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No influence of everolimus on mycophenolic acid area under the concentration–time curve: Limited sampling strategy for mycophenolic acid in Japanese kidney transplant recipients treated with tacrolimus, mycophenolate mofetil, steroid, and everolimus

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Kidney transplantation, everolimus, mycophenolic acid, limited sampling strategy, drug-drug interaction

Abbreviations:
AUC; area under the concentration-time curve, CNIs; calcineurin inhibitors, CyA; cyclosporine A, dnDSA; *de novo* donor-specific antibody, eGFR; estimated glomerular filtration rate, ECLIA; electrochemiluminescence immunoassay, EVR; everolimus, IMPDH; inhibition of the monophosphate dehydrogenase, KTx; Kidney transplantation, LSS; limited sampling strategy, MMF; mycophenolate mofetil , MPA; mycophenolic acid, MPAG; mycophenolic acid glucuronide, mPSL; methylprednisolone, mTOR-i; mammalian target of rapamycin inhibitor, PETINIA; particle-enhanced turbidimetric inhibition immunoassay, RMSE; root-mean-square error, SRL; sirolimus, Tac; tacrolimus, TDM; therapeutic drug monitoring, /D; per dose

Tables: 4

Figures: 3 (color –No)

Abstract

Background

Despite a growing need for everolimus (EVR) to reduce calcineurin inhibitor toxicity in kidney transplantation (KTx), EVR influence on the pharmacokinetics of mycophenolic acid (MPA), a mycophenolate mofetil (MMF) active metabolite, is obscure, and no suitable limited sampling strategy (LSS) for MPA when EVR is concomitantly present exists. We aimed to investigate EVR influence on MPA pharmacokinetics in KTx.

Methods

This study complied with all principles of the Helsinki Declaration. Twenty patients were initially administered tacrolimus, MMF, and methylprednisolone, then received EVR four months after KTx. Approximately four weeks before and after EVR administration, the estimated value of the area under the concentration–time curve for MPA from 0–12 h (MPA-AUC0-12) was calculated using MPA blood concentration just before, and 1, 2, 4, and 6 h after, MMF administration. We compared several MPA pharmacokinetics parameters before and after EVR addition and determined the best estimation equation for LSS of MPA-AUC0-12.

Results

Although MPA-C6 per dose (MPA-C6/D) significantly decreased after EVR addition [from (3.4 ± 2.2) ng/mL/g to (2.5 ± 0.9) ng/mL/g], MPA-C0/D, -C1/D, -C2/D, -C4/D, and MPA-AUC0-12/D showed no significant change. MPA-AUC0-12/D did not correlate with EVR-AUC0-12/D. The best estimation equation for LSS of MPA-AUC0-12 by two time points was [(2.94 × C2) + (5.09 × C4) + 5.32] (R^2 = 0.73) and [(5.70 × C0) + (1.39 × C1) + 22.45] (R^2 = 0.72) before and after EVR addition, respectively.

Conclusions

EVR can be safely combined with MMF after KTx once our results have been re-evaluated.

Introduction

Improvements in immunosuppressive therapies have dramatically reduced acute rejection episodes and improved allograft survival in patients who have undergone kidney transplantation (KTx) in the last several decades [1]. Mainstream immunosuppressive therapy includes calcineurin inhibitors (CNIs), such as cyclosporine A (CyA) or tacrolimus (Tac); however, long-term use of CNIs may cause chronic nephrotoxicity. Consequently, reducing chronic CNI toxicity has become a critical objective in KTx [2]. To avoid chronic CNI toxicity in KTx, and to preserve long-term graft function, recent efforts have focused on utilizing a mammalian target of rapamycin inhibitor (mTOR-i), such as everolimus (EVR) or sirolimus, in immunosuppressive protocols to eliminate or minimize CNI. Most attempts have successfully demonstrated improved graft function [2-4]. By contrast, a study has reported that *de novo* donor-specific antibody (dnDSA) development correlates with Tac trough levels and the recipient's individualized alloimmune risk, which is another main obstacle to long-term survival [5]. Furthermore, a study reported the importance of mycophenolate mofetil (MMF) to avoid dnDSA production in KTx [6].

MMF, a prodrug of mycophenolic acid (MPA), is widely used to prevent rejection of kidney grafts by inhibiting T and B lymphocyte proliferation [7]. In KTx, the MMF dose is determined based on therapeutic

drug monitoring (TDM); the rate of rejection and side effects after KTx correlate with the area under the concentration–time curve from 0 to 12 h for MPA (MPA-AUC0-12) [8-10]. In clinical settings, many limited sampling strategies (LSSs) that estimate MPA-AUC0-12 using the fewest possible blood collection points have been reported [11,12]. On the other hand, MPA pharmacokinetics are reportedly affected by graft function, serum albumin concentration, and time lapsed after KTx [9,13]. Moreover, in view of drug–drug interactions, CyA was reported to reduce MPA exposure by inhibiting MPA enterohepatic recirculation [14].

Although there is a growing need for EVR to reduce CNI toxicity in KTx, and the possibility that EVR and MMF are co-administered may become more frequent, the influence of EVR on MPA pharmacokinetics has scarcely been reported, and a suitable LSS for MPA in the presence of concomitant EVR has not been elucidated. At our institution, we apply quadruple immunosuppression comprising Tac, MMF, EVR, and methylprednisolone (mPSL) to patients who have recently received KTx to prevent both CNI toxicity and dnDSA production, thereby achieving excellent long-term graft function. The objective of this study was to assess the effect of EVR on the pharmacokinetic parameters of MMF and to develop the best LSS with the consideration of co-administered EVR in patients who have had KTx.

Methods

Patients

Twenty adult patients who underwent living donor KTx between August 2019 and March 2021 at Kobe University Hospital were included in this study. All patients were treated with combination immunosuppressive therapy consisting of Tac (Graceptor Capsules®, Astellas Pharma Inc., Tokyo, Japan), MMF (Cellcept®, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan), and mPSL (Medrol®, Pfizer Japan Inc., Tokyo, Japan) beginning a few days before KTx and started receiving EVR (Certican®, Novartis Pharmaceuticals Inc., Tokyo, Japan) at nearly four months after KTx. Patients who experienced a rejection episode before EVR addition, and patients who were administered EVR later than six months after KTx, were excluded from this study. The patients were prospectively planned to undergo pharmacokinetic analysis for Tac, MMF, and EVR before and after EVR addition. The protocol of this study complied with the Declaration of Helsinki and was approved by the Ethics Committee of Kobe University Hospital; all patients provided written informed consent.

Immunosuppression and sample collection

The immunosuppression protocol and timing of blood sample collection for pharmacokinetic analysis of each drug are shown in Figure 1. All patients received combination immunosuppression comprising Tac,

MMF, EVR, and mPSL. Postoperatively, Tac was first administered by intravenous infusion for 3 d. Then, oral administration of the once-daily prolonged-release preparation targeting trough whole blood concentration (C0) at 8 ng/mL in the first month after KTx, 5–8 ng/mL from the second month to the fourth month, and 4 ng/mL after the second pharmacokinetic analysis were evaluated. MMF was started twice daily at 30 mg/kg/d, which was opportunely reduced depending on adverse effects, such as anemia, infection, or gastrointestinal disorder before the first pharmacokinetic analysis. Patients were also treated with mPSL (500 mg bolus on the day of KTx, tapering to 4 mg by six weeks after KTx) and basiliximab (Simulect® i.v. injection; Novartis Pharmaceuticals) at 20 mg/body on the day of, and 4 d after, KTx. In patients with ABO incompatibility, rituximab (Rituxan® i.v. infusion; Chugai Pharmaceutical Co., Ltd.) at 150 mg/m^2 was administered twice preoperatively. At approximately four months after kidney transplantation, patients started to receive EVR at an initial dose of 1.5 mg/d. The dose was adjusted to target EVR-C0 within 3–8 ng/mL. At approximately two weeks before the initiation of EVR, the first pharmacokinetic analysis, measurement of Tac and MPA blood concentrations immediately before administration and 1, 2, 4, and 6 h after administration of each drug, was performed (Tac- and MPA-C0, -C1, -C2, -C4, and -C6, respectively). The doses of Tac and MMF were fixed between the first and second pharmacokinetic analyses, except that the dose of Tac was slightly adjusted to maintain a suitable Tac-C0.

The second pharmacokinetic analysis was then performed for Tac, MPA, and EVR, after we confirmed that the EVR dose was properly adjusted to achieve the targeted EVR-C0.

MPA assay and pharmacokinetic analysis

In the first pharmacokinetic analysis before the initiation of EVR, and the second pharmacokinetic analysis after the initiation of EVR, blood samples were collected in EDTA tubes just before drug administration and 1, 2, 4, and 6 h after the administration of each drug.

Whole blood concentrations of Tac and EVR were determined using the electrochemiluminescence immunoassay (ECLIA) technique on the Cobas 8000 e801® (Roche Diagnostics K.K., Tokyo, Japan). The lower limit of quantification for Tac and EVR was 0.2 μg/mL. Plasma was separated by centrifugation at 1700 × *g* for 10 min. Serum MPA concentration was determined using the particle-enhanced turbidimetric inhibition immunoassay (PETINIA) on a Dimension EXL 200® (Siemens Healthcare Diagnostics K.K., Tokyo, Japan). The lower limit of quantification for MPA was 0.2 μg/mL.

The values of Tac-AUC0-24, EVR-AUC0-12, and MPA-AUC0-12 were calculated using Tac, EVR, and MPA-C0, -C1, -C2, -C4, and -C6, and a linear trapezoidal rule. The LSS for MPA was developed, and each estimation equation comprised two blood-sampling points out of MPA-C0, -C1, -C2, -C4, and -C6.

Statistical analysis

All results are expressed as mean ± standard deviation (SD). The Shapiro–Wilk test was used to confirm normal distribution of data. The Wilcoxon signed-rank test was used to evaluate statistically significant differences in the pharmacokinetic parameters of Tac and MPA before and after EVR addition. Single linear regression analysis was performed to determine the relationship between MPA-AUC0-12 and all factors, including EVR-AUC0-12 and Tac-AUC0-24. The LSS for MPA was developed using multiple comparison analysis before and after EVR addition. Each estimation equation was developed using multiple comparison analysis and comprised two blood sampling points out of MPA-C0, -C1, -C2, -C4, and -C6. Precision was evaluated using Spearman's rank correlation test, root-mean-square error (RMSE), correlation coefficient (R^2) using the least squares method, and correlation between the measured AUC and estimated AUC.

All statistical analyses were performed using the EZR software, a modified version of R commander designed to add statistical functions (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [15], which is for the R commander software (version 4.0.0; R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics and clinical data

The baseline characteristics of the 20 patients are presented in Table 1. Mean age of the recipients was (47.1 ± 8.3) years. Mean intervals from KTx to the first MPA pharmacokinetic analysis, from KTx to the initiation of EVR, and from KTx to the second MPA pharmacokinetic analysis were (92.4 ± 5.7) d, (128.5 ± 27.9) d, and (157.4 ± 30.2) d, respectively. The results of Tac and EVR pharmacokinetics and laboratory data before and after EVR addition are summarized in Table 2. Mean eGFR (according to the modified equation for Japanese patients proposed by the Japanese Society of Nephrology), serum albumin, and hemoglobin before and after EVR addition were (50.1 ± 11.6) mL/min/1.73 m^2 and (49.2 ± 10.6) mL/min/1.73 m^2 for eGFR, (6.0 ± 7.3) g/dL and (4.4 ± 0.8) g/dL for serum albumin, and (12.5 ± 1.0) g/dL and (12.8 ± 1.4) g/dL for hemoglobin, respectively, and no significant changes were observed (p=0.33, 0.35, and 0.10, respectively). No patient experienced rejection episodes during observation.

Values of mean Tac-AUC0-24 per dose (Tac-AUC0-24/D) before and after EVR addition were (50.4 ± 20.6) ng·h/mL/mg and (49.7 ± 21.4) ng·h/mL/mg, respectively; no significant change was observed (p=0.80). In all patients, EVR-C0 was within the target range (3–8 ng/mL) at the time of the second pharmacokinetic analysis, and mean EVR-AUC0-12/D was (63.7 ± 16.9) ng·h/mL/mg.

Mycophenolic acid pharmacokinetics analysis

The mean plasma MPA concentration–time curve and pharmacokinetic profile before and after EVR addition are shown in Table 2 and Figure 2. Mean MPA-C6/D significantly decreased from (3.4 ± 2.2) ng/mL/g to (2.5 ± 0.9) ng/mL/g after EVR addition (p = 0.04). By contrast, mean MPA-C0/D [(2.4 ± 1.5) ng/mL/g vs. (2.8 ± 1.6) ng/mL/g], -C1/D [(7.1 ± 5.3) ng/mL/g vs. (10.5 ± 7.1) ng/mL/g], -C2/D [(6.8 ± 2.7) ng/mL/g vs. (8.1 ± 3.5) ng/mL/g], and -C4/D [(4.8 ± 2.9) ng/mL/g vs. (4.2 ± 2.2) ng/mL/g] before and after EVR addition exhibited no significant change (p=0.42, 0.10, 0.16, and 0.24, respectively). MPA-AUC0-12/D values before and after EVR addition were (48.4 ± 19.8) ng/mL/g and (50.5 ± 15.5 ng/mL/g), respectively; no significant change was observed (p=0.58, Figure 3). Moreover, no correlation was observed between MPA-AUC0-12/D and EVR-AUC0-12/D using linear regression analysis (R^2 = −0.047) (Table 3). There was also no correlation between MPA-AUC0-12/D and other clinical variables, including Tac-AUC0-24.

LSS for MPA before and after everolimus addition

Based on the MPA pharmacokinetic profile, we also determined the best estimation equation for LSSs for MPA-AUC0-12 before and after EVR addition. The derived formulas to predict MPA-AUC0-12 at two time points are listed in Table 4. Before EVR administration, the best LSS for MPA-AUC0-12 was described by the equation using C2 and C4, and the estimation formula was [(2.94 × C2) + (5.09 × C4) + 5.32] (R^2 =

0.73). By contrast, the model based on C0 and C1 provided the highest estimation after EVR addition, and the estimation formula was [(5.70 × C0) + (1.39 × C1) + 22.45] (R^2 = 0.72).

Discussion

MMF has been widely used as a part of the immunosuppressive regimen in KTx without any known nephrotoxicity [16]. Orally administered MMF is rapidly absorbed and hydrolyzed *in vivo* to the active compound MPA [8], which suppresses the proliferation of T and B lymphocytes by depleting the deoxyguanosine triphosphate pool for DNA synthesis through the inhibition of the monophosphate dehydrogenase (IMPDH). MPA is then excreted as inactivated MPA glucuronide (MPAG, reaction catalyzed by UDP-glucuronosyltransferase) in the urine and bile, and undergoes enterohepatic recirculation as MPAG, partially recover MPA in the intestine [7].

Similar to other immunosuppressive drugs, such as Tac and EVR, MPA displays large intra- and inter-patient pharmacokinetic variability; therefore, systemic exposure to MPA should be monitored in the treatment of patients who have had kidney transplants. In a study evaluating MPA exposure in KTx, the rate of rejection and side effects were reported to correlate with AUC0-12 [8,10]. Although trough concentrations of Tac and EVR are correlate well with AUC0-24 and AUC0-12 and are used to evaluate

drug efficacy, MPA-C0 is not useful for adjusting the MMF dose to achieve optimal clinical outcomes because it has little correlation with MPA-AUC0-12 [17]. However, routine determination of MPA-AUC0-12 for 12 h dose intervals, which requires frequent sampling of blood and may impose a mental and physical burden on both the patient and medical staff. An LSS that estimates AUC0-12 using the fewest possible blood collection points, solves this problem and makes it possible to estimate MPA-AUC0-12 within a few hours.

MPA pharmacokinetics are altered by graft function and serum albumin concentration [13]. Studies have reported that oral MPA clearance increases with increased time post-operation [8,9]. Thus, a suitable LSS may differ depending on the clinical situation. In addition to the importance of TDM in immunosuppressive treatments for KTx, certain drug–drug interactions should be considered for the dose adjustment of each drug, especially when multiple immunosuppressive drugs are co-administered. For example, CyA moderately increases EVR blood levels by 2.7-fold [18]. This pharmacokinetic interaction is likely due to the inhibition of EVR metabolism by CyA. However, differing results concerning the effect of EVR on Tac pharmacokinetics have been reported [19–22]. Although EVR is widely used as a part of a multiple immunosuppression regimen, clinical evidence about the pharmacokinetic interaction between MMF and EVR is lacking, especially in human participants.

In this study, we characterized the pharmacokinetics of MPA in patients who were initially treated with Tac, MMF, and mPSL, then subsequently began receiving EVR, in order to investigate the influence of EVR on the pharmacokinetics of MPA and determine the optimal LSS to exert the best efficacy of MMF after EVR co-administration. Our analysis of MPA pharmacokinetics MPA-AUC0-12/D did not change after EVR administration to KTx patients who were initially treated with triple immunotherapy, despite the decrease in MPA-C6/D. Additionally, no significant correlation was observed between MPA-AUC0-12/D and EVR-AUC0-12/D after addition of EVR to the regimen. These results suggest that EVR has no significant influence on the clinical efficacy of MPA, either intra- or inter-individually.

We then found the most suitable equation in the two-point LSS for MPA-AUC0-12 shifted from the formula using C2 and C4 to the formula using C0 and C1 after beginning EVR administration. Although this result implied that LSS should be re-evaluated after beginning EVR, the same LSS using C2 and C4 is efficient to estimate the clinical usefulness of MMF after the initiation of EVR ($R^2 = 0.658$, $p = 0.000417$), based on the fact that the target range of MPA-AUC0-12 was 30–60 μg·h/mL and the standard deviation values in this study. Thus, changing the dose of MMF when adding EVR to the triple immunosuppressive therapy comprising Tac, MMF, and mPSL is not required.

CyA has been reported to reduce MPA exposure by inhibiting MPA enterohepatic recirculation [14]. By contrast, whether Tac has any influence on the enterohepatic recirculation of MPA is controversial [23]. Although details are limited, several hypotheses have been proposed to explain the mechanism of interaction between MPA and CyA. For example, Kobayashi et al. reported that CyA reduces MPA enterohepatic recirculation by inhibiting the multidrug resistance-associated protein 2 transporter, which leads to reduced intestinal reabsorption of MPA after breakdown of MPAG by the intestinal flora [24]. Other reports presented a different mechanism by which CyA reduces the reabsorption of MPAG by hepatic cells from the circulating blood by inhibiting the organic anion transporting polypeptide (OATP)-1B3 and OATP-1B1 which, consequently, decreases the amount of enterohepatic circulation [25,26].

Regarding the interaction between EVR and MMF, Cattaneo et al. compared MPA pharmacokinetics in 21 patients treated with sirolimus (SRL), mammalian target of rapamycin inhibitors, as with EVR, or CyA in addition to MMF. In their report, the mean MPA-AUC0-12 was higher in SRL- than in CyA-treated patients. The pharmacokinetic profile of SRL-treated, but not CsA-treated, patients showed a second peak. Moreover, SRL and CsA had different effects on MPA metabolism [27]. Aurelija et al. conducted a comparative analysis between MMF with CsA or EVR treatment groups. MPA-AUC0–12 exposure was 43% higher in patients treated with a medium dose of MMF and EVR than in patients treated with a medium

dose of MMF and CsA [28]. They concluded that CyA has an impact on the main MPA pharmacokinetic parameters in a CyA dose-dependent manner, whereas EVR mildly influenced or did not affect MPA pharmacokinetic parameters.

Consistent with these reports, the results of our study showed that EVR did not affect MPA-AUC0-12 in the consecutive analysis of MPA pharmacokinetics before and after EVR addition. An alternative hypothesis for the decreased MPA-C6 after EVR addition, despite constant MPA-C0, -C1, -C2, -C4, and AUC0-12, is that EVR might not affect the absorption, distribution, and elimination of MPA, but has a slightly suppressive effect on MPA recirculation. That is, it could partly inhibit the enterohepatic recirculation of MPA, which falls short of an apparent MPA-AUC0-12 decrease.

This study has some limitations. First, our study involved a small number of patients, and more accurate data may be obtained in larger study groups. Second, the value of the MPA concentrations in this study may be slightly overestimated by the serum acyl-glucuronide metabolite (AcMPAG) concentration because the antibody used to measure MPA concentration by PETINIA has cross-reactivity with AcMPAG [29]. Thus, the universality of MPA pharmacokinetic analysis in this study may be limited. Third, MPA-AUC0-12 was determined based on a relatively few points of serum MPA concentration. We used five points (C0, C1, C2,

C4, and C6) as a surrogate for AUC because it was the upper limit to conciliate patient burden and study efficacy under clinical conditions. Of course, more frequent measurement of MPA concentration may help in assessing AUC more precisely. We believe that our 5-point equation, including MPA-C6, is sufficient to practically assess enterohepatic recirculation.

In conclusion, although our results must be interpreted within the context of the limitations, we demonstrated that EVR did not influence MPA-AUC0-12/D in the consecutive analysis of MPA pharmacokinetics, and that MMF and EVR can be safely combined with immunosuppressive therapy at therapeutic doses. In addition, it may not be essential to change the dose of MMF after the addition of EVR after KTx. Whether to change LSS for MMF after EVR addition should be considered in a larger study.

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Figure legends

Figure 1. Immunosuppressive protocol and timing of pharmacokinetic analysis of the drugs. Rituximab was alternatively administered to patients with ABO incompatibility. Tac, tacrolimus; MMF, mycophenolate mofetil; MPA, mycophenolic acid; mPSL, methylprednisolone; EVR, everolimus; KTx, kidney transplantation; C0, C1, C2, C4, C6; blood concentrations measured immediately before administration and 1, 2, 4, and 6 h after administration, respectively; Pre-EVR PK analysis; pharmacokinetics analysis performed before EVR addition, Post-EVR PK analysis; pharmacokinetics analysis performed after EVR addition.

Figure 2. Mean plasma mycophenolic acid (MPA) concentration-time curves before and after everolimus (EVR) addition. The concentration of MPA is plotted as the value per dose. Mean MPA-C6 per dose (MPA-C6/D) after EVR addition was significantly lower than that before EVR addition (2.5 ± 0.9 vs. 3.4 ± 2.2 ng/mL/g; $p = 0.04$). Mean MPA-C0/D, -C1/D, -C2/D, and -C4/D before and after EVR addition exhibited no significant change (p=0.42, 0.10, 0.16, and 0.24, respectively). Pre-EVR; data before EVR addition, Post-EVR; data after EVR addition. MPA-C0, -C1, -C2, -C4, and -C6; blood concentrations of

mycophenolic acid measured immediately before administration and 1, 2, 4, and 6 h after administration. Statistical significance was set at $p < 0.05$. The error bar denotes standard deviation.

Figure 3. Comparison of MPA-AUC0-12 per dose before and after everolimus (EVR) addition. Pre-EVR: Data before EVR addition, Post-EVR: Data after EVR addition. MPA, mycophenolic acid; EVR, everolimus; AUC, area under the concentration–time curve. Statistical significance was set at $p < 0.05$. The error bar denotes standard deviation.

Table 1. Patient characteristics

Recipients	
Age (y)	47.1 ± 8.3
Sex (male/female)	15/5
BMI (kg/m^2)	23.1 ± 3.4
Primary disease DKD/IgAN/ADPKD/others or unknown	5/3/2/10
ABO blood type compatibility (C/I)	14/6
PEKT/Non-PEKT	11/9
Interval between the first PK analysis and KTx (d)*	92.4 ± 5.7
Interval between the initiation of EVR and KTx (d)**	128.5 ± 27.9
Interval between the second PK analysis and KTx (d)***	157.4 ± 30.2
Donors	
Age (year)	58.0 ± 10.8
Sex (male/female)	6/14
Living-related donor/deceased donor	20/0

*Mean interval from kidney transplantation to first pharmacokinetics analysis of tacrolimus and mycophenolic acid. **Mean interval from kidney transplantation to everolimus (EVR) addition. ***Mean interval from kidney transplantation to second pharmacokinetics analysis of tacrolimus, mycophenolic acid, and EVR, BMI; body mass index, DKD; diabetic kidney disease, IgAN; IgA nephropathy, ADPKD; autosomal dominant polycystic kidney disease, C/I; Compatible/Incompatible, PEKT; Preemptive kidney transplantation, PK; pharmacokinetics, KTx; kidney transplantation

Table 2. Results of tacrolimus (Tac), mycophenolic acid (MPA), and everolimus (EVR) pharmacokinetics and laboratory data before and after EVR addition

	pre-EVR	post-EVR	*p*-value
Tac			
C0/D (ng/mL/mg)	1.3 ± 0.6	1.2 ± 0.6	0.08
AUC0-24/D (ng・h/mL/mg)	50.4 ± 20.6	49.7 ± 21.4	0.80
MPA			
C0/D (ng/mL/g)	2.4 ± 1.5	2.8 ± 1.6	0.42
C1/D (ng/mL/g)	7.1 ± 5.3	10.5 ± 7.1	0.10
C2/D (ng/mL/g)	6.8 ± 2.7	8.1 ± 3.5	0.16
C4/D (ng/mL/g)	4.8 ± 2.9	4.2 ± 2.2	0.24
C6/D (ng/mL/g)	3.4 ± 2.2	2.5 ± 0.9	0.04
AUC0-12/D (ng・h/mL/g)	48.4 ± 19.8	50.5 ± 15.5	0.58
EVR			
C0/D (ng/mL/mg)	-	3.4 ± 0.8	-
AUC0-12/D (ng・h/mL/mg)	-	63.7 ± 16.9	-
Laboratory data			
Hemoglobin (g/dL)	12.5 ± 1.0	12.8 ± 1.4	0.10
Aspartate transaminase (IU)	18.2 ± 5.3	19.5 ± 5.3	0.21
Alanine transaminase (IU)	17.3 ± 7.8	17.4 ± 7.6	0.95
Serum albumin (g/dL)	6.0 ± 7.3	4.4 ± 0.8	0.35
Total bilirubin (mg/dL)	0.61 ± 0.23	0.62 ± 2.0	0.75
eGFR (mL/min/1.73 m^2)	50.1 ± 11.6	49.2 ± 10.6	0.33

Pre-EVR; Data before EVR addition, Post-EVR; Data after the EVR addition, C0, C1, C2, C4, C6; blood concentration of each drug measured immediately before administration and 1, 2, 4, and 6 h after administration, AUC; area under the concentration–time curve, /D; per dose, eGFR; estimated glomerular filtration rate. Statistical significance was set at $p < 0.05$.

Table 3. Correlation between MPA-AUC0-12/D and EVR-C0/D, EVR-AUC0-12/D, and other clinical parameters

	Simple liner regression				
	Coefficient (β)	*t*-value	*p*-value	95% CI	adjusted R2
EVR-C0/D	−4.719	−1.117	0.278	−13.587 to 4.149	0.012
EVR-AUC0-12/D	−0.083	−0.376	0.710	−0.549 to 0.382	−0.047
Tac-C0/D	−2.649	−0.441	0.664	−15.260 to 9.960	−0.044
Tac-AUC0-24/D	−0.076	−0.451	0.657	−0.434 to 0.281	−0.043
BMI (kg/m^2)	−1.778	−1.735	0.001	−3.930 to -0.374	0.095
Age (y)	0.778	1.993	0.061	−0.042 to 1.599	0.135
Hemoglobin (g/dL)	−7.026	−2.182	0.042	−13.789 to -0.262	0.165
Aspartate transaminase (IU)	−0.443	−0.652	0.522	−1.872 to 0.985	−0.031
Alanine transaminase (IU)	−0.717	−1.584	0.130	−1.669 to 0.233	−1.584
Serum albumin (g/dL)	5.731	1.366	0.188	−3.080 to 14.543	0.043
Total bilirubin (mg/dL)	−7.922	−0.449	0.658	−44.968 to 29.123	−0.043
eGFR (mL/min/1.73m^2)	0.147	0.428	0.673	−0.575 to 0.869	−0.044

95% CI; 95% confidence interval, MPA; mycophenolic acid, Tac; tacrolimus, EVR; everolimus, C0; blood concentration of each drug measured immediately before, AUC; area under the concentration-time curve, /D; per dose, eGFR; estimated glomerular filtration rate, BMI; body mass index

Statistical significance was set at $p < 0.05$. Statistical significance is defined as $t > 2.0$ or $t<-0.2$

Table 4. Estimation equation using two time-points for limited sample strategy of MPA-AUC0-12 before and after everolimus (EVR) addition

	Pre-EVR				Post-EVR			
Sampling point	Estimated AUC	r2	*p*	RMSE	Estimated AUC	r2	*p*	RMSE
C0,C1	3.23*C0 + 1.70*C1+30.73	0.52	< 0.01	11.48	5.70 * C0 + 1.39 * C1 + 22.45	0.72	< 0.01	10.15
C0,C2	3.14*C0 + 2.39*C2+26.18	0.34	0.01	13.41	4.07 * C0 + 2.83 * C2 + 17.73	0.71	< 0.01	10.35
C0,C4	1.21*C0 + 4.25*C4+28.27	0.39	< 0.01	12.96	6.21*C0 + 1.50*C4+31.30	0.27	0.06	16.57
C0,C6	3.13*C0 + 6.81*C6+19.73	0.60	< 0.01	10.49	6.14*C0 + 6.62*C6+19.63	0.38	0.01	15.24
C1,C2	1.50*C1 + 1.84*C2+27.37	0.48	< 0.01	11.96	0.83*C1 + 2.33*C2+25.13	0.71	< 0.01	10.34
C1,C4	1.49*C1 + 4.02*C4+20.72	0.68	< 0.01	9.37	1.65*C1 + 3.82*C4+19.29	0.67	< 0.01	11.01
C1,C6	1.44*C1 + 6.06*C6+19.84	0.72	< 0.01	8.72	1.52*C1 + 7.95*C6+16.28	0.68	< 0.01	10.85
C2,C4	2.94*C2 + 5.09*C4+4.37	0.73	< 0.01	8.52	3.17*C2 + 2.04*C4+17.67	0.65	< 0.01	11.35
C2,C6	2.19*C2 + 6.58*C6+12.55	0.64	< 0.01	9.96	3.05*C2 + 5.36*C6+13.21	0.69	< 0.01	10.77
C4,C6	2.96*C4 + 5.10*C6+19.65	0.58	< 0.01	10.77	1.77*C4 + 6.88*C6+30.16	0.13	0.16	18.02

Pre-EVR; Data before the EVR addition, Post-EVR; Data after the EVR addition, C0, C1, C2, C4, C6; blood concentration of each drug measured immediately before administration and 1, 2, 4, and 6 h after administration, RMSE; root-mean-square error, AUC; area under the concentration-time curve.

Figure 1

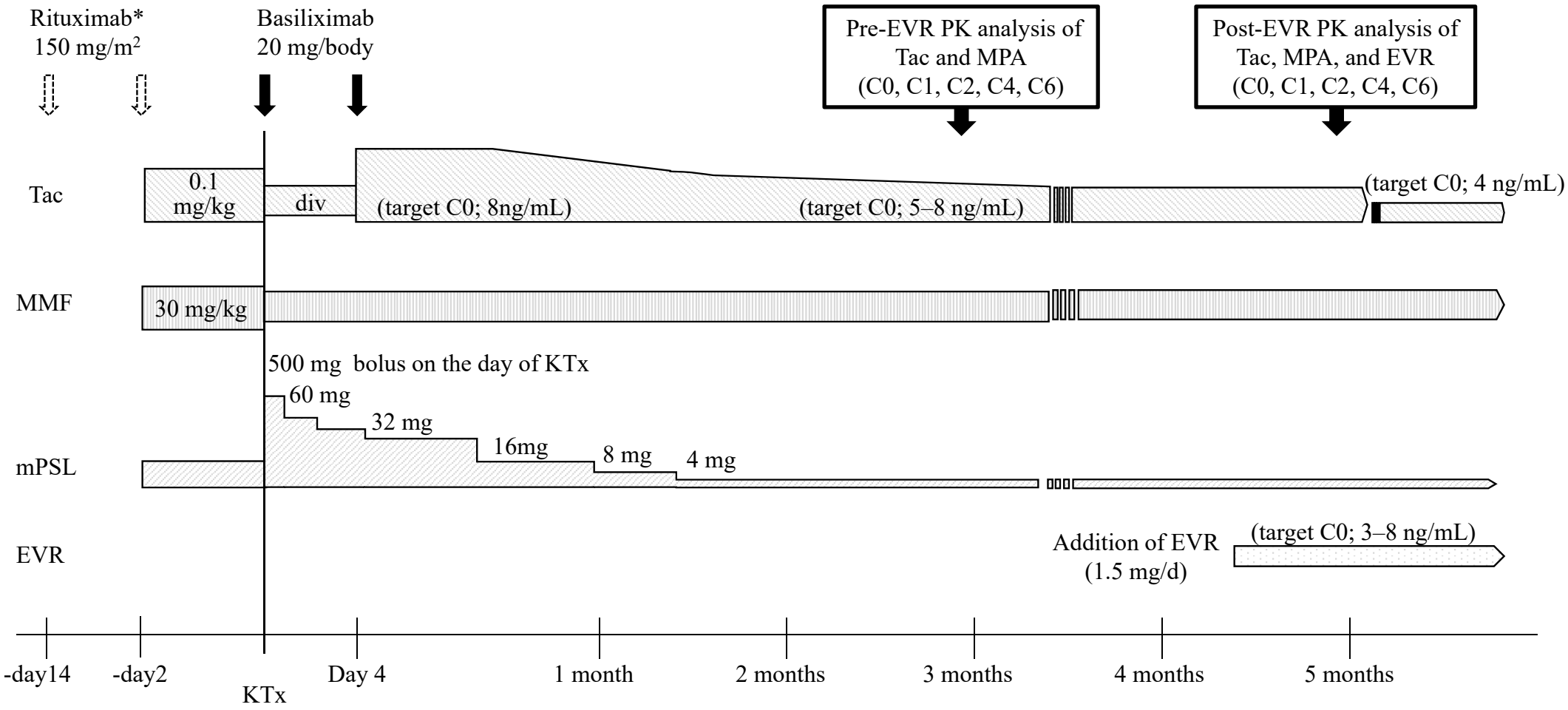

Rituximab*
150 mg/m2
Basiliximab
20 mg/body
Pre-EVR PK analysis of
Tac and MPA
(C0, C1, C2, C4, C6)
Post-EVR PK analysis of
Tac, MPA, and EVR
(C0, C1, C2, C4, C6)
Tac
0.1
mg/kg
div
(target C0; 8ng/mL)
(target C0; 5–8 ng/mL)
(target C0; 4 ng/mL)
MMF
30 mg/kg
500 mg bolus on the day of KTx
60 mg
32 mg
16mg
8 mg
4 mg
mPSL
EVR
Addition of EVR
(1.5 mg/d)
(target C0; 3–8 ng/mL)
-day14
-day2
KTx
Day 4
1 month
2 months
3 months
4 months
5 months

Figure 2

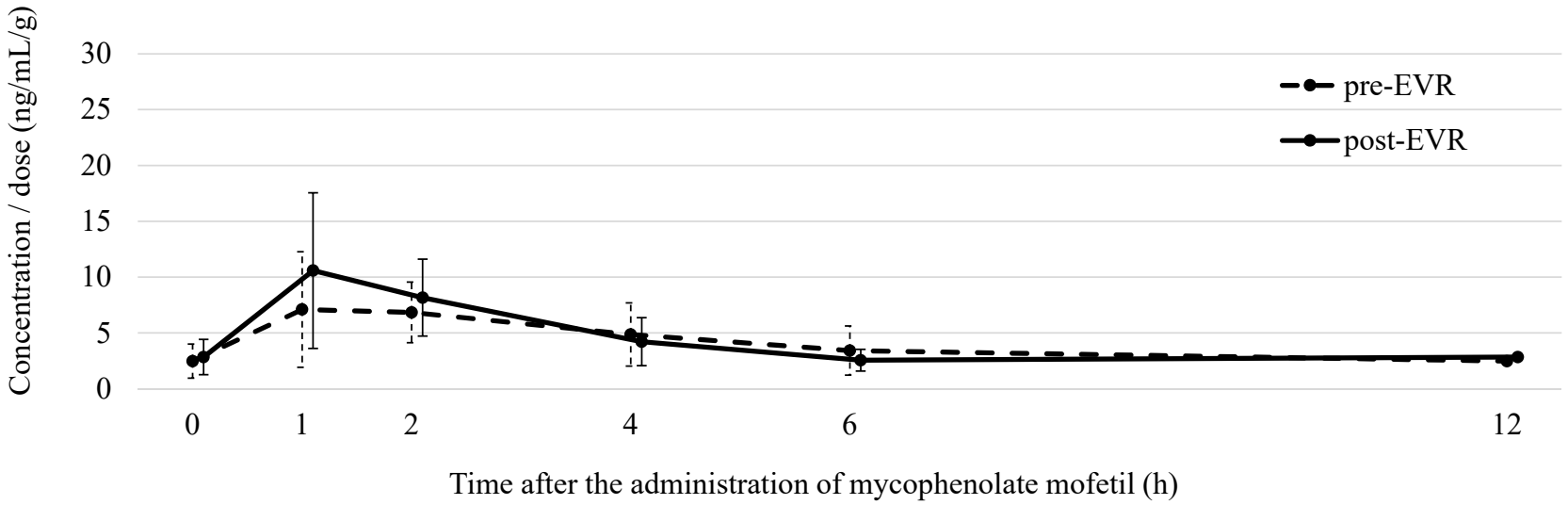
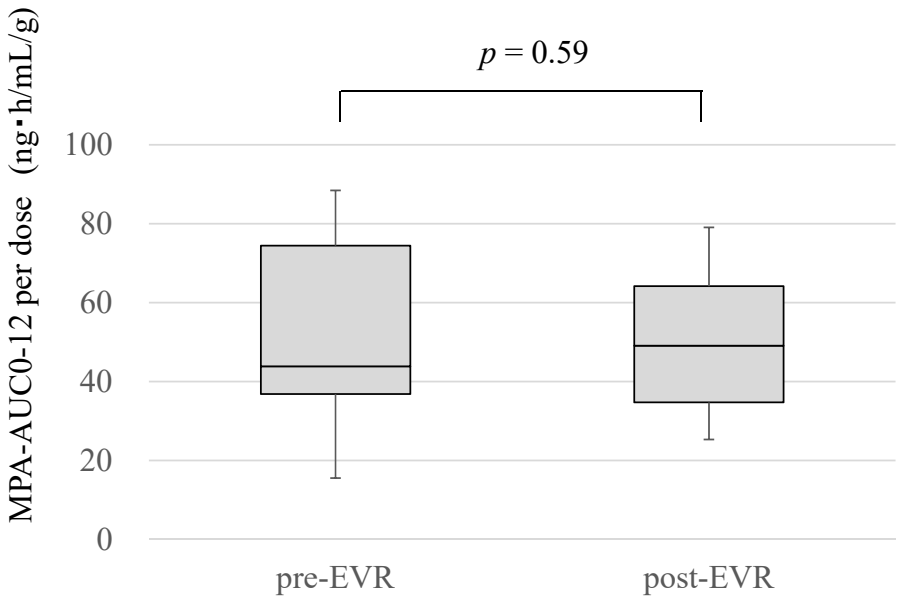

Concentration / dose (ng/mL/g)
30
25
20
15
10
5
0
0
1
2
4
6
12
Time after the administration of mycophenolate mofetil (h)
pre-EVR
post-EVR

Figure 3


p = 0.59
MPA-AUC0-12 per dose (ng・h/mL/g)
100
80
60
40
20
0
pre-EVR
post-EVR