Population pharmacokinetics and pharmacodynamics of mycophenolic acid using prospective data in patients undergoing hematopoietic stem cell transplantation

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Running heading:

Population analysis of mycophenolic acid

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Abstract

Mycophenolate mofetil (MMF), a prodrug of mycophenolic acid (MPA), is used to suppress *graft-versus-host* disease in patients undergoing hematopoietic stem cell transplantation (HCT). The purpose of this study was to construct a population pharmacokinetic and pharmacodynamic model in HCT patients for individualized MPA therapy. Blood samples were obtained from 49 HCT patients after starting MMF therapy. Population pharmacokinetic and pharmacodynamic parameters were obtained using the program NONMEM. MPA was described via a 1-compartment model with a first order elimination, and 30.9% of MPA glucuronide (MPAG) was found in the enterohepatic circulation. Inosine-5'-monophosphate dehydrogenase (IMPDH) activity was modeled as a maximal inhibitory model with a half-maximal inhibitory concentration (IC_{50}) of 3.59 μg/mL against MPA concentrations. Simulations based on the obtained pharmacokinetic and pharmacodynamic parameters revealed that decreased creatinine clearance increases the MPAG concentration followed by an increased MPA concentration; therefore, IMPDH activity decreases. Diarrhea decreases the enterohepatic circulation of MPAG and consequently reduces MPA concentration. The IC_{50} for MPA exhibited a positive association with C-reactive protein. Dosage adjustment based on plasma MPA concentration is required especially for patients with renal dysfunction and/or diarrhea.

Introduction

Mycophenolate mofetil (MMF) is clinically used to suppress *graft-versus-host* disease (GVHD) in patients undergoing hematopoietic stem cell transplantation (HCT) and acute rejection after solid organ transplantation[1,2]. Mycophenolic acid (MPA), an active form of MMF, is metabolized by glucuronosyltransferases in the liver. MPA glucuronide (MPAG) and MPA acyl glucuronide (AcMPAG) are primarily produced by UGT1A9 and 2B7, respectively [3]. While MPAG is an inactive metabolite, AcMPAG exhibits pharmacological activity *in vitro* and is potentially responsible for the toxicity of MPA [4]. The majority of MPA metabolites are eliminated via the urine and partial elimination also occurs in the bile mediated by multidrug resistance associated protein 2 (MRP2) followed by the entero-hepatic recirculation [5].

The pharmacokinetics (PK) of MPA exhibits a large inter- and intra-individual variability, and it is recommended that the area under the concentration-time curve (AUC) of MPA be monitored for individualized therapy in solid organ transplant recipients[6, 7]. Recently, Arai et al. [8] proposed that MPA drug monitoring was necessary for the effective prophylaxis of acute GVHD undergoing cord blood stem cell transplantation (CBT). However, information regarding the optimal dose of MMF or the target range for MPA concentrations in HCT patients is limited [9].

MPA selectively inhibits inosine-5'-monophosphate dehydrogenase (IMPDH) and suppresses the proliferation of B and T lymphocytes [10]. IMPDH exists as two isoforms derived from different genes [11, 12], and recombinant proteins of IMPDH2 is 4.8-fold more sensitive to MPA than IMPDH1[13]. The area under the effect curve (AUEC) of IMPDH activity on day 21 after HCT was reportedly associated with both non-relapse and overall mortality [14]. Therefore, the measurement of IMPDH activity in peripheral blood mononuclear cells (PBMCs) in addition to monitoring the AUC of MPA is considered to be an effective predictor of the clinical outcome of MPA therapy.

The PK of MPA is influenced by serum albumin, renal dysfunction, total bilirubin, age, co-administration with cyclosporine, and dose [15-19]. In addition, the incidence of acute rejection in the first year post-transplantation was significantly lower in carriers of SNPs for *IMPDH1 -106 G>A* and *125 G>A* compared with the respective wild-type individuals[20]. The SNP for *IMPDH2 3757 T>C* was associated with a significantly higher IMPDH activity following the MMF intake, despite of no difference in the MPA exposure between groups[21]. The SNP for *MRP2 -24C>T* was associated with a significantly higher dose-corrected MPA trough levels at later time points after transplantation[22]. The SNP for *UGT2B7 -842G>A* resulted in a significantly higher AUC of AcMPAG at 1 and 3 months post-transplantation in patients with renal transplantation[23].

In this study, the effects of the patient characteristics including previously proven genetic polymorphisms were examined using a population PK and pharmacodynamics (PD) analysis. Effects of covariates were quantitatively evaluated by the simulation to examine the clinical significance of these covariates.

Subjects and Methods

Study design

A total of 49 adult Japanese HCT patients between March 2013 and August 2016 were included in the study. Acute GVHD prophylaxis comprised tacrolimus (Prograf™, Astellas Pharma Inc., Tokyo, Japan) and MMF (Cellcept™, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) in CBT, plus short-term methotrexate (Methotrexate™, Pfizer Japan Inc., Tokyo, Japan) in bone marrow transplant (BMT) or peripheral blood stem cell transplantation (PBT). MMF was orally administered at 10 mg/kg every 8 h (30 mg/kg/day), and was initiated on day −1 after CBT or on day 7 after BMT and PBT, except in one patient administered 15 mg/kg every 12 h. No potentially interacting drugs including cyclosporine or foods with MPA were co-administered.

Pre-transplant recipient DNA was used to determine *UGT2B7* *−842C>T* (rs7439366) and *MRP2* *−24C>T* (rs717620) genotypes. Approximately five weeks after MMF administration commenced,

post-transplant donor DNA as well as pre-transplant recipient DNA were used to determine *IMPDH1 −106G>A* (rs2278294), *IMPDH1 125G>A* (rs2278293), and *IMPDH2 3757T>C* (rs11706052) genotypes. Blood samples were collected immediately before, 1, 2, 4, and 8 h after the first and third weeks after MMF administration commenced, plus blood sampling at 12 h after MMF administration in one patient administered 15 mg/kg every 12 h. This clinical study was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine and Kyoto University Hospital. Written informed consent was obtained from all patients included in the study.

Analytical methods

Total plasma concentrations of MPA, MPAG, and AcMPAG were analyzed using LC-MS/MS according to the previously reported method [24]. The lower limits of quantification (LLOQ) were 0.05, 0.2, and 0.02 μg/mL for MPA, MPAG, and AcMPAG, respectively. PBMC samples were used to measure the IMPDH activity according to the previous method [24]. The IMPDH activity was calculated based on the XMP produced, which was normalized to the intracellular AMP. The LLOQ were 50 nM for both XMP and AMP. The data for AMP under the LLOQ were excluded from the analysis due to extremely low white blood cell counts after the transplantation.

Population PK/PD analysis

A population PK analysis was conducted using NONMEM. The overview of the basic PK/PD model for MPA is shown in Fig. 1. Since only the oral data were available, the relative bioavailability (F) of MPA was assumed to be 1. The model was parameterized using clearances for MPA, MPAG, and AcMPAG ($CL_{MPA,}$ CL_{MPAG}, and CL_{AcMPAG}), as well as the volume of distribution for MPA, MPAG, and AcMPAG ($V_{MPA,}$ V_{MPAG}, and V_{AcMPAG}). It was assumed that MPA was metabolized to MPAG and AcMPAG by a first-order process, in which 99 % and 1 % of MPA was converted to MPAG and AcMPAG, respectively, because the ratio of AUC_{0-8} for MPAG to AcMPAG was approximately 99:1 in this study. The enterohepatic circulation (EHC) was tested as a first-order process (K_{EHC}) from the central compartment of each metabolite to the gastrointestinal tract. For the comparison, 2-compartment model with EHC, and the lag time and the transit compartment models in the absorption process were tested [25]. Additionally, EHC modeling by presuming a hypothetical gall bladder compartment was tested [26, 27].

Interindividual and interoccasion variability (IIV and IOV) in the PK/PD parameters were modeled using an exponential error model [28]. The estimation for the IOV was as follows: occasions 1 and 2 pertained to one and three weeks after MMF administration commenced, respectively. The influence of each covariate on the population mean parameters was evaluated by the stepwise forward inclusion and backward elimination method, and significance levels were 1 % and 0.1 % (6.63 and 10.8 with freedom

of 1 assuming a chi-square distribution), respectively. The tested covariates for the PK parameters included body weight, gender, stem cell source, age, aspartate aminotransferase, serum albumin, total bilirubin, creatinine clearance (CL_{CR}), dose of MMF, diarrhea, and investigated genotypes for MRP2 and UGT2B7 of recipient. Diarrhea was defined as the occurrence of loose, muddy or watery stool, or more than five times per a day of fecal frequency in case of not recording the fecal condition.

Continuous variables were normalized by each population median using the following power function model:

$$\theta_i = \theta_{pop} \times \left(\frac{COV_i}{COV_{med}}\right)^{\theta_{cov}} \quad (1)$$

where θ_i is the individual model-predicted PK parameter (e.g. CL_{MPA}) for an individual with covariate value of COV_i. θ_{pop} represents the population mean for the parameter θ, COV_{med} represents the population median of the covariate, and θ_{cov} represents the covariate effect. For dichotomous variables, the value of COV_i is typically set to 0 for the normal classification and 1 for the other classifications in each individual as follows:

$$\theta_i = \theta_{pop} \times \theta_{cov}^{COV_i} \quad (2)$$

After the final population PK model was obtained, the relationship between the MPA concentrations and IMPDH activity was explored graphically and modeled using a direct sigmoid inhibitory maximum effect model as followed:

$$E = E_0 \times \left(1 - \frac{C_{MPA}^{\gamma}}{IC_{50,MPA}^{\gamma} + C_{MPA}^{\gamma}}\right) \quad (3)$$

where E_0, $IC_{50,MPA}$, and γ represent baseline of IMPDH activity, half-maximal inhibitory MPA concentration, and the Hill coefficient to be estimated [29], and C_{MPA} represents the MPA concentration. To investigate the effect of the AcMPAG concentration on IMPDH activity, an additional inhibitory effect model was tested [30]. The tested covariates for the PD parameters included the stem cell source, reduced-intensity conditioning, gender, age, serum albumin, C-reactive protein (CRP), and investigated genotypes (*IMPDH1* and *2*) of donor or recipient. In the value of CRP was under the LLOQ (<0.2 mg/mL), this value was converted to 0.1 due to the difficulty of the calculation. Goodness-of-fit and prediction-corrected visual predictive check plots were used for internal validation [31]. For prediction-corrected visual predictive check plots, the final PK/PD model was used to simulate original data sets at 1000 times compared with the observed data.

Simulation study

The effects of statistically significant covariates on the PK/PD of MPA were evaluated by the simulation using the final population parameters. The dose was fixed to 500 mg every 8 h for all simulations. In the simulation for the effect of each covariate, other covariates were fixed to the median value of each covariate and without diarrhea. The AUC_{0-8} or $AUEC_{0-8}$ were calculated using the linear trapezoidal method.

Results

Patient characteristics

The patient characteristics and the distribution of each genotype before and five weeks after the transplantation are summarized in Table 1. All of the observed genotype distributions were consistent with Hardy-Weinberg equilibrium.

Population PK modeling

In total, 522 concentration data for MPA, MPAG, and AcMPAG, respectively, were analyzed. Five samples with MPA concentrations under the LLOQ were replaced with half of the LLOQ [32], and included in the analysis. 2-compartment model improved the model fitting compared with 1-compartment model

without EHC. However, after an inclusion of EHC process, 2-compartment model did not improve the model fitting compared with 1-compartment model (ΔOBJ = −8.12), and a terminal elimination rate constant was not correctly estimated. Therefore, the PK of MPA was finally described by 1-compartment model with first order absorption and elimination, and was affected by the EHC of MPAG. An inclusion of the absorption lag-time did not improve the model fitting. Transit compartment model was not adopted owing to the high computational intensity required, although the model fit was significantly improved. Although the inclusion of EHC of AcMPAG in the model brought a statistically significant model improvement, CL_{AcMPAG} was not correctly estimated. Therefore, the model including the EHC of only MPAG was selected. EHC modeling by presuming a hypothetical gall bladder compartment did not improve the model fitting compared with first-order EHC model. The simultaneous inclusion of IOVs for Ka, F, K_{EHC}, CL_{MPAG}, and CL_{AcMPAG} significantly improved the model fitting (ΔOBJ = −721). The final PK parameters and its relative standard error (RSE) are presented in Table 2. Figure 2 shows the inter-occasional parameters for one and three weeks after MMF administration commenced.

After the evaluation of each covariate, the serum albumin revealed a significant negative association with CL_{MPA} and V_{MPA}. CL_{CR} exhibited a significant positive relationship with both CL_{MPAG} and CL_{AcMPAG}, and K_{EHC} in the patients showing diarrhea was 0.375-fold lower than that without diarrhea (Table 2). An

inclusion of IIV was tested for all the MPA PK parameters and an exclusion of IIV was tested after inclusion of IOV. After all, IIVs on V_{MPA} and F were retained in the final model, and shrinkage values of them were 27.6% and 16.8%, respectively. The ratio for the EHC of MPAG was estimated to be 30.9% (EHC (%) = $K_{EHC}/(K_{EHC} + CL_{MPAG}/V_{MPAG}) \times 100$) in patients with CL_{CR} of 112 mL/min without diarrhea.

PD modeling

A total of 460 IMPDH activity data from 49 patients were used for the PD model building following the PK modeling process. The 62 IMPDH activity data were excluded due to AMP under the LLOQ. The IMPDH activity was described with the inhibitory E_{max} model using the MPA concentrations. The Hill coefficient was fixed to 1 by the statistical selection (ΔOBJ = −3.00). The additive inhibitory effect model for AcMPAG did not significantly improve the model fitting. An inclusion of IOV on E_0 significantly improved the model fitting (ΔOBJ = −200). The value of IC_{50} for MPA revealed a positive association with CRP (ΔOBJ = −11.4). No polymorphisms were identified as significant covariates in the PK/PD model. The final PD parameters with RSE are shown in Table 2. The IIV for IC_{50} was 81.2%, and its shrinkage was 29.7%. The goodness-of-fit and prediction-corrected visual predictive check

demonstrated that the population PK/PD model accurately predicted the observed MPA and its metabolites concentrations, as well as IMPDH activity (Figs. 3 and 4).

Simulation study

A total of 1,000 data sets in each group were simulated under the several renal functions with or without diarrhea (Fig. 5). The $AUC_{0\text{-}8}$ of MPAG and AcMPAG significantly increased according to the decreased CL_{CR} compared with those for 120 mL/min. The $AUC_{0\text{-}8}$ of MPA also significantly increased according to the decreased CL_{CR}. The $AUEC_{0\text{-}8}$ of IMPDH significantly decreased from 339 to 215 $\mu mol \cdot h \cdot sec^{-1} \cdot mol\ AMP^{-1}$ with a decrease in CL_{CR} from 120 to 10 mL/min; however, a large interindividual variability was noted. In addition, the diarrhea significantly decreased the $AUC_{0\text{-}8}$ of both MPA and AcMPAG in every CL_{CR}, but did not affect the $AUC_{0\text{-}8}$ of MPAG. The $AUEC_{0\text{-}8}$ of IMPDH with diarrhea was significantly higher than that without diarrhea in the case of CL_{CR} under 60 mL/min.

The $AUC_{0\text{-}8}$ of MPA significantly decreased with a reduction in serum albumin, although the $AUEC_{0\text{–}8}$ of IMPDH did not significantly change (Fig. 6). At a MPA concentration of 3.59 μg/mL, which is equal to the population mean of IC_{50} in the case of CRP of 1.2 mg/dL, the IMPDH activity is 1.34-fold

223 higher in patients with CRP of 10 mg/dL, compared with that for CRP of 1.2 mg/dL. The $AUEC_{0-8}$ of

224 IMPDH also significantly increases as the CRP rises.

225

226 **Discussion**

227 Patients undergoing HCT generally have intestinal mucosal damage due to a myeloablative or

228 reduced intensity conditioning regimen prior to HCT [33]. Indeed, MPA concentrations in HCT patients are

229 generally lower than those of organ transplant patients despite an equivalent dose of MMF [34]. In addition,

230 leukopenia and co-administered antibiotics induce the destruction of intestinal flora, leading to diarrhea.

231 The diarrhea decreased the reabsorption of MPA in the gastro-intestinal tract, and consequently decreased

232 the MPA concentration. In this study, IMPDH activity in CL_{CR} under 60 mL/min with diarrhea was

233 significantly higher compared to those in the same CL_{CR} without diarrhea in the same MPA dosing,

234 secondary to the PK changes.

235 In the early phase after transplantation, HCT patients suffer from renal impairment due to

236 thrombotic microangiopathy, which is an adverse effect caused by calcineurin inhibitors and high-dose

237 chemotherapy [35]. Since MPA metabolites are excreted into the urine, the clearance of MPA metabolites

238 have been reported to decrease in association with lower renal function [14, 36]. In the simulation using the

239 final PK/PD parameters, MPA concentration will be increased with a decreased CL_{CR}, due to the
240 enhanced EHC of MPAG. Moreover, the IMPDH activity in CL_{CR} under 30 mL/min was significantly
241 lower than that in 120 mL/min. Therefore, particular attention regarding extra-immunosuppression in
242 response to MPA is needed for patients with severe renal dysfunction.

243 The target range of MPA exposure may be influenced by changes in serum albumin, since the free
244 fraction of MPA was 1–2% [37]. In the PD analysis, we speculated that the serum albumin had a significant
245 effect on the IC_{50} value of MPA due to the changed unbound fraction of MPA; however, we were unable
246 to conclusively demonstrate this effect due to a large inter- and intra-individual variability in IMPDH
247 activity. Therefore, changes in the serum albumin in clinical situations might not have a significant effect
248 on IMPDH activity, although it can affect the PK of MPA.

249 Interestingly, CRP exhibited a positive association with the IC_{50} for MPA. Patients undergoing HCT
250 suffered from various symptoms caused by an infection and/or the excessive production of inflammatory
251 cytokines on the days around the engraftment [38, 39]. Indeed, the median of CRP in one week after starting
252 the MMF therapy (3.0 mg/dL) was significantly higher than that in three weeks after the therapy (0.8
253 mg/dL). Moreover, IMPDH1 is constitutively expressed in normal leukocytes, whereas IMPDH2 is
254 up-regulated in neoplastic and replicating cells[40, 41]. These findings suggest the reasons why CRP

exhibited a positive association with the IC_{50} for MPA. Whether the elevated CRP value reflects infection or excessive production of inflammatory cytokines remains to be examined.

The simultaneous inclusion of IIVs and IOVs for PK/PD parameters significantly improved the model fitting. HCT patients have single or multiple damages due to the conditioning regimen, infection or excessive production of inflammatory cytokines[33, 38, 39, 42]. In addition, the recovery rate of organic and hematopoietic functions following HCT showed a large variability. Therefore, the IIV and IOV in the PK/PD of MPA in HCT patients should be large.

The present study included some limitations. The PK of MPA is usually expressed as 2-compartment model, and/or sometimes includes more sophisticated models [26, 27]. In this study, the model fitting was not improved by using any sophisticated models. The examined model largely depends on the experimental design, and we would like to pick up clinical significant covariates on the PK of MPA in a routine clinical care as shown in the previous our study [8]. Additionally, although our population size was modest, the estimated parameters, such as the ratio of EHC of MPAG or the IC_{50} for MPA, were similar to those of previous reports [14]. Therefore, the constructed PK/PD model for MPA was considered to be appropriate. The effects of covariates extracted in the present study should be examined by 2-compartment model using more rich sampling data in a future.

In conclusion, we successfully constructed a population PK/PD model of MPA in patients undergoing HCT. Renal dysfunction, diarrhea, and CRP are clinically significant factors affecting the PD of MPA in the same dosing regimen. Dosage adjustment based on plasma MPA concentration is required especially for patients with renal dysfunction and/or diarrhea.

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Figure legends

Fig. 1 Overview of the basic pharmacokinetic and pharmacodynamic model characterizing mycophenolic acid (MPA), MPA glucuronide (MPAG), MPA acylglucuronide (AcMPAG), and inosine-5'-monophosphate dehydrogenase (IMPDH). GI, gastrointestinal tract; Ka, first-order absorption rate constant; CL_{MPA}, clearance of MPA; CL_{MPAG}, clearance of MPAG; CL_{AcMPAG}, clearance of AcMPAG; V_{MPA}, volume of distribution of MPA; V_{MPAG}, volume of distribution of MPAG; V_{AcMPAG}, volume of distribution of AcMPAG; K_{EHC}, first-order rate constant of enterohepatic circulation; FM_1, fraction of MPA converted to MPAG.

Fig. 2 Interoccasional pharmacokinetic and pharmacodynamic parameters between one and three weeks after initiation of mycophenolate mofetil (MMF) therapy. (A) The first-order absorption rate constant (Ka); (B) relative bioavailability (F); (C) clearance of MPAG (CL_{MPAG}); (D) clearance of AcMPAG (CL_{AcMPAG}); (E) first-order rate constant for the enterohepatic circulation (K_{EHC}); (F) baseline IMPDH activity (E_0).

Fig. 3 Goodness-of-fit plots of the observed *versus* population predictions (A-D) or individual predictions (E-H) using the final model. (A and E) MPA concentrations; (B and F) MPAG concentrations; (C and G) AcMPAG concentrations; (D and H) IMPDH activity. Each dotted line denotes the line of identity.

Fig. 4 Prediction corrected visual predictive check plots. All open circles represent the observed concentrations or IMPDH activities (prediction corrected). (A-D) one week after initiation of MMF therapy; (E-H) three weeks after initiation of MMF therapy. The solid line represents the median of the observed data. The dotted line represents the observed 5^{th} and 95^{th} percentiles. The shaded area denotes the simulation-based 95% confidence interval for the median or the 5^{th} and 95^{th} percentiles.

Fig. 5 Simulation for the effects of creatinine clearance (CL_{CR}) and diarrhea on the pharmacokinetics and pharmacodynamics of MPA in typical patients based on the final population model. (A) $AUC_{0\text{-}8}$ of MPA; (B) $AUC_{0\text{-}8}$ of MPAG; (C) $AUC_{0\text{-}8}$ of AcMPAG; (D) $AUEC_{0\text{–}8}$ of IMPDH activity. The dose of MMF was fixed to 500 mg every 8 h. Each box plot represents the 5^{th} percentile, lower quartile, median, upper quartile, and 95^{th} percentile values obtained from 1000 simulated data sets. *; $p < 0.05$; ***; $p < 0.001$, significantly different from the group with a CL_{CR} of 120 mL/min by the Kruskal-waillis test following

by the Dunn test. †; $p < 0.05$;†††; $p < 0.001$, significantly different from the same CL_{CR} without diarrhea by the Kruskal-waillis test following by the Dunn test.

Fig. 6 Simulation for the effects of serum albumin (A and B) and C-reactive protein (CRP) (C and D) on the pharmacokinetics and pharmacodynamics of MPA in typical patients based on the final population model. The dose of MMF was fixed to 500 mg every 8 h. (A) $AUC_{0\text{-}8}$ of MPA; (B) $AUEC_{0\text{-}8}$ of IMPDH activity; (C) the relationship between the MPA plasma concentration and IMPDH activity. The dotted, thin, and thick lines represent 0.1, 1.2, and 10 mg/dL of CRP, respectively. The vertical line represents the MPA concentration of 3.59 μg/mL (the population mean of IC_{50} in the case of CRP 1.2 mg/dL); (D) $AUEC_{0\text{-}8}$ of IMPDH activity. Each box plot represents the 5^{th} percentile, lower quartile, median, upper quartile, and 95^{th} percentile values obtained from 1000 simulated data sets. ***; $p < 0.001$, significantly different from the group of 4.2 mg/dL of serum albumin by the Kruskal-waillis test following by the Dunn test. †††; $p < 0.001$, significantly different from the group of 0.1 mg/dL of CRP by the Kruskal-waillis test following by the Dunn test.