Limited Sampling Strategy for Mycophenolic Acid in Japanese Heart Transplant Recipients

— Comparison of Cyclosporin and Tacrolimus Treatment —

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Background The purpose of the study was to characterize the pharmacokinetics of mycophenolic acid (MPA) in Japanese heart transplant recipients and to find the time point that has the best correlation with the MPA area under the plasma concentration curve (AUC).

Methods and Results Twenty-two Japanese recipients treated with mycophenolate mofetil were evaluated in the study. Approximately 9 months after transplantation, the area under the MPA serum concentration-time curve from 0 to 12 h (AUC_{0–12h}) was evaluated. The MPA AUC_{0–12h} values in the cyclosporine (CsA) and tacrolimus (FK) groups ranged from 13.11 to 50.98 µg·h/ml and from 39.19 to 93.18 µg·h/ml, respectively. Fourteen models were developed and analyzed for their ability to estimate the MPA AUC_{0–12h} based on a limited number of samples in the CsA group. Sixteen models were developed in the FK group. The best model for predicting the full MPA AUC_{0–12h} in the CsA group was a 3-time-point model that included C_{0h}, C_{1h} and C_{2h} (r², 0.96; mean prediction error, 0.15±7.85%); a 2-time-point model that included C_{0h} and C_{2h} (r², 0.94; mean prediction error, 0.495±10.35%) was also reliable. In the FK group, a 3-time-point model that included C_{1h}, C_{2h} and C_{4h} (r², 0.73; mean prediction error, 2.73±17.09%) was the best model for predicting the full MPA AUC_{0–12h}, but it was not reliable in clinical practice.

Conclusion A 3- (C_{0h}, C_{1h} and C_{2h}) and a 2-time-point model (C_{0h} and C_{2h}) are useful for predicting the full MPA AUC_{0–12h} in Japanese heart transplant recipients treated with CsA but not with FK. (Circ J 2007; 71: 1022–1028)

Key Words: Heart; Mycophenolate mofetil; Pharmacokinetics; Transplantation

Mycophenolate mofetil (MMF) is widely used for the prevention of acute rejection after organ transplantation^{1–3}. Following oral administration, MMF is rapidly and extensively absorbed and is hydrolyzed to mycophenolic acid (MPA), the active immunosuppressive agent^{4–7}. Pharmacokinetic studies of MPA in transplant patients showed a large variability in pharmacokinetic parameters including the area under the plasma concentration curve (AUC), time to peak plasma concentration (t_{max}) and maximal plasma concentration (C_{max})^{8–11}. Several studies showed a significant relationship between the MPA AUC and the occurrence of acute rejection^{6–8,12–14}. A low AUC in the early post-operative period is associated with a high incidence of rejection during the first 6 months¹². Recent reports suggest that a target of 30–60 µg·h/ml might be suitable during both the early post-transplant period and

maintenance therapy in heart transplant recipients^{7,11,14–16}. However, the routine measurement of AUC in clinical practice is very impractical and would be cost prohibitive; therefore, development of an abbreviated sampling strategy for reliable estimation of the MPA AUC is required.

The pharmacokinetics of MPA in Japanese heart transplant recipients has not been previously characterized. The purpose of this study was to characterize the pharmacokinetics of MPA in Japanese heart transplant recipients receiving concomitant cyclosporine (CsA) or tacrolimus (FK), and to find the time point that correlates best with the MPA AUC.

Methods

Patients

Twenty-two Japanese recipients were enrolled in the present study. The individuals underwent heart transplantation at the National Cardiovascular Center (13 recipients), Japan and the overseas hospital, University of California, Los Angeles, CA (9 recipients), USA between May 1999 and November 2006. The participants consisted of 21 recipients with dilated cardiomyopathy and 1 recipient in the dilated phase of hypertrophic cardiomyopathy. They were treated with MMF in addition to corticosteroids and CsA or FK as immunosuppressants. The pharmacokinetics and clinical efficacy of MPA were evaluated approximately 9

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Table 1 Characteristics of Study Patients

	Cyclosporine group	Tacrolimus group	p value
Number of patients	11	11	
Gender (M/F)	10/1	6/5	
Age (years)*	39.1±13.1 (15–62)	29.5±13.5 (9–55)	0.10
Weight (kg)*	55.0±6.5 (45.7–67.25)	49.6±8.5 (33.45–63.55)	0.11
Post-transplant time (days)*	273.7±12.1 (249–290)	267±23.9 (227–305)	0.42
Creatinine (mg/dl)*	0.97±0.26 (0.55–1.42)	0.76±0.21 (0.5–1.16)	0.091
Dose of immunosuppressants			
Cyclosporine (mg·kg ⁻¹ ·day ⁻¹)*	4.34±1.30 (2.60–6.54)		
Tacrolimus (mg·kg ⁻¹ ·day ⁻¹)*		0.095±0.058 (0.04–0.22)	
Prednisolone (mg)*	8.9±5.2 (2.5–20.0)	6.5±1.6 (5.0–10.0)	0.16

*Mean ± SD (range).

months after heart transplantation. There were no recipients with severe gastrointestinal disorders. All research procedures were conducted according to the clinical research guidelines in our institute. All patients gave written informed consent for disclosure of their clinical data.

Medications and Therapeutic Drug Monitoring

The dose of CsA or FK was applied according to the original protocol of National Cardiovascular Center!⁷ CsA (Neoral, Novartis Pharma K.K., Tokyo, Japan) was initially administered at a dose of 6mg·kg⁻¹·day⁻¹ in 2 divided doses. Thereafter, doses of CsA were adjusted to achieve trough levels of 350 to 450ng/ml during the first month, 250 to 350ng/ml at 2–3 months, 200 to 300ng/ml at 4–12 months and 100 to 250ng/ml for 13 or more months after transplantation. FK (Prograf, Astellas Pharma K.K., Tokyo, Japan) was initially administered at a dose of 0.05 mg·kg⁻¹·day⁻¹ in 2 divided doses. Thereafter, FK was administered to achieve a target trough blood level of 13 to 15ng/ml during the first 3 months, 10 to 15ng/ml at 4–5 months and 5 to 10ng/ml for 6 or more months after transplantation. In addition, the doses of CsA and FK were adjusted based on the AUC during hospital admission for periodic biopsy. Plasma concentrations of both CsA and FK were measured by fluorescence polarization immunoassay (TDx, Abott Japan Co, LTDA).

MMF (Cellcept, Chugai Pharma K.K., Tokyo, Japan) was initially administered at a dose of 1 g in 2 divided doses and then a 2 to 3 g maintenance dose in accordance with the leukocyte count. Subsequent doses of MMF were based on the AUC of MPA and were adjusted during admission for scheduled biopsy.

The areas under the serum concentration time curve from 0 to 12h (AUC_{0–12h}) of MPA approximately 9 months after transplantation were evaluated. We collected blood samples before dosing and at 1, 2, 4, 6, and 12h after dosing from recipients taking MMF and CsA concomitantly. In addition, blood samples were obtained before dosing and at 1, 2, 4, 6, 8 and 12h after dosing from recipients taking MMF and FK concomitantly. Approximately 1 ml of blood was collected each time from an arm vein by a disposable syringe and was transferred to a vacuum blood-collection tube. The samples were centrifuged, and harvested serum was frozen at –30° until analysis.

Measurement of Plasma MPA Concentration

Serum concentrations of MPA were measured by reverse-phase high performance liquid chromatography with the use of a minor modification of the method of Tsina et al¹⁸ with indomethacin as an internal standard. In brief, in

this modified method, an octadecylsilane, C18 column (250×46mm inside diameter, SHIMADZU, Kyoto, Japan) was used, and the absorbance of the effluent from the column was measured at 254nm. The mobile phase consisted of a mixture of acetonitrile and 0.05 mol/L phosphate solution (vol/vol, 60:40). With this method, the detectable concentration of MPA was 50ng/ml, and the inter-day and intra-day coefficients of variation were less than 5%.

Pharmacokinetics Data Analysis

The MPA AUC_{0–12h} values were calculated by trapezoidal approximation. The time to maximum concentration (t_{max}) and maximum concentration (C_{max}) were derived directly from the measured values. Non-compartmental analysis was used to determine the mean residence time (MRT).

Limited Sampling Strategy Development

We searched for predictive models of the MPA AUC_{0–12h} using a multiple regression analysis based on a limited number of samples. These analyses produced equations of the form: AUC = $\alpha_1 C_1 + \dots + \alpha_n C_n + \beta$, where α_n and β are coefficients and n is the number of samples (n≤3). Data were analyzed with Statcel 2 (Excel, Visual Basic for Applications for Windows). The final limited-sampling strategy models were used to calculate the prediction error (%) in each patient using the following equation: ((estimated AUC – measured AUC)/measured AUC)×100. The variance inflation factor was calculated to check for collinearity of the models.

Results

Patient Characteristics

The characteristics of the study recipients are presented in Table 1. A total of 22 recipients were evaluated (16 male and 6 female; mean age±SD, 39.1±13.1 years (CsA group), 29.5±13.5 years (FK group); mean weight±SD, 55.0±6.5 kg (CsA group), 49.6±8.5 kg (FK group); mean post-transplant time±SD, 273.7±12.1 days (CsA group), 267±23.9 (FK group); mean serum creatinine±SD, 0.97±0.26 mg/dl (CsA group), 0.76±0.21 (FK group)). One patient had high serum creatinine in the CsA group. The mean CsA and FK dose was 4.34±1.30 mg·kg⁻¹·day⁻¹ and 0.095±0.058 mg·kg⁻¹·day⁻¹, respectively. The mean prednisolone dose in the CsA and FK groups was 8.9±5.2 mg/day and 6.5±1.6 mg/day, respectively.

MPA Pharmacokinetics in CsA- and FK-Treated Heart Transplant Recipients

Serum concentration-time profiles of MPA in the CsA

Fig 1. Mycophenolic acid (MPA) plasma concentration-time profile of Japanese heart transplant patients for tacrolimus (FK) group (Top) and cyclosporine (CsA) group (Bottom).

Table 2 MPA Pharmacokinetic Parameters in Heart Transplantation Patients (Cyclosporine Group and Tacrolimus Group)

	Cyclosporine group	Tacrolimus group	p value
MMF dose ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$)	25.42 ± 14.17 (9.29–58.82)	34.01 ± 14.95 (14.95–60.42)	0.18
t_{max} (h)	1.73 ± 0.90 (1–4)	2.18 ± 1.54 (1–6)	0.41
C_{max} ($\mu\text{g}/\text{ml}$)	8.82 ± 4.10 (2.78–15.90)	14.23 ± 7.43 (6.75–30.80)	0.04
MRT(h)	3.76 ± 0.77 (2.56–5.54)	4.75 ± 0.93 (3.57–6.40)	0.01
$\text{AUC}_{0-12\text{h}}$ ($\mu\text{g} \cdot \text{h}/\text{ml}$)	32.57 ± 13.07 (13.11–50.98)	58.55 ± 17.51 (39.19–93.18)	<0.01

Mean $\pm$ SD (range).

MPA, mycophenolic acid; MMF, mycophenolate mofetil; t_{max} , time to maximum concentration; C_{max} , maximum concentration; MRT, mean residence time; AUC, area under the concentration-time curve.

and FK groups are depicted in Fig 1. The pharmacokinetic profiles of MPA were characterized by an early increase of MPA concentration with the first peak concentration reached at approximately 1 to 2 h after dosing. In the FK group, a secondary plasma peak of MPA occurred 8 h after administration. The pharmacokinetic parameters of MPA in the CsA and FK groups are presented in Table 2. The mean MMF dose in the CsA and FK groups was $25.42 \pm 14.17 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ and $34.01 \pm 14.95 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$, respectively. There was no difference in the mean dose of MMF between the CsA and FK groups. The MPA $\text{AUC}_{0-12\text{h}}$ values in the CsA and FK groups ranged from 13.11 to $50.98 \mu\text{g} \cdot \text{h}/\text{ml}$ (mean $\pm$ SD, $32.57 \pm 13.07 \mu\text{g} \cdot \text{h}/\text{ml}$) and from 39.19 to $93.18 \mu\text{g} \cdot \text{h}/\text{ml}$ (mean $\pm$ SD, $58.55 \pm 17.51 \mu\text{g} \cdot \text{h}/\text{ml}$), respectively. The mean MPA $\text{AUC}_{0-12\text{h}}$ in the FK group was significantly higher than in the CsA group ($p < 0.01$). The correlations between the dose of MMF per body weight and $\text{AUC}_{0-12\text{h}}$ are shown in Fig 2. Signifi-

cant correlations between the dose of MMF per body weight and $\text{AUC}_{0-12\text{h}}$ of MPA were observed in the both the CsA and FK groups. The mean t_{max} in the CsA and FK groups was $1.73 \pm 0.90 \text{ h}$ (1–4 h), $2.18 \pm 1.54 \text{ h}$ (1–6 h), respectively. The coefficient of variation of t_{max} in the CsA and FK groups was 52.4% and 70.5%, respectively. The mean MPA C_{max} in the CsA and FK groups was $8.82 \pm 4.10 \mu\text{g}/\text{ml}$ and $14.23 \pm 7.43 \mu\text{g}/\text{ml}$, respectively. The mean MPA MRT in the CsA and FK groups was $3.76 \pm 0.77 \text{ h}$ and $4.75 \pm 0.93 \text{ h}$, respectively. The mean MPA MRT in the FK group was significantly longer than in the CsA group ($p = 0.01$). In the CsA group, there were 5 recipients who had an MPA $\text{AUC}_{0-12\text{h}} < 30 \mu\text{g} \cdot \text{h}/\text{ml}$, but no recipient experienced International Society for Heart & Lung Transplantation (ISHLT) Grade III rejection. In the FK group, there was no recipient who had an MPA $\text{AUC}_{0-12\text{h}} < 30 \mu\text{g} \cdot \text{h}/\text{ml}$, and no recipient experienced ISHLT Grade III rejection. In the FK group, there were 5 recipients who had an MPA

Fig 2. Relationship between mycophenolate mofetil (MMF) dose·kg⁻¹·day⁻¹ and mycophenolic acid (MPA) AUC_{0-12h} for tacrolimus (FK) group (closed circle) and cyclosporine (CsA) group (open circle).

Table 3 Multiple Regression Analysis to Correlate Abbreviated MPA AUC Values With AUC Values Calculated Using the Full Set of 12 Timed MPA Concentrations (CsA Group)

Model	Sampling times (h)	Model equation	r ²	Prediction error (%)				VIF						
				Mean±SD	Within±15%	<-15%	>15%	C _{0h}	C _{1h}	C _{2h}	C _{4h}	C _{6h}	C _{12h}	
1	0	8.73C _{0h} +25.11	0.29	15.77±48.21	1 (9.1)	5 (45.5)	5 (45.5)							
2	1	1.40C _{1h} +23.14	0.36	14.27±44.60	5 (45.5)	2 (18.2)	4 (36.3)							
3	2	2.67C _{2h} +13.38	0.46	11.30±35.83	10 (90.9)	0 (0)	1 (9.1)							
4	4	4.00C _{4h} +21.39	0.28	31.57±6.97	9 (81.8)	1 (9.1)	1 (9.1)							
5	6	8.18C _{6h} +20.24	0.43	12.94±39.31	3 (27.3)	3 (27.3)	5 (45.5)							
6	12	13.64C _{12h} +18.93	0.54	11.90±42.13	4 (36.3)	3 (27.3)	4 (36.3)							
7	0,1	15.34+8.92C _{0h} +1.43C _{1h}	0.66	7.53±27.91	5 (45.5)	2 (18.1)	4 (36.3)	1.00	1.00					
8	0,2	-0.51+11.47C _{0h} +3.24C _{2h}	0.94	0.49±10.35	10 (90.9)	0 (0)	1 (9.1)	1.04		1.04				
9	0,4	21.31+5.36C _{0h} +2.39C _{4h}	0.35	14.42±42.57	10 (90.9)	0 (0)	1 (9.1)	1.74			1.74			
10	0,6	19.27-4.90C _{0h} +11.60C _{6h}	0.44	12.68±40.79	3 (27.3)	3 (27.3)	5 (45.5)	5.55				5.55		
11	0,12	18.80-2.38C _{0h} +15.81C _{12h}	0.55	12.02±43.02	5 (45.5)	3 (27.3)	3 (27.3)	2.78						2.78
12	0,1,2	0.10+11.15C _{0h} +0.42C _{1h} +2.80C _{2h}	0.96	0.15±7.85	11 (100)	0 (0)	0 (0)	1.68	1.06	1.61				
13	0,2,4	-0.23+12.70C _{0h} +3.36C _{2h} -0.80C _{4h}	0.94	0.36±9.80	10 (90.9)	0 (0)	1 (9.1)	1.23		2.04	2.08			
14	1,2,4	1.28+1.91C _{1h} +0.26C _{2h} +5.91C _{4h}	0.95	0.77±7.94	10 (90.9)	0 (0)	1 (9.1)		2.21	2.02	1.43			

CsA, cyclosporine; VIF, variance inflation factor. Other abbreviations see in Table 2.

Table 4 Multiple Regression Analysis to Correlate Abbreviated MPA AUC Values With AUC Values Calculated Using the Full Set of 12 Timed MPA Concentrations (FK Group)

Model	Sampling times (h)	Model equation	r ²	Prediction error (%)				VIF						
				Mean±SD	Within±15%	<-15%	>15%	C _{0h}	C _{1h}	C _{2h}	C _{4h}	C _{6h}	C _{8h}	C _{12h}
1	0	1.53C _{0h} +55.48	0.01	7.96±30.17	6 (54.5)	3 (27.3)	2 (18.2)							
2	1	-0.23C _{1h} +60.75	0.01	8.03±30.64	3 (27.3)	3 (27.3)	5 (45.5)							
3	2	1.66C _{2h} +41.16	0.65	3.50±20.06	6 (54.5)	2 (18.2)	3 (27.3)							
4	4	2.97C _{4h} +44.54	0.32	5.84±26.97	5 (45.5)	2 (18.2)	4 (36.3)							
5	6	3.03C _{6h} +49.93	0.10	7.03±27.03	4 (36.3)	3 (27.3)	4 (36.3)							
6	8	5.90C _{8h} +36.99	0.22	5.71±22.48	6 (54.5)	1 (9.1)	4 (36.3)							
7	12	1.57C _{12h} +53.61	0.04	7.57±28.68	3 (27.3)	3 (27.3)	5 (45.5)							
8	0,1	57.83+1.14C _{0h} -0.17C _{1h}	0.01	7.97±30.42	3 (27.3)	3 (27.3)	5 (45.5)	1.16	1.16					
9	0,2	34.87+2.95C _{0h} +1.69C _{2h}	0.68	3.26±19.57	6 (54.5)	2 (18.2)	3 (27.3)	1.01		1.01				
10	0,4	47.63-2.01C _{0h} +3.17C _{4h}	0.33	5.80±26.71	4 (36.3)	3 (27.3)	4 (36.3)	1.13			1.13			
11	0,6	48.52+0.79C _{0h} +2.97C _{6h}	0.10	7.01±26.99	4 (36.3)	3 (27.3)	4 (36.3)	1.02				1.02		
12	0,8	39.36-2.93C _{0h} +6.86C _{8h}	0.24	5.48±21.92	6 (54.5)	1 (9.1)	4 (36.3)	1.27					1.27	
13	0,12	50.41+1.58C _{0h} +1.581C _{12h}	0.05	7.49±28.42	3 (27.3)	3 (27.3)	5 (45.5)	1.00						1.00
14	0,1,2	26.93+4.17C _{0h} +0.49C _{1h} +1.77C _{2h}	0.70	2.97±18.28	7 (63.6)	1 (9.1)	3 (27.3)	1.08	1.20	1.24				
15	0,2,4	35.03+2.39C _{0h} +1.59C _{2h} +0.43C _{4h}	0.68	3.24±19.48	6 (54.5)	2 (18.2)	3 (27.3)	1.688		1.882	1.3			
16	1,2,4	23.56+1.05C _{1h} +1.25C _{2h} +2.53C _{4h}	0.73	2.73±17.09	6 (54.5)	1 (9.1)	4 (36.3)		2.21	1.645	3.105			

FK, tacrolimus. Other abbreviations see in Tables 2,3.

AUC_{0-12h} > 60 μg·h/ml, and in the CsA group, there was no recipient who had an MPA AUC_{0-12h} > 60 μg·h/ml. No recipient had severe side effects in either group.

Limited Sampling Strategy

The correlations between MPA concentrations at various time points and the full MPA AUC_{0-12h} values and prediction errors for the abbreviated AUC_{0-12h} profiles in the CsA and FK groups are summarized in Tables 3 and 4. Fourteen

Fig 3. Linear regression plots of mycophenolic acid (MPA) area under the plasma concentration curve (AUC) values predicted by regression model 12 in cyclosporine (CsA) group (open circle; 3 samples is 0-h, 1-h and 2-h MPA concentration), 16 in tacrolimus (FK) group (closed circle; 3 samples; 1-h, 2-h and 4-h MPA concentrations) vs the corresponding each 11 MPA AUC values calculated from the full sets of samples by the linear trapezoidal rule.

Fig 4. Prediction errors for the abbreviated mycophenolic acid (MPA) area under the plasma concentration curve (AUC) profiles. model 12 in cyclosporine (CsA) group (open circle; 3 samples is 0-h, 1-h and 2-h MPA concentration), 16 in tacrolimus (FK) group (closed circle; 3 samples; 1-h, 2-h and 4-h MPA concentrations) vs the corresponding each 11 MPA AUC values calculated from the full sets of samples by the linear trapezoidal rule.

models were developed and analyzed for their ability to estimate the MPA AUC_{0-12h} based on a limited number of samples in the CsA group. Sixteen models were developed in the FK group. The collinearity check for these models was not violated if the VIF of each model was smaller than 10. Fig 3 shows the MPA AUC values predicted using regression model 12 in the CsA group (model 16 in the FK group) plotted against the corresponding 11 MPA AUC values calculated from the full sets of 12 timed samples by the linear trapezoidal rule. Fig 4 shows prediction errors for the abbreviated MPA AUC profiles (model 12 in the CsA group and 16 in the FK group) plotted against the corresponding 11 MPA AUC values calculated from the full set of 12 timed samples by the linear trapezoidal rule. The best model for predicting the full MPA AUC_{0-12h} in the CsA group was a 3-time-point model (model 12; C_{0h}, C_{1h}, C_{2h}; $r^2 = 0.96$) with a mean prediction error of $0.15 \pm 7.85\%$ (Table 3, Figs 3 and 4). The estimated prediction errors fell within $\pm 15\%$ in 100% of the profiles (11/11) with this model. The 2-sample model that gave the best r^2 value (0.94) was model 8 (C_{0h}, C_{2h}) with a mean prediction error of $0.49 \pm 10.35\%$. At this model, 90.9% of profiles (10/11) had an estimated prediction error within $\pm 15\%$. The highest coefficient of determination between the MPA AUC_{0-12h} and a single concentration was observed with C_{12h} ($r^2 = 0.54$). The mean prediction error was $11.90 \pm 42.13\%$ and the estimated prediction error fell within $\pm 15\%$ in only 36.3% of the profiles (4/11). The best model for predicting the full MPA AUC_{0-12h} in the FK group was a 3-time-point model (model 16; C_{1h}, C_{2h}, C_{4h}; $r^2 = 0.73$) with a mean prediction error of $2.73 \pm 17.09\%$ (Table 4, Figs 3 and 4). The esti-

mated prediction errors fell within $\pm 15\%$ in 54.5% of the profiles (6/11) with this model. The 2-sample model that gave the best r^2 value (0.68) was model 9 (C_{0h}, C_{2h}) with a mean prediction error of $3.26 \pm 19.57\%$. At this model, 54.5% profiles (6/11) had an estimated prediction error within $\pm 15\%$. The highest coefficient of determination between the MPA AUC_{0-12h} and a single concentration was observed with C_{2h} ($r^2 = 0.65$). The mean prediction error was $3.50 \pm 20.06\%$ and the estimated prediction error fell within $\pm 15\%$ in 54.5% of the profiles (6/11).

Discussion

The present study showed that the best model for predicting the full MPA AUC_{0-12h} was a 3-time-point model that included C_{0h}, C_{1h} and C_{2h} in the CsA group. The 2-time-point model that included C_{0h} and C_{2h} was also useful for predicting the full MPA AUC_{0-12h}. However, reliable models for a limited sampling strategy could not be obtained in the FK group. The measurement of the MPA AUC using a full set of samples requires considerable personnel time, laboratory resources and large quantities of blood. To support therapeutic drug monitoring of MPA in clinical practice, limited-sampling strategies should be developed for estimation of the MPA AUC. The r^2 values of other 3-time-point models (C_{0h}, C_{2h}, C_{4h}; C_{1h}, C_{2h}, C_{4h}) in the CsA group were 0.94 and 0.95, respectively. The mean estimated prediction errors for these models were 0.36 ± 9.80 and 0.77 ± 7.94 , respectively. These models could also be used for predicting the full MPA AUC_{0-12h}. In contrast, the r^2 values of 3-time-point models (C_{0h}, C_{1h}, C_{2h}; C_{0h}, C_{2h},

C_{4h}; C_{1h}, C_{2h}, C_{4h}) in the FK group were 0.70, 0.68 and 0.73, respectively. The mean estimated prediction errors for these models were 2.97 ± 18.28 , 3.24 ± 19.48 and 2.73 ± 17.09 , respectively. Therefore, these models should not be used for predicting the full MPA AUC_{0–12h}. In the 2-sample model (C_{0h}, C_{2h}) with the best r^2 value (0.94) in the CsA group, the estimated prediction errors fell within $\pm 15\%$ in 90.9% of the profiles (10/11). This model is also suitable for predicting the full MPA AUC_{0–12h}. On the contrary, in the 2-sample model (C_{0h}, C_{2h}) with the best r^2 value (0.68) in the FK group, the estimated prediction errors fell within $\pm 15\%$ in 54.5% of the profiles (6/11). Therefore, this model is not suitable for predicting the full MPA AUC_{0–12h}. A single-point method would be useful to support therapeutic drug monitoring of MPA in the clinical management of our recipients; however, a reliable single-point method for predicting the full MPA AUC_{0–12h} was not obtained in either the CsA or FK groups. Moreover, reliable models for a limited-sampling strategy were not obtained in the FK group.

Cho et al reported that MPA concentrations before and at 1 and 8 h after dosing were positively correlated with the AUC in kidney transplant recipients treated with CsA.¹⁹ Pawinski et al reported that a 3-sample model with sampling before and at 0.5 and 2 h after dosing was the best model for predicting the full MPA AUC in kidney transplant recipients treated with FK (r^2 value of 0.862 and a prediction error of $6.1 \pm 19.0\%$).²⁰ In another report, an r^2 value of 0.946 was obtained by a 3-sample model with sampling at 20 min, 1 and 3 h after dosing in kidney transplant recipients treated with CsA.²¹ The discrepancy among the results of these previous studies might partly be explained by variation of the transplant organ (kidney or heart), concomitant drug therapy and the sampling times used to determine the full MPA AUC. It has been reported that a significant decrease of the MPA AUC and an increase of the oral apparent clearance are observed in renal-impaired recipients.⁸ The suggested mechanism for these phenomena is a uremia-induced increase of the MPA-free fraction, leading to a temporary increase in the clearance of this restrictively-cleared drug. Thus, the MPA absorption profile might be different between kidney and heart transplant recipients. Cho et al collected blood samples before dosing and at 0.5, 1, 2, 4, 6 and 8 h after dosing.¹⁹ Pawinski et al collected blood samples before dosing and at 0.5, 1, 2, 3, 4, 6, 8, 9, 10, 11, and 12 h after dosing.²⁰ Le Guellec et al collected blood samples before dosing and at 20 min, 40 min, 1, 1.5, 2, 3, 4, 6 and 9 h after dosing.²¹ In our study, blood samples were collected before dosing and at 1, 2, 4, 6, and 12 h after dosing in the CsA group and at 1, 2, 4, 6, 8 and 12 h after dosing in the FK group. Differences in the limited sampling strategies recommended in these studies could be attributable to sampling time differences.

In our study, reliable models for prediction of the MPA AUC were obtained in the CsA group, but not in the FK group. This suggests that concomitant drug therapy might be an important factor for predicting the MPA AUC. The difference in the results between the CsA and FK groups was attributed to distinct MPA pharmacokinetics. It has been reported that a secondary plasma peak of MPA attributed to enterohepatic circulation occurs 6 to 12 h after administration in recipients treated with FK.²² In contrast, CsA might inhibit the transport of 7-O-MPA glucuronide, an inactive metabolite of MPA, into the bile and reduce the enterohepatic recirculation of MPA; therefore, a secondary

plasma peak of MPA does not occur when MMF is used concomitantly with CsA.²²

There was no recipient who experienced ISHLT Grade III rejection in the present study. No serious adverse effects were observed in 22 recipients enrolled in the study, including the recipients with an MPA AUC_{0–12h} $> 60 \mu\text{g} \cdot \text{h/ml}$. Consequently, the present study could not determine whether the target range of 30–60 $\mu\text{g} \cdot \text{h/ml}$ for the MPA AUC is suitable for reducing the risk of acute rejection and adverse effects. Further studies should be conducted on the relationship between the MPA AUC and the risk of rejection and adverse effects in Japanese heart transplant recipients. For this purpose, the development of a limited sampling strategy for estimation of the MPA AUC in Japanese heart transplant recipients is desirable. The 3-time-point model that included C_{0h}, C_{1h} and C_{2h} in the CsA group was useful for predicting the full MPA AUC_{0–12h}. In addition, the 2-time-point model that included C_{0h} and C_{2h} in the CsA group was also reliable for predicting the full MPA AUC_{0–12h}. Although our study is limited in that a small number of recipients were evaluated, it is the first study to characterize the pharmacokinetic parameters of MPA in Japanese heart transplant recipients. A more detailed study is necessary to verify the assumption that the 2- and 3-time-point models for predicting the MPA AUC are valuable in Japanese heart transplant recipients treated with CsA. Furthermore, limited sampling strategies for predicting the MPA AUC need to be developed in FK-treated transplant recipients.

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