Title

Pharmacokinetics of mycophenolic acid after haplo-hematopoietic stem cell transplantation in Japanese recipients

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Abstract

Purpose:

Mycophenolate mofetil (MMF), a mycophenolic acid (MPA) prodrug, is used to prevent graft-versus-host disease (GVHD) in hematopoietic stem cell transplantation (HSCT). Although previous studies have reported that enterohepatic circulation (EHC) of MPA, which is usually observed in MMF-treated patients, does not occur in HSCT patients, it is unclear what happens in haploidentical–HSCT (haplo-HSCT) patients, who are using post-transplant cyclophosphamide. This study was conducted to investigate MPA pharmacokinetics in haplo-HSCT patients.

Methods:

Seventeen haplo-HSCT patients, who received MMF for GVHD prophylaxis, were enrolled in this study. We collected blood samples on days 14 and 28, and plasma MPA concentrations were measured by high-performance liquid chromatography; pharmacokinetic parameters such as area under the curve (AUC), mean residence time (MRT), and apparent oral clearance (CL/F) were measured with moment analysis. We also evaluated EHC as $AUC_{6\text{-}12h}/AUC_{0\text{-}12h}$.

Results:

There was no significant difference in MPA pharmacokinetic parameters between days

14 and 28. There was also no difference between the pharmacokinetic parameter changes and diarrhea. Additionally, varying plasma MPA concentrations suggested that MPA EHC did not occur.

Conclusion:

In this study, we revealed the pharmacokinetics of MMF in Japanese haplo-HSCT recipients. Additionally, our study demonstrated that MPA EHC might not occur in Japanese haplo-HSCT recipients.

Keywords:

mycophenolate mofetil (MMF), mycophenolic acid (MPA), haploidentical hematopoietic stem cell transplantation (haplo-HSCT), enterohepatic circulation (EHC)

Introduction

Mycophenolate mofetil (MMF) is an ester prodrug of immunosuppressant mycophenolic acid (MPA). MPA is the active form of MMF, interfering with cell proliferation by inhibiting inosine monophosphate dehydrogenase (IMPDH), thereby blocking de novo purine synthesis in T and B cell lymphocytes.[1] One MMF pharmacokinetic feature is the effect of enterohepatic circulation (EHC) on MPA-glucuronide (MPAG), mediated by multidrug resistance-associated protein 2 (MRP2), which is responsible for biliary excretion of MPAG and subsequent MPA EHC.[2] MPA is primarily metabolized in the liver by uridine diphosphate glucuronosyl transferases (UGTs) and transformed into phenolic MPAG. It is then excreted into bile, subsequently hydrolyzed in the intestine, and reabsorbed as MPA.

MMF is widely used to prevent acute rejection of solid organ transplantation, and therapeutic targets for plasma MPA levels have been recommended, with trough level and area under the curve (AUC) from 0 to 12 h of 1–3.5 μg/ml and 30–60 μg·h/ml, respectively.[3]

On the other hand, hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for hematological malignancies. HLA-matched related or unrelated donors are the first choice, but not necessarily found for all patients. HLA haploidentical-

HSCT (haplo-HSCT) is an alternative treatment in the absence of an HLA-matched donor.[4] Although haplo-HSCT was associated with increased graft-versus-host disease (GVHD) and graft rejection incidence, strategies such as post-transplant cyclophosphamide (PTCy), which is combined with a calcineurin inhibitor and MMF, have been developed over the last few decades to overcome HLA barriers.[5-7] MMF is used as prophylaxis and GVHD treatment in HSCT. Although several studies have shown MPA pharmacokinetics and the relationship between MPA exposure and HSCT clinical outcomes,[8-14] MPA pharmacokinetics is still obscure, especially in haplo-HSCT.

In this study, we investigated the pharmacokinetics of MPA administered for GVHD prophylaxis in Japanese haplo-HSCT patients.

Methods

Patients

Haplo-HSCT patients aged 16 years and above, who were administered MMF for GVHD prophylaxis in between July 2017 and March 2018, were enrolled in this prospective study. This study was approved by the Institutional Review Board at Hokkaido University Hospital (approval number: 016–0250) and written informed consent was obtained from all patients who decided to participate in this study after receiving adequate explanation.

Conditioning regimens and graft-versus-host disease prophylaxis

Figure 1 shows the study outline. Myeloablative conditioning (MAC) regimens were either fludarabine (Flu) 30 mg/m^2/day on days -6 to -4 plus total body irradiation (TBI) 4 Gy/day on days -3 to -1 or a combination of Flu 30 mg/m^2/day on days -6 to -2, busulfan (BU) 3.2 mg/kg/day on days -6 to -3 and TBI 4 Gy on day -1. The reduced-intensity conditioning (RIC) regimen was a combination of Flu 30 mg/m^2/day on days -6 to -2, BU 3.2 mg/kg/day on days -4 and -3 and TBI 4 Gy on day -1. GVHD prophylaxis consisted of high-dose cyclophosphamide (CY), 50 mg/kg/day in MAC or 40 mg/kg/day in RIC on days 3 and 4 and MMF 15 mg/kg twice daily (maximum 2,000 mg/day) plus tacrolimus 0.02–0.03 mg/kg/day starting on day 5. MMF was orally administered until day 30 and tapered, while tacrolimus was adjusted to maintain therapeutic levels of 10–15 ng/mL by continuous intravenous infusion, tapered, and switched to oral medication, depending on the patient's condition until day 180.

Blood Sampling and MPA concentration measurement

Blood samples were collected at 0, 1, 2, 4, 6, 8, and 12 h after the morning dose on day 14 as the time of graft engraftment in general and day 28 as the before time of MMF tapering from day 30 according to the protocol, after haplo-HSCT, in ethylenediaminetetraacetic acid tubes. These samples were centrifuged at 14,000 ×g for

15 min at 4°C and the plasma was stored at -80°C until analysis. Each plasma sample (50 μL) was mixed with methanol (50 μL) in a centrifuge tube. Then, 0.1 mmol/L H_3PO_4 in CH_3CN 100 μL was added as a protein precipitant. The Naproxen (5 μg/mL) dissolved in methanol was used internal standard. The samples were vortexed and centrifuged at 14,000 ×g for 15 min at 4°C. One hundred μL of supernatant was subsequently loaded onto a high-performance liquid chromatography (HPLC) column with a guard column.

MPA plasma concentrations were detected using HPLC, as previously reported.[15] MPA standards were obtained from Wako (Osaka, Japan). LC-10ADLP (Shimazu, Kyoto, Japan) includes an isocratic pump, a diode array detector, and an automatic sampling system. An ERC-ODS-1161 column (6×100 mm; Yokohamarika Co, Yokohama, Japan) was used for separation. The mobile phase consisted of 60 mmol/L H_3PO_4 and CH_3CN (60:40, v/v), column temperature was 55°C, and flow rate 1.0 mL/min. The detector wavelength was 215 nm. The validated assay was linear in the range of 0.25–16 μg/mL for plasma MPA concentration and correlation coefficient was always >0.99. Accuracy and precision were evaluated by use of percent coefficient of variation (%CV) and percent relative error (%RE), respectively. The inter-day and intra-day %CV were less than ±15% and %RE was less than 15%.

After quantification, we used MOMENT (EXCEL), which can perform moment analysis

to calculate pharmacokinetic parameters.[16] Non-compartmental analysis was used to determine several pharmacokinetic parameters, including AUC, area under the moment curve (AUMC), and mean residence time (MRT). AUC was estimated using the linear trapezoidal rule and apparent steady-state concentration (C_{ss}), and apparent clearance (CL/F) were determined as $AUC_{0\text{-}12h}/12$, and dose/$AUC_{0\text{-}12h}$, respectively. Additionally, we evaluated EHC as $AUC_{6\text{-}12h}$/ $AUC_{0\text{-}12h}$ according to previous reports.[17-19]

Evaluation

The primary endpoint was to investigate pharmacokinetics of MPA between days 14 and 28 after haplo-HSCT in Japanese recipients, and the secondary endpoint was to clarify relationship between the MPA pharmacokinetic parameter changes and diarrhea. Toxicity was evaluated based on the Common Toxicity Criteria for Adverse Events (version 5.0) of the National Cancer Institute. Acute GVHD was diagnosed and graded based on traditional criteria.[20] Recovery from diarrhea was defined as a down-grade from days 14 to 28. Worse or no grade change in case of diarrhea was defined as non-recovery.

Statistical Analyses

The Wilcoxon signed-rank test was used to compare MPA pharmacokinetic parameters and laboratory values between days 14 and 28. The Fisher's exact test was used to compare the relationship between diarrhea grade and MPA pharmacokinetic parameters.

All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is an R graphical user interface (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.[21] *P* value < 0.05 was considered statistically significant.

Results

Patient Characteristics

A total of 17 patients were enrolled in this study. Patient characteristics at baseline are shown in Table 1. In most patients, serum albumin levels, and liver and renal functions were normal.

MPA Pharmacokinetics in haplo-HSCT

Individual plasma concentrations of $MPA_{0\text{-}12h}$ on days 14 and 28 are shown in Figure 2a and b. MPA pharmacokinetic parameters showed a large inter-patient variation on days 14 and 28 (Table 2, Supplemental Table 1). There was no significant difference in any MPA pharmacokinetic parameters and laboratory values, except serum creatine (p = 0.047) between days 14 and 28.

Relationship between diarrhea and MPA pharmacokinetic parameters

We evaluated diarrhea severity on days 14 and 28. Of the 17 patients, 7, 4, and 6 experienced grades 1, 2, and 3 diarrhea on day 14, respectively, whereas all patients had grade 1 diarrhea on day 28. We also assessed the relationship between recovery from diarrhea and MPA $AUC_{0\text{-}12h}$ increased, MRT extension, and CL/F decreased (Table 3). As a result, there was no statistical relationship in any of the parameters.

Variation in EHC parameter

EHC incidence was assessed. $AUC_{6\text{-}12h}$/ $AUC_{0\text{-}12h}$, which was defined as an EHC index, was approximately 0.2 on both days (Table 2). Moreover, there was no correlation between EHC increase and recovery from diarrhea (Table 3).

Discussion

This study demonstrated MPA pharmacokinetics for GVHD prophylaxis in Japanese haplo-HSCT recipients. We initially hypothesized that there would be some changes in MPA pharmacokinetic parameters between days 14 and 28. It is known that MPA plasma concentration and AUC are lower in HSCT patients, compared to those in solid organ transplant patients and healthy individuals receiving MMF 1.0 g twice daily.[8,9] This study showed that plasma MPA concentration and $AUC_{0\text{-}12h}$ were low, previously reported in HSCT patients. Moreover, there was no significant difference in the MPA

pharmacokinetic parameters between days 14 and 28; however, we speculated that MPA $AUC_{0\text{-}12h}$ on day 28 would be higher than that on day 14. Possible factors affecting MPA pharmacokinetics are reportedly due to renal and hepatic function, albumin level, etc.[1] Hepatic function and albumin levels were similar in both days. Contrarily, serum creatine level was significantly higher on day 28 than on day 14, but clinically within normal range. We thought that other factors were believed to influence MPA pharmacokinetics.

Next, we assumed that haplo-HSCT-mediated diarrhea might have induced the results. We considered that diarrhea had influenced the reduction in MPA absorption and reabsorption by disrupting the intestinal flora because of heavy use of some antibacterial agents.[22] However, there was no correlation between recovery from diarrhea and MPA pharmacokinetics in this study.

We evaluated whether EHC occurred in haplo-HSCT patients. Potential factors affecting MPA EHC could be pharmacogenetic factors and drug-drug interactions.[1] MPA is primarily metabolized in the liver by UGTs and transformed into phenolic MPAG. Its EHC is MRP2-mediated.[23] There are various single nucleotide polymorphisms (SNPs) in *UGT* and *MRP2* gene promoter regions influencing MPA pharmacokinetics.[24] Two previous studies demonstrated that T275A and C2152T SNPs of *UGT1A9* were associated with significantly lower MPA AUC in renal transplantation, resulting in MPA EHC

reduction,[25,26] although they are rare in Japanese.[27] Therefore, it is difficult to explain that there was no difference in MPA $AUC_{0\text{-}12h}$ between days 14 and 28.

MPA concentration-time profile generally has two peak concentrations: maximum within 1–2 h post-administration and 6–12 h post-administration due to resorption from EHC in healthy subjects and solid organ transplant patients.[28-31] When patients were administered MMF in combination with cyclosporine, which is derived from hepatic MRP2 inhibition,[2] MPA exposure is approximately 30–40 % lower than when given alone or with tacrolimus.[1] Contrarily, all patients were administered tacrolimus with MMF and not dosed any MRP2 inhibitor in this study. Additionally, concomitant treatment with MMF and pantoprazole significantly decreased MPA peak concentration and AUC to 25 % by decreasing its dissolubility in heart transplant patients.[32] It is possible to speculate that $AUC_{0\text{-}12h}$ was reduced since all patients in this study used proton pump inhibitor (PPI). However, it is difficult to evaluate its possibility since it is general for HSCT recipients to use them. Moreover, MPA $AUC_{0\text{-}12h}$ on day 28 would increase more, compared to day 14, even if all patients were administered any PPI. For these reasons, we thought that the possibility of drug-drug interaction is also low.

A previous study on renal transplantation reported that the MPA AUC increased at a later MMF administration stage than at the initial, and MPA EHC was confirmed.[29] Contrarily,

previous studies have reported no MPA EHC in HSCT patients.[8,33,34] They speculated that the mucosal damage associated with conditioning regimens such as TBI and chemotherapy, and GVHD in HSCT might have induced the results. Thus, we assessed the MPA concentration-time profiles on days 14 and 28 using $AUC_{6\text{-}12h}/AUC_{0\text{-}12h}$ as an EHC measure, although $AUC_{6\text{-}12h}$ consists of both normal absorption and resorption. As a result, $AUC_{6\text{-}12h}/AUC_{0\text{-}12h}$ which was approximately 0.3–0.4 when patients were administered MMF with tacrolimus in solid organ transplantation was calculated to be approximately 0.2 on both days 14 and 28 in this study, suggesting that MPA EHC did not occur in haplo-HSCT recipients.[17-19,25] Additionally, it is common for solid organ transplantation recipients to be administered PPIs like HSCT recipients. We speculated that plasma MPA concentration did not increase on day 28 owing to the absence of MPA EHC. Moreover, there was no relationship between recovery from diarrhea and MRT extension, $AUC_{6\text{-}12h}/AUC_{0\text{-}12h}$ increase, and no patient had gastrointestinal GVHD during the evaluation period. Therefore, we thought that although the possibility that MPA EHC did not appear owing to intestinal disorder in haplo-HSCT was low, transporter expression in patients might have been decreased by severe chemotherapy.

It is considered that MRP2 expression and activity are influenced by specific drugs, foods, health supplements and disease state.[35,36] A previous study reported that

methotrexate downregulates hepatic MRP2 expression levels depending on its concentration in rats.[37] Although we initially suspected intestinal disorders related to MPA EHC disappearance, we hypothesized that chemotherapy prior to HSCT, conditioning chemotherapy, TBI, or HSCT itself might have suppressed hepatic MRP2 expression and/or activity. We strongly suspect this possibility. Therefore, further studies are required to clarify whether chemotherapy used for hematologic diseases affects hepatic MRP2 expression and/or activity. Actually, as shown in Figure 2, we considered that EHC was observed in a few patients. We speculated that multiple factors such as chemotherapy intensity, pharmacogenetic factors and drug-drug interactions might have synergistically influenced on EHC. In the future, we are going to investigate whether EHC arises in what kind of patients.

Our study has some limitations. First, this study was conducted using a relatively small population sample at a single institution. Second, we did not measure concentration levels of plasma MPAG directly circulating in enterohepatic. In addition, we did not measure the unbound MPA concentration that might have affected pharmacodynamics because Giaccone et al have reported that MPA had linear pharmacokinetics and no association is seen between unbound MPA C_{ss} and serum albumin.[9] We intend to evaluate the relationship between MPA pharmacokinetic parameters and clinical outcomes in haplo-

HSCT recipients in future studies. In that case, we will consider measuring the unbound MPA concentration because the relationship between unbound MPA C_{ss} and CMV reactivation has been demonstrated.[9] Third, some patients registered in this study may not tolerate oral MMF due to nausea, anorexia, and oral mucositis, although we had confirmed that medical adherence of all patients was fine as per the medical records.

Conclusion

There was no significant difference in MPA pharmacokinetic parameters between days 14 and 28 in this study. Additionally, our study demonstrated the possibility that MPA EHC does not occur in Japanese haplo-HSCT recipients. This is the first MPA pharmacokinetic study in Japanese haplo-HSCT patients, who were administered MMF.

Declarations

Funding: Not applicable

Conflict of Interest: K.U., Y.S., Y.T., J.S., T.T., and M.S. have no conflicts of interest.

Ethical approval: All procedures in this study were performed, per the ethical standards of the institutional and/or national research committee. They also conformed to the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Authors contributions:

Participated in research design: K.U., Y.S., Y.T., J.S., T.T., and M.S.

Conducted experiments: K.U.

Performed data analysis: K.U., Y.S., and Y.T.

Wrote or contributed to the writing of the manuscript: K.U., Y.S., Y.T., J.S., T.T., and M.S.

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Table 1. Patient Demographics

	n =17
Sex (Male/Female)	11/6
Median age (years) [range]	50 [20–65]
Median body weight (kg) [range]	57.9 [34–81]
Type of disease	
AML	5
ALL	2
MDS	4
EB	1
ATL	1
MF	1
CML	1
CTCL	1
TLBL	1
Conditioning regimens	
MAC (Flu+TBI)	9
MAC (Flu+BU4+TBI)	1
RIC (Flu+BU2+TBI)	7
Median Laboratory values [range]	
Serum albumin (g/dL)	3.3 [2.6–4.3]
Aspartate transferase (U/L)	21 [11–102]
Alanine transferase (U/L)	28 [9–101]
Total bilirubin (mg/dL)	0.8 [0.4–1.9]
Serum creatine (mg/dL)	0.45 [0.3–0.99]
Blood urea nitrogen (mg/dL)	10 [5–22]

AML: acute myeloid leukemia, ALL: acute lymphoblastic leukemia, MDS: myelodysplastic syndrome, EB: Epstein-Barr virus, ATL: adult T cell leukemia, MF:

myelofibrosis, CML: chronic myeloid leukemia, CTCL: cutaneous T-cell lymphoma, TLBL: T-cell lymphoblastic lymphoma, MAC: myeloablative conditioning, RIC: reduced-intensity conditioning, Flu: fludarabine, TBI: total body irradiation, and BU: busulfan

Range indicates minimum to maximum level of each parameter.

Table 2. Comparison of MPA PK parameters and laboratory values in haplo-HSCT recipients between days 14 and 28

	day14 (n =17)	day28 (n =17)	*p* value
Pharmacokinetic parameters Median [range]			
$AUC_{0\text{-}12h}$ (μg・h/mL)	10.31 [0.89–22.54]	11.67 [6.81–36.23]	0.23
$AUC_{6\text{-}12h}$ (μg・h/mL)	1.92 [0.05–9.07]	2.12 [0.07–16.98]	0.46
$AUC_{6–12h}/AUC_{0–12h}$	0.20 [0.02–0.41]	0.18 [0.01–0.47]	1.00
C_{ss} (μg/mL)	0.86 [0.07–1.88]	0.97 [0.57–3.02]	0.23
AUMC (μg・h^2/mL)	37.19 [0.89–118.58]	39.05 [12.92–220.12]	0.19
MRT (h)	3.75 [1.02–5.44]	3.61 [1.51–6.08]	1.00
CL/F (L/h)	97.06 [33.28–559.62]	78.52 [25.84–146.94]	0.13
Dose/body weight (mg/kg)	14.29 [12.22–18.12]	13.99 [11.79–18.35]	0.86
Laboratory values Median [range]			
Serum albumin (g/dL)	3.2 [2.3–4.2]	3.5 [2.5–4.3]	0.47
Total bilirubin (mg/dL)	0.6 [0.3–1.7]	0.5 [0.3–3.9]	0.57
Serum creatine (mg/dL)	0.47 [0.29–0.97]	0.54 [0.39–0.94]	0.047*

$AUC_{0\text{-}12\,h}$: area under the curve from 0 to 12 h, $AUC_{6\text{-}12\,h}$: area under the curve from 6 to 12 h, C_{ss}: steady-state concentration, AUMC: area under the moment curve, MRT: mean residence time, CL/F: apparent oral clearance, CL: clearance, F: oral bioavailability, and *: $P < 0.05$

$AUC_{6–12h}/AUC_{0–12h}$ was used as the surrogate marker of enterohepatic circulation.

Range indicates minimum to maximum level of each parameter.

Table 3. Relationship between recovery from diarrhea and Mycophenolic acid pharmacokinetic parameter changes

	Recovery (n =10)	Non-recovery (n =7)	*p* value
$AUC_{0\text{-}12\ h}$			
Increase	6	4	1.00
Not increase	4	3	
MRT			
Extension	5	4	1.00
Not extension	5	3	
CL/F			
Decrease	6	4	1.00
Not decrease	4	3	
$AUC_{6–12h}/AUC_{0–12h}$			
Increase	6	2	0.34
Not increase	4	5	

$AUC_{0\text{-}12\ h}$: area under the curve from 0 to 12 h, MRT: mean residence time, CL/F: apparent oral clearance, CL: clearance, and F: oral bioavailability

Increased/decreased pharmacokinetic parameters were defined as higher or lower on day 28, compared to day 14.

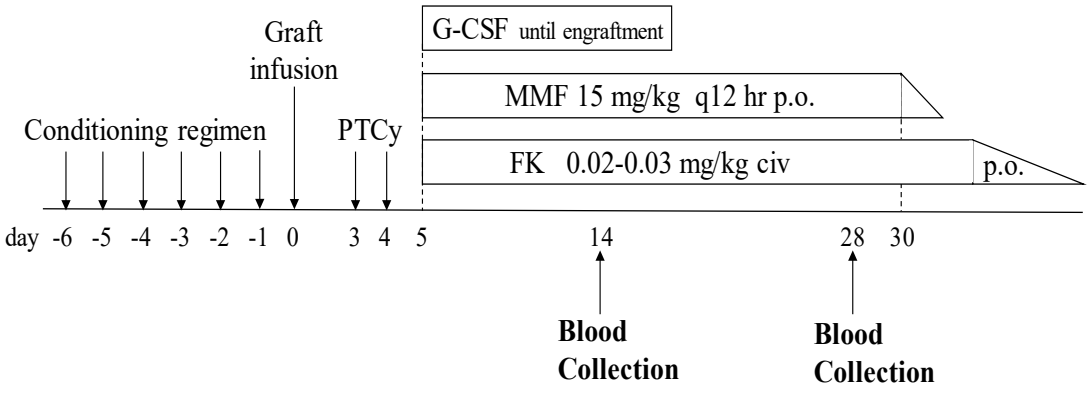
Supplemental Table 1. Individual MPA pharmacokinetic parameters on days 14 and 28

No.	Day14					Day28				
	$AUC_{0\text{-}12\ h}$ (μg·h/mL)	$AUC_{6\text{-}12\ h}$ (μg·h/mL)	AUMC (μg·h^2/mL)	MRT (h)	CL/F (L/h)	$AUC_{0\text{-}12\ h}$ (μg·h/mL)	$AUC_{6\text{-}12\ h}$ (μg·h/mL)	AUMC (μg·h^2/mL)	MRT (h)	CL/F (L/h)
1	19.49	7.86	106.06	5.44	38.47	8.96	3.28	40.77	4.76	83.69
2	15.17	4.39	68.22	4.50	65.92	11.67	2.12	40.77	3.49	85.66
3	9.90	1.69	37.19	3.75	100.92	9.02	1.62	32.55	3.60	110.84
4	10.93	3.33	46.98	4.30	68.63	29.03	10.58	144.53	4.98	25.84
5	18.69	2.46	55.89	2.99	53.49	19.04	5.19	75.10	3.94	52.52
6	8.02	1.24	23.56	2.94	124.60	12.73	0.56	25.48	1.99	78.52
7	2.00	0.06	6.22	3.11	373.27	7.90	1.72	34.02	4.30	94.99
8	7.50	1.92	31.42	4.19	100.06	10.63	1.40	35.13	3.30	70.57
9	1.95	0.20	5.18	2.65	512.06	7.71	2.30	34.80	4.52	129.9
10	22.54	0.48	44.39	1.97	33.28	14.72	3.89	67.45	4.58	50.97
11	5.17	1.64	24.80	4.80	193.34	11.17	1.35	39.05	3.51	89.52
12	7.85	2.68	35.33	4.51	127.41	15.83	2.45	48.43	3.06	63.17
13	22.09	9.07	118.58	5.37	45.27	36.23	16.9	220.12	6.08	27.6
14	0.89	0.05	0.89	1.02	559.62	15.46	0.79	23.20	1.50	32.35
15	22.41	4.58	79.84	3.56	33.47	17.50	3.43	68.26	3.91	42.85
16	11.89	0.45	24.74	2.08	84.13	11.48	1.41	30.86	2.69	87.10
17	10.30	2.09	44.36	4.31	97.06	6.81	0.07	12.92	1.91	146.94

$AUC_{0\text{-}12\ h}$: area under the curve from 0 to 12 h, $AUC_{6\text{-}12\ h}$: area under the curve from 6 to 12 h, AUMC: area under the moment curve,

MRT: mean residence time, CL/F: apparent oral clearance, CL: clearance, and F: oral bioavailability

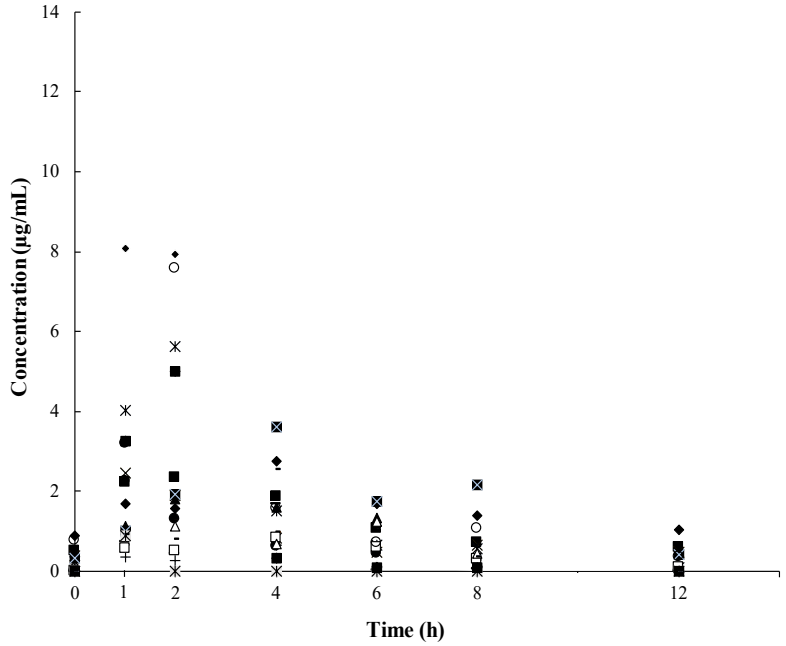
Figure 1. Scheme of this study



PTCy: post-transplantation cyclophosphamide, G-CSF: granulocyte-colony stimulating factor, MMF: mycophenolate mofetil, FK: tacrolimus, civ: continuous intravenous infusion, and p.o.: per os

Figure 2. Plasma mycophenolic acid concentration-time profiles of 17 patients on days (a) 14 and (b) 28

(a)



(b)