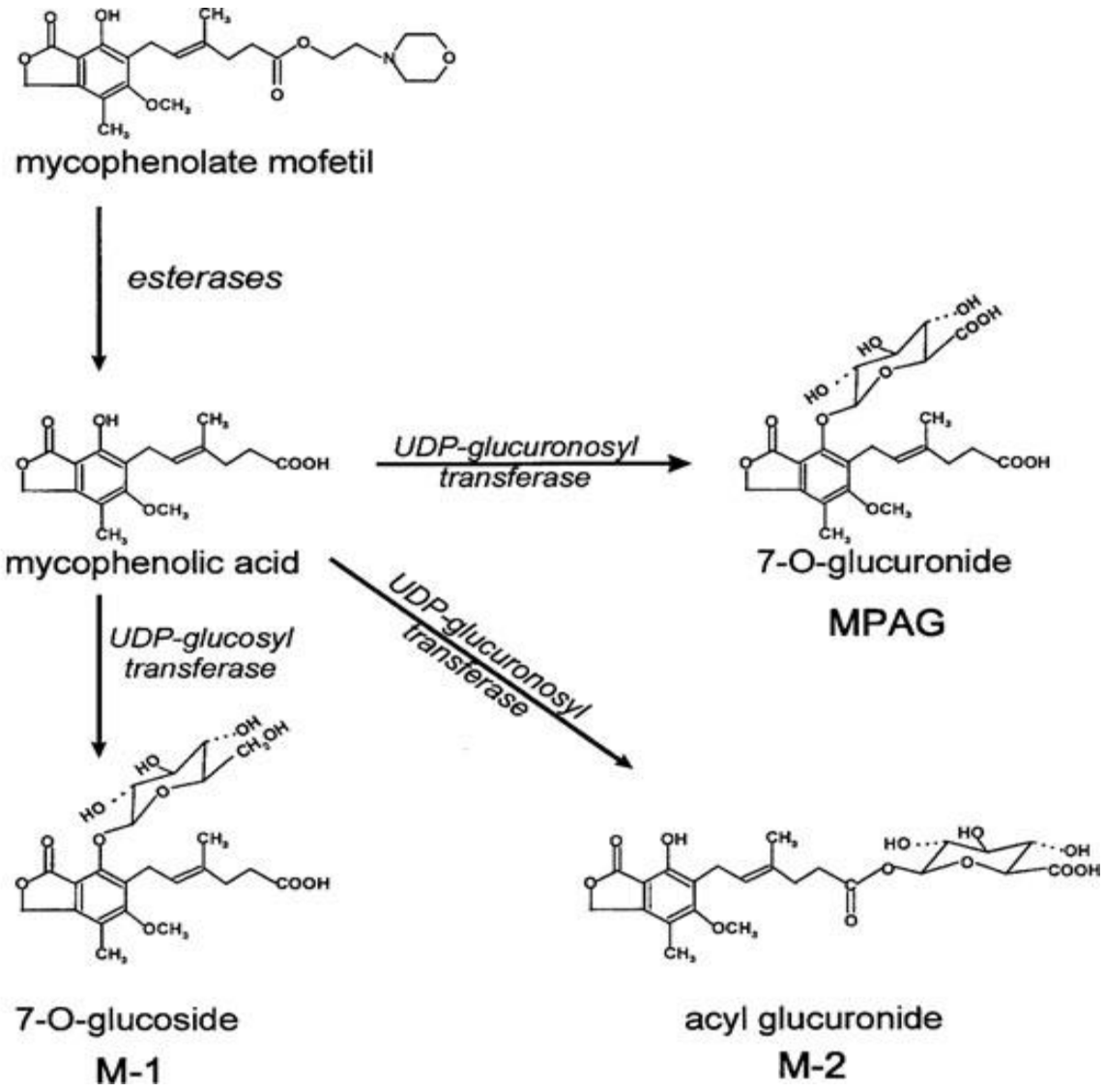


Figure 1.2

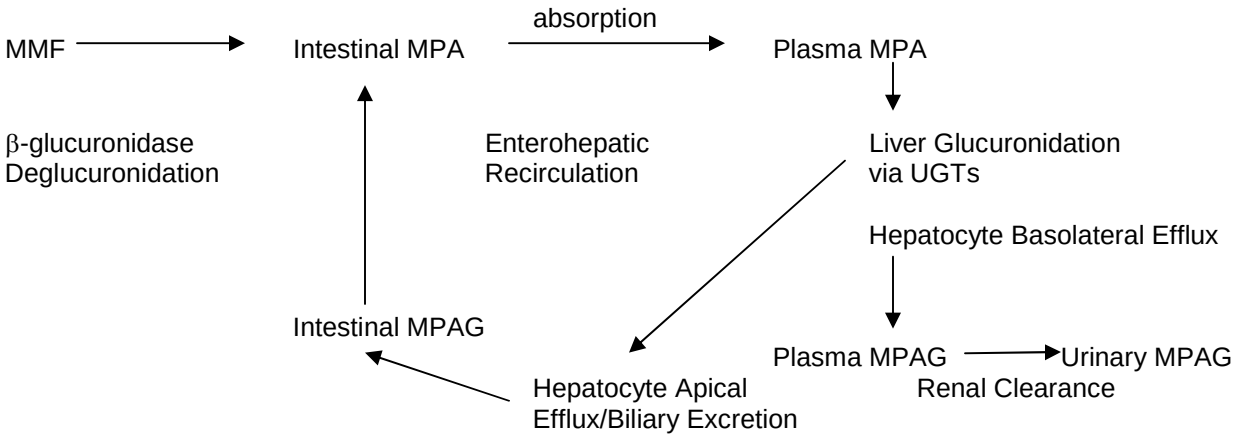
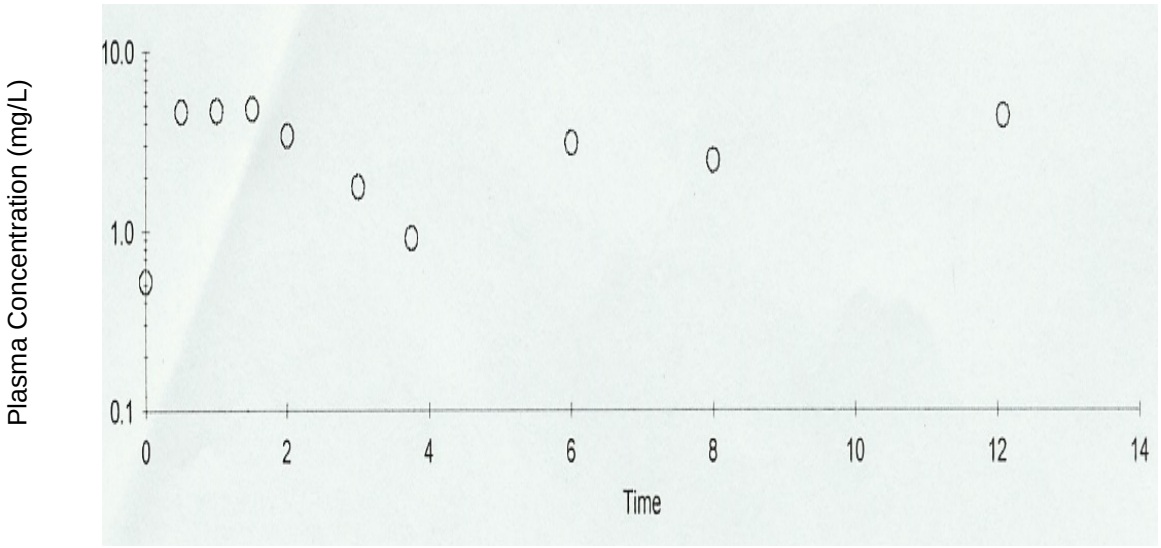

MMF
Intestinal MPA
absorption
Plasma MPA
β-glucuronidase
Deglucuronidation
Enterohepatic
Recirculation
Liver Glucuronidation
via UGTs
Hepatocyte Basolateral Efflux
Intestinal MPAG
Plasma MPAG
Urinary MPAG
Renal Clearance
Hepatocyte Apical
Efflux/Biliary Excretion

Figure 1.3



Chapter 2

Pharmacokinetics of Mycophenolic Acid in Patients with Lupus Nephritis

Melanie S. Joy, Pharm.D., FCCP,[1] Tandrea Hilliard, B.S.,[1] Yichun Hu, M.S.,[1] Susan L. Hogan, Ph.D.,[1] MPH, Mary Anne Dooley, M.D.,[1] Ronald J. Falk, M.D.,[1] Philip C. Smith, PhD[2]. School of Medicine, University of North Carolina at Chapel Hill, UNC Kidney Center and Division of Nephrology and Hypertension,[1] and Eschelman School of Pharmacy,[2] Chapel Hill, NC

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With permission from: Joy MS, Hilliard T, Hogan SL, Dooley MA, Falk RJ, Smith PC. Pharmacokinetics of mycophenolic acid in patients with lupus nephritis. Pharmacotherapy 2009;29(1):7-16.

Abstract

Lupus nephritis is associated with urinary protein excretion, hypoalbuminemia, and renal function declines, which may impact the pharmacokinetics (PK) of mycophenolic acid (MPA).

The primary study objective was to evaluate and describe the PK of MPA and its glucuronide (MPAG) in lupus nephritis. Secondary objectives were to determine the single and/or multiple effects of clinical parameters (urinary protein excretion, serum albumin, and creatinine clearance) and demographic variables (age, race, and gender) on total and unbound MPA and MPAG PK.

Plasma and urine were collected for 24-hours and assayed by HPLC with UV detection. Noncompartmental PK analysis was performed using WinNonlin v4.1. Statistics included descriptive analyses, urivariate and multiple regression tests, and T-test or nonparametric equivalent.

Time to maximal concentration (0.5 to 8 hrs) was variable. Unbound MPA was 2.6±1.9% and oral clearance (Cl/F 343 ± 200 mL/min) was ~ 2-fold higher than previously reported. Multiple regression showed MPA Cl/F was predicted by creatinine clearance (Clcr) and serum albumin (MPA lnCl/F = 5.358 + 0.0092 (Clcr) – 0.078 (ranked albumin), R^2 51.1%, p = 0.0195). UP:Cr ≥ 1 g/d had lower trough and area under the curve (AUC_{0-12}) and higher Cl/F versus UP:Cr < 1 g/d. Serum albumin < 4 g/dL had higher MPA Cl unbound and MPAG Clr_{0-12} versus serum albumin ≥ 4g/dL. Recycling AUC (AUC_{6-12}) and equally gender and age predicted renal clearance of MPAG.

Clcr and serum albumin were identified as primary contributors to MPA exposure and should be considered when evaluating dosages. The results of future studies should clarify the interactions of other variables on drug exposure and treatment responses. Clinicians need to be mindful of clinical changes that occur throughout the course of lupus nephritis in order to maintain efficacy and reduce toxicity from MPA therapy.

Introduction

Mycophenolic acid (MPA) has been used as an immunosuppressant agent to prevent renal transplant rejection since 1995. As there is inherent variability in mycophenolic acid pharmacokinetics within transplant patients, several researchers have sought to describe mycophenolic acid variations that occur from the early post-transplant period to several months after transplant.[1-3] More recently, it has been suggested that therapeutic plasma monitoring of mycophenolic acid may help to improve immunosuppressive outcomes. [4] Area under the plasma concentration time curve from 0 to 12 hours (AUC_{0-12}) of 30 to 60 μg h/L and trough plasma concentrations (Ctr) of 1.0 to 3.5 μg/mL are suggested as targets for combination immunosuppressive therapy (MPA plus cyclosporine and steroids) in kidney and heart transplant patients. [4-5] These concentrations are based on high performance liquid chromatography (HPLC) assays. Target ranges for MPA in single or double agent therapies or for use in autoimmune diseases have not been established.

Since 1999, mycophenolic acid therapy has been evaluated for efficacy in patients with lupus nephritis. [6-9] Similar to renal transplant recipients, glomerular disease patients often have diminished renal function manifest as reductions in estimated glomerular filtration rate (eClcr). However, glomerular disease patients also commonly have protein in the urine and alterations in serum albumin. Both urinary protein and decreased serum albumin (in addition to altered eClcr) conceivably could lead to pharmacokinetic alterations of highly protein bound drugs such as MPA in patients with glomuerulonephritis. Hence, a comprehensive evaluation of total and free MPA pharmacokinetics in lupus nephritis patients on stable therapy is warranted. Analyses of the impact of alterations in urinary protein, serum albumin, and eClcr on pharmacokinetics could provide patient-specific factors that may be important for individualized dosing.

The primary purpose of this study was to evaluate the total and free pharmacokinetics of MPA and its phenolic O-glucuronide (MPAG) in patients with lupus nephritis. The secondary

objectives were to determine the effects of clinical parameters (urinary protein excretion (UP:Cr), serum albumin, and eClcr) and demographic variables (age, race, gender) on total and unbound MPA and MPAG pharmacokinetics.

Methods

Patients

Patients with biopsy confirmed lupus nephritis receiving maintenance therapy with MPA were evaluated for study enrollment. Patients were required to be on a stable MPA dose for at least two weeks. Concomitant therapy with other immunosuppressants was allowed and recorded. Patients were fasting at study initiation and were fed a standard diet in the research unit throughout the study period. The following clinical data was measured at the time of the study or abstracted from the medical record: eClcr, UP:Cr, serum albumin, and serum creatinine. The study and consent form was approved by the University's Institutional Review Board and patient consent was required prior to participation.

Pharmacokinetic Study

Patients were admitted to the General Clinical Research Unit (GCRC) to participate in a 24-hour inpatient stay for pharmacokinetic analysis. Baseline blood was drawn for a trough plasma concentration. The patients were then instructed to take their morning oral dose of MPA. Additional plasma samples (7.5 mL) were obtained at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours. Urine was collected during the following intervals: 0-6, 6-12, and 12-24 hours into acidified (15 mL 6 N HCl) collection containers. Heparinized blood samples were immediately centrifuged for 10 minutes at 4C, plasma transferred to plastic screw top tubes and stored at -80 until assay. Urine volume for each collection time period was recorded, and 2 mL aliquots were stored at -80C until assay. Unbound plasma fraction was determined by filtration via a Centrifree® Micropartition device (Millipore, Ireland) with a filter cut-point of 30,000 daltons. Temperature and centrifugation conditions were optimized to enable filtration of 10% of the total plasma volume. The unbound fraction was assessed at the time point corresponding to the Cmax and

evaluated in spiked plasma separately. The unbound concentrations were then calculated as unbound fraction multiplied by total concentration. Linearity of binding throughout the evaluated concentration ranges was assumed. Samples were assayed by HPLC using a variation on the methods of Wiwattanawongsa, et al [10], using methanol-formic acid 0.1% isocratic mobile phase (52:48) at a flow rate of 1.0 mL/min, suprofen as the internal standard, and UV detection at 250 nm. The HPLC system consisted of a Hewlett-Packard series 1050 pump/injector, Hewlett-Packard Series 1050 UV detector, and Axxiom ODS column (150 x 4.6 mm I.D., 5 micrometers). 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 5-1500 μg/mL, respectively. MPAG concentrations were represented in terms of MPA-equivalents by multiplying each MPAG concentration by 0.646 (molecular mass of MPA to MPAG) and reported in μg/mL. The amount of MPA available from a dose of the prodrug was estimated as 72% of the dose (molecular mass of MPA to MPA mofetil). This calculation was used to determine the amount of drug excreted in the urine in reference to the dose of MPA actually administered from mycophenolate mofetil.

Pharmacokinetic Analysis

Noncompartmental pharmacokinetic analysis of total and unbound MPA and MPAG was conducted using WinNonlin v4.1 (Pharsight, Mountain View CA) linear up-log down for AUC determination. The following parameters were reported: concentration maximum (Cmax), time to maximum concentration (Tmax), concentration trough (Ctr) at 12 and 24 hours, area under the plasma concentration time curve from 0-12 hours (AUC_{0-12}), apparent oral clearance (Cl/F), apparent renal clearance (Cl_R/F), and mean residence time (MRT). For the purpose of pharmacokinetic evaluations we made the assumption that F = 1, since others have reported bioavailability of close to 1. [2] AUC_{12-24} and AUC_{6-12} were calculated. The AUC_{6-12} was used to estimate entero-hepatic recycling as performed by others. [11-12] Urine analysis was performed

by multiplying the concentration by volume for each collection period (0-6, 6-12, and 12-24 hours). Amount excreted in the urine (Ae) was computed for the 0-12 hour time frame by adding the Ae for the first two collection intervals. Apparent Cl_R/F for the 0-12 hour time frame was calculated by Ae_{0-12}/ AUC_{0-12}.

Statistics

Descriptive analyses for pharmacokinetic parameters, demographic variables and laboratories included means, standard deviations, and medians as appropriate. Univariate assessments of the key clinical characteristics (serum albumin, UP:Cr, eClcr, age, race, gender, steroid dose) versus each pharmacokinetic parameter of interest (MPA Cl/F, MPA Cl_R/F, MPA unbound clearance (Clunb), MPAG Cl_R/F, MPA AUC_{0-12}, MPA AUC_{6-12}, MPAG AUC_{0-12}) were assessed by Spearman Rank correlations. The correlations and resultant p values from the univariate assessments were analyzed for possible inclusion into a multiple regression model for prediction of the pharmacokinetic parameters of interest. All data that failed normality testing were transformed by various functions to ensure normality was attained. Model building consisted of using multiple regression analysis with forward addition of variables as well as backward elimination, noting any significant changes in coefficients of the primary predictors as well as the R^2 and p value resulting from the various models. The final model was selected based on significance of each variable on predicting the dependent variables in the model as well as the overall R^2. Race (white and non-white) and gender (female and male) were coded as 1 and 2, respectively.

Comparisons between clinical groups based on urinary protein excretion (< 1 g/day vs ≥ 1 g/day), serum albumin (< 4 g/dL vs ≥ 4 g/dL), age (< 40 yrs vs ≥ 40 yrs), race (white vs nonwhite), and gender (female vs male) were analyzed by the nonparametric Mann Whitney Test. Upon review of our data, it was not possible to compare eClcr groups as there was no meaningful cut-point value for evaluation.

Results

A total of 18 biopsy-confirmed lupus nephritis patients completed 21 full twenty-four hour MPA/MPAG pharmacokinetic evaluations. We report the results for the 18 discrete patients. The patient demographic composition included age 36 ± 9 years, 83% female, 60% non-Caucasian, and weight 82.3 ± 22 kg. The non-Caucasian patients consisted of 7 African American, 2 Asian, and 2 Native American. All patients were receiving the mycophenolate mofetil prodrug of MPA (Cellcept®, Roche). The average MPA daily dose was 1860 ± 764 mg and this was represented by twice daily dosing in all but one patient who received 1000 mg three times daily. The distribution of doses given twice daily were 500 mg (n = 6), 750 mg (n = 1), 1000 mg (n = 7), and 1500 mg (n = 4). eClcr was used as the assessment of GFR in this study. [13] The mean (± standard deviation) clinical laboratory results at baseline were serum creatinine 1.1 ± 0.8 mg/dL, UP:Cr 1.3 ± 2.2, eClcr 114 ± 49 mL/min, and serum albumin 3.9 ± 0.4 g/dL. Fifty percent (n = 9) of patients were receiving concomitant glucocorticoids, with a mean ± SD daily dose of 11.4 ± 8.9. No other immunosuppressants were prescribed. Two patients were prescribed oral contraceptives.

Mycophenolic Acid Pharmacokinetics

A representative concentration vs time profile for steady state MPA and MPAG concentrations in our lupus nephritis patients is presented in Figure 1. The mean (± standard deviation) pharmacokinetic parameters for patients with lupus nephritis are provided in Table 1. In order to eliminate differences secondary to body size, the apparent oral clearance (Cl/F) data was adjusted to a 70 kg patient based on a scaling method that uses a power of 0.75. [14] The Cl/F of 343 ± 200 mL/min suggests that MPA is a moderate extraction ratio drug whose metabolism would be impacted by changes in unbound fraction. While the mean percentage of free MPA was 2.6 ± 1.9, five patients (28%) had free MPA percentages that were greater (range 2.9 to 6.3%). The mean MPA area under the plasma concentration time curve ($AUC_{0\text{-}12}$) in our

lupus patients was outside the range of 30 to 60 mg hr/L recommended in the first six months post renal transplant, [15] with 39% of patients exceeding and 22% failing to achieve this range. Examination of the AUC_{6-12} to the AUC_{0-12} suggested that recycling accounted for 37% (± 16%) of the AUC reflected from the first daily dosing interval.

The mean MPA trough (Ctr) at 12 hours exceeded the range of 1.0 to 3.5 μg/mL that is recommended in transplant patients [15], with 28% of patients below and 33% above this target, respectively. The Ctr that resulted after the first 12 hours was ~20% less than the Ctr following the second dosing interval, however the difference was not significant. The time to maximal concentration (Tmax) varied in the range of 0.5 to 8 hours and would not have been appreciated in shortened sampling schemes. A three hour AUC profile would have under-represented exposure over the dosing interval.

As suggested previously [2], the clearance of MPA is primarily the result of systemic metabolism to MPAG. The apparent renal clearance (Cl_R/F) for MPA represented ~ 1% of the Cl/F. The Cl_R/F of nonmetabolized MPA was 1.8 ± 1.4 mL/min, which was ~ 2% of the eClcr in the evaluated patients. The kidneys contributed to the excretion of 1% of the total MPA dose, assuming all MPAG formed was via the liver. The amount of MPA in the urine over the 0-12 hour interval (4.8 ± 3.3 mg) was ~25% less than the amount in the 12-24 hour interval (6.5 ± 9.1 mg), despite the dosages being consistent, but this was not significant. The eClr was similar between the 0-12 hour and 12-24 hour dosing intervals.

Mycophenolic Acid Glucuronide Pharmacokinetics

The MPAG pharmacokinetic results are presented in Table 2.1. The MPAG Ctr after the first 12 hours was ~15% less than the Ctr following the second dosing interval. A calculated AUC ratio of MPAG to MPA resulted in a metabolic ratio (MR) of 7.1 ± 4.8.

The renal clearance of MPAG was 53.5 ± 52.3 mL/min, which was 44% of the Clcr. The kidneys contributed to the elimination of 96% of the total MPA dose through excretion of the

metabolite, MPAG. Hence, the kidneys were responsible for eliminating ~97% of the total dose of MPA. The remaining MPA was likely eliminated secondary to excretion of the acyl-MPAG metabolite by the kidneys (not measured) as well as by biliary secretion of MPAG that is not recycled. The amount of MPAG in the urine over the 0-12 hour interval (565 $\pm$ 310 mg) was ~28% more than the amount in the 12-24 hour interval (441 $\pm$ 341 mg), despite the dosages being consistent. The Clr was similar between the 0-12 hour and 12-24 hour dosing intervals.

Unbound Pharmacokinetics

Our patient data showed that 2.5% and 9.3% of MPA and MPAG, respectively, were unbound in the plasma. Since the unbound MPAG was less than that reported previously [16], we reviewed our data with normal plasma that was spiked with MPA and MPAG either alone or in combination. The blank plasma that was spiked separately demonstrated similar percentages to that found in our patient data. The combination drug and metabolite spiked plasma showed an increase in unbound percentage of 4% and 11% for MPA and MPAG, respectively, suggesting competitive binding to albumin as reported previously.

Since the normal percentage of unbound MPA is ~2%, if one aims for a total Ctr of 1.0 to 3.5 μg/mL then an unbound target would be 0.02 to 0.07 μg/mL. Likewise, if suggested total AUC goals are 30 to 60 μg h/mL, then unbound AUC goals would be 0.6 to 1.2 μg h/mL. Our data showed mean unbound Ctr levels (0.1 μg/mL at 12 and 0.13 μg/mL at 24 hours) that were greater than suggested, with 44.4% of patients within the range. With regard to unbound AUC, the mean exposure was greater than the upper range of 1.2 μg h/mL in 33% of our lupus patients.

Regression

Multiple regression was performed to determine which clinical factor (UP:Cr, eClcr, serum albumin, age, race, gender, steroid dose) had the most effect on pharmacokinetic parameters for MPA (Cl_R/F, Cl/F, AUC $_{0-12}$, AUC $_{6-12}$) and MPAG (Cl_R/F, AUC $_{0-12}$). MPAG clearance

parameters were included as increased MPAG may result in enhanced recycling and subsequent increases in MPA exposure. Models were constructed by forward selection and backward elimination schemes employing the pharmacokinetic parameter as the Y factor and clinical variables as the X factors. AUC_{6-12} was also included as an X factor when Cl_R/F variables were assessed. The eClcr and serum albumin were the two clinical parameters contributing to MPA Cl/F. Ln MPA Cl/F = 5.3585 + 0.0092 (eClcr) – 0.0776 (ranked serum albumin), R^2 51.1%, p = 0.0195; eClcr p = 0.0265, serum albumin p = 0.0586. The regression equation for MPA AUC_{0-12} demonstrated similar results, which is expected given the reciprocal relationship between Cl/F and AUC_{0-12}. For the MPAG Cl_R/F analyses, the AUC_{6-12} was consistent in models that controlled for either gender or age. These two models were: 1) Ln MPAG Cl_R/F = 6.6009 – 1.3519 (gender) – 0.5257 (ln AUC_{6-12}), R^2 39.9%, p = 0.0282; race p = 0.0405, ln AUC_{6-12} p = 0.0687, and 2) Ln MPAG Cl_R/F = 13.1896 – 2.2901 (ln age) – 0.5105 (ln AUC_{6-12}), R^2 39.9%, p = 0.0300; ln age p = 0.0434, ln AUC_{6-12} p = 0.0776. No significant predictors of AUC_{6-12} or Cl_R/F for MPA were found.

Comparison Between Groups Based on Clinical Laboratories

Given the importance of albumin in the regression model for Cl/F and AUC_{0-12} and the prevalence of increased UP:Cr in glomerulonephritis patients with reduced serum albumin concentrations, we wanted to explore the differences in PK parameters by distinct clinical groupings. (Table 2.2) UP:Cr was selected as a clinical variable secondary to the high plasma protein binding characteristics of MPA and MPAG. It is conceivable that highly protein bound drugs may be eliminated in the urine bound to protein in patients with proteinuria and/or they may be preferentially eliminated by metabolism secondary to increased unbound fraction. A cut-point value of 1 g/day was selected based on the premise that UP:Cr less than 1 g/day would be less likely to alter PK. The MPA data shows that Cl/F was significantly increased (790 mL/min vs 305 mL/min, p = 0.0464) and Ctr_{12} and AUC_{0-12} were both significantly reduced in

the high protein excretion group (0.88 μg/mL vs 5.0 μg/mL; p = 0.012 and 33.2 μg h/mL vs 91.9 μg h/mL; p = 0.018, respectively).

Since MPA and MPAG are highly bound to serum albumin, albumin was also selected for evaluation. (Table 2.2). Several findings of this analysis were of borderline significance. The MPA Cl_R/F was found to be increased nearly 2-fold in the low serum albumin group (p = 0.073). This finding would be expected given that renal clearance would be directly related to eClcr as well as the unbound fraction of MPA. $Cl_{unbound}$ was found to be increased in the low albumin group and this finding was of borderline significance (p = 0.051). Although the renal clearance was enhanced 2-fold, the overall contribution of the kidneys to clearance was low given that only 3% of a MPA dose is normally eliminated unchanged in the urine. [16] MPAG Cl_R/F was increased in patients with reduced albumin (p = 0.053), reducing the amount of MPAG available for recycling to MPA and potentially leading to reduced MPA exposure. With regard to MPA AUC values, we found slightly increased MPA AUC_{0-12} in our high albumin group (p = 0.128), reflecting the reciprocal changes in Cl/F.

The differences in pharmacokinetic variables between age grouping (< 40 years vs ≥ 40 years), race (white vs nonwhite), and gender (female vs male) were also evaluated (data not shown in Table 2.2). The MPA MRT was found to be greater in younger patients (21.6 hrs vs 8.23 hrs; p=0.066), but this did not result in a significant p value. Additionally, the MPAG Cl_R/F_{0-12} was found to be increased 6-fold in females as opposed to males (66.5 mL/min vs 10.7 mL/min; p 0.047). The eClcr, however, was only ~21% greater in females than males.

Discussion

Our study is the first published report that has focused on describing the pharmacokinetic disposition of MPA and its metabolite MPAG after chronic therapy in patients with lupus nephritis. Additionally, in order to achieve clinical relevance to our work, we have described relevant patient laboratory data that were found to portend variations in pharmacokinetic

disposition. Our multivariate regression assessments for prediction of Cl/F and AUC_{0-12} implicated serum albumin and eClcr as the main contributors. Although there is some degree of correlation between serum albumin and UP:Cr, there is also a fair amount of variability between the two measures in individual patients. The combined, correlative contribution of UP:Cr and serum albumin cannot, however be fully evaluated. Hence, it is prudent to assess both the serum albumin and UP:Cr when evaluating initial dosing for highly protein bound drugs such as MPA. The multivariate regression assessment of MPAG Cl_R/F determined that log AUC_{6-12} was contributing with gender and age also contributing equally, although in a separate fashion.

The resulting MPA PK parameters for patients with lupus nephritis appear to be comparable with that what has been reported for renal transplant recipients, with the exception of Cl/F, which is up to 2 -fold greater in the lupus nephritis population. Reasons for enhanced Cl/F include increased systemic metabolism secondary to either up-regulated glucuronidation (single nucleotide polymorphisms in the UGT1A9 promoter or steroids), increased MPA unbound fraction (available for hepatic extraction/metabolism), or enhanced renal excretion. Regarding glucocorticoids, patients receiving concurrent steroids had similar Cl/F estimates as patients who were not receiving steroids. Also, steroid dose did not contribute to the Cl/F in the regression analysis. We are currently evaluating the contribution of genotype as a factor in altering MPA clearance. The unbound fraction, implicated as a variable leading to increased drug availability for metabolism is important in our patients given that 40% had albumin concentrations that were < 4 g/dL. The regression analysis for Cl/F implicated serum albumin as a predictive variable.

Enhanced renal clearance could occur secondary to increased free drug available or due to loss of protein bound MPA with the urinary protein, both cases resulting in an increase in Cl/F. However, when we evaluated Cl_R/F between patients with UP:Cr < 1 g/day and those with UP:Cr ≥ 1 g/day, the Cl_R/F results were similar. It is plausible that the magnitude of difference in Clr was under-appreciated based on our selected cut-point for UP:Cr of 1 g/day. Further review

of our data shows a confounding effect of serum albumin levels; while 29% of our UP:Cr < 1 g/day had low albumin levels, 75% of our UP:Cr ≥ 1 g/day had low albumin levels.

A previous study of 16 autoimmune disease patients (containing six lupus erythematosis patients) who received 1 g MPA every 12 hours reported a mean MPA AUC_{0-12} of 70.6 ± 28.7 μg h/mL, which was comparable to our study. [17] However, it is not clear whether the previous study normalized AUC data to weight or body size to enable appropriate assessments. The MPAG AUC_{0-24} (2017.2 ± 1124 μg h/mL) was 2-fold higher than what would have been predicted in our study based on extrapolation of the AUC_{0-12} data. MPAG is minimally active pharmacologically and it is important in enterohepatic recycling and MPA exposure. While it was expected that eClcr would predict the MPAG Cl_R/F secondary to MPAG being a polar metabolite that is primarily excreted by the kidneys, our distribution of kidney function did not encompass late stage CKD patients to enable a display of these relationships. Previous clearance data from renal transplant patients has shown MPAG plasma clearance to be highly correlated (R^2 0.86) with estimated glomerular filtration rate (eClcr) and the mean Cl_R/F values for MPAG in patients with mild, moderate and severe kidney disease were reported as 21.7, 10.0, and 5.0 mL/min, respectively. [18] Hence, a patient with severe kidney disease could have a 4-fold reduction in MPAG clearance, resulting in an increase in MPA AUC through recycling. Our regression model suggested that ln AUC_{6-12} along with ln age and male gender were predictors for decreased MPAG Cl_R/F. An increase in recycling AUC predicted a reduction in Cl_R/F of MPAG since less drug would be available as the polar, renally excreted metabolite. An increase in age predicted a decrease in MPAG Cl_R/F, which would support (indirectly) a role of eClcr. Most of our patients spanned the second to the fourth decade and thus the effects of age on eClcr were not appreciated. Refinements and validation of our model will require addition of representative patients with more severe reductions in eClcr to fully understand the role of renal function.

A study in renal transplant recipients used a multivariate analysis and demonstrated that 24% of the MPA Cl/F could be explained by proteinuria (yes/no), eClcr, and diabetes mellitus. [19] Our data showed that 51% of MPA Cl/F could be explained by serum albumin and eClcr, two readily measured clinical laboratories. The contribution of eClcr to MPA Cl/F was unexpected given the low percentage of MPA (1-3%) that is normally excreted by the kidneys. However, patients with diminished eClcr have been documented to exhibit decreased hepatic metabolism postulated to be due to the CKD state itself or the effect of CKD on the accumulation of endogenous substrates. [20] In subjects with both decreased albumin and decreased eClcr, the MPA AUC lowering effect of reduced albumin (more drug available for metabolism) may be balanced by an increased AUC effect secondary to a reduced eClcr. [21-22] Along another pathway, states of inflammation can have variable effects on drug metabolizing enzymes and transporters. [23-24]

Regression models for a quantitative prediction of the Cl/F based on the serum albumin and Clcr, when validated, could be used to guide dosage regimens. For example, in our current model, for each 20 mL/min decrease Clcr, one would expect a decrease in Cl/F of about 30 mL/min assuming a stable serum albumin of 4.4 g/dL and an increase of about 180 mL/min assuming a concomitant reduction in the serum albumin to 2.9 g/dL. Hence, the effects of moderate reductions in serum albumin would have fairly significant effects on increasing Cl/F versus moderate reductions in Clcr. Since increases in proteinuria often result in concomitant reductions in serum albumin, the combined contributions could enhance the Cl/F of MPA even further. However, more patients with significant proteinuria are needed to provide a more definitive conclusion regarding the contribution of proteinuria (somewhat independent of serum albumin) to Cl/F for MPA. Although requiring additional validation, the regression equation for AUC_{0-12} could enable calculations of dosage modifications depending on the targeted MPA AUC_{0-12} with the assumption of linearity within the clinically obtained plasma concentrations.

While we report the contribution of serum albumin and estimated glomerular filtration rate (via eClcr) to MPA clearance, there are some limitations to our research. As noted previously, our patients had relatively preserved eClcr, with only three patients presenting with more severe kidney disease (stages 2 and 3). The full contribution of reductions in eClcr to alterations in clearance would require assessment across the spectrum of kidney disease. Similarly, since only two patients in our dataset were nephrotic (UP:Cr > 3.5 g/d), the full contribution of UP:Cr to clearance may actually be under-recognized based on our dataset with less significant degrees of proteinuria. Additionally, the combined role of albumin and urinary protein to elimination of highly bound drugs in patients with glomerular diseases requires rigorous assessments. Future analyses of our data include assessment of the contribution of genotype for drug metabolizing enzymes and transporters to drug clearance and outcomes and analysis of the contribution of Ctr and AUC to patient outcomes. We hope to better define appropriate concentration or exposure targets for lupus nephritis patients.

Conclusions

MPA therapy in lupus nephritis patients, as opposed to use in renal transplantation is further complicated by urinary protein excretion and hypoalbuminemia, in addition to altered eClcr. Serum albumin and eClcr appear to be the primary contributors to clearance estimates of MPA and should be accounted for when dosing MPA. Similarly, clinical changes that are associated with either response to therapy or progression of disease may necessitate future adjustments to therapy to maintain efficacy and/or reduce toxicity. MPA therapy individualization is possible in lupus nephritis and the results of such interventions require prospective assessments. The acceptable AUC target for MPA therapy will need to be defined specifically for patients with lupus nephritis to enhance clinical outcomes.

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Table 2.1

Pharmacokinetic Parameters in Patients with Lupus Nephritis

Mycophenolic Acid Parameters	
Tmax (hrs)	1.69 ± 1.86
Cmax (μg/mL)*	21.0 ± 16.2
Ctr_{12} (μg/mL)*	4.06 ± 5.15
Lambda (hr^{-1})	0.11 ± 0.07
MRT (hrs)	16.3 ± 19.9
AUC_{MPA} 0-12 (μg hr/mL)#	78.8 ± 74.1
AUC_{MPA} 6-12 (μg hr/mL)#	33.2 ± 39.0
MPA Cl/F (mL/min)+	343 ± 200
MPA Cl_R/F 0-12 (mL/min)+	1.85 ± 1.42
Ae 0-12 (mg)	4.81 ± 3.34
Ae 12-24 (mg)	6.53 ± 9.10
MPA free (%)	2.56 ± 1.97
Mycophenolic Acid Glucuronide Parameters	
Tmax (hrs)	3.36 ± 3.56
Cmax (μg/mL)*	55.1 ± 42.7
Ctr_{12} (μg/mL)*	28.2 ± 25.2
Lambda (hr^{-1})	0.08 ± 0.05
AUC_{MPAG} 0-12 (μg hr/mL)#	518 ± 460
MPAG:MPA	7.09 ± 4.76
MPAG Cl_R/F 0-12 (mL/min)+	53.5 ± 52.3
Ae 0-12 (mg)	656 ± 310
Ae 12-24 (mg)	441 ± 341
MPAG free %	9.30 ± 5.23

Free Mycophenolic Acid Parameters

Cmax (μg/mL)*	0.44 ± 0.54
Ctr_{12} (μg/mL)*	0.10 ± 0.15
Ctr_{24} (μg/mL)*	0.13 ± 0.25
AUC_{MPA} 0-12 (μg hr/mL)#	1.76 ± 2.60
MPA Cl/F (L/min)+	27.4 ± 30.5

* normalized to a 1000 mg dose

+ scaled to a body size of 70 kg using 0.75 power

dose-normalized to 1000 mg and weight normalized to 70 kg

Table 2.2

Clinical Grouping of Patients and Pharmacokinetics

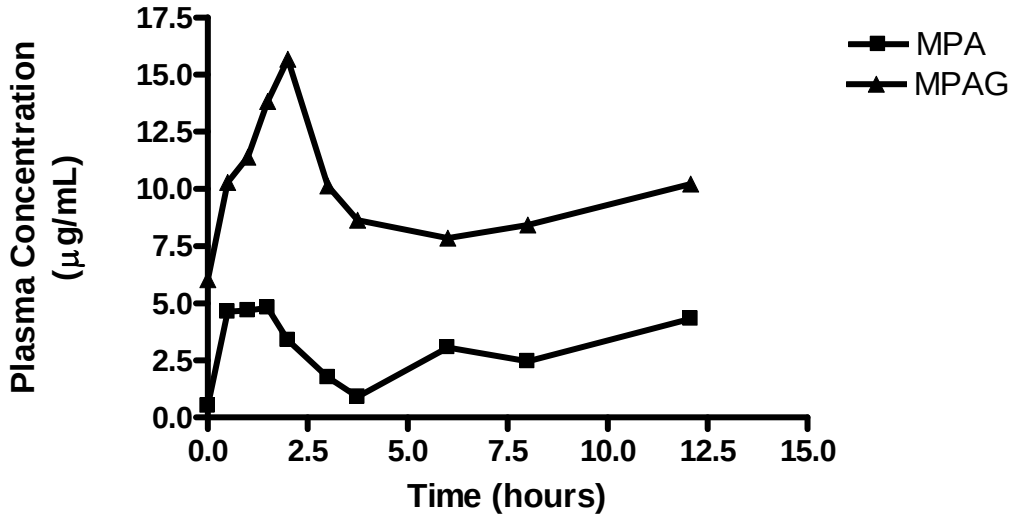
PK Parameter	**Mean (SD)**		**P-value**
	UP:Cr < 1 g/day	*UP:Cr ≥ 1 g/day*	
	(n = 14)	*(n = 4)*	
MPA % Unbound	2.09 (1.64)	4.10 (2.42)	0.2017
MPA Ctr_{12} (μg/mL)	4.97 (5.53)	0.88 (0.22)	0.0118
MPA AUC_{0-12} (μg hr/mL)	91.9 (79.6)	33.2 (9.87)	0.0176
MPA Cl/F (mL/min)	305 (146)	790 (423)	0.0464
MPA Cl_R/F_{0-12} (mL/min)	1.70 (1.37)	3.35 (2.14)	0.1630
MPA $Cl_{unbound}$ (mL/min)	32695 (40245)	21565 (5982)	0.6235
MPA MRT (hrs)	19.8 (22.4)	6.41 (2.60)	0.0176
MPAG AUC_{0-12} (μg hr/mL)	564 (497)	355 (294)	0.4418
MPAG Clr_{0-12} (mL/min)	53.1 (47.8)	68.0 (79.14)	0.6235
Metabolic ratio	6.40 (4.37)	9.52 (6.00)	0.2327

	Albumin < 4 g/dL	*Albumin ≥ 4 g/dL*	
	(n = 7)	*(n = 7)*	
MPA % unbound	2.20 (2.09)	3.35 (2.41)	0.4452
MPA Ctr_{12} (μg/mL)	4.38 (7.85)	4.26 (3.51)	0.3176
MPA AUC_{0-12} (μg hr/mL)	80.4 (112)	85.7 (48.8)	0.1282
MPA Cl/F (mL/min)	522 (408)	342 (238)	0.4557
MPA Cl_R/F_{0-12} (mL/min)	2.98 (1.71)	1.46 (1.55)	0.0728
MPAG AUC_{0-12} (μg h/mL)	280 (262)	769 (538)	0.0728
MPA MRT (hrs)	13.9 (4.50)	32.8 (29.9)	0.1061
MPAG Clr_{0-12} (mL/min)	80.7 (61.3)	36.4 (37.8)	0.0530
Metabolic ratio	5.19 (3.44)	9.04 (5.19)	0.3176
MPA $Cl_{unbound}$ (L/min)	36.0 (30.6)	30.7 (51.3)	0.0513

Figure Legends

Figure 2.1. Representative 12-hour Mycophenolic Acid (MPA) and Mycophenolic Acid Glucuronide (MPAG) Plasma Concentration Versus Time Curve in a Lupus Nephritis Patient.

Figure 2.1


Plasma Concentration (μg/mL)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
Time (hours)
MPA
MPAG

Chapter 3

Influence of Clinical and Demographic Variables on Mycophenolic Acid Pharmacokinetics in Anti-Neutrophil Cytoplasmic Antibody (ANCA) Associated Vasculitis

Melanie S. Joy, Pharm.D., FCCP,[1] Tandrea Hilliard, B.S.,[1] Yichun Hu, M.S.,[1] Susan L. Hogan, Ph.D., MPH,[1] Jinzhao Wang, B.S.,[1] Ronald J. Falk, M.D.,[1] Philip C. Smith, PhD[2]. School of Medicine, University of North Carolina at Chapel Hill, UNC Kidney Center and Division of Nephrology and Hypertension,[1] and Eshelman School of Pharmacy,[2] Department of Molecular Pharmaceutics, Chapel Hill, NC

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With permission: Joy MS, Hilliard T, Hu Y, Hogan SL, Wang J, Falk RJ, Smith PC. Influence of clinical and demographic variables on mycophenolic acid pharmacokinetics in ANCA-associated vasculitis. Annals of Pharmacotherapy 2009;Jun;43(6):1020-7. Epub 2009 Jun 2.

Abstract

Background: Mycophenolic acid (MPA) is used off-label to treat many forms of glomerulonephritis.

Objectives: The objectives were to evaluate the pharmacokinetics of MPA and its glucuronide (MPAG) in anti-neutrophil cytoplasmic antibody (ANCA) vasculitis patients with kidney manifestations and to determine effects of clinical (urinary protein excretion, serum albumin, and creatinine clearance) and demographic (age, race, gender) variables on MPA and MPAG pharmacokinetics.

Methods: Twenty-three patients at steady state on MPA were evaluated. Plasma and urine samples were collected over 24 hours. Analyses included noncompartmental pharmacokinetics and statistics including Mann Whitney test and univariate/multiple regression.

Results: MPA clearance (Cl/F 288 ± 154 mL/min) was ~2-fold higher than previously reported from transplant patients and predicted by weight and race (ranked MPA Cl/F = -11.766 + 0.2035 (wt) + 4.9578 (race), R^2 41.8%, p = 0.0045). Estimated creatinine clearance (eClcr) < 60 mL/min resulted in higher MPA exposure; total AUC_{0-12} and AUC_{6-12}, as well as unbound AUC_{0-12}. The metabolic ratio ($MPAG_{AUC}$:MPA_{AUC}) of 8.67±5.57 was lower than previously reported in kidney transplant recipients.

Conclusions: Diminished kidney function (e.g. eClcr<60 mL/min) demonstrated enhanced MPA and MPAG exposure in ANCA vasculitis patients. However, unlike kidney transplant recipients, patients with ANCA vasculitis had enhanced Cl/F and diminished metabolic ratio, suggesting the need to comprehensively evaluate the role of disease-specific factors on MPA pharmacokinetics.

Introduction

Mycophenolic acid (MPA) is used off-label for immune-mediated disorders and is FDA approved for transplant rejection. [1-6] Three studies evaluated the efficacy of MPA in small vessel vasculitis. [7-9] We reported 3-fold improvements in disease activity (Birmingham Vasculitis Activity Score (BVAS)) in patients experiencing disease relapse compared to those defined as treatment resistant. [9] While patients with kidney manifestations of vasculitis are similar to renal transplant recipients in that they can have alterations in glomerular filtration rate, they also often have altered serum albumin, urinary protein, and markers of inflammation.

Since inter-individual variability in MPA pharmacokinetics has been documented in transplant recipients, therapeutic plasma monitoring has been suggested to improve immunosuppressive outcomes. [10-13] Area under the plasma concentration time curve from 0-12 hours (AUC_{0-12}) of 30-60 μg h/mL and trough plasma concentrations of 1-3.5 μg/mL were suggested as targets for triple combination immunosuppressive therapy in kidney and heart transplant patients. [13-14] Target ranges for MPA in autoimmune kidney diseases such as lupus nephritis and anti-neutrophil cytoplasmic antibody (ANCA) vasculitis have not been established.

The primary purpose of this study was to evaluate the pharmacokinetics of total and free MPA and its phenolic O-glucuronide (MPAG) metabolite in ANCA vasculitis. The secondary objectives were to determine the effects of clinical parameters (urinary protein to creatinine excretion ratio (UP:Cr), serum albumin, and estimated creatinine clearance (eClcr)) and demographic variables (age, race, gender) on pharmacokinetics.

Materials and Methods

Patients

Patients with biopsy confirmed ANCA vasculitis receiving MPA therapy (as mycophenolate mofetil [Cellcept®, Roche, NJ]) and at steady state were eligible. Concomitant therapies with other immunosuppressants were permitted and recorded. Patients entered the General Clinical Research Unit (GCRC) for 24-hours to assess the pharmacokinetics or MPA at the dose and

interval they were prescribed. Patients were fasting at study initiation and were fed a standardized diet. eClcr (calculated by the Cockroft and Gault equation [15]), UP:Cr, serum albumin, and serum creatinine were recorded/obtained. The study was approved by the University's Institutional Review Board in accordance with the Declaration of Helsinki.

Pharmacokinetic Study

After obtaining baseline blood for measurement of a trough plasma concentration (Ctr), patients were instructed to take their morning dose of MPA. Plasma samples (7.5 mL) were obtained at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours and immediately centrifuged for 10 minutes at 4C, aliquoted and transferred to plastic screw top tubes and stored at -80 until assay. Urine was collected at 0-6, 6-12, and 12-24 hour intervals into acidified (15 mL 6 N HCl) collection containers, volumes recorded, and 2 mL aliquots were stored at -80C until assay. Plasma and urine samples were processed and assayed as described previously. [16-17] Unbound plasma fraction was determined as previously described. [17] 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 5-1500 μg/mL, respectively. MPAG concentrations were represented in terms of MPA-equivalents by multiplying the MPAG concentration by 0.646 (molecular mass of MPA to MPAG) and reported in μg/mL. The amount of MPA available from a dose of mycophenolate mofetil was estimated as 72% of the dose.

Pharmacokinetic Analysis

Noncompartmental pharmacokinetic analysis of total and unbound MPA and total MPAG was conducted using WinNonlin v4.1 (Pharsight, Mountain View CA). Concentration maximum (Cmax), time to maximum concentration (Tmax), concentration trough (Ctr) at 12 hours, area under the plasma concentration time curve from 0-12 hours (AUC_{0-12}), apparent oral clearance (Cl/F), apparent renal clearance (Cl_R/F), and mean residence time (MRT) were recorded. Both

concentration and AUC parameters were dose-normalized and the clearance data were adjusted to a 70 kg patient based on a scaling method using a power of 0.75. [18] We made the assumption that bioavailability (F) was equal to 1.0 as reported previously. [11] AUC_{12-24} and AUC_{6-12} were calculated, and the latter was used to estimate apparent entero-hepatic recycling. [19-20] Amount of MPA and MPAG in urine over each collection was determined by multiplying concentration by volume. MPA and MPAG amounts in urine (Ae) were computed for 0-12 hours by adding the Ae for the first two intervals. Cl_R/F for the 0-12 hour time was calculated by Ae_{0-12}/ AUC_{0-12}.

Statistics

Descriptive analyses for pharmacokinetic and demographic variables and laboratories included means, standard deviations, and medians as appropriate. Bivariate assessments of the key characteristics (serum albumin, UP:Cr, eClcr, age, weight, race, prednisone dose) versus each pharmacokinetic parameter of interest (MPA Cl/F, AUC_{0-12}, AUC_{6-12}, Ctr_{12}, Cl_R/F, Cl_{unb}, $AUC_{Unb\ 0-12}$, and MPAG AUC_{0-12}, Cl_R/F) were assessed by Spearman Rank correlations. The effect of cyclosporine on MPA could not be directly assessed secondary to only three patients receiving the drug. The correlations and resultant p values from the biivariate assessments were analyzed for possible inclusion into multiple regression models that predicted pharmacokinetic parameters. Correlations with $p<0.15$ were selected for incorporation into the multiple regression models. Variables were transformed to insure that each followed a normal distribution. Model building for analysis of determinants of the parameters identified above consisted of multiple linear regression analysis with forward addition of variables as well as backward elimination, noting influences on the coefficients of the primary predictors. The final model was selected based on significance of each variable on predicting the parameters in the model as well as the overall R^2 of the model.

Comparisons between clinical groups based on eCrCl (< 60 mL/min vs ≥ 60 mL/min) and UP:Cr (< 500 vs ≥ 500) were analyzed by nonparametric Mann Whitney Test. It was not

possible to compare serum albumin groups as there were no significant deviations from the laboratory normal reference range.

Results

Twenty-three biopsy-confirmed ANCA small vessel vasculitis patients completed the MPA/MPAG pharmacokinetics study. Patient demographics included age 53±14 years, 57% female, 78% Caucasian, and weight of 87±19 kg. Non-Caucasian races consisted of African-American (n=3), Asian (n=1) and Other (n=1). All patients were receiving the mycophenolate mofetil prodrug of MPA with the exception of one patient (prescribed mycophenolate sodium), who was included in the analyses as the pharmacokinetics were similar. The average MPA daily dose was 1489 ± 596 mg with dosing divided twice daily in all but one patient who was dosed once daily. The distribution of doses were 250mg (n=1), 500mg (n=9), 750mg (n=4), 1000mg (n=8), and 1500mg (n=1). The mean (± standard deviation) clinical laboratory results were serum creatinine 1.3±0.6 mg/dL (range 0.7 to 3.4), UP:Cr 0.42±0.50 (range 0.04 to 1.87), eClcr 84.4±40.1 mL/min (range 18.3 to 182.2), and serum albumin 4.4±0.40 g/dL (range 3.6 to 5.2). Thirty percent (n = 7) of patients were receiving concomitant glucocorticoids and 13% (n = 3) were receiving cyclosporine.

Mycophenolic Acid Pharmacokinetics

A concentration versus time profile for one patient at steady state MPA and MPAG concentrations over 12 hours is presented in Figure 3.1. The mean pharmacokinetic parameters for patients with ANCA vasculitis are provided in Table 3.1. The scaled Cl/F of 288±154 mL/min suggests that MPA is a moderate extraction ratio drug whose metabolism could be impacted by changes in unbound fraction. The mean percentage of free MPA was 1.0±0.6%, with all patients having free fractions of ≤2.4%, similar to expected free fraction. [11-21] The MPA AUC_{0-12} was outside the 30-60μg hr/mL range that was recommended in kidney transplant patients within the first six month period post transplant, [22] with 22% (n=5) of patients

above and 30% (n=7) below this range. Examination of the AUC_{6-12} to the AUC_{0-12} suggested that recycling accounted for 34±10% of the AUC, which is within the published range. [21-22]

The Ctr at 12 hours exceeded the range of 1.0 to 3.5μg/mL recommended in transplant patients [22], with 22% (n=5) above this target. The Tmax varied from 0.5 to 5 hours, severely limiting applicability of shortened plasma collections for AUC determination.

The MPA Cl_R/F represented 2% of the Cl/F, consistent with previous reports.[21] As suggested previously [11], the clearance of MPA is primarily the result of systemic metabolism to MPAG. The Cl_R/F of MPA was 5.8±5.8 mL/min, which was 9% of the eClcr in our patients.

Mycophenolic Acid Glucuronide Pharmacokinetics

The MPAG pharmacokinetic results are reported in Table 3.1. A calculated AUC_{0-12} ratio of MPAG to MPA resulted in a metabolic ratio (MR) of 8.7± 5.6, less than previously reported in renal transplant recipients. [23]

The renal clearance of MPAG was 33.7±34.9 mL/min, representing 40% of the eClcr. The kidneys contributed to the elimination of 97% of the MPA dose primarily through excretion of MPAG. The amount of MPAG in the urine over the 0-12 hour interval (513±285 mg) was more than the amount in the 12-24 hour interval (378±257 mg), $p = 0.017$. The Cl_R/F was also greater in the 0-12 hour (33.7±34.9 mL/min) versus 12-24 hour dosing interval (28.4±36.9 mL/min), $p = 0.0043$.

Unbound Pharmacokinetics

Our data showed that 1.0% and 13% of MPA and MPAG, respectively, were unbound in the plasma. Since the unbound MPAG was less than that reported previously [21], we performed studies with MPA and/or MPAG spiked heparinized plasma. [17] The plasma that was spiked separately demonstrated similar unbound percentages to that found in our ANCA patient data, the combination of drug and metabolite resulted in an increase in unbound percentage of MPA

and MPAG. This may be suggestive of competitive binding to albumin as has been reported previously. [24]

Since the normal percentage of unbound MPA is 2%, if one aims for a total MPA Ctr of 1.0 to 3.5 μg/mL then an unbound target would be 0.02 to 0.07μg/mL. [21] Likewise, if suggested total AUC goals are 30 to 60 μg h/mL, then unbound AUC goals would be 0.6 to 1.2 μg h/mL. Mean unbound Ctr levels were 0.04±0.06μg/mL (consistent at both the 12 and 24 hour time points), with five patients exceeding the range and thirteen patients below the range; resulting in only 22% of all patients falling within the targeted kidney transplant range. With regard to unbound AUC, the mean exposure was greater than the upper range of 1.2 μg h/mL in only one patient, but was less than the targeted range in 15 patients.

Regression

The multiple regression model for MPA Cl/F revealed that race and weight contributed; ranked Cl/F= -11.766+0.2035(wt)+4.9578(race), R^2 41.8%, p=0.0045. The $AUC_{6\text{-}12}$ showed the following relationship: Ln MPA $AUC_{6\text{-}12}$= 3.706 – 0.0094 (eClcr), R^2 36.86%, p=0.0021. In analysis of MPA Cl_R/F, the $AUC_{6\text{-}12}$ was the only significant contributing variable: ranked MPA Cl_R/F= 30.2674 – 6.2733 (ln $AUC_{6\text{-}12}$); R^2 33.2%, p=0.004. Regression assessment of the predictors for unbound MPA clearance indicated that eClcr and age were important: Ranked Cl_{unb}= 16.055 + 0.0601 (eClcr) – 0.1994 (age); R^2 52.3%, p=0.0013. MPAG Cl_R/F analysis showed that race and prednisone dose contributed. Ln MPAG Cl_R/ F= 2.6645 + 1.1799 (race) – 0.3041 (ranked prednisone dose); R^2 88.0%, p=0.0143.

Comparison Between Groups Based on Clinical Laboratories

The analysis of differences in pharmacokinetic by clinical grouping of UP:Cr (< 500 vs ≥ 500) and eClcr (<60 mL/min vs ≥ 60 mL/min) were assessed. (Table 3.2) There was a considerable distribution of eClcr across the population (low 18.3 mL/min and high 182 mL/min). Since MPAG is eliminated via the renal route, reductions in renal elimination would be predicted

to have more direct effects on MPAG with secondary effects on MPA due to potential enhanced MPA AUC $_{6\text{-}12}$, reflective of enterohepatic recycling.(Table 3.2)

The MPA Ctr were 3-fold higher in patients with reduced eClcr compared to higher eClcr (6.9± 6.8 vs 2.7±1.8, respectively), p=0.0301. The $AUC_{6\text{-}12}$ demonstrated 3-fold higher values in low vs high eClcr grouping (35.9±27.0 vs 16.7±8.8mg h/L, respectively), p=0.0149. The MPA $AUC_{0\text{-}12}$ was 2-fold greater in the low versus high eClcr grouping (95.0±66.9 vs 52.5±22.8 mL/min, respectively, p=0.0225). The MPA AUC $_{0\text{-}12}$ unbound values were significantly higher in the low eClcr group (1.29±0.61 vs 0.59±0.56 mg h/mL, p=0.0318), suggesting the presence of more pharmacologically active drug. The MPA Cl unbound was 3-fold reduced in the low eClcr group, with a trend toward statistical significance, which may be suggestive of reduced metabolism and/or eClcr. While the Cl_R/F MPAG was not statistically different between groups, the MPAG AUC $_{0\text{-}12}$ was enhanced 2-fold in the low eClcr patient group (959±664 vs 404±336 mg hr/L, p 0.0135).

UP:Cr was selected as a clinical variable secondary to the high plasma protein binding characteristics of MPA and MPAG. To enable at least five observations per group, a cut-point of 500 mg/day was selected. None of the pharmacokinetic parameters were statistically significant between the high and low UP:Cr grouping. Only four patients had UP:Cr > 1.0 g/day, preventing a comparison that may be more likely to be clinically relevant.

Discussion

While descriptions of the pharmacokinetics of MPA in kidney transplant patients are abundant, there is a paucity of data in autoimmune diseases that affect the kidney. Our study was conducted to comprehensively evaluate the pharmacokinetics of MPA and MPAG after chronic therapy in ANCA-associated vasculitis patients. Additionally, we wanted to understand the relevance of clinical and demographic variables in predicting pharmacokinetic parameters. eClcr was positively predictive for MPA Cl_{unb} and negatively predictive for MPA $AUC_{6\text{-}12}$. Race

was found to positively predict both MPA Cl/F and MPAG Cl_R/F, whereby non-Caucaisn race had higher clearances, suggesting an influence on both metabolism and renal clearance. Prednisone dose was negatively associated with MPAG Cl_R/F, suggesting an influence on active renal secretion. Unfortunately, the influences of UP:Cr and serum albumin on pharmacokinetic variables were not able to be fully assessed secondary to limited distribution of UP:Cr and relatively conserved values of serum albumin. A previous MPA study in lupus nephritis showed that at a UP:Cr of ≥1 g/day, Ctr and AUC_{0-12} were significantly reduced and Cl/F was significantly increased. [17] We previously reported higher MPA $Cl_{unbound}$ and MPAG Cl_R/F in lupus nephritis patients with serum albumin levels < 4g/dL vs those with levels ≥ 4g/dL.[17]

Creatinine clearance significantly affected pharmacokinetics of MPA and MPAG in ANCA-associated vasculitis. Although MPA itself is not highly eliminated by the kidneys, exposure was markedly enhanced in the low eClcr grouping; with the dosing interval (AUC_{0-12}), enterohepatic recycling (AUC_{6-12}), and unbound ($AUC_{0-12unb}$) exposures being significantly greater. Since MPAG is primarily eliminated by renal excretion, reductions in eClcr may predispose patients to higher levels of MPAG, which, through recycling can increase systemic exposure to MPA. These results suggest that patients with diminished kidney function can reach targeted MPA exposure ranges with lower dosages; minimizing adverse events. Lower unbound MPA (e.g. AUC) would not be predicted to be increased through a purely restrictive clearance mechanism and our patients were not hypoalbuminemic, hence our data may suggest the influence of additional factors affecting plasma concentrations in patients with glomerular kidney diseases. Assessment of MPA Ctr values showed a consistent 2-3 fold higher value in patients with a eClcr<60mL/min compared to eClcr>60mL/min. When we performed a post-hoc ANOVA to evaluate for the differences in pharmacokinetics based on eClcr groupings, we found that significant differences in Ctr, recycling AUC, unbound AUC, and unbound clearance were all

demonstrated between the stage 3 /4 vs 1 group. Only unbound clearance was found to also be significant between the stage 3 /4 vs stage 2 group.

The pharmacokinetics of MPA in ANCA patients are comparable with renal transplant patients, with the exception of Cl/F, which is about 2 -fold greater in vasculitis. Reasons for enhanced Cl/F can include increased systemic metabolism secondary to either up-regulated glucuronidation, increased MPA unbound fraction, or enhanced renal excretion. Regarding enhanced glucuronidation, patients receiving concurrent steroids (enzyme inducers) had similar Cl/F estimates to patients who were not receiving steroids (data not shown). We are currently evaluating the contribution of enhanced catalysis polymorphisms in the uridine diphosphate glucuronosyltransferase (UGT) enzymes as factors altering MPA clearance. The metabolic ratio, a reflection of metabolite to parent AUC was 8.67±5.57 in our study, considerably less than the 25.6±8.7 that was previously reported in kidney transplant recipients. [23] The unbound fraction was relatively normal (~1%) in our patients as they had essentially normal serum albumin concentrations (3.6 to 5.2 g/dL). Enhanced renal clearance can result from increased eClcr, loss of highly protein bound drugs with urinary protein, or enhanced secretory transport mechanisms. Our ANCA-vasculitis patients had a mean eClcr of 84mL/min with a range between 18 and 182mL/min. Although renal elimination of MPA is limited, enhanced eClcr could result in increased clearance secondary to renal clearance of the polar metabolite MPAG. Enhanced renal clearance secondary to loss of the highly protein bound MPA with the urinary protein could also account for an increase in Cl/F. However, when we evaluated Cl_R/F between patients with UP:Cr <500 mg/day and those with UP:Cr ≥500 mg/day, the results were similar. The magnitude of differences in Cl_R/F between UP:Cr groups may have been underappreciated based on our selected cut-point. MPAG is a substrate for MRP2, an efflux transporter found on the luminal surface of the proximal tubule. Theoretically, single nucleotide polymorphisms in

this transport gene can result in enhanced activity and could increase the renal excretion of MPAG, limiting the effect of recycling.

Renal transplant recipients, similar to our ANCA-vasculitis patients generally have reductions in eClcr. A previous publication used a multivariate analysis and demonstrated that 24% of the MPA Cl/F could be explained by proteinuria (yes/no), glomerular filtration rate, and diabetes mellitus. [25] Unfortunately, the range of proteinuria required to designate a yes versus no categorization was not reported. Our regression data showed that eClcr was predictive for both Cl_{unb} and $AUC_{6\text{-}12}$. Previous data from our laboratory in lupus nephritis showed that 51% of MPA Cl/F could be explained by eClcr and serum albumin, two readily measured clinical laboratory measures. [17] The contribution of race to MPA Cl/F in our vasculitis patients requires assessment of genotype as a confounding variable as genomic effects have been shown to influence the pharmacokinetics of MPA. [26] eClcr would generally be predicted to contribute little to MPA Cl/F secondary to the low percentage of MPA (1-3%) that is normally excreted by the kidneys. Hence, non-renal clearance, through metabolism of MPA to MPAG would comprise the largest bulk of the Cl/F for MPA. Our regression analyses demonstrated that $AUC_{6\text{-}12}$ was the only significant predictor of MPA Cl_R/F demonstrating an influence of MPA plasma concentration on Cl_R/F. The regression models are important as they provide insights into the mechanisms that may underlie the alterations in pharmacokinetics seen in disease states such as ANCA-associated vasculitis. This is particularly important since there is a paucity of published research in medication off-label disease groups, whereby there can be extensive variations in medication handling versus in the diseases where the drugs were FDA approved.

Unlike our ANCA-vasculitis patients, our previous report of MPA pharmacokinetics in lupus nephritis patients showed higher UP:Cr and lower serum albumin and eClcr. [17] The key differences in pharmacokinetics of MPA and MPAG between these studies included enhanced MPA MRT, MPA Cl_R/F, metabolic ratio (MPAG AUC:MPA AUC), unbound MPAG %, and MPA Cl/F_{unb}, and reduced MPA $AUC_{0\text{-}12}$, MPA $AUC_{6\text{-}12}$, MPAG Cl_R/F, MPA Ctr_{unb}, MPA AUC_{unb}, and

normal free MPA % in the ANCA versus lupus nephritis population. This data generally demonstrates lesser MPA exposure in the ANCA-vasculitis patients as opposed to patients with lupus nephritis. While lower MPA dosages in the ANCA-vasculitis patients could reflect reduced exposure, our data was dose-normalized to eliminate the dose effect. The effects of single nucleotide polymorphisms in UGT enzymes requires assessment in these autoimmune diseases, especially since environmental exposures are thought to play a role in their etiology and since the UGT enzymes play a role in the body's natural defense against environmental toxins. Additionally, the role of inflammation on MPA pharmacokinetics in autoimmune diseases requires evaluation as a potential disease component that may modify drug metabolism and transport.

While we report the contribution of eClcr to MPA clearance and exposure in a model of ANCA-vasculitis, there are some limitations to our research. As noted previously, our patients had preserved serum albumin concentrations, preventing the full assessment of the contribution of reductions in serum albumin on clearance. Similarly, since only four patients in our dataset had UP:Cr of ~ 1g/day and none had nephrotic range proteinuria (UP:Cr > 3.5 g/day), the full contribution of UP:Cr to clearance may actually be under-recognized. Future analyses of our data include assessment of the contribution of genotype for drug metabolizing enzymes and transporters on clearance and outcomes, as well as the analysis of the contribution of Ctr and AUC to patient outcomes. Our studies in autoimmune-related kidney diseases are important as they provide a framework to understand the contributions of disease-related and unrelated factors to MPA exposure. A goal of our future work is to better define appropriate MPA concentration or exposure targets for ANCA vasculitis patients.

Conclusions

MPA therapy in glomerular diseases such as ANCA-vasculitis can be complicated by urinary protein excretion, hypoalbuminemia, and reductions in eClcr. Assessment of pharmacokinetic alterations based on eClcr demonstrated enhanced MPA and MPAG exposure in patients with

reductions in eClcr, the most significant effects appreciated in patients with eClcr < 60 mL/min. Regression models demonstrated the demographic variables nonCaucasian race, increased weight, and decreased age were predictors of decreased MPA exposure (AUC) and/or increased clearance. Approaches to comprehensively evaluate the influence of clinical and demographic factors on MPA pharmacokinetics are needed in order to begin to identify variable that could be used to individualize treatment strategies for patients with ANCA-associated vasculitis.

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Table 3.1

Pharmacokinetic Parameters in Patients with Anti-neutrophil Cytoplasmic Antibody (ANCA)-Associated Vasculitis

Mycophenolic Acid (MPA) Parameters	
Tmax (hrs)	1.46 ± 1.24
Cmax (μg/mL)[a]	21.5 ± 20.3
Ctr_{12} (μg/mL)[a]	3.99 ± 4.32
Lambda (hr^{-1})	0.07 ± 0.04
MRT (hrs)	27.2 ± 36.2
$AUC_{MPA\ 0\text{-}12}$ (μg hr/mL)[a]	65.4 ± 44.4
$AUC_{MPA\ 6\text{-}12}$ (μg hr/mL)[a]	22.6 ± 18.3
MPA Cl/F (mL/min)[b]	288 ± 154
MPA $Cl_R/F_{\ 0\text{-}12}$ (mL/min)[b]	5.77 ± 5.80
$Ae_{\ 0\text{-}12}$ (mg)	13.6 ± 12.2
MPA free (%)	1.02 ± 0.66
Mycophenolic Acid Glucuronide (MPAG) Parameters	
Tmax (hrs)	2.51 ± 1.44
Cmax (μg/mL)[a]	74.3 ± 58.9
Ctr_{12} (μg/mL)[a]	35.1 ± 32.3
Lambda (hr^{-1})	0.07 ± 0.04
$AUC_{MPAG\ 0\text{-}12}$ (μg hr/mL)[a]	573 ± 515
MPAG:MPA ratio	8.67 ± 5.57
MPAG $Cl_R/F_{\ 0\text{-}12}$ (mL/min)[b]	33.7 ± 34.9
$Ae_{\ 0\text{-}12}$ (mg)	513 ± 285
MPAG free %	12.9 ± 7.0

Free Mycophenolic Acid (MPA) Parameters

Cmax (μg/mL)[a]	0.22± 0.24
Ctr_{12} (μg/mL)[a]	0.04 ± 0.06
$AUC_{MPA\ 0\text{-}12}$ (μg hr/mL)[a]	0.76 ± 0.64
MPA Cl/F (L/min)[b]	37.0 ± 29.6

a normalized to a 1000 mg dose

b scaled to a body size of 70 kg using 0.75 power

Ae – amount excreted in the urine

AUC – area under the plasma concentration time curve

Cmax – maximal plasma concentration

Cl/F – oral clearance

Cl_R/F – renal clearance

Ctr_{12} – trough plasma concentration at 12 hours

MRT – mean residence time

Tmax – time to maximal concentration in plasma

Table 3.2

Clinical Grouping of Patients and Pharmacokinetics (by eClcr and UP:Cr)

Pharmacokinetic Parameter	eClcr Status [Mean (SD)]		P-value
	Clcr < 60 mL/min	Clcr ≥ 60 mL/min	
	(n = 7)	(n = 16)	
MPA % Unbound	0.9 (0.7)	1.1 (0.7)	0.5979
MPA Ctr_{12} (μg/mL) [a]	6.88 (6.79)	2.72 (1.81)	0.0301
MPA AUC_{0-12} (μg hr/mL) [a]	95.0 (66.9)	52.5 (22.8)	0.0225
MPA AUC_{6-12} (μg hr/mL) [a]	35.9 (27.0)	16.7 (8.8)	0.0149
MPA Cl/F (mL/min) [b]	210 (86.7)	323 (167)	0.1089
MPA Cl_R/F_{0-12} (mL/min) [b]	3.36 (3.07)	6.77 (6.48)	0.3403
MPA $Cl_{unbound}$ (mL/min) [b]	15928 (6376)	43539 (31045)	0.0670
MPA $AUC_{0-12unb}$ [a] (mL/min)	1.29 (0.608)	0.592 (0.562)	0.0318
MPA MRT (hrs)	36.9 (35.9)	23.4 (36.8)	0.4523
MPAG AUC_{0-12} (μg hr/mL)	959 (664)	404 (336)	0.0135
MPAG Cl_R/F_{0-12} (mL/min) [b]	21.8 (20.2)	38.9 (39.1)	0.2490
MPAG AUC_{0-12}/MPA AUC_{0-12}	10.7 (6.19)	7.77 (5.24)	0.2776

	UP:Cr Status [Mean (SD)]		
	UP:Cr < 500 mg/day	*UP:Cr ≥ 500 mg/day*	*P value*
	(n = 15)	*(n = 6)*	
MPA % unbound	0.99 (0.65)	1.21 (0.78)	0.5160
MPA Ctr_{12} (μg/mL) [a]	3.07 (1.79)	6.56 (7.87)	0.3809
MPA AUC_{0-12} (μg hr/mL) [a]	55.8(22.3)	84.5 (80.6)	0.7910
MPA AUC_{6-12} (μg hr/mL) [a]	18.9 (9.18)	32.2 (32.6)	0.1532
MPA Cl/F (mL/min) [b]	300(163)	285 (165)	0.9699
MPA Cl_R/F_{0-12} (mL/min) [b]	5.47 (6.08)	7.31 (6.23)	0.5693
MPA $Cl_{unbound}$ (L/min) [b]	43.5 (33.5)	19.7 (12.5)	0.1450
MPA $AUC_{0-12unb}$ (μg hr/mL) [a]	0.82 (0.74)	1.25 (0.82)	0.1859
MPAG AUC_{0-12} (μg h/mL)	467 (366)	860 (798)	0.7333
MPA MRT (hrs)	24.2 (37.8)	43.4 (36.8)	0.1859
MPAG Cl_R/F_{0-12} (mL/min) [b]	28.1 (19.5)	45.9 (62.0	0.7910
Metabolic ratio	8.46 (5.57)	9.95 (6.48)	0.3403

a normalized to a 1000 mg dose

b scaled to a body size of 70 kg using 0.75 power

Abbreviations:

Ae – amount excreted in the urine

AUC – area under the plasma concentration time curve

Cmax – maximal plasma concentration

Cl/F – oral clearance

Cl_R/F – renal clearance

Ctr_{12} – trough plasma concentration at 12 hours

eClcr – estimated creatinine clearance

MPA – mycophenolic acid

MPAG – mycophenolic acid glucuronide

MRT – mean residence time

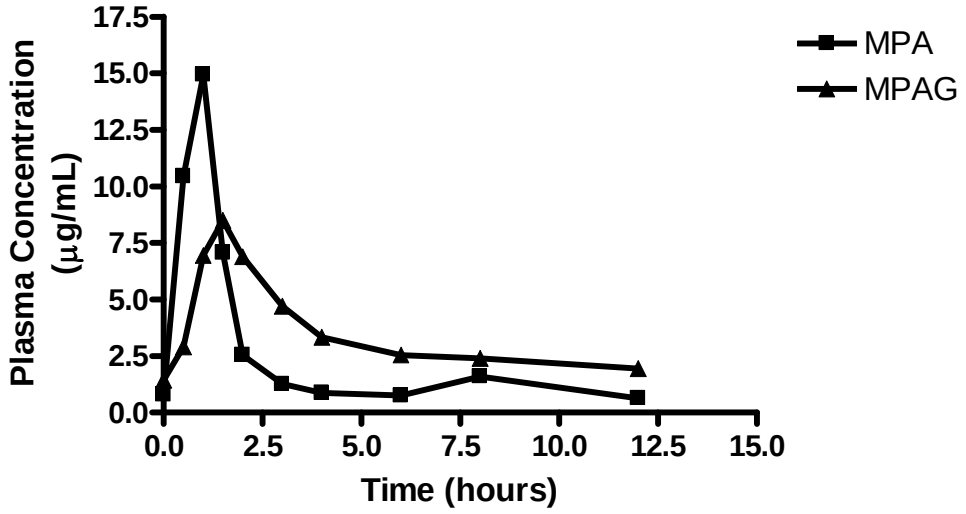
Tmax – time to maximal concentration in plasma

UP:Cr – urinary protein to creatinine ratio

Figure Legends

Figure 3.1 Mycophenolic Acid and Mycophenolic Acid Glucuronide (MPAG) 12-hour Plasma Concentration versus Time Curve in a Small Vessel Vasculitis Patient.

Figure 3.1


Plasma Concentration (µg/mL)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
0.0
2.5
5.0
7.5
10.0
12.5
15.0
Time (hours)
MPA
MPAG

Chapter 4

Population Pharmacokinetics of Mycophenolic Acid and Metabolites in Patients with Glomerulonephritis

Melanie S. Joy, Pharm.D., Ph.D.[1,2] and Wai-Johnn Sam, Ph.D.[3]

University of North Carolina, School of Medicine, UNC Kidney Center,[1] and Eshelman School of Pharmacy,[2] Chapel Hill, NC and University of Rhode Island, College of Pharmacy, Department of Biomedical and Pharmaceutical Sciences, Kingston, RI[3]

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Abstract

Background and Objective: Mycophenolic acid (MPA) is an inosine monophosphate dehydrogenase inhibitor used as immunosuppressive therapy for induction and maintenance of remission in glomerulonephritis due to systemic lupus erythematosus and small vessel vasculitis. The objective of the current study was to develop a population pharmacokinetic model for MPA and its two metabolites, MPA glucuronide (MPAG) and acyl-MPA glucuronide (AcMPAG) in patients with glomerulonephritis.

Methods: Thirty-nine patients with glomerulonephritis and receiving mycophenolate mofetil were recruited to participate in a 24-hour pharmacokinetic study. Blood was collected at times 0, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours and urine was collected over the intervals of 0-6, 6-12, and 12-24 hours. Plasma and urine samples were assayed for MPA and MPAG by high-performance liquid chromatography (HPLC), and for AcMPAG by liquid chromatography / mass spectrometry (LC/MS). Population pharmacokinetic analysis and covariate model building were evaluated using Non-linear Mixed Effect Modeling software (NONMEM, version 6.2.0, ICON Development Solutions, Ellicott City, MD).

Results: The final model for MPA and it's metabolites consisted of 9 discrete compartments; 1) depot gastrointestinal, 2) central MPA, 3) peripheral MPA, 4) gallbladder, 5) MPA urine, 6) MPAG central, 7) MPAG urine, 8) AcMPAG central, and 9) AcMPAG urine compartment. The MPA population mean estimates for apparent non-renal clearance (Cl_{NR}/F) and apparent central volume of distribution were 14.3 L/hr and 21.1 L, respectively. The mean population estimate for apparent renal clearance (Cl_R/F) was dependent on estimated creatinine clearances (eClcr); 0.0975 L/hr for eClcr $\leq$80 mL/min and 0.157 L/hr for eClcr > 80 mL/min. Covariate analyses identified the following significant effects: eClcr on $CL_{NR,MPA}/F$ ($P<0.001$), eClcr (with a cut-off value at 80 ml/min) on $CL_{R,MPA}/F$ ($P<0.025$), serum albumin on $CL_{NR,MPA}/F$ ($P<0.01$), eClcr on $CL_{R,MPAG}/F$ ($P<0.001$) and eClcr on $CL_{R,AcMPAG}/F$ ($P<0.001$). Evaluation of the final model by

visual predictive check showed that most of the observed values were within the 95th percent prediction interval generated from 100 simulations of the final model.

Conclusion: The current population pharmacokinetic model demonstrated two key covariates, eClcr and serum albumin influenced the renal and nonrenal components of Cl/F in patients with glomerulonephritis, suggesting patients with these diseases would have highly altered MPA exposures.

Introduction

The pharmacokinetics of mycophenolic acid (MPA), the pharmacologically active component of mycophenolate mofetil (Cellcept®, Roche, Nutley, NJ) are well described in transplant recipients and population pharmacokinetic models are reported. [1-8] However, there is considerable lack of consensus in the transplant community surrounding optimal limited pharmacokinetic sampling strategies to monitor therapy, selection of optimal targets for exposure, and/or trough plasma concentrations. [9,10] Much of this conflict is the result of the large inter- and intra-individual variability and unexplained error in pharmacokinetic predictions. [9-11] Development of therapeutic drug monitoring is the goal for MPA since several publications have reported relationships between exposure and treatment-related outcomes in transplant patients. [12-19]

The knowledge and applicability of MPA pharmacokinetics data from transplant populations to other kidney diseases are limited despite its use in induction and maintenance regimens for glomerulonephritis including systemic lupus erythematosus (SLE) [20-24] and small vessel vasculitis (SVV). [25-27] However, unlike kidney transplant patients who receive a 3-4 drug immunosuppressive regimen, glomerulonephritis patients receive only 1-2 immunosuppressive drugs. Previous results from noncompartmental pharmacokinetic analyses have suggested altered disposition of MPA in glomerulonephritis, [28,29] a finding that is not surprising given urinary protein losses, serum protein reductions, kidney function declines, and inflammation. Compartmental pharmacokinetic modeling approaches in patients with glomerulonephritis[30] and data supporting relationships between exposure and/or trough plasma concentrations and outcomes is currently lacking in glomerulonephritis.

The aim of the current study was to develop a population pharmacokinetic model for MPA and its two metabolites [MPA glucuronide (MPAG) and acyl-MPA glucuronide in patients with

glomerulonephritis using plasma and urine data (AcMPAG)] and followed by covariate assessments to determine covariates which influence its pharmacokinetics.

Methods

Patients and Samples

Patients with glomerulonephritis from SLE or SVV and receiving MPA as mycophenolate mofetil (Cellcept®, Roche, New Jersey) for at least 2 weeks on a stable dose, were recruited to participate in a pharmacokinetic study approved by the institution's Biomedical Institutional Review Board. Details of these studies and results from noncompartmental pharmacokinetics for MPA and MPAG were previously described. [28,29] Briefly, blood samples were collected at times 0, 0.5, 1.0, 1.5, 2, 3, 4, 6, 8, 12, and 24 hours and urine was collected from 0-6, 6-12, and 12-24 hours. Plasma and urine samples were assayed for MPA and MPAG by a high-performance liquid chromatography (HPLC) with ultraviolet detection assay. [31] 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 5-1500 μg/mL, respectively. The AcMPAG metabolite was assayed in plasma and urine by liquid chromatography / mass spectrometry (LC/MS). Plasma and urine standard curves for AcMPAG were linear over the range of 0.01-50 μg/mL and 1-500 μg/mL, respectively. MPAG and AcMPAG concentrations were represented in terms of MPA-equivalents by multiplying the MPAG and AcMPAG concentration by 0.646 (molecular mass of MPA to MPAG/AcMPAG) and reported in mcg/mL. The amount of MPA available from a dose of the prodrug (mycophenolate mofetil) was estimated as 72% of the dose (molecular mass of MPA to mycophenolate mofetil).

Demographic data (age, weight, race, gender), clinical data (serum creatinine, serum albumin, urinary protein to creatinine ratio), and genotype data for single nucleotide polymorphisms relevant for MPA metabolism (uridine glucuronosyltransferase genes; *UGTs, e.g. UGT2B7 C802T*, *UGT1A7 T622C*) or transport (multidrug resistance gene; *MDR1/ABCB1,*

e.g. MDR1 C3435T, and *MDR1 C1236T*) were abstracted from the medical record or research database, where applicable. Kidney function was assessed by estimated creatinine clearance (eClcr) calculated by the Cockroft-Gault equation. [32]

Population Pharmacokinetic Analysis

Pharmacokinetics of MPA, MPAG, and AcMPAG were evaluated using Non-linear Mixed Effect Modeling software (NONMEM Version 6.2.0, ICON Development Solutions, Ellicott City, MD). Initial visual inspection of semi-logarithmic plasma concentration-time plots for MPA, MPAG and AcMPAG demonstrated bi-exponential and mono-exponential decay patterns (Figure 1), consistent with a two-compartmental pharmacokinetic model for MPA and one-compartment pharmacokinetic models for MPAG and AcMPAG. Pharmacokinetic models were parameterized in terms of apparent clearances and volumes with the subroutines ADVAN6 TRANS1 and incorporated a gallbladder compartment to account for enterohepatic recycling of MPA via MPAG. The enterohepatic recycling process was modeled by introducing a rate constant describing the transfer from the MPAG central compartment to a gallbladder compartment. During gallbladder emptying, MPAG was transferred and converted back to the parent MPA in the depot compartment. Double precision and first-order conditional estimation (FOCE) were used. Inclusion of urine data allowed estimation of apparent renal clearance (CL_R/F) and apparent nonrenal clearance (CL_{NR}/F). Both MPAG and AcMPAG pharmacokinetics were modeled as a central metabolite compartment for plasma that was connected to the central MPA compartment. Each metabolite compartment had a non-reversible elimination pathway to a urine compartment, and an additional elimination pathway such as through enterohepatic recycling through the gallbladder compartment for MPAG.

Intersubject variability in structural model parameters was estimated by an exponential error model (Equation 1).

$$P_j = \theta \cdot e^{\eta j} \qquad (1)$$

Where P_j is the individual value for P in the j^{th} individual, θ is the population mean value of the pharmacokinetic parameter P (e.g. CL/F, V_c/F, etc), and η_j is a random error term (the difference between the typical value and individual value).

Residual variability $\varepsilon_{ij,k}$ (k=1, 2, 3, 4, 5, 6), which is the discrepancy between the individual observed ($C_{obs,\ ij}$) i^{th} plasma or urine concentration measured in the j^{th} individual for the MPA, MPAG, and AcMPAG and the respective individual model-predicted plasma or urine concentrations ($C_{pred,\ ij}$) in the natural logarithm domain and was modeled according to an additive error model (Equations 2-7).

$$\text{Ln}\ (C_{obs,\ ij}) = \text{Ln}\ (C_{pred,\ ij}) + \varepsilon_{ij,1} \text{ for MPA plasma} \quad (2)$$

$$\text{Ln}\ (C_{obs,\ ij}) = \text{Ln}\ (C_{pred,\ ij}) + \varepsilon_{ij,2} \text{ for MPAG plasma} \quad (3)$$

$$\text{Ln}\ (C_{obs,\ ij}) = \text{Ln}\ (C_{pred,\ ij}) + \varepsilon_{ij,3} \text{ for AcMPAG plasma} \quad (4)$$

$$\text{Ln}\ (C_{obs,\ ij}) = \text{Ln}\ (C_{pred,\ ij}) + \varepsilon_{ij,4} \text{ for MPA urine} \quad (5)$$

$$\text{Ln}\ (C_{obs,\ ij}) = \text{Ln}\ (C_{pred,\ ij}) + \varepsilon_{ij,5} \text{ for MPAG urine} \quad (6)$$

$$\text{Ln}\ (C_{obs,\ ij}) = \text{Ln}\ (C_{pred,\ ij}) + \varepsilon_{ij,6} \text{ for AcMPAG urine} \quad (7)$$

Random effect parameters η and ε were assumed to be symmetrically distributed with 0 mean and variances of ω^2 and σ^2, respectively. Different pharmacokinetic models were tested and the best structural model was chosen based on goodness-of-fit criteria including diagnostic plots, minimum objective function value (MOFV) after accounting for the number of fitted parameters, precision, and physiological plausibility of parameter estimates.

Covariate Model Building

Covariate models were created [33] to evaluate for the influence of patient demographics (age, weight, gender), clinical status (serum creatinine, eClcr, serum albumin, urinary protein to creatinine ratio), and genotypes (*UGT2B7 C802T*, *UGT1A7 T622C*, *MDR1 C3435T*, and *MDR1 C1236T*) on the pharmacokinetic parameters.

For continuous covariates (age, weight, serum creatinine, eClcr, serum albumin, urinary protein to creatinine ratio), Equation 8 was used:

$$P = \theta * (\text{covariate}/\text{median covariate})^{\theta_{covariate}} \quad (8)$$

where θ is the population mean value of P for a patient with the median covariate value and $\theta_{covariate}$ is the estimated effect for the covariate on P. For some continuous covariates which influenced P only below a critical cutoff value, the covariate model was modified as shown in Equations 9 and 10:

$$P = \theta_1 * (\text{covariate}/\text{median covariate})^{\theta_{covariate}} \text{ for covariate} \leq \text{cutoff value} \quad (9)$$

$$P = \theta_2 \quad \text{for covariate} > \text{cutoff value} \quad (10)$$

where θ_1 is the population mean value of P for a patient with the median covariate value below or equal to the cutoff value, $\theta_{covariate}$ is the estimated effect of the covariate on P below or equal to the cutoff value, and θ_2 is the population mean of P for a patient with a covariate value above the cutoff value. The critical cutoff values were determined graphically from the plots of the posthoc pharmacokinetic parameter estimates versus covariates.

For categorical covariates (race, gender, genotypes) on P was modeled according to Equations 11 and 12:

$$P = \theta \quad \text{for reference covariate} \quad (11)$$

$$P = \theta * \theta_{covariate} \quad \text{for investigated covariate} \quad (12)$$

where θ is the population mean value of P (e.g. CL/F, V_c/F, etc), $\theta_{covariate}$ is the estimated fractional change in θ for the investigated covariate. Likelihood ratio tests to compare hierarchical models were performed by comparing differences in MVOF between models to χ^2 distributions with degrees of freedom equal to the difference in the number of parameters A reduction in MVOF of >3.84 (1 degree of freedom) from the base or previous model to the current model was designated as statistically significant at $p<0.05$.

The incorporation of covariates in the final model was determined by stepwise forward addition followed by backward elimination. During forward addition, covariates at the $p < 0.05$ level were included in the model, and during backward elimination, covariates at the $p < 0.01$ level were retained in the model.

Predictive Ability

A visual predictive check was employed to evaluate the predictability of the model. One hundred data sets were simulated each for plasma and urine MPA, MPAG, and AcMPAG from the final model. The observed data were superimposed with the 2.5^{th}, 50^{th}, and 97.5^{th} percentiles of the simulated data calculated at each time point.

Results

The characteristics from the combined set of 39 lupus nephritis and ANCA-associated vasculitis patients are presented in Table 4.1. The patients were predominantly Caucasian (60%) and African-American (28%) race. A minimal to moderate level of kidney dysfunction was present; eClcr 91.3±45.7 mL/min and urinary protein to creatinine ratio 0.8±1.6, with conserved serum albumin (4.2±0.5 g/dL). Approximately 40% of patients were receiving double immunosuppressant therapy with glucocorticoids (31%) or cyclosporine (8%).

A full steady-state 12-hour plasma concentration vs time profile was generated for all 39 patients. The entire dataset produced a total of 444 MPA, 441 MPAG, and 362 AcMPAG plasma and a total of 130 MPA, 130 MPAG (n=130), and 71 AcMPAG urine concentrations. Figure 4.1 shows the observed steady state plasma concentration vs time profiles for MPA, MPAG, and AcMPAG after orally administered mycophenolate mofetil and demonstrate secondary peaks between 4 and 12 hours consistent with enterohepatic recycling of MPA.

Similar to the previous work of MPA disposition in kidney transplant recipients [1], a 2-compartment model with enterohepatic recycling, first-order absorption, and linear elimination was selected as the base model. While several patient plasma concentration time curves

demonstrated an absorption lag time, its inclusion into models resulted in a reduction of the MVOF but with the cost of decreased precision of other data parameters and was therefore not incorporated. Duration of gallbladder emptying was fixed at 0.01 hours. [4,8] Due to insufficient data collected around the secondary peak, the transfer rate constant of MPAG from the gallbladder to the depot compartments (k_{41}) was fixed at 67.5 hr^{-1} [8]. The final model parameters are presented in Table 4.2. Figure 4.2 is a schematic representation of the final model employing plasma and urine concentration data for MPA, MPAG, and AcMPAG.

The covariates were examined to determine their relationship with eta values for apparent Clr/F, apparent Clnr/F, and the central compartment volume (V_c/F). Stepwise forward addition identified the following significant covariate effects: eClcr on $CL_{NR,MPA}$/F (ΔMVOF=-19.602, P<0.001), eClcr (with a cut-off value at 80 ml/min/1.73m^2) on $CL_{R,MPA}$/F (ΔMVOF=-8.803, P<0.025), serum albumin on $CL_{NR,MPA}$/F (ΔMOF=-6.627, P<0.01), eClcr on $CL_{R,MPAG}$/F (ΔMVOF=-18.699, P<0.001) and eClcr on $CL_{R,AcMPAG}$/F (ΔMVOF=-11.033, P<0.001). All these covariates remained significant (p<0.01) during backward elimination.

Table 4.2 shows the population parameter estimates and covariate relationships for MPA, MPAG, and AcMPAG for the final model. In general, parameters were estimated with acceptable precision (7-53% relative standard error, %RSE). eClcr ≤80 mL/min had a covariate effect on $Cl_{R,MPA/F}$ (Equation 13).

$$CL_{R\ MPA\ creatinine\ clearance \le 80}\ /F\ L/hr = 0.0975\ L/hr * (eClcr/54.93)^{1.33} \quad (13)$$

For apparent CL_{NR}/F, eClcr had a positive effect, while serum albumin was found to have an inverse effect (Equation 14).

$$CL_{NR\ MPA}\ L/hr = 14.3\ L/hr * (eClcr/88.54)^{0.831}\ (albumin/4.2)^{-1.35} \quad (14)$$

For MPAG and AcMPAG, increased eClcr resulted in increased apparent CL_R/F for each respective metabolite. (Equations 15 and 16).

$$CL_{R\ MPAG}\ L/hr = 1.77\ L/hr * (eClcr/88.54)^{0.641} \quad (15)$$

$$CL_{R\ AcMPAG}\ L/hr = 1.75\ L/hr * (eClcr /88.54)^{1.00} \quad (16)$$

None of the *UGT2B7, UGT1A7,* and *MDR1* genotypes were found to be significant in the final model.

Model diagnostic plots for plasma MPA, MPAG and AcMPAG data are shown in Figures 4.3, 4.5 and 4.7, respectively. Model diagnostic plots for urine MPA, MPAG and AcMPAG data are shown in Figures 4.4, 4.6 and 4.8, respectively. These plots showed that our comprehensive models adequately described the data. The results of the visual predictive check evaluation for plasma and urine MPA, MPAG, and AcMPAG are presented in Figures 4.9 and 4.10, respectively. Most of the observations are contained within the 95th % prediction intervals. This analysis suggests that the final model provided an adequate fit to the data.

Table 4.3 demonstrates the predicted population values for MPA Cl/F, CL_R/F, and CL_{NR}/F in a glomerulonephritis population exhibiting selected values for eClcr and serum albumin that are clinically relevant.

Discussion

The current study reported a population pharmacokinetic analysis of MPA and its metabolites MPAG and AcMPAG, in a group of patients with glomerulonephritis secondary to SVV and SLE. This study was necessary to investigate the influence of patient-level characteristics including kidney function (e.g. eClcr) and kidney structure (urinary protein to creatinine ratio), and serum protein (serum albumin concentration) that are altered in glomerulonephritis. Additionally, demographic and genotype variables were investigated for their influence on MPA pharmacokinetics. The population approach enabled us to estimate mean pharmacokinetic parameters, inter-individual variability, residual variability, and covariate effects. As opposed to kidney transplant recipients, little is known about pharmacokinetic variability of MPA in glomerulonephritis, despite being used off-label for this indication for almost a decade.

As compared to the previous study in kidney transplant patients, the glomerulonephritis population had higher population mean (%RSE) absorption rate constant (Ka) [1.16 hr^{-1} (15.2%) vs 0.67 hr^{-1} (24.8%)], higher apparent intercompartmental clearance (Q/F) [23.4 L/hr (16.4%) vs 8.11 L/hr (24.2%)], and lower V_C/F. [21.1 L (34.1%) vs 25.9 L (34.9%)]. [1] In the current study, we evaluated mycophenolate mofetil versus mycophenolate sodium, [1] which may have accounted for variability in Ka. Other MPA population models in kidney transplant patients have reported population mean Ka estimates that range from 2.27 hr^{-1} to 4.1 hr^{-1} [5,7,34], which are also greater than the current estimate. Additionally, mean population estimates for V_C/F from 10.3 to 97.7 L [4,5,7,35] , consistent range with the current study. The population mean (%RSE) V_P/F was substantially higher in the current study over what was previously reported [1240 L (23.4%) vs 39.6 L (86.9%)] in kidney transplant patients, suggesting a larger degree of uncertainty with this estimate. [1] In the current study, we collected urine samples, which enabled estimation of the Cl_R/F component to apparent oral clearance (CL/F). Two population mean Cl_R/F estimates for MPA were provided based on two categories of eClcr; levels ≤80 mL/min and > 80 mL/min. The Cl_R/F estimate was nearly 2-fold higher in patients with eClcr values of > 80 mL/min versus ≤80 mL/min. Apparent renal clearance estimates of MPAG and AcMPAG were an order of magnitude greater than MPA estimates. As would be expected, the Cl_{NR}/F estimate (%RSE) for MPA was significantly greater [14.3 L/hr (8.04%)] than the Cl_R/F estimates [0.0975 L/hr (20.8%) and 0.157 L/hr (20.5%)] as MPA is primarily metabolized by the liver. The previously published MPA pharmacokinetic models did not measure urine concentrations and hence did not provide estimates for the Cl_R/F. Previous studies have reported ranges for MPA CL/F of 11.9 L/hr to 33 L/hr. [4,5,7,35] A recent publication in 38 patients with glomerulonephritis receiving mycophenolate mofetil reported higher mean (%RSE) V_C/F [52.4 L (17%)], higher Ka [6.2 hr^{-1} (22%)], lower V_P/F [262 L (5%)], and lower Q/F [16.2 L/hr (22%)] than our current study. [30] Differences between the glomerulonephritis populations were a higher percentage of females, more diverse racial

make-up, higher kidney function (eClcr), and a lower percentage of patients on concomitant glucocorticoids in the current versus earlier study.

The volumes of the central metabolite compartments are not uniquely identifiable in this analysis. A recent study, however, estimated the central MPAG compartment apparent volume as 4.4 L. [8] If we make the same assumption for our MPAG compartment volume, the percentage of MPAG clearance that underwent recycling through the gallbladder in glomerulonephritis patients would be estimated as 17.9%. A previous model had gallbladder being filled continuously from the central compartment, but many parameters in the model were required to be fixed secondary to insufficient data collection surrounding the occurrence of recycling. [30] The percentage of MPA clearance that underwent recycling through gallbladder was fixed at 37%, and this recycling was attributed solely to the parent MPA. [30] Another study reported that 29.1% of total absorbed MPA was recycled from MPAG. [8] The current model shows that AcMPAG undergoes rapid reversible interconversion with the parent MPA in plasma. This is consistent with recent animal data from our laboratory which suggests that AcMPAG is actually cleaved to MPA by nonspecific esterases within the liver (data not shown). This is in contrast to MPAG, which is thought to be cleaved by β-glucuronidases in the intestine. [36]

In the current study, the final structural model that fit the MPA pharmacokinetic data obtained from the glomerulonephritis patients consisted of nine compartments. This model is slightly more complex than the six compartment model we previously employed in kidney transplant recipients. [1] The higher complexity mainly resulted from the incorporation of urine compartments for MPA, MPAG, and AcMPAG. The previously published model in patients with glomerulonephritis was different than our current and previous models as it did not employ MPAG or AcMPAG plasma compartments, had two separate absorption compartments representing a short and lag time, exhibited a different gallbladder component, and did not incorporate urine compartments. [30] Other published structural models for MPA in renal

transplant patients include a 4-compartment model with a gastrointestinal compartment [3,4], and a 5-compartment model with incorporation of a gallbladder compartment. [8]

It is known that there is a large degree of interpatient variability in the pharmacokinetic parameters of MPA in kidney transplant patients. [19] Studies have reported CL/F interpatient variability in the range of 28% to 41% [5,6] Interpatient variability in the V_C/F has been reported to range from 18% to 87.8% in other studies. [1,3] In this study, estimated interpatient variability for MPA pharmacokinetic parameters (%RSE) were as follow: $CL_{R,MPA}$/F [72.5% (30.9%)], $CL_{NR,MPA}$/F [39.7% (19.1%)], and V_c/F [143% (50.7%)]. This large variability supports the therapeutic monitoring of MPA in patients with glomerulonephritis. Residual error analysis for plasma data demonstrated the greatest error [standard deviation (%RSE)] in MPA [1.81 µg/mL (18.7%)] followed by AcMPAG [1.54 µg/mL (7.83%)] and MPAG [1.50 µg/mL (7.54%)]. Residual error analysis for urine data demonstrated the greatest error in MPA [2.49 µg/mL (16.6%)] followed by MPAG [1.77 µg/mL (14.0%)] and AcMPAG [1.31 µg/mL (24.6%)]. The reasonable residual error estimates likely were reflective of the sensitive assay methods used.

Covariate modeling demonstrated a significant effect of eClcr on increasing MPA Cl_R/F (covariate coefficient 1.33) in patients with eClcr values of ≤80 mL/min and increasing MPA Cl_{NR}/F (covariate coefficient 0.831). As demonstrated in Table 4.3, a glomerulonephritis patient with eClcr of 60 mL/min would have a 3-fold higher MPA Cl_R/F than a patient with an eClcr of 30 mL/min (0.11 L/hr vs 0.04 L/hr). Serum albumin concentrations were also found to influence Cl_{NR}/F, with decreased serum albumin resulting in increased Cl_{NR}/F (covariate coefficient -1.35). An increase in eClcr from 30 to 60 mL/min in the presence of a normal serum albumin (4.4 g/dL) would double the calculated Cl_{NR}/F from 5.5 to 9.7 L/hr. For the same increase in eClcr, patients with reduced serum albumin to 2.5 g/dL would have a doubling of the calculated CL_{NR}/F, above that demonstrated at each level of eClcr in patients with normal serum albumin concentrations (from 11.7 to 21 L/hr). Regarding effects on $AUC_{0\text{-}tau}$; for the group of patients with normal serum albumin concentrations, the $AUC_{0\text{-}tau}$ decreased from eClcr values of 30 to

120 mL/min (131 to 41.4 mg hr/L). When these same patients also had serum albumin values reduced to 2.5 g/dL, the $AUC_{0\text{-}tau}$ was reduced another 2-fold at each level of eClcr (from 61.2 to 19.4 mg hr/L). While MPA $AUC_{0\text{-}tau}$ targets are not defined for glomerulonephritis, if one were to target the $AUC_{0\text{-}tau}$ values suggested for renal transplant recipients (30 to 60 mg hr/L) as a starting point for therapy, the covariate effects would result in many patients either above or below MPA $AUC_{0\text{-}tau}$ targets. In particular, patients with low serum albumin and patients with low eClcr would be at highest risk. As unbound MPA $AUC_{0\text{-}tau}$ may be more relevant to target in patients with these clinical manifestations, future assessments should address these unbound targets. However, the relative contribution of unbound levels to efficacy versus availability for elimination would dictate the relevance of unbound levels. Creatinine clearance was also found to positively influence the Cl_R/F of MPA's metabolites (MPAG and AcMPAG) as well. Glomerulonephritis patients with decreased eClcr would be expected to have at least transiently increased AcMPAG and MPAG concentrations prior to any recycling processes. Overall, increased metabolism could result in increased MPA exposure through recycling.

Creatinine clearance has been reported as a significant covariate in MPA Cl/F in previous population pharmacokinetic models. [3,5] van Hest, et al, reported increased Cl/F of MPA with reduced values of eClcr in a kidney transplant population using a population pharmacokinetic model of MPA which does not incorporate enterohepatic recycling of MPA since all the patients were on concomitant cyclosporine, which is known to inhibit biliary excretion of MPA. [5] However, since urinary concentrations of MPA were not obtained, estimation of a Cl_R/F and Cl_{NR}/F were not feasible. The previous authors surmised that kidney disease results in reductions in protein binding of MPA to serum albumin secondary to uremic competitors as well as MPAG accumulation secondary to loss of kidney function; both factors contributing to increased Cl/F. [5] In contrast, de Winter et al reported a positive correlation of eClcr and MPA Cl/F in patients with autoimmune disease. [30] The authors attributed the difference in correlation by the concomitant cyclosporine, which inhibits the enterohepatic recycling of MPA via MPAG.

Decreased kidney function will lead to reduced renal clearance of MPAG and increased biliary excretion. As a result, more MPAG will undergo enterohepatic recirculation and conversion back to MPA. Our kidney disease model, e.g. glomerulonephritis, would be predicted to result in reductions in protein binding of MPA secondary to reductions in serum albumin due to kidney losses and also due to accumulation of metabolites (MPAG and AcMPAG) secondary to the loss of kidney function. Since we measured MPA and metabolites in the urine, the Cl_R/F component could be estimated and the Cl_{NR}/F component could also be calculated. Our results suggest that the Cl_{NR}/F component of MPA Cl/F is influenced to a greater extent than Cl_R/F in patients with glomerulonephritis. Studies into the influences of the systemic diseases that result in glomerulonephritis on phase II drug metabolizing processes may elucidate the role of serum albumin versus alterations in *UGTs* on nonrenal clearance.

Conclusions

This study reported a population pharmacokinetic model for MPA and its glucuronide metabolites in patients with glomerulonephritis secondary to SLE and SVV. Unlike previous models of MPA pharmacokinetics, our model was developed with extensive plasma and urine sample collections from a well-defined population of patients. The resulting parameter estimates were considerably different than those obtained in many of the previous publications of kidney transplant patients receiving MPA. Two covariates, eClcr and serum albumin, influenced the renal and nonrenal components to apparent clearance. The clinical relevance of the current study can be realized when using the population parameters to simulate $AUC_{0\text{-}tau}$ values under scenarios of altered creatinine clearance and/or altered serum albumin. We demonstrated that patients with glomerulonephritis would have highly altered MPA exposures when one includes assessment of covariates on renal and nonrenal apparent clearance estimates. Future work will elucidate unbound exposures and relevance to efficacy, toxicity, and metabolic pathways.

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Table 4.1

Study Patient Characteristics (n=39)

Age (years)	46.9±14.8
Weight (kg)	85.7±20.9
Gender (male/female)	11/28
Race n (%)	
Caucasian	23 (59)
African-American	11 (28)
American-Indian	2 (5)
Asian	2 (5)
Other	1 (3)
Serum albumin (g/dL)	4.2±0.5
Serum creatinine (mg/dL)	1.3±0.7
Estimated Creatinine Clearance (mL/min)[a]	91.3±45.7
Urinary protein to creatinine ratio	0.8±1.6
Concomitant glucocorticoids (%)	12 (31)
Concomitant cyclosporine (%)	3 (8)
Mycophenolate mofetil dose (mg)	827±325

a – estimated by Cockroft and Gault equation [32]

Data presented as mean±standard deviation

Table 4.2. Final parameter estimates of the population modeling

Model parameter (units)	Estimate (%RSE)	Interindividual variability, CV% (%RSE)
Mycophenolic Acid (MPA)		
Fixed effects		
ka (hr^{-1})	1.16 (15.2)	
$CL_{R, MPA}$/F [eCLCR ≤ 80 mL/min] (L/hr)	0.0975 (20.8)	72.5 (30.9)
$CL_{R, MPA}$/F [eCLCR > 80 mL/min] (L/hr)	0.157 (20.5)	72.5 (30.9)
$CL_{NR, MPA}$/F (L/hr)	14.3 (8.04)	39.7 (19.1)
V_C/F (L)	21.1 (34.1)	143 (50.7)
V_P/F (L)	1240 (23.4)	
Q/F (L/hr)	23.4 (16.4)	
Covariate coefficient		
Effect of creatinine clearance on $CL_{R, MPA}$/F [eCLCR ≤ 80 mL/min], eCRCL_ $CL_{R, MPA}$/F [a]	1.33 (33.2)	
Effect of creatinine clearance on $CL_{NR, MPA}$/F, eCRCL_ $CL_{NR, MPA}$/F [b]	0.831 (18.5)	
Effect of albumin on $CL_{NR, MPA}$/F, ALB_ $CL_{NR, MPA}$/F [c]	-1.35 (31.5)	
Residual error estimates (standard deviation)		
MPA, plasma (μg/mL)	1.81 (18.7)	
MPA, urine (μg/mL)	2.49 (16.6)	
Mycophenolic Acid Glucuronide (MPAG)		
Fixed effects		
$CL_{R, MPAG}$/F (L/hr)	1.77 (12.7)	71.8 (25.4)
FM1*	0.271 (14.9)	72.7 (37.7)
k_{84} (hr^{-1})	0.0878 (53.2)	
Covariate coefficient		
Effect of creatinine clearance on $CL_{R, MPAG}$/F, eCRCL_ $CL_{R, MPAG}$/F [d]	0.641 (37.0)	
Residual error estimates (standard deviation)		
MPAG, plasma (μg/mL)	1.50 (7.54)	
MPAG, urine (μg/mL)	1.77 (14.0)	
Acyl-mycophenolic acid glucuronide (AcMPAG)		
Fixed effects		
$CL_{R, AcMPAG}$/F (L/hr)	1.75(18.2)	95.9 (29.5)
FM2*	0.0142 (24.4)	80.4 (25.9)

k_{102} (hr^{-1})	1.63 (40.3)	
Covariate coefficient Effect of creatinine clearance on $CL_{R, AcMPAG}/F$, eCRCL_ $CL_{R, AcMPAG}/F$ [e]	1.00 (31.4)	
Residual error estimates (standard deviation) AcMPAG, plasma (μg/mL) AcMPAG, urine (μg/mL)	 1.54 (7.83) 1.31 (24.6)	

Abbreviations:

coefficient of variation, CV; estimated creatinine clearance, eClcr; percent relative standard error, % RSE; absorption rate constant, k_a; apparent renal clearance of MPA, $CL_{R, MPA}/F$; apparent non-renal clearance of MPA, $CL_{NR, MPA}/F$; apparent volume of central compartment, V_C/F; apparent volume of peripheral compartment, V_P/F; apparent renal clearance of MPAG, $CL_{R, MPAG}/F$; apparent renal clearance of AcMPAG, $CL_{R, AcMPAG}/F$; ratio of fraction of MPA metabolized to MPAG to volume of distribution of MPAG, FM1*; ratio of fraction of MPA metabolized to AcMPAG to volume of distribution of AcMPAG, FM2*; rate constant for the transfer of MPAG from central to gall bladder compartment, k_{84}; rate constant for the transfer of AcMPAG from central to MPA central compartment, k_{102};

[a] $CL_{R, MPA}/F_{individual} = CL_{R, MPA}/F\ [(eCRCL/54.93)^{CRCL\_CLR,MPA}] \times EXP(\eta\ CL_{R, MPA}/F)$;

[b] $CL_{NR, MPA}/F_{individual} = CL_{NR, MPA}/F\ [(eCRCL/88.54)^{CRCL\_CLNR,MPA}] \times EXP(\eta\ CL_{NR, MPA}/F)$;

[c] $CL_{NR, MPA}/F_{individual} = CL_{NR, MPA}/F\ [(ALB/4.2)^{ALB\_CLNR,MPA}] \times EXP(\eta\ CL_{NR, MPA}/F)$;

[d] $CL_{R, MPAG}/F_{individual} = CL_{R, MPAG}/F\ [(eCLCR/88.54)^{CLCR\_CLR,MPAG}] \times EXP(\eta\ CL_{R, MPAG}/F)$;

[e] $CL_{R, AcMPAG}/F_{individual} = CL_{R, AcMPAG}/F\ [(eCLCR/88.54)^{CLCR\_CLR,AcMPAG}] \times EXP(\eta\ CL_{R, AcMPAG}/F)$

Table 4.3

Glomerulonephritis Patient Predictions for Mycophenolic Acid Apparent Clearance Terms Based on Creatinine Clearance and Serum Albumin

	CL_{NR}/F (L/hr)	CL_R/F (L/hr)	CL/F[a] (L/hr)	$AUC_{0\text{-}Tau}$[b] (mg hr/L)
Creatinine Clearance [c]				
30 mL/min	5.46	0.04	5.5	131
60 mL/min	9.72	0.11	9.83	73.2
120 mL/min	17.3	0.16	17.5	41.3
Creatinine Clearance [d]				
30 mL/min	11.7	0.04	11.8	61.2
60 mL/min	20.8	0.11	20.9	34.4
120 mL/min	37.0	0.16	37.2	19.4
Serum Albumin [e]				
2.0 g/dL	50.1	0.16	50.2	14.3
3.0 g/dL	28.9	0.16	29.1	24.8
4.4 g/dL	17.9	0.16	18.1	39.9

Serum Albumin [f]				
2.0 g/dL	15.8	0.04	15.9	45.4
3.0 g/dL	9.14	0.04	9.18	78.4
4.4 g/dL	5.46	0.04	5.50	131

a: $CL/F = CL_R/F + CL_{NR}/F$

b: 1000 mg mycophenolate mofetil dose is 720 mg mycophenolic acid dose

c: serum albumin 4.4 g/dL

d: serum albumin 2.5 g/dL

e: creatinine clearance 120 mL/min

f: creatinine clearance 30 mL/min

Creatinine clearance estimated by the Cockroft and Gault equation [32]

Abbreviations

$AUC_{0\text{-}tau}$ – area under the plasma concentration time curve during a dosing interval

Cl/F – apparent total oral clearance

CL_{NR}/F – apparent nonrenal clearance

Cl_R/F – apparent renal clearance

Figure Legends

Figure 4.1. Observed Plasma Concentration Versus Time After Dose. Figure shows observed plasma concentration versus time after dose for a). mycophenolic acid (MPA), b). mycophenolic acid glucuronide (MPAG), and c). acyl-mycophenolic acid glucuronide (AcMPAG).

Figure 4.2. Final Compartment Model for Mycophenolic Acid (MPA), Mycophenolic Acid Glucuronide (MPAG), and Acyl-mycophenolic Acid Glucuronide (AcMPAG) Plasma and Urine Data. Abbreviations: mycophenolic acid, MPA; mycophenolic acid glucuronide, MPAG; acylmycophenolic acid glucuronide, AcMPAG; absorption rate constant, k_a; apparent renal clearance of MPA, $CL_{R,\ MPA}/F$; apparent non-renal clearance of MPA, $CL_{NR,\ MPA}/F$; compartment, CMT; apparent volume of central compartment, V_C/F; apparent volume of peripheral compartment, V_P/F; apparent renal clearance of MPAG, $CL_{R,\ MPAG}/F$; apparent renal clearance of AcMPAG, $CL_{R,\ AcMPAG}/F$; ratio of fraction of MPA metabolized to MPAG to volume of distribution of MPAG, FM1*; ratio of fraction of MPA metabolized to AcMPAG to volume of distribution of AcMPAG, FM2*; rate constant for the transfer of MPAG from central to gall bladder compartment, k_{84}; rate constant for the transfer of AcMPAG from central to MPA central compartment, k_{102}; rate constant for the transfer of MPAG from gallbladder to depot; k_{41}.

Figure 4.3. Mycophenolic Acid in Plasma Goodness-of-Fit Plots. (Upper left and right panels) Natural logarithmic-transformed population and individual predicted plasma mycophenolic acid (MPA) concentration *vs* natural logarithmic-transformed observed plasma MPA concentration. (Lower left and right panels) Natural logarithmic-transformed population predicted plasma MPA concentration and time after dose *vs* weighted residuals (WRES).

Figure 4.4. Mycophenolic Acid in Urine Goodness-of-Fit Plots. (Upper left and right panels) Natural logarithmic-transformed population and individual predicted urine mycophenolic acid (MPA) concentration *vs* natural logarithmic-transformed observed urine MPA concentration.

(Lower left and right panels) Natural logarithmic-transformed population predicted urine MPA concentration and time after dose *vs* weighted residuals (WRES).

Figure 4.5. Mycophenolic Acid Glucuronide in Plasma Goodness-of-Fit Plots. (Upper left and right panels) Natural logarithmic-transformed population and individual predicted plasma mycophenolic acid glucuronide (MPAG) concentration *vs* natural logarithmic-transformed observed plasma MPAG concentration. (Lower left and right panels) Natural logarithmic-transformed population predicted plasma MPAG concentration and time after dose *vs* weighted residuals (WRES).

Figure 4.6. Mycophenolic Acid Glucuronide in Urine Goodness-of-Fit Plots. (Upper left and right panels) Natural logarithmic-transformed population and individual predicted urine mycophenolic acid glucuronide (MPAG) concentration *vs* natural logarithmic-transformed observed urine MPAG concentration. (Lower left and right panels) Natural logarithmic-transformed population predicted urine MPAG concentration and time after dose *vs* weighted residuals (WRES).

Figure 4.7. Acyl-Mycophenolic Acid Glucuronide in Plasma Goodness-of-Fit Plots. (Upper left and right panels) Natural logarithmic-transformed population and individual predicted plasma mycophenolic acid acyl glucuronide (AcMPAG) concentration *vs* natural logarithmic-transformed observed plasma AcMPAG concentration. (Lower left and right panels) Natural logarithmic-transformed population predicted plasma AcMPAG concentration and time after dose *vs* weighted residuals (WRES).

Figure 4.8. Acyl-Mycophenolic Acid Glucuronide in Urine Goodness-of-Fit Plots. (Upper left and right panels) Natural logarithmic-transformed population and individual predicted urine mycophenolic acid acyl glucuronide (AcMPAG) concentration *vs* natural logarithmic-transformed observed urine AcMPAG concentration. (Lower left and right panels) Natural logarithmic-transformed population predicted urine AcMPAG concentration and time after dose *vs* weighted residuals (WRES).

Figure 4.9. Visual Predictive Check for Plasma A) MPA, B) MPAG and C) AcMPAG.

Observed data (●) compared to the 97.5th (upper dotted line), 50th (middle solid line) and 2.5th (lower dotted line) percentiles of the simulated (100) data sets.

Figure 4.10. Visual Predictive Check for Urine A) MPA, B) MPAG and C) AcMPAG.

Observed data (●) compared to the 97.5th (upper dotted line), 50th (middle solid line) and 2.5th (lower dotted line) percentiles of the simulated (100) data sets.

Figure 4.1

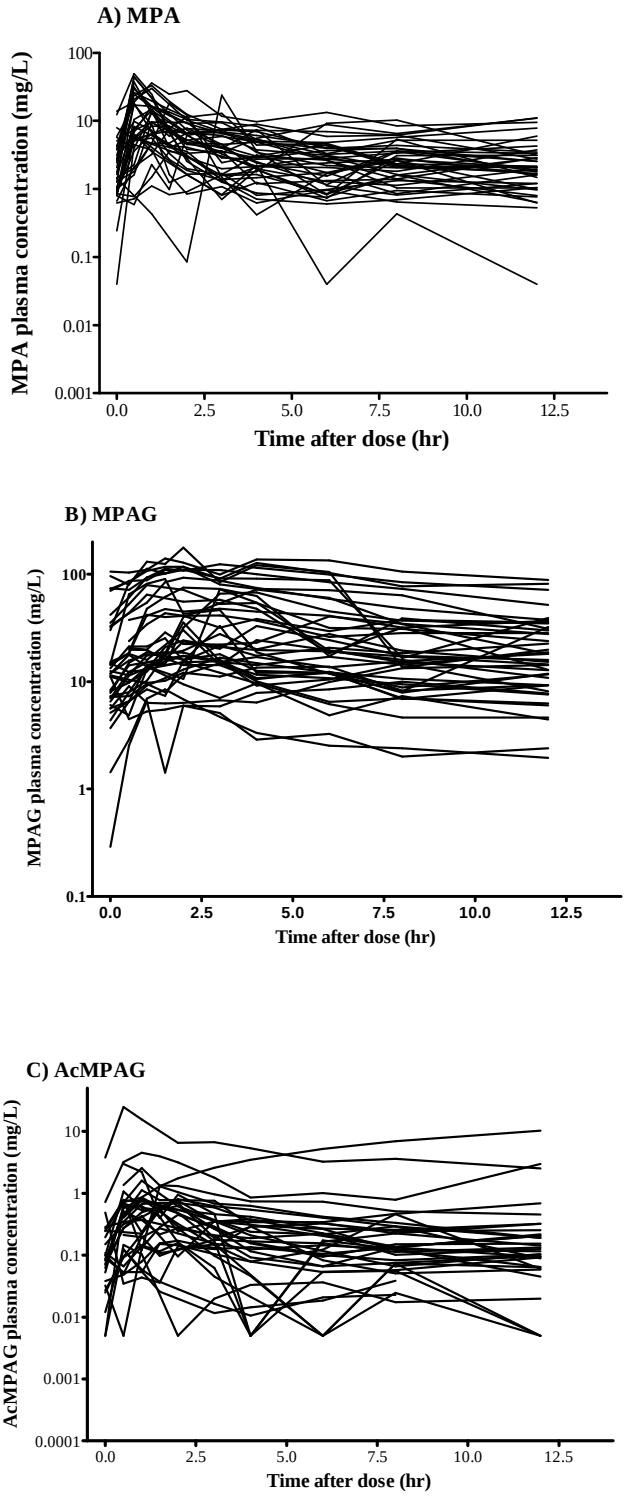
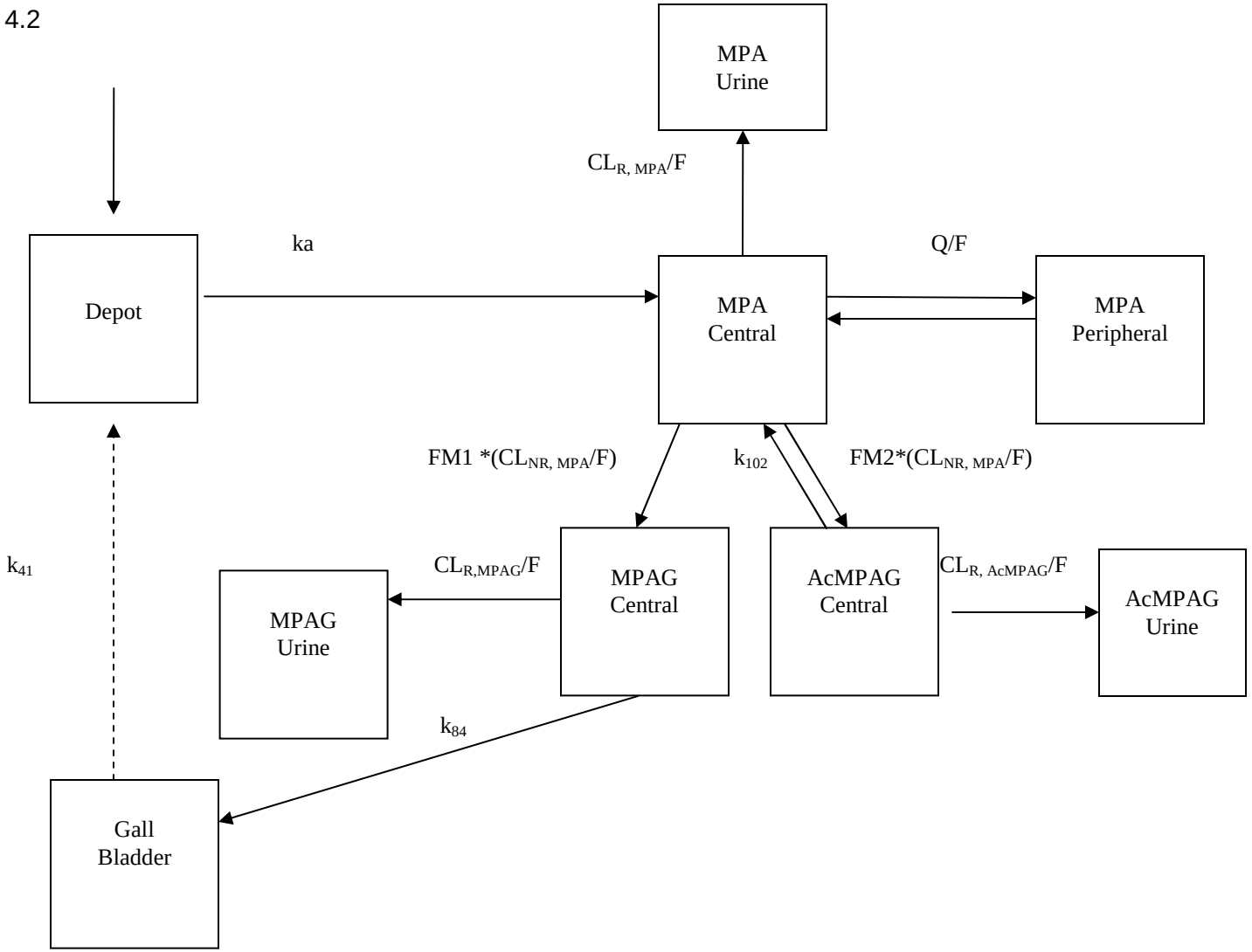

A) MPA
MPA plasma concentration (mg/L)
100
10
1
0.1
0.01
0.001
0.0
2.5
5.0
7.5
10.0
12.5
Time after dose (hr)
B) MPAG
MPAG plasma concentration (mg/L)
100
10
1
0.1
0.0
2.5
5.0
7.5
10.0
12.5
Time after dose (hr)
C) AcMPAG
AcMPAG plasma concentration (mg/L)
10
1
0.1
0.01
0.001
0.0001
0.0
2.5
5.0
7.5
10.0
12.5
Time after dose (hr)

Figure 4.2


MPA
Urine
$CL_{R, MPA}/F$
ka
Q/F
Depot
MPA
Central
MPA
Peripheral
FM1 *($CL_{NR, MPA}$/F)
k_{102}
FM2*($CL_{NR, MPA}$/F)
k_{41}
$CL_{R,MPAG}/F$
MPAG
Central
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$CL_{R, AcMPAG}/F$
AcMPAG
Urine
MPAG
Urine
k_{84}
Gall
Bladder

Figure 4.3

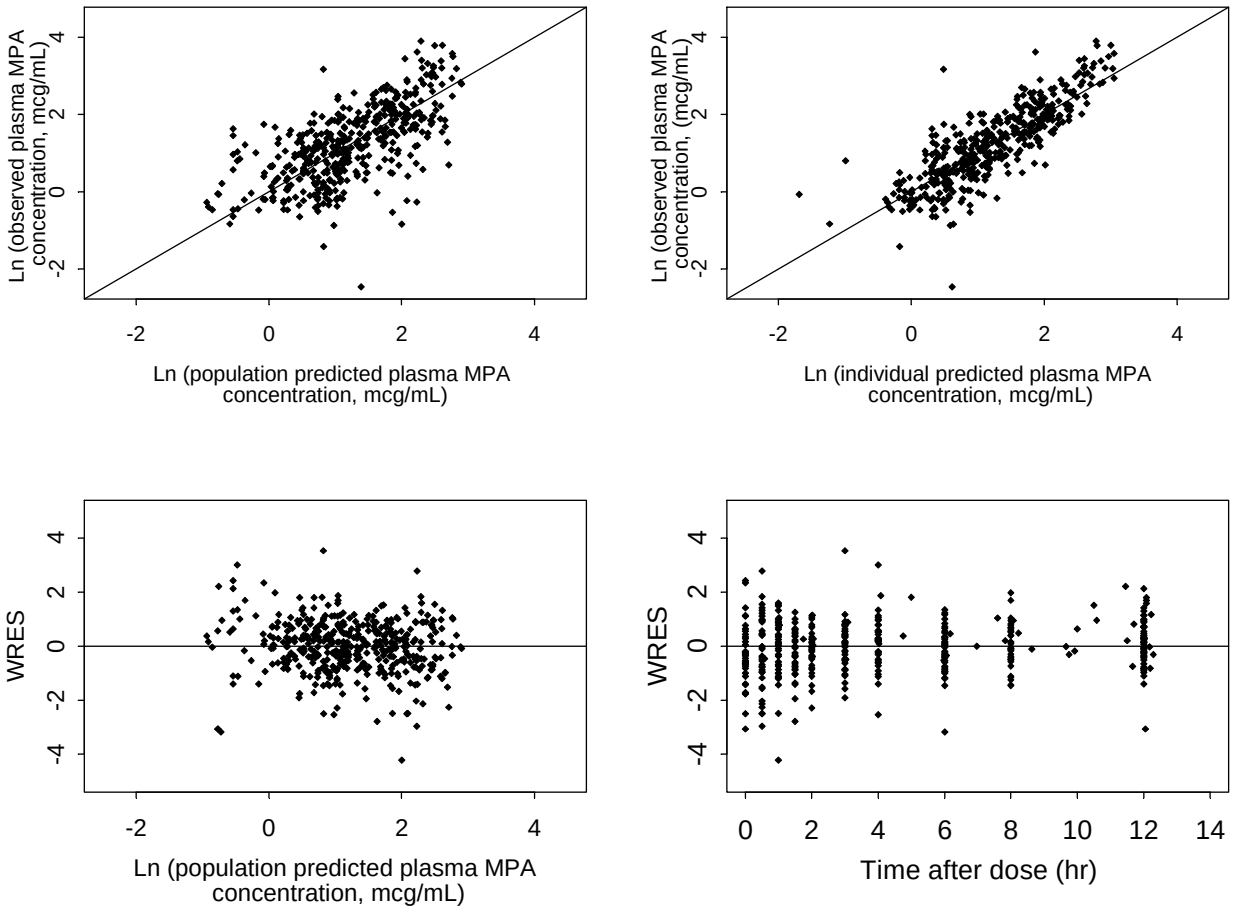

Ln (observed plasma MPA concentration, mcg/mL)
Ln (population predicted plasma MPA concentration, mcg/mL)
Ln (observed plasma MPA concentration, (mcg/mL)
Ln (individual predicted plasma MPA concentration, mcg/mL)
WRES
Ln (population predicted plasma MPA concentration, mcg/mL)
WRES
Time after dose (hr)

Figure 4.4

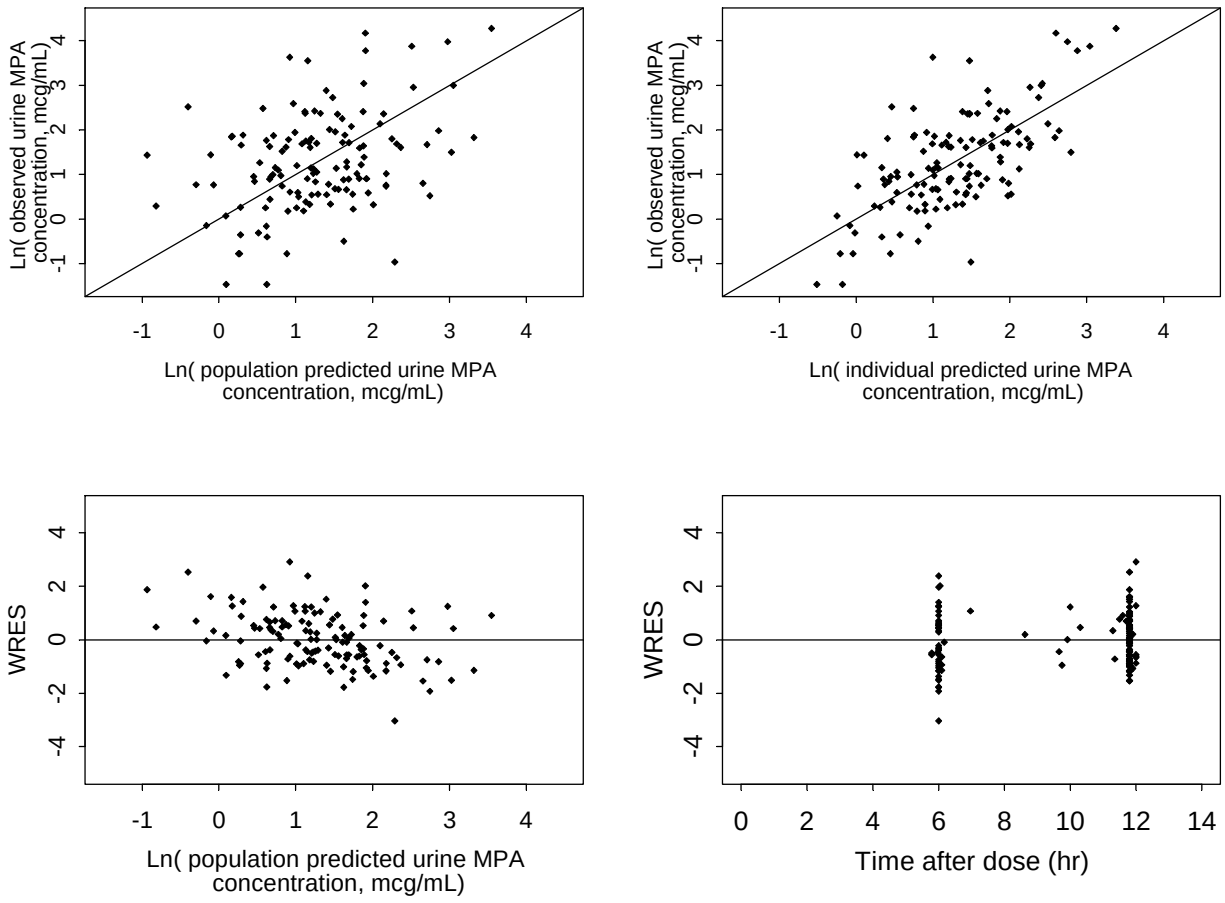

Ln(observed urine MPA concentration, mcg/mL)
Ln(population predicted urine MPA concentration, mcg/mL)
Ln(observed urine MPA concentration, mcg/mL)
Ln(individual predicted urine MPA concentration, mcg/mL)
WRES
Ln(population predicted urine MPA concentration, mcg/mL)
WRES
Time after dose (hr)

Figure 4.5

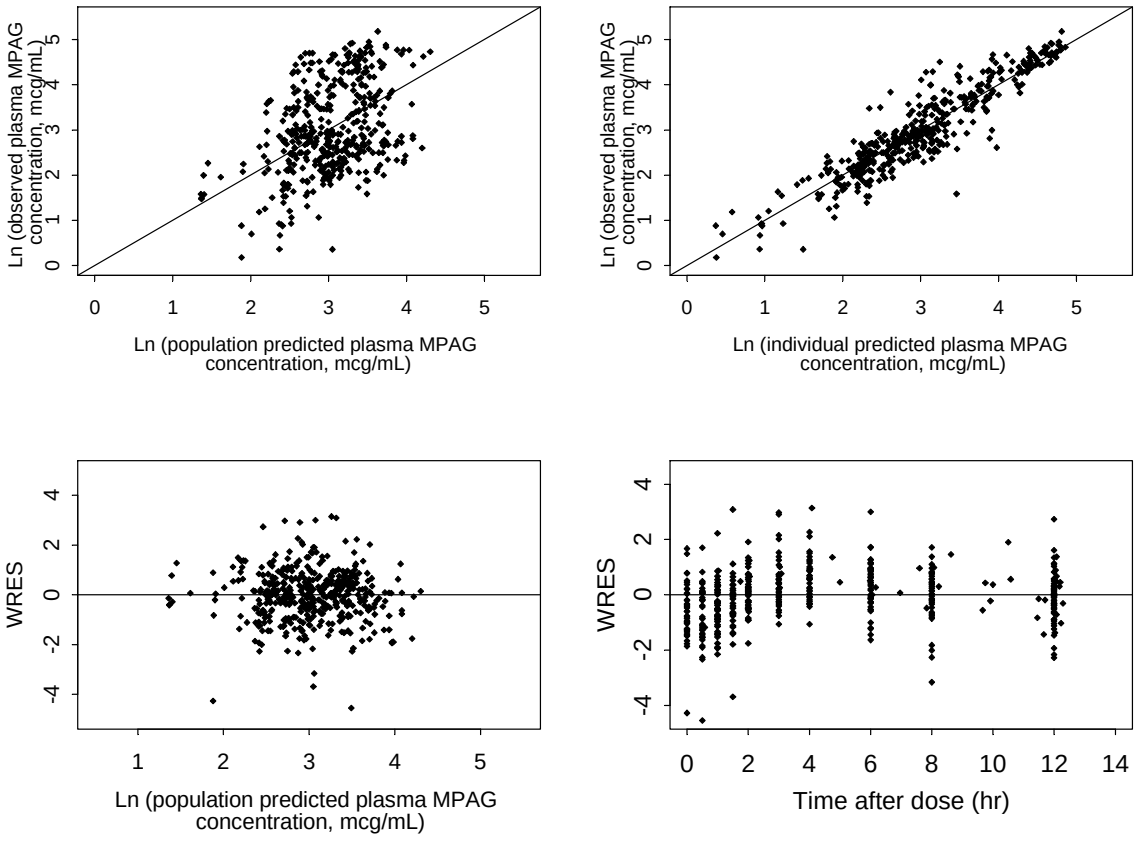

Ln (observed plasma MPAG concentration, mcg/mL)
Ln (population predicted plasma MPAG concentration, mcg/mL)
Ln (observed plasma MPAG concentration, mcg/mL)
Ln (individual predicted plasma MPAG concentration, mcg/mL)
WRES
Ln (population predicted plasma MPAG concentration, mcg/mL)
WRES
Time after dose (hr)

Figure 4.6

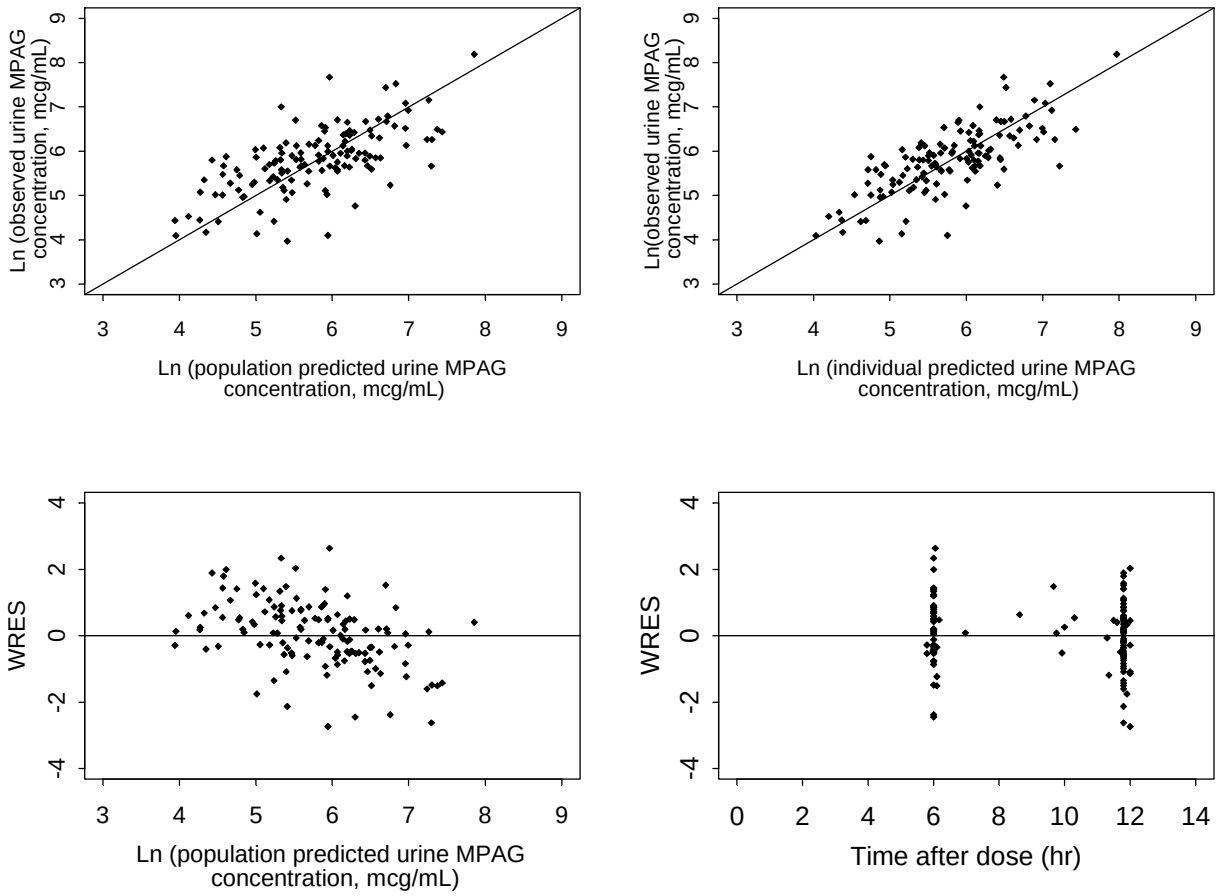

Ln (observed urine MPAG concentration, mcg/mL)
Ln (population predicted urine MPAG concentration, mcg/mL)
Ln(observed urine MPAG concentration, mcg/mL)
Ln (individual predicted urine MPAG concentration, mcg/mL)
WRES
Ln (population predicted urine MPAG concentration, mcg/mL)
WRES
Time after dose (hr)

Figure 4.7

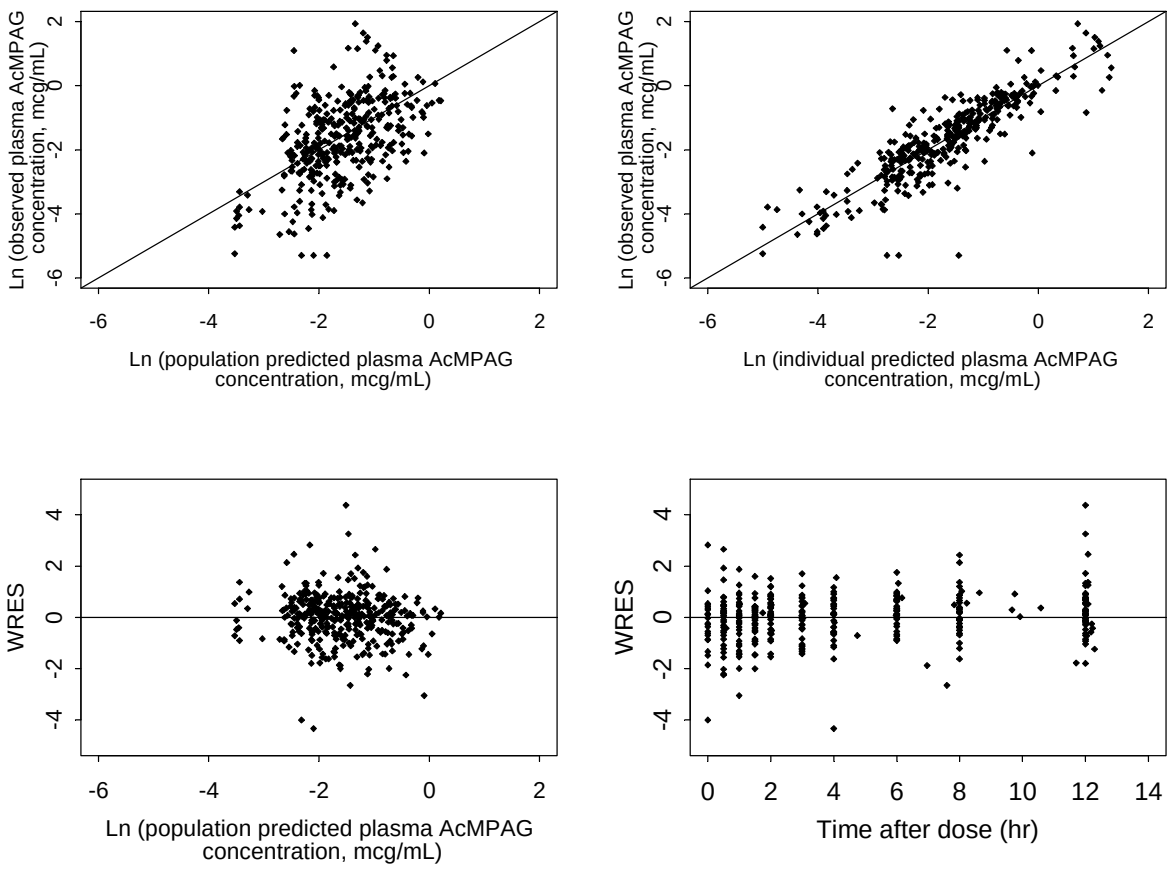

Ln (observed plasma AcMPAG concentration, mcg/mL)
Ln (population predicted plasma AcMPAG concentration, mcg/mL)
Ln (observed plasma AcMPAG concentration, mcg/mL)
Ln (individual predicted plasma AcMPAG concentration, mcg/mL)
WRES
Ln (population predicted plasma AcMPAG concentration, mcg/mL)
WRES
Time after dose (hr)

Figure 4.8

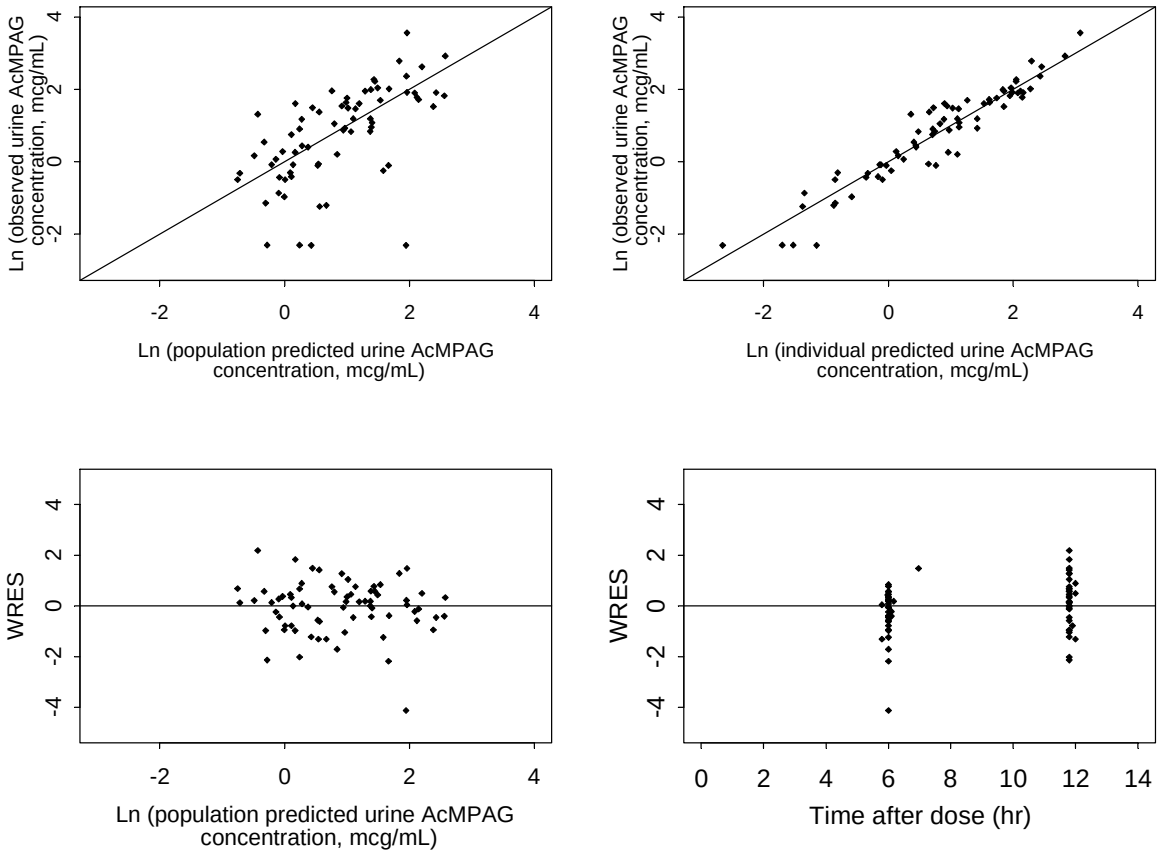

Ln (observed urine AcMPAG concentration, mcg/mL)
Ln (population predicted urine AcMPAG concentration, mcg/mL)
Ln (observed urine AcMPAG concentration, mcg/mL)
Ln (individual predicted urine AcMPAG concentration, mcg/mL)
WRES
Ln (population predicted urine AcMPAG concentration, mcg/mL)
WRES
Time after dose (hr)

Figure 4.9

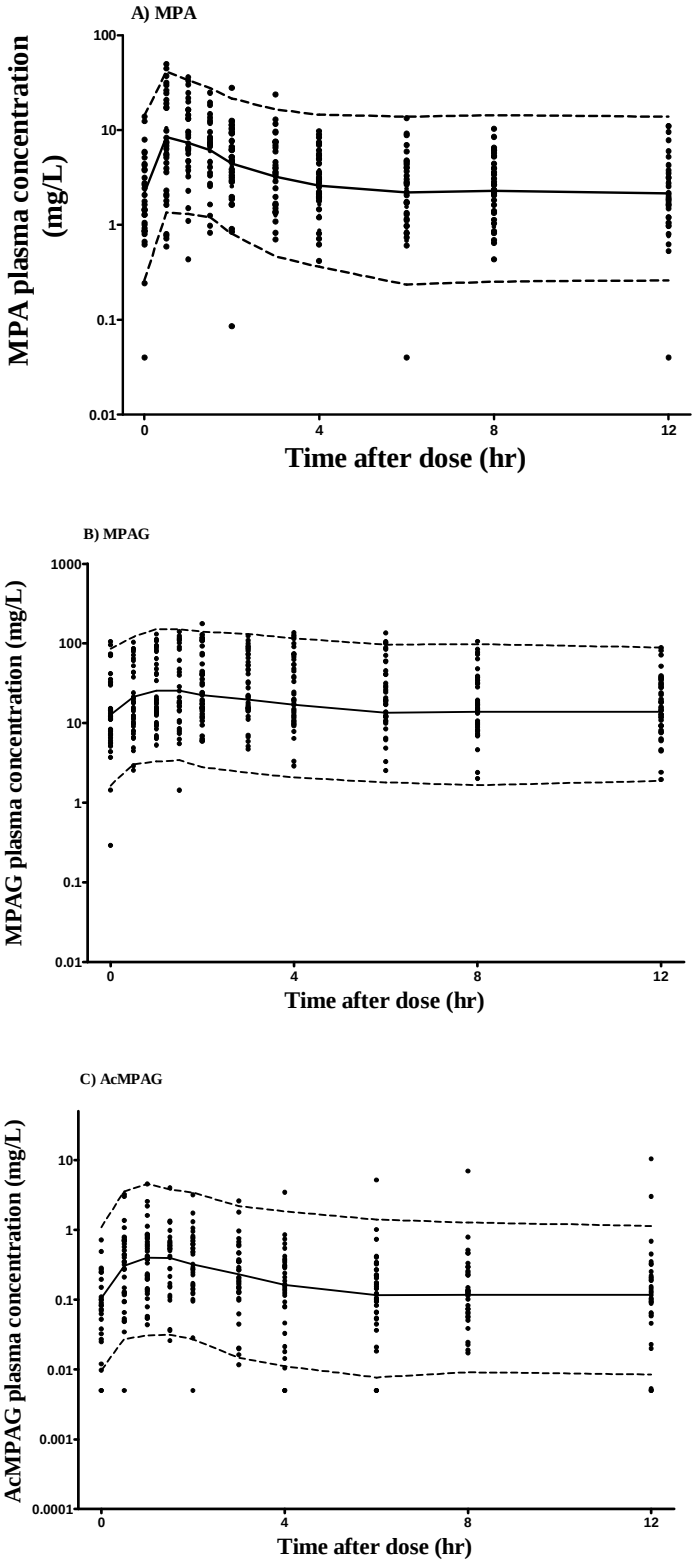
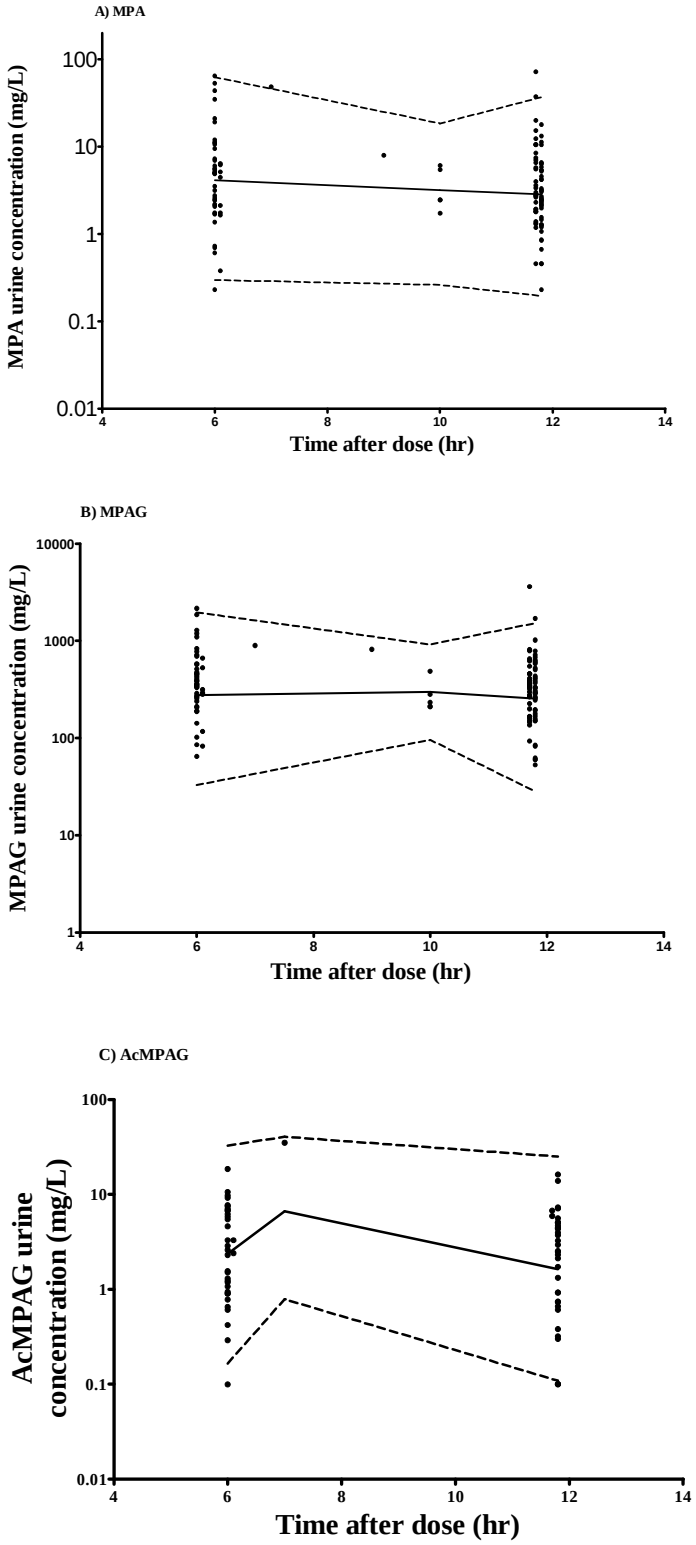

A) MPA
MPA plasma concentration (mg/L)
100
10
1
0.1
0.01
0
4
8
12
Time after dose (hr)
B) MPAG
MPAG plasma concentration (mg/L)
1000
100
10
1
0.1
0.01
0
4
8
12
Time after dose (hr)
C) AcMPAG
AcMPAG plasma concentration (mg/L)
10
1
0.1
0.01
0.001
0.0001
0
4
8
12
Time after dose (hr)

Figure 4.10


A) MPA
MPA urine concentration (mg/L)
100
10
1
0.1
0.01
4
6
8
10
12
14
Time after dose (hr)
B) MPAG
MPAG urine concentration (mg/L)
10000
1000
100
10
1
4
6
8
10
12
14
Time after dose (hr)
C) AcMPAG
AcMPAG urine concentration (mg/L)
100
10
1
0.1
0.01
4
6
8
10
12
14
Time after dose (hr)

Chapter 5

EFFECTS OF URIDINE GLUCURONOSYLTRANSFERASE 2B7 AND 1A7 PHARMACOGENOMICS AND PATIENT CLINICAL PARAMETERS ON STEADY STATE MYCOPHENOLIC ACID PHARMACOKINETICS IN GLOMERULONEPHRITIS

Melanie S. Joy, PharmD,[1,2] Tammy Boyette, BS,[1] Yichun Hu, MS,[1] Jinzhao Wang, BS,[1] Mary La,[1] Susan L. Hogan, PhD,[1] MPH, Paul W. Stewart, PhD,[3] Ronald J. Falk, MD.,[1] Mary Anne Dooley, MD,[1] Philip C. Smith, PhD,[2] University of North Carolina, School of Medicine, UNC Kidney Center,[1] Eschelman School of Pharmacy,[2] and Gillings School of Public Health,[3] Chapel Hill, NC

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Abstract

Background: Mycophenolic acid (MPA) is an immunosuppressant used in the treatment of glomerulonephritis and transplantation. MPA is metabolized by several uridine diphosphate glucuronosyltransferases (UGTs) and several transporters are responsible for uptake and efflux of MPA and its metabolites. Data concerning the influence of clinical covariates and polymorphisms in drug metabolizing genes and transporter genes on the pharmacokinetics of MPA have not been described in glomerulonephritis.

Aim: The role of pharmacogenomics, clinical and demographic parameters on pharmacokinetic predictions was evaluated in patients receiving mycophenolic acid (MPA). In particular, the study focused on the influence of polymorphisms in the less-well described gene-pharmacokinetic relationships pertaining to MPA.

Methods: A cohort study design of patients with glomerulonephritis secondary to lupus nephritis and anti-neutrophil cytoplasmic antibody (ANCA) small vessel vasculitis was employed. Forty-six patients with lupus nephritis and anti-neutrophil cytoplasmic antibody (ANCA) small vessel vasculitis and receiving MPA were recruited from the nephrology clinic. The study assessed the relative single and combined roles of genomic, clinical, and demographic characteristics on pharmacokinetic (PK) parameters using general linear models. The study focused on single nucleotide polymorphisms in *UGT1A7, UGT2B7* and *ABCB1/MDR1*; all of which have limited data available concerning relevance to MPA disposition.

Measurements: All patients had PK assessments for MPA and its glucuronide metabolites (MPAG and AcMPAG). Genotyping was performed for known variants of UGTs (*UGT1A9, UGT1A7, UGT2B7*), and multidrug resistance protein (*ABCB1/MDR1),* involved in MPA disposition. Analyses included PK, as well as 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 MPA apparent oral clearance. *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 MPA apparent oral clearance ($p<0.0001$). 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.

Limitations: The main limitation to this study was small sample size to enable a robust assessment of the effects of all planned genotypes on MPA PK parameters.

Conclusions: Clinical and demographic parameters (especially kidney function and urinary protein) were 2-4 times more important in MPA disposition than genotypes and explained 30% to 40% of the PK parameters.

Introduction

Autoimmune related kidney diseases such as systemic lupus erythematosus (SLE) and anti-neutrophil cytoplasmic antibody (ANCA) small vessel vasculitis (SVV) are treated with a myriad of 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 clearance through clearance of bound drugs. 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 as compared to the kidney transplant population could lead to variability in pharmacokinetics above and beyond that which would be predicted from studies employing the later 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 described. The *UGT1A9 T-275A* and *C-2152T*

promotor 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 descrbied 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 were comprised of 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 know 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 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 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 for 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 *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.

Methods

Research Subjects

Patients with biopsy confirmed SLE or ANCA SVV vasculitis with kidney manifestations and receiving maintenance therapy on a stable dose of MPA (Cellcept®, Roche, New Jersy) for at least two weeks were evaluated for enrollment. These patients participated in a 24-hour MPA pharmacokinetics evaluation approved by the Biomedical Institutional Review Board and conducted in the inpatient clinical research center. 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 hours and urine was collected from 0-6, 6-12, and 12-24 hours. Plasma and urine samples were assayed for MPA and MPAG by a 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 5-1500 µg/mL, respectively. The AcMPAG metabolite was assayed in plasma and urine by liquid chromatography/mass spectrometry (LC/MS). Plasma and urine standard curves for AcMPAG were linear over the range of 0.01-50 µg/mL and 1-500 µg/mL, respectively.

Clinical data was abstracted from medical charts and included serum creatinine (SCr), estimated creatinine clearance (ClCr) 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, Inc., Valencia, CA, USA). Genotyping was conducted for several published *UGT1A9, UGT1A7,* and *UGT2B7* SNPs reported to result in alterations in MPA metabolism.(Table 5.1) [3-5,7,8] Additionally, *ABCB1/MDR1* polymorphismss were evaluated secondary to published data suggesting a role of the P-glycoprotein transporter in MPA disposition. [11] (Table 5.1) Genotyping assessments for *UGT1A7 T622C* (c___287260_10, Applied Biosystems, Foster City, CA) and *MDR1 C1236T* (c___7586662_10, Applied Biosystems, Foster City, CA) 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, Foster City, CA. Allelic discrimination was assessed for all Applied Biosystems products using 5 µL of TaqMan Universal PCR Master Mix, No AmpErase UNG (2X) (Applied Biosystems), 0.25 µL (of 40X assay) or 0.5 µL (of 20X assay), 10 to 20 ng genomic DNA and a total reaction volume of 10 µL per the manufacturer's instructions. The reactions were cycled with an initial denaturation of 95^{o}C for 10 minutes followed by 50 cycles of 92^{o}C for 15 seconds, and then 60^{o}C for 1.5 minutes 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 5.2 in order that they could serve as positive controls for the former assays. Genotyping for *UGT1A9 G8A*, *UGT1A9 C-2152T*, and *UGT2B7 C802T* was conducted by Polymorphic DNA Technologies, Inc (Alameda, CA). 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-square test procedure for testing of deviation from Hardy-Weinberg equilibrium.

Putative relationships between pharmacokinetic outcomes and patient characteristics were explored using descriptive methods (e.g., estimation of spearman 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. The genotypes of interest focused on 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*.

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, Inc., Cary, NC.)

Results

Noncompartmental pharmacokinetic data (for MPA, MPAG, and acyl-MPAG), demographic data, and clinical data were available for 27 SVV patients and 19 SLE patients. (Table 5.3) 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 laboratories: 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 5.4. 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 5.5. For those models evaluating only clinical and demographical factors, the fit (R^2) ranged from ~0.10 to ~0.32. Noteable 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 on 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 5.5 were assessed in multivariate models to predict the combined influence of these parameters on pharmacokinetics. (Table 5.6) 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 for 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 later 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 for

increased AUC $_{0\text{-}12}$ (R^2 0.2950) and AUC $_{6\text{-}12}$ (R^2 0.3420). Additionally, decreased Scr and female gender were predictors for 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) were also important in predicting the AcMPAG AUC and renal clearance. Increased AUC $_{0\text{-}12}$ was predicted by Caucasian race and decreasing eClCr (R^2 0.4542), while AUC $_{6\text{-}12}$ was predicted by increased age and Scr (R^2 0.4092). Both decreased serum albumin and Scr were predictive for increased AcMPAG renal clearance (R^2 0.4239).

Discussion

In this study, we sought to characterize the roles of clinical and demographic factors in glomerulonephrits, as well as genomic alterations in selected *UGTs* (*1A9, 1A7*, and *2B7*) and *ABCB1*/*MDR1* on 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 than where the drug was originally approved, there is a potential for pharmacokinetic alterations that may require dosing changes to enable an appropriate exposure (AUC) that optimizes outcomes and minimizes adverse effects. This is particularly relevant for glomerulonephritis where unlike kidney transplant patients with primarily 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] as compared to 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 by less well described polymorphisms in *UGT2B7*, *UGT1A7* and *ABCB1*/*MDR1*. The resultant multivariable models explained 30 to 50% of MPA's pharmacokinetic outcomes. Genomic factors alone explain about 10% of MPA's pharmacokinetic outcomes.

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 5.5 and 5.6) suggested a primary importance of kidney function, through either SCr or eClcr, on 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, that 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 MPA and metabolites pharmacokinetics. An elevated UP:Cr predicted reduced exposures (AUCs) and increased oral clearance for MPA. Two previous publications by our group also highlights 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, a UP:Cr increase from 0.5 to 3.5 at a stable SCr of 2 mg/dL (176.8 moles/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 moles/L to 442 moles/L) would result in a tripling of the AUC_{0-12} (from 76 µg 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 exposures (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 moles/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.

Females 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 suggests that either there exists an added effect of female gender above 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 females have a lower SCr value for level of kidney function as compared to males, the later 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 to 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 the 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 the influences on human MPA pharmacokinetics. [3,4,26] The *UGT2B7 C802T* has been purported to result in increases of 25% in total AUC and 48% in unbound AUC for MPA, as well as increases in maximal plasma concentrations and urinary AcMPAG. [3,4,26] Our univariate models employing only genotype showed increased recycling (AUC_{6-12}) and decreased renal clearance for MPA in the variant homozygous group. Increased oral clearance, decreased AUC_{0-12} and AUC_{6-12}, and increased renal clearance was demonstrated in patients exhibiting heterozygosity for *UGT2B7 C802T*. Increased AcMPAG renal clearance was also demonstrated in the *UGT2B7* heterozygous group. 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 as compared to 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, [27] suggesting a greater contribution of the metabolic enzyme toward 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] In our univariate assessments analyzing only genotype variables, genotypes heterozygosity for the UGT1A7 variant contributed toward increased oral clearance and decreased maximal plasma concentration values. While there are currently no human studies demonstrating effects of *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 resulting in decreased clearance. We failed to detect any effects of *ABCB1*/*MDR1 C1236T* and

C3435T on altering 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 between 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 on 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 may have additional alterations in

MPA disposition. Lastly, as we 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- to 4-times more of the variability in MPA pharmacokinetic variables than did the genotype covariates. Our data suggests the need for further research and larger pharmacogenomic studies in glomerulonephritis to adequately assess the contributions of genetic- and disease- related perturbations on MPA metabolism and transport.

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Table 5.1

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

Abbreviations

MDR – multidrug resistance

UGT – uridine diphosphate glucuronosyltransferase

Table 5.2

Sequencing and 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

Abbreviations

MDR – multidrug resistance

UGT – uridine diphosphate glucuronosyltransferase

Table 5.3

Demographics, Clinical and Pharmacokinetic Data (Mean ± standard deviation)

Data represents 27 small vessel vasculitis and 19 lupus nephritis prospective patients (total n = 46)

Data provided as mean (sd); range

Age (years)	46.0 (15.0); 22-78			
Race (W/B/O)	27/13/6			
Gender (M/F)	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			
Pharmacokinetics	MPA_{total}	MPA_{free}	MPAG	AcMPAG
	(n = 46)	(n = 46)	(n = 46)	(n = 41)
C_{max} (μg/mL)	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)	4.11 (4.46)	0.07 (0.11)	31.7 (27.8)	0.28 (0.63)
AUC_{0-12} (μg h/mL)	66.3 (43.8)	1.07 (1.57)	498 (433)	3.88 (4.80)
Cl/F (mL/min)	305 (173)	31.7 (28.6)[a]	N/A	N/A
AUC_{6-12} (μg h/mL)	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)

a – L/min

Abbreviations

AcMPAG – acyl MPAG

AUC_{0-12} – area under the plasma concentration time curve from 0-12 hours

AUC_{6-12} – area under the plasma concentration time curve from 6-12 hours

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

$T_{1/2}$ – half life

Table 5.4

Genotype Frequency Distributions **(frequency (n))**

		Small Vessel Vasculitis n = 27	Lupus Nephritis n = 19
UGT1A9			
G8A	G/G	1.0 (28)	1.0 (19)
	G/A	0 (0)	0 (0)
	A/G	0 (0)	0 (0)
C98T	C/C	0.96 (27)	1.0 (19)
	C/T	0.04 (1)	0 (0)
	T/T	0 (0)	0 (0)
C-2152T	C/C	0.96 (27)	1.0 (19)
	C/T	0.04 (1)	0 (0)
	T/T	0 (0)	0 (0)
T-275A	T/T	0.96 (27)	1.0 (19)
	T/A	0.04 (1)	0 (0)
	A/A	0 (0)	0 (0)
UGT1A7			
T622C	T/T	0.48 (13)	0.47 (9)
	T/C	0.52 (14)	0.47 (9)

	C/C	0 (0)	0.06 (1)
UGT2B7			
C802T	C/C	0.30 (8)	0.47 (9)
	C/T	0.44 (12)	0.37 (7)
	T/T	0.26 (7)	0.16 (3)
ABCB1/MDR1			
C1236T	C/C	0.36 (10)	0.53 (10)
	C/T	0.50 (14)	0.47 (9)
	T/T	0.14 (4)	0 (0)
C3425T	C/C	0.32 (9)	0.37 (7)
	C/T	0.54 (15)	0.58 (11)
	T/T	0.14 (4)	0.05 (1)

Abbreviations

ANCA – anti-neutrophil cytoplasmic antibody

MDR – multidrug resistance

UGT – uridine diphosphate glucuronosyltransferase

Table 5.5

Final Univariate Models for the Separate Effects of Clinical, Demographic, and Genotype Parameters on the Prediction of Pharmacokinetic Outcomes

Dependent Variable *	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
	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

- The natural logarithmic transformation was used for all dependent variables except for acyl MPAG MR.

Abbreviations

AUC_{0-12} – area under the plasma concentration time curve from 0-12 hours

AUC_{6-12} – area under the plasma concentration time curve from 6-12 hours

AUC_{unb} – unbound area under the plasma concentration time curve from 0-12 hours

eClcr – estimated creatinine clearance

Cl/F – oral clearance

Cl_{unb} – unbound oral clearance

Cl_R/F – renal 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

UGT – Uridine diphosphate glucuronosyltransferase

Table 5.6

Final Multivariable Linear Models of the Combined Effects of Genotype, Clinical, and Demographic Parameters on Pharmacokinetics

Dependent Parameters*	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		

* 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).

Abbreviations

AUC_{0-12} – area under the plasma concentration time curve from 0-12 hours

AUC_{6-12} – area under the plasma concentration time curve from 6-12 hours

AUC_{unb} – unbound area under the plasma concentration time curve from 0-12 hours

eClcr – estimated creatinine clearance

Cl/F – oral clearance

Cl_{unb} – unbound oral clearance

Cl_R/F – renal 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

UGT – Uridine diphosphate glucuronosyltransferase

Chapter 6

Expression Patterns for Drug Metabolizing Enzyme and Transporter Transcripts in Glomerulonephritis Patients

Melanie S. Joy, PharmD,[1,2] Jinzhao Wang, BS,[1] Yichun Hu, MS,[1] Susan L. Hogan, MPH, PhD,[1] Gloria A. Preston, PhD,[1] Kim L.R. Brouwer, PharmD, PhD,[2] Philip C. Smith, PhD[2], Ronald J Falk, MD[1]. University of North Carolina, School of Medicine, Division of Nephrology, Kidney Center,[1] and Eshelman School of Pharmacy,[2] Chapel Hill, NC

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Introduction

The mRNA expression patterns of drug metabolizing enzymes and transporters in peripheral blood cells (neutrophils, lymphocytes, and monocytes) may be important in patient responses to treatments for glomerulonephritis since the target of the pharmacological agents (e.g. mycophenolic acid and cyclophosphamide) are the lymphocytes (B and T lymphocytes). For mycophenolic acid, the active therapeutic component is transformed to inactive glucuronide metabolites after administration. The pharmacologically active 4-hydroxycyclophosphamide metabolite of the prodrug cyclophosphamide is first formed by phase I metabolism through cytochrome P450 enzymes and further converted to the phosphoramide mustard. As exposure of the lymphocytes to the active species of a medication is critical for pharmacological effects, the balance and direction between exposure to parent drug versus metabolite is necessary to enhance efficacy and reduce toxicity.

Alterations in expression of drug metabolizing enzymes or transporters in lymphocytes could affect the exposure of these cells to pharmacologically active components such as mycophenolic acid and 4-hydroxycyclophosphamide. Regarding drug transport, enhanced activity and/or expression of cellular efflux genes and their respective proteins relative to uptake would be predicted to reduce the intracellular concentration of therapeutic entities, assuming active processes guide exposure. Decreased activity and/or expression of export genes relative to uptake would be expected to increase intracellular drug concentrations. For metabolism, expression of drug metabolizing enzymes within the lymphocyte may modulate the exposure of the tissue to active (4-hydroxycyclophosphamide) versus inactive (mycophenolic acid glucuronide) pharmacologic moieties. While studies have described the presence or absence of uridine diphosphate glucuronosyltransferase (*UGT*) mRNA in various solid organs (liver, kidney, intestine, lung, stomach, brain, breast, prostate, heart, adrenals, bladder, ovary, uterus, and testis) within rats and humans [1-4] , the peripheral blood cells have been largely ignored for drug metabolism genes and limited studies have reported mRNA expression of selected

transporters.[5,6] Furthermore, there is currently limited information regarding expression of drug transporter genes or drug metabolizing genes in patients representing specific disease models or in selected tissues that are important as the targeted pharmacological site of action.

Several exogenous and endogenous factors may be responsible for altering mRNA expression and subsequent exposure to therapeutic agents at their active site. Inducers of transport and metabolism have been shown to concordantly increase activity and mRNA expression within hepatocytes. [7] mRNA expression of drug transporters has been reported to be affected by inflammatory condictions (e.g. rheumatoid arthritis, ulcerative colitis, ischemia-reperfusion injury) and upon direct exposure to inflammatory cytokines (e.g. TNF-α, IL-6). [8-11] Gender specific effects on *UGT* mRNA expression in tissues (liver, kidney, lung, intestine, brain, nose) have been documented in mice. [12,13] A genotype dependent down-regulation of mRNA expression and protein function has also been reported, [14] whereby wild-type and heterozygotes for the *C3435T* single nucleotide polymorphism in the multidrug resistance protein gene (*ABCB1; MDR1*) exhibited less relative mRNA expression in peripheral blood mononuclear cells as compared to the homozygous variant genotype. [14] This scenario would imply that the intracellular concentration of active therapeutic agent would be enhanced in patients without the variant/variant genotype.

The purpose of the current study was two-fold; 1) to evaluate mRNA expression patterns of drug metabolizing enzyme genes (*UGT1A7, UGT1A9, UGT2B7, CYP2C9, CYP2B6, CYP3A4)* and transporter genes (*ABCB1*, *ABCC2, ABCG2*, and *SLCO1A2*) in leukocytes of patients with glomerulonephritis secondary to systemic lupus erythematosus (SLE) or small vessel vasculitis (SVV), and 2) to evaluate the relationships between mRNA expression and patient-level data (including common genotypes for drug metabolizing enzymes and transporters) to understand the effects of metabolic processing and transport of cyclophosphamide and mycophenolic acid.

Methods

Specimens

Patients with glomerulonephritis secondary to SLE (n=36) and SVV (n=35) who participated in prospective pharmacokinetic studies to evaluate oral mycophenolic acid [15,16] and intravenous cyclophosphamide [17] had 15mL blood drawn into multiple ethylenediaminetetraacetic acid (EDTA) vacutainer tubes. Leukocytes were isolated from blood by incubation (11 minutes) in a hypotonic red cell lysis buffer, followed by centrifugation and a wash with Hank's balanced salt solution (HBSS). The leukocytes were subsequently lysed in RNA Stat 60 solution and stored at -70C for up to 2 weeks until processing.

mRNA Isolation

The mRNA isolation procedure consisted of adding 200μL chloroform for phase separation. The aqueous phase (containing the mRNA) was added to a solution of isopropanol and centrifuged. The pellet was then washed with 1mL 75% ethanol, re-suspended in 100μL nuclease free water (Promega, Madison, WI), and centrifuged. Four microliters RNA secure 25X (Ambion, Austin, TX) was added to each sample. The RNeasy kit and protocol (Qiagen, Valencia, CA) was used for the remainder of the mRNA preparation. Briefly, after adding Buffer RLT, β-Mercaptoethanol, and 100% ethanol to the samples, the mRNA solution was applied to an RNeasy mini spin column for purification. mRNA was re-treated with RNA secure at 1X (Ambion, Austin, TX) after the column elution. mRNA was quantified by evaluation of the absorbance at 260 nm and 280 nm using a spectrophotometer. The mRNA integrity was determined by visualization of the 28S and 18S mRNA bands using 0.5 μg mRNA on a 1% agarose gel stained with Sybr Gold (Molecular Probes, Eugene, OR). mRNA was stored at -70C.

Evaluation of Transcript Levels

An aliquot of each patient's mRNA was converted to cDNA via the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). A 20μL reaction was prepared that included; 2μL of 10x RT Buffer; 0.8μL of 25x dNTP Mix (100mM); 2μL of 10x RT Random Primers; 1μL of MultiScribe Reverse Transcriptase; 4.2μL of Nuclease-free water and 10μL of mRNA. The plate was placed in a thermal cycler under the profile; 25℃ for 10 minute, 37℃ for 120 minutes, 85℃ for 5minutes, and 4℃ for infinity .

Pre-designed assays containing primers and probes were purchased from Applied Biosystems (Foster City, CA) for assessment of transcript levels of the targeted metabolizing enzymes (*UGT1A7, UGT1A9, UGT2B7, CYP3A4, CYP2C9,* and CYP*2B6*) and transporters (*ABCB1, ABCC2, ABCG2,* and *SLCO1A2*); *UGT1A7* (Hs02517015_s1), *UGT2B7* (Hs02556232_s1), *UGT1A9* (Hs02516855_sH), *CYP3A4* (Hs00604506_M1), *CYP2C9* (Hs00426397_m1), *CYP2B6* (Hs00167937_g1), *ABCC2* (Hs00166123_m1), *ABCB1* (Hs00184500_m1), *ABCG2* (Hs01053795_m1), and *SLCO1A2* (Hs01072338_m1). Cytochrome C oxidase was used as the normalization (housekeeping) gene. The forward and reverse primers were designed using Primer Express software (Applied Biosystems, Foster City, CA). The forward primer (TGGCATCTGGAGGTGGTGTT) and reverse primer (GTCCAGTCCCTTTGCAGC) were purchased from Applied Biosystems (Foster City, CA). Sybr 1:400 was used as the probe in the cytochrome c oxidase assay (Molecular Probes, Leiden, Netherlands).

Taqman® PCR was performed on an Applied Biosystems PRISM 7900 HT sequence detection system (Applied Biosystems,Foster City, CA). The duplicate 10μL reactions were performed in MicroAmp Optical 384 well plates. For the commercial assays, the reaction mixture was composed of 40ng (4μL) of cDNA; 0.5μL of 20x probe and primer (Applied Biosystems, Foster City, CA), 0.5μL nuclease-free water, and 5μL of 2x Universal PCR Master

Mix (Applied Biosystems, Foster City, CA). For the cytochrome C oxidase assay, the reaction mixture was composed of 40ng (4μL) of cDNA; 0.1μL of 5uM forward primer; 0.1μL of 5μM reverse primer; 0.3μL of 1:400 dilution Sybr Green (Molecular Probes, Leiden Netherlands); 0.5μL nuclease-free water, 5μl of 2x Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The thermal cycling conditions were; 50℃ for 2 minutes, 95℃ for 10minutes, 95℃ for 15 seconds in 50 cycles, 60℃ for 1hour.

Genotype Assessments

A 5 mL whole blood sample was collected into an EDTA containing vacutainer tube and genomic DNA was isolated using a Flexigene Qiagen kit (Qiagen, Inc., Valencia, CA, USA). Genotyping was conducted for several published *UGT* single nucleotide polymorphisms (*UGT1A9, UGT1A7,* and *UGT2B7*) relevant for alterations in metabolism, [18-22] and *ABCB1/MDR1* relevant for transport of mycophenolic acid [23] . Genotyping was also conducted for polymorphisms in some cytochrome P450 genes (*CYP2B6, CYP3A4, CYP2C9)* relevant for alterations in cyclophosphamide metabolism. [24-26] Genotyping assessments for *UGT1A7 T622C* (c287260-10), *ABCB1 C1236T* (c7586662-10), *CYP2B6 C1459T*(c30634242), and *CYP2B6 G516T* (c22275631) were conducted using commercially available assays (Applied Biosystems, Foster City, CA). Genotyping for *UGT1A9 C98T*, *UGT1A9 T-275A*, *CYP2B6 A785G* and *ABCB1 C3435T* was conducted using custom assays manufactured by Applied Biosystems, Foster City, CA. Genotyping for *UGT1A9 G8A*, *UGT1A9 C-2152T*, and *UGT2B7 C802T* was conducted by Polymorphic DNA Technologies, Inc (Alameda, CA).

Allelic discrimination was assessed for all Applied Biosystems products using 5 μL of TaqMan Universal PCR Master Mix, No AmpErase UNG (2X) (Applied Biosystems), 0.25 μL (of 40X assay) or 0.5 μL (of 20X assay), 1 to 20 ng genomic DNA and a total reaction volume of 10 μL per the manufacturer's instructions. The reactions were cycled with an initial denaturation of 95^{o}C for 10 min followed by 50 cycles of 92^{o}C for 15 sec, and then 60^{o}C for 1.5 minutes on an

Applied Biosystems 7900 Taqman PCR instrument. Genotypes for polymorphisms in *ABCC2, ABCG2* and *SLCO1A2* were not assessed.

Data Analyses

Stored mRNA from healthy controls; HC (n=10), untreated SLE nephritis patients; LC (n=5) and untreated SVV with nephritis; VC (n=5) patients were used as study and disease controls, respectively. The Ct values (the fractional cycle at which the fluorescence intensity equals the threshold fluorescence; inversely related to the abundance of transcript in a sample) were computed for each sample. Subsequently ΔCt values were calculated for each sample by subtracting the Ct value for the housekeeping gene (cytochrome C oxidase) from the Ct value for the gene of interest. In order to calculate fold-change, the 2^-ΔΔCt were computed. The ΔΔCt values were calculated by subtracting the ΔCt of a selected healthy control from the ΔCt of each discrete sample. The fold-change was calculated by dividing the individual 2^-ΔΔCt values by the average of the 2^-ΔΔCt values for healthy control samples.

Transcript fold-change in each of the five groups (SVV,VC, SLE, LC, HC) were computed and recorded as mean±standard deviation. Significant differences of the median fold-change values among patient groups were determined using Kruskal Wallis nonparametric ANOVA. A post-ANOVA Dunn's Multiple Comparison's test was used to determine differences in median transcript expression. Patient level data that was evaluated for relationships with transcript fold-change were: disease (SVV vs SLE), treatment (cyclophosphamide vs mycophenolic acid), gender, race (Caucasian vs non-Caucasian), and genotype (*UGT1A7, UGT1A9, UGT2B7, CYP2C9, CYP3A4, CYP2B6*, and *ABCB1*). The expression values were converted to the log 10 and linear regression was used to evaluate these former relationships. Spearman correlation analysis was used to evaluate relationships between fold-change expression values by disease, genotypes, treatments, gender, and race within disease groups. Spearman correlation analyses were conducted to evaluate for relationships between continuous mycophenolic acid and

cyclophosphamide pharmacokinetic variables; area under the plasma concentration time curve (AUC), trough plasma concentration (Ctr), oral/systemic clearance, renal clearance, and transcript expression. Wilcoxon two-samples tests were used to assess for relationships of *SLCO1A2* transcript expression between gender, race, disease, and treatment. P values of < 0.05 was considered statistically significant. Statistical analyses were performed using InStat v3.0 (GraphPad, San Diego, CA) and SAS Statistical Software, Version 9.1 (SAS Institute, Inc., Cary, NC).

Results

The description of SLE and SVV study subjects who donated blood for gene transcript analyses are provided in Table 6.1. This information was not available (demographics) or did not apply (treatment) to the three control groups. The transcript of transporter genes (*ABCC2, ABCB1, and ABCG2)* were expressed in the leukocytes of 92% to 98% of subjects. Figure 6.1 is a representative amplification plot of the *ABCB1* transporter transcripts. The transcript of *SLCO1A2* was expressed in only 50% of subjects. Regarding the drug metabolizing enzyme genes, the transcript of *UGT1A9*, *UGT1A7*, and *UGT2B7* were expressed in ~50% of subjects, while the *CYP2B6* transcript was expressed in 94% of subjects. Figure 6.2 is a representative amplification plot of the *UGT1A7* transcript. The *CYP3A4* and *CYP2C9* genes were not appreciably expressed in the leukocytes of the evaluated subjects. Fold-change values for each gene in each patient group (SVV, VC, SLE, LC, HC) are recorded as mean±SD in Table 6.2. Differences were noted in expression of *UGT1A7*, *ABCB1*, and *ABCC2* across the evaluated patient populations. Regarding *UGT1A7*, the SVV (0.17±0.42; $p<0.05$) and SLE (0.03±0.10; $p<0.05$) groups had statistically lower expression values than the HC subjects (0.79±2.02). For *ABCB1*, the SLE group had significantly lower mean expression values (0.33±0.21; $p<0.05$) than the HC group (1.00±0.82). For the *ABCG2* gene, the SVV group had lower mean expression values (0.17±0.14; $p<0.05$) than the HC subjects (1.00±1.82). Differences in

expression of *ABCC2* approached statistical significance, with the VC patients (2.02±1.13) exhibiting higher expression than the SVV patients (1.06±1.11; p=0.05).

Genotype frequencies for the *UGT1A7, UGT2B7, ABCB1*, and *CYP2B6* single nucleotide polymorphisms evaluated in the 67 treated SLE and SVV patients are shown in Table 6.3. Genotype frequencies for all evaluated polymorphisms were in Hardy-Weinberg equilibrium. Genotype analyses are not reported for the *UGT1A9* polymorphisms that were planned to be evaluated secondary to their extremely low frequency in this glomerulonephritis population.

Several important findings resulted from the evaluation of the relationships between transcript expression and patient-level data. (Table 6.4) However, none of the relationships resulted in R^2 values of greater than 0.10 secondary to the dichotomous nature of the patient-level data. Among the SVV and SLE groups receiving treatment with either mycophenolic acid or cyclophosphamide, *ABCC2* expression was different by race (1.26±1.82 Caucasian versus 1.37±0.86 non-Caucasian; p=0.049); *CYP2B6* expression was different by treatment (2.07±2.94 cyclophosphamide versus 0.45±0.50 mycophenolic acid; p=0.010). Results of borderline significance were *ABCB1* expression by *ABCB1 C3435T* genotype (0.43±0.55 wildtype versus 0.63±0.88 variants; p=0.076), *ABCC2* expression by disease type (1.20±1.50 SVV versus 1.43±1.29 SLE; p=0.078), and *ABCG2* expression within SLE patients by gender (0.34±0.34 female versus 0.11±0.07 male; p=0.074). Assessments of relationships between *UGT* or *SLCO1A2* expression and patient-level variables were not attempted secondary to the higher percentage of subjects with absent transcript in leukocytes. Additionally, too few subjects exhibited the evaluated single nucleotide polymorphisms in the *UGT1A9* gene to enable evaluation with transcript expression.

Assessments of relationships between transcript expression and pharmacokinetic parameters for mycophenolic acid and cyclophosphamide were evaluated by patient treatment to ascertain whether clinically relevant medication effects were demonstrated. For patients

receiving cyclophosphamide, significant negative correlations were noted between *ABCC2* expression and cyclophosphamide clearance (r^2 -0.449; p=0.041), and 4-hydroxycyclophosphamide AUC (r^2-0.536; p=0.012). For patients receiving mycophenolic acid, significant negative correlations were noted between *ABCG2* gene expression and mycophenolic acid Ctr (r^2-0.378; p=0.043). No other correlations were noted.

Discussion

The current study is the first to describe expression of drug metabolizing enzyme and drug transporter transcript in the leukocytes of patients with kidney disease secondary to glomerulonephritis. This research is relevant as therapies for the treatment of glomerulonephritis are directed primarily toward the peripheral blood cell lymphocyte populations. This study selectively assessed only those genes thought to be involved in the transport and metabolism of the two primary glomerulonephritis treatments; mycophenolic acid and cyclophosphamide. Our results showed leukocyte expression of the *ABCC2, ABCB1, ABCG2* transcripts in ~90% and S*LCO1A2* transcript in ~50% of patients with glomerulonephritis, respectively. The expression of genes for the drug metabolizing enzymes *UGT1A9*, *UGT1A7*, and *UGT2B7* were demonstrated in the leukocytes of ~50% of patients. However, the leukocyte expression of *CYP2B6* was evident in >90% of patients while *CYP3A4* and *CYP2C9* expression was virtually absent. Treatment-related differences in expression were assessed in mycophenolic acid- versus cyclophosphamide-treated patients. Our results showed that cyclophosphamide-treated glomerulonephritis patients had 4-fold higher expression for *CYP2B6* (2.07±2.94 vs 0.45±0.50; p=0.010) than mycophenolic acid-treated patients. While it is tempting to attribute this finding to induction of gene transcription by cyclophosphamide, this scenario is unlikely since previous doses had been administered at least 30 days prior, doses were lower (0.8±0.2 g/m^2) than reported for enzyme induction [27], and blood was obtained prior to and not after the next planned dose. We cannot rule-out the possibility, however, that

concomitant daily glucocorticoid therapy could have induced expression of *CYP2B6*. Significant differences in transporter transcript expression by race (*ABCC2*), disease (*ABCC2, ABCG2*), and genotype (*ABCB1*) were also found. Significant relationships in cyclophosphamide clearance/4-hydroxycyclophosphamide AUC (*ABCC2*) and mycophenolic acid Ctr (*ABCG2*) were also found.

Evaluation of expression in *ABCG2, ABCB1*, and *ABCC1* transcript in lymphocytes and monocytes of healthy patients [6] previously showed cell type dependent expression only in *ABCB1* transcript, with greater expression of *ABCB1* in lymphocytes (lymphocytes 9.67±5.53 versus monocytes 0.821±0.263). Since we did not assess expression in individual cell types (lymphocytes, neutrophils, monocytes), and neutrophils normally out-number lymphocytes by a factor of two to three, it is conceivable that a reasonable expression of the *UGT* and *SLCO1A2* genes in the lymphocytes may have been obscured by a dilutional effect of other cells in patients with reduced transcript expression. Albermann et al, reported the relative order of ABC-transporter gene expression in peripheral blood mononuclear cells as *ABCC1> ABCG2> ABCB1> ABCC2*.[5] While we did not assess *ABCC1*, the relative order of magnitude in expression for glomerulonephritis patients was *ABCC2>SLCO1A2>ABCB1=ABCG2* for SLE and *SLCO1A2>ABCC2>ABCB1>ABCG2* for SVV. These data suggest that the MRP2 and OATP transporters most pertinent to overall mycophenolic acid dispositionhave the highest expressed transcripts, e.g. *ABCC2* and *SLCO1A2* within the leukocytes of SLE and SVV patients (when they are in fact expressed). The role of MRP2 and OATP in leukocyte transport of mycophenolic acid and its metabolites have not yet been assessed in leukocyte cell-based studies.

Differences in mean transcript expression among the subject groups were found in the present study. A notable finding was that healthy controls had higher expression of *UGT1A7* relative to SVV and SLE patients, higher *ABCB1* expression than SLE patients, and higher

ABCG2 expression than SVV patients. This data would imply that the transport activity and/or capacity though the proteins encoded by *ABCB1* (P-glycoprotein) and *ABCG2* (breast cancer resistance associated protein; BCRP), are reduced in patients with SLE or SVV, possibly allowing higher intracellular concentrations of transported substrates. However, we don't currently know what threshold levels of transcript are necessary to have sufficient activity to exhibit a normal versus reduced transport phenotype. Regarding medications used in the treatment of glomerulonephritis, mycophenolic acid is suggested to be a substrate of P-glycoprotein [23,28,29], and BCRP [30], and glucocorticoids are known substrates for P-glycoprotein.[31,32] Regarding drug metabolizing enzymes, mycophenolic acid is a substrate for UGT1A7[33] and since SLE and SVV patients have reduced *UGT1A7* transcript expression relative to healthy normals, our patients would be predicted to have lower turnover of mycophenolic acid through metabolism within the leukocytes, with the assumption that gene expression correlates significantly with protein expression within these cells. However, only ~50% of our patients expressed *UGT1A7* in leukocytes. The affinity of mycophenolic acid for UGT1A7 is reportedly greater than the affinity for UGT1A9 [33], but the overall relative contribution of UGT1A7 to mycophenolic acid metabolism has not been reported.

We were interested in exploring the effects of patient-level factors on transcript expression in the SLE and SVV patients. These factors (disease, treatment, race, gender, and genotype) were included as existing data in the literature supported these evaluations. [7,12,14,34-36] Regarding disease type, the SLE patients had consistently higher expression of both the *ABCC2 and ABCG2* gene as compared to the SVV patients. Higher expression of *ABCC2* and *ABCG2* would be predicted to reduce intracellular exposure to mycophenolic acid in the SLE patients as compared to SVV patients if active transport modulates expression more than passive equilibrium with plasma. While we did not measure this directly or in a separate *in vitro* cell-based study, our previous pharmacokinetic publications [15,16] do support higher systemic (extracellular) exposures in SLE vs SVV patients.

Exposures to concurrent treatments can influence expression of drug metabolizing enzyme transcripts. An *in vitro* experiment employing CaCo2 cells demonstrated a suppression of *UGT2B7* transcript expression after exposure to retinoids. [37] Several publications have reported suspected isotretinoin-induced vasculitis, [38-40] and we have preliminary data suggesting an increased relative risk of SVV in patients with single nucleotide polymorphisms in *UGT2B7* (associated with a decreased metabolic activity phenotype). A study in mice demonstrated inducibility of liver and intestinal *UGT1* and *UGT2* transcript by microsomal enzyme inducers of specific transcription factors (arylhydrocarbon receptor, constitutive androstane receptor, pregnane X receptor, peroxisome proliferators-activated receptor alpha, and NF-E2 related factor 2). [36] Additionally, a study employing rat and human hepatocytes showed induction of *UGT* transcript by arylhydrocarbon receptor ligands (3-methylcholantrine, β-naphthoflavone, and omeprazole). [7] In this same study, human expression of *ABCB1* was induced with phenobarbital and rifampin, *ABCB3* was induced with fenofibrate, and *SLCOA* was induced by pregnenalone-16 carbonitrile and omeprazole. Differential transcript expression (inducing agent) was demonstrated in rat hepatocytes; *ABCB11* (dexamethasone), *ABCB2* (dexamethasone), *ABCC2* (pregnenalone-16 carbonitrile, dexamethasone), *ABCC3* (3-methylchoantrine, β-naphthoflavone, and omeprazole), and *SLCO1A2* (pregnenalone-16 carbonitrile, dexamethasone, pregnenalone-16 carbonitrile). While our patients did not receive most of these compounds, glucocorticoids were prescribed in 36% of mycophenolic acid-treated and 86% of cyclophosphamide-treated patients. Based on this previous data, glucocorticoids could be predicted to induce *ABCC2* and possibly *SLCO1A2*. This presumption is compatible with the finding of high expression of both transcripts in our SLE and SVV patients.

Since recent publications have reported gender divergent effects on *UGT* transcript and tissue expression in mice [12,13] and reduced activity of UGTs females, [41] we wanted to evaluate the gender-stratified expression of our evaluated genes in the glomerulonephritis population.

While none of these assessments reached statistical significance, a trend was noted in female patients having 3-fold higher expression of *ABCG2* than males. This finding is interesting as females compose the majority of SLE patients and we also found higher expression of *ABCG2* in this disease group; implying that a disease-gender interaction may be confounding. Regarding race effects, the expression of *ABCC2* in leukocytes was found to be lower in Caucasian than non-Caucasian SLE and SVV patients. The non-Caucasian group comprise the majority of SLE patients and these patients are disproportionately African-American. African-American SLE patients have worse treatment related outcomes[42] and it is plausible that reduced intracellular concentrations of therapies may be contributing.

The role of genotype on expression of *ABCB1* was recently reported in a study that isolated peripheral blood cells from healthy subjects and incubated them *in vitro* with lipopolysaccharide (LPS).[14] The investigators evaluated the effect of acute inflammation by LPS as compared to baseline, on *ABCB1* transcript expression. The authors stratified their study results according to patient genotype at the *ABCB1* nucleotide base location 3435. The results showed decreased *ABCB1* expression in the blood of patients exhibiting the C/C (wildtype) and C/T (heterozygote) genotypes and no effects in those with the T/T genotype. However, the published data concerning P-glycoprotein activity in patients who are homozygous wildtype versus homozygous variant for the *ABCB1 C3435T* polymorphism are conflicting. [43] In the current study, we found higher *ABCB1* expression in patients who exhibited the C/T and T/T genotypes as compared to the wildtype (C/C) genotype, a finding consistent with the literature. [14,43]

Since drug therapy may alter drug metabolizing enzymes and transporters and we had existing data on mycophenolic acid and cyclophosphamide/4-hydroxycyclophosphamide pharmacokinetics in our glomerulonephritis patients, we evaluated for correlations with leukocyte transcript expression. In providing plausible explanation for these findings, it is necessary to make the assumption that leukocyte expression correlated directly with liver and/or kidney protein expression. *ABCC2* significantly negatively correlated with both

cyclophosphamide clearance and 4-hydroxycyclophosphamide AUC, suggesting enhanced clearance of the former and enhanced exposure to the later when the *ABCC2* transcript is reduced. Since cyclophosphamide's metabolism is quite complicated and 4-hydroxycyclophosphamide is highly reactive, information regarding transport must be inferred from assessments of other downstream metabolites. It has been suggested that MRP2, MRP4, and possibly BCRP2 contribute to the disposition of 4-hydroxycyclophosphamide. [44] The protein of *ABCC2*, e.g. MRP2 is localized to the apical (bile cannilicular) membrane of liver and serves to efflux organic anions from hepatocytes. Decreased MRP2 protein in liver would be hypothesized to result in reduced loss of 4-hydroxycyclophosphamide from the liver and enhanced opportunity for efflux through MRP4 at the basolateral membrane, with increased AUC. We found a negative correlation between *ABCG2* expression and mycophenolic acid trough concentrations. This data would imply that increased BCRP protein and/or activity would result in increased loss of mycophenolic acid glucuronide by urinary excretion, resulting in a decrease in Ctr. The interplay between drug metabolism and transport should be considered when evaluating and predicting overall effects on drug disposition. [45]

Conclusions

The current study showed differential expression patterns of drug metabolizing enzyme and transporter transcripts in patients with glomerulonephritis as compared to healthy control subjects. Treatment and demographic variables were associated with significant differences in expression. This study adds to the sparse literature describing the transcript expression of drug transporters in leukocytes and focuses on a disease in which patients receive therapies targeted to the lymphocytes. Additionally, this study provides initial information pertaining to expression of drug metabolizing enzyme transcripts in leukocytes. This basic knowledge is required as transcript and ultimately protein expression of drug metabolizing enzymes and transporters can modulate the exposure to active pharmacologic moieties in the blood and tissues. This inital data may guide future investigations into mechanisms for altered responses in order to improve

patient-related exposures to therapies targeting leukocytes and to support efforts to measure protein expression in tissues by absolute quantitative methods such as mass spectroscopy. [46] It will be necessary to test the current study's findings in another cohort of patients to determine the generality of these associations. Large prospectively designed studies with serial expression profiles will be necessary to validate cause and affect relationships.

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Table 6.1

Demographics of Glomerulonephritis Patients

Data presented as n (percentage)

	Small vessel vasculitis	Systemic lupus erythematosus
	(n=35)	(n=36)
Race (%)		
Caucasian	25 (71%)	8 (22%)
Non-Caucasian	10 (29%)	28 (78%)
Gender (%Female)	20 (57%)	28 (78%)
Treatment (%)		
Cyclophosphamide	7 (20%)	15 (42%)
Mycophenolic acid	28 (80%)	21 (58%)

Table 6.2

Transcript Values in the Evaluated Groups (mean±SD)

	SVV (n=35)	SVV-Control (n=5)	SLE (n=36)	SLE-Control (n=5)	HC (n=10)
UGT1A9	0.98±2.24	NA	0.62±1.27	0.34±0.27	0.94±1.73
UGT2B7	2.46±6.38	0.52±0.00	2.13±4.87	1.35±1.78	1.00±1.64
UGT1A7	0.17±0.42[a]	0.27±0.00	0.03±0.10[b]	0.22±0.21	0.79±2.02
CYP2B6	0.50±0.57	0.15±0.12	1.49±2.55	0.50±0.62	1.0±0. 99
ABCB1	0.65±0.96	0.54±0.60	0.33±0.21[c]	0.45±0.31	1.00±0.82
ABCC2	1.06±1.11 [d]	2.02±1.13	1.35±1.21	1.60±1.08	1.00±0.41
ABCG2	0.17±0.14 [e]	0.01±0.0	0.31±0.33	0.10±0.07	1.0±1.82
SLCO1A2	1.45±3.68	NA	0.47±0.75	0.01±0	0.84±0.99

a – SVV < HC; p<0.05

b – SLE < HC; p<0.05

c – SLE < HC; p<0.05

d – SVV < SVV-control; p=0.05

e - SVV < HC; p<0.05

Abbreviations

ABCB1 – multidrug resistance protein

ABCC2 – multidrug resistance-associate protein

ABCG2 – breast cancer resistance protein

ANCA – antineutrophil cytoplasmic antibody

CYP – cytochrome P450

HC – healthy control

NA – not applicable

SLCO1A2 – organic anion transporting polypeptide

SLE – systemic lupus erythematosus

SVV – small vessel vasculitis

UGT – uridine-glucuronosyltransferase

Table 6.3

Genotype Frequency Distributions (frequency (n))

		SLE and SVV Patients
UGT1A7		
T622C	T/T	0.53 (35)
	T/C	0.42 (28)
	C/C	0.05 (3)
UGT2B7		
C802T	C/C	0.39 (26)
	C/T	0.42 (28)
	T/T	0.19 (13)
CYP2B6		
C1459T	C/C	0.82 (55)
	C/T	0.15 (10)
	T/T	0.03 (2)
G516T	G/G	0.49 (33)
	G/T	0.43 (29)
	T/T	0.08 (5)
ABCB1		
C3435T	C/C	0.34 (23)
	C/T	0.55 (37)
	T/T	0.11 (7)
C1236T	C/C	0.43 (29)
	C/T	0.49 (33)
	T/T	0.08 (5)

Abbreviations

ABCB1 – multidrug resistance protein

CYP – cytochrome P450

UGT – uridine-glucuronosyltransferase

Table 6.4

Relationships Between Transcript Expression and Patient-Level Data In Subjects with Systemic Lupus Erythematosus and Small Vessel Vasculitis

Transcript Variable	Patient-Level Variable	Parameter Esitmate	P value
ABCB1	Gender	0.070	0.542
	Race	0.061	0.558
	Treatment	-0.049	0.660
	Disease	0.152	0.144
	ABCB1 C3435T genotype	-0.194	0.078
	ABCB1 C1236T genotype	-0.092	0.385
ABCC2	Gender	0.113	0.203
	Race	-0.157	0.049
	Treatment	0.113	0.184
	Disease	-0.141	0.078
ABCG2	Gender	0.224	0.093
	Race	-0.070	0.562
	Treatment	0.058	0.657
	Disease	0.040	0.831
CYP2B6	Gender	0.140	0.531
	Race	-0.196	0.330
	Treatment	0.537	0.010
	Disease	-0.142	0.483
	CYP2B6 A785G genotype	0.049	0.906
	CYP2B6 C1459T genotype	-0.166	0.533
	CYP2B6 G516T genotype	0.083	0.680

Transcript expression results were log 10 transformed for analyses.

Figure Legends

Figure 6.1 – Real-time RT-PCR for Quantification (amplification plot) of *ABCB1* mRNA in Leukocytes of Patients with Glomerulonephritis Secondary to Systemic Lupus Erythematosus and Small Vessel Vasculitis. *ABCB1* was expressed in 95% of patients.

Figure 6.2 – Real-time RT-PCR for Quantification (Amplification Plot) of *UGT1A7* mRNA in Leukocytes of Patients with Glomerulonephritis Secondary to Systemic Lupus Erythematosus and Small Vessel Vasculitis. *UGT1A7* was expressed in 50% of patients.

Figure 6.1

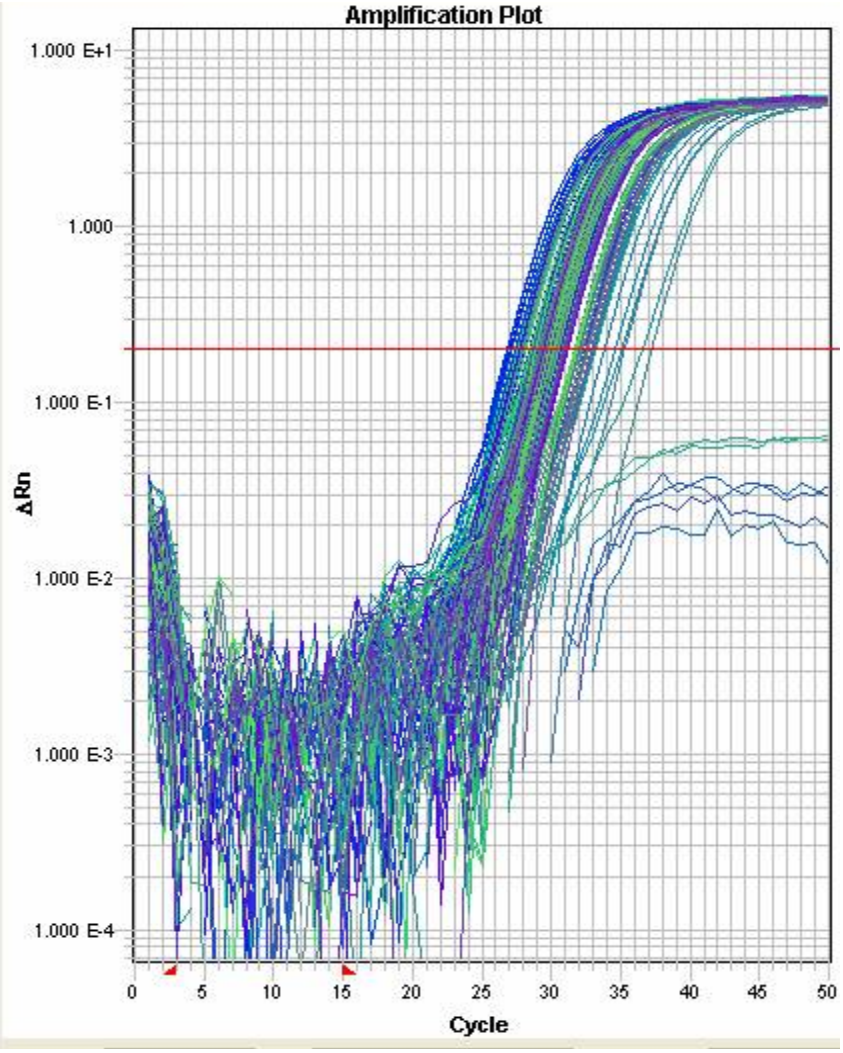
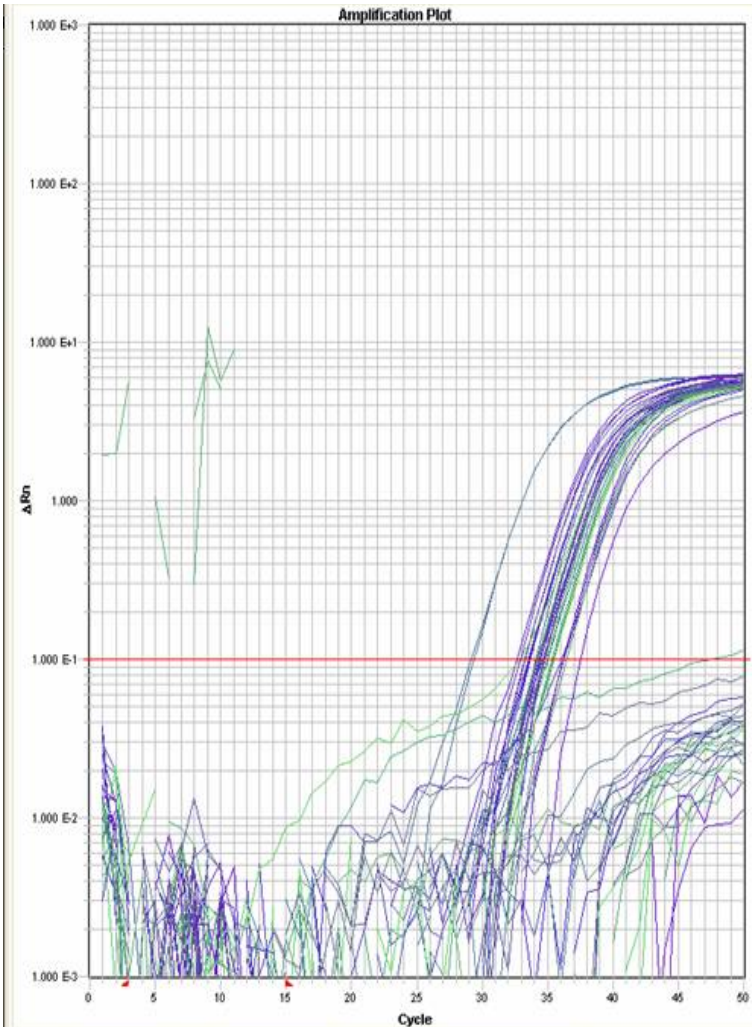

Amplification Plot
1.000 E+1
1.000
1.000 E-1
1.000 E-2
1.000 E-3
1.000 E-4
ΔRn
0
5
10
15
20
25
30
35
40
45
50
Cycle

Figure 6.2


Amplification Plot
1.000 E+3
1.000 E+2
1.000 E+1
1.000
1.000 E-1
1.000 E-2
1.000 E-3
ΔRn
0
5
10
15
20
25
30
35
40
45
50
Cycle

Chapter 7

DETERMINANTS OF KIDNEY OUTCOMES TO MYCOPHENOLIC ACID-BASED THERAPIES IN GLOMERULONEPHRITIS

Melanie S. Joy, PharmD,[1,2] Jinzhao Wang, BS,[1] Yichun Hu, MS,[1] Tandrea Hilliard, MPH,[1] Tammy Boyette, BS,[1] Susan L. Hogan, PhD,[1] MPH, Paul Stewart, PhD,[3] Tamara Gregory,[1] Gabrielle Y. Galloway,[1] Mary Anne Dooley, MD,[1] Keisha Gibson, MD,[1] Sophia Lionaki,, MD,[1] Caroline Jennette, BS,[1] Philip C. Smith, PhD,[2] Ronald J. Falk, MD.,[1] University of North Carolina at Chapel Hill, [1]School of Medicine, UNC Kidney Center, [2]Eschelman School of Pharmacy, and [3]School of Public Health, Chapel Hill, NC

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Introduction

The pharmacologically active immunosuppressive agent mycophenolic acid, is used off-label for the treatment of autoimmune-mediated glomerulonephritis, e.g. systemic lupus erythematosus (SLE) and small vessel vasculitis (SVV). Several studies now published regarding the SLE nephritis population support the efficacy and safety of mycophenolic acid for induction and maintenance regimens. [1-6] There also appears to be mounting evidence supporting the improvement in kidney outcomes in African-American SLE patients receiving mycophenolic acid based regimens as opposed to those containing cyclophosphamide. [6,7] Data concerning outcomes to mycophenolic acid therapy for SVV patients are more limited and consist of mostly small studies.[8-11] A recently completed, but unpublished larger trial (IMPROVE) compared maintenance therapy with azathioprine versus mycophenolate mofetil in 175 patients with SVV. There is currently a paucity of data that enables clinicians to predict which glomerulonephritis patients will respond most or least favorably to mycophenolic acid therapy. Additionally, there is currently no solid evidence supporting any targeted mycophenolic acid plasma concentrations or exposures that are most optimal for producing favorable kidney outcomes in patients with glomerulonephritis.

Patients with glomerulonephritis can have alterations in serum albumin, kidney function (glomerular filtration rate (eGFR)), and urinary protein excretion, all of which may alter drug disposition and could influence therapy responsiveness. Additionally, studies in the transplant literature have reported wide inter-patient variability in pharmacokinetics, limiting the applicability of one patient's data to another. [12,13] Previous mycophenolic acid pharmacokinetic studies by our group in patients with SLE nephritis and antineutrophil-cytoplasmic antibody (ANCA) SVV patients have been published. [14,15] The reports demonstrated greater urinary protein excretion and lower serum albumin in the SLE nephritis population [14], and more severe kidney dysfunction as defined by creatinine clearance in the SVV patients [15]. A consistent finding in both SLE and SVV population studies was an increased oral clearance of

mycophenolic acid as compared to reports in kidney transplant recipients. The increased oral clearance in SLE nephritis patients was associated with increased creatinine clearance and decreased serum albumin. [14] Assessment of oral clearance according to urinary protein excretion as a marker for kidney structure abnormalities showed enhanced clearance with urinary protein excretion values of $\geq$ 1g/day. [14] Enhanced clearance has the potential for reducing plasma concentrations and overall exposure to therapeutic agents. Positive relationships between plasma concentrations and/or subsequent exposure and outcomes may require assessment of patient level clinical data to guide therapy decisions and optimize treatment-related outcomes.

In addition to the influence of clinical data on altered mycophenolic acid pharmacokinetics, the presence and influence of single nucleotide polymorphisms (SNPs) in the uridine diphosphate glucuronosyltransferase metabolizing enzymes (UGTs) have been reported in kidney transplant patients. [16-21] Single nucleotide polymorphisms at the *UGT1A9* promotor have been associated with enhanced metabolism [16,20], while other non-promoter *UGT* SNPs are associated with enhanced exposure to MPA, [16] suggesting a reduction in metabolism. Reports linking SNPs in drug metabolism genes to altered mycophenolic acid pharmacokinetics or outcomes in the glomerulonephritis population are currently lacking. Since the SVV population is primarily Caucasian and the SLE population is represented by African-Americans as well as Caucasians, it is feasible that pharmacogenomic factors may be contributing at different levels to therapeutic outcomes in the two forms of glomerulonephritis. Data concerning the frequency of *UGT* SNPs in the glomerulonephritis population as compared to other reference populations also requires assessment to begin to evaluate for any disease-gene association. This is an intriguing area for exploration as the UGT enzymes exist in the body primarily for detoxification of environmental chemicals and toxins and this may be relevant in diseases such as SLE and SVV, as both diseases are proposed to have environmental causes. [22-24] Data from the cancer literature report disease-*UGT* associations and cancer risks. [25-28]

In the current study, we sought to evaluate for predictors of outcomes to mycophenolic acid therapy in glomerulonephritis patients with SLE and SVV. The specific outcomes of interest included; attainment of a composite outcome (dialysis, transplantation, death), changes in serum creatinine (SCr), changes in estimated glomerular filtration rate (eGFR), and changes in urinary protein to creatinine excretion ratio (UP:Cr). We also investigated the influence of genetic polymorphisms in MPA drug metabolizing enzyme genes (*UGT1A7*, *UGT1A9*, *UGT2B7*) and a transporter gene (*ABCB1/MDR1*) associated with efflux of MPA [31], on mycophenolic acid therapy outcomes, as well as risk factors for SLE or SVV. We also explored associations of mRNA expression patterns of metabolizing enzyme genes and transporter genes in leukocytes and outcomes. Lastly, we evaluated pharmacokinetic variables representing drug exposure, as defined by area under the plasma concentration time curve (AUC) and trough plasma concentrations (Ctrough), to assess their relationships with treatment outcomes.

Methods

Research Subjects

A population of 85 patients with glomerulonephritis due to SLE and SVV who were receiving or who had received therapy with mycophenolic acid were enrolled in the study. This treatment population had existing long-term follow-up consents in place through the Glomerular Disease Collaborative Network (GDCN) and subsequently had clinical and demographic follow-up data available. A subgroup of this population (n=45) was actively recruited to participate in a mycophenolic acid pharmacokinetics evaluation requiring an inpatient visit to the clinical research center. Details concerning the design, conduct and results from the pharmacokinetic studies were recently reported. [14,15] Results for mycophenolic acid exposure (dose normalized $AUC_{0\text{-}Tau}$, where Tau is the dosing interval) and dose-normalized trough plasma concentrations (Ctrough) were abstracted for evaluation of relationships with treatment outcomes. These later patients also had blood obtained, processed, and assayed for mRNA expression.

Four cohorts of 269 patients (SVV, SLE, rheumatoid arthritis, and healthy controls) were evaluated for assessment of the frequency of common genetic variants in the MPA drug metabolizing enzyme genes *UGT1A9*, *UGT1A7*, and *UGT2B7* and the drug transporter gene multidrug resistance protein (*ABCB1/MDR1*). Patients with biopsy confirmed SLE or SVV with kidney manifestations were included in the SLE and SVV cohorts. Patients with rheumatoid arthritis, an autoimmune disease without kidney manifestations, and healthy control patients with no kidney disease and no autoimmune disease were included into these later two respective control cohorts.

For all SVV and SLE subjects, data was abstracted from medical charts and included kidney biopsy activity and chronicity scores, proteinase 3 (PR3)/myeloperoxidase (MPO) antineutrophil cytoplasmic antibody (ANCA) status, WHO classification of SLE nephritis [29] , glucocorticoid dose (if applicable), mycophenolate mofetil dose at time of evaluation, and duration of disease follow-up. Serum creatinine and UP:Cr were collected at time of biopsy, time of treatment, and time of last available follow-up. Estimated glomerular filtration rate (eGFR) was calculated by the four variable Modification of Diet and Renal Disease Equation. [30] Abstracted demographic data included age, weight, race, and gender. The study and consent forms were approved by the University's Institutional Review Board and patient consent was required prior to participation.

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, Inc., Valencia, CA, USA). Genotyping was conducted for several published SNPs in *UGT1A9, UGT1A7, UGT2B7* and *MDR1/ABCB1,* all previously reported to result in alterations in mycophenolic acid metabolism and/or transport. [16-18,20,21,31] Data regarding the assays and conditions for genotyping assessments have previously been reported. [32] All genotyping results were coded as 0 (wildtype/wildtype), 1 (heterozygote), or 2 (variant/variant).

mRNA Expression Analyses

For mRNA expression analyses, a 15mL blood sample was obtained from multiple ethylenediaminetetraacetic acid (EDTA) vacutainers. Leukocytes were isolated from whole blood by incubation (11 minutes) in a lysis buffer, followed by centrifugation and a wash with Hank's balanced salt solution (HBSS). The leukocytes were subsequently suspended in RNA Stat 60 solution and stored at -70C for up to 2 weeks until processing. The procedures for mRNA isolation and cDNA conversions have been previously reported. [33] Pre-designed assays containing primers and probes were purchased from Applied Biosystems (Foster City, CA) for assessment of transcript expression of the targeted metabolizing enzymes (*UGT1A7, UGT1A9, UGT2B7, CYP3A4, CYP2C9,* and CYP*2B6*) and transporters (*ABCB1, ABCC2, ABCG2,* and *SLCO1A2*); *UGT1A7* (Hs02517015_s1), *UGT2B7* (Hs02556232_s1), *UGT1A9* (Hs02516855_sH), *CYP3A4* (Hs00604506_M1), *CYP2C9* (Hs00426397_m1), *CYP2B6* (Hs00167937_g1), *ABCC2* (Hs00166123_m1), *ABCB1* (Hs00184500_m1), *ABCG2* (Hs01053795_m1), and *SLCO1A2* (Hs01072338_m1). Cytochrome C oxidase was used as the normalization (housekeeping) gene. The forward and reverse primers were designed using Primer Express software (Applied Biosystems, Foster City, CA). The forward primer (TGGCATCTGGAGGTGGTGTT) and reverse primer (GTCCAGTCCCTTTGCAGC) were purchased from Applied Biosystems (Foster City, CA). Sybr 1:400 was used as the probe in the assays (Molecular Probes, Leiden, Netherlands). Data concerning the specific assay conditions was previously reported. [33]

Statistical Analysis Strategy and Methods

Descriptive statistical methods were applied to the demographic (age, race, gender), clinical (SCr, eGFR, UP:Cr), pharmacokinetic data ($AUC_{0\text{-}Tau}$, Ctrough), and genotype data (*UGT1A9 G8A, C98T, C-2152T, T-275A, UGT2B7 C802T, UGT1A7 T622C,* and *MDR1 C1236T* and *C3435T*) to provide summary tabulations of frequencies, means, standard deviations, and ranges.

Differences in demographic and clinical variables were assessed between SLE and SVV disease groups by unpaired T Test with Welch Correction for continuous variables and Fisher's Exact Test for categorical variables. Absolute and percent changes in outcome clinical measures (absolute changes in SCr, eGFR, and UP:Cr) were assessed between disease groups by Wilcoxan Two Sample Tests. Kaplan Meier curves were generated to test for composite survival probability between disease groups employing the composite outcome of dialysis, transplantation, or death.

For each SNP, tabulated cohort-specific allelic frequencies were used in a chi-square test of the null hypothesis, "no differences among the four cohorts (SVV, SLE, rheumatoid arthritis, healthy control)." The observed genotype frequencies for each defined locus were used in a chi-square test procedure for testing of the null hypothesis, "no deviation from Hardy-Weinberg equilibrium." Differences in genotype frequencies between disease groups versus healthy control group were evaluated by Fisher's Exact Test. The logistic procedure was used to evaluate the odds of having SVV, SLE, or rheumatoid arthritis based on genotype, with wildtype/wildtype as the comparator genotype. Relationship between specific genotypes and absolute changes in eGFR, SCr, and UP:Cr were assessed by the Kruskal-Wallis Test.

Relationships between mRNA expression and pharmacokinetics with composite kidney outcomes were assessed by the Wilcoxan Two-Sample test. Spearman Correlation Coefficients were used to assess relationships between the absolute changes in eGFR, SCr, and UP:Cr and gene expression and pharmacokinetics.

Following descriptive graphical examinations of the relationships between the outcomes and the various patient variables, simple linear models were fitted for each outcome variable conditional on selected clinical, demographic, or genotype variables. Variable selection algorithms (e.g., stepwise selection, backward elimination) were also applied to construct a multivariable linear model for each outcome variable conditional on clinical, demographic, or genotype predictors. For each outcome variable, a final model was selected based on

considerations of the statistical significance of the candidate predictor variables and the overall model R^2 . All statistical computations were performed using SAS System software (Version 9.1, SAS Institute, Inc., Cary, NC.)

Results

Demographic and combined clinical data from 85 patients with glomerulonephritis receiving therapy with mycophenolic acid were available; 37 with SLE and 48 with SVV.(Table 7.1) The SLE patients were younger (39±11 versus 54±16 years; p<0.0001) and had a higher percentage of African American patients (46% versus 10%; p=0.0003) than SVV patients. Baseline serum creatinine (2.5±2.3 versus 1.5±1.3 mg/dL; p=0.0247) was higher in the SVV patients and UP:Cr (2.8±3.4 vs 1.2±1.6; p=0.0450) was higher in the SLE patients. The patients received an average daily mycophenolate mofetil dose of 1600±820 mg and total therapy duration/exposure was 1.3±2.1 years. A total of 96% of patients had exposure to glucocorticoids and 85% had exposure to cyclophosphamide throughout their disease course. Total duration of glomerulonephritis follow-up was 4.6±3.6 years. Available data (75% of patients) concerning biopsy staining patterns for ANCA showed; PR3 (n=22; 61%) and MPO (n=14; 36%) sero-positivity. Data concerning SLE nephritis classification by WHO criteria was available for 73% of mycophenolic acid-treated patients; Class 3, (22%), Class 4 (67%), and Class 5 (22%), with some patients having a mixed classification. Scores from SLE biopsies for activity and chronicity were 7±4 and 3±2, respectively. For the SLE cohort, Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [34] (3.6±4.2) and Damage Index (DI) [35] (0.9±1.4) scores were reported. For the SVV cohort, Birmingham Vasculitis Assessment Scores (BVAS) [36] (0.59±1.2) and Vasculitis Damage Index (VDI) [37] (2.2±1.7) scores were reported.

Absolute changes and percentage changes in SCr, eGFR, and UP:Cr were calculated at disease diagnosis, during mycophenolic acid therapy, and final follow-up for the 85 patients. Figure 7.1 and Table 7.2 demonstrate actual values for serum creatinine, eGFR, and UP:Cr at

disease diagnosis, treatment, and follow-up. As noted in Table 7.2, the SVV patients had statistically significant lower eGFR and increased SCr as compared to SLE patients at each evaluation period. Overall, the SVV patients had higher serum creatinine and lower eGFR at all time points as compared to the SLE patients. The SCr increased by 0.2±41% and decreased by 6.4±60.7% in SLE and SVV patients (p=0.1837) over time, respectively, from disease diagnosis to follow-up for an overall change of -3.3±52.4%. The absolute change in SCr between SVV (-0.60±2.5 mg/dL) and SLE (-0.04±0.9 mg/dL) patients was not statistically significant (P=0.1207). A total of 22 (32.0%) patients with available paired serum creatinine results (biopsy and follow-up) had at least a 50% increase in serum creatinine from diagnosis to the end of follow-up. The eGFR increased by 93.2±302% and 8.6±44% in SVV and SLE patients (p=0.0755), respectively, from disease diagnosis to follow-up for an overall change of 55.3±229%. The absolute change in eGFR between SVV (11.1±25.1 mL/min/1.73m^2) and SLE (0.2±29.3 mL/min/1.73m^2) patients was not statistically significance (P=0.2635).

The SLE patients had higher UP:Cr as compared to the SVV patients at all time points, although these differences were not statistically significant.(Table 7.2) The UP:Cr decreased by 11.0±106% and increased by 28.5±273% in SLE and SVV patients (p=0.5882), respectively, for an overall increase of 9.7±209%. Absolute changes in UP:Cr for SLE (-1.3±3.5) and SVV (-0.2±2.2) patients were not different (p=0.6505). A total of 7 (16.7%) of patients with available paired UP:Cr results had at least a 50% increase in UP:Cr from diagnosis to follow-up. These 50% increases in UP:Cr and SCr data suggest that up to ~32% of patients had at least a partial disease relapse during the course of their maintenance therapy with mycophenolic acid.

Kaplan Meier survival curves for the composite outcome of hemodialysis, transplantation, or death were generated for patients receiving mycophenolic acid. (Figure 7.2) Survival estimates were similar between the SLE and SVV patients (p=0.1100). These data show a 2-year and 5-year estimated kidney survival in SLE patients of 100% and 90.3%, respectively while receiving

mycophenolic acid. The data for SVV patients were similar, with 2-year and 5-year estimated kidney survival of 83.9% and 79.9%, respectively.

A total of 269 discrete DNA samples were available for genotyping assessments. The patient groups and numbers included; 101 patients with SVV, 67 patients with SLE, 26 patients with rheumatoid arthritis, and 75 healthy controls. The allelic and genotype frequencies are reported in Tables 7.3 and 7.4, respectively. The expected vs observed genotype frequencies within each patient cohort were in Hardy Weinberg Equilibrium. Fisher's Exact test demonstrated differences between the disease cohorts for the *UGT2B7* SNP (p=0.0002) and for the *UGT1A7* SNP (p=0.0123). Genotype frequencies across disease cohorts for the *UGT2B7* variant/variant genotype were; healthy control (0), rheumatoid arthritis (0), SLE (0.08), and SVV patients (0.17). For the *UGT1A7* variant/variant genotype, frequencies across disease cohorts were; SVV (0.07), SLE (0.15), healthy control (0.12), and rheumatoid arthritis patients (0). Additional analyses controlling for race were performed to assess disease-genotype associations.(Table 7.5) When controlling for race, the odds of having SVV was 3.073 (C.I. 1.530-6.172, p=0.0016) when patients were classified as being either heterozygote or variant/variant genotype for *UGT2B7 C802T*. The odds of having SVV was reduced to 0.414 (C.I. 0.215-0.796, p=0.0082) when patients were heterozygotes or variant/variant genotype for *UGT1A7 T622C*.

The influence of *UGT* and *ABCB1* genotypes on the absolute changes in eGFR, SCr, and UP:Cr from diagnosis to follow-up were also examined.(Table 7.6) For glomerulonephritis patients who received mycophenolic acid, a significant genotype-change in kidney function parameter UP:Cr was found. There was a statistical trend between *UGT1A7* variant/variant genotype (p=0.0706) and increased overall UP:Cr from diagnosis to follow-up as compared to wildtype and heterozygotes, who had an overall reduction in change in UP:Cr. A statistically significant finding was shown in *ABCB1 C3435T* genotype (p=0.0409) with lesser increases or actual decreases in UP:Cr seen in the variant/variant and heterozygote groups. No significant

effects of genotypes on SCr or eGFR were noted for patients receiving mycophenolic acid. We were limited in our assessment of the effects of genotypes on composite outcomes in patients receiving MPA secondary to the limited number of composite outcomes in this group. We found that being heterzygote or variant/variant for *UGT2B7 C802T* resulted in a reduction in composite outcome, although the p value was 0.0983. When grouping both mycophenolic acid- and cyclophosphamide-treated glomerulonephritis patients by genotype status for *ABCB1 C1236T, ABCB1 C3435T, UGT1AT 7622C,* and *UGT2B7 C802T* SNPs, no significant differences in composite kidney outcomes were demonstrated.

Pharmacokinetic and mRNA expression data were available for a subset of 45 glomerulonephritis patients who received mycophenolic acid; 27 SVV and 18 SLE patients. We planned to evaluate the relationships between mycophenolic acid exposure (dose normalized $AUC_{0\text{-}Tau}$, dose normalized Ctrough) and kidney outcomes. Since the $AUC_{0\text{-}Tau}$ (64.4±50 mcg h/mL versus 68.9±42.7 mcg h/mL) and Ctrough (4.1±5.5 mcg/mL versus 4.3±4.1 mcg/mL) values were similar in SLE and SVV patients, respectively, the two disease groups were combined. As there were too few patients in this subgroup who exhibited the composite outcomes of dialysis, transplantation, or death, the results focused on the correlations in changes in eGFR, SCr, and UP:Cr from diagnosis to follow-up with $AUC_{0\text{-}tau}$ and Ctrough. No significant correlations were demonstrated in kidney function changes and exposure to mycophenolic acid.

As there were no statistical differences in transcript expression of metabolizing enzymes (*UGT1A9*, *UGT2B7*, *UGT1A7*) and transporters (*ABCC2*, *ABCG2*, *SLCO1A2*) between the SLE and SVV disease groups, they were combined for analyses of the relationship to kidney outcomes (changes in eGFR, serum creatinine, and UP:Cr from diagnosis to follow-up). No significant correlations were demonstrated in kidney function changes and transcript expression of drug metabolizing enzymes and transporters in peripheral blood cells in patients exposed to mycophenolic acid therapy. Analysis of composite outcomes based on transcript expression

patterns in the leukocytes of the combined patients receiving either cyclophosphamide or mycophenolate mofetil resulted in *ABCB1* transcript expression that was lower (0.2±0.2) in patients who had composite outcomes versus those who did not (0.6±0.8); p=0.0150.

Discussion

The goal of the current study was to identify predictors of kidney outcomes in a well-characterized population of patients with glomerulonephritis due to either SLE or SVV who were treated with mycophenolic acid. The purpose for this study was to generate a personalized tool kit that clinicians could use to select the candidates for mycophenolic acid therapy who would be predicted to have the most benefit in terms of improvement in prevention of kidney function. This is particularly relevant for patients with SLE and SVV, since drugs including mycophenolic acid are used off-label and were never evaluated in glomerulonephritis patients as a whole via rigorous clinical development studies that would have included pharmacokinetic elucidation, drug dosing scheme assessments, safety evaluations, and efficacy evaluations. Our previous work, in fact, demonstrated altered pharmacokinetics of mycophenolic acid in patients with SLE and SVV as compared to a population of kidney transplant patients, the later subjects in whom the drug has FDA approval. [14,15,38,39] Several clinical factors including urinary protein excretion, serum creatinine, weight, and race, and to a lesser extent, genomic factors including single nucleotide polymorphisms in *UGT2B7* and *UGT1A7,* were found to predict mycophenolic acid pharmacokinetic outcomes including oral clearance and trough plasma concentrations. [32] Regarding pharmacogenomic factors and treatment outcomes, we report in this publication that changes in UP:Cr over the treatment course with mycophenolic acid were significantly worsened in patients with the *UGT1A7 C622T* polymorphism and improved with the *MDR1 C3435T* polymorphism. Additionally, the expression of the *MDR1* transcript in the leukocytes was reduced in patients who experienced the composite kidney outcome of dialysis, transplantation or death. The data also demonstrated a relationship between SVV disease and the *UGT2B7 C802T* polymorphism. We unexpectedly found a higher risk of SVV disease in

patients with the *UGT2B7 C802T* polymorphism. However, patients with the polymorphism and glomerulonephritis tended to have improved composite outcomes. As mycophenolic acid is a substrate for UGT2B7, decreased liver activity of this enzyme would be predicted to increase systemic exposure to mycophenolic acid, leading to improved kidney outcomes. Our previous report did in fact, show increased MPA exposure in patients who were homozygous for the C802T polymorphism in *UGT2B7*. [32] We also showed decreased MPA renal clearance in these homozygous patients that could be reflective of a combination of decreased metabolism to the acyl glucuronide metabolite and decreased hydrolysis of this later metabolite in the urine. However, the hydrolysis of acyl metabolite in urine has not been previously elucidated.

Since most of the study population was enrolled into the Glomerular Disease Collaborative Network's database within our institution, the patients were well characterized and had outcome measures readily available. However, since the population consisted of both SLE and SVV patients, differences in baseline clinical measures and demographics were found. Predictably, the SLE group was younger and consisted of a higher percentage African-American race than the SVV group. Regarding baseline clinical laboratories, the SVV group had higher SCr and the SLE group had higher UP:Cr. Activity indices were higher and damage indices were lower for the SLE versus SVV patients using their respective SLEDAI/DI and BVAS/VDI assessment tools, respectively.

Evaluation of changes in the kidney outcome parameters of eGFR, SCr, and UP:Cr during mycophenolic acid therapy demonstrated differences between disease groups. The SVV patients had changes that favored non-statistically significant decreases in SCr and increases in eGFR over a mean follow-up period of 4.6±3.6 years; albeit the increase in eGFR was on the order of 11 mL/min/1.73m^2. The SLE patients had changes in UP:Cr that favored an order of magnitude decrease (-1.3) over the SVV patients (-0.2), although these changes were not statistically significant. A 50% increase in SCr and/or UP:Cr, suggesting at least a partial

disease relapse on mycophenolic acid therapy was demonstrated in 32% and 17% of patients, respectively.

Previous small studies of mycophenolic acid maintenance therapy in patients with SVV have reported stabilization of kidney function and remission at 15 months. [11] In one recent study of Asian patients with ANCA vasculitis being treated for induction of remission, 78% of mycophenolic acid-treated and 47% of cyclophosphamide-treated patients had complete remission, [9] suggesting a better resonse to mycophenolic acid versus cyclophosphamide therapy in Asian patients. Additionally, 44% of patients receiving mycophenolic acid recovered kidney function. [9] Lanford, et al reported a relapse rate of 43% for Wegener's Granulomatosus patients receiving mycophenolic acid. [10] Previous reports in the SLE population have reported relapse rates of between 19% and 46% in patients receiving mycophenolic acid for maintence of remission. [2,3] These previous studies in SLE and SVV have reported relapse rates on mycophenolic acid therapy that were similar to our own data. We also assessed changes in urinary parameters and composite outcomes by race (data not shown), the results of which did not demonstrate any differences in these outcomes. We did not evaluate Asians as a group, however, since there were limited patients in this race category. Five year kidney survival was similar between glomerulonephritis patients treated with mycophenolic acid; 90% for SLE and 80% for SVV. Other publications have not reported estimated 5-year kidney survival based on mycophenolate mofetil treatment for maintenance of remission.

A previous meta-analysis in SLE nephritis reported a reduction in relative risk for all-cause mortality (RR 0.709; CI 0.373-1.347) and kidney failure (RR 0.453; CI 0.183-1.121) in patients who received either mycophenolic acid or intravenous cyclophosphamide therapies for induction. [40] Most recently, the results from the ASPREVA Lupus Management Study, which compared mycophenolate mofetil (dosing target of 3 g/day) to intravenous cyclophosphamide (monthly dosing target of 0.5 to 1 g/m^2) for induction of remission were published. [6] These results showed similar primary outcomes in each treatment arm. The specific outcomes

assessed were: decreases in UP:Cr to <3.0 in patients with nephrotic range proteinuria, or decreases by ≥50% in patients with sub-nephrotic proteinuria, and stabilization (±25%) or improvement in SCr at 24 weeks. The primary efficacy end-point was reached by 64% of mycophenolate mofetil-treated patients and 57% of cyclophosphamide-treated patients. [6] Overall, 52% of patients showed a ≥50% decrease in UP:Cr and 56% showed a ≥50% improvement in SCr from diagnosis while on mycophenolic acid therapy. Our data seems consistent with the results from the ASPREVA study. An important finding from the ASPREVA trial was that the racial group categorized as "other" and primarily comprised of African-American had a statistically significant reduction in efficacy with cyclophosphamide (38%) as opposed to similar efficacy with Caucasians in patients treated with mycophenolate mofetil (60%).[6] In the age of personalized medicine, this finding suggests that mycophenolic acid may be preferred over cyclophosphamide for African-American SLE patients. Another recent study reported clinical responses in only ~50% of Hispanic patients with SLE being treated with either mycophenolate mofetil or cyclophosphamide, [41] presenting an opportunity to evaluate the comparable efficacy of other therapies in this patient group. [41] Studies to evaluate the etiologies for differences in response to selected therapies are warranted to enable a comprehensive individualized therapy approach in glomerulonephritis.

The influence of genetic polymorphisms in MPA drug metabolizing enzyme (*UGT1A7*, *UGT1A9*, *UGT2B7*) and transporter (*MDR1)* genes on therapy outcomes were assessed in order to evaluate pharmacogenomics/genetics as a tool for individualizing therapy in glomerulonephritis patients receiving mycophenolic acid. A rheumatoid arthritis and healthy control cohort were included in our genomic evaluations to facilitate our understanding of allelic frequencies in SLE and SVV patients as compared to another autoimmune disease that does not afflict the kidneys and healthy patients. This information was pertinent for our understanding of the association between autoimmune kidney diseases and alterations in metabolizing genes and transporters. This hypothesis was reasonable given that numerous drugs and

environmental substances are substrates of these enzymes and transporter proteins. Based on the genetic background of the study population, an adequate frequency for polymorphisms in the *UGT1A7*, *UGT2B7*, and *MDR1* genes enabled the planned assessments. Some statistically significant differences across disease cohorts were demonstrated for *UGT1A7* and *UGT2B7*. The SVV cohort had a higher frequency of the *UGT2B7 C802T* variant/variant than healthy controls or rheumatoid arthritis cohorts (where it was absent in both cohorts), and had a 2-fold increase in the polymorphism frequency over the frequency in the SLE cohort. The *UGT1A7 T622C* variant/variant was found in a similar frequency in the SLE and healthy control populations, a 2-fold lower frequency was found in the SVV cohort, and the polymorphism was absent in the rheumatoid arthritis cohort.

The polymorphisms that we evaluated have been purported to have various effects on the pharmacokinetics of mycophenolic acid and its metabolites and this could partly explain treatment-related outcomes. The *UGT2B7 C802T* polymorphism has been purported to result in a 25% increase in mycophenolic acid area under the plasma concentration time curve of total as well as unbound drug, and increases in $C_{max,}$ suggesting a phenotype of reduction in UGT2B7 enzyme activity. [16,17] We previously showed decreased renal clearance and increased AUC of mycophenolic acid in patients who were heterozygotes for the *UGT2B7 C802T* polymorphism. [32] Since renal clearance is a component of total clearance, this finding of enhanced AUC is likely reflective of a reduction in the renal and nonrenal components of total clearance. A study compared plasma concentrations after an oral mycophenolate mofetil dose in *mdr1* and *mrp2* deficient mice to clarify the roles of each transporter in MPA disposition. The results showed increased brain concentrations of MPA in the *mdr1* deficient, but not the *mrp2* deficient mice, suggesting the possibility of mycophenolic acid being a substrate for P-glycoprotein. [31] Additionally, a slight reduction in plasma concentration was seen only in the sampling time just after dose in the *mdr1* deficient mice, but no effect on overall disposition was demonstrated. We previously reported that *UGT1A7 T622C* heterozygosity predicted increased

oral clearance and decreased Ctrough of mycophenolic acid, but we did not have adequate numbers of patients who were variant/variant to assess this genotype. [32] In this study, we expanded our previous research by assessing patient-related outcomes to mycophenolic acid therapy in accordance with genotype. In mycophenolic acid-treated patients, we noted differences in the absolute changes of the UP:Cr in relationship to *MDR1 C3435T* and *UGT1A7 T622C* genotype. Patients who were heterozygous or homozygous for the *UGT1A7* variant had a relative increase or only slight decrease in UP:Cr, suggesting a detrimental effect of this *UGT1A7* SNP on mycophenolic acid efficacy as measured by urinary protein excretion.

Patients who were heterozygous or homozygous variant for the *MDR1 C3435T* polymorphism had reductions or lesser increases in UP:Cr while receiving mycophenolic acid therapy, suggesting a beneficial effect of the *MDR1* SNP on immunosuppressant efficacy as measured by urinary protein excretion. P-glycoprotein, the translated protein product of *MDR1* is present in the tubules of the kidneys [42] and is proposed to be engaged in the transport of mycophenolic acid [31]. Enhanced activity of P-glycoprotein via the polymorphism would be hypothesized to enhance the renal elimination of MPA by either MPA itself or its glucuronide metabolites. The efficacy of MPA, however, should be viewed from its site of action in the lymphocytes and not at the kidney level. Hence, while renal P-glycoprotein may enhance renal elimination of MPA or its metabolites, this aspect cannot be translated directly to any mechanistic effects of the drug at the level of the kidney. Any true effects of MPA on surrogate measures of kidney function, such as urinary protein excretion or glomerular filtration rate are likely mediated through the circulating lymphocytes. Various publications in other disease states have described treatment-related outcomes based on polymorphisms in drug metabolizing enzymes and transporters. [43-50] Polymorphisms in *MDR1* at the nucleotide 3435 position and disease outcomes have been assessed in epilepsy, transplantation, and breast cancer. [43,44,49] While no effects of *MDR1* genotype on epilepsy outcomes were found, cardiac transplant patients prescribed standard triple-drug combination of cyclosporine, azathioprine,

and prednisolone and who were wildtype homozygous had a 1.8 times increased risk for having a rejection event in the first 12 months. [44] The presence of the variant/variant *MDR1 C3435T* genotype predicted clinical response for locally advanced breast cancer to anthracycline therapy. [49] These data follow the same positive direction of response as our current study in glomerulonephritis; demonstrating a beneficial effect of the variant/variant genotype on outcomes. Single nucleotide polymorphisms in other drug metabolizing enzymes have also been associated with various treatment-related outcomes. [45-48,50-52]

Since the SVV cohort exhibited higher frequencies of the evaluated *UGT2B7* polymorphism, we assessed whether this polymorphism increased the risk of autoimmune disease in SVV, SLE, and rheumatoid arthritis patients. Since the frequency of the *UGT2B7* variant allele has not been reported in African-American patients and since SLE and SVV patient populations are different in terms of racial composition, any analysis to evaluate for a gene-disease association requires controlling for race. When we controlled for race, we found an increased Odds Ratio (3.073; C.I. 1.53-6.17) for having SVV when exhibiting heterozygosity or homozygous variant for the *UGT2B7* polymorphism. Additionally, our data showed a reduction in Odds Ratio (0.414; C.I. 0.21-0.80) for SVV when patients were heterozygous or homozygous variant for the *UGT1A7* polymorphism. Previous studies have reported increases in the risk of colorectal, breast, bladder, and orolaryngeal cancers in patients with various SNPs in the *UGTs*, including the *UGT2B7 C802T* polymorphism. [25,27,28,53,54] Polymorphisms in *MDR1* have also been found to be predictive of end-stage kidney disease progression, regulation of the aldosterone system, and susceptibility to inflammatory bowel disease. [55-57] The *UGT2B7* polymorphism is a non-synonymous SNP resulting in a histidine to tyrosine (H268Y) amino acid change and is expressed in various tissues including the kidney, lung, liver, breast, brain, and intestine. [58] A previous study showed a lower activity of the variant protein toward detoxification/ glucuronidation of the tobacco carcinogen NNAL. [59] Endogenous bile acids and steroids, as well as therapeutic agents including nonsteroidal anti-inflammatory agents, retinoic acid, and

estradiol are substrates for UGT2B7. Our data is intriguing given that there are proposed environmental exposure risk factors for SVV, [22-24] and the UGT enzymes play a central detoxification role. Hence polymorphisms in genes encoding metabolizing enzymes that result in reduced detoxification efficacy or efficiency would be hypothesized to be risk factors for disease. We previously published a review on drug exposure associated SVV [60] and many of the medications in that report are moieties known to be substrates for metabolism by UGT2B7.

Pharmacokinetic variables representing drug exposure and leukocyte mRNA expression patterns of metabolizing enzymes and transporters were evaluated in the current study to assess their relationships with treatment outcomes. We wanted to evaluate the relationship of mycophenolic acid AUC and Ctrough plasma concentrations with outcomes since there is ongoing debate in the kidney transplant community surrounding this issue. In fact, there is advocacy for an AUC of 30 to 60 mg hr/L [61-64], and a Ctrough concentration of at least 1 to 3.5 mg/L [65,66] when patients are receiving triple therapy immunosuppression for prevention of kidney transplant rejection. These recommendations were based on evaluation of acute rejection rates in randomized trials that aimed to assess this outcome based on mycophenolic acid exposure. Since patients with glomerulonephritis are typically treated with only single or at most, double immunosuppressive drugs, it is conceivable that targeted mycophenolic acid AUC and Ctrough concentrations should be considerably higher.

In our previous publication, we identified several patient-level variables that influenced mycophenolic acid pharmacokinetics: increased UP:Cr and weight and decreased SCr were predictors of increased oral clearance; Caucasian race and elevated SCr were predictors of higher mycophenolic Ctrough concentrations [32]. A recent publication in autoimmune glomerulonephritis patients receiving mycophenolic acid reported 29% of Ctrough concentrations of < 3mg/L were from patients with active disease, whereas only 2% of Ctrough concentrations ≥3mg/L were from patients with active disease, suggesting a critical ctrough concentration that is associated with disease activity. [67] Additionally, remission maintenance

was persistent in patients with Ctrough concentrations of ≥3.5mg/L. [67] Unfortunately, we did not detect any significant correlations between either AUC or Ctrough plasma concentrations and outcomes as defined by changes in eGFR, SCr, or UP:Cr. Additionally, we evaluated for differences in AUC or Ctrough by patients who had worsened versus improved UP:Cr and eGFR and still failed to appreciate any differences (data not shown). We were also unable to assess for composite kidney outcomes due to too few patients exhibiting these outcomes in patients who had mycophenolic acid pharmacokinetics assessed. Possible reasons for failure to obtain any significant findings between mycophenolic acid pharmacokinetics and outcomes include: 1) the true lack of such a relationship in glomerulonephritis patients, 2) failure to follow patients for a sufficiently long enough period of time, and/or 3) failure of the measured pharmacokinetics to represent the patients' actual exposures throughout the course of their treatment, secondary to different doses being prescribed and different durations of time spent at different doses.

Previous studies have reported that patterns of mRNA expression within leukocytes are important for predicting outcomes and treatment responses in various diseases. [68-70] We have evaluated patterns of expression for drug metabolizing enzyme and transporter transcript in peripheral blood cells of glomerulonephritis patients undergoing therapy with mycophenolic acid or cyclophosphamide. [33] We were interested in whether the expression in glomerulonephritis patients correlated with treatment outcomes as there were differences in expression in several genes between cohorts. Matched mRNA expression and genotype data was not significantly correlated in the SVV and SLE patients, but there was a borderline result for *MDR1 C3435T* genotype and *MDR1* transcript expression; with wildtype genotype having lower expression than variant genotypes. Unfortunately, matched data was not available for healthy controls and no expression data was available for any of our rheumatoid arthritis patients. We were unable to demonstrate any significant relationships between kidney outcomes of eGFR, SCr, or UP:Cr and mRNA expression in mycophenolic acid treated patients. When we assessed composite

kidney outcomes in our entire cohort of patients (receiving either mycophenolic acid or cyclophosphamide), we showed that expression of *MDR1* was significantly reduced in patients with the composite outcomes of death, dialysis, or transplantation. This finding is somewhat confusing given some previous research that has suggested decreased *MDR1* expression and enhanced intracellular accumulation of a P-glycoprotein probe substrate. [71] Additionally, in our own previous work in assessment of kidney toxicity in transplant recipients, increased nephrotoxicity was shown in patients with reduced immunohistochemical staining for P-glycoprotein; with the possibility of enhanced intra-tubular concentrations of pharmacologically active nephrotoxins. [42] For mycophenolic acid, enhanced lymphocyte exposure and pharmacological effects would be predicted in conditions of reduced expression of P-glycoprotein. However, there is often promiscuity among transporters for various substrates and the multi-drug resistance associated proteins (MRPs) are known to transport the glucuronide metabolite of mycophenolic acid. Therefore, a reduction in one transporter and an increase in another may negate the effects that would be attributed to one single transport protein. Recent research has suggested that reduced P-glycoprotein expression can reduce the release of intracellular cytokines including interferon gamma, interleukin 2, interleukin 4, and tumor necrosis factor alpha, [72] and this may be at least partly responsible for worsened outcomes in inflammatory autoimmune diseases such as SLE and SVV.

Conclusions

The current study sought to identify patient, genomic, and/or pharmacokinetic factors that may influence outcomes to mycophenolic acid therapy in patients with glomerulonephritis. The outcomes of interest were changes in kidney function parameters (eGFR, SCr, and UP:Cr) as well as composite outcomes (dialysis, death, transplantation). While there were no differences in mycophenolic acid treatment-related outcomes by race, the *UGT1A7* polymorphism was associated with worsened UP:Cr and the *MDR1 C3435T* polymorphism was associated with improve UP:Cr. The most intriguing finding was the association of SVV disease with *UGT2B7*

C802T polymorphism. We are currently assessing this polymorphism in a larger set of autoimmune disease patients to confirm our results. Assessment of relationships between drug exposure or trough plasma concentrations and outcomes did not yield any significant findings. This research demonstrates the complex relationships between disease risks and/or outcomes and individualized factors such as genotype in patients with glomerulonephritis.

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Table 7.1

Demographics, Clinical and Pharmacokinetic Data (Mean (standard deviation)) for Glomerulonephritis Patients Treated with Mycophenolic Acid

	All	SLE	SVV
	n=85	n=37	n=48
Age (years)	47 (16.0)	39 (11)	54 (16)
Race (C/AA/O)	55/22/8	15/17/5	40/5/3
Gender (M/F)	28/57	6/31	22/26
SCr (mg/dL) at Biopsy	2.1 (2.0)	1.5 (1.3)	2.5 (2.3)
eGFR (mL/min) at Biopsy	57.6 (40.8)	75.2 (46.8)	41.9 (26.4)
UP:Cr at Biopsy	2.0 (2.7)	2.8 (3.4)	1.2 (1.6)
Daily Dose (mg/day)	1600 (820)	1622 (975)	1505 (685)
Present or previous steroids(%)	96%	93%	98%
Previous cyclophosphamide(%)	85%	81%	88%
Duration of Follow-up (yrs)	4.6 (3.6)	5.0 (4.0)	4.3 (3.2)

Abbreviations

C/AA/O – Caucasian/African-American/Other

eGFR – estimated glomerular filtration rate

SCr – serum creatinine

SLE – systemic lupus erythematosus

SVV – small vessel vasculitis

UP:Cr – urinary protein to creatinine ratio

Table 7.2:
Distribution for eGFR, serum creatinine, and UP:Cr between disease groups in patients treated with mycophenolic acid

	Disease	N	obs	Mean	Std	Median	P values*
eGFR_diagnosis							0.0007
	SVV	48	39	41.9	26.4	38.7	
	SLE	37	32	75.2	46. 8	77.9	
eGFR_treatment							0.0009
	SVV	48	44	55.2	24.6	52.3	
	SLE	37	35	84.9	42.7	89.3	
eGFR_follow-up							0.0184
	SVV	48	46	53.9	27.5	49.0	
	SLE	37	35	73.8	39.3	73.7	
SCr diagnosis							0.0012
	SVV	48	39	2.5	2.3	1.8	
	SLE	37	32	1.5	1.3	1.1	
SCr treatment							0.0013
	SVV	48	44	1.6	0.9	1.4	
	SLE	37	35	1.2	0.8	0.9	
SCr follow-up							0.0452
	SVV	48	46	1.8	1.4	1.4	
	SLE	37	35	1.5	1.3	1.0	
UP:Cr diagnosis							0.0712
	SVV	48	24	1.2	1.6	0.6	
	SLE	37	24	2.8	3.4	1.0	
UP:Cr treatment							0.1080
	SVV	48	40	0.8	1.2	0.3	
	SLE	37	33	1.3	1.9	0.6	
UP:Cr follow-up							0.1075
	SVV	48	36	0.8	1.6	0.2	
	SLE	37	31	1.4	2.1	0.4	

P values were calculated by Wilcoxon two sample test.

Abbreviations

eGFR – estimated glomerular filtration rate

SCr – serum creatinine

SLE – systemic lupus erythematosus

SVV – small vessel vasculitis

UP:Cr – urinary protein to creatinine ratio

Table 7.3

Allelic Frequency Distributions

		Study Cohorts			
		SVV	**SLE**	**Healthy Control**	**R. Arthritis**
		(n=101)	*(n=67)*	*(n=75)*	*(n=26)*
UGT1A9					
G8A	G	1.0	1.0	1.0	1.0
	A	0.0	0.0	0.0	0.0
C98T	C	0.99	0.99	0.99	1.0
	T	0.01	0.01	0.01	0.0
C-2152T	C	0.97	0.96	0.97	0.94
	T	0.03	0.04	0.03	0.06
T-275A	T	0.97	0.94	0.96	0.90
	A	0.03	0.06	0.04	0.10
UGT1A7					
T622C	T	0.78	0.78	0.63	0.71
	C	0.22	0.22	0.37	0.29
UGT2B7					
C802T	C	0.64	0.74	0.79	0.81
	T	0.36	0.26	0.21	0.19
MDR1/ABCB1					
C1236T	C	0.60	0.60	0.56	0.69
	T	0.40	0.40	0.44	0.31
C3425T					
	C	0.56	0.66	0.56	0.58
	T	0.44	0.34	0.44	0.42

Abbreviations

MDR1/ABCB1 – multi-drug resistance gene

R. Arthritis – rheumatoid arthritis

SLE – systemic lupus erythematosus

SVV – small vessel vasculitis

UGT – uridine diphosphate glucuronosyltransferase

Table 7.4

Genotype Frequency Distributions		**(frequency (n))**			
		Vasculitis	**SLE**	**Healthy Control**	**Rheumatoid Arthritis**
		n = 101	**n = 67**	**n = 75**	**n = 26**
UGT1A9					
G8A	G/G	1.0 (79)	1.0 (51)	1.0 (70)	1.0 (26)
	G/A	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
	A/G	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
C98T	C/C	0.98 (98)	0.98 (64)	0.99 (72)	1.0 (26)
	C/T	0.02 (2)	0.02 (1)	0.01 (1)	0.0 (0)
	T/T	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
C-2152T	C/C	0.94 (74)	0.92 (47)	0.94 (67)	0.88 (23)
	C/T	0.06 (5)	0.08 (4)	0.06 (4)	0.12 (3)
	T/T	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
T-275A	T/T	0.94 (95)	0.88 (57)	0.91 (64)	0.81 (21)
	T/A	0.06 (6)	0.12 (8)	0.09 (6)	0.19 (5)
	A/A	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)

UGT1A7					
T622C[a]	T/T	0.62 (62)	0.45 (29)	0.39 (27)	0.42 (11)
	T/C	0.31 (31)	0.40 (26)	0.49 (34)	0.58 (15)
	C/C	0.07 (7)	0.15 (10)	0.12 (9)	0.0 (0)
UGT2B7					
C802T[b]	C/C	0.44 (34)	0.55 (28)	0.58 (41)	0.62 (16)
	C/T	0.39 (30)	0.37 (19)	0.42 (30)	0.38 (10)
	T/T	0.17 (13)	0.08 (4)	0.0 (0)	0.0 (0)
MDR1/ABCB1					
C1236T	C/C	0.38 (37)	0.45 (30)	0.33 (23)	0.38 (10)
	C/T	0.44 (42)	0.46 (31)	0.47 (33)	0.62 (16)
	T/T	0.18 (17)	0.09 (6)	0.20 (14)	0.0 (0)
C3425T	C/C	0.29 (28)	0.43 (29)	0.33 (23)	0.27 (7)
	C/T	0.54 (52)	0.45 (30)	0.46 (32)	0.62 (16)
	T/T	0.17 (16)	0.12 (8)	0.21 (15)	0.11 (3)

a: p=0.0123 for differences across groups

b: p=0.0002 for differences across groups

Abbreviations:

MDR1 – multi-drug resistance gene

UGT – uridine diphosphate glucuronosyltransferase

Table 7.5

The Odds of Autoimmune Diseases Among the *UGT2B7* and *UGT1A7* Genotype Groups When Controlling for Race

Disease Group	Predictor		OR (95% CI)*	P Values*
Vasculitis	Race	2 vs. 1	0.908(0.387~2.130)	0.8248
	UGT2B7 C802T	1 or 2 vs. 0	3.073(1.530~6.172)	0.0016
	Race	2 vs. 1	0.823(0.374~1.811)	0.6277
	MMF_UGT1A7 T622C	1 or 2 vs. 0	0.414(0.215~0.796)	0.0082
	Race	2 vs. 1	1.011(0.456~2.241)	0.9790
	MDR1 C1236T	1 vs. 0	0.792(0.383~1.636)	0.5282
		2 vs. 0	0.658(0.266~1.628)	0.3649
	Race	2 vs. 1	1.027(0.457~2.311)	0.9484
	MDR1 C3435T	1 vs. 0	1.242(0.583~2.644)	0.5746
		2 vs. 0	0.730(0.284~1.877)	0.5132
Lupus	Race	2 vs. 1	7.011(3.017~16.292)	<0.0001
	UGT2B7 C802T	1 or 2 vs. 0	1.434(0.620~3.320)	0.3998
	Race	2 vs. 1	6.702(3.000~14.963)	<0.0001
	UGT1A7 T622C	1 vs. 0	0.971(0.419~2.249)	0.9455
		2 vs. 0	1.854(0.544~6.310)	0.3235
	Race	2 vs. 1	6.763(2.970~15.399)	<0.0001
	MDR1 C1236T	1 vs. 0	0.951(0.406~2.227)	0.9080
		2 vs. 0	0.642(1.189~2.197)	0.4827
	Race	2 vs. 1	9.104(3.546~23.373)	<0.0001
	MDR1 C3435T	1 vs. 0	1.909(0.703~5.179)	0.2043
		2 vs. 0	1.127(0.319~3.980)	0.8531
RA	Race	1 vs. 2	1.057(0.355~3.146)	0.9201
	UGT2B7 C802T	1 or 2 vs. 0	0.887(0.348~2.265)	0.8025
	Race	2 vs. 1	1.003(0.324~3.106)	0.9961
	UGT1A7 T622C	1 or 2 vs. 0	0.922(0.352~2.416)	0.8687
	Race	2 vs. 1	1.044(0.334~3.268)	0.9411
	MDR1 C1236T	1or 2 vs. 0	0.769(0.286~2.067)	0.6032

	Race	2 vs. 1	1.088(0.327~3.622)	0.8903
	MDR1 C3435T	1 vs. 0	1.592(1.516~4.905)	0.4183
		2 vs. 0	0.565(0.112~2.840)	0.4880

P value and odds were calculated by Logistic model

Genotypes were categorized as: 0 for wildtype/wildtype, 1 for heterozygote, and 2 for variant/variant

Abbreviations

MDR1/ABCB1 – multi-drug resistance gene;UGT – uridine diphosphate glucuronosyltransferase

Table 7.6
Mean±Standard Deviation Changes in eGFR, SCr, and UP:Cr by Genotype Category in Glomerulonephritis Patients Receiving Mycophenolic Acid

	SNP/Genotype	Delta eGFR	Delta SCr	Delta UP:Cr
UGT1A7 T622C	WT/WT	1.7±26.1	-0.2±1.2	-1.1±3.8[a]
	Heterozygote	11.5±28.9	-0.6±2.4	-0.7±1.5
	Variant/Variant	-3.6±26.4	0.7±2.0	1.4±2.7
UGT2B7 C802T	WT/WT	24.0±31.7	-0.1±1.3	-0.2±2.1
	Heterozygote	4.7±19.5	-0.3±0.6	-1.6±4.0
	Variant/Variant	6.7±36.5	-0.0±0.9	-0.4±0.3
MDR1 C1236T	WT/WT	12.4±31.3	-0.7±2.9	-0.1±2.5
	Heterozygote	1.8±27.9	-0.3±1.0	-1.5±3.1
	Variant/Variant	7.8±13.7	0.4±2.0	0.7±4.0
MDR1 C3435T	WT/WT	14.4±31.6	-0.9±3.0	0.6±1.8[b]
	Heterozygote	4.5±27.6	-0.3±0.8	-2.0±3.3
	Variant/Variant	-2.0±17.5	0.6±1.7	0.3±2.9

a: p=0.0706

b: p=0.0409

Data represents absolute changes in parameters from diagnosis to last follow-up

Abbreviations

eGFR – estimated glomerular filtration rate

MDR1 – multidrug resistance

SCr – serum creatinine

UGT – uridine diphosphate glucuronosyltransferase

UP:Cr – urinary protein to creatinine ratio

WT – wildtype

Figure Legends

Figure 7.1: Clinical Measures in Patients with Glomerulonephritis Treated with Mycophenolic Acid. Figure represents changes from diagnosis to follow-up in patients with glomerulonephritis treated with mycophenolate mofetil. A.Serum creatinine; B.Urinary protein to creatinine ratio (UP:Cr); C. estimated glomerular filtration rate (eGFR).

Figure 7.2. Kaplan Meier Survival Curves for Composite Outcomes (Dialysis, Death, or Transplantation) During Mycophenolic Acid Treatment. Abbreviations are: eGFR; estimated glomerular filtration rate, SLE; systemic lupus erythematosus, SVV; small vessel vasculitus. SLE is demonstrated by the red/top line and SVV is demonstrated by the blue/bottom line.

Figure 7.1A.

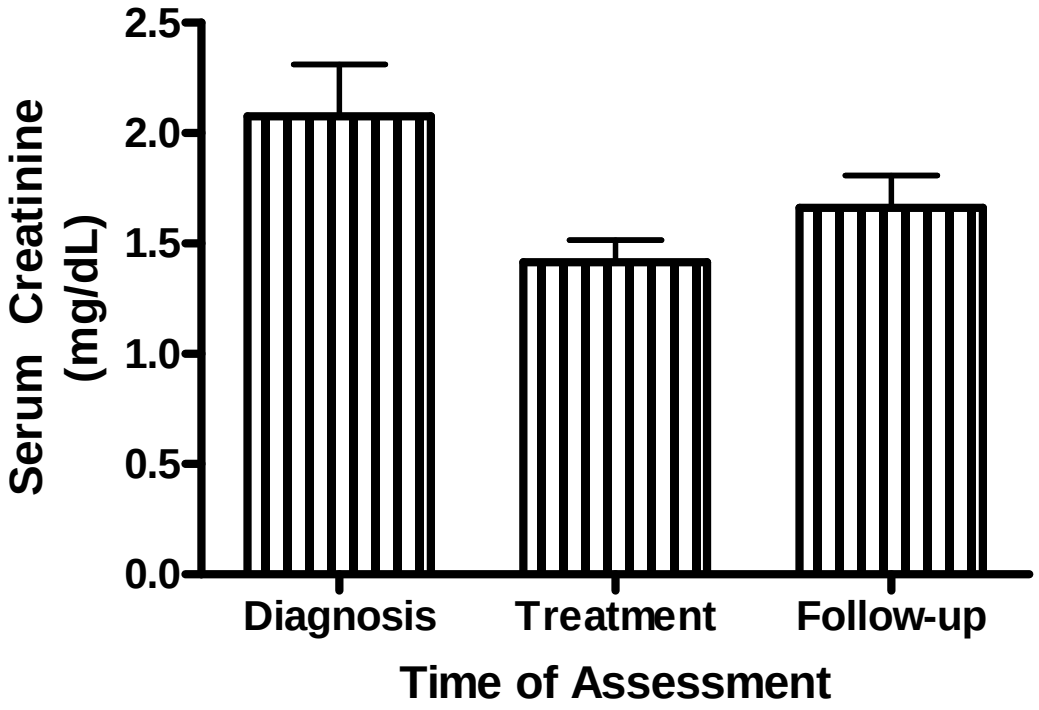

Serum Creatinine (mg/dL)
2.5
2.0
1.5
1.0
0.5
0.0
Diagnosis
Treatment
Follow-up
Time of Assessment

Figure 7.1B.

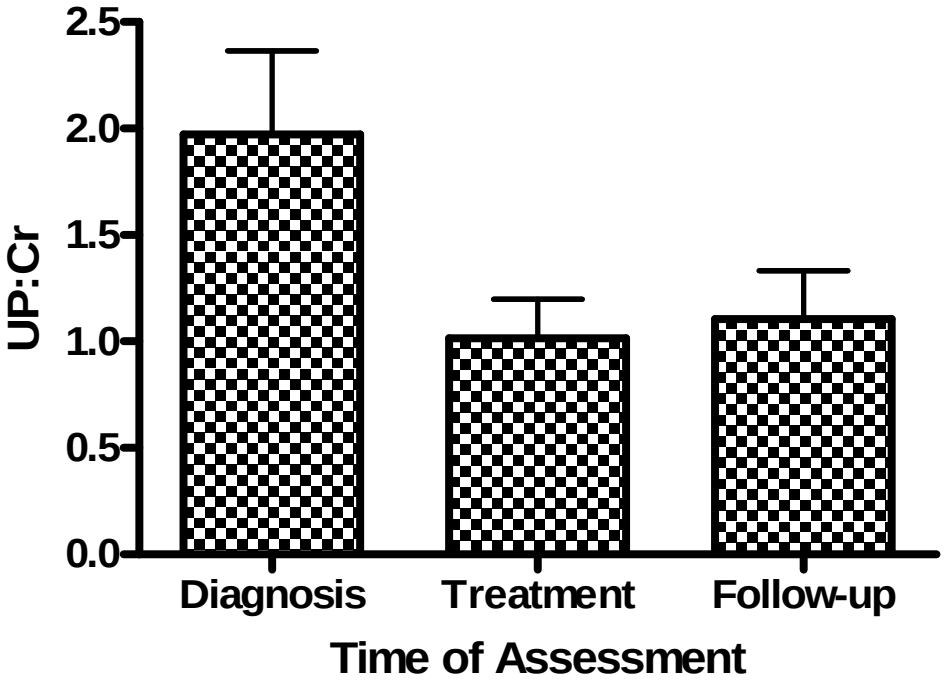

2.5
2.0
1.5
1.0
0.5
0.0
UP:Cr
Diagnosis
Treatment
Follow-up
Time of Assessment

Figure 7.1C.

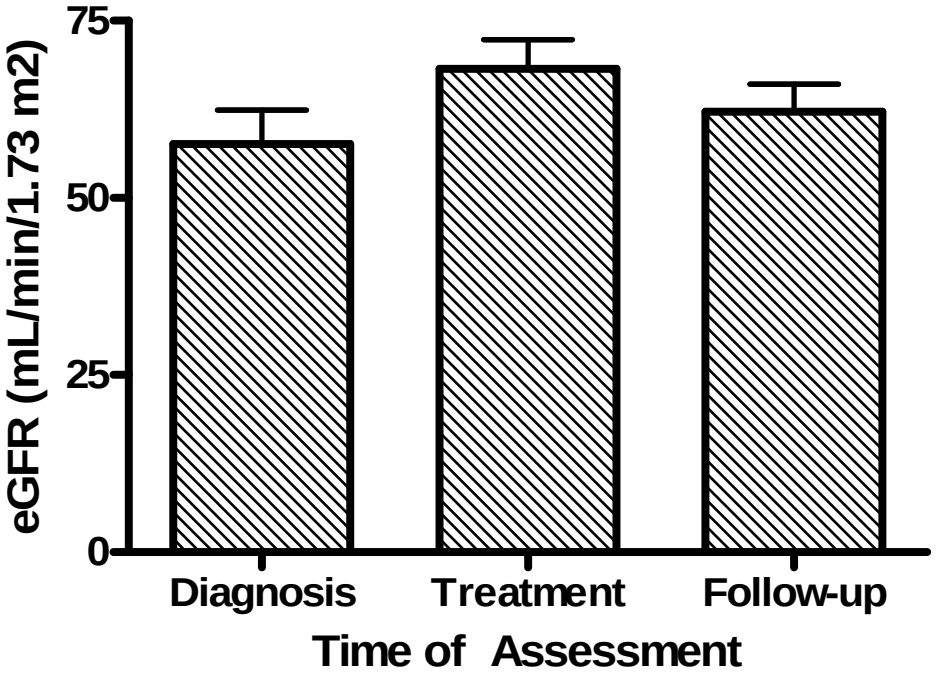
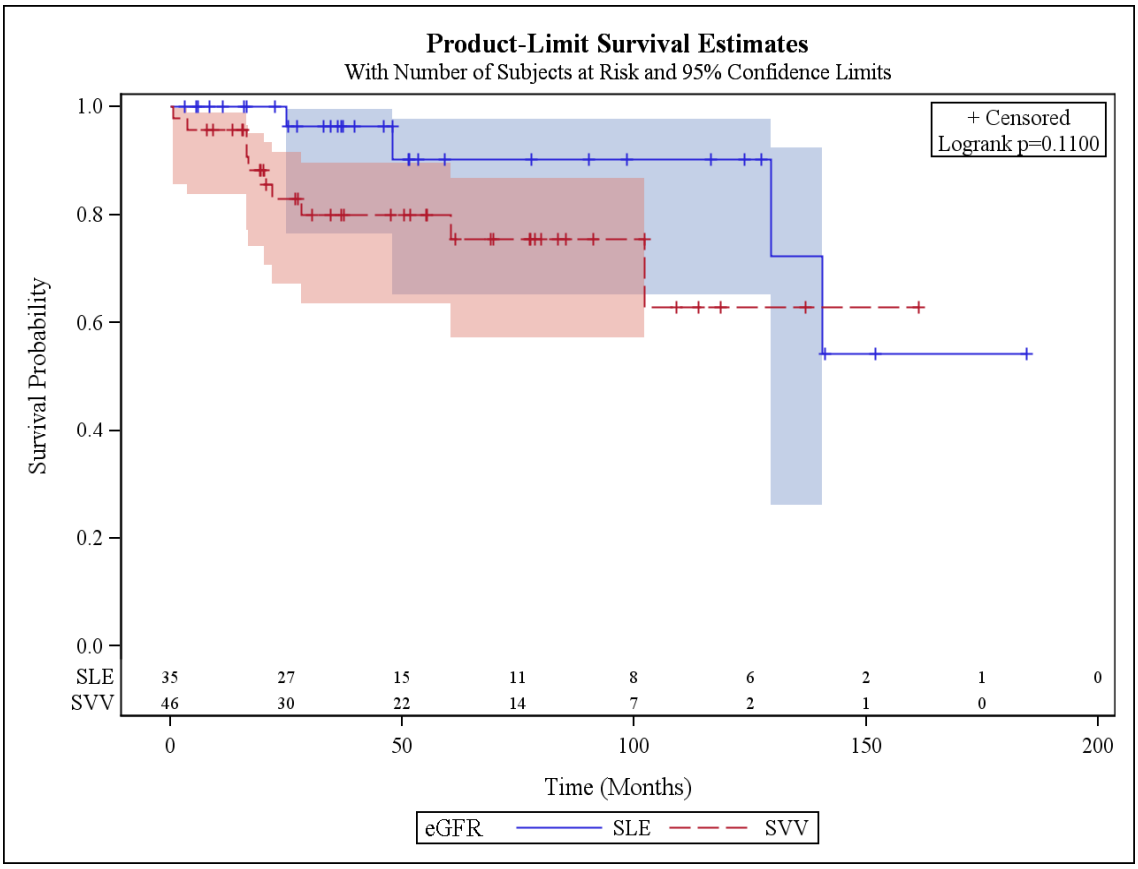

75
50
25
0
eGFR (mL/min/1.73 m2)
Diagnosis
Treatment
Follow-up
Time of Assessment

Figure 7.2


Product-Limit Survival Estimates
With Number of Subjects at Risk and 95% Confidence Limits
+ Censored
Logrank p=0.1100
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
SLE 35 27 15 11 8 6 2 1 0
SVV 46 30 22 14 7 2 1 0
0
50
100
150
200
Time (Months)
eGFR SLE SVV

Chapter 8

Conclusions

This dissertation project sought to evaluate pharmacokinetic and pharmacogenomic factors that may be associated with altered outcomes to mycophenolic acid (MPA) therapy in glomerulonephritis patients with small vessel vasculitis (SVV) and systemic lupus erythematosus (SLE). The goal of this research was to understand treatment responses to MPA in these former patient groups in order to implement strategies to improve outcomes. The central hypothesis was that the metabolism and transport of MPA are different in individual patients with glomerulonephritis and these differences account for variations in systemic or tissue exposure and thus influence renal outcomes. Three specific objectives were developed to investigate the hypothesis.

Objective 1. Evaluate the pharmacokinetic parameters for MPA in subjects with SVV and SLE with variable levels of kidney function as reported by glomerular filtration rate, proteinuria, and disease activity. Develop a population pharmacokinetic model for MPA in glomerulonephritis.

Objective 2. Evaluate the pharmacokinetics for the glucuronide metabolities of MPA; e.g. mycophenolic acid glucuronide (MPAG) and acyl-mycophenolic acid glucuronide (AcMPAG) in the SVV and SLE patients as a function of variable kidney function as reported by glomerular filtration rate and proteinuria. Incorporate metabolite plasma and urine data into a population pharmacokinetic model in glomerulonephritis.

Objective 3. Assess genotype frequencies at sites of known single nucleotide polymorphisms in uridine diphosphate glucuronosyltransferases (UGTs) *1A9, 1A7*, and *2B7,* and multidrug resitance gene (*MDR1/ABCB1)* and evaluate for associations with MPA pharmacokinetics and disease outcomes in glomerulonephritis patients. Determine mRNA expression patterns for the drug metabolizing genes *UGT1A9, UGT1A7, UGT2B7,* and transporter genes *ABCB1, ABCC2*, and *SLCO1A2* in leukocytes of glomerulonephritis patients and their associations with pharmacokinetics and disease outcomes.

The underlying rationale for evaluating MPA and metabolite pharmacokinetics in patients with glomerulonephritis was due to the disease-associated clinical findings of urinary protein excretion, hypoalbuminemia, and kidney function decline and the lack of published data that have described the impact of these clinical scenarios on the pharmacokinetics of many pharmaceutical agents. Noncompartmental pharmacokinetic results for MPA from these studies showed highly increased apparent oral clearance (Cl/F 343 ± 200 mL/min in SLE and 288 ± 154 mL/min in SVV) values that were 2-fold higher than previously reported in kidney transplant recipients. Multiple regression analyses in SLE patients showed that MPA apparent oral clearance was predicted by creatinine clearance (Clcr) and serum albumin (MPA lnCl/F = 5.358 + 0.0092 (Clcr) – 0.078 (ranked albumin), R^2 51.1%, p = 0.0195). Patients with urinary protein to creatinine ratios ≥ 1 g/d had lower trough concentrations and area under the curve (AUC_{0-12}) values, and higher apparent oral clearance compared to patients with urinary protein to creatinine ratios < 1 g/d. Patients with serum albumin < 4 g/dL had higher MPA apparent unbound clearance and MPAG apparent renal clearance values versus patients with serum albumin ≥ 4g/dL. Area under the plasma concentration time curve during the period of enterohepatic recycling (e.g. AUC_{6-12}), gender, and age all contributed toward the prediction of MPAG apparent renal clearance. For SVV, weight and race were predictive for MPA apparent oral clearance (ranked MPA Cl/F = -11.766 + 0.2035 (wt) + 4.9578 (race), R^2 41.8%, p = 0.0045). Creatinine clearance (Clcr) < 60 mL/min resulted in higher MPA exposure as assessed by total AUC_{0-12}, AUC_{6-12}, and unbound AUC_{0-12}. Additionally, the ratio of metabolite to MPA exposure ($MPAG_{AUC}$:MPA_{AUC}) of 8.7±5.6 was lower than previously reported in renal transplant recipients. In summary, the noncompartmental analyses showed that higher creatinine clearance and decreased serum albumin were identified as primary contributors to increased MPA apparent oral clearance and decreased exposure in SLE. Higher body weight and Caucasian race were primary contributors to increased MPA apparent oral clearance and

decreased exposure in SVV. Additionally, SVV patients with creatinine clearance <60 mL/min versus ≥ 60 mL/min had enhanced MPA exposure. These findings are important as they encourage clinicians to be mindful of clinical changes that occur throughout the disease courses of SLE and SVV that may subsequently alter MPA pharmacokinetics and exposure.

A population pharmacokinetic modeling approach to MPA in the entire cohort of patients with glomerulonephritis was developed to enable estimates of key pharmacokinetic parameters including renal clearance, nonrenal clearance, and central volume, and to further investigate the influence of covariates including measures of kidney function, serum albumin, demographic variables and genotype for single nucleotide polymorphisms. The population approach also enabled estimation of MPA inter-individual variability and residual variability. The final pharmacokinetic model was composed of nine compartments and included terms to describe biliary drug clearance. The model fit the data well as demonstrated by the generated goodness of fit plots. Unlike previous models of MPA pharmacokinetics, the model was developed with extensive plasma and urine sample collections from a well-defined population of glomerulonephritis patients. The resulting parameter estimates were considerably different than those obtained by other investigators who evaluated kidney transplant patients receiving MPA. As with the noncompartmental analysis, two key covariates, estimated creatinine clearance and serum albumin, influenced the renal and nonrenal components of MPA clearance. Creatinine clearance ≤80 mL/min had a positive effect on MPA renal clearance resulting in a mean (%RSE) covariate coefficient of 1.33 (33.2). For the nonrenal clearance component, creatinine clearance had a positive effect (covariate coefficient of 0.831 (18.5)), while serum albumin had a negative effect (covariate coefficient of -1.35 (31.5)). Creatinine clearance also had a positive influence on MPAG and AcMPAG renal clearance estimates. Through simulations of typical clinic patients with variations in serum albumin and creatinine clearance, it was demonstrated that patients with glomerulonephritis would have highly altered MPA exposures than what would

be concluded without weighing these factors in the calculation of the renal and nonrenal clearance pathways for MPA. Future work will be needed to elucidate unbound MPA exposures and relevance to efficacy, toxicity, and metabolic pathways. The current population model estimates can now be employed in validation glomerulonephritis populations.

After assessing the noncompartmental and population pharmacokinetics of MPA and metabolites, the role of pharmacogenomics, alone and in combination with clinical and demographic parameters on pharmacokinetic predictions in the entire cohort of glomerulonephritis patients receiving MPA was evaluated. Genotyping was performed for known variants of UGTs reported to be primary enzymes for MPA metabolites (*UGT1A9, UGT1A7, UGT2B7*), and known variants for *MDR1/ABCB1* that could potentially alter MPA disposition. For assessment of genotype influence on pharmacokinetics, both *UGT2B7* heterozygosity and *UGT1A7* heterozygosity predicted increased MPA apparent oral clearance. *UGT1A7* heterozygosity also predicted lower MPA trough plasma concentrations. Since the numbers of patients in the homozygous variant groups were small relative to the heterozygous groups, the clear effects of homozygosity were not able to be fully assessed. Only *UGT2B7* heterozygosity remained in multivariate models, where it predicted enhanced apparent renal clearance. The reason for disparity in genotype covariate effects between these regression models and the population models are not apparent. In future studies, it will be necessary to further investigate the role of the kidneys as a key component to apparent oral clearance through the UGT2B7 metabolizing enzyme. Future pharmacogenomic validation assessments will require numbers of patients.

Patient-level clinical and demographic data were contributory in both univariate and multivariate models. In multivariate assessments, higher urinary protein excretion, lower serum creatinine, and increased weight predicted greater MPA apparent oral clearance. White race and higher serum creatinine predicted higher MPA trough plasma concentrations. Higher exposure to MPA was predicted by reduced levels of urinary protein excretion and higher serum

creatinine concentrations. In summary, clinical and demographic parameters explained 30% to 50% of MPA pharmacokinetics, while genetic polymorphisms explained only about 10%. Unfortunately, we were limited in our ability to fully assess genetic polymorphisms in *UGT1A9* secondary to the low frequency encountered in the glomerulonephritis population. Hence the potential importance of the *UGT1A9* polymorphisms in MPA disposition within the glomerulonephritis cohort may not be fully appreciated.

Since immunosuppressive drugs including MPA have their pharmacological site of action at the level of the leukocytes, and limited data was available concerning mRNA expression of drug transporters and drug metabolizing enzymes in leukocytes, this area of research was pursued within the dissertation research. In addition to describing mRNA expression patterns in SLE and SVV patients, exploratory analyses related to prediction of pharmacokinetics and outcomes, and relationships to genotypes were also assessed. Drug transporter transcripts (*ABCC2, ABCB1*, and *ABCG2*) were found in the leukocytes of most patients with glomerulonephritis, with the exception of *SLCO1A2*, which was expressed in only half of subjects. Regarding drug metabolizing transcripts, *UGT1A7, UGT1A9*, and *UGT2B7* were expressed in 50% of subjects' leukocytes, *CYP2B6* was expressed in over 90% of subjects, and *CYP3A4* and *CYP2C9* were not expressed. This data would imply that active drugs such as MPA could undergo local metabolism within the leukocyte to the inactive MPAG in about half of patients, perhaps limiting overall exposure to MPA. Alternatively, patients without the expression of *UGTs* would be hypothesized to have enhanced MPA local leukocyte exposure, which would be predicted to lead to higher efficacy, but with the risk of enhanced toxicity. Since protein expression was not assessed, the direct link between transcript and protein expression cannot be defined absolutely. Newly developed absolute quantitative mass spectroscopy methods will be employed in future work to more clearly define the relationship between transcript and protein expression. Additionally, research evaluations to elucidate MPA turnover in lymphocytes is planned. Other relevant findings resulting from the mRNA expression studies were: differential

expression patterns of drug metabolizing enzyme and transporter transcripts in patients with glomerulonephritis as compared to healthy control subjects, and differences in expression according to pharmacologic treatment, disease type, race, and possibly genotype. This initial research will guide future investigations into transcript-mediated mechanisms for altered efficacy and toxicity to pharmacological therapies.

In the final efforts of this research program, the determinants of kidney outcomes in glomerulonephritis patients receiving mycophenolate mofetil therapy were evaluated. Changes in estimated glomerular filtration rate, serum creatinine, and urinary protein excretion from diagnosis to follow-up, and the composite outcome of dialysis, transplantation, or death were assessed. Both the SVV and SLE patients had absolute reductions in serum creatinine and increases in estimated glomerular filtration rate from diagnosis to follow-up, with the SVV patients having a higher magnitude of beneficial change. However, the SLE patients as compared to the SVV patients, exhibited a greater reduction in urinary protein excretion while receiving mycophenolate mofetil therapy. From the data, a 32% relapse rate, as defined by at least a 50% increase in serum creatinine, and a 17% relapse rate, as defined by at a least a 50% increase in urinary protein excretion was inferred. Both rates were evaluated from the period of diagnosis to follow-up. The results demonstrated similar 2- and 5- year estimated composite survival rates for the SVV and SLE patients.

Genetic factors appeared to contribute to SVV disease, as well as MPA outcomes in the entire glomerulonephritis cohort. The odds of SVV disease was greater in patients who were classified as heterozygous or variant homozygous for the *UGT2B7 C802T* polymorphism. The odds of SVV disease was reduced in patients who were classified as heterozygous or variant homozygous for the *UGT1A7 T622C* polymorphism. A trend toward statistical significance was found between the *UGT1A7 T622C* variant homozygous genotype and worsened urinary protein excretion. Additionally, glomerulonephritis patients who were heterozygous or variant homozygous for *ABCB1/MDR1 C3435T* had a more favorable urinary protein excretion

response than patients who were wildtype variants. A trend toward reductions in composite outcomes were noted in glomerulonephritis patients who were heterozygous or variant homozygous for the *UGT2B7 C802T* polymorphism. Trends in outcome differences to mycophenolate mofetil therapy were not detected based on drug exposure (as defined by area under the plasma concentration time curve or trough plasma concentration) or leukocyte expression of drug metabolizing enzyme or transporter transcripts. Future work will focus on more clearly defining the role of genetic determinants to therapy outcomes in patients with glomerulonephritis.

In summary, the work presented in this dissertation has considerably advanced the understanding of the disposition of MPA and its metabolites in patients with glomerulonephritis, a disease consisting of several clinical manifestations including urinary protein excretion, hypoalbuminemia, and reductions in kidney function. Consistent with this work evaluating MPA, and additional work by the author, glomerulonephritis patients have increased apparent oral clearance of highly protein bound small molecule drug moieties. The noncompartmental pharmacokinetics and linear statistical modeling approaches employed in this research demonstrated a contribution of increased creatinine clearance and decreased serum albumin on increasing apparent oral clearance. Population compartmental modeling demonstrated that the renal component to clearance was impacted by creatinine clearance, while the nonrenal clearance component was impacted by serum albumin. The developed population model can now be used in validation work within a larger cohort of glomerulonephritis patients to predict MPA pharmacokinetics. Additionally, the statistical models developed within the work can be used prospectively to target defined pharmacokinetic goals for MPA therapy. While it was somewhat disappointing to find only a relatively small effect of genetic polymorphisms on disposition of MPA and metabolites, a finding of a gene-disease link was particularly intriguing. Ongoing work in a larger subset of patients are planned to validate the association between

SVV disease and the condition of being *UGT2B7 C802T* heterozygous and homozygous variant. This finding could have implications for developing guidelines for exposure to targeted therapeutic agents that are metabolized by UGT2B7 in patients who are deemed to be “at-risk” individuals for SVV. Additionally, further work is warranted in defining the contribution of kidney localized UGT2B7 toward metabolism of MPA and other substrates. Lastly, the contribution of leukocyte localized drug metabolizing and drug transporting gene transcripts toward overall MPA exposure and outcomes is an appealing area for further investigation.