Population Pharmacokinetics of Unbound Mycophenolic Acid in Pediatric and Young Adult Patients Undergoing Allogeneic Hematopoietic Cell Transplantation

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Mycophenolate mofetil (MMF) is an immunosuppressant routinely used in allogeneic hematopoietic cell transplantation (alloHCT) to promote stem cell engraftment and prevent acute graft vs host disease. Administered as a prodrug, MMF is converted by esterases to the active moiety, mycophenolic acid (MPA). The impact of clinical covariates on unbound MPA exposure was investigated with a population pharmacokinetic approach. Pharmacokinetic data were obtained from routine area under the curve (AUC) monitoring of unbound MPA drug levels in 36 pediatric (n = 31) and young adult (n = 5) patients undergoing alloHCT for a variety of malignant and nonmalignant disorders. Unbound MPA pharmacokinetics were well described by a 2-compartment model with linear elimination and first-order absorption. The important clinical covariates affecting unbound MPA pharmacokinetics were weight, estimated creatinine clearance, and total bilirubin. Unbound MPA clearance was reduced, and exposure ($AUC_{0\text{-}8}$) increased in individuals with decreased renal function. In individuals with severe hepatic dysfunction (total bilirubin >10 mg/dL) unbound MPA clearance was approximately 3-fold lower compared with patients with normal to mild hepatic impairment. In alloHCT recipients with renal dysfunction or severe hepatic injury, dose reductions may be necessary to prevent toxicity and ensure optimal immunosuppression.

Keywords: *Mycophenolate; mycophenolic acid; pharmacokinetics; hematopoietic cell transplantation; acute graft vs host disease; pediatric*

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Mycophenolate mofetil (MMF) is an immunosuppressive agent commonly used in allogeneic hematopoietic cell transplantation (alloHCT) to prevent acute graft vs host disease (aGVHD) and promote stem cell engraftment. MMF is an ester prodrug that is rapidly and extensively (95%) hydrolyzed by esterases found in the blood, gut wall, liver, and other tissues to the active moiety, mycophenolic acid (MPA). MPA is a reversible inhibitor of inosine monophosphate dehydrogenase, a key enzyme involved in the de novo purine synthesis pathway of both B and T lymphocytes, leading to decreased lymphocyte proliferation. MPA is extensively bound to serum albumin at approximately 97% in patients with normal renal and hepatic function, whereas only the unbound fraction of MPA is pharmacologically active.[1] MPA is glucuronidated by UDP glucuronosyltransferase (UGT) enzymes to the primary inactive metabolite, MPA 7-O-glucuronide (MPAG),

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which is also highly protein bound (82%).[1] The glucuronide is then either excreted into the urine via active tubular secretion or carried back into the intestinal lumen via bile through multidrug-resistant protein (MRP) transporters, specifically MRP2.[2] In the intestine, MPAG can be converted back into MPA and reabsorbed back into systemic circulation through enterohepatic recycling.[2]

Unbound MPA pharmacokinetics (PK) display wide inter- and intrapatient variability in plasma concentrations in both adult and pediatric alloHCT.[3-6] Although unbound MPA exposure is highly variable in adult and pediatric alloHCT patients, factors influencing variability have not yet thoroughly been evaluated. Acute renal dysfunction is a common complication of alloHCT, occurring to some degree in up to 93% of patients by day 100 of transplantation.[7] The effect of renal function on pharmacologically active unbound MPA drug concentrations is unclear. Several studies performed in solid organ transplantation have reported an increase in unbound MPA exposure in the presence of renal dysfunction,[8-10] whereas in other studies unbound concentrations were unchanged.[11-12] Contributing to the complexity of unbound drug exposure is severe treatment-related hepatic dysfunction or veno-occlusive disease, which may occur with or without renal failure in alloHCT recipients.[13]

Dose reductions of MMF are often considered in alloHCT patients with renal dysfunction over concerns that elevated unbound MPA exposure may lead to leukopenia and presumably graft failure. These dose modifications are thought to be warranted based on the association of increased risk of leukopenia in pediatric renal transplant recipients with an unbound MPA area under the curve ($AUC_{0\text{-}12}$) greater than 400 ng·h/mL.[14] However, at most centers the routine therapeutic monitoring of MMF therapy is performed by measurement of total MPA plasma concentrations (a combined measurement of bound and unbound drug) rather than the determination of only unbound MPA levels. This is concerning since prior studies have shown a weak correlation between total and unbound MPA concentrations.[3] Additionally, hematologic toxicity has been demonstrated in several case reports where total MPA exposure was within the typical range for solid organ transplantation (30-60 μg·h/mL) but unbound MPA exposure was extremely high.[15-17] Moreover, in an adult population undergoing alloHCT, a relationship between unbound MPA exposure and risk of aGVHD has been demonstrated. Subjects with an unbound MPA $AUC_{0\text{-}12}$ of less than 300 ng·h/mL had a higher cumulative incidence of aGVHD grades II-IV compared with those with higher exposure.[3,5] Identifying clinical covariates that significantly influence unbound MPA exposure is important for the development of better dosing strategies for immunosuppressive therapy in alloHCT. The objective of this study was to evaluate patient-specific covariates as contributors to the variability of unbound MPA exposure in pediatric and young adult alloHCT recipients, as this has not previously been reported.

PATIENTS AND METHODS

This study used PK data obtained from routine area under the curve (AUC) monitoring of unbound MPA drug levels in 36 pediatric (n = 31) and young adult (n = 5) patients who had received an alloHCT at the University of Minnesota between January 2008 and May 2009. Patients were eligible to be included in the PK analysis if they had undergone a related or unrelated alloHCT, were to receive MMF (Cellcept Roche, South San Francisco, California) and cyclosporine (CSA) for aGVHD immunosuppression, and had unbound MPA concentration data available for analysis. Mycophenolate therapy was initiated at 15 mg/kg (maximum 1 g) every 8 hours for patients weighing less than 45 kg. For patients weighing equal to or greater than 45 kg, MMF was started at 1500 mg every 12 hours. Because part of routine clinical care included in the transplantation protocols, unbound MPA plasma concentrations were therapeutically monitored beginning at day +2 (steady state), and dose adjustments were made to maintain an $AUC_{0\text{-}8}$ of 200 to 250 ng·h/mL and $AUC_{0\text{-}12}$ of 300-350 ng·h/mL. In all patients, MMF therapy was initiated intravenously (IV) on day –3 and continued IV for at least 7 days post transplant. Following this, if the patients were able to take oral medications without difficulty, they were converted at a 1:1 ratio to oral MMF therapy. Intravenous CSA also began on day –3 at a dose of 2.5 mg/kg every 12 hours and was adjusted to maintain whole blood trough concentrations of 200 to 400 ng/mL. Patients were converted to oral CSA once they were able to tolerate oral medications.

Subjects included in this analysis received a variety of different alloHCT preparative regimens. Briefly, chemotherapy included 1 of the following combinations: (1) alemtuzumab, busulfan, cyclophosphamide, (2) alemtuzumab, clofarabine, melphalan, or (3) anti-thymocyte globulin, cyclophosphamide,

and fludarabine. For those containing busulfan, lorazepam was administered as seizure prophylaxis. Premedication for anti-thymocyte globulin and alemtuzumab was methylprednisolone and hydrocortisone, respectively. All patients received antibiotic prophylaxis and gut decontamination with a fluoroquinolone beginning on day –1.

This study was approved by the University of Minnesota Institutional Review Board, and all patients provided written informed consent to participate in routine therapeutic monitoring of unbound MPA as part of their specific transplant protocol.

Pharmacokinetic Sampling

Intensive PK sampling was performed with both oral and IV administration of MMF, beginning day +2 post transplant and thereafter at the discretion of the clinical team following dose modifications or when clinically indicated. All PK sampling occurred under steady-state conditions. When PK sampling was performed following IV administration, MMF was infused over 2 hours at a constant rate and samples were obtained at 0, 2, 4, 6, 8, and 12 hours (12-hour sample omitted for patients following an 8-hour dosing regimen). Following an oral dose of mycophenolate, PK samples were collected at 0, 1, 2, 4, 6, 8, and 12 hours (12-hour sample omitted for patients following an 8-hour dosing regimen). Two milliliters of blood was collected at each sampling time through a central venous catheter and placed in an EDTA tube. Within 60 minutes of collection, all samples were centrifuged at 3400 rpm for 10 minutes at 4°C and the plasma was removed. Plasma samples were kept at 4°C and processed in batches for unbound MPA analysis within 48 hours of PK sampling. Clinical data were collected on each day of PK sampling and included estimates for body size, renal function, and hepatic function. Creatinine clearance was estimated in pediatric patients (≤17 years of age) by the Schwartz method and in adults by the Cockcroft-Gault equation using ideal body weight.

Plasma samples were analyzed by the University of Minnesota Medical Center Drug Analysis and Biochemical Genetics Laboratory using a validated reverse-phase high-performance liquid chromatography assay with tandem mass spectrometry as previously described.[18] The assay was linear in the range of 1 to 200 ng/mL. Plasma samples with concentrations above the upper limit of linearity were diluted and reassayed. Samples with unbound MPA levels reported below the lower limit of quantification (1 ng/mL) were excluded from further analysis. The inter- and intraday variabilities of the assay were 8.5% to 10.1% and 2.6% to 4.6%, respectively.

Base Model Development

Pharmacokinetic model development using unbound MPA concentration–time data was carried out by the nonlinear mixed-effect modeling program NONMEM (version VII, ICON Development Solutions, Ellicott City, Maryland) with the interface Perl-speaks-NONMEM. Diagnostic graphics and postprocessing of NONMEM output and simulations were performed using the statistical software R. The first-order conditional estimation method with interaction was used throughout the model-building process to estimate PK parameters and variability. Model development was guided by exploratory analysis of the data, changes in the NONMEM objective function value (OFV), diagnostic plots, and the potential biological plausibility of a relationship between clinical covariates and drug exposure. A standard 2-compartment PK model with first-order absorption and linear elimination was assumed (NONMEM subroutine ADVAN4 TRANS4). Given the limited number of concentrations collected following oral dosing of MMF (number of individual profiles = 2), the absorption rate constant (K_a) was fixed to previously reported values, and bioavailability (F) was assumed to be unity. Using standard principles of allometric scaling, weight was built into the base model a priori and scaled to a reference patient having a weight of 20 kg. All PK parameters were assumed to be log-normally distributed and defined as

$$PK_i = TVPK \times exp(\eta_{PK})$$

where *TVPK* is the typical population value of PK parameter, PK_i is the individual PK parameter, and η_{PK} is the interindividual variability (IIV) with a mean of zero and estimated variance of ω^2. Residual unexplained variability (RUV) was explored using the proportional model

$$C_{obs,ij} = C_{pred,ij} \times (1 + \varepsilon_{ij})$$

where $C_{obs,ij}$ and $C_{pred,ij}$ are the *j*th observed and predicted plasma concentrations in the *i*th individual, and ε_{ij} is the proportional RUV, which is assumed to be normally distributed with a mean of zero and estimated variance of σ^2. Although many subjects did have intensive sampling on more than 1 occasion,

no attempt was made to model between-visit variability. The model was parameterized in terms of clearance (CL), volume of distribution in the central compartment (Vc), volume of distribution in the peripheral compartment (Vp), and intercompartmental clearance (Q).

Covariate Model Development

Patient-specific factors considered for covariate testing included age, serum creatinine, creatinine clearance (CrCL), blood urea nitrogen, albumin, alkaline phosphatase, total bilirubin, alanine transaminase, and the day PK sampling post stem cell infusion. For individuals who underwent PK sampling recorded as greater than 100 days post stem cell infusion (exact day not provided, n = 3), a fixed value of 100 was entered into the model. Continuous variables were normalized by the median or a clinically relevant approximation to the median and incorporated into the model using a power function as follows:

$$TVPK = \theta_{PK} \times \left(\frac{CrCL}{median_{CrCL}} \right)^{\theta_{CrCL,PK}}$$

where *TVPK* is typical population value of the parameter, θ_{PK} is the PK parameter for a subject with a CrCL of median value, and $\theta_{CrCL,PK}$ is an exponent accounting for a continuous covariate effect on θ_{PK}. For covariates such as alkaline phosphatase, alanine transaminase, and total bilirubin, in which clinically significant alterations in drug exposure do not typically occur until a threshold is achieved (eg, 3-5 times the upper limit of normal), several clinically relevant cut points were evaluated and then entered into the model as dichotomous covariates. For example,

$$TVPK = \theta_{PK} \times \left(\theta_{TBIL,PK} \right)^{TBIL}$$

where *TBIL* is given the value of zero if total bilirubin is 10 mg/dL or less and a value of 1 if total bilirubin is more than 10 mg/dL. Thus, θ_{PK} is the PK parameter for a patient with TBIL 10 mg/dL or less and $\theta_{TBIL,PK}$ is the fractional change of θ_{PK} for a patient with total bilirubin greater than 10 mg/dL.

The final PK model was built through the process of forward selection and backward elimination of clinical covariates. The likelihood ratio test was used to assess the significance of all covariates in the final model. During forward selection, covariates were univariately tested and deemed significant if the OFV decreased by at least 3.8 (χ_2, $P \leq .05$, $df = 1$) with its inclusion in the model. During backward elimination, significance of the covariates was confirmed by removing one at a time from the full model and required an increase in the OFV of at least 10.8 (χ^2, $P \leq .001$, $df = 1$) to remain in the model.

Model Evaluation

To evaluate the precision of the final model parameter estimates, a nonparametric bootstrap was performed, and a visual predictive check was performed to assess the ability of the final PK model to predict the observations. A total of 1000 bootstrap data sets were generated by repeated sampling with replacement from the original data and the final PK model fitted to each of the bootstrap data sets. The median and the 2.5th and 97.5th percentiles were then obtained for each PK parameter and compared with the final model PK estimates. Stratified bootstrap was implemented to restrict the procedure to ensure that resampling had the same proportion of patients with a total bilirubin 10 mg/dL or less and patients with a total bilirubin greater than 10 mg/dL. For the visual predictive check, 100 data sets using the covariate distributions from the original data set were simulated using the parameter estimates from the final model, and the median and 5th and 95th percentiles were compared with observed concentrations.

Unbound MPA Exposure Measure

The AUC of unbound MPA was derived from the empirical Bayes estimates of individual CL (AUC = Dose/CL). The dose of MMF was determined based on a standard IV dose of 15 mg/kg and converted to MPA equivalents.

RESULTS

Demographics of Patients

Intensive PK sampling was performed in 36 patients (31 children, 5 young adults) for a total of 87 individual PK profiles. Among the 36 study subjects, 55% (n = 20) underwent intensive PK sampling on more than 1 occasion. The median day that post-transplant PK sampling occurred relative to day of stem cell infusion was 13 (range, 2-100+). The majority (85/87) of the individual time–concentration profiles were obtained after IV dosing of MMF. Seventy-five percent (n = 27) of study subjects underwent transplantation for the treatment of an inherited metabolic disorder including X-linked

adrenoleukodystrophy (n = 15), Hurler's syndrome (n = 5), Wolman's disease (n = 2), and other (n = 5). A diagnosis of hemoglobinopathies and hematologic malignancies was the reason for transplantation in 7 and 2 subjects, respectively. The majority of study subjects were male (69%). Eighty percent (n = 29) of individuals were younger than 12 years of age with a median (range) age 5 years (0.17-36). Median weight was 19.1 kg (4.4-99.5) with 86% (n = 31) of subjects weighing less than 45 kg. Renal function on the day of PK sampling was widely variable among subjects, with a median CrCL of 80 mL/min (24-336), serum creatinine of 0.6 mg/dL (0.17-3.39), and blood urea nitrogen of 38 mg/dL (7-161). Of the 36 subjects, 58% (n = 21) displayed some degree of renal impairment on the day of PK sampling. The proportions of individuals classified with mild (50-80 mL/min), moderate (30-50 mL/min), and severe (<30 mL/min) renal impairment as defined by the FDA regulatory guidelines for PK studies in patients were 30% (n = 11), 25% (n = 9), and 2.7% (n = 1), respectively.[19] Laboratory tests indicating the level of hepatic function (albumin 2.3 g/dL [1.9-4.1], alanine transaminase 126 IU/L [10-711], and alkaline phosphatase 18 IU/L [4-359]) were also highly variable in study subjects. Median total bilirubin was 1.1 mg/dL (0.1-26.8), with 6 individuals (totaling 6 intensive PK sets) having a total bilirubin greater than 10 mg/dL (16.7%) at the time of PK sampling. The suspected cause of hyperbilirubinemia in subjects with a total bilirubin greater than 10 mg/dL included veno-occlusive disease (n = 2), aGVHD (n = 1), and disease-related (Wolman's disease, n = 2).

Population PK Model Building

Figure 1 displays the observed dose-normalized unbound MPA plasma time–concentration profiles for all patients. The range of observed concentrations was 1 to 773 ng/mL. A total of 417 quantifiable concentrations were available for population PK modeling and were best described with a 2-compartment base model with first-order absorption and linear elimination. Nineteen plasma samples were excluded from PK analysis because unbound MPA concentrations fell below the level of quantification (1 ng/mL). During forward selection, CrCL and total bilirubin were identified as significant covariates for unbound MPA CL decreasing the objective function value by 154.1 and 59.7, respectively. When backward elimination was performed, both CrCL and total bilirubin increased the objective function to maintain statistical significance and were retained

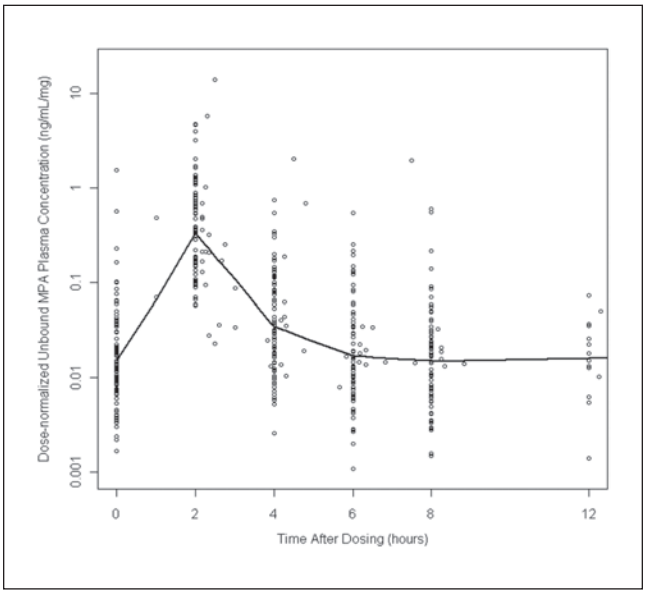

Figure 1. Dose-normalized unbound mycophenolic acid (MPA) plasma concentration vs time profiles.

in the model. The final covariate model for unbound MPA CL was

$$CL_i = \theta_{CL} \cdot \left(\frac{Weight_i}{20kg}\right)^{0.75} \cdot \left(\frac{CrCL_i}{80ml/min}\right)^{\theta_{CrCL,CL}} \cdot \left(\theta_{TBIL,CL}\right)^{TBIL} \cdot exp(\eta_{CL})$$

The population PK parameters estimates and their relative standard errors (%) from the final model are presented in Table I. The goodness-of-fit plots for the base and final model showed clear improvement with good distribution of population-predicted concentrations around the line of unity indicating that the data were adequately described by the final model (see online Figure 1, available at http://jcp.sagepub.com/supplemental/). All conditional weighted residuals fell within 3 standard deviations, demonstrating good predictability of the model. No trend in the residuals was observed.

Model Evaluation

The median PK parameter estimates and 95% confidence intervals from the bootstrap analysis are presented in Table I. Median estimates of PK parameters, interpatient variability, and residual unexplained variability derived from the bootstrap analysis were all within 2% of the typical values derived from the original

Table I Final Population PK Model Parameter Estimates and Bootstrap Results

Population PK Parameters	Units	Final Model Results: Typical Value Estimates[a]	Final Model Results: RSE, %	Bootstrap Results: Median Value	Bootstrap Results: 95% Confidence Interval
k_a, first-order rate constant	h^{-1}	4 (fixed)	—	—	—
θ_{CL}, clearance	L/h	711	11.7	714	619-848
$\theta_{CrCL,CL}$, exponent accounting for a continuous CrCL effect on CL		0.677	23.2	0.671	0.488-0.872
$\theta_{TBIL,CL}$, proportional change for a patient with total bilirubin >10 mg/dL		0.543	9.9	0.539	0.469-0.633
V_c, volume of distribution (central)	L	367	17.9	364	226-488
Q, distribution clearance	L/h	113	25.3	114	86-190
V_p, volume of distribution (peripheral)	L	795	18.4	785	631-1007
Interpatient variability, %CV[b]					
CL		37.0	24.7	36.3	30.0-42.8
V_c		51.6	48.9	51.5	10.6-77.8
V_p		31.3	80.3	31.5	13.6-66.7
Residual unexplained variability, %CV		56.1	13.8	55.7	51.0-60.4

CL, clearance; CrCL, creatinine clearance; CV, coefficient of variation; PK, pharmacokinetic; RSE, relative standard error.
a. Median typical value of the PK parameter in the final model.
b. Presented as CV% (the ratio of the standard deviation to the mean).

population PK analysis. These results demonstrate that the model was able to estimate PK parameters with a reasonably high degree of precision. Figure 2 presents the visual predictive check of the final model. The median of 100 simulated data sets captured the median of the original observed PK data well.

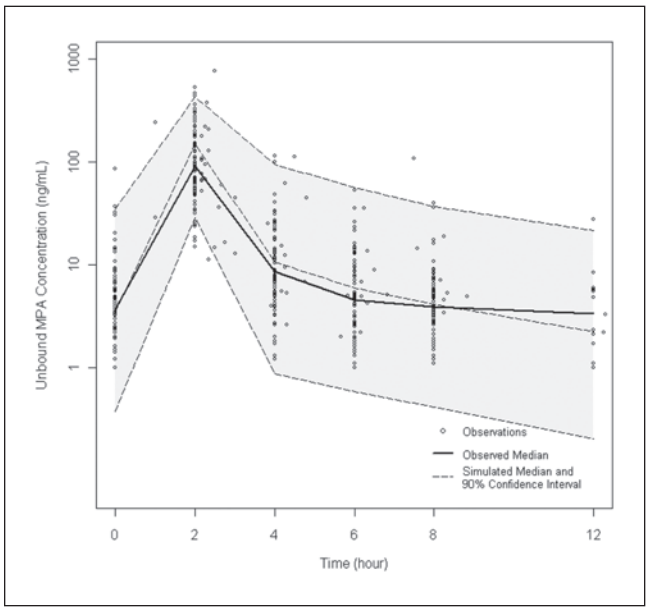

Figure 2. Visual predictive check of the final model. MPA, mycophenolic acid.

Effect of Covariates on Unbound MPA Pharmacokinetics

Important patient-specific covariates found to affect unbound MPA PK were body weight, CrCL, and total bilirubin. Table II displays the typical values for unbound MPA CL (normalized to weight) and predicted unbound MPA $AUC_{0\text{-}8}$ stratified by weight and renal function for individuals with a total bilirubin 10 mg/dL or less receiving a standard dose of MMF (15 mg/kg). Weight categories (10 kg, 20 kg, 30 kg, and 40 kg) are presented similar to the original data set and are largely representative of a pediatric population (<45 kg). Levels of renal function were selected based on regulatory guidelines for PK studies in patients with impaired renal function.[19] As weight increased, weight-normalized unbound MPA CL decreased, leading to increases in exposure. With standard dosing of MMF (15 mg/kg), smaller children (<10 kg) with normal renal function (>80 mL/min) were predicted to have drug exposure below the desired therapeutic range of $AUC_{0\text{-}8}$ 200-250 ng·h/mL. Conversely, an individual receiving standard dosing of MMF with a weight 40 kg and normal renal function was predicted to have drug exposure above the desired therapeutic range. Renal impairment to any degree (mild, moderate, or severe) was

Table II Model Predicted Unbound Mycophenolic Acid AUC_{0-8} With Standard Dosing of Mycophenolate Mofetil (15 mg/kg) Stratified by Weight and Renal Function[a]

Level of Renal Function[b]	Weight, kg	Dose, mg[c]	CL, L/h/kg[d]	AUC_{0-8}, ng·h/mL[e]
Normal renal function (CrCL = 110 mL/min)	10	150	52	195
	20	300	44	232
	30	450	40	257
	40	600	37	276
Mild impairment (CrCL = 80 mL/min)	10	150	42	242
	20	300	36	288
	30	450	32	318
	40	600	30	342
Moderate impairment (CrCL = 50 mL/min)	10	150	31	333
	20	300	26	395
	30	450	23	438
	40	600	22	470
Severe impairment (CrCL = 30 mL/min)	10	150	22	470
	20	300	18	559
	30	450	17	618
	40	600	15	664

AUC, area under the curve; CL, clearance; CrCL, creatinine clearance.
a. Individual's total bilirubin is assumed to be less than or equal to 10 mg/dL.
b. As defined the FDA Guidance for Industry: Pharmacokinetics in Patients with Impaired Renal Function.[19]
c. Standard dose of 15 mg/kg.
d. Typical unbound mycophenolic acid CL normalized to weight (kg).
e. AUC is displayed in mycophenolic acid equivalents and estimated by AUC = dose/clearance.

predicted to increase drug exposure above the targeted therapeutic range across all weight groups except in small children (<10 kg) with mild renal impairment. Approximately a 2-fold increase in unbound MPA AUC_{0-8} was predicted when CrCL decreased from normal renal function (>80 mL/min) to 30 mL/min (severe renal impairment).

Hepatic function was also an important predictor of unbound MPA CL. Figure 3 presents a box and whisker plot of individual predicted unbound MPA CL against total bilirubin. Unbound MPA CL was lower (median 286 L/h [32-725] vs 828 [119-3165]) in subjects with a total bilirubin greater than 10 mg/dL compared with 10 mg/dL or less (P = .004). This represents nearly a 3-fold reduction in unbound CL in subjects with severe hepatic impairment (bilirubin ≥3 times the upper normal limit).

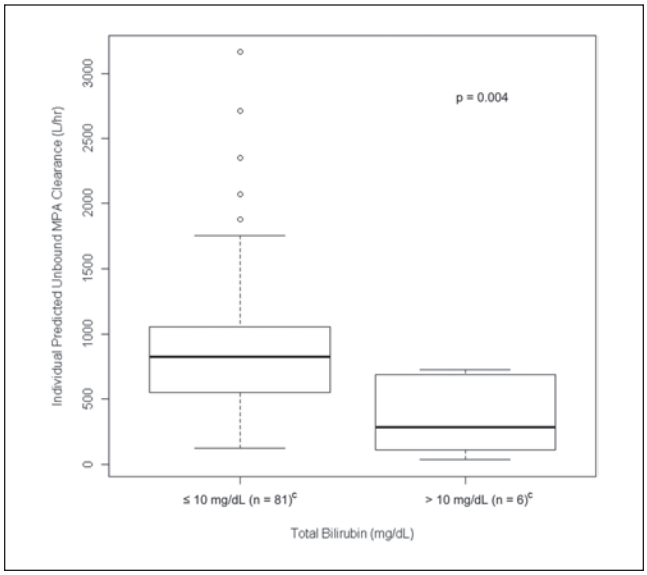

Figure 3. Plots of the effect of total bilirubin on clearance of unbound mycophenolic acid (MPA).[a,b]
a. Whiskers represent all data points falling within 1.5 times the interquartile range.
b. p-value determined by Welch two-sample t-test.
c. n = number of pharmacokinetic profiles.

DISCUSSION

This is the first study to report the PK results of the therapeutic drug monitoring of unbound MPA for the prevention of graft rejection and aGVHD in children and young adult patients undergoing alloHCT. Prior

to these results, very little information was available regarding the pharmacologically active component, unbound MPA, to help guide decisions for dosing MMF, particularly in children. Moreover, unbound MPA exposure is highly variable, and factors influencing variability had not been thoroughly investigated. In this study, we developed a population PK model of unbound MPA CL using intensive time–concentration data obtained from routine unbound AUC monitoring. Plasma concentrations of unbound MPA were well described by a 2-compartment model with first-order absorption and linear elimination. We identified weight, estimated CrCL, and total bilirubin as important clinical covariates affecting unbound MPA CL. These results may be used to help guide clinicians and inform better dosing decisions for MMF in children and adolescents undergoing alloHCT.

There have been contradictory reports regarding the effects of renal function on unbound MPA exposure.[8-12] In our model, CrCL was significantly correlated with unbound MPA CL. When renal function declined, unbound MPA CL (adjusted for weight) was reduced. This is consistent with several previously published studies in solid organ transplantation reporting elevated unbound MPA concentrations in patients with significant renal dysfunction.[8-10,20,21] Increased MPA exposure in the presence of renal impairment is most likely due to reduced nonrenal CL of unbound MPA.[22,23] The nonrenal CL of MPA is mediated in the liver primarily through UDP glucuronosyltransferases.[24] Increasing evidence suggests that acute renal failure (or injury) may cause significant alterations in the nonrenal CL of drugs extensively metabolized by the liver.[23,25,26] The specific mechanisms that lead to reduced hepatic CL in renal dysfunction are still not well understood. The accumulation of azotemic or uremic molecules may have a direct impact on the expression or activity of metabolic enzymes.[23,26] There is evidence to suggest that renal failure leads to reduced phase II enzymatic activity, including glucuronidation, presumably through the presence of uremic toxins.[23,27,28] Other mechanisms that may also contribute to reduced hepatic CL of unbound MPA CL in patients with acute renal failure include transplant-related inflammation or drug interactions (enzyme inhibitors).

To our knowledge, this is the first study to demonstrate that severe hepatic dysfunction may lead to impaired unbound MPA CL and elevated drug exposure. Our analysis showed that total bilirubin is an important predictor of unbound MPA CL. In subjects with severe hepatic dysfunction (total bilirubin >10 mg/dL), unbound MPA CL was approximately 3-fold lower when compared with those with a total bilirubin 10 mg/dL or less. Several pathological changes in the liver can lead to decreased hepatic metabolic capacity, including a reduction in total liver mass, loss of functioning cells, impaired uptake of drugs across the capillary endothelium, and reduced blood flow.[29-31] Impaired glucuronidation has been demonstrated in patients with severe cirrhosis, and UGT enzymes may be inhibited in patients with active inflammatory conditions.[29,32] Multiple clinical factors or disease states that are common following alloHCT could impair glucuronidation or reduce liver blood flow, including drug-induced hepatic dysfunction or veno-occlusive disease.[13,33] Of the 36 subjects included in this analysis, only 6 individuals had a total bilirubin levels greater than 10 mg/dL. Confirmatory studies including a larger number of subjects with variable hepatic function are needed to better define these relationships.

Body weight was found to be a significant covariate affecting unbound MPA CL. Based on the population PK analysis, the predicted CL of unbound MPA normalized to weight is higher in smaller children and declines with increasing body weight. Our data indicate that for children less than 10 kg, higher doses may be required to achieve exposure similar to that in adults known to prevent aGVHD. The effect of weight on the CL of unbound MPA is not surprising, as smaller or younger children often require higher weight-normalized doses of drugs compared with older adolescents or adults because of increased renal CL, particularly when elimination occurs through both glomerular filtration and active tubular secretion via transporters.[34] Expression of UGT enzymes involved in MPA metabolism, including UGT1A9 and UGT2B7, undergoes significant changes, increasing gradually over the first 2 years of life.[35,36] The median age of subjects in this study was 5 years (range, 0.17-36), with only 36% (n = 13) of subjects younger than 2 years of age. Future studies should be pursued that include a larger number of children less than 2 years of age, as these analyses may yield important information about the relationship between unbound MPA PK and the ontogeny of hepatic glucuronidation.

In the present analysis, we found no impact of serum albumin on unbound MPA CL. Albumin concentrations less than 3.1 g/dL have been associated with significantly higher unbound MPA concentrations in renal transplantation.[1] Elevated unbound MPA exposure has been reported in pediatric renal transplant recipients with decreased serum albumin

levels.[1] A lack of correlation between unbound MPA and albumin in this study may be due to several factors, including the limited range of serum albumin concentrations among subjects, the severity of disease or clinical status, or the impact of coadministered medications on albumin binding.

Although the PK parameters were well estimated in our final model, the residual unexplained variability was considerably high (56%). This suggests that other clinical or patient-specific factors not tested in this analysis study may be determinants of unbound MPA CL. Potential factors include changes in the patient's clinical status or differences in coadministered medications at the time of sampling. Future studies are needed to evaluate the impact of coadministered medications on unbound MPA PK through induction or inhibition of UGT and MRP2. This is particularly relevant given that alloHCT recipients may receive 10 to 20 other medications concurrently with MMF. Corticosteroids, used in the treatment of aGVHD, have been shown to induce the expression of several UGT enzymes and may lead to enhanced unbound MPA CL.[2] CSA, a known inhibitor of MPR2, leads to decreased biliary excretion of MPAG and enterohepatic recycling of MPA.[2] Kidney transplant recipients receiving CSA have lower MPA concentrations compared with those receiving tacrolimus, sirolimus, or MMF alone.[2] Although all subjects were receiving CSA, exposure may be highly variable depending on clinical status. Similarly, quinolones are known substrates for MPR2 and may alter unbound MPA hepatic CL and renal excretion though competition with MPAG or MPA for binding sites.[37] Patients began receiving gut decontamination with a fluoroquinolone on day −1, but the duration of therapy was unknown. Other potential factors unexplored in this analysis include genetic variants of genes involved in MPA metabolism and disposition. Several allelic variants of UGT enzymes are known to alter in vitro enzymatic activity or modify MPA pharmacokinetics in the setting of renal transplantation.[38] The impact of several MRP2 genetic variants on MPA exposure has also been investigated, with variable results reported.[38] Given the complexity of alloHCT recipients and likelihood of concomitant medications that may mask or confound variant effects, genetic studies of unbound MPA exposure may prove difficult in alloHCT and will require careful consideration. Finally, chemotherapy, infection, and disease states such as aGVHD may alter unbound MPA disposition through induction or inhibition of drug-metabolizing enzymes or transporters, and the influence on UGT and MPR2 should also be explored in alloHCT.

The primary goal of this work was to evaluate patient-specific covariates as contributors to the variability of unbound MPA exposure in pediatric and young adult alloHCT recipients. Identifying clinical covariates that significantly influence unbound MPA exposure is important to provide better dosing strategies for immunosuppressive therapy in alloHCT. However, it must be acknowledged the therapeutic targets for mycophenolate therapy in pediatric alloHCT remain poorly defined. In our previous study, an unbound MPA $AUC_{0\text{-}12}$ less than 300 ng·h/mL week 1 post transplant in adult alloHCT recipients was associated with greater risk of developing aGVHD.[3] Increased risk of leukopenia and infection has been shown in solid organ recipients with an unbound MPA $AUC_{0\text{-}12}$ greater than 400 ng·h/mL.[14] Several case reports have shown neutropenia or engraftment failure in patients with high unbound MPA and severe organ dysfunction, and additional studies suggest that elevated unbound MPA concentrations are associated with adverse effects.[15-17,39] Therefore, pharmacokinetic–pharmacodynamic studies in pediatric alloHCT are urgently needed to better understand the relationships between unbound MPA exposure and clinical outcomes and to define therapeutic targets. Given the small number of subjects and significant heterogeneity among subjects, the relationships between pharmacokinetic parameters and clinical outcomes were not evaluated in this analysis.

In conclusion, we developed a population PK model of unbound MPA CL in children and young adult alloHCT recipients. Unbound MPA CL was reduced and exposure increased in subjects as CrCL declined. In individuals with severe hepatic dysfunction (total bilirubin >10 mg/dL), unbound MPA CL was approximately 3-fold lower compared with patients with normal to mild hepatic impairment. These results demonstrate that dose reductions may be necessary in alloHCT recipients with renal dysfunction and severe hepatic injury to prevent drug-induced toxicity and ensure optimal immunosuppression.

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