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A Population Pharmacokinetic Analysis for Enteric-coated Mycophenolate Sodium Using Non-linear Mixed Effect Model in Korean Kidney Transplant Recipients

비선형 혼합효과 모형을 이용한 신이식 후 장용정 마이코페놀레이트의 집단약동학

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이 논문을 약학박사 학위논문으로 제출함
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Abstract

A Population Pharmacokinetic Analysis for Enteric-coated Mycophenolate Sodium Using Non-linear Mixed Effect Model in Korean Kidney Transplant Recipients

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Mycophenolic acid (MPA) has been used as an immunosuppressive agent to prevent rejection events as a combination with calcineurin inhibitor (CNI) and corticosteroids in renal transplantation. Since the first original mycophenolate mofetil (MMF) was introduced in 1995, the post-transplant rates of rejection decreased from ~40% to 20%. However, gastrointestinal adverse events are frequently observed in renal transplant recipients treated with MMF. To abrogate these adverse drug events and improve the clinical

outcome, enteric-coated mycophenolate sodium (EC-MPS, Myfortic®) was developed.

Although EC-MPS and MMF showed similar efficacy and safety profiles, the MPA pharmacokinetic (PK) parameters after administration of EC-MPS and MMF were different as a result of the enteric-coating formulation[1-3]. For instance, the lag-time varied much more in EC-MPS treated patients, resulting in unpredictable PK profiles. Moreover, ethnic differences in the prevalence of the metabolic enzymes and transporters may affect PK alterations. Nevertheless, fixed doses have been used empirically according to patient's body weight and concurrent medications in the clinical setting because there are no dosing strategies and prediction model established. In some studies, therapeutic drug monitoring (TDM) is recommended as a tool to optimize MPA treatment in renal transplant recipients. The MPA area under the total plasma concentration time curve (AUC) is a better predictor of the risk of rejection than trough concentration of MPA. But, AUC measurements have limitations to perform for practical reasons which the variability in MPA exposure is wide compared to the therapeutic window and is influenced by many factors[4].

The objective of this study was to develop a population PK model and to evaluate the influence of genetic and clinical factors on the MPA PK of EC-

MPS in Korean renal transplant recipients. And we aimed to design the optimum dosing strategies for this population, or individual patient considering the variability issues discussed in our population PK model.

Patients over 18 years of age, who were primary recipient of cadaveric or living-related kidney transplants, and who were maintained on 180 to 720 mg twice daily EC-MPS as part of a double or a triple immunosuppressive regimen with stable serum creatinine values, were asked to give informed consent to be qualified for inclusion in this study. Assuming the usual 80% power and 5% type I error, the range of sample size was 34-43.

For PK analysis, plasma samples were taken predose, 0.5, 1, 2, 3, 4, and 6 hour after morning EC-MPS administration. Total MPA concentrations were measured using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method in Chemical Laboratory of Seoul National University Hospital. Demographic and laboratory data, including renal function, weight, and genotypes of transporter and metabolic enzyme, were collected from electronic medical records. All patients were genotyped for *UGT1A7-9*, *UGT2B7*, *ABCC2*, and *SLCO1B*.

Population PK analysis of EC-MPS was performed using non-linear mixed-effects modeling (NONMEM). 1 and 2 compartment models with first-order or non-linear elimination were tested to fit the MPA concentration-time data.

The data were best described using a 2-compartment model with a lag-time, first-order absorption and first-order elimination. The entero-hepatic recirculation was described with a separate metabolic compartment.

Fixed effects of body weight, age, gender, donor type, graft weight, concomitant medications, biochemistry parameters as well as genotypes, which may influence the PK of MPA, were investigated. All covariates were incorporated into the model in such a way as to preserve positive PK parameters using additive, proportional, exponential, and/or power approaches. After clinical and genetic factors were evaluated using a stepwise covariate method, we selected clinically relevant covariates considering covariate effects. Population PK data were analyzed with NONMEM 7.2 using first-order conditional estimation with interaction (FOCE+I). In modeling, the minimum value of objective function (OFV) can be used as a criterion for model selection. A decrease in the OFV >3.84 or an increase in the OFV >6.64 shows a significant improvement of a nested model with one degree of freedom of p <0.05 and p <0.01, respectively. Model adequacy was further evaluated by using “goodness-of-fit (GOF)” plots through the use of Xpose 4 in R software ver. 3.0.2.

The final model was validated to accuracy and robustness by non-parametric bootstrap with resampling and prediction corrected visual predictive check

(pcVPC). Resampling was repeated 2,000 times and the 5^{th} and 95^{th} percentile values of estimated parameters from the bootstrap procedure were compared with those estimated from the original data set. And we plotted the observed concentrations and 90% prediction intervals of simulated concentrations versus time from 200 pcVPC results. The entire procedure was performed using Perl-speaks-NONMEM (PsN) ver. 3.6.2. And next, we performed a Monte Carlo simulation to explore the optimal dose in several simulation scenarios. The AUCs according to the several virtual scenarios were simulated based on a sample of 1,000 patients and compared with recommended AUC range from previous studies.

As a result, total 34 patients and 166 MPA plasma concentrations were included. A time lagged 2-compartment (central and metabolic compartment) with a flip-flop model best describes the PK of MPA. Population parameters of apparent clearance (CL/F), central and metabolic compartment volume of distribution (V_c/F and V_p/F), and absorption rate constant were estimated as 9.3 L/h, 42 and 60.3 L, and 1.24 hr^{-1}, respectively. The covariate analysis identified lower creatinine clearance (CLcr) and *SLCO1B1* 388A>G variant genotype were correlated with lower MPA clearance, on the contrary, *UGT1A9* -118dT variant had decreased distribution of MPA, contributing to lower absorption. The median estimates resulting from the bootstrap

procedure are similar to the population estimates of the final model. This validation method showed a good agreement between the simulated and observed concentrations at all sampling time points.

Finally, we performed the model-based simulations in order to define optimal dose to achieve target $AUC_{0\text{-}12h}$. A simulation of each 1,000 patients showed that the new dosing strategy resulted in a higher success of achieving the target $AUC_{0\text{-}12h}$ in the 30-60 mg.h/L. Furthermore, in most of cases, when considering to *UGT1A9*, *SLCO1B1* genotype, and renal function, MPA 540 mg twice daily is the optimum dose to reach a target $AUC_{0\text{-}12h}$.

In conclusion, CLcr, *UGT1A9* and *SLCO1B1* genotypes seem to be promising parameters to predict the pharmacokinetics with flip-flop phenomenon of EC-MPS in transplant recipient having stable renal function. This model on clinical practice may help prevent overexposure and to achieve a proper AUC in Korean population.

Keywords: EC-MPS, population pharmacokinetics, flip-flop phenomenon, nonlinear mixed-effect modeling (NONMEM), renal transplantation, genetic polymorphism

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I. Introduction

1 Kidney Transplantation in Korea

1.1 Current Status of Kidney Transplantation

When kidneys fail, there are three treatment choices including hemodialysis, peritoneal dialysis, and kidney transplantation. In Korea, dialysis is more common than transplantation, but the interest in transplantation has increased steadily in patients with end stage renal disease as the treatment of choice [Figure 1].

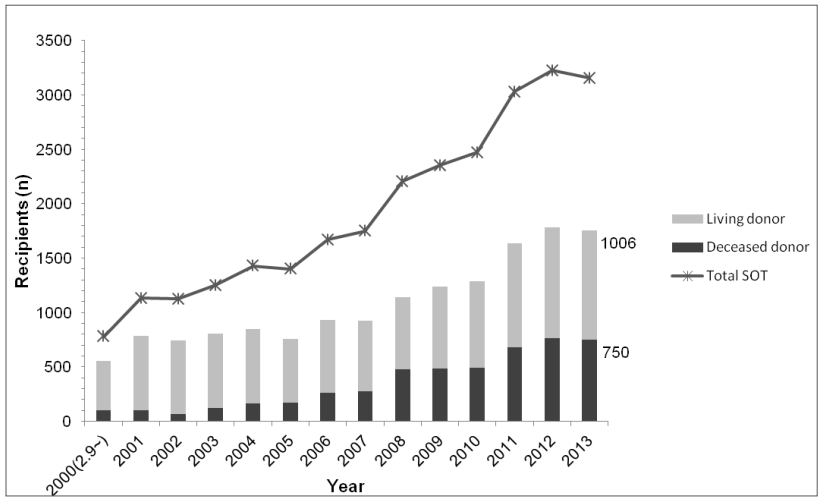

Figure 1 Annual number of kidney transplantation from living donor and deceased donor in Korea, 2000-2013 (SOT: solid organ transplantation) (KONOS, 2014)

A kidney transplant is a surgical procedure to place a functioning kidney from a donor into a person whose kidneys no longer function properly. Kidneys from a living donor have significantly better long-term survival than kidneys from a deceased donor. However, patients with advanced kidney failure who do not have the option of a living donor transplant join the waiting list for a deceased donor. Korea was a country where living donor kidney transplantation dominated. However, in report from the Korean Network for Organ Sharing: Organ transplantation statistics (KONOS), the number of Korean deceased donor kidney transplantation cases has remarkable increased over the past 10 years (2004 vs. 2013, 19.27% vs. 42.71%, respectively)[5,6].

1.2 General Approach to Treatment after Kidney Transplantation

A multidrug immunosuppression approach is rational to work on different immunologic targets. The use of a multiple agent regimen may allow for pharmacologic activity at several key steps in the T-cell replication process and lower doses of individual agents, thereby reducing the severity of dose-related adverse effects. In general, there are three stages of clinical immunosuppression: induction therapy, maintenance therapy, and treatment of an established rejection episode.

Although induction therapy may not be uniformly used, in almost every setting, patients receive IV methylprednisolone and basiliximab intraoperatively. The most

common maintenance immunosuppressive agents can be divided into four classes: (1) the calcineurin inhibitors (CNIs) (cyclosporine or tacrolimus), (2) anti-metabolites or antiproliferatives (azathioprine or mycophenolic acid derivatives), (3) mammalian target of rapamycin (mTOR) inhibitors (sirolimus or everolimus), and (4) corticosteroids. Immunosuppressive regimens vary among transplantation centers but most often include a CNI and a corticosteroid, with or without an anti-metabolite agent. Selection of appropriate immunosuppressive regimens should be patient specific, taking into account the pharmacologic properties, adverse-event profile, and potential drug-drug interactions, as well as the patient's preexisting diseases, and risk of rejection.

1.2.1 Calcineurin Inhibitors

Cyclosporine and tacrolimus are the two CNIs currently used for most solid-organ transplant recipients. The introduction of CNIs significantly improved the outcomes in terms of patient and graft survival and more than 80% of transplant recipients receive CNIs[7].

CNIs block T-cell proliferation by inhibiting the production of IL-2 and other cytokines by T cells. Cyclosporine and tacrolimus bind to unique cytoplasmic immunophilins cyclophilin and FK-binding protein-12 (FKBP12), respectively. The drug–immunophilin complex inhibits the action of calcineurin, an enzyme that activates the nuclear factor of activated T cells, which is, in turn, responsible for

the transcription of several key cytokines necessary for T-cell activity, including IL-2. IL-2 is a potent growth factor for T cells and ultimately is responsible for activation and clonal expansion.

Early phase acute nephrotoxicity is dose dependent and frequently accompanied by clinical manifestations including elevated serum creatinine and blood urea nitrogen levels, hyperkalemia, hyperuricemia, mild proteinuria, and a decreased fractional excretion of sodium. Because the clinical features of acute renal allograft rejection and calcineurin inhibitor nephrotoxicity may overlap considerably, it is often difficult to differentiate CNI nephrotoxicity from renal allograft rejection. Since this CNI nephrotoxicity is recognized as the leading cause of renal dysfunction, it is important of monitoring calcineurin inhibitor trough blood levels and reducing the CNI dosage, and avoiding other nephrotoxins (e.g., aminoglycosides, amphotericin B, and nonsteroidal antiinflammatory agents) when possible.

1.2.2 Anti-metabolites

Antimetabolites have been used since the early days of transplantation because they prevent proliferation of lymphocytes. Azathioprine, a prodrug for 6-mercaptopurine (6-MP), has been used as an antimetabolite in combination with corticosteroids since the earliest days of the modern transplantation era, however, its use has dramatically declined with the availability of newer immunosuppressants, mycophenolate mofetil (MMF), morpholinoethyl ester of

mycophenolic acid (MPA).

The immunosuppressive effect of MPA is exerted through noncompetitive binding to inosine monophosphate dehydrogenase, the key enzyme responsible for guanosine nucleotide synthesis via the de novo pathway. Inhibition of inosine monophosphate dehydrogenase results in decreased nucleotide synthesis and diminished DNA polymerase activity, ultimately reducing lymphocyte proliferation. Although the MPA has considerable effect for prevention of acute renal allograft rejection after transplantation, gastrointestinal adverse effects are a major concern which may require a dose reduction or discontinuation.

Enteric-coated mycophenolate sodium (EC-MPS) is an advanced formulation of MPA that delivers the active moiety MPA. It has been developed to help protect the upper gastrointestinal tract. It is implied that a reduction of adverse drug effects as well as a reduction of dose may improve efficacy and compliance[8,9].

1.2.3 Corticosteroids

Corticosteroids have been used since the beginning of the modern transplantation era. Despite their many adverse events, they continue to be a cornerstone of immunosuppression regimens in many transplant centers with 70% of kidney transplant patients receiving corticosteroids at the time of hospital discharge. The most commonly used corticosteroids in transplantation are methylprednisolone and prednisone. Corticosteroids block cytokine activation by binding to corticosteroid

response elements, thereby inhibiting IL-1, IL-2, IL-3, IL-6, -interferon, and tumor necrosis factor- synthesis. Additionally, corticosteroids interfere with cell migration, recognition, and cytotoxic effector mechanisms.

2 Inter-individual Variability in Mycophenolate Response

2.1 Clinical Pharmacokinetics of Mycophenolate

Mycophenolate mofetil is indicated for the prevention of organ transplant rejection in adults and renal transplant rejection in children over 2 years. The data presented in previous paper are only related to the MMF formulation and not to the EC-MPS[1-3]. EC-MPS is an advanced formulation delivering MPA. However, the latter has different and more variable pharmacokinetics, and the algorithms to predict MPA exposure developed for MMF cannot be used for EC-MPS.

MPA is metabolized by the UDP-glucuronosyltransferase (UGT) enzymes to a major metabolite, 7-O-glucuronide (MPAG), and a minor acyl glucuronide (AcMPAG); MPAG undergoes biliary excretion via multidrug resistance protein 2 (MRP2) and solute carrier organic anion transporter (SLCO) mediated transport into the intestine[10-12]. In humans, renal elimination is comprised of 3% unchanged MPA and 87-91% of the dose excreted as glucuronides; primarily as MPAG (~87%) and secondarily as AcMPAG (1%).

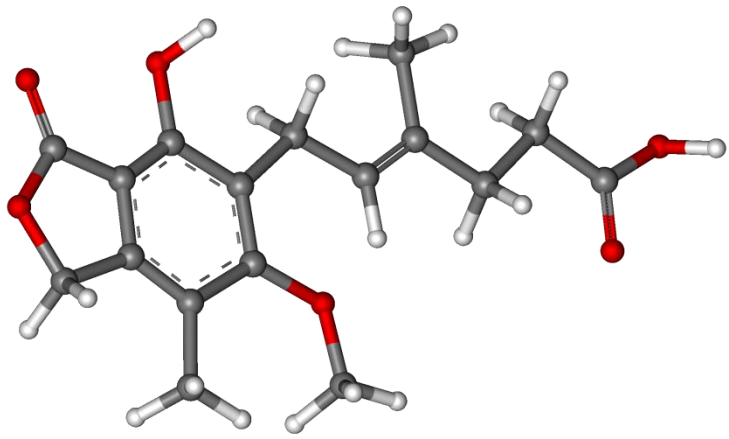

Figure 2 Chemical structures of mycophenolic acid

Systematic (IUPAC) name: (4E)-6-(4-Hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl)-4-methylhex-4-enoic

2.2 Clinical and Genetic Effects on Inter-individual Variability of PK Parameters

2.2.1 Clinical Covariates Impacting PK of Mycophenolate

Gender

To say the conclusion first, the studies about relationship between gender and PK parameters give conflicting results. Borrows *et al* found increased MPA predose levels in female renal transplant recipients compared to males (p =0.002)[13] and, in van Hest *et al* study, males have an 11% higher MPA clearance than females[14]. However, other studies including meta-analysis found no effect of gender on the MPA PK[15-17]. A possible effect has been suggested to result from a competitive inhibition of UGT enzymes by estrogen[18].

Ethnicity

African-American renal transplant patients have been recognized to be at higher risk for early acute rejection episodes. For this reason, in AA renal transplant patients a higher MMF dose is needed to produce a significant benefit in acute rejection compared with Caucasians (1.5 g twice daily and 1 g twice daily, respectively)[19]. This difference in clinical outcome between African-American and Caucasian patients cannot be explained by a difference in MPA exposure, as no significant differences in the MPA PK were found.

Body Weight

Le Guellec *et al* and Staatz *et al* study found that bodyweight was positively correlated with oral MMF clearance[20,21]. However, Other large trials of van Hest *et al*, Kuypers *et al*, and Borrows *et al* studies did not show a correlation between bodyweight and MPA PK[13,16,17]. These results suggest that, even if bodyweight does influence MPA PK, the amount of effect will not reach clinical relevance.

Kidney Function

In general, renal dysfunction leads to decreased MPA concentrations, increased metabolites, MPAG, concentrations and increased free MPA fractions. Increased serum creatinine and decreased glomerular filtration rate (GFR) are associated with decreased MPA predose levels[13]. Renal dysfunction leads to an increase in free fraction of MPA exposure due to elevated active metabolite MPAG levels[22,23]. Similarly, in a population pharmacokinetic model, reduced creatinine clearance (CLcr) correlated significantly with increased MPA clearance[14].

2.2.2 Implication of Genetic Variability of *UGT*, *ABCC2*, and *SLCO1B* on Mycophenolate PK Parameters

To explore factors contributing to inter-individual variability in drug response, there is a growing interest in the impact of gene polymorphisms of enzymes and transporters[24,25]. Numerous genetic variants of UGT and SLCO are known to be

related with significantly increased dose-adjusted MPA trough levels in stable renal transplant recipients[26]. Baldelli *et al* suggested that patients with the *UGT1A9* -440C/-331T allele may need high dose than -440TT/-331CC genotype[27]. In Van Schaik *et al* and Kuypers *et al* studies, patients with the *UGT1A9* -275A/-2152T allele displayed up to a 50% lower MPA area under the curve from 0 to 12 h ($AUC_{0\text{-}12h}$) and may need a dose 1.5 times higher than those with the -275TT or -2152CC genotype[28,29]. Zhang *et al* reported that another promoter SNP *UGT1A9**1b (-118delT), which has been shown to enhance glucouronidation[30,31] was associated with increased enterohepatic circulation of MPA[32]. Dose-adjusted AUC was higher in patients carrying at least one allele without T deletion (-118dT10/10 and 9/10)[32,33]. Meanwhile, Picard *et al* study determined the roles of the *SLCO1B1* and *1B3* polymorphisms in the PK of MPA. Dose-adjusted MPA exposure was increased because of reduced enterohepatic cycling in patients carrying the *SLBO1B1* variants[34-36]. In addition to such genotypes, clinical factors can influence MPA concentrations, including renal function, albumin level, concomitant immunosuppressants, and time after transplantation[10,16,37-39].

3 Strategies for Reducing Inter-individual Differences

3.1 Non-linear Mixed Effect Analysis

Unlike conventional PK studies, population PK analysis using non-linear mixed effect analysis allows an estimate of the means and variances of PK parameters directly in the population of interest as well as the relationship between these parameters and specific patient covariates with a minimum of blood sampling.

3.2 Individual Dosing Strategy

Many studies on the establishment of an individualized immunosuppressant dosage regimen have been published, but most have not considered the abovementioned clinical and genetic factors nor have taken concomitant medicines with immunosuppressive agents into account. The most common and practical method is by measuring trough blood concentrations and $AUC_{0\text{-}12h}$. MPA $AUC_{0\text{-}12h}$ is a better predictor than trough concentration of clinical response[16,38-40]. The target MPA $AUC_{0\text{-}12h}$ range in renal transplant recipients is 30-60 mg.h/L when combined with cyclosporine[37]. However, since the target is applicable during the early stage after transplantation, there is no evidence in stable renal transplant recipients, one year post transplantation.

II. Objective

The objective of this study was to develop a population PK model of oral EC-MPS in stable renal transplant recipients and to investigate the potential contributing factors including genetic polymorphisms to the inter-individual variability in PK parameters of MPA. Furthermore, as a secondary objective, model-based optimal AUC-targeted dosing strategy was explored in stable renal transplant patients.

III. Methods

1 Patients and Data Collection

De novo adult kidney transplant recipients with stable renal function treated with EC-MPS (Myfortic® ; Novartis Pharma AG, Basel, Switzerland) were included at a tertiary teaching hospital in Korea from June 2011 to June 2012. Patients younger than 18 years of age and taking mTOR inhibitors, sirolimus and everolimus, were excluded.

Patient demographic information and laboratory data were collected on the date of sampling from electronic medical record (EMR). This study was conducted in accordance with the Declaration of Helsinki and the protocol was reviewed and approved by the Ethics Committee and Institutional Review Board (IRB) of the research institute. Written informed consent was obtained from each patient.

2 Immunosuppressive Regimen

All patients received a fixed dose of EC-MPS twice per day concomitant with a CNI and corticosteroid. More than half of the patients received a low dose of steroids (2.5-7.5 mg prednisolone) as co-medication and five patients (15%) used cyclosporine as concomitant CNI. Because of all patients having stable renal function, the oral tacrolimus dose was adjusted to achieve a target trough level of 4-6 ng/mL and cyclosporine levels were maintained around 100 ng/mL.

3 Sampling Procedure and Analytic Methods

3.1 Limited Sampling Strategy

Serial concentration-time samples (5 mL per sample) for analysis of the pharmacokinetic of MPA were collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes by venipuncture pre-dose and at 0.5, 1, 2, 4, and 6 hours after administration. In some patients, the time points of sample drawing were different because of protocol violations, but these sparse concentration-time profiles were still suitable for pharmacokinetic analysis. Three to five blood samples were collected in each patient (3 blood samples collected at: pre-dose, 2, 4 h after in 2 patients; 5 blood samples collected at: pre-dose, 1, 2, 4, 6 h or pre-dose,

0.5, 1, 2, 6 h after dosing in 28 and 4 patients, respectively).

After collection, EDTA blood samples were immediately centrifuged at 3000 g, and the plasma was stored frozen at -80 ℃ until analysis. MPA plasma concentrations were measured using a validated ultraperformance of the liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique in Seoul National University Hospital.

3.2 Genotyping Analysis

W genotyped thirteen common polymorphisms of the three enzymes (UGT1A7, UGT1A9, and UGT2B7) and three transporters (ABCC2, SLCO1B1, and SLCO1B3) affecting MPA kinetics. Genotypes of *UGT1A7* (387T>G, 391C>A) and *UGT1A9* -118delT were determined by direct sequencing. Multiplexed SNaPshot for *UGT1A9* (-331C>T, -440T>C), *UGT2B7* -138G>A, *ABCC2* (-24C>T, 1249G>A, 3972C>T), *SLCO1B1* (388A>G, 521T>C), and *SLCO1B3* (334T>G, 699G>A) were performed.

PCR method was used to amplify the four ARRB1 fragments with UCSC In-Silico PCR. The purified PCR products were sequenced using a BigDye Terminator Cycle Sequencing Kit and an ABI 3730xl automated sequencer (Applied Biosystems, Foster City, CA, USA). Mutation analyses were performed using Phred, Phrap, Consed, Polyphred 5.04 software. We screened the genotyping using single base primer extension assay using ABI PRISM SNaPShot Multiplex kit

(ABI, Foster City, CA, USA) according to the manufacturer's recommendation. Table 1 shows the primer sets and Tm used for the SNaPshot assay. The Hardy-Weinberg equilibrium was evaluated via the chi-square test and linkage disequilibrium (LD) between SNPs was analyzed using the SNPAnalyzer 2.0 (Istech Inc., South Korea).

Table 1 Primer sets and Tm for the SNaPshot assay

Gene	SNPname (rs number)	Strand		Primer Sequence	Tm	Additive
UGT1A7	T387G	Forward	Forward	CACCATTGCGAAGTGCAT	60	-
	(rs17868323)		Reverse	TTCTTAATGTGCTAAAGGGGAGA		
UGT1A7	C391A	Forward	Forward	CACCATTGCGAAGTGCAT	60	-
	(rs17863778)		Reverse	TTCTTAATGTGCTAAAGGGGAGA		
UGT1A9	del T	Forward	Forward	AGGCGAGCCCCAATTTAG	60	-
	(rs3832043)		Reverse	GGCAAAGCCACAGGTCAG		
UGT1A9	C-331T	Forward	Forward	AGGCGAGCCCCAATTTAG	60	-
	(rs2741046)		Reverse	GGCAAAGCCACAGGTCAG		
			Genotyping	gctttaatcaaatttact		
UGT1A9	T-440C	Forward	Forward	GGATGGGGGCAGTCCTAT	60	-
	(rs2741045)		Reverse	TGGAATTTGTCCCAGAGCA		
			Genotyping	tgttttctttgcttagag		
UGT2B7	G-138A	Forward	Forward	ggctctccaactgattgtt	55	-
	(rs73823859)		Reverse	AATTCCCAGAGCTAAAGCA		
			Genotyping	tcYttgccatccacatgctcagact		
ABCC2	C-24T	Reverse	Forward	CAGGAAACAGCTATGACCactggtgagtctccctgtcc	60	Betaine
	(rs717620)		Reverse	TGTAAAACGACGGCCAGTaaggcaattttgcgactagc		
			Genotyping	GCATGATTCCTGGACTGCGTCTGGAAY		

Table 1 Primer sets and Tm for the SNaPshot assay (Cont'd)

Gene	SNPname (rs number)	Strand		Primer Sequence	Tm	Additive
ABCC2	G1249A	Reverse	Forward	TTTGTCCATGGGTCCTAATTT	55	Betaine
	(rs2273697)		Reverse	ATGAAGTTGGTCACATCCATG		
			Genotyping	GACATCAGGTTCACTGTTTCTCCAA		
ABCC2	C3972T	Reverse	Forward	TACCGACCTGAGCTGGATC	55	Betaine
	(rs3740066)		Reverse	CATCCAGGCCTTCCTTCA		
			Genotyping	CTCCACCTACCTTCTCCATGCTACC		
SLCO1B1	A388G	Reverse	Forward	ggggaagataatggtgcaaa	60	-
	(rs2306283)		Reverse	cggcaggtttatcatccagt		
			Genotyping	GTCGATGTTGAATTTTCTGATGAAT		
SLCO1B1	T521C	Reverse	Forward	cagcataagaatggactaatacacc	50	-
	(rs4149056)		Reverse	TGGACCAATCATTGCTATTG		
			Genotyping	TCCACGAAGCATATTACCCATGAAC		
SLCO1B3	T334G	Forward	Forward	TCTTTGAGGGAAGGTACAA	55	-
	(rs4149117)		Reverse	AAAAGCCATGATAAATAAAGAA		
			Genotyping	TATGGGAACTGGAAGTATTTTGACA		
SLCO1B3	G699A	Reverse	Forward	CTGGATCTACCCTTGAAAT	55	-
	(rs7311358)		Reverse	GATTATTAATGGATTTATTTCCTAC		
			Genotyping	GATCTACATATCCAATATCCACGTA		

4 Population Pharmacokinetic Analysis

Population PK model was developed in the following steps: 1) a most appropriate structural model was built with residual errors using both fixed and random effects, and 2) potential covariates were explored based MPA absorption, distribution, metabolism, and elimination (ADME), and 3) final developed model was validated by comparison of simulated and experimental data.

4.1 Structural Model

One- or two- compartment with or without lag time PK models were compared as structural model. Drug disposition was compared at a combination of zero- or first-order absorption with linear or non-linear elimination. And we considered the flip-flop kinetic model in order to reflect the characteristics of suspended released. Since EC-MPS bioavailability (F) could not be determined, the values of clearance (CL) and volume of distribution (V) each corresponded to the ratios CL/F and V/F, respectively. The inter-individual variability of PK parameters was estimated using proportional and exponential models. Individual deviation is independent and normally distributed with mean 0 and variance ω^2. And additive, proportional and exponential models were tested for residual errors.

4.2 Covariate Model

Covariates to explain inter-patient, inter-occasion, and residual variability of PK were the following: sex, age, body weight, immunosuppressant dose and trough level, hemoglobin, hematocrit, blood urea nitrogen, serum creatinine (Scr), glomerular filtration rate (GFR), calculated creatinine clearance (CLcr), total protein, albumin, total cholesterol, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and genetic polymorphisms.

Initially, we tested covariates correlation and selected for inclusion into the candidate models to eliminate multicollinearity in advance. And then, parameter-covariate relations were identified for a systematic covariate search by applying stepwise covariate modeling (SCM) method. Continuous covariates were estimated using the additive, proportional, and exponential models and categorical covariates were the exponential and power function models.

All genotypes were tested for heterogeneity under the dominant, recessive, and additive models. In modeling from both forward inclusion and backward elimination of SCM, the minimum value of objective function (OFV) can be used as a criterion for model selection. If the difference in OFV between two nested models is larger than the critical value from a chi-squared distribution with degrees of freedom equal to the difference in the number of estimated parameters, the models are significantly different from each other. A decrease in the OFV >3.84 of forward selection and an increase in the OFV >6.64 of backward elimination shows

a significant improvement of a nested model with one degree of freedom of p <0.05 and p <0.01, respectively. All NONMEM analyses were carried out using the first-order conditional estimation method with the interaction option (FOCE+I).

Furthermore, covariate effects were explored using the effect size estimation with 95% interval of posterior distribution. Clinically important change was defined as difference greater or less than 20% of median value. Additionally, we have performed bootstrap analyses of the SCM (Boot-SCM, n=200), as a tool for analyzing type I error of covariate inclusion and identifying significant covariates. Boot-SCM datasets were generated by resampling from the original dataset with a p <0.05 for both the forward and backward process.

Model adequacy was further evaluated by using "goodness-of-fit (GOF)" plots and values of random effects variances. To analyze the graphical GOF extensive plotting was available through the use of Xpose 4 in R software ver. 3.0.2 (The R Foundation for Statistical Computing, Vienna, Austria).

4.3 Final Model Validation

As an internal validation method, to evaluate the accuracy and robustness of the final model, we performed the resampling techniques of bootstrap and visual predictive check (VPC). Resampling was repeated 2,000 times and the 5^{th} and 95^{th} percentile values of estimated parameters from the bootstrap procedure were compared with those estimated from the original data set. Furthermore, VPC data

sets (n=200) were simulated from the original data set using the final model[41]. Since the dose of EC-MPS was variable to target a certain trough level and the final model included covariates, we performed the prediction corrected VPC (pcVPC). Per time point, the median simulated concentration and 90% prediction intervals were compared graphically with the observed concentrations. Bootstrap and VPC were performed with Perl-speaks-NONMEM (PsN) version 3.6.2.

5 Individual Dosing Strategy based on Simulation

We performed a Monte Carlo simulation to explore the optimal dose in several simulation scenarios for a hypothetical population. Random data was generated from the model's probability distribution using the R program. For each population comprised of 1,000 subjects, plasma concentration-time profiles were simulated using NONMEM after giving 4 doses of 180, 360, 540, and 720 mg. We calculated the $AUC_{0\text{-}12h}$ using the trapezoidal rule and compared the percentage of subjects achieving the target range.

6 Statistical Analysis

Statistical test were performed with the software package PASW 21 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive analyses for demographic and pharmacokinetic variables included means, standard deviations, and medians

(range) as appropriate. A *p*-value of 0.05 was considered statistically significant.

All analysis was performed using the non-linear mixed effects modeling program, NONMEM ver. 7.2.0 (ICON Development Solutions, Ellicott City, MD, USA). The GOF was assessed by graphical diagnostics using Xpose 4 in R software ver. 3.0.2 (The R Foundation for Statistical Computing, Vienna, Austria).

IV. Results

1 Patient Characteristics

The characteristics of 34 patients included in this study are listed in Table 2. Of the patients, 23 were male (67.7%) and the median age was 47.5 years (24-66 years) with median time post-transplant of 67.7 months (8.6 to 160.6 months). All recipients except 5 patients were co-treated with tacrolimus (mean doses 1.40 ± 0.57 mg twice daily and mean trough level 3.83 ± 1.71 ng/mL); the remaining 5 patients with cyclosporine (mean doses 75 ± 25 mg twice daily and mean trough level 110.80 ± 96.53 ng/mL). Each patient participated in at least three PK sampling at different time points after oral administration. Figure 3 shows the observed MPA plasma concentration-time points for EC-MPS treated patients. 18 (52.94%) of the profile of patients, MPA predose concentrations were higher than the MPA concentration in the subsequent 0.5 hour after administration. Mean $AUC_{0\text{-}12h}$ was 35.99 mg.h/L, with a standard deviation as 44.92 mg.h/L. Time to reach peak concentration (T_{max}) of MPA is 2 hr and mean C_{max} (peak concentration) was 12.29 μg/mL. Allelic frequencies of the genotypes are shown in Table 3. Each genotype did not deviate from the Hardy-Weinberg equilibrium (p >0.05). No variant alleles of the *UGT2B7* -138G>A was observed and homozygous variants of the *UGT1A9* -331C>T, -440T>C, *SLCO1B1* 521T>C, *ABCC2* 1249G>A.

Table 2 Characteristics of the 34 Korean adult renal transplant recipients tacking EC-MPS

Patient Characteristics	**Value**
No. of patients	34
Male	23 (67.65)
Age (year) [ac]	47.5 (24-66)
Body weight (kg) [bc]	64.52 ± 13.07 (43-98.7)
Post-operative days [a]	1889 (242-4498)
Cause of kidney disease	
Diabetes Mellitus	4 (11.76)
Hypertension	5 (14.71)
Glomerulonephritis	13 (38.24)
Others	4 (11.76)
Unknown	8 (23.53)
Donor type	
Living donor	18 (52.94)
Deceased donor	16 (47.06)
Donor age (year) [ac]	35.5 (24-68)
Graft weight (g) [bc]	156.86 ± 29.97 (94-209)

a. median (range)

b. mean ± S.D. (range)

c. at time of transplantation

Table 3 Genotype frequencies of *UGT1A7*, *UGT1A9*, *UGT2B7*, *SLCO1B1*, *SLCO1B3*, and *ABCC2* and Hardy-Weinberg Equilibrium in renal transplant recipients

Gene	Variant	Genotype	%	Major allele (MAF)	*P*-value
UGT1A7	387T>G	TT	21.43	G (0.482)	0.70
	(rs17868323)	TG/GT	53.57		
		GG	25.00		
	397C>A	CC	21.43	A (0.482)	0.70
	(rs17863778)	CA/AC	53.57		
		AA	25.00		
UGT1A9	-331C>T	CC	0	T (0.071)	0.68
	(rs2741046)	CT/TC	4 (14.29)		
		TT	24 (85.71)		
	-440T>C	TT	0	C (0.071)	0.68
	(rs2741045)	TC/CT	4 (14.29)		
		CC	24 (85.71)		
	-118delT (9>10)	TT	7 (25.00)	T (0.500)	-
	(rs3832043)	T-	14 (50.00)		
		--	7 (25.00)		
UGT2B7	-138G>A	GG	28 (100)	G (0)	-
	(rs73823859)	GA/AG	0		
		AA	0		
SLCO1B1	388A>G	AA	2 (7.14)	G (0.250)	0.80
	(rs2306283)	AG/GA	10 (35.71)		
		GG	16 (57.14)		

MAF, minor allele frequency

Table 3 Genotype frequencies of *UGT1A7*, *UGT1A9*, *UGT2B7*, *SLCO1B1*, *SLCO1B3*, and *ABCC2* and Hardy-Weinberg Equilibrium in renal transplant recipients (Cont'd)

Gene	Variant	Genotype	%	Major allele (MAF)	*P*-value
SLCO1B1	521T>C	TT	21 (75.00)	T (0.125)	0.45
	(rs4149056)	TC/CT	7 (25.00)		
		CC	0		
SLCO1B3	334T>G	TT	1 (3.57)	G (0.179)	0.89
	(rs4149117)	TG/GT	8 (28.57)		
		GG	19 (67.86)		
	699G>A	GG	1 (3.57)	A (0.179)	0.89
	(rs7311358)	GA/AG	8 (28.57)		
		AA	19 (67.86)		
ABCC2	-24C>T	CC	9 (32.14)	C (0.375)	0.12
	(rs717620)	CT/TC	17 (60.71)		
		TT	2 (7.14)		
	1249G>A	GG	26 (92.86)	G (0.036)	0.84
	(rs2273697)	GA/AG	2 (7.14)		
		AA	0		
	3972C>T	CC	8 (28.57)	C (0.393)	0.07
	(rs3740066)	CT/TC	18 (64.29)		
		TT	2 (7.14)		

MAF, minor allele frequency

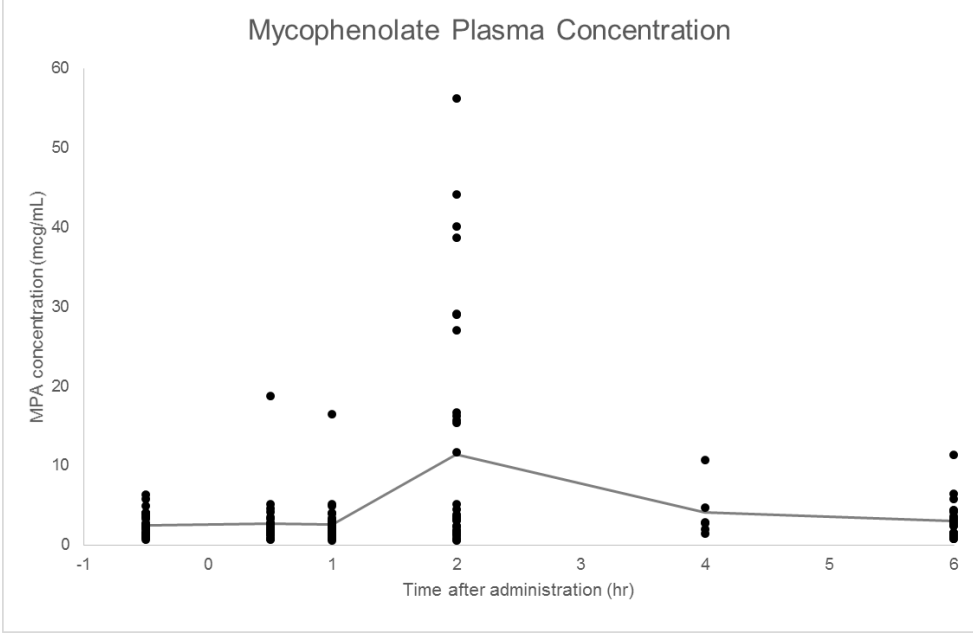

Figure 3 Observed MPA plasma concentration versus time data for all patients treated with EC-MPS

2 Population Pharmacokinetic Modeling and Evaluation

2.1 Structural Model

A total of 166 plasma concentrations were above the lower limit of quantification and available for population modeling. MPA PK was best described with a time-lagged 2-compartment with first-order absorption and elimination (ADVAN6) [Figure 4]. In our model, peripheral compartment means a metabolic compartment considering entero-hepatic recirculation. And the PK profile was shown to follow a flip-flop kinetic model in accordance with sustained release type. Interindividual variability was estimated exponentially and a proportional error model was used to account for the residual variability.

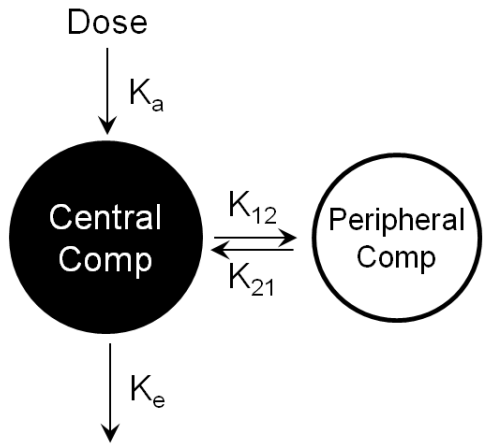

Figure 4 Minimal two-compartment PK model

The model consists of a central compartment and a peripheral compartment (metabolic compartment). Lag is the time immediately before absorption and accommodates variable gastric emptying.

2.2 Covariate Model

The covariates were examined to determine their linear relationship with eta value for apparent CL/F, K_a, V_c/F, and V_p/F. In correlation test between covariates from full covariate model, *ABCC2* polymorphisms (-24C>T, 1249G>A) were positively correlated with *UGT1A9* polymorphisms (-331C>T, -440T>C) (all correlation coefficients >0.7) and excluded. And *UGT1A7* 387T>G and 391C>A had the same frequency distribution, one of the two, *UGT1A9* 387T>G was excluded.

During the forward selection processes of SCM, the following covariates produced a significant decrease in OFV: *UGT1A7* 391C>A polymorphisms on V_c/F; *SLCO1B1* 388A>G SNP on CL/F; Scr and *UGT1A9* -118delT on K_a; and CLcr on V_p/F. When effect size estimates using a full covariate model based on forward selection results, *UGT1A9* -118delT and CLcr were statistically significant effect with clinically important, but, *SLCO1B1* 388A>G, *UGT1A7* 391C>A, 387T>G SNPs, and Scr were only statistically significant. Therefore, CLcr and *UGT1A9* -118delT polymorphism were selected at first [Figure 5]. And then, in the backward elimination process, *SLCO1B1* 388A>G on CL/F, *UGT1A9* -118delT on K_a, and CLcr on V_p/F were retained because other covariates had not impact on the increase OFV in p <0.01 from the full model. No covariates significantly influenced V_c/F, Q/F or lag time of EC-MPS. Similarly, result of Boot-SCM was consistent and showed that *SLCO1B1* 388A>G, *UGT1A7* 387T>G, *UGT1A9* 188 delT, and Scr had high inclusion rate of more than 10%.

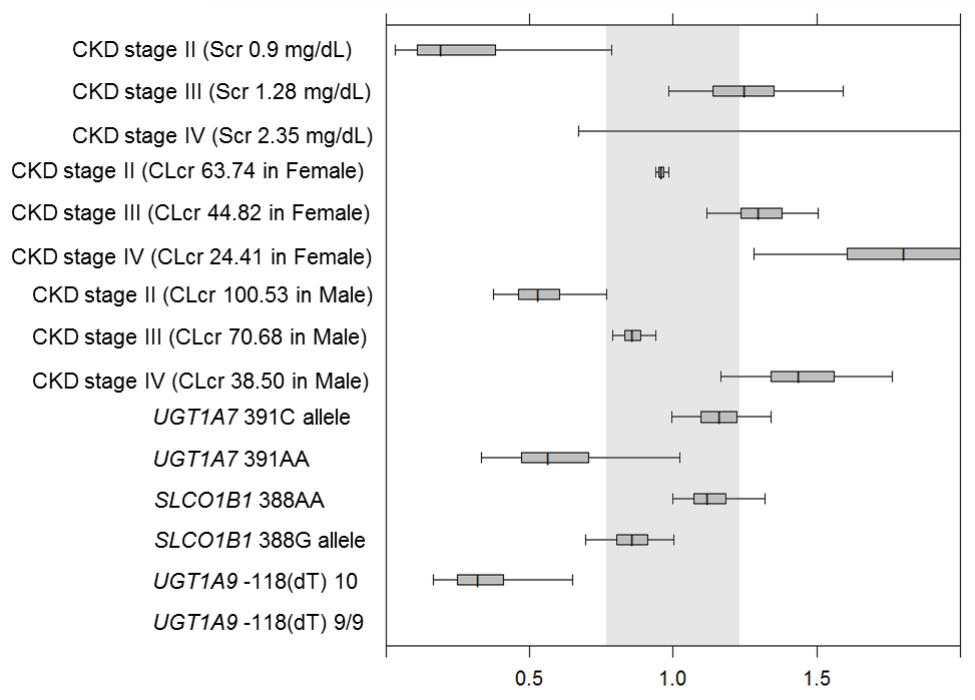

Figure 5 Covariate effects on EC-MPS PK parameters

The distributions (5^{th} and 95^{th} percentiles) of the 2,000 nonparametric bootstrap estimates are provided as box with error bar for covariate values. The shaded gray areas represent the 80-125% range.

2.3 Final Model

Finally, the relationship between PK parameters and covariates was expressed with the equation:

$$CL/F \text{ (L/hr)} = 10.2 \times 0.689^{SLCO1B1*}$$

$$V_c/F \text{ (L)} = 38.5$$

$$V_p/F \text{ (L)} = 58.1 \times e^{-0.0178 \cdot (CLcr - 65.42)}$$

$$\text{Ka } (\text{hr}^{-1}) = 0.265 + 1.31 \times \text{e}^{3.36 \cdot (\text{UGT1A9})**}$$

* *SLCO1B1* 388AA, SLCO1B1=0; G allele, SLCO1B1=2.33

** *UGT1A9* -118delT 10/10 or 9/10, UGT1A9=-0.25; -118delT 9/9, UGT1A9=0.75

The predictive performance of the final model developed is reported in Table 4. And GOF plots of final model are shown in Figure 6 and plots of individual concentration-time profiles were showed in Figure 7.

The typical population values CL/F, V_c /F, K_a, Q/F, V_p/F, and lag-time were 9.3 L/hr, 42 L, 1.24 hr^{-1}, 37.4, 60.3 L, and 1.79 hr, respectively. The population coefficients of variation (CV%) for CL/F, V_c/F, and K_a were 72.7%, 160.9%, and 216.6%, respectively. The squared coefficient of correlation between random effects for CL/F and V_c /F was 0.996. The intrapatient variance was 0.175.

Table 4 Population pharmacokinetic parameters of EC-MPS

Pharmacokinetic Parameter		Population Mean	RSE (%)	Inter-individual variability (%)	Bootstrap (n=2,000)	
					5th percentile	95th percentile
CL/F	Θ_1	9.3	17	72.7	6.52	12.1
SLCO1B1 388A>G	Θ_2	0.696	13	-	0.515	0.876
Vc/F	Θ_3	42	29	160.9	22.2	61.8
Ke	Θ_1/Θ_3	0.221	-	-	-	-
Ka	Ke+ Θ_4	1.24	39	216.6	0.103	2.38
UGT1A9 -118dT (9>10)	Θ_5	2.58	21	-	0.266	4.89
Q/F	Θ_6	37.4	40	-	-0.352	75.2
Vp/F	Θ_7	60.3	14	-	38.4	82.2
CLcr	Θ_8	-0.017	28		-0.0311	-0.00288
Lag time	Θ_9	1.79	4	-	1.65	1.93
$\omega^2_{CL/F}$	η_1	0.529	20	-	0.116	0.941
$\omega^2_{Vc/F}$	η_2	2.59	12	-	1.19	3.98
ω^2_{Ka}	η_3	4.69	12	-	1.42	7.96
σ^2	ε_1	0.175	10	-	0.111	0.239
Random residual variability (%)		-	-	41.8	-	-

RSE, relative standard error; CL, clearance; F, bioavailability; Vc, central volume of distribution; Ke, elimination rate constant; Ka, absorption rate constant; Q, intercompartment clearance; Vp, peripheral volume of distribution; Scr, serum creatinine; ω^2, variance of the interindividual random effects; σ^2, variance of the residual random effects

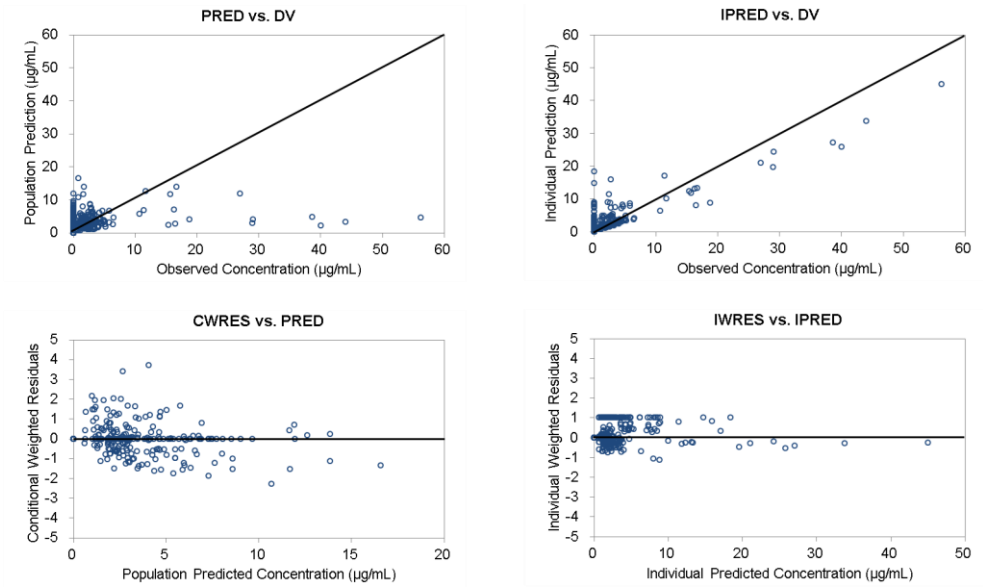

Figure 6 Model performance and diagnostic plots for final model

Upper left, Population prediction (PRED) versus observed concentration (DV); upper right, individual prediction (IPRED) versus observed concentration; bottom left, population conditional weighted residual (CWRES) versus population prediction; bottom right, individual weighted residual (IWRES) versus individual prediction.

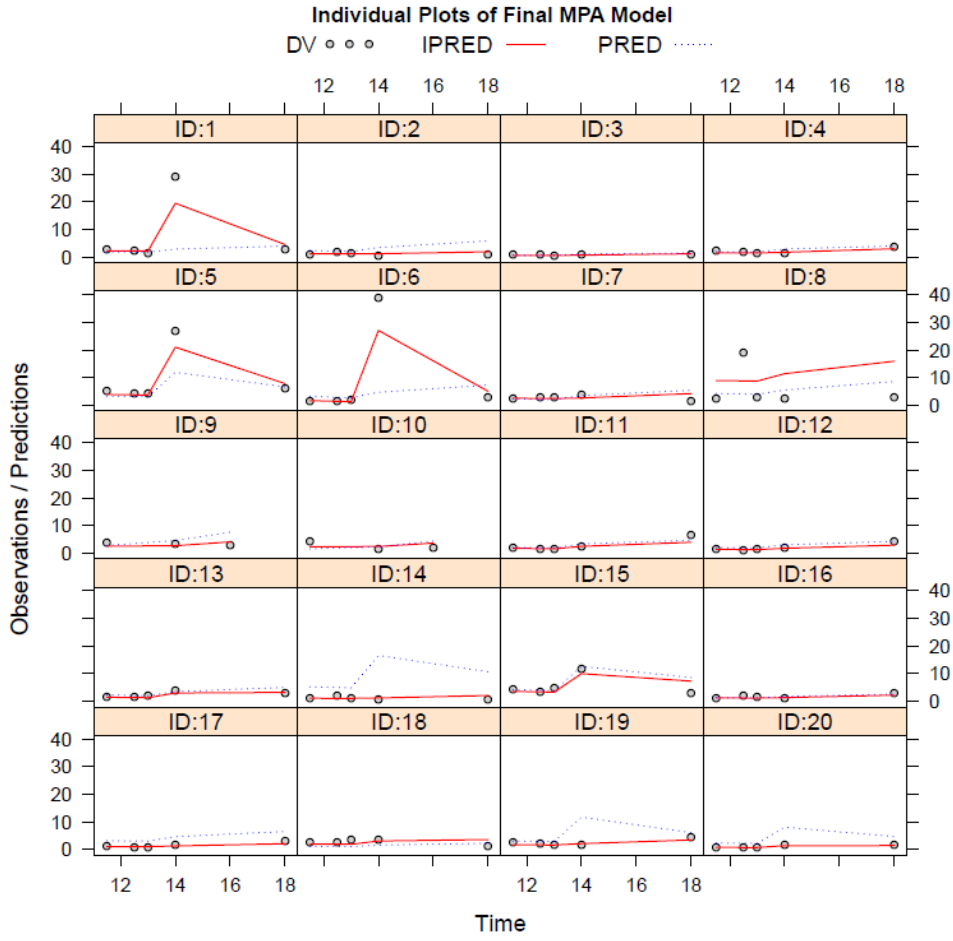

Figure 7 Individual plots of individual predictions and observations versus time

The dashed line is individual prediction of MPA and the circle is observed concentration of MPA. The solid line is individual prediction of MPA.

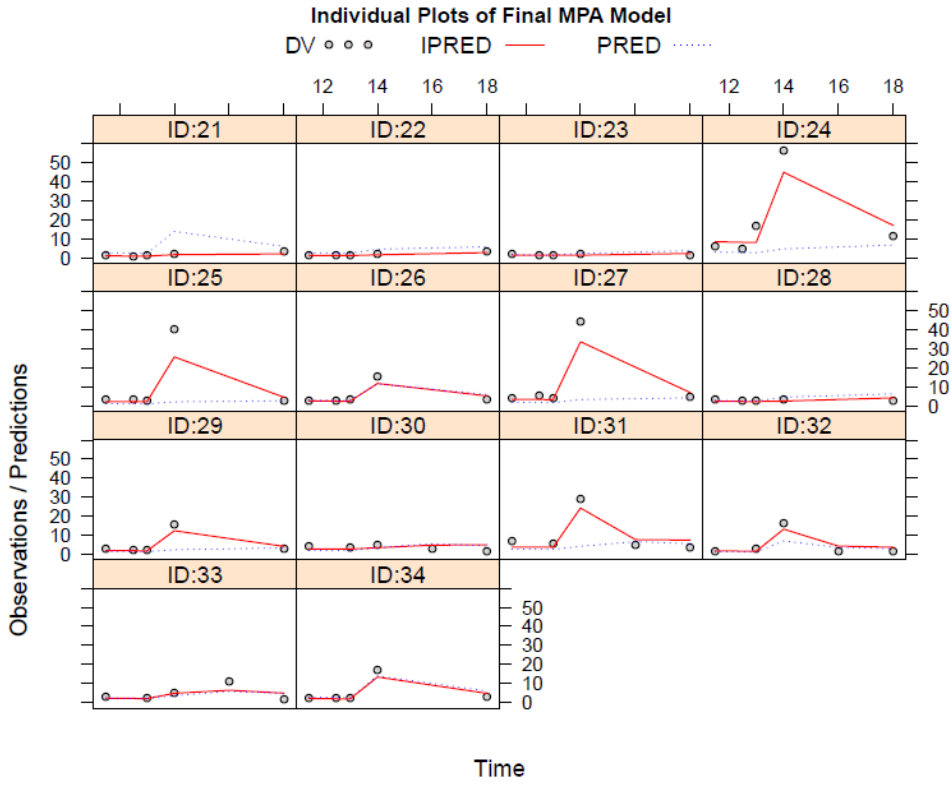

Figure 7 Individual plots of individual predictions and observations versus time

The dashed line is individual prediction of MPA and the circle is observed concentration of MPA. The solid line is individual prediction of MPA (Cont'd).

2.4 Model Validation

For the bootstrap and pcVPC, the data of MPA fit well within the 5^{th} to 95^{th} percentiles [Table 4]. As shown in Figure 8 the model resulted in more accurate and precise prediction of MPA than the base model.

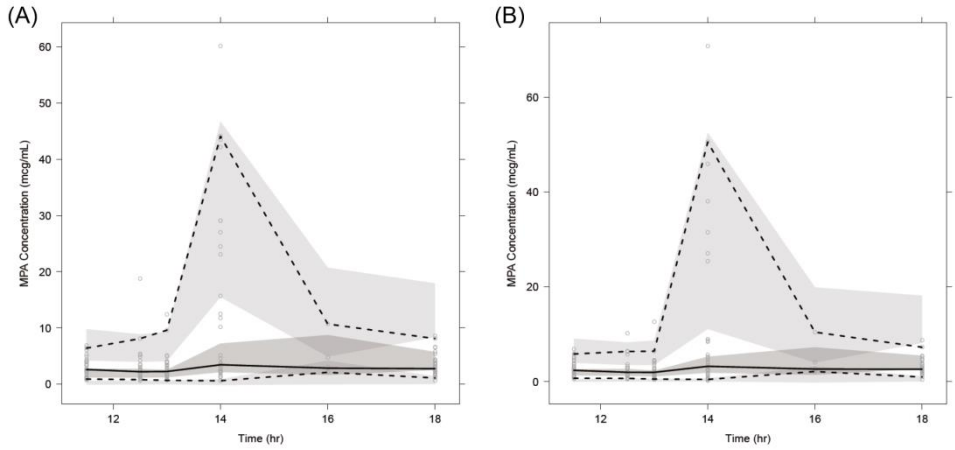

Figure 8 Prediction-corrected VPC for the (A) base and (B) final PK model

The dashed lines represent the 5^{th} and 95^{th} percentiles of prediction-corrected concentrations. The solid lines represent 50^{th} percentiles of prediction-corrected concentrations. The semitransparent field represents a simulation-based 95% confidence interval.

3 Individual Dosing Strategy based Simulation

To evaluate the clinical utility of final model we created 32 scenarios considering combination of significant factors such as *UGT1A9* -118dT (10 vs. 9/9), *SLCO1B1* 388A>G (AA vs. G allele), and renal function, as reflected by CLcr (90 vs. 60 mL/min) in each dosing regimen. Detailed descriptions and data are provided in the Table 5.

In the model assumption, scenarios “Dose 180 mg” and “Dose 360 mg” showed a lower mean $AUC_{0\text{-}12h}$ (20-40 mg.h/L) than high dosage of EC-MPS [Figure 9]. These simulated data indicate that the most appropriate dose for stable renal transplant recipient is 540 mg every 12 hours. Among them, some patients with *SLCO1B1* 388G allele had lower blood levels while others with 388AA genotype or low CLcr level had higher levels with the same dosing.

Table 5 Summary of 32 scenarios for simulations with final covariates

Scenario #	Dose	*UGT1A9* -118(dT)	*SLCO1B1* 388A>G	CLcr
1	180 mg	dT(10/10 or 9/10)	388AA	60
2	180 mg	dT(10/10 or 9/10)	388AA	90
3	180 mg	dT(10/10 or 9/10)	388G allele	60
4	180 mg	dT(10/10 or 9/10)	388G allele	90
5	180 mg	dT(9/9)	388AA	60
6	180 mg	dT(9/9)	388AA	90
7	180 mg	dT(9/9)	388G allele	60
8	180 mg	dT(9/9)	388G allele	90
9	360 mg	dT(10/10 or 9/10)	388AA	60
10	360 mg	dT(10/10 or 9/10)	388AA	90
11	360 mg	dT(10/10 or 9/10)	388G allele	60
12	360 mg	dT(10/10 or 9/10)	388G allele	90
13	360 mg	dT(9/9)	388AA	60
14	360 mg	dT(9/9)	388AA	90
15	360 mg	dT(9/9)	388G allele	60
16	360 mg	dT(9/9)	388G allele	90
17	540 mg	dT(10/10 or 9/10)	388AA	60
18	540 mg	dT(10/10 or 9/10)	388AA	90
19	540 mg	dT(10/10 or 9/10)	388G allele	60
20	540 mg	dT(10/10 or 9/10)	388G allele	90
21	540 mg	dT(9/9)	388AA	60
22	540 mg	dT(9/9)	388AA	90

Table 5 Summary of 32 scenarios for simulations with final covariates (Cont'd)

Scenario #	Dose	*UGT1A9* -118(dT)	*SLCO1B1* 388A>G	CLcr
23	540 mg	dT(9/9)	388G allele	60
24	540 mg	dT(9/9)	388G allele	90
25	720 mg	dT(10/10 or 9/10)	388AA	60
26	720 mg	dT(10/10 or 9/10)	388AA	90
27	720 mg	dT(10/10 or 9/10)	388G allele	60
28	720 mg	dT(10/10 or 9/10)	388G allele	90
29	720 mg	dT(9/9)	388AA	60
30	720 mg	dT(9/9)	388AA	90
31	720 mg	dT(9/9)	388G allele	60
32	720 mg	dT(9/9)	388G allele	90

CLcr, creatinine clearance (mL/min)

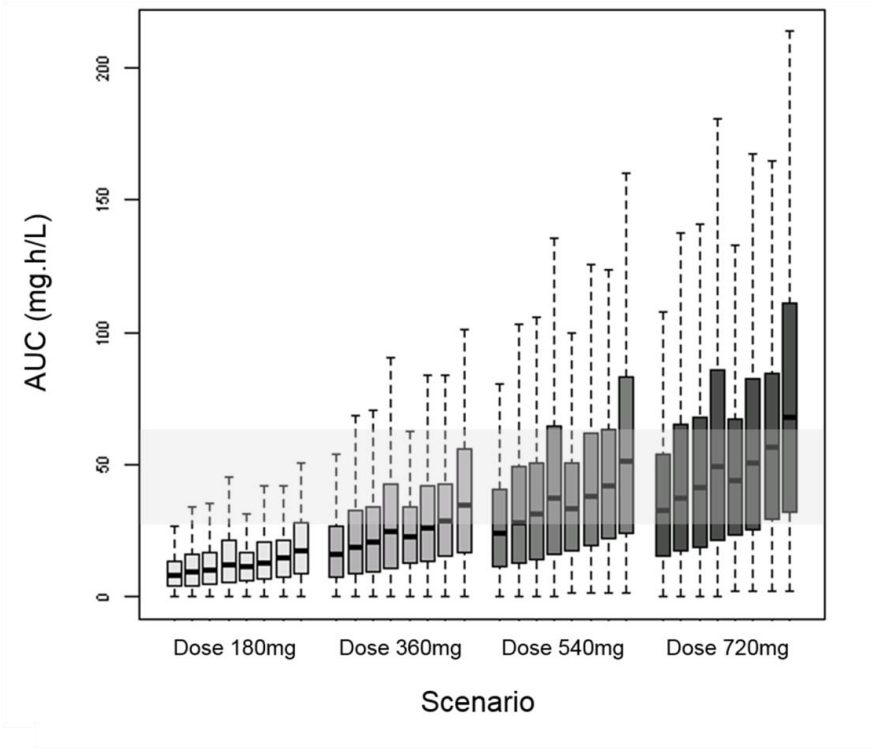

Figure 9 Simulation results of AUC changes in scenarios including different dosing schedule

Posterior estimates of parameters in thirty-two scenarios were simulated. For each scenario, 1,000 subjects were simulated. The shaded gray areas represent the target $AUC_{0\text{-}12h}$ range of 30-60 mg.h/L.

V. Discussion

In this study, we investigated the population PK characteristics of EC-MPS (Myfortic®) and the relationship between PK parameters and various covariates including genetic polymorphisms, clinical, and demographic factors in Korean adult kidney transplant recipients with stable renal function.

This population PK model was best described as a two-compartment model with time-lagged first order absorption and elimination. In our model, peripheral compartment means a metabolic compartment considering entero-hepatic recirculation. The latest research results of MPA studies have been described using time-lag or Erlang distribution for modeling ‘delayed’ absorption. In Henin *et al* study, as another method to explain the delayed PK, the transit time model is considered in simulating the enteric-coated drug[42]. Therefore, we compared the time-lagged model and the time-transit model considering the formulation, and the GOF was better in the time-lagged model. Moreover, this article is the first to estimate the absorption and elimination of EC-MPS with flip-flop kinetics. Generally, flip-flop phenomenon is usually due to drugs with prolonged absorption periods, or apparent sustained absorption profiles[43]. Although enteric-coated formulation can be absorbed completely but very slowly, thus leading to flip-flop kinetics, it was never considered before. In the flip-flop kinetic model, the absorption phase K_a is controlled by the elimination phase K_e. If the Ka is faster than K_e, K_a can be parameterized by adding some other constant C, equal to K_e + C,

and vice versa[43]. Therefore, we developed the structural model to manage flip-flop kinetics as follows, and K_e influenced K_a:

$$K_e = CL/V$$

$$K_a = K_e + \Theta \ (\Theta, \text{estimated constant})$$

In the final model, CLcr as renal function was included to decrease the OFV significantly. Myfortic® is excreted in urine more than 60% as mycophenolic acid glucuronide and 3% as unchanged. This means that the clearance of MPA is dependent on renal function after transplantation[13]. Van Hest *et al* suggested that reduced CLcr correlated significantly with decreased MPA clearance in population PK model[16]. Therefore, impaired renal function may result in greatly reduced elimination of MPA, leading to high AUC. These results are consistent with suggestions of Cellcept® insert paper where dose is not to exceed the 1 g twice daily recommended in impaired renal function (GFR <25 mL/min/1.73 m^2).

Pharmacogenetic considerations may be important as a cause of inter-individual variability of MPA. UGT1A7, 1A8, and 1A9 enzymes are believed to be major isoforms involved in MPA gluconidation, in contrast, UGT2B7 plays a role in metabolism to the AcMPAG[11,12]. In our study, *UGT1A9* -118dT (9>10) polymorphism has demonstrated a significant effect on MPA absorption and distribution. UGT1A9 is likely the main isoform intestine and liver. A possible explanation for this effect of *UGT1A9* promoter region SNP-induced changes in

MPA exposure is variant genotype down-regulating intestinal UGT1A9 activity and relatively lowers net absorption of MPA[29]. Secondly, evaluation studies of genetic variation in *UGT1A9* identified that *UGT1A9* polymorphisms are associated with glucuronidation and clearance of MPA[31,44]. However, in Asian *UGT1A9* -275A>T or -2152T>C SNPs occur with a minimum allele frequency of <5% and were not included in our study. The *UGT* gene polymorphisms are associated with a different AcMPAG:MPA ratio. As an example, in carriers of *UGT2B7*-138G>A, AcMPAG/MPA ratios was 9.3-12.3 fold higher than noncarriers[45]. In other words, *UGT1A9* genetic variations lead to a decrease MPA glucuronidation and correspondingly enhanced the formation of AcMPAG. Because AcMPAG is more rapidly eliminated than MPAG, it is reasonable that *UGT1A9* variant affects decreased AUC of MPA. Genetic variations of transporters as well as metabolism enzymes influence drug elimination.

In our study, patients carrying the *SLCO1B1* 388G allele had lower CL/F as compared to those with AA genotype. *SLCO1B1* 388A>G variation occurs more frequent in Asian (G allele frequency greater than 60%)[46,47]. As well known, since *SLCO1B1* 388A>G genetic variation reduces drug uptake activity up to 50%, it plays important roles in drug clearance and oral bioavailability.

Meanwhile, although multiple studies have investigated the effect of an *ABCC2* -24C>T polymorphism on MPA exposure, the genotype had no impact on the PK in our study[34,48]. Other factors of the CNI and concomitant drugs as well as weight, age, and albumin level did not shown significant improvement and none of them

was selected in this study.

We simulated and compared the AUC changes in each scenario which created to take into account covariates derived from the model. The therapeutic range of the $AUC_{0\text{-}12h}$ (30 to 60 mg.h/L) was used in this study to estimate the proportion of patients achieving the range. The proportion outside of the cutoff range helps us to predict the possibility of therapeutic failure such as graft rejection or adverse responses. In our data, to achieve the target range of $AUC_{0\text{-}12h}$ 540 mg every 12 hours may be required. However, since these patients are at low immunological risk because of beyond one year after transplantation, it is necessary to reduce the AUC target. Kornberg *et al* found that reduced-dose regimen of mycophenolate was safe and effective without increasing the immunological risks in patients with CNI-based long-term immunosuppression[49]. And Sarangi *et al* and Kuypers *et al* suggested that patients in the high range group (MPA $AUC_{0\text{-}12h}$ >60 mg.h/L) had significantly higher incidence of diarrhea and bone marrow toxicity as compared to lower range group ($AUC_{0\text{-}12h}$ <30 mg.h/L)[50,51]. Therefore, clinical trials are needed to assess the utility of target AUC range in associating drug exposure with long-term graft outcomes or adverse events within different time scales after transplantation.

In this study, to develop a mechanism-based PK model, we decided the sampling time points at pre-dose, 0.5, 1, 2, 4, and 6 hr after dose. A previous systematic review for limited sampling strategy (LSS) and Ana *et al* concluded time points at 1, 2, and 4 hr after dosing provided the most reliable and accurate estimation of the

MPA AUC in renal transplant recipients[52,53]. Another limitation of this study was that we could not include a lot of patients; however, this does not pose a statistical problem because the number of patients corresponded to the planned sample size. To overcome these limitations, several methods were used for identifying significant covariates. First, SCM was performed for building of a covariate model. Second, we have explored the covariates clinically significant using full covariate modeling. This is the unique study in terms of assessing both clinical and statistical significance of covariate effects. Additionally, we have performed bootstrap analyses of the SCM (boot-SCMs), as a tool for analysing type I error of covariate inclusion, correlations and interactions between covariate inclusions, selection bias and bias in covariate estimates.

Although this study has demonstrated the covariate effect consistent results of previous studies in transplant recipients, more prospective studies are required to establish the benefit of genotyping in predicting the clinical outcomes to further improve EC-MPS therapy.

VI. Conclusion

In conclusion, the population PK analysis of EC-MPS has suggested that *UGT1A9* and *SLCO1B1* genetic variations and CLcr seem to be promising parameters to predict MPA PK considering the flip-flop phenomenon. And the simulation results may help predict optimal dose to achieve a proper AUC in a Korean population.

References

1. Arns W, Breuer S, Choudhury S, et al. Enteric-coated mycophenolate sodium delivers bioequivalent MPA exposure compared with mycophenolate mofetil. Clinical transplantation 2005;19:199-206.
2. Budde K, Bauer S, Hambach P, et al. Pharmacokinetic and pharmacodynamic comparison of enteric-coated mycophenolate sodium and mycophenolate mofetil in maintenance renal transplant patients. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2007;7:888-98.
3. Budde K, Glander P, Kramer BK, et al. Conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium in maintenance renal transplant recipients receiving tacrolimus: clinical, pharmacokinetic, and pharmacodynamic outcomes. Transplantation 2007;83:417-24.
4. van Gelder T, Shaw LM. The rationale for and limitations of therapeutic drug monitoring for mycophenolate mofetil in transplantation. Transplantation 2005;80:S244-53.
5. ESRD Registry Committee KSoN. Current Renal Replacement Therapy in Korea - Insan Memorial Dialysis Registry. 33rd Annual Autumn Meeting of the Korean Society of Nephrology; 2013.
6. Organ transplantation statistics: Korean Network for Organ Sharing; 2014 January 13, 2014.

7. 2007 Annual Report of the U.S. Organ Procurement and Transplantation Network and the Scientific Registry of Transplant Recipients. Transplant Data 1996–20062007.
8. Salvadori M, Holzer H, de Mattos A, et al. Enteric-coated mycophenolate sodium is therapeutically equivalent to mycophenolate mofetil in de novo renal transplant patients. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2004;4:231-6.
9. Budde K, Glander P, Diekmann F, et al. Enteric-coated mycophenolate sodium: safe conversion from mycophenolate mofetil in maintenance renal transplant recipients. Transplantation proceedings 2004;36:524S-7S.
10. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of mycophenolate in solid organ transplant recipients. Clinical pharmacokinetics 2007;46:13-58.
11. Bernard O, Tojcic J, Journault K, Perusse L, Guillemette C. Influence of nonsynonymous polymorphisms of UGT1A8 and UGT2B7 metabolizing enzymes on the formation of phenolic and acyl glucuronides of mycophenolic acid. Drug metabolism and disposition: the biological fate of chemicals 2006;34:1539-45.
12. Picard N, Ratanasavanh D, Premaud A, Le Meur Y, Marquet P. Identification of the UDP-glucuronosyltransferase isoforms involved in mycophenolic acid phase II metabolism. Drug metabolism and disposition: the biological fate of

chemicals 2005;33:139-46.

13. Borrows R, Chusney G, James A, et al. Determinants of mycophenolic acid levels after renal transplantation. Therapeutic drug monitoring 2005;27:442-50.
14. van Hest RM, van Gelder T, Vulto AG, Mathot RA. Population pharmacokinetics of mycophenolic acid in renal transplant recipients. Clinical pharmacokinetics 2005;44:1083-96.
15. Pescovitz MD, Guasch A, Gaston R, et al. Equivalent pharmacokinetics of mycophenolate mofetil in African-American and Caucasian male and female stable renal allograft recipients. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons 2003;3:1581-6.
16. van Hest RM, Mathot RA, Pescovitz MD, Gordon R, Mamelok RD, van Gelder T. Explaining variability in mycophenolic acid exposure to optimize mycophenolate mofetil dosing: a population pharmacokinetic meta-analysis of mycophenolic acid in renal transplant recipients. Journal of the American Society of Nephrology : JASN 2006;17:871-80.
17. Kuypers DR, Claes K, Evenepoel P, et al. Long-term changes in mycophenolic acid exposure in combination with tacrolimus and corticosteroids are dose dependent and not reflected by trough plasma concentration: a prospective study in 100 de novo renal allograft recipients. Journal of clinical pharmacology 2003;43:866-80.
18. Morissette P, Albert C, Busque S, St-Louis G, Vinet B. In vivo higher

glucuronidation of mycophenolic acid in male than in female recipients of a cadaveric kidney allograft and under immunosuppressive therapy with mycophenolate mofetil. Therapeutic drug monitoring 2001;23:520-5.

19. Neylan JF. Immunosuppressive therapy in high-risk transplant patients: dose-dependent efficacy of mycophenolate mofetil in African-American renal allograft recipients. U.S. Renal Transplant Mycophenolate Mofetil Study Group. Transplantation 1997;64:1277-82.
20. Le Guellec C, Bourgoin H, Buchler M, et al. Population pharmacokinetics and Bayesian estimation of mycophenolic acid concentrations in stable renal transplant patients. Clinical pharmacokinetics 2004;43:253-66.
21. Staatz CE, Duffull SB, Kiberd B, Fraser AD, Tett SE. Population pharmacokinetics of mycophenolic acid during the first week after renal transplantation. European journal of clinical pharmacology 2005;61:507-16.
22. Shaw LM, Korecka M, Aradhye S, et al. Mycophenolic acid area under the curve values in African American and Caucasian renal transplant patients are comparable. Journal of clinical pharmacology 2000;40:624-33.
23. Shaw LM, Mick R, Nowak I, Korecka M, Brayman KL. Pharmacokinetics of mycophenolic acid in renal transplant patients with delayed graft function. Journal of clinical pharmacology 1998;38:268-75.
24. Levesque E, Delage R, Benoit-Biancamano MO, et al. The impact of UGT1A8, UGT1A9, and UGT2B7 genetic polymorphisms on the pharmacokinetic profile of mycophenolic acid after a single oral dose in

healthy volunteers. Clinical pharmacology and therapeutics 2007;81:392-400.

25. Satoh S, Tada H, Murakami M, et al. Circadian pharmacokinetics of mycophenolic Acid and implication of genetic polymorphisms for early clinical events in renal transplant recipients. Transplantation 2006;82:486-93.
26. Suzuki H, Sugiyama Y. Single nucleotide polymorphisms in multidrug resistance associated protein 2 (MRP2/ABCC2): its impact on drug disposition. Advanced drug delivery reviews 2002;54:1311-31.
27. Baldelli S, Merlini S, Perico N, et al. C-440T/T-331C polymorphisms in the UGT1A9 gene affect the pharmacokinetics of mycophenolic acid in kidney transplantation. Pharmacogenomics 2007;8:1127-41.
28. van Schaik RH, van Agteren M, de Fijter JW, et al. UGT1A9 -275T>A/-2152C>T polymorphisms correlate with low MPA exposure and acute rejection in MMF/tacrolimus-treated kidney transplant patients. Clinical pharmacology and therapeutics 2009;86:319-27.
29. Kuypers DR, Naesens M, Vermeire S, Vanrenterghem Y. The impact of uridine diphosphate-glucuronosyltransferase 1A9 (UGT1A9) gene promoter region single-nucleotide polymorphisms T-275A and C-2152T on early mycophenolic acid dose-interval exposure in de novo renal allograft recipients. Clinical pharmacology and therapeutics 2005;78:351-61.
30. Girard H, Court MH, Bernard O, et al. Identification of common polymorphisms in the promoter of the UGT1A9 gene: evidence that UGT1A9 protein and activity levels are strongly genetically controlled in the liver.

Pharmacogenetics 2004;14:501-15.

31. Yamanaka H, Nakajima M, Katoh M, et al. A novel polymorphism in the promoter region of human UGT1A9 gene (UGT1A9*22) and its effects on the transcriptional activity. Pharmacogenetics 2004;14:329-32.
32. Zhang WX, Chen B, Jin Z, et al. Influence of uridine diphosphate (UDP)-glucuronosyltransferases and ABCC2 genetic polymorphisms on the pharmacokinetics of mycophenolic acid and its metabolites in Chinese renal transplant recipients. Xenobiotica; the fate of foreign compounds in biological systems 2008;38:1422-36.
33. Guo D, Pang LF, Han Y, et al. Polymorphisms of UGT1A9 and UGT2B7 influence the pharmacokinetics of mycophenolic acid after a single oral dose in healthy Chinese volunteers. European journal of clinical pharmacology 2013;69:843-9.
34. Miura M, Satoh S, Inoue K, et al. Influence of SLCO1B1, 1B3, 2B1 and ABCC2 genetic polymorphisms on mycophenolic acid pharmacokinetics in Japanese renal transplant recipients. European journal of clinical pharmacology 2007;63:1161-9.
35. Michelon H, Konig J, Durrbach A, et al. SLCO1B1 genetic polymorphism influences mycophenolic acid tolerance in renal transplant recipients. Pharmacogenomics 2010;11:1703-13.
36. Picard N, Yee SW, Woillard JB, et al. The role of organic anion-transporting polypeptides and their common genetic variants in mycophenolic acid

pharmacokinetics. Clinical pharmacology and therapeutics 2010;87:100-8.

37. Cox VC, Ensom MH. Mycophenolate mofetil for solid organ transplantation: does the evidence support the need for clinical pharmacokinetic monitoring? Therapeutic drug monitoring 2003;25:137-57.
38. Knight SR, Morris PJ. Does the evidence support the use of mycophenolate mofetil therapeutic drug monitoring in clinical practice? A systematic review. Transplantation 2008;85:1675-85.
39. Arns W, Cibrik DM, Walker RG, et al. Therapeutic drug monitoring of mycophenolic acid in solid organ transplant patients treated with mycophenolate mofetil: review of the literature. Transplantation 2006;82:1004-12.
40. Hale MD, Nicholls AJ, Bullingham RE, et al. The pharmacokinetic-pharmacodynamic relationship for mycophenolate mofetil in renal transplantation. Clinical pharmacology and therapeutics 1998;64:672-83.
41. Jadhav PR, Gobburu JV. A new equivalence based metric for predictive check to qualify mixed-effects models. The AAPS journal 2005;7:E523-31.
42. Henin E, Bergstrand M, Standing JF, Karlsson MO. A mechanism-based approach for absorption modeling: the Gastro-Intestinal Transit Time (GITT) model. The AAPS journal 2012;14:155-63.
43. Yanez JA, Remsberg CM, Sayre CL, Forrest ML, Davies NM. Flip-flop pharmacokinetics--delivering a reversal of disposition: challenges and opportunities during drug development. Therapeutic delivery 2011;2:643-72.

44. Bernard O, Guillemette C. The main role of UGT1A9 in the hepatic metabolism of mycophenolic acid and the effects of naturally occurring variants. Drug metabolism and disposition: the biological fate of chemicals 2004;32:775-8.

45. Ting LS, Benoit-Biancamano MO, Bernard O, Riggs KW, Guillemette C, Ensom MH. Pharmacogenetic impact of UDP-glucuronosyltransferase metabolic pathway and multidrug resistance-associated protein 2 transport pathway on mycophenolic acid in thoracic transplant recipients: an exploratory study. Pharmacotherapy 2010;30:1097-108.

46. Jada SR, Xiaochen S, Yan LY, et al. Pharmacogenetics of SLCO1B1: haplotypes, htSNPs and hepatic expression in three distinct Asian populations. European journal of clinical pharmacology 2007;63:555-63.

47. Jindal C, Kumar S, Choudhari G, Goel H, Mittal B. Organic anion transporter protein (OATP1B1) encoded by SLCO1B1 gene polymorphism (388A>G) & susceptibility in gallstone disease. The Indian journal of medical research 2009;129:170-5.

48. Miura M, Kagaya H, Satoh S, et al. Influence of drug transporters and UGT polymorphisms on pharmacokinetics of phenolic glucuronide metabolite of mycophenolic acid in Japanese renal transplant recipients. Therapeutic drug monitoring 2008;30:559-64.

49. Kornberg A, Kupper B, Hommann M, Scheele J. Introduction of MMF in conjunction with stepwise reduction of calcineurin inhibitor in stable liver

transplant patients with renal dysfunction. International immunopharmacology 2005;5:141-6.

50. Sarangi SC, Reeta KH, Agarwal SK, Kaleekal T, Guleria S, Gupta YK. A pilot study on area under curve of mycophenolic acid as a guide for its optimal use in renal transplant recipients. The Indian journal of medical research 2012;135:84-91.
51. Kuypers DR, de Jonge H, Naesens M, et al. Current target ranges of mycophenolic acid exposure and drug-related adverse events: a 5-year, open-label, prospective, clinical follow-up study in renal allograft recipients. Clinical therapeutics 2008;30:673-83.
52. Bruchet NK, Ensom MH. Limited sampling strategies for mycophenolic acid in solid organ transplantation: a systematic review. Expert opinion on drug metabolism & toxicology 2009;5:1079-97.
53. Sanchez Fructuoso AI, Perez-Flores I, Calvo N, et al. Limited-sampling strategy for mycophenolic acid in renal transplant recipients reciving enteric-coated mycophenolate sodium and tacrolimus. Therapeutic drug monitoring 2012;34:298-305.

국 문 초 록

비선형 혼합효과 모형을 이용한 신이식 후 장용정 마이코페놀레이트의 집단약동학

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장기이식 환자에서 이식 후 거부반응을 예방하기 위해 투여하는 삼중요법 중 마이코페놀레이트(mycophenolic acid, MPA)는 칼시뉴린 저해제와 함께 이식 후 생존율을 급격하게 향상시켰다. MPA의 초기 개발 제형인 마이코페놀레이트 모페틸(mycophenolate mofetil, MMF)에 대해서는 많은 약동학 연구들이 이루어져왔으며 개인간 약물반응 차이에 영향을 미치는 요인들도 규명되었다. 그러나 MMF는 전구약물(prodrug)로, 체내에서 모페틸 염이 떨어져 MPA로 존재하고, MPA는 간과 소장에서 대사되면서 활성형 대사체인 acyl-MPAG가 생성되는데, 이로 인해 위장관 자극이 증가하여 설사와 같은 부작용이

발생할 수 있다. 이에 따라 MMF의 위장관계 부작용 발생 위험을 감소시켜 약물의 흡수를 안정적으로 유지하기 위해 마이코페놀레이트 장용정(enteric-coated mycophenolate sodium, EC-MPS, Myfortic®) 제제가 개발되었으며, 이는 결과적으로 이식 후 장기(graft) 생존율을 향상시키는 결과를 나타냈다. 그러나 장용정 제제의 특성 상 기존의 MMF와 약동학적 특성이 달라 개인간 약물반응 예측이 어렵다. 뿐만 아니라 MPA의 체내 대사와 수송에 영향을 미치는 유전형이 인종간 차이가 크고, 특히 아시아인에서 변이형의 빈도가 유의하게 높은 유전형의 경우 타 인종과의 약동학적 차이가 나타날 수 있다. 그러나 이렇듯 개인간 약물반응 차이가 존재하는 것으로 알려져 있음에도 불구하고 임상에서는 체중과 병용 약물에 따라 경험적으로 용량을 설정하여 사용하고 있어 개인맞춤 약물요법의 실현을 위해 약물반응을 정량화하고 예측할 수 있는 계량적 모델 수립이 요구된다. 또한 타 인종에서는 MPA의 치료목표로 농도곡선하면적(Area under the curve, AUC)을 측정하여 맞추도록 권고하고 있지만, 아직까지 한국인에서 임상적 유용성이 평가된 바 없어 그대로 적용하기에 제한이 있다. 따라서 본 연구에서는 신장이식 후 안정기에 도달한 환자들에서 EC-MPS의 집단약동학적 특성을 평가하고, 개인간 EC-MPS의 약동학 파라미터의 차이에 기여하는 요인을 규명하며, 치료 목표범위로 알려진 AUC_{0-12h} target의 유용성을 검증하고자 한다.

본 연구에서는 만 18세 이상의 성인 신장이식 환자 중 이식 후 1년이

지나 비교적 신장기능이 안정화되어 있고, EC-MPS를 포함한 면역억제요법을 받는 환자를 대상으로 하였다. 5%의 유의수준에서 80%의 검정력을 갖도록 하는 목표 피험자수는 34~43명이었다. 집단약동학적 파라미터 산출을 위해 EC-MPS 투여직전과 투여 후 30분, 1, 2, 4, 6 시간 후 중의 4번 이상을 채혈하였으며, 개인간 차이를 최소화하기 위해 오전 중 채혈하였고, 약물농도 분석 오차를 최소화하기 위해 서울대병원의 진단검사실을 이용하여 LC-MS/MS로 농도를 분석하였다. EC-MPS의 집단약동학에 영향을 미치는 요인을 규명하고자 인구학적 정보와 신기능, 체중 등과 같은 임상 변수, 그리고 약물의 대사 및 수송에 영향을 미치는 효소와 수송체의 유전적 다형성 정보를 수집하였다. 유전형 분석은 직접 염기서열분석과 SNaPshot 방법으로 분석하였고, 모든 유전형에 대해 일배체형(haplotype) 분석도 시행하였다. 집단약동학적 모델은 1구획, 2구획 및 선형 소실(linear elimination)과 비선형 소실(non-linear elimination) 등을 모두 평가하였고, 장용정의 특성을 고려하여 흡수 지연시간(lag-time)을 고려하였으며, 개인간 차이(interindividual error)는 additive, proportional, exponential model을 모두 평가하여 가장 최적의 모델을 기본으로 하였다. 집단약동학적 특성에 영향을 미치는 공변량을 고려하기 위해 고정효과(fixed effect)와 무작위효과(random effect)를 고려하여 모델을 도출하였다. 고정효과 요인 분석으로 체중, 신장, 성별, 나이, 이식 전 투석여부, 공여신의 무게, serum creatinine (Scr), glomerular filtration rate (GFR), 산출된 creatinine clearance (CLcr),

hemoglobin (Hb), hematocrit (Hct), WBC, platelet, total bilirubin (T.bil), liver function (AST/ALT), total protein, albumin 및 약물유전형(*UGT1A7, UGT1A9, UGT2B7, ABCC2, SLCO1B1* 및 *SLCO1B3*) 등을 고려하여 유의한 영향을 미치는 공변량을 도출하였다. 모델링은 NONMEM version 7.2를 이용하여 분석하였고, 상호작용을 고려한 일차조건부추정(FOCE with interaction) 방법으로 추정하였다. 모델의 적합도는 적합도(goodness of fit)를 시각화 한 그래프와 목적함수값(objective function value, OFV)을 기준으로 전진선택법(forward selection)에서 3.84 이상(P <0.05) 감소하거나 후진제거법(backward elimination)에서 6.64(P <0.01) 이상 증가할 때 적합한 것으로 평가하였다. 공변량의 선정 단계에서는 OFV의 차이뿐만 아니라 통계적 유의성과 임상적 유의성을 함께 고려하기 위해 후보 변수들의 효과크기(effect size)를 계산하여 유의한 변수들을 선정하였다. 추가적으로 공변량의 선정 단계에서 반복 추정으로 발생하는 제 1형 오류를 최소화하기 위해 200회의 재추출 기반 단계적 공변량 탐색도 시행하여, 최종적으로 선정된 공변량의 통계적 근거도를 높이고자 하였다.

최종 모델의 정확성과 강건성(robustness)을 평가하기 위하여 시뮬레이션 기반의 Bootstrap 재추출 기법과 구축된 모델의 예측력을 시각적으로 평가하는 visual predictive check (VPC)를 시행하였다. 분석에 포함한 환자들에게 동일한 용량의 EC-MPS를 투여하지

않았으므로 보정된 값인 prediction corrected VPC(pcVPC)로 평가하였으며 Bootstrap과 pcVPC는 모두 Perl speaks NONMEM (PsN) version 3.6.2. 프로그램을 이용하였다. Bootstrap 과정은 서로 다른 무작위 추출로 2000번 시행하였으며, bootstrap 데이터 세트에서 각각의 약동학 파라미터의 5^{th}, 95^{th} 백분율 값 내의 범위에 final model의 약동학 파라미터가 포함되는지 확인하였다. pcVPC는 200번 시행하였으며, 실제 관측값과 시뮬레이션된 예측값을 그래프를 통해 비교·평가하였다. 마지막으로 개발된 모델의 임상적 적합성 및 예측력 평가를 위해 최종 모델에서 도출된 공변량을 토대로 가상 용량을 투여했을 때의 AUC를 평가하여 기존에 알려진 MPA의 목표 AUC에 도달하는 환자의 비율 및 목표범위의 임상적 유용성을 평가하였다.

결과적으로 총 34명을 모집하여 인구학적 특성과 유전형 분석을 시행하였고, 모든 유전형에서 Hardy-Weinberg 평형을 따랐다. 34명에서 채혈한 166개의 혈중농도를 가지고 집단약동학적 분석으로 약물의 청소율 CL (L/hr), 분포용적 V_c (L), 흡수속도상수 K_a (hr^{-1})를 계산하였고, 추가적으로 개인별 0에서 12시간까지의 분포곡선 하 면적 AUC_{0-12h} (mg.h/L)와 최고혈중농도 C_{max} (ng/mL)를 산출하였다. EC-MPS는 2 구획 모델로, 중심구획과 말초구획(또는 대사구획[metabolic compartment])으로 하였고 선형 흡수와 소실의 특성을 가지며 흡수 시 시간지연(lag-time)이 있었다. 또한 EC-MPS는 장용정의 특성상 소실속도상수(elimination rate constant, K_e)와 K_a가 혼재되어 있는

flip-flop 현상을 나타내므로 K_e을 토대로 K_a를 예측하였다. 최종 집단약동학 모델에서 약물의 소실속도와 중심구획 및 대사구획의 겉보기 분포용적은 각각 9.3 L/hr와 42 및 60.3 L로 기존 문헌과 유사한 값을 보였고, 흡수속도와 소실속도는 각각 1.24와 0.221 hr^{-1}로 나타났다.

공변량 분석의 결과로, 약물의 배설(CL/F)에 영향을 미치는 요인으로 *SLCO1B1* 388A>G 유전형이 도출되었고, 유전적으로 변이형인 사람에서는 약물의 배설이 감소하였다. 또한 흡수속도(K_a)에 영향을 미치는 요인으로 *UGT1A9* -118dT 유전형이 도출되었으며, 말초구획의 겉보기 분포용적(V_p/F)에는 CLcr이 유의한 영향을 미치는 것으로 나타났다. *UGT1A9*의 변이형이 MPA의 흡수에 영향을 미치는 이유는 위장관에 있는 UGT1A9 효소 때문으로 생각되며, *UGT1A9*에 변이형인 사람에서는 대사가 적게 나타나 상대적으로 흡수가 증가하게 된다. 또한 간에서 *UGT1A9* 391C>A에서 A 변이형인 사람에게서 *UGT1A9*의 대사기능이 감소하여 MPAG의 생성이 감소하고, 상대적으로 Ac-MPAG의 생성이 증가하여 약물의 제거율이 증가함으로써 흡수속도가 감소하였다. 신기능을 대변하는 CLcr은 대사구획에서의 약물의 제거와 관련되어 있어, CLcr이 높은 사람에서는 말초구획에서의 제거가 증가하여 말초구획의 겉보기 분포용적이 감소하게 된다. 최종적으로 수립된 모델의 검증에서 최종 모델의 약동학적 파라미터 값은 Bootstrap의 5^{th}, 95^{th} 백분율 범위에 포함되고 pcVPC 결과도 모델이 잘 예측하여 집단약동학 모델로서 적합한 것으로

나타났다. 추가적으로 EC-MPS의 치료 목표 AUC의 유용성을 평가한 결과, *UGT1A9*, *SLCO1B1*, 및 CLcr을 고려하여 가상의 용량으로 생성한 32개의 시나리오에서 외국과 달리 한국에서는 목표 AUC_{0-12h}인 30-60 mg.h/L에 도달하기 위한 필요 용량은 일반적으로 540 mg이었으며, 개인별 도출된 요인을 고려하여 맞춤 용량을 설정할 수 있었다.

본 연구의 결과로 EC-MPS의 집단약동학적 파라미터를 산출하였고, 약동학에 영향을 미치는 요인으로 신기능, *UGT1A9* 및 *SLCO1B1*의 유전적 다형성을 규명하였고, 예측력 평가에서도 모델의 타당성이 검증되었으며, 모델에 기반한 시뮬레이션으로 목표 AUC_{0-12h} 범위에 도달하기 위한 용량을 산출하였다.

주요어 : 마이코페놀레이트 장용정(EC-MPS), 집단약동학, flip-flop 현상, 비선형혼합효과 모델링(NONMEM), 신장이식, 유전적 다형성

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