Limited Sampling Strategy for the Estimation of Mycophenolic Acid and its Acyl Glucuronide Metabolite Area under the Concentration-Time Curve in Japanese Lung Transplant Recipients

Masaki Tanaka 1, Masafumi Kikuchi 1,2, Shinya Takasaki 1, Tensei Hirasawa 2, Kensuke Shigeta 2, Aoi Noda 1, Miki Akiba 3, Yasushi Matsuda 4, Hisashi Oishi 4, Tetsu Sado 4, Masafumi Noda 4, Yoshinori Okada 3,4, Nariyasu Mano 1,2, Hiroaki Yamaguchi 1,2,a

1Department of Pharmaceutical Sciences, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai, Japan. 2Faculty of Pharmaceutical Sciences, Tohoku University, 6-3 Aramaki-Aza-Aoba, Aoba-ku, Sendai, Japan. 3Department of Organ Transplantation Center, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai, Japan. 4Department of Thoracic Surgery, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai, Japan.

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ABSTRACT - Purpose: The dose of mycophenolate mofetil (MMF) used to prevent rejection after lung transplantation is often adjusted based on the 12-hour area under the concentration-time curve (AUC0-12) of mycophenolic acid (MPA). A limited sampling strategy (LSS) is useful to define the pharmacokinetic (PK) profiles of MPA and mycophenolic acid acyl glucuronide (AcMPAG). Therefore, this study aimed to design a LSS based on multiple linear regression for estimating the AUC0-12 of MPA and AcMPAG at the minimum blood sampling points in Japanese lung transplant patients with concomitant tacrolimus. Methods: Forty-five lung transplantation recipients were enrolled in a PK study of MPA, mycophenolic acid glucuronide (MPAG), and AcMPAG. The plasma MPA, MPAG, and AcMPAG concentrations were determined just before and at 0.5, 1, 2, 4, 8, and 12 hours after dosing. The AUC0-12 of MPA and AcMPAG was calculated using a linear trapezoidal rule from the plasma concentration of each blood sampling time. LSS was used to develop models for estimated AUC in the model group (n = 23) and was evaluated in the validation group (n = 22). Results: The best three time-point equation was 4.04 + 1.64 · C1 + 3.08 · C4 + 5.17 · C8 for MPA, and -0.13 + 3.01 · C1 + 3.51 · C4 + 5.74 · C8 for AcMPAG. The prediction errors (PE) and the absolute prediction errors (APE) were within the clinically acceptable ± 5% and 15% range, respectively (MPA: PE = 2.00%, APE = 11.66%, AcMPAG: PE = 0.98%, APE = 14.69%). The percentage of estimated AUC0-12 within ± 15% of the observed AUC0-12 was 77.27% for MPA and 81.82% for AcMPAG. Conclusion: LSS using three time-point (C1, C4, and C8) provides the most reliable and accurate simultaneous estimation of the AUC0-12 of MPA and AcMPAG in Japanese lung transplant patients.

INTRODUCTION

Mycophenolate mofetil (MMF) is rapidly hydrolyzed in vivo to the immunosuppressant mycophenolic acid (MPA), which reversibly inhibits inosine 5'-monophosphate dehydrogenase, an enzyme involved in the de novo synthesis of guanosine in lymphocytes (1, 2). Subsequently, MPA is predominantly metabolized to a pharmacologically inactive mycophenolic acid glucuronide (MPAG) and pharmacologically active mycophenolic acid acyl glucuronide (AcMPAG) by uridine diphosphate glucuronosyltransferase (3). On the other hand, MPAG is hydrolyzed back to MPA during enterohepatic recirculation, and its contribution to the total MPA exposure is approximately 40% (4). MMF is administered in combination with a calcineurin inhibitor, such as tacrolimus or cyclosporine, and steroid to reduce the risk of rejection after lung transplantation (5). Several studies have reported that the 12-hour area under the concentration-time curve (AUC0-12) of MPA is a useful pharmacokinetic parameter for predicting clinical efficacy and rejection (6, 7). Therefore, the dose of MMF is often adjusted based on the AUC0-12 of MPA.

Corresponding Author: Hiroaki Yamaguchi, Ph.D. Department of Pharmaceutical Sciences, Tohoku University Hospital, 1-1 Seiryo-machi, Aoba-ku, Sendai, Japan. Email: hiroaki.yamaguchi@med.id.yamagata-u.ac.jp Present address: Yamagata University Graduate School of Medical Science/Department of Pharmacy, Yamagata University Hospital, 2-2-2, Iida-nishi, Yamagata, 990-9585, Japan.
Recently, Zegarska et al. have been reported that AcMPAG concentrations in liver transplant recipients is related to the development of bacterial infection (8). In addition, Yoshimura et al. have been reported that the cutoff values of AcMPAG AUC\textsubscript{0-24} for successful gastrointestinal acute graft-versus-host disease prevention in hematopoietic stem cell transplant patients were 15.6 μg·hr/mL (9). Accordingly, therapeutic drug monitoring (TDM) of AcMPAG is considered important in ensuring the safety and effectiveness of MMF treatment in both clinical practice and research.

The continuous measurement of MPA and AcMPAG AUC\textsubscript{0-12} based on multiple blood sampling points increases the patient's burden. For this reason, a limited sampling strategy (LSS) estimating the AUC\textsubscript{0-12} based on a limited number of blood samples is essential for defining the PK profiles of MPA and AcMPAG. However, LSS that simultaneously evaluates the AUC\textsubscript{0-12} of MPA and AcMPAG has not been reported. This study aimed to design a LSS based on multiple linear regression for estimating the AUC\textsubscript{0-12} of MPA and AcMPAG at the minimum blood sampling points in Japanese lung transplant patients with concomitant tacrolimus.

**MATERIALS AND METHODS**

**Patients**

This study was a single-center prospective study, was performed in Tohoku University Hospital from December 2016 to December 2017. The inclusion criteria were as follows: age ≥18 years, after lung transplantation, use of MMF, and the ability and willingness to provide written informed consent. We did not set the exclusion criteria. The chronic diseases that lead to lung transplantation were lymphangioleiomyomatosis (n=16), interstitial pneumonia (n=9), pulmonary hypertension (n=7), bronchiectasis (n=3), Eisenmenger syndrome (n=3), pulmonary emphysema (n=2), diffuse panbronchiolitis (n=2), cystic fibrosis (n=1), and other pulmonary disease (n=2). Forty-five transplant recipients were enrolled in a study investigating the pharmacokinetics of MPA, MPAG, and AcMPAG. This study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine (approval number: 2017-1-096). All patients were provided written informed consent.

**Immunosuppression regimen**

All patients received MMF, tacrolimus, and prednisolone as a basic triple immunosuppressive regimen in lung transplantation. MMF (CellCept\textsuperscript{®}, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) was administered by intubation from the day 2 after surgery, and it was switched to oral administration after tube was withdrawn. MMF was administered at 1000 mg/day (body weight < 60 kg) or 1500 mg/day (body weight ≥ 60 kg). The dose of MMF was adjusted so that the white blood cell count was 4000 or more. The dose was reduced if abdominal symptoms were present. Tacrolimus was adjusted to maintain a target concentration of 10 to 14 ng/mL within 6 months after transplantation, 9 to 13 ng/mL from 7 months to 1 year after transplantation, and 8 to 10 ng/mL afterward. Prednisolone was orally administered at 1 mg/kg/day after transplantation, and then tapered to a fixed maintenance dose of 5 mg/day by 6 months after transplantation.

**Assay of plasma MPA, MPAG, and AcMPAG concentrations**

MPA was purchased from Sigma-Aldrich (Tokyo, Japan), MPAG, AcMPAG, and MPA-\textsuperscript{2}H\textsubscript{3} (internal standard, IS) were from Toronto Research Chemicals (Toronto, Ontario, Canada). The plasma concentrations of MPA, MPAG, and AcMPAG was measured as previously described, with modifications (10, 11). Briefly, blood collected in heparin tubes was centrifuged at 1580 × g for 10 minutes to separate the plasma. To stabilize AcMPAG, 10% acetic acid was added to 20 μL per milliliter of plasma (11). Thirty-microliters of acidified plasma were mixed with 30 μL of 1 μg/mL MPA-\textsuperscript{2}H\textsubscript{3} dissolved in acetonitrile for use as an internal standard substance. In addition, 120 μL of acetonitrile was added to denatured proteins and centrifuged at 15,000 × g for 5 minutes. To prepare a sample for injection, 200 µL of water was added to 100 μL of this supernatant. For LC/ESI-MS/MS analysis, a LCMS-8050 triple quadrupole mass spectrometer coupled with a Nexera X2 UHPLC system (Shimadzu, Kyoto, Japan) was used. Nexera X2 UHPLC consisted of a vacuum degasser, two solvent delivery systems, an autosampler, and a column oven. Chromatographic separation was achieved using an Inertsil C8-3 column (150 × 2.1 mm i.d., 3 μm, GL Sciences, Tokyo, Japan), which was maintained at 40°C. The mobile phase consisted of solution A (0.1% formic acid in water) and solution B (0.1% formic acid in acetonitrile), which formed the following gradient: 45% B (0–2 min); 45–70% B (2–5.4 min); 45% B (5.4–6.5 min). The flow rate of the mobile phase was 0.3 mL/min. The...
LCMS-8050 was equipped with an electrospRAY ionization source operating in positive and negative ion detection mode. During selected reaction monitoring, the m/z transitions 321.25→303.25, 495.15→319.15, 495.15→319.15, and 324.30→306.30 monitored MPA, MPAG, AcMPAG, and MPA→2H3, respectively. Three controls (0.15, 1.5, 15 μg/mL for MPA, 1.2, 12, 120 μg/mL for MPAG, and 0.03, 0.3, 3 μg/mL for AcMPAG, respectively) were used for quality control. Linearity was achieved with a correlation coefficient (R2) > 0.995 (0.1 to 20 µg/mL for MPA, 0.8 to 160 µg/mL for MPAG, and 0.02 to 4 µg/mL for AcMPAG, respectively). The intra- and inter-day precisions were less than 14.2%, and accuracy was ± 11.2%.

Pharmacokinetic analyses and AUC0-12 calculation
The plasma concentrations of MPA, MPAG, and AcMPAG were determined just prior to dosing and 0.5, 1, 2, 4, 8, and 12 hours after administration. The pharmacokinetic parameters and AUC0-12 of MPA, MPAG, and AcMPAG were analyzed by a non-compartmental model from the plasma concentration of each blood sampling time (Phoenix WinNonlin software Version 7.0 [Certara USA, Inc., Princeton, NJ, USA]).

STATISTICAL ANALYSIS
According to the previous report (12-14), the patients were randomly assigned to two groups at a 1:1 ratio: the model group (n = 23) and the validation group (n = 22). An AUC0-12 prediction formula was derived using multiple regression analysis with the model group AUC0-12 as a dependent variable and the plasma concentration at each blood sampling time as an explanatory variable. A maximum of three concentrations were used for the clinically convenient limited sampling strategy. In the validation group, the predictive performance of the LSS was analyzed with linear regression, correlation coefficient (r), prediction error (PE) (%), absolute prediction error (APE) (%), and the percentage of estimated AUC within ± 15% of the observed AUC as previous reported (15). The two error parameters were calculated using the following equations:

\[ PE(\%) = \frac{100}{n} \cdot \frac{\sum(AUC_{predicted} - AUC_{observed})}{AUC_{observed}} \]

\[ APE(\%) = \frac{100}{n} \cdot \frac{\sum|AUC_{predicted} - AUC_{observed}|}{AUC_{observed}} \]

where n is the number of patients. According to the previous report (15-17), the acceptable percentage limits of PE and APE were defined to be ± 5% and 15%, respectively. The Bland–Altman test was used to evaluate the agreement between the observed and estimated AUC, and the fixed range was defined as the mean ± 1.96SD. Continuous variables (expressed as mean ± SD) were compared using the t test, and categorical variables were compared using the Fisher's exact test. The significance level was set at P < 0.05. We used SAS Version 9.4 (SAS Institute Inc., Cary, NC, USA) for statistical analysis.

RESULTS
Forty-five patients (18 males and 27 females) were included in this study and the patients were randomly assigned to two groups at a 1:1 ratio: the model group (n = 23) and the validation group (n = 22). Patient characteristics are summarized in Table 1. The mean age was 44.4 ± 11.6 years and the mean body weight was 51.5 ± 11.6 kg. There were no significant differences in sex, laboratory test results, concomitant medications affecting the pharmacokinetics of MPA (proton pump inhibitor, magnesium oxide, ciprofloxacin), MMF dose, pharmacokinetic parameters of MPA and metabolites, tacrolimus dose, or the post-transplant period between the model group and the validation group.

The mean plasma concentration-time profiles of MPA, MPAG, and AcMPAG in the model and validation groups are shown in Figure 1. These curves followed a similar tendency, whereby the peak was reached after 2 hours for MPA and 4 hours for MPAG and AcMPAG. Among these compounds, MPA and AcMPAG are related to clinical outcomes; therefore, we focused on both MPA and AcMPAG.
Table 1. Baseline characteristics of patients

|                        | All Patients (n = 45) | Model Group (n = 23) | Validation Group (n = 22) | P      |
|------------------------|-----------------------|----------------------|---------------------------|--------|
| Age (yr)               | 44.4 ± 11.6           | 45.5 ± 11.6          | 43.1 ± 11.8               | 0.497  |
| Sex (Male/Female)      | 18/27                 | 8/15                 | 10/12                     | 0.236  |
| Body weight (kg)       | 51.5 ± 11.6           | 53.2 ± 12.6          | 49.6 ± 10.3               | 0.298  |
| Serum creatinine (mg/dL)| 1.01 ± 0.34           | 1.03 ± 0.40          | 0.99 ± 0.27               | 0.684  |
| Blood urea nitrogen (mg/dL) | 19.4 ± 7.0        | 19.3 ± 7.8           | 19.5 ± 6.2                | 0.927  |
| Estimated glomerular filtration rate (mL/min/1.73 m²) | 60.9 ± 23.2       | 58.5 ± 20.5          | 63.4 ± 26.0               | 0.487  |
| γ-Glutamyltransferase (U/L) | 31.3 ± 18.3         | 28.9 ± 21.8          | 33.8 ± 13.8               | 0.371  |
| Alanine aminotransferase (U/L) | 12.9 ± 7.1         | 11.3 ± 5.0           | 14.6 ± 8.6                | 0.128  |
| Aspartate aminotransferase (U/L) | 16.6 ± 4.4         | 15.7 ± 2.9           | 17.6 ± 5.4                | 0.137  |
| Total protein (mg/dL)  | 6.29 ± 0.51           | 6.27 ± 0.55          | 6.31 ± 0.48               | 0.779  |
| Serum albumin (mg/dL)  | 3.96 ± 0.36           | 3.97 ± 0.39          | 3.95 ± 0.33               | 0.891  |
| White blood cell count (×10^3/μL) | 6.38 ± 2.14       | 6.23 ± 2.17          | 6.54 ± 2.16               | 0.633  |
| Neutrophil count (×10^3/μL) | 4.30 ± 1.86          | 4.16 ± 1.89          | 4.45 ± 1.86               | 0.602  |
| Concomitant medications |                       |                      |                           |        |
| Proton pump inhibitor   | 43                    | 22                   | 21                        | 1.000  |
| Magnesium oxide         | 11                    | 5                    | 6                         | 1.000  |
| Ciprofloxacin           | 1                     | 1                    | 0                         | 1.000  |
| MMF dose (range) (mg/day) | 789 ± 402            | 826 ± 415            | 750 ± 393                 | 0.532  |
| MPA AUC_{0-12} (μg·hr/mL) | 32.2 ± 11.8          | 32.8 ± 13.0          | 31.5 ± 10.6               | 0.713  |
| Dose-normalized MPA AUC_{0-12} [((μg·hr/mL)/g)] | 51.8 ± 35.6       | 43.1 ± 13.4          | 60.9 ± 48.5               | 0.098  |
| MPA T_{max} (hr)        | 2.53 ± 2.00           | 2.09 ± 1.90          | 3.00 ± 2.05               | 0.128  |
| MPA C_{max} (μg/mL)     | 6.98 ± 3.50           | 7.03 ± 3.76          | 6.94 ± 3.29               | 0.931  |
| MPAG AUC_{0-12} (μg·hr/mL) | 404 ± 288            | 457 ± 349            | 350 ± 199                 | 0.210  |
| Dose-normalized MPAG AUC_{0-12} [((μg·hr/mL)/g)] | 617 ± 516             | 556 ± 259            | 681 ± 692                 | 0.423  |
| MPAG T_{max} (hr)       | 3.53 ± 1.87           | 3.13 ± 1.29          | 3.95 ± 2.28               | 0.147  |
| MPAG C_{max} (μg/mL)    | 49.7 ± 34.0           | 53.4 ± 39.3          | 45.9 ± 27.8               | 0.469  |
| AcMPAG AUC_{0-12} (μg·hr/mL) | 3.23 ± 2.60       | 3.32 ± 2.56          | 3.14 ± 2.69               | 0.819  |
| Dose-normalized AcMPAG AUC_{0-12} [((μg·hr/mL)/g)] | 5.78 ± 8.41        | 4.39 ± 3.34          | 7.23 ± 11.50              | 0.262  |
| AcMPAG T_{max} (hr)     | 3.41 ± 2.21           | 3.11 ± 1.66          | 3.73 ± 2.68               | 0.355  |
| AcMPAG C_{max} (μg/mL)  | 0.55 ± 0.39           | 0.55 ± 0.39          | 0.54 ± 0.41               | 0.987  |
| Tacrolimus dose (mg/day)| 2.81 ± 1.74           | 3.04 ± 1.63          | 2.56 ± 1.85               | 0.356  |
| Tacrolimus trough concentration (ng/mL) | 9.12 ± 2.07       | 9.41 ± 2.35          | 8.82 ± 1.73               | 0.345  |
| Post-transplant period (day) | 1460 ± 934          | 1404 ± 983           | 1518 ± 899                | 0.686  |

Continuous variables (expressed as mean ± SD) were compared using the t test, and categorical variables were compared using the Fisher's exact test. MMF: mycophenolate mofetil, MPA: mycophenolic acid, MPAG: mycophenolic acid glucuronide, AcMPAG: mycophenolic acid acyl glucuronide.
Multiple linear regression analyses of the MPA and AcMPAG AUC\textsubscript{0-12} are shown in Table 2. The highest correlation coefficient between MPA AUC\textsubscript{0-12} and plasma MPA concentrations was at C\textsubscript{1}, C\textsubscript{4}, and C\textsubscript{8} for three time points (MPA AUC\textsubscript{0-12} = 4.04 + 1.64·C\textsubscript{1} + 3.08·C\textsubscript{4} + 5.17·C\textsubscript{8}, r = 0.923, P < 0.001).

Conversely, the highest correlation coefficient between AcMPAG AUC\textsubscript{0-12} and plasma AcMPAG concentrations was obtained at three time points, C\textsubscript{2}, C\textsubscript{4}, and C\textsubscript{12} (AcMPAG AUC\textsubscript{0-12} = 0.28 + 1.96·C\textsubscript{2} + 3.44·C\textsubscript{4} + 4.64·C\textsubscript{12}, r = 0.990, P < 0.001).

**Figure 1.** Mycophenolic acid (MPA) (A), mycophenolic acid glucuronide (MPAG) (B), and mycophenolic acid acyl glucuronide (AcMPAG) (C) concentration-time profiles.
Considering the combination of the three time-point models, it was two models (C₁, C₄, C₈ and C₂, C₄, C₈) that PE and APE were clinically acceptable within ±5% and 15%, respectively.

Moreover, the percentage of estimated AUC₀-₁₂ within ±15% of the observed AUC₀-₁₂ was higher in model (C₁, C₄, C₈) than in model (C₂, C₄, C₈). Therefore, the LSS of MPA and AcMPAG with the most convenient and conventional sampling times were for C₁, C₄, and C₈ (MPA AUC₀-₁₂ = 4.04 + 1.64·C₁ + 3.08·C₄ + 5.17·C₈, AcMPAG AUC₀-₁₂ = -0.13 + 3.01·C₁ + 3.51·C₄ + 5.74·C₈). In the
validation group, the PE and APE of this models were within the clinically acceptable range (MPA: PE = 2.00%, APE = 11.66%, AcMPAG: PE = 0.98%, APE = 14.69%). The percentage of estimated AUC within ± 15% of the observed AUC was 77.27% for MPA and 81.82% for AcMPAG. The correlation between the measured and estimated AUC of MPA and AcMPAG for LSS in Figure 2 also suggested a good estimation (MPA: $r = 0.923$, $P < 0.001$, AcMPAG: $r = 0.955$, $P < 0.001$). The Bland–Altman analysis revealed a mean bias of 2.00% for MPA AUC and 0.98% for AcMPAG. Only three plotted differences exceeded the fixed range of the mean ± 1.96 SD in the estimation using $C_1$, $C_4$, and $C_8$ (Figure 3).

DISCUSSION

In this study, we showed that the AUC of MPA and AcMPAG using three sampling points ($C_1$, $C_4$, and $C_8$) for limited sampling strategies (MPA AUC$_{0-12}$ = 4.04 + 1.64 $C_1$ + 3.08 $C_4$ + 5.17 $C_8$, AcMPAG AUC$_{0-12}$ = -0.13 + 3.01 $C_1$ + 3.51 $C_4$ + 5.74 $C_8$). The line represents the mean bias and the dashed lines represent ± 1.96 standard deviation (SD) of the mean bias.

When the trough concentration was included at blood sampling points within 4 hours, the blood sampling points with the greatest correlations were ($C_0$, $C_2$, $C_4$) (MPA AUC$_{0-12}$ = 4.91 + 3.06 $C_0$ + 2.03 $C_2$ + 3.39 $C_4$, $r = 0.820$, $P < 0.001$, AcMPAG AUC$_{0-12}$ = -0.12 + 5.84 $C_0$ + 2.76 $C_2$ + 3.60 $C_4$, $r = 0.955$, $P < 0.001$). The PE of this models was within the clinically acceptable range (MPA: 2.43%, AcMPAG: 0.98%), but the APE was exceeded 15% (MPA: 16.32%, AcMPAG: 19.87%).
patients.

Both immunoassays and chromatographic methods are available for therapeutic drug monitoring of MPA. Although immunoassays are widely used in clinical laboratories due to ease of adopting such methods on automated analyzers, immunoassay such as a particle-enhanced turbidimetric inhibition immunoassay (PETINIA) shows cross-reactivity with AcMPAG. In fact, we have revealed an average positive bias of 26.3% in the PETINIA compared to that with LC-MS/MS in lung transplant patients (18). Furthermore, MPAG and AcMPAG can only be measured by LC-MS/MS. Therefore, plasma MPA, MPAG, and AcMPAG concentrations were quantified by LC-MS/MS in this study.

The pharmacokinetics of MPA and its metabolites are reported to be affected by the administration of concomitant drugs, and differ between lung and heart transplant patients (19-23). Therefore, the development of the LSS of MPA and AcMPAG should consider concomitant medication as well as the transplanted organ. Proton pump inhibitor or magnesium oxide reduces the solubility of MMF and decreases the drug exposure of mycophenolic acid (24, 25). In addition, ciprofloxacin reduces plasma MPA concentration because of noncompetitive inhibition of deconjugation of MPAG by intestinal β-glucuronidase (26). However, there were no significant differences in baseline characteristics of patients including concomitant drugs between the model group and the validation group. The mean MPA, MPAG, and AcMPAG concentration–time profiles of all patients, the model group, and the validation group followed the same trend.

The primary enzymes involved in MPA glucuronidation are uridine-diphosphate glucuronosyltransferase (UGT) 1A9, 2B7, 1A8, and 1A7 (27). In addition, UGT1A9 -275T>A and -2152C>T polymorphism that affect the pharmacokinetics of MPA have been reported in Caucasians (28). Nevertheless, maximum MPA concentration, time to reach the maximum MPA concentration, AUC ratio MPAG/MPA, and AUC ratio AcMPAG/MPA were similar to those previously reported (29).

Considering the correlation coefficient, PE, APE, and the percentage of estimated AUC within ±15% of the observed AUC, the most convenient and conventional sampling times were obtained with the three time-point (C1, C4, and C8) equations. Therefore, the three time-point (C1, C4, and C8) equations were developed (MPA AUC0-12 = 4.04 + 1.64·C1 + 3.08·C4 + 5.17·C8, r = 0.923, P < 0.001, AcMPAG AUC0-12 = -0.13 + 3.01·C1 + 3.51·C4 + 5.74·C8, r = 0.955, P < 0.001). Compared to previous reports (30, 31), the three time-point equations developed in this study have a relatively better predictive performance (MPA: PE = 2.00%, APE = 11.66%, AcMPAG: PE = 0.98%, APE = 14.69%). Moreover, about 80% of the estimated AUC was within ±15% of the observed AUC.

LSS in lung transplantation patients was previously reported by Ting et al (12). Those authors developed LSS by two time points (C0 and C2) in the same number of patients for cyclosporine and tacrolimus in combination; however, the correlation coefficient of the two time points (C0 and C2) was 0.699 in this study, and a good correlation was not observed (data not shown). Cyclosporine inhibits the biliary transporter ATP-binding cassette, subfamily C, member 2 (ABCC2), resulting in reduced enterohepatic re-circulation of MPAG/MPA (18-20), but tacrolimus has no such effect. For this reason, pharmacokinetics of MPA, MPAG, and AcMPAG are significantly different between cyclosporine and tacrolimus (29). All patients received tacrolimus, suggesting that the influence of C8 or C12 derived from the enterohepatic re-circulation of MPAG/MPA was greater than that reported by Ting et al (2006).

Tacrolimus is used more commonly after lung transplantation due to its effect at reducing the risk of bronchiolitis obliterans syndrome and low levels of rejection as well as control of persistent rejection (32, 33). Therefore, the formula used here to estimate AUC0-12 may be useful to adjust the dose of MMF in patients treated with tacrolimus after lung transplantation.

Blood sampling 8 hours after MMF administration is not practical because it restrains outpatient's activities for a long time. The same multiple regression method was performed with the variables within 2 hours post-dose (C0, C0.5, C1, and C2), but the correlation coefficient of the three time points was not sufficient (data not shown). When the trough concentration is included at blood sampling points within 4 hours, there was a good correlation with the three time-point equation (C0, C2, and C4) (MPA: r = 0.820, P < 0.001, AcMPAG: r = 0.969, P < 0.001). However, the APE exceeded 15%, and should therefore be carefully evaluated. Recently, we developed a LC-MS/MS method for the quantification of MPA, MPAG, and AcMPAG in dried blood spot samples (10). The dried blood spot method makes it possible to collect blood sample 8 hours after MMF administration without restraining
the outpatients. Therefore, application of the dried blood spot method to outpatients may permit TDM using three-point blood sampling (C₁, C₄, and C₈).

This study has some limitations that should be considered. All cases were Japanese lung transplant patients taking tacrolimus, and it is not evaluated by other races. In addition, no collected datapoint between 4 and 8 hours after MMF intake potentially related to the enterohepatic re-circulation of MPAG/MPA was not collected. Moreover, the PK profiles were obtained at approximately 4 years post-transplant, and genetic polymorphism for MPA and AcMPAG metabolism were not analyzed. We also need to consider using population PK modeling to make AUC estimation based on limited sampling more robust and enhance its prediction performance.

CONCLUSION

We established a formula to estimate the AUC₀₋₁₂ of MPA and AcMPAG by LSS in Japanese lung transplant patients with concomitant tacrolimus. The best three time-point equation was

\[4.04 + 1.64 \cdot C₁ + 3.08 \cdot C₄ + 5.17 \cdot C₈\]

for MPA, and

\[-0.13 + 3.01 \cdot C₁ + 3.51 \cdot C₄ + 5.74 \cdot C₈\]

for AcMPAG. It could be a useful tool to utilized for clinical practice and research.

CONFLICT OF INTEREST

No conflicts of interest to disclose.

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ABBREVIATIONS

MMF: mycophenolate mofetil
MPA: mycophenolic acid
MPAG: mycophenolic acid glucuronide
AcMPAG: mycophenolic acid acyl glucuronide
AUC₀₋₁₂: 12-hour area under the concentration-time curve
TDM: therapeutic drug monitoring
LSS: limited sampling strategy
PE: prediction error
APE: absolute prediction error
UGT: uridine-diphosphate glucuronosyltransferase

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