Mechanism-Based Enterohepatic Circulation Model of Mycophenolic Acid and Its Glucuronide Metabolite: Assessment of Impact of Cyclosporine Dose in Asian Renal Transplant Patients

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Mycophenolic acid (MPA) is mainly metabolized to MPA-glucuronide (MPAG), which may be reconverted to MPA following enterohepatic circulation (EHC). A physiologically realistic EHC model was proposed to estimate and assess the impact of cyclosporine (CsA) dose on the extent of EHC of MPA and MPAG. After the first oral dose of mycophenolate mofetil (MMF), the MPA and MPAG plasma concentration-time data of 14 adult renal transplant patients (12 receiving concomitant CsA and prednisolone and 2 receiving only concomitant prednisolone without CsA) were analyzed by individual pharmacokinetic modeling using a proposed 5-compartment drug and metabolite EHC model with a time-varying gallbladder emptying process. Simulations were performed to assess the influence of the time of bile release after dosing (T_{bile}) and the gallbladder emptying interval (τ_{gall}) on the EHC process. The extent of EHC for both MPA and MPAG tended to be lower in the group receiving CsA coadministration and decreased with increasing total body weight–adjusted CsA dose. Simulations revealed that T_{bile} and τ_{gall} influenced the time of occurrence and maximum concentration of the second peak, as well as the extent of EHC, for MPA and MPAG.

Keywords: *Mycophenolic acid; pharmacokinetic modeling; enterohepatic circulation; renal transplant; cyclosporine*

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Mycophenolate mofetil (MMF), the ester prodrug of mycophenolic acid (MPA), is a potent immunosuppressant used in combination with a calcineurin inhibitor (cyclosporine [CsA][1-3] or tacrolimus[4-7]) and corticosteroids for the prophylaxis of organ rejection in renal, cardiac, and hepatic transplant recipients. After oral administration, MMF is rapidly absorbed and hydrolyzed to MPA, the active immunosuppressive entity, by esterases present in the gut wall, liver, and possibly lung and peripheral tissues.[8] MPA is extensively metabolized by uridine diphosphate glucuronosyltransferases (UGTs) in the liver, kidney, and gastrointestinal tract (GIT) mainly into its inactive metabolite, MPA-glucuronide (MPAG).[8,9] MPAG is primarily excreted by the kidneys and may undergo enterohepatic circulation (EHC) to be converted back to MPA by bacterial beta-glucuronidases in the GIT.[8,10] This EHC process results in the occurrence of a secondary peak in the plasma MPA concentration-time profile that is observed around 4 to 12 hours postdose,[11] as well as in the plasma MPAG concentration-time profile that is less pronounced and delayed in relation to the MPA secondary peak.[10] The contribution of EHC to MPA exposure has been estimated to range from 10% to 61%, with a mean of 37%, in healthy individuals.[8,10]

To describe the complex pharmacokinetics (PK) of MPA and MPAG, one should have a PK model

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incorporating the EHC process. To date, 2 EHC models have been developed for PK analysis of MPA based on the population approach.[12,13] The first model by Funaki[12] was a simplified 3-compartment EHC model based on a 1-compartment disposition model for MPA and included the central, gut, and gallbladder compartments. This model assumed MPA as the recirculating entity and bile release as a bolus at the time of discharge from the gallbladder.[12] However, it is known that MPA undergoes EHC primarily as its conjugate metabolite MPAG[8,10] and that bile release does not physiologically occur as a bolus. Instead, bile release occurs over time, typically taking around 1 hour for complete gallbladder emptying in the presence of adequate amounts of fat in the meal for humans.[14] The other reported EHC model by Cremers et al[13] was a 4-compartment EHC model comprising the gut, central, and peripheral compartments for MPA, as well as an additional central compartment for MPAG. This is an improvement over the former model as it has taken into account that the recirculating entity is MPAG and that MPA exhibits biexponential elimination, which has been well described by most 2-compartment population PK models.[15-20] However, in this latter model, EHC was modeled as a continuous process,[13] which was physiologically unrealistic as gallbladder emptying occurs intermittently at irregular intervals and commences when food digestion starts to occur in the upper GIT about 30 minutes after a meal.[14]

This study was therefore undertaken to propose an improved EHC model that was physiologically more realistic to better describe the PK of MPA and MPAG, particularly the EHC process. In contrast to the 2 earlier models by Funaki[12] and Cremers et al,[13] which have been developed for population modeling analysis, the improved EHC model that is proposed in the current study was fit to each participant separately. The objectives of this study were to quantitatively describe the EHC of MPA and MPAG, as well as to characterize and assess the impact of CsA dose on the extent of EHC of MPA and MPAG after oral administration of the first dose of MMF in Asian renal transplant recipients.

METHODS

Study Design

This was part of a prospective, open-labeled, single-center PK study conducted in Singapore General Hospital (SGH). This study was approved by the institutional review board at SGH, and all patients were recruited after written informed consent. This article presents a post hoc analysis study to characterize the EHC process of MPA and MPAG by PK modeling.

Patients

Renal transplant recipients who were newly started on MMF (CellCept, Roche Pharmaceuticals, Basel, Switzerland) after conversion from azathioprine to MMF therapy were recruited into an acute dose study to evaluate MPA and MPAG PK. Patients were excluded from the study if they had (1) severe gastrointestinal disorders that interfered with their ability to receive or absorb oral medication, (2) severe diarrhea (more than 5 watery stools per day), (3) liver disease (seropositivity for hepatitis B surface antigen or anti–hepatitis C antibody or elevated alanine aminotransferase [ALT] and/or aspartate aminotransferase [AST] levels more than 3 times normal), and/or (4) cholecystitis, cholecystectomy, or other gallbladder disorders. Patients were also excluded if they were taking antacids but were included if they were consuming H2 receptor blockers (n = 1) or proton pump inhibitors (n = 4).

Demographic and Biochemical Data Collection

Pertinent demographic data were collected, including age, gender, race, and weight; concomitant medications and dosing regimen; interval posttransplant; nature of transplant (deceased or live donor); and any concurrent underlying medical condition(s). In addition, the results of any routine clinical laboratory tests that were carried out on the day of PK investigation, such as serum creatinine, serum albumin, serum total bilirubin, serum alkaline phosphatase, and serum ALT and AST, were also recorded.

Blood Sampling

Venous blood samples (3 mL) were collected into ethylenediaminetetraacetic acid–containing Vacutainer tubes over a 12-hour period at the targeted time points of 0 (predose), 0.5, 1, 1.5, 2, 6, and 12 hours after administration of the first dose of MMF (morning dose) under supervision. Food and fluid intake by the study participants were only allowed without special restrictions, after collection of the 0-hour blood sample and immediately after MMF administration. For each blood sampling time

point, ±10 minutes were allowed. The exact times of MMF administration and blood sampling were recorded. The blood samples were centrifuged at 3000 rpm for 10 minutes at 25°C using a UNIVERSAL 32R centrifuge (Hettich, Germany) to harvest plasma that was transferred to polypropylene tubes and stored at –20°C until analysis.

Sample Analysis

Plasma samples were analyzed for the concentrations of both total MPA and total MPAG simultaneously based on the previously developed and validated high-performance liquid chromatography method.[21] The intraday imprecision for measurements of MPA and MPAG in plasma at 304 nm was less than 2.5% and 8.2%, respectively; the interday imprecision was not more than 8.6% and 10.0%, respectively.[21] The accuracy of the assay of total MPA (0.5-40 mg/L) and total MPAG (10-400 mg/L) in plasma samples was within 86.0% to 104% and 89.9% to 115%, respectively.[21]

Compartmental Pharmacokinetic Analysis

Pharmacokinetic Model

MPA and MPAG plasma concentration-time data were fitted to a 5-compartment drug and metabolite EHC model, as shown in Figure 1. This model was modified from a 4-compartment model by Cremers et al[13] and an EHC model by Gabrielsson and Weiner.[22] This model includes 2 compartments for MPA; 1 compartment for its metabolite, MPAG; and 2 additional compartments—namely, the gallbladder and gut compartments—to incorporate the EHC process. The differential equations describing the compartmental mass transfer for the model, as well as a glossary of the terms used in the figure and the equations, are presented in Table I.

In this 5-compartment drug and metabolite EHC model (Figure 1, Table I), the MMF dose is administered orally and rapidly hydrolyzed into MPA in the gut. MPA is absorbed after a lag time, t_{lag}, and the absorption is described by a first-order absorption rate constant, k_a. MPA distributes between the central and peripheral compartments, which is parameterized by intercompartmental clearance, Q. The elimination of MPA occurs via 2 pathways: renal excretion and metabolism mainly to MPAG, with the corresponding first-order rate constants being k_r and k_m, respectively. Once formed, MPAG is assumed to distribute readily throughout the central compartment. MPAG is eliminated from the central compartment by 2 pathways: renal and biliary excretion, with the corresponding first-order rate constants being $k_{r,m}$ and k_{bile}, respectively. The EHC process is represented in the model by 2 processes—namely, the biliary excretion of MPAG and gallbladder emptying. The bile is emptied intermittently from the gallbladder into the gut and assumed to occur only once in each dosing interval. At the time of release of bile from the gallbladder after dosing (T_{bile}), MPAG is released to the gut over the time interval of bile release (τ_{gall}), with a zero-order release rate of MPAG from the gallbladder compartment, $\frac{A_{bile}}{\tau_{gall}}$. In the gut, MPAG is rapidly and completely reconverted back to MPA, which is reabsorbed into the central compartment.

The following assumptions were made in the development of this model: (1) MMF was completely and rapidly hydrolyzed to form MPA immediately after oral administration, so the de-esterification of MMF was not included in the model; (2) only one bile release, and hence only one EHC process, occurred per dose interval; (3) bile release from the gallbladder occurred only at a certain time after dosing, T_{bile}, and over a particular gallbladder emptying interval, τ_{gall}; (4) all minor metabolic pathways were disregarded as the major metabolic pathway of MPA was the glucuronidation of MPA to its main primary metabolite, MPAG; and (5) MPAG was totally and rapidly hydrolyzed to MPA in the gut immediately after bile release, followed by complete absorption of MPA.

Computer Fitting of Model

The PK modeling was performed using the WinNonlin Professional software (Version 5.0.1, Pharsight Corporation, Cary, North Carolina). The differential equations (equations (1)-(5), Table I) were written in FORTRAN notation for the 5-compartment drug and metabolite EHC model (Figure 1). For each study participant, these equations were fit to the observed plasma concentration-time data of both total MPA and total MPAG simultaneously by nonlinear least squares regression analysis based on the Nelder-Mead simplex minimization algorithm with iterative reweighting by the reciprocal of the square of the predicted plasma concentration. The MMF doses and MPAG plasma concentrations were all converted to MPA equivalents for the PK modeling.

The initial estimates of the parameters V_1, V_2, V_m, Q, k_a, k_f, k_{bile}, k_r, $k_{r,m}$, t_{lag}, T_{bile}, and τ_{gall} were varied to minimize the weighted residual sum of squares

Table I Differential Equations Describing the Compartmental Mass Transfer for the 5-Compartment Drug and Metabolite EHC Model

Differential equations for the five-compartment model

$$\frac{dC_1}{dt} = \begin{cases} 0 & \text{when } t \le t_{lag} \quad (1a) \\ \frac{(FDk_a e^{-k_a(t-t_{lag})} + k_a A_{gut} - k_m C_1 V_1 - QC_1 + QC_2 - k_r C_1 V_1)}{V_1} & \text{when } t > t_{lag} \quad (1b) \end{cases}$$

$$\frac{dC_2}{dt} = \frac{(QC_1 - QC_2)}{V_2} \quad (2)$$

$$\frac{dC_m}{dt} = \frac{(k_m C_1 V_1 - k_{r,m} C_m V_m - k_{bile} C_m V_m)}{V_m} \quad (3)$$

$$\frac{dA_{bile}}{dt} = \begin{cases} k_{bile} C_m V_m - \frac{A_{bile}}{\tau_{gall}} & \text{when } T_{bile} \le t \le (T_{bile} + \tau_{gall}) \quad (4a) \\ k_{bile} C_m V_m & \text{when } t < T_{bile} \text{ or } t > (T_{bile} + \tau_{gall}) \quad (4b) \end{cases}$$

$$\frac{dA_{gut}}{dt} = \begin{cases} \frac{A_{bile}}{\tau} - k_a A_{gut} & \text{when } T_{bile} \le t \le (T_{bile} + \tau_{gall}) \quad (5a) \\ -k_a A_{gut} & \text{when } t < T_{bile} \text{ or } t > (T_{bile} + \tau_{gall}) \quad (5b) \end{cases}$$

where

F	bioavailability
D	oral MMF dose (expressed in MPA equivalents)
C_1	concentration of MPA in the central compartment
C_2	concentration of MPA in the peripheral compartment
C_m	concentration of MPAG (expressed in MPA equivalents) in the central compartment for MPAG
V_1	volume of central compartment for MPA[a]
V_2	volume of peripheral compartment for MPA[a]
V_m	volume of central compartment for MPAG[a]
A_{gut}	amount of MPA in the gut compartment
A_{bile}	amount of MPAG (expressed in MPA equivalents) in the gallbladder compartment
Q	intercompartmental clearance of MPA[a]
k_a	absorption rate constant of MPA
k_m	formation rate constant of MPAG
k_{bile}	biliary excretion rate constant of MPAG
k_r	renal excretion rate constant of MPA
$k_{r,m}$	renal excretion rate constant of MPAG
t_{lag}	lag time for absorption
T_{bile}	time of bile release after dosing
τ_{gall}	gallbladder emptying interval

EHC, enterohepatic circulation; MPA, mycophenolic acid; MPAG, MPA-glucuronide; MMF, mycophenolate mofetil.
a. The clearance and volume terms are apparent oral values (ie, Q/F, V/F), but for simplicity, F is not shown in the notations.

(WRSS) and Akaike information criterion (AIC) and to increase the correlation coefficient (R) between the predicted and observed data for each fit. The best fit of model to the observed plasma concentration-time data of total MPA and total MPAG for each study participant was one with the lowest possible WRSS and AIC values and the largest possible R value. To mimic the physiological condition of bile release, we restricted τ_{gall} to be between 0.5 and 3 hours, whereas T_{bile} was selected to be around mealtime whenever possible. The parameter estimates for V_1, V_2, V_m, Q, k_a, k_f, k_{bile}, k_r, $k_{r,m}$, t_{lag}, T_{bile}, and τ_{gall} were obtained from the best model fit of the observed plasma concentration-time data for each study participant.

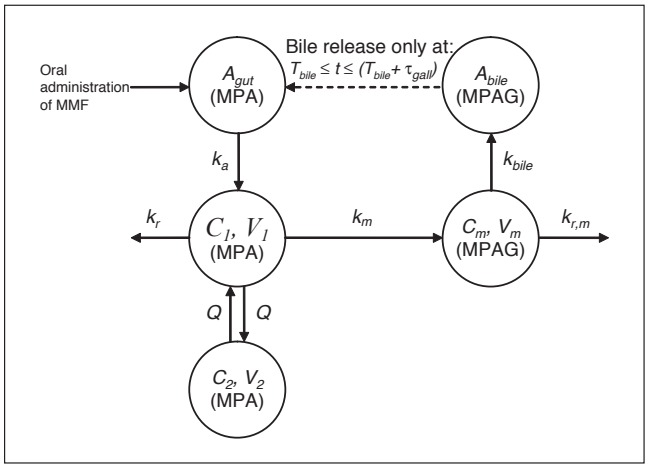

Figure 1. A 5-compartment drug and metabolite EHC model describing the pharmacokinetics of MPA and MPAG after oral administration of MMF. The terms are defined in Table I.

The apparent oral clearance of MPA (CL_{MPA}) and MPAG (CL_{MPAG}), apparent formation clearance of MPA to MPAG (CL_f), apparent biliary clearance of MPAG ($CL_{MPAG,bile}$), apparent volume of distribution for MPA (V_d), biliary excretion half-life of MPAG ($t_{1/2,MPAG,bile}$), and elimination half-lives of MPA ($t_{1/2,MPA}$) and MPAG ($t_{1/2,MPAG}$) were calculated according to the following equations:

$$CL_{MPA} = (k_r + k_m) \times V_1, \quad (6)$$

$$CL_{MPAG} = (k_{r,m} + k_{bile}) \times V_m, \quad (7)$$

$$CL_f = k_m \times V_1, \quad (8)$$

$$CL_{MPAG,bile} = k_{bile} \times V_m, \quad (9)$$

$$V_d = V_1 + V_2, \quad (10)$$

$$t_{1/2,MPAG,bile} = \frac{\ln 2}{k_{bile}}, \quad (11)$$

$$t_{1/2,MPA} = \frac{\ln 2}{\lambda_{Z,MPA}}, \quad (12)$$

$$t_{1/2,MPAG} = \frac{\ln 2}{\lambda_{Z,MPAG}}, \quad (13)$$

where $\lambda_{Z,MPA}$ and $\lambda_{Z,MPAG}$ are the first-order overall elimination rate constants of MPA and MPAG, respectively, estimated by least squares regression of values in the terminal log-linear portion of the respective model-fitted MPA and MPAG PK profiles.

The area under the model-fitted plasma concentration-time curve from time 0 to infinity with ($AUC_{\infty,\ with\ EHC}$) and without ($AUC_{\infty,\ without\ EHC}$) EHC and the extent of EHC were estimated based on the following equations using the linear trapezoidal method for the calculation of AUC values:

$$AUC_{\infty,with\ EHC} = \int_0^{\infty} C(t)dt, \quad (14)$$

where C(t) is the plasma concentration at time t of the model-fitted PK profile with 0.1-hour interval concentrations.

$$AUC_{\infty,without\ EHC} = AUC_{EHC\ start} + \frac{C_{EHC\ start}}{\lambda_Z}, \quad (15)$$

where $AUC_{EHCstart}$ is the area under the model-fitted plasma concentration-time curve with 0.1-hour interval concentrations from time 0 to the time of start of EHC, and $C_{EHCstart}$ is the plasma concentration at the time of start of EHC.

$$\text{Extent of EHC}\,(\%) = \frac{AUC_{\infty,\ with\ EHC} - AUC_{\infty,\ without\ EHC}}{AUC_{\infty,\ with\ EHC}} \times 100\%. \quad (16)$$

To test the applicability of the present 5-compartment drug and metabolite EHC model to fit MPA concentration-time data demonstrating different features of MPA PK, we fitted the model to MPA data obtained from individual concentration-time profiles from 3 different adult renal transplant recipients receiving CsA-MMF-prednisone immunosuppressive therapy reported by Shum et al.[15]

Computer Simulation of the Model

As intensive blood sampling between 6 and 12 hours was not possible in the present study, the exact time of occurrence and duration of the second peaks for MPA and MPAG could not be determined by visual inspection of the observed plasma concentration-time profiles. Hence, simulations were carried out with the WinNonlin Professional software. The total MPA and total MPAG plasma concentration-time curves for each study participant were simulated using the 5-compartment drug and metabolite EHC model by varying the 2 parameters, T_{bile} and τ_{gall}, to assess their influence on the EHC process in each individual participant. The values for the other parameters (V_1, V_2, V_m, Q, k_a, k_m, k_{bile}, k_r, $k_{r,m}$, t_{lag}) used in the simulations were obtained from the best model fit of the observed plasma concentration-time data for each study participant as described in the previous section. These parameters were kept constant while simulations were performed

by varying either T_{bile} or τ_{gall}. The values for T_{bile} and τ_{gall} were varied from 3 to 11 hours and 0.1 to 4 hours, respectively. When employed as constants, values for T_{bile} and τ_{gall} were set at their respective values obtained from the initial model fitting like the other parameters. Data points were generated at a 0.1-hour interval from 0 to 12 hours.

For each study participant, the simulated profiles that displayed close fit with most of the observed concentration-time data were selected for evaluation of the influences of T_{bile} and τ_{gall} on the extent of EHC for both MPA and MPAG. The extent of EHC of the simulated plasma concentration-time curves for each study participant was determined using equations (14) to (16).

Statistical Analysis

Statistical analyses were performed using SPSS 13.0 (SPSS, Inc, Chicago, Illinois). The 1-sample Kolmogorov-Smirnov test was used to test for normality. As some demographic data and PK data were normally distributed and some were not, data were all expressed as mean ± standard deviation (SD) and median (range) to allow a better comparison of the data. Comparisons of demographics data and PK data between the 2 independent groups receiving CsA-MMF-prednisolone and MMF-prednisolone, respectively, were performed using the independent-samples *t* test with Levene's test for homogeneity of variances to test for violation of the assumption of equal variance for normally distributed data, and they were performed using the Mann-Whitney test for nonnormally distributed data. Correlation analysis was carried out to determine the relationships between the extent of EHC for MPA or MPAG and total body weight–adjusted CsA dose (mg/kg/d). A *P* value of less than .05 was considered statistically significant.

RESULTS

Patient Demographics

Fourteen renal transplant patients without known gastrointestinal or hepatobiliary disorders who had been newly converted from azathioprine to MMF therapy were recruited into and analyzed in this study. Of these 14 study participants, 12 were receiving CsA-MMF-prednisolone immunosuppressive therapy, whereas 2 were receiving calcineurin inhibitor–sparing immunosuppressive regimen comprising only MMF and prednisolone. All study participants had received twice-daily MMF doses of 500, 750, or 1000 mg as clinically indicated. Study participants receiving concomitant CsA (Neoral, Novartis Pharmaceuticals) had undergone CsA dose adjustments according to 2-hour CsA levels and clinical circumstances as previously described.[23] All participants were also on oral sulfamethoxazole-trimethoprim for prophylaxis against *Pneumocystis jiroveci* infection. Other common oral medications administered to most of the patients included lipid-lowering agents, antihypertensives, and calcium supplements. The demographic characteristics of the study participants are summarized in Table II. Glomerular filtration rate was estimated based on serum creatinine, age, gender, and race using the abbreviated Modification of Diet in Renal Disease (aMDRD) formula.[24] All patients involved in this study were at least 1 month posttransplant, with the majority of patients being at least 1 year posttransplant. There were no statistically significant differences ($P > .05$) between the 2 patient groups in terms of age, total body weight, interval posttransplant, total body weight–adjusted MMF dose, prednisolone dose, and all biochemical data, as presented in Table II.

Model Fitting

For each study participant, the 5-compartment drug and metabolite model was fitted to the observed plasma concentration-time data of both total MPA and total MPAG simultaneously. Examples of the best fit of model to the observed plasma concentration-time data for 2 typical participants receiving CsA-MMF-prednisolone and MMF-prednisolone, respectively, are presented in Figure 2. For the model-fitted PK profiles of total MPAG (Figure 2), the plasma MPAG concentrations previously converted to MPA equivalents for model fitting had been recalculated back to actual plasma MPAG concentrations. The model-fitted PK profiles for all study participants exhibited a smaller secondary MPA peak between 5 and 12 hours postdose and a slightly delayed secondary MPAG peak following it.

The parameter estimates for V_1, V_2, V_m, Q, k_a, k_f, k_{bile}, k_r, $k_{r,m}$, t_{lag}, T_{bile}, and τ_{gall} providing the best fit to the data set of each participant are summarized in Table III. Upon comparison of the parameter estimates between the CsA-MMF-prednisolone and MMF-prednisolone groups, the estimate for T_{bile} was significantly smaller in the latter ($P < .05$); however, there were no statistically significant differences in the remainder of the parameters.

Table II Characteristics of Study Population

Characteristics	CsA-MMF-Prednisolone (n = 12)	MMF-Prednisolone (n = 2)
Male, n (%)	9 (75.0)	1 (50.0)
Ethnicity, n (%)		
Chinese	9 (75.0)	1 (50.0)
Malay	1 (8.3)	1 (50.0)
Indian	1 (8.3)	0 (0)
Eurasian	1 (8.3)	0 (0)
Donor source, n (%)		
Deceased	11 (91.7)	2 (100)
Live related	1 (8.3)	0 (0)
Twice daily dose of MMF, n (%)		
500 mg	3 (25.0)	1 (50.0)
750 mg	5 (41.7)	1 (50.0)
1000 mg	4 (33.3)	0 (0)
Age, y	46.0 ± 7.1	41.5 ± 0.7
	46.5 (33.0-57.0)	41.5 (41.0-42.0)
Total body weight, kg	70.3 ± 15.0	51.7 ± 6.1
	69.3 (47.2-98.5)	51.7 (47.4-56.0)
Interval posttransplant, mo	52.1 ± 43.8	104 ± 146
	56.1 (1.0-120.2)	104 (1.1-207)
Serum creatinine, μmol/L	189 ± 69	500 ± 378
	176 (94-309)	500 (232-767)
Serum albumin, g/L	32.1 ± 6.9	31.5 ± 5.0
	33.5 (19.0-44.0)	31.5 (28.0-35.0)
Serum total bilirubin, μmol/L	13.3 ± 6.1	13.0 ± 7.1
	13.5 (4.0-26.0)	13.0 (8.0-18.0)
Serum alkaline phosphatase, u/L	69.2 ± 29.9	44.5 ± 16.3
	62.0 (33.0-142.0)	44.5 (33.0-56.0)
Serum alanine aminotransferase, u/L	29.0 ± 18.2	22.0 ± 2.8
	23.5 (12.0-73.0)	22.0 (20.0-24.0)
Serum aspartate aminotransferase, u/L	25.0 ± 11.6	23.5 ± 2.1
	21.0 (11.0-43.0)	23.5 (22.0-25.0)
Estimated glomerular filtration rate, mL/min[a]	39.3 ± 18.2	14.3 ± 9.99
	38.3 (14.5-79.0)	14.3 (7.2-21.3)
Total body weight–adjusted MMF dose, mg/kg per dose	11.1 ± 2.5	12.0 ± 2.0
	10.8 (6.2-15.3)	12.0 (10.5-13.4)
Total body weight–adjusted CsA dose, mg/kg/d	3.68 ± 1.34	—
	3.21 (2.03-6.12)	
Prednisolone dose, mg/d	13.0 ± 7.7	27.5 ± 24.7
	(6.0-28.0)	27.5 (10.0-45.0)

All data are expressed as mean ± SD, median (range), unless specified otherwise. CsA, cyclosporine; MMF, mycophenolate mofetil.
a. Glomerular filtration rate was estimated using the aMDRD formula.[24]

The apparent clearance values and elimination half-lives of MPA and MPAG, as well as V_d, calculated based on the corresponding parameter estimates for each participant, are shown in Table IV. There was a trend toward smaller apparent clearance values and longer elimination half-lives of MPA and MPAG, as well as larger V_d, for the group receiving CsA-MMF-prednisolone as compared to that receiving MMF-prednisolone. However, the differences were not statistically significant except for the total body weight–normalized $CL_{MPAG,bile}$, which was significantly smaller in the former group ($P < .05$).

The extent of EHC for MPA and MPAG, respectively, was assessed by the contribution of EHC to AUC_∞ and a comparison of the AUC_∞ values with and without EHC (Table V). There were no statistically significant differences in the extent of EHC and AUC_∞ values with and without EHC, as well as

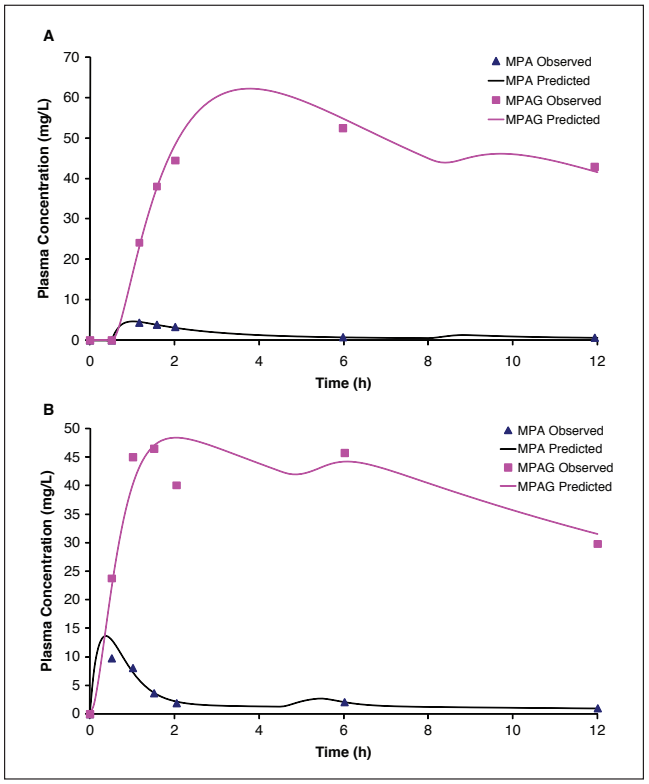

Figure 2. Best fit of model to the observed plasma concentration-time data of total mycophenolic acid (MPA) and total MPA-glucuronide (MPAG) for 2 typical participants after oral administration of the first dose of mycophenolate mofetil (MMF) in combination with (A) concomitant prednisolone and cyclosporine (CsA) and (B) concomitant prednisolone without CsA, respectively.

dose-normalized (in terms of mg per kg total body weight) AUC_{∞} values with and without EHC, between the 2 groups receiving CsA-MMF-prednisolone and MMF-prednisolone, respectively. Nonetheless, the AUC_{∞} values for both MPA and MPAG tended to be higher in patients with concomitant CsA immunosuppression as compared to those without, and the extent of EHC for both MPA and MPAG tended to be lower in the former (Table V). Further analysis revealed decreasing trends in the extent of EHC for both MPA and MPAG with increasing total body weight–adjusted CsA dose, which were not confounded by renal function, suggesting increasing degrees of EHC inhibition with increased CsA doses (Figure 3). These correlations were statistically significant (MPA: r^2 = 0.44, P = .009; MPAG: r^2 = 0.37, P = .022; Figure 3).

The concentration-time profiles of total MPA from 3 different adult renal transplant recipients receiving CsA-MMF-prednisone immunosuppressive therapy for at least 5 days as reported by Shum et al[15] and the best fit of the present 5-compartment EHC model to these data are presented in Figure 4. Despite a lack of MPAG data for these reported patients, the proposed model demonstrated its applicability to fit MPA concentration-time data displaying different features of MPA PK after repeated oral dosing—namely, a lag time in absorption, a complex absorption process, and a markedly significant EHC process, as seen in these 3 participants, respectively.[15] However, because of the absence of MPAG data in these participants, the parameter estimates associated with MPAG obtained during model fitting would have to be interpreted with caution (Table VI).

Model Simulation

The effects of changes in T_{bile} and τ_{gall} on the simulated concentration-time profiles were separately examined for each study participant. Examples of the simulated concentration-time profile produced by varying T_{bile} and τ_{gall} are presented in Figures 5 and 6, respectively. The plasma MPAG concentrations previously converted to MPA equivalents for model fitting had been recalculated back to actual plasma MPAG concentrations in Figures 5 and 6.

The time of occurrence of the second peak was dependent on both T_{bile} and τ_{gall}. For all study participants, the peak times of the second peak of both MPA ($t_{max2,MPA}$) and MPAG ($t_{max2,MPAG}$) increased when T_{bile} or τ_{gall} increased. Nonetheless, varying T_{bile} had negligible influence on the maximum concentration of the second peak of MPA ($C_{max2,MPA}$). On the contrary, the $C_{max2,MPA}$ decreased with increasing τ_{gall} for all patients. A similar trend was observed for the maximum concentration of the second peak of MPAG ($C_{max2,MPAG}$), which decreased with either an increase in T_{bile} or τ_{gall} for all study participants.

The influences of T_{bile} and τ_{gall} on the extent of EHC for both MPA and MPAG were determined by evaluating the extent of EHC of selected simulated profiles that displayed close fit with most of the observed concentration-time data for each individual participant. Overall, the extent of EHC for MPA and MPAG varied from 0.50 to 1.35 times and 0.71 to 1.46 times of the best fit of model, respectively, upon varying the T_{bile} within the range of 3 to 11 hours. The effect of changes in τ_{gall} on the extent of EHC was less prominent as compared to that in T_{bile}. The extent of EHC for MPA and MPAG varied from 0.93 to 1.12 times and 0.83 to 1.35 times of the best model-fitted profile, respectively, when τ_{gall} was varied within the range of 0.1 to 4 hours.

Table III Parameter Estimates Providing the Best Fit of the 5-Compartment Drug and Metabolite EHC Model to the Observed Plasma Concentration-Time Data of MPA and MPAG

Parameter	CsA-MMF-Prednisolone (n = 12)	MMF-Prednisolone (n = 2)
V_1, L	15.1 ± 10.9	7.6 ± 4.4
	11.9 (1.19-37.7)	7.6 (4.5-10.8)
V_1, L/kg	0.210 ± 0.147	0.154 ± 0.103
	0.187 (0.025-0.538)	0.154 (0.081-0.227)
V_2, L	188 ± 154	68 ± 39
	141 (31-593)	68 (41-96)
V_2, L/kg	2.72 ± 2.20	1.37 ± 0.92
	2.45 (0.51-8.48)	1.37 (0.72-2.02)
V_m, L	3.74 ± 1.53	6.42 ± 4.16
	3.64 (1.93-7.06)	6.42 (3.48-9.36)
V_m, L/kg	0.056 ± 0.026	0.120 ± 0.066
	0.053 (0.020-0.119)	0.120 (0.073-0.167)
Q, L/h	20.2 ± 11.9	9.7 ± 11.0
	16.8 (4.8-49.6)	9.7 (1.9-17.5)
Q, L/h/kg	0.301 ± 0.194	0.202 ± 0.236
	0.236 (0.068-0.730)	0.202 (0.035-0.369)
k_a, h^{-1}	1.57 ± 1.04	2.61 ± 0.39
	1.32 (0.51-3.58)	2.61 (2.34-2.89)
k_m, h^{-1}	1.69 ± 2.12	3.31 ± 3.45
	1.21 (0.34-8.10)	3.31 (0.87-5.75)
k_{bile}, h^{-1}	0.123 ± 0.086	0.128 ± 0.065
	0.106 (0.011-0.250)	0.128 (0.082-0.174)
k_r, h^{-1}	0.117 ± 0.111	0.106 ± 0.135
	0.087 (0.001-0.325)	0.106 (0.010-0.201)
$k_{r,m}$, h^{-1}	0.127 ± 0.105	0.023 ± 0.014
	0.110 (0.012-0.327)	0.023 (0.013-0.032)
t_{lag}, h	0.126 ± 0.198	0.230 ± 0.325
	0.000 (0.000-0.517)	0.230 (0.000-0.459)
T_{bile}, h	9.42 ± 1.22	6.00 ± 2.12
	10.00 (6.50-11.00)	6.00 (4.50-7.50)*
τ_{gall}, h	1.42 ± 0.82	0.72 ± 0.26
	1.09 (0.51-2.64)	0.72 (0.53-0.90)

All data were expressed as mean ± SD, median (range). The volume and clearance terms are apparent oral values (ie, V/F, Q/F), but for simplicity, F is not shown in the notations. CsA, cyclosporine; EHC, enterohepatic circulation; MPA, mycophenolic acid; MPAG, MPA-glucuronide; MMF, mycophenolate mofetil.

*Statistically different from CsA-MMF-prednisolone group ($P < .05$).

DISCUSSION

MPA exhibits a complex PK profile as it may undergo EHC via its inactive metabolite MPAG to be reconverted back to the active MPA.[8,10] Therefore, PK analysis by traditional 1- or 2-compartmental models may not adequately describe the PK of MPA and MPAG or appropriately estimate the λ_Z and hence the $t_{1/2}$ of such a drug that undergoes EHC. As such, a more complex model that incorporates EHC via MPAG, as previously presented by Cremers et al,[13] would better describe the PK of MPA and MPAG. However, this previous model, which was developed for population modeling analysis, suffers from the drawback of describing the EHC as a continuous process that is physiologically unrealistic and unable to depict the second peaks of MPA and MPAG because of EHC in the model-fitted PK profiles. An attempt to apply this model to characterize the EHC process in the recruited patients in the present study was similarly unable to demonstrate the second peaks of MPA and MPAG. Furthermore, this model made an extreme assumption that biliary excretion of MPAG is completely inhibited by CsA, and thus the EHC process

Table IV Apparent Clearance Values and Elimination Half-Lives of MPA and MPAG and Apparent Volume of Distribution for MPA Obtained Based on the 5-Compartment Drug and Metabolite EHC Model

Parameter	CsA-MMF-Prednisolone (n = 12)	MMF-Prednisolone (n = 2)
CL_{MPA}, L/h	15.0 ± 5.6 13.2 (8.5-27.2)	18.2 ± 12.5 18.2 (9.4-27.0)
CL_{MPA}, L/h/kg	0.219 ± 0.080 0.218 (0.119-0.389)	0.340 ± 0.201 0.340 (0.198-0.483)
CL_{MPAG}, L/h	0.847 ± 0.390 0.943 (0.270-1.421)	0.859 ± 0.298 0.859 (0.649-1.070)
CL_{MPAG}, L/h/kg	0.0124 ± 0.0063 0.0116 (0.0041-0.0261)	0.0164 ± 0.0038 0.0164 (0.0137-0.0191)
CL_f, L/h	13.2 ± 5.1 11.6 (5.9-21.9)	17.7 ± 11.9 17.7 (9.3-26.1)
CL_f, L/h/kg	0.191 ± 0.072 0.193 (0.086-0.313)	0.331 ± 0.191 0.331 (0.196-0.466)
$CL_{MPAG,bile}$, L/h	0.406 ± 0.334 0.364 (0.038-1.223)	0.686 ± 0.115 0.686 (0.604-0.767)
$CL_{MPAG,bile}$, L/h/kg	0.0057 ± 0.0045 0.0051 (0.0007-0.0175)	0.0132 ± 0.0007* 0.0132 (0.0128-0.0137)
V_d, L	203 ± 161 156 (41-631)	76 ± 43 76 (45-106)
V_d, L/kg	2.93 ± 2.31 2.56 (0.57-9.02)	1.53 ± 1.02 1.53 (0.81-2.25)
$t_{1/2,MPAG,bile}$, h	15.8 ± 21.4 6.7 (2.8-65.4)	6.2 ± 3.2 6.2 (4.0-8.5)
$t_{1/2,MPA}$, h	21.1 ± 26.8 11.6 (3.8-101.9)	13.4 ± 2.9 13.4 (11.4-15.5)
$t_{1/2,MPAG}$, h	19.7 ± 23.6 11.4 (4.7-91.2)	10.1 ± 1.7 10.1 (8.9-11.4)

All data were expressed as mean ± SD, median (range). The clearance and volume terms are apparent oral values (ie, CL/F, V/F), but for simplicity, F is not shown in the notations. CsA, cyclosporine; EHC, enterohepatic circulation; MPA, mycophenolic acid; MPAG, MPA-glucuronide; MMF, mycophenolate mofetil.
*Statistically different from CsA-MMF-prednisolone group ($P < .05$).

that was parameterized by the rate transfer constant describing biliary excretion of MPAG was being omitted in patients with CsA coadministration.[13] However, results from previous studies have demonstrated that the inhibition of EHC by CsA was not complete,[25,26] with CsA resulting in only about 17.5% reduction in the biliary excretion of MPAG.[26] Hence, this present study proposes an improved simultaneous MPA and MPAG 5-compartment EHC model that incorporates a separate time-varying gallbladder emptying process as an integral component of EHC to mimic physiological condition and is applied to the PK analysis based on individual modeling for all patients regardless of CsA coadministration.

Because of the infeasibility of intensive blood sampling in the present study, the second peaks for MPA and MPAG resulting from EHC could not be captured by the observed plasma concentration-time data for the study participants except for 1 patient without CsA coadministration (Figure 2B). Nonetheless, visual inspection of the observed plasma MPA concentration-time data of all the remaining 13 study participants revealed that the MPA plasma concentration at the 12-hour sampling time point was higher than that at 6 hours for most patients, and this strongly suggested the occurrence of EHC even in participants receiving concomitant CsA immunosuppression. It was thus necessary to perform PK modeling to characterize the EHC process in each individual study participant to better understand the complex PK of MPA and MPAG. The application of the proposed simultaneous MPA and MPAG model has enabled this PK analysis. The developed model allowed for the maximum number of PK parameters

Table V AUC_{∞} Values With and Without EHC and the Extent of EHC for MPA and MPAG Based on the 5-Compartment Drug and Metabolite EHC Model

Parameter	CsA-MMF-Prednisolone (n = 12)	MMF-Prednisolone (n = 2)
MPA		
$AUC_{\infty \text{ with EHC}}$, mg·h/L	45.8 ± 15.9 43.6 (24.9-73.4)	34.9 ± 13.7 34.9 (25.2-44.5)
$AUC_{\infty, \text{ without EHC}}$, mg·h/L	41.6 ± 14.7 38.6 (20.7-67.6)	29.9 ± 13.2 29.9 (20.6-39.2)
Dose-normalized $AUC_{\infty \text{ with EHC}}$, (mg·h/L)/(mg/kg)	5.74 ± 2.07 5.30 (3.15-9.32)	4.13 ± 2.24 4.13 (2.55-5.71)
Dose-normalized $AUC_{\infty, \text{ without EHC}}$, (mg·h/L)/(mg/kg)	5.21 ± 1.86 4.79 (2.61-8.39)	3.56 ± 2.08 3.56 (2.08-5.03)
Extent of EHC, %	9.2 ± 4.5 9.5 (1.8-17.1)	15.0 ± 4.4 15.0 (11.9-18.1)
MPAG		
$AUC_{\infty \text{ with EHC}}$, mg·h/L	1250 ± 1160 1030 (508-3660)	968 ± 32 968 (990-945)
$AUC_{\infty, \text{ without EHC}}$, mg·h/L	1160 ± 799 975 (465-3520)	836 ± 73 836 (785-888)
Dose-normalized $AUC_{\infty \text{ with EHC}}$, (mg·h/L)/(mg/kg)	152 ± 77 124 (65-324)	111 ± 22 111 (95-127)
Dose-normalized $AUC_{\infty, \text{ without EHC}}$, (mg·h/L)/(mg/kg)	141 ± 74 114 (59-311)	97 ± 24 97 (79-114)
Extent of EHC, %	7.7 ± 4.9 7.3 (0.5-18.8)	13.6 ± 4.6 13.6 (10.4-16.9)

All data were expressed as mean ± SD, median (range). CsA, cyclosporine; EHC, enterohepatic circulation; MPA, mycophenolic acid; MPAG, MPA-glucuronide; MMF, mycophenolate mofetil.

that could be estimated given the number of data points available per participant. Although a number of assumptions were made during the development of this 5-compartment drug and metabolite EHC model as mentioned earlier, the model has demonstrated its applicability to successfully characterize the complex PK profile, including the EHC process, of MPA and MPAG for all the 14 renal transplant recipients in this study who received their first dose of MMF with concomitant prednisolone and with or without CsA.

Following oral administration in healthy volunteers, the mean terminal half-lives of MPA and MPAG, which reflect both metabolic elimination and EHC, have been reported to be similar and around 16 hours,[8,10,27] and the mean apparent plasma clearance and volume of distribution of MPA are about 193 mL/min (or 11.58 L/h) and 4.0 L/kg, respectively.[27] The results obtained from this present study (Table IV) are in accordance with these reported data. The application of the proposed EHC model for PK analysis has facilitated the estimation of these parameters that could not be determined appropriately using noncompartmental analysis or traditional 1- or 2-compartmental models for drugs undergoing EHC.

The observation of a significantly smaller total body weight–normalized $CL_{MPAG,bile}$ for the group of patients with CsA coadministration (CsA-MMF-prednisolone group) as compared to that for the group without CsA (MMF-prednisolone group; Table IV) is consistent with the reported PK drug interaction of CsA-mediated inhibition of the hepatic canalicular transporter, multidrug resistance-associated protein 2 (MRP2), which is responsible for biliary excretion of MPAG.[25,26,28] As a result, a higher MPAG AUC_{∞} value in the former group would be expected, as was previously reported,[29] and this was similarly observed in the present study, although the difference was not significant (Table V). In addition, the inhibition of MRP2-dependent biliary excretion of MPAG by CsA would disrupt the EHC process and likely decrease MPA AUC_{∞} in the former group, as observed in an animal study.[29] However, an opposite though nonsignificant observation was seen in the present study (Table V), and this may be due

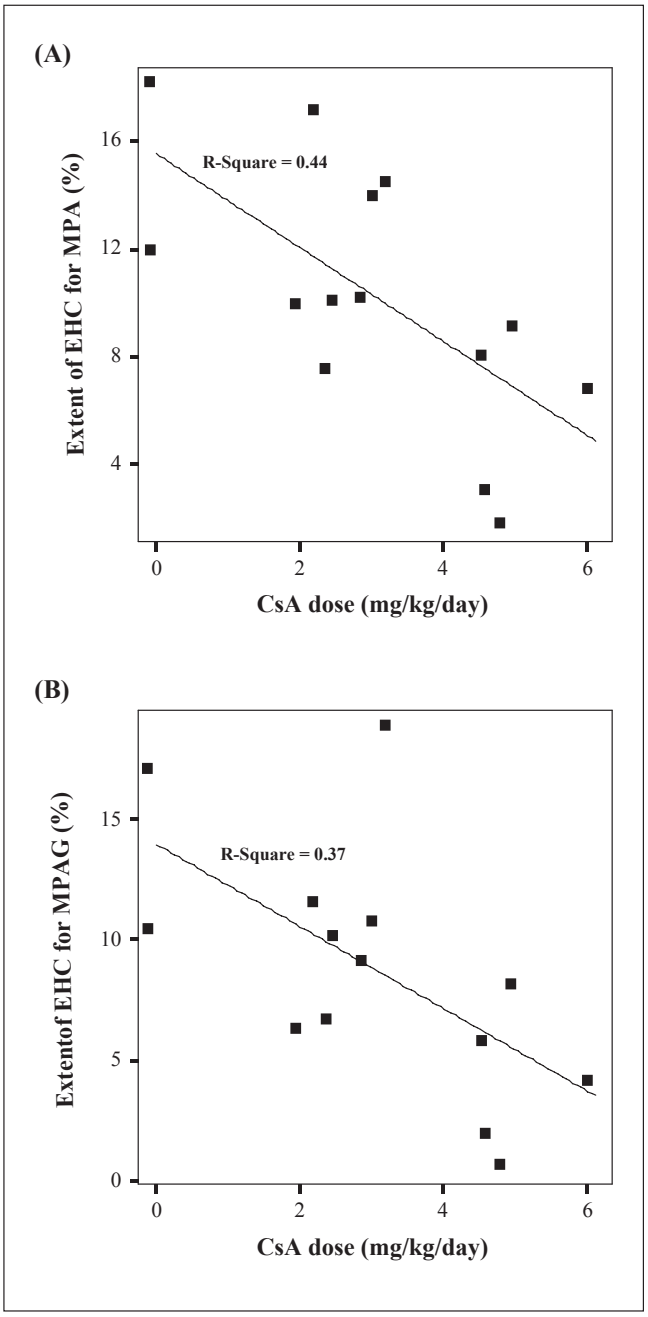

Figure 3. Correlations of the extent of enterohepatic circulation (EHC) for (A) mycophenolic acid (MPA) and (B) MPA-glucuronide (MPAG) with total body weight–adjusted cyclosporine (CsA) daily dose.

to the inhibition of the hepatic glucuronidation of MPA by CsA, which is another likely mechanism of the CsA-MMF drug interaction previously suggested.[26] Nonetheless, any observed differences in the PK parameters between the groups with and without

Table VI Parameter Estimates Providing the Best Fit of the 5-Compartment Drug and Metabolite EHC Model to the Observed Plasma Concentration-Time Data of MPA for 3 Adult Renal Transplant Recipients Receiving CsA-MMF-Prednisone Immunosuppression as Reported by Shum et al[15]

Parameter	Patient (A)[a]	Patient (B)[b]	Patient (C)[a]
V_1, L	11.0	24.1	36.4
V_2, L	38.6	73.1	30.5
V_m, L	1.12	0.908	0.559
Q, L/h	8.39	0.128	6.25
k_a, h^{-1}	2.64	1.05	0.557
k_m, h^{-1}	1.82	0.909	0.648
k_{bile}, h^{-1}	0.0596	0.0615	0.336
k_r, h^{-1}	0.00379	0.0723	0.00248
$k_{r,m}$, h^{-1}	0.0102	0.0315	0.0181
t_{lag}, h	0.943	1.69	0.214
T_{bile}, h	5.00	7.00	9.00
τ_{gall}, h	1.40	0.908	0.515

The volume and clearance terms are apparent oral values (ie, V/F, Q/F), but for simplicity, F is not shown in the notations. CsA, cyclosporine; EHC, enterohepatic circulation; MPA, mycophenolic acid; MMF, mycophenolate mofetil.
a. Pharmacokinetic profile on day 5 posttransplantation.
b. Pharmacokinetic profile on day 28 posttransplantation.

CsA coadministration in this study are not conclusive as the sample sizes of both groups were small, especially for the latter group in which there were only 2 patients. Furthermore, the current study is a post hoc PK modeling analysis and is not powered or designed to detect such differences. Future prospective studies investigating PK differences in MPA between patients with and without CsA coadministration and involving larger sample sizes may be proposed to draw more conclusive results.

Previous studies have reported the contribution of EHC to MPA exposure[8,10] but not the contribution of EHC to MPAG exposure. In this present study, the contribution of EHC to both MPA and MPAG exposure could be estimated using the proposed 5-compartment drug and metabolite model for MPA and MPAG. Although statistical significance was not reached, it was observed that the extent of EHC for both MPA and MPAG tended to be lower in patients receiving concomitant CsA immunosuppression than those without CsA coadministration (Table V). This is consistent with the reported inhibition of biliary excretion of MPAG by CsA, which decreased the EHC process.[25,26,29] In addition, it is interesting to note that the extent of EHC for both MPA and MPAG decreased with increasing total

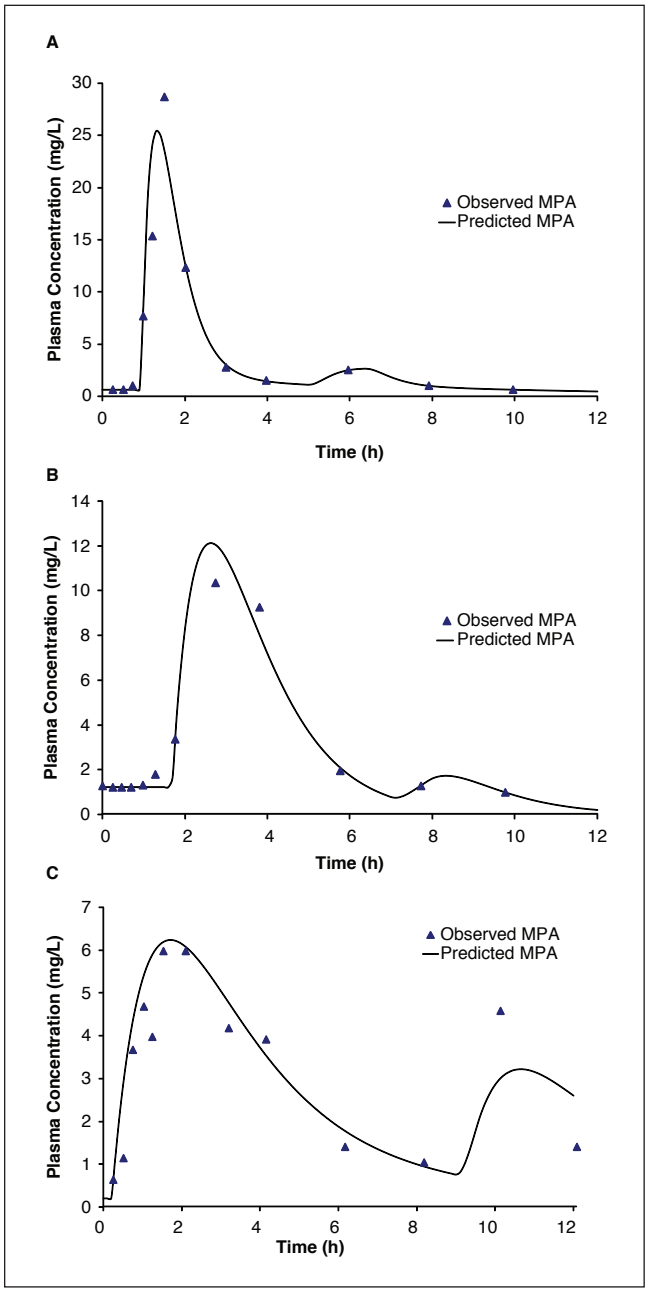

Figure 4. Best fit of model to the observed plasma concentration-time data of total mycophenolic acid (MPA) after an oral dose of mycophenolate mofetil (MMF) in 3 adult renal transplant recipients receiving cyclosporine (CsA)-MMF-prednisone for at least 5 days, demonstrating (A) a lag time in absorption, (B) a complex absorption process, and (C) a markedly significant enterohepatic circulation (EHC) process, respectively. These data were obtained from a study by Shum et al.[15]

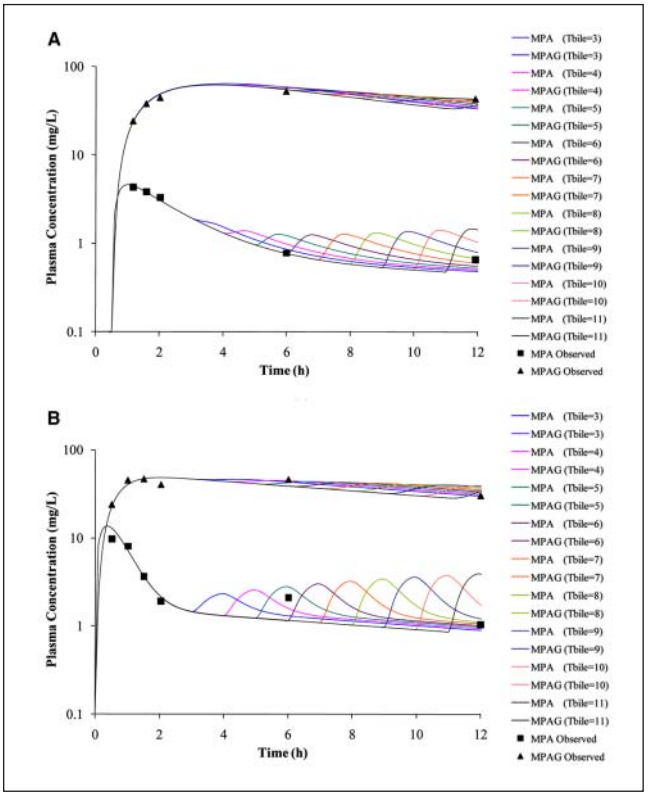

Figure 5. Influence of T_{bile} on plasma concentration-time profiles of total mycophenolic acid (MPA) and total MPA-glucuronide (MPAG) for 2 typical participants after oral administration of the first dose of mycophenolate mofetil (MMF) in combination with (A) concomitant prednisolone and cyclosporine (CsA) and (B) concomitant prednisolone without CsA, respectively.

body weight–adjusted CsA dose in the present study (Figure 3). This observation, which has not been previously reported in the literature, suggests that CsA-mediated inhibition of MPAG biliary excretion is dose dependent. The effect of the dose of CsA prescribed (mg/kg/d) on the disposition of MPA and MPAG is noteworthy as it may have an impact on the clinical outcome (safety and efficacy) of MMF therapy in patients receiving concomitant CsA immunosuppression.

As compared with previous studies,[8,10] the contribution of EHC to MPA exposure in all the current renal transplant patients analyzed (Table V) was much lower than or toward the lower end of the previously reported range of 10% to 61% (mean 37%) in healthy individuals,[8,10] even after considering the variation in the extent of EHC as a result of changes

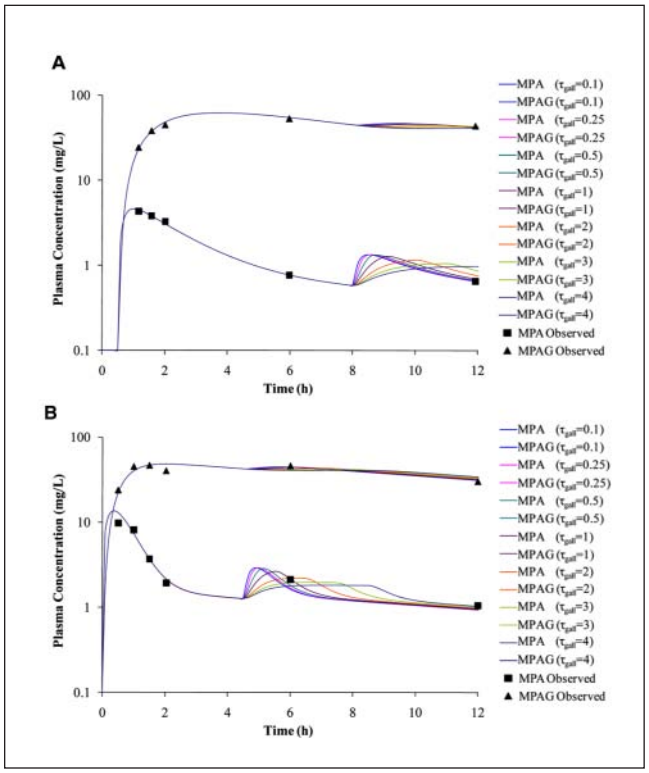

Figure 6. Influence of τ_{gall} on plasma concentration-time profiles of total mycophenolic acid (MPA) and total MPA-glucuronide (MPAG) for 2 typical participants after oral administration of the first dose of mycophenolate mofetil (MMF) in combination with (A) concomitant prednisolone and cyclosporine (CsA), and (B) concomitant prednisolone without CsA, respectively.

in the T_{bile} or τ_{gall} in the study participants based on simulations performed. These results strongly suggest that the influence of EHC on MPA exposure in the present Asian study participants is not significant. This is also supported by another study on Asian renal transplant recipients, specifically of Indian ethnicity, whereby the contribution of EHC on steady-state MPA exposure was also demonstrated to be insignificant in Indian participants receiving MMF and prednisolone, with CsA (mean 4.4% ± 2.9% for patients more than 1 year posttransplant) or without CsA (mean 5.1% ± 3.7% and 7.8% ± 10.9% for patients less than and more than 1 year posttransplant, respectively).[30] Furthermore, most population PK studies on renal transplant recipients reported that the second peak due to EHC was relatively small[16,19] or that EHC did not appear to influence the PK of MPA to a large extent,[17] thus resulting in the EHC process not being incorporated in these population PK models.[16,17,19]

In addition to the adequacy of the proposed 5-compartment EHC model to characterize the PK of both MPA and MPAG simultaneously in all the 14 participants in the present study, further evaluation of its applicability was carried out on literature reported data. However, due to the lack of actual observed plasma concentration-time data of both MPA and MPAG for renal transplant recipients from the literature, this evaluation could only be performed on the actual MPA data available from 3 individual concentration-time profiles reported by Shum et al,[15] which had a sufficient number of observations per PK profile for the model fitting. The proposed model was evaluated to adequately fit MPA plasma concentration-time data, even in the absence of MPAG data, of individual renal transplant patients on multiple dosing of MMF, as obtained from the study by Shum et al.[15] Despite the feasibility of such an application, it would be preferable to apply this proposed model to PK profiles containing both MPA and MPAG plasma concentration-time data to increase the reliability of the parameter estimates obtained.

In the present study, the applicability of the developed 5-compartment drug and metabolite EHC model to characterize the complex PK of MPA and MPAG simultaneously without intensive blood sampling suggests a potential clinical application of this model to predict MPA PK based on limited sampling. To assess the feasibility of this approach, we would need larger studies to determine the breadth of the expected PK variability that could not be captured with the relatively small patient cohort of 14 participants in this study. It would also be of clinical value to extend the use of this EHC model to other patient groups, including pediatric patients, patients receiving MMF with other concomitant medications such as tacrolimus and sirolimus, patients of other ethnicity, and patients receiving other types of organ transplants to aid in the estimation of MPA PK parameters in various groups of patients. Thus, further studies would be warranted to look into these aspects to evaluate such potential clinical applications of the current proposed EHC model.

CONCLUSION

This study presents a 5-compartment drug and metabolite EHC model, which incorporates a physiologically realistic time-varying gallbladder emptying process for the characterization of the complex PK of MPA and MPAG. The application of this model to the simultaneous PK analysis of both MPA

and MPAG in 14 renal transplant recipients after oral administration of the first dose of MMF has provided greater insight to the EHC process and its influence on the disposition kinetics of MPA and MPAG in these participants with or without CsA coadministration. The extent of EHC for both MPA and MPAG was found to decrease with increasing total body weight–adjusted CsA dose, suggesting a dose-dependent impact on CsA-mediated inhibition of MPAG biliary excretion.

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