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Pharmacokinetics and Pharmacodynamics of Once-Daily Tacrolimus Compared with Twice-Daily Tacrolimus in the Early Stage After Living Donor Liver Transplantation

Short title: PK and PD of Once-Daily Tacrolimus

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Abstract

BACKGROUND: This study investigates the pharmacokinetics and pharmacodynamics of tacrolimus using the once-daily (OD) formulation in the early stage after living donor liver transplantation (LDLT) in comparison with those using the twice-daily (TD) formulation.

METHODS: Nine patients undergoing primary LDLT and treated with the OD tacrolimus formulation were included. The trough blood concentration (C_0) of tacrolimus was monitored every day for three weeks after LDLT. A time-course study of the blood tacrolimus concentrations and calcineurin (CN) phosphatase activity in peripheral blood mononuclear cells was performed three weeks after LDLT. Pharmacokinetic and pharmacodynamic parameters were compared with previously reported data using the TD formulation.

RESULTS: The inter-individual variability in the daily dose of tacrolimus was significantly larger in the OD formulation than in the TD formulation ($P < 0.001$). In the time-course study, the tacrolimus blood concentrations at 4, 8, and 12 hours post-administration and the area under the concentration-time curve from 0 to 24 hours ($AUC_{0–24}$) in the OD group were significantly higher than in the TD group, although the C_0 was equivalent. In addition, the C_0 was not significantly correlated with the $AUC_{0–24}$ in the OD formulation. The apparent

clearance and the pharmacodynamic parameters examined were not significantly different between the OD and TD groups.

CONCLUSIONS: The C_0 monitoring of the OD formulation may not be optimal in patients at the early stage after LDLT since the C_0 was not correlated with the $AUC_{0–24}$. If clinicians target the same C_0 using the OD and TD formulations, the exposure of tacrolimus can be higher in the OD formulation, and excessive immunosuppression should be noted. Particular attention should be paid to the patients in the early stage after LDLT in the use of the OD oral formulation of tacrolimus.

KEYWORDS: tacrolimus; living donor liver transplantation (LDLT); once-daily formulation (OD); pharmacokinetics; calcineurin (CN) activity

After living donor liver transplantation (LDLT), tight control of immunosuppressive therapy is necessary to prevent acute rejections. The calcineurin (CN) inhibitor tacrolimus is one of the primary immunosuppressants used for the prevention of acute rejection after LDLT. In this context, therapeutic drug monitoring of tacrolimus is recommended to adjust the dosage, because of its narrow therapeutic range and its highly inter-individually variable pharmacokinetics.[1, 2, 3] In previous studies based on the twice-daily (TD) formulation of tacrolimus, the area under the curve (AUC) after the oral administration had a nearly linear relationship with the trough blood concentration (C_0). Therefore, the C_0 of tacrolimus is usually monitored to adjust the dosage of this drug[4].

The TD formulation was first developed with oral formulations of tacrolimus. Later, the once-daily (OD) prolonged-release formulation was developed to provide a more convenient formulation that was intended to improve patient adherence, and since then, several OD products have become available worldwide. Pharmacokinetic comparisons between the TD and OD formulations have been reported in a few studies mostly focused on the renal transplant field.[5, 6] The AUC and C_0 of tacrolimus in the OD formulation were shown to be nearly the same as in the TD formulation with no difference in the average dose of tacrolimus in the early stage after renal transplantation.[5] However, in a study using another

extended-release OD product, the C_0 per daily dose ratio was higher in the OD formulation than in the TD formulation after renal transplantation.[6] Therefore, the pharmacokinetics of tacrolimus using the OD formulation may differ depending on the prolonged-release mechanisms.

In the liver transplant field, there have been several studies on the conversion of the tacrolimus formulation from TD to OD in the stable stage after transplantation.[7] However, studies using OD tacrolimus formulations in the early stage after LDLT are limited. In patients in the early stage after LDLT, the inter-individual variability of tacrolimus pharmacokinetics can be more substantial than in LDLT patients in the stable stage or renal transplant patients. Liver function depends on the degree of regeneration and recovery of the grafted liver, and may affect tacrolimus metabolism.[8] Moreover, the absorption of tacrolimus may be low due to reduced movement of the small intestine in these patients[9, 10]. Therefore, information on tacrolimus pharmacokinetics using the OD formulation is needed in patients in the early stage after LDLT.

The measurement of CN phosphatase activity in peripheral blood mononuclear cells (PBMCs) is a pharmacodynamic biomarker used to evaluate the immunosuppressive effect of CN inhibitors[11]. The CN activity at the trough time point was suggested to be a surrogate

indicator of overall CN activity throughout the dosing intervals after administration of the TD tacrolimus formulation in LDLT patients[12]. In this study, we investigated the pharmacokinetics and pharmacodynamics of tacrolimus using the OD formulation with simultaneous measurements of blood tacrolimus concentrations and CN activity in the early stage after LDLT and compared the data with previously reported data of the TD formulation[12].

Materials and Methods

STUDY DESIGN

Nine patients, who had undergone primary LDLT at the Division of Hepato-Biliary-Pancreatic Surgery and Transplantation in Kyoto University Hospital between October 2010 and February 2012 and who were treated with the OD formulation (Graceptor; Astellas Pharma, Tokyo, Japan), were included in this study. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki as reflected in *a priori* approval by the Kyoto University Graduate School and Faculty of Medicine and Kyoto University Hospital Ethics Committee. Written informed consent was obtained from each patient.

Immunosuppressive therapy with tacrolimus was started in the morning on the day after the LDLT. The first oral daily dose of the OD formulation was 10 mg and was started at nine o'clock in the morning. The initial daily dose in two patients was decreased by the decision of an attending surgeon. The dose was adjusted to target the blood tacrolimus concentration according to the trough measurement for three weeks after the transplantation. The target trough concentration was set between 10 and 15 ng/mL on postoperative days (PODs) 1-7, between 8 and 12 ng/mL on PODs 8-14, and between 6 and 10 ng/mL after POD 15. Patients treated with the OD tacrolimus formulation also received mycophenolate mofetil and methylprednisolone, according to a standard immunosuppressive regimen.

For comparison, we used previously reported data from 13 patients who had undergone LDLT between November 2007 and March 2009 and were treated with tacrolimus using the TD formulation (Prograf; Astellas Pharma, Tokyo, Japan) at nine o'clock in the morning and evening[12]. The recommended first oral dose of tacrolimus in the TD formulation group was 0.05 mg/kg/day. After that, the dose of tacrolimus was adjusted to the target trough concentration as described for patients receiving the OD formulation. No patients received potent inhibitors of CYP3A4 during the study period.

ANALYTICAL METHODS

Blood samples were taken every day before the morning administration of tacrolimus. Six additional samples were taken on a single day between PODs 19-23 (three weeks after LDLT) at 1, 2, 4, 8, 12, and 24 hours after the tacrolimus administration. The tacrolimus concentration was measured using the Architect i1000SR (Abbott Japan, Tokyo, Japan). Samples were assayed as soon as possible after the blood collection. Otherwise, they were stored at -20 °C until the assay could be performed.

CN phosphatase activity in PBMCs was measured using the remainder of the blood sample after the blood concentration measurement, as a pharmacological biomarker of tacrolimus. The assay of CN phosphatase activity in PBMCs was performed using a γ-^{32}P-labeled regulatory subunit type II phosphopeptide as a substrate, according to a previously described procedure.[13] On the day of transplantation, a blood sample was obtained from some patients to determine the baseline CN activity before the administration of tacrolimus.

PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

The AUC from 0 to 24 hours ($AUC_{0–24}$) after tacrolimus administration was calculated according to the trapezoidal rule in the OD formulation. For the comparison, $AUC_{0–24}$ in the TD formulation was calculated by twice the value of $AUC_{0–12}$ that was calculated according to the trapezoidal rule, because there was no tacrolimus concentration data obtained after 12

hours in the time-course study in the TD formulation[12]. The highest observed concentration and associated time point were defined as the maximum drug concentration (C_{max}) and the time at which the maximum concentration occurred (T_{max}), respectively. The apparent clearance (CL/F) was calculated by dividing the corresponding daily dose on each study day by the $AUC_{0–24}$. The area under the CN activity-time curve from 0 to 24 hours ($AUA_{0–24}$) after the tacrolimus administration was also calculated according to the trapezoidal rule in the OD group. The AUA_{0-24} in the TD formulation was also calculated as twice the value of $AUA_{0–12}$. The greatest observed CN inhibition, which caused a nadir of CN activity, and its associated time point were defined as CN_{nadir} and T_{nadir}, respectively. The relationship between the blood tacrolimus concentration and CN activity in PBMCs was analyzed using the following maximum inhibitory effect (E_{max}) model:

$$CN = E_{max} - E_{max} \times C / (EC_{50} + C)$$

where CN is the CN activity at the blood concentration (C); E_{max} is the maximum inhibitory effect attributable to the drug, which is assumed to be the same as the baseline activity; EC_{50} is the blood concentration that gives a half-maximal effect. The fixed parameters, E_{max} and EC_{50}, were estimated using the nonlinear mixed effects modeling program NONMEM 7.2.0 (ICON Development Solutions, Ellicott City, MD, USA). Inter-individual variability for E_{max}

and EC_{50} and residual variability were assumed to be a log-normal distribution and normal distribution, respectively[13].

STATISTICAL ANALYSIS

Data are presented as the mean ± the standard deviation (SD). Statistical analyses were performed using the statistical software package GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). The statistical significance of the difference in mean values between the two groups was analyzed using an unpaired t-test if the variances of the two groups were similar. Otherwise, the Mann-Whitney's U test was used for the analysis. The variance between the two groups was analyzed using an F-test. The role of gender, ABO blood type, and primary disease was assessed using a Chi-square test. Pearson's correlation coefficient (r) was used to estimate the correlation between the tacrolimus blood concentration in the respective time point and AUC_{0-24} after administration. A value of $P < 0.05$ was considered to be statistically significant.

Results

STUDY POPULATION

Pharmacokinetic and pharmacodynamic data from nine LDLT patients treated with the OD tacrolimus formulation were compared with previously reported data from 13 patients treated

with the TD formulation[12]. The patient's demographic characteristics are summarized in Table 1. The mean age in the OD group was significantly younger than in the TD group (51 vs. 58 years old, $P = 0.048$). The mean initial daily dose of tacrolimus in the OD group was approximately five-times larger than in the TD group (0.153 vs. 0.032 mg/kg/day, respectively), although the mean maintenance daily dose of tacrolimus three weeks after LDLT were not significantly different between the two groups.

COMPARISON OF DAILY DOSE AND EXPOSURE

The mean daily dose of tacrolimus in the OD group gradually decreased in the early post-transplant period, after that it tended to increase, and then reached a plateau, although the mean daily dose of tacrolimus in the TD group was almost constant for three weeks after the transplantation (Fig. 1A). The inter-individual variability of the daily dose in the OD group was significantly larger than in the TD group ($P < 0.001$). The doses and trough concentrations of tacrolimus on POD 1 were significantly higher in the OD group compared with those in the TD group (Fig. 1A, B). Similar trough concentrations between the two formulations were obtained by day 4 after dose adjustments.

TIME PROFILES OF THE BLOOD TACROLIMUS CONCENTRATIONS AND CN ACTIVITIES

Figure 2 shows the time-course profiles of the tacrolimus blood concentrations and CN activities three weeks after LDLT. The tacrolimus blood concentrations at 4, 8, and 12 hours post-administration in the OD group were significantly higher than in the TD group, although the C_0 was nearly equivalent (Fig. 2A). The tacrolimus blood concentrations adjusted by the daily dose per body weight were not significantly different between the two groups (Fig. 2B). The CN activities gradually decreased 4 hours after the administration, and after that gradually increased as the tacrolimus blood concentrations decreased (Fig. 1C). The CN activity returned to pre-dose levels 12 hours post-dose in the TD group or 24 hours post-dose in the OD group. The CN activity at each time was not significantly different between the two groups.

Table 2 shows the pharmacokinetic and pharmacodynamic parameters of tacrolimus in the OD and TD formulations. The median of T_{max} in the OD group was significantly greater than in the TD group. The C_{max}, C_{max}/C_0, and $AUC_{0–24}$ were significantly greater in the OD group compared with those in the TD group. The CL/F and the examined pharmacodynamic parameters were not significantly different between the OD and TD groups.

Correlation between each blood concentration and the AUC of tacrolimus

As mentioned in the previous report[12], the C_0 was highly correlated with the $AUC_{0–12}$ after the administration of the TD formulation. However, the correlation between the $AUC_{0–24}$ and C_0 was not significant (r^2 = 0.189, P = 0.282) in the OD formulation (Fig. 3A). Instead, the blood concentrations at 4, 8, 12, and 24 hours post-dose (C_4, C_8, C_{12}, and C_{24}, respectively) showed a significant correlation with the $AUC_{0–24}$ as the r^2 value was > 0.5, and C_{12} showed the highest correlation (r^2 = 0.995, P < 0.001).

RELATIONSHIP BETWEEN THE CN ACTIVITIES AND BLOOD CONCENTRATIONS

The CN activities in PBMCs were inhibited in a concentration-dependent manner by tacrolimus in both the OD and TD formulations (Fig. 4A). By applying the E_{max} model to data obtained from 22 patients using the OD and TD formulations, the EC_{50} was calculated as 21.8 ng/mL (95% confidence interval (CI), 10.3-33.3) and the E_{max} as 62.7 pmol/min/mg protein (95% CI, 53.7-71.7) using the nonlinear mixed effects model. Inter-individual variabilities for EC_{50} and E_{max} were 75.4% and 23.6% as a coefficient of variation %, respectively. Residual variability was 3.61 pmol/min/mg protein as a standard deviation.

The CN activity at pre-dose (CN_0) in the TD group showed a strong correlation with the $AUA_{0–24}$ ($r^2 = 0.965$, $P < 0.001$; Fig. 4B). In the OD group, the CN_0 was also well correlated with the $AUA_{0–24}$ ($r^2 = 0.775$, $P = 0.009$). The slope of two linear regressions was similar.

Discussion

Unexpectedly, the C_0 was not correlated with the $AUC_{0–24}$ after administration of the tacrolimus OD formulation in the early stage after LDLT, showing that the C_0 monitoring may not be optimal for these patients. If clinicians target the same C_0 using the OD and TD formulations, the exposure of tacrolimus, namely the $AUC_{0–24}$, can be substantially higher in the OD formulation than in the TD formulation, and excessive immunosuppression may occur.

While the C_0 and $AUC_{0–24}$ were not significantly correlated, the C_{24} was well correlated with the $AUC_{0–24}$ in the OD formulation (Fig. 3). The morning dose of tacrolimus was changed from the previous morning dose in some patients according to the tacrolimus concentration measurement. Although the C_{24} reflects the $AUC_{0–24}$ of tacrolimus after the morning dose, the C_0 reflects the $AUC_{0–24}$ of tacrolimus after the morning dose on the previous day. Therefore, the C_{24}, but not the C_0, was well correlated with the $AUC_{0–24}$. In fact, on the day of the time-course study, six out of nine patients in the OD formulation took a different tacrolimus

dose compared with the dose on the previous day. It was reported that the $AUC_{0–24}$ was well correlated with the C_0 both in the TD and OD tacrolimus formulations in the stable liver transplant recipients[14]. In addition, the C_0 has shown a good correlation with the AUC_{0-24} in the TD and OD formulations in the early stage of renal transplantation[15]. Control of the tacrolimus trough concentrations and the daily dose adjustments of the OD formulation are considered to be manageable in the early stage of renal transplantation. In the early stage after LDLT, since the pharmacokinetics and the daily dose of tacrolimus are variable in the OD formulation, it is difficult to predict the $AUC_{0–24}$ of tacrolimus using the C_0 even three weeks after LDLT.

The daily maintenance dose of tacrolimus in the OD formulation was almost twice that in the TD group (Fig. 1 and Table 1), although the C_0 was nearly equivalent (Fig. 1 and Table 2). These results were in agreement with the previous report, showing that when *de novo* liver transplant patients were administered tacrolimus as the OD or TD formulation, the dose of OD formulation became larger than that of TD formulation to get the same C_0[16]. Additionally, the $AUC_{0–24}$ tended to be higher in the OD formulation under the same C_0 (Fig. 2), consistent with the previous report in the early stage of liver transplantation[16]. Since the CL/F in the TD and OD formulations were similar (Table 2), we considered that the bioavailability of the two

formulations was similar. A higher dose per time and day in the OD formulation made a higher peak concentration and higher AUC_{0-24} in the OD group despite the same trough concentration. Similarly, a significant decrease in the tacrolimus C_0 was observed one month after a 1:1 conversion from the TD to OD formulation in long-term stable liver transplant recipients.[17, 18] Previous reports have demonstrated a safe conversion to OD formulation without increasing the risk of liver dysfunction or rejection.[19, 20] Taking these findings into consideration, the target trough concentration might be decreased in the OD formulation to prevent excess exposure at the treatment initiation or the conversion to the OD formulation in LDLT patients.

There were no differences in the relationship between the CN activity and tacrolimus concentrations depending on the tacrolimus formulations (Fig. 4A), so we can expect the same degree of immunosuppression in the setting of the same target AUC of OD and TD formulations. However, since there is a large inter-individual variability in the pharmacodynamics of tacrolimus, the AUC measurements alone are insufficient to predict the pharmacodynamic effects. Measuring the CN activity in addition to the concentrations of tacrolimus can be effective in deciding the individual pharmacotherapy[11].

In our previous report[12], the correlation between the C_0 and the $AUC_{0–12}$ was shown in the TD formulation three weeks after LDLT, and the C_0 was suggested to be a good factor reflecting the AUC. In this study, we reanalyzed this using the same TD pharmacokinetic data: the relationship between the $AUC_{0–24}$ (y), calculated by doubling the $AUC_{0–12}$, and C_0 (x) was shown to be $y = 25.5 * x + 23.4$ in the TD formulation, whereas in the OD formulation, the relationship between the $AUC_{0–24}$ (y) and C_{12} (x) was shown to be $y = 25.3 * x – 14.3$. The slope in the OD formulation was almost equal to the relationship mentioned above between the $AUC_{0–24}$ and C_0 in the TD formulation. This could mean that the C_{12} is a useful marker reflecting the $AUC_{0–24}$ in the OD formulation, and monitoring the C_{12} to target the similar range of C_0 in the TD formulation can prevent excessive exposure to tacrolimus in patients at an early stage of LDLT dosed OD. However, this suggestion should be validated by an additional study using a larger number of patients, and monitoring of C_{12} might be quite impractical.

This study included some limitations. It should be noted that the concentrations of tacrolimus in whole blood were measured using the Abbott Architect assay in the OD formulation. In the TD formulation, the tacrolimus concentrations were measured using high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS), as previously reported[21].

Although the values obtained from the Abbott Architect and LC-MS/MS methods have been suggested to be well correlated[22], immunoassays usually have a positive bias compared with LC-MS/MS method[23]. In addition, OD and TD studies were performed separately, encompassing very small numbers of patients (9 *versus* 13), with stable doses in the TD group and more variable doses in the OD group. ~~and dose in the TD being quite stable, while in the OD group being quite variable.~~ Therefore, special attention should be paid to the interpretation of the present pharmacokinetic comparison between the OD and TD groups.

Conclusion

If clinicians target the same C_0 using the OD and TD formulations, systemic exposure to tacrolimus can be substantially higher after administration of the OD formulation than of the TD formulation, and excessive immunosuppression may occur in the early stage after *de novo* LDLT. Tacrolimus therapy using the OD formulation in the early stage of LDLT is associated with unstable pharmacokinetics even at three weeks after LDLT, which makes the usage of C_0 monitoring less reliable. Particular attention should be paid to the patients in the early stage after LDLT when these receive a tacrolimus OD oral formulation.

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FIGURE LEGENDS

FIGURE 1. Comparison of the daily dose (A) and trough concentrations (B) of tacrolimus between once-daily (OD; closed circles) and twice-daily (TD; open circles) formulations[12]. Each symbol represents the mean ± the standard deviation (SD). *$P < 0.05$, ***$P < 0.001$, significantly different compared with the TD group. POD: postoperative day.

FIGURE 2. Time-course of the tacrolimus blood concentrations (A), dose-corrected tacrolimus blood concentrations (B), and calcineurin (CN) phosphatase activities in peripheral blood mononuclear cells (C) three weeks after LDLT using OD (closed circles) and TD (open circles) formulations[12]. Each symbol represents the mean ± SD. *$P < 0.05$, **$P < 0.005$, significantly different compared with the twice-daily group.

FIGURE 3. Correlation between the area under the concentration-time curve from 0 to 24 h ($AUC_{0–24}$) and tacrolimus blood concentration at pre-dose, 1, 2, 4, 8, 12, and 24 h after the morning administration (C_0, C_1, C_2, C_4, C_8, C_{12}, and C_{24}, respectively) using the OD tacrolimus formulation. Solid and dotted lines show the linear regression line and its 95% confidence intervals, respectively.

FIGURE 4. Relationships between the CN phosphatase activity and tacrolimus blood concentrations (A), and between the area under the CN activity-time curve from 0 to 24 h

($AUA_{0–24}$) and the CN activity at pre-dose (CN_0) (B). Each point shows a different time point three weeks after LDLT. Closed and open circles represent data after the administration of the OD and TD formulations[12], respectively. A solid curve in panel A shows the predicted CN phosphatase activity *versus* the blood tacrolimus concentration profile according to the maximum inhibitory effect model as determined using the nonlinear mixed effects modeling program. Solid and dashed lines in panel B show linear regression line of the OD and TD formulations, respectively.

TABLE 1. Demographic characteristics of the study population.

Characteristic	**OD**	**TD**	***P*-value**
Gender (male/female)	6/3	7/6	0.548 [1]
Age (years)	51 ± 9	58 ± 6	0.048 [2]
Grafted liver weight (g)	522 ±144	616 ± 155	0.188 [2]
GRWR (%)	0.926 ± 0.211	0.952 ± 0.182	0.766 [2]
Body weight (kg)	59.8 ± 10.1	63.4 ± 9.8	0.435 [2]
Initial dose (mg/kg/day)	0.153 ± 0.051	0.032 ± 0.009	<0.001 [2]
Dose in the time-course study (mg/kg/day)	0.098 ± 0.088	0.052 ± 0.032	0.120 [2]
ABO blood group match			0.390 [1]
Identical	7	7	

Compatible	3	5	
Incompatible	0	2	
Primary disease			0.287 [1]
Hepatitis B virus infection	1	5	
Hepatitis C virus infection	4	4	
Primary biliary cirrhosis	1	3	
Others	4	2	

OD: once daily, TD: twice daily, GRWR: graft-to-recipient weight ratio

Data are presented as the number of patients or as the mean value ± standard deviation (SD).

P-value was calculated using the Chi-square[1] or unpaired t-test [2]. Data from the TD group were the same in the previous study[12].

TABLE 2. Pharmacokinetic and pharmacodynamic parameters of tacrolimus three weeks after the transplantation

Parameters	**OD (n=9)**	**TD (n=13)**	***P*-value**
Pharmacokinetic parameters			
C_0 (ng/mL)	9.0 ± 4.5	7.7 ± 2.6	0.426 [1]
T_{max} (h)	4 (0–12)	2 (0–4)	0.034 [2]
C_{max} (ng/mL)	22.3 ± 8.6	12.5 ± 4.7	0.005 [1]
C_{max} / C_0	2.46 ± 0.94	1.67 ± 0.47	0.026 [1]
$AUC_{0–24}$ (ng h/mL)	330 ± 103 [a]	220 ± 76	0.016 [1]
CL / F (L/h/kg)	0.319 ± 0.205 [a]	0.267 ± 0.213	0.612 [1]
Pharmacodynamic parameters			
CN_0 (pmol/min/mg protein)	40.5 ± 7.4 [b]	47.0 ± 10.8	0.195 [1]
T_{nadir} (h)	4 (0–12) [b]	4 (2–4) [c]	0.122 [2]

CN_{nadir} (mg/min/mg protein)	32.8 ± 9.4 [b]	39.3 ± 9.7 [c]	0.198 [1]
$AUA_{0–24}$ (pmol h/min/mg protein)	924 ± 178 [b]	1021 ± 228 [c]	0.376 [1]

C_0: Blood concentration pre-dose, C_{max}: maximum blood concentration, T_{max}: time corresponding to C_{max}, $AUC_{0–24}$: area under the concentration-time curve from 0 to 24 h, CL/F: apparent clearance, CN_0: calcineurin activity pre-dose, CN_{nadir}: calcineurin activity at maximum inhibition, T_{nadir}: time corresponding to CN_{nadir}, AUA_{0-24}: Area under the calcineurin activity-time curve from 0 to 24 h.

Data are given as the mean ± SD, or as the median with the minimum-maximum in parentheses. The values of $AUC_{0–24}$ and $AUA_{0–24}$ in the TD group was calculated as two times the $AUC_{0–12}$ and $AUA_{0–12}$, respectively.

The *P*-value was calculated using the unpaired t-test[1] or the Mann-Whitney's U test [2].

[a] n = 8 patients, [b] n = 7 patients, [c] n = 12 patients

Fig. 1

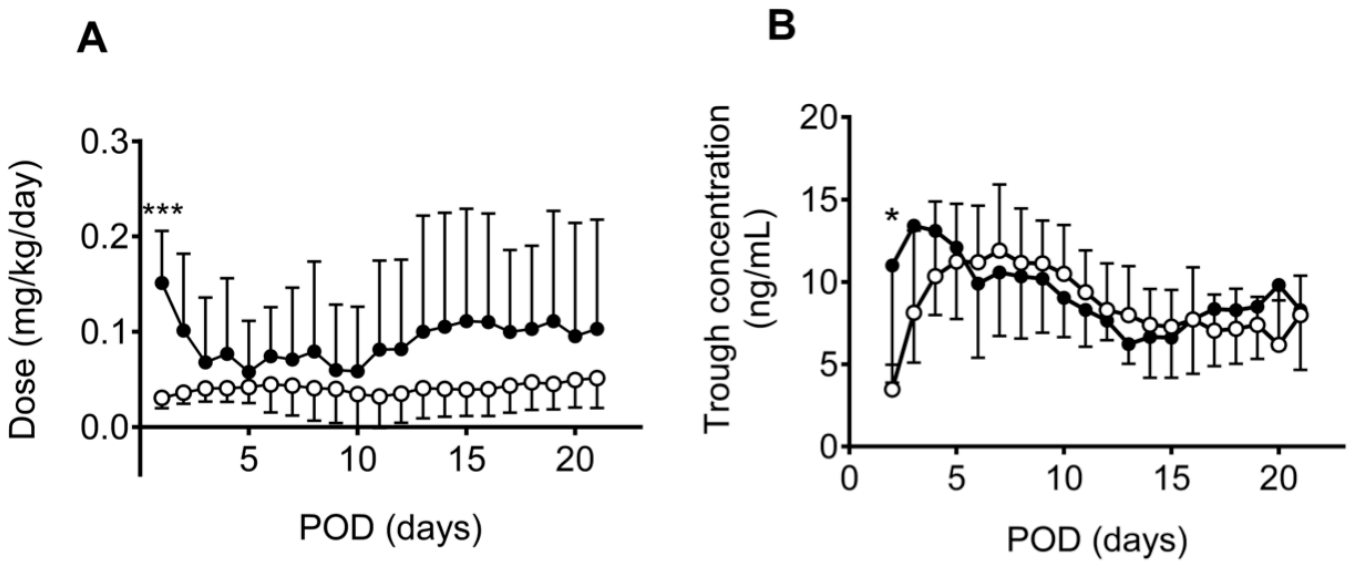

A
Dose (mg/kg/day)
0.3
0.2
0.1
0.0
***
5
10
15
20
POD (days)
B
Trough concentration (ng/mL)
20
15
10
5
0
*
0
5
10
15
20
POD (days)

Fig. 2

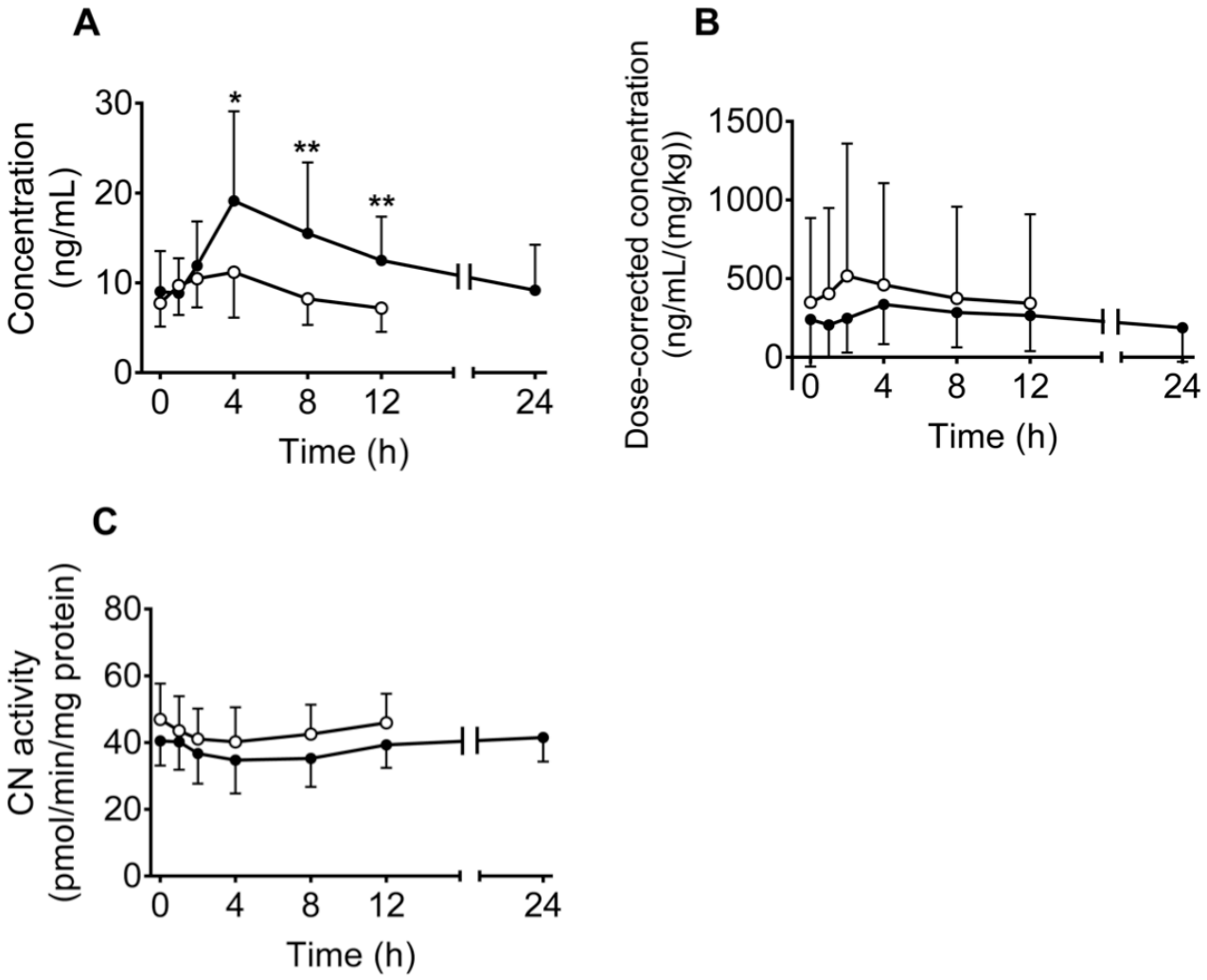

A
Concentration (ng/mL)
30
20
10
0
*
**
**
0
4
8
12
24
Time (h)
B
Dose-corrected concentration (ng/mL/(mg/kg))
1500
1000
500
0
0
4
8
12
24
Time (h)
C
CN activity (pmol/min/mg protein)
80
60
40
20
0
0
4
8
12
24
Time (h)

Fig. 3

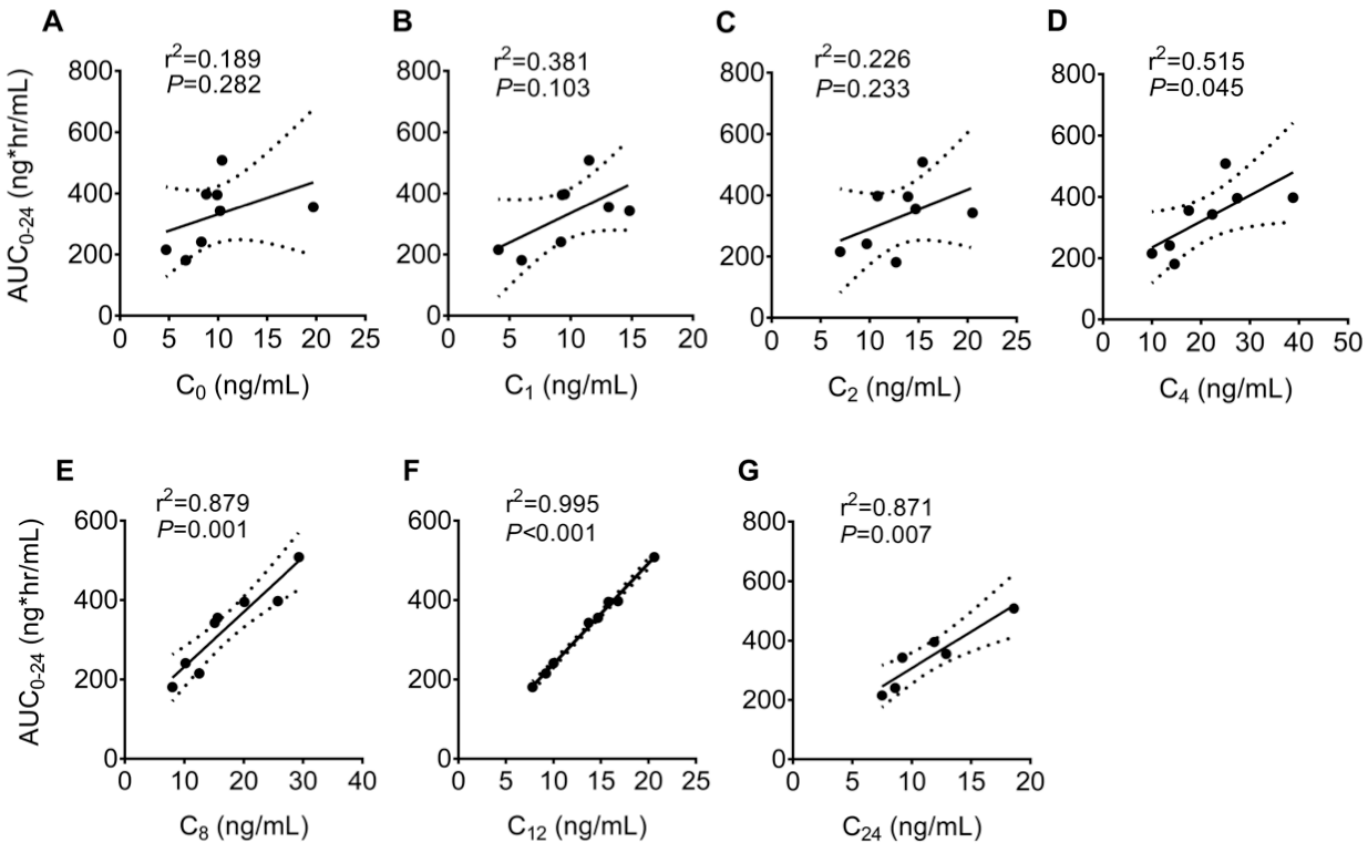
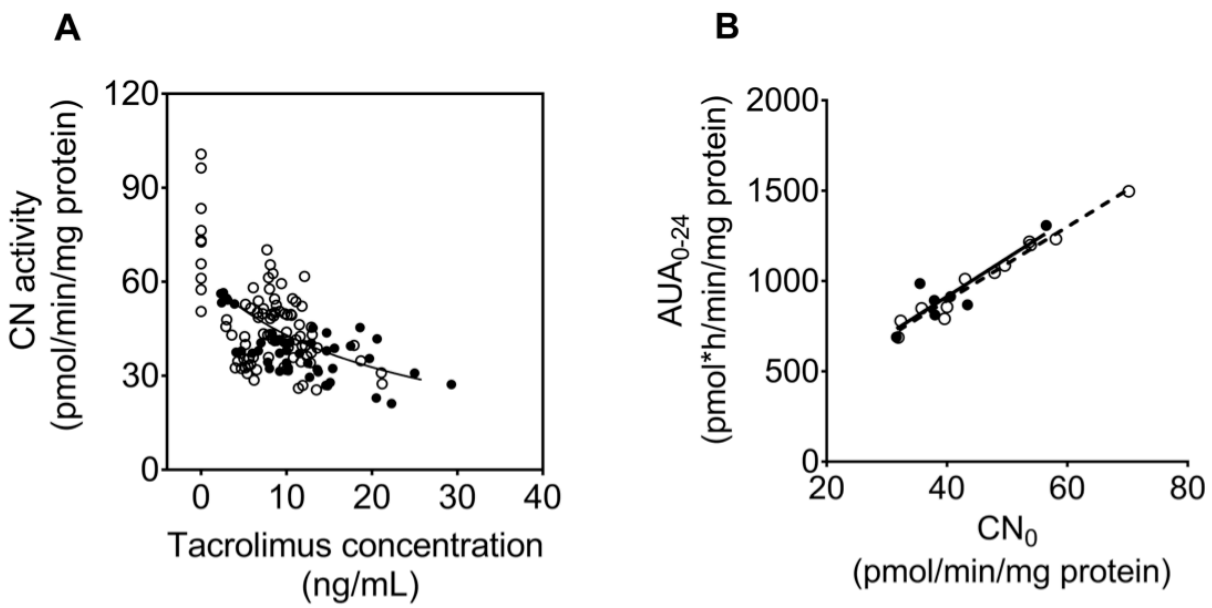

A
r²=0.189
P=0.282
AUC0-24 (ng*hr/mL)
C0 (ng/mL)
B
r²=0.381
P=0.103
C1 (ng/mL)
C
r²=0.226
P=0.233
C2 (ng/mL)
D
r²=0.515
P=0.045
C4 (ng/mL)
E
r²=0.879
P=0.001
AUC0-24 (ng*hr/mL)
C8 (ng/mL)
F
r²=0.995
P<0.001
C12 (ng/mL)
G
r²=0.871
P=0.007
C24 (ng/mL)

Fig. 4


A
CN activity
(pmol/min/mg protein)
120
90
60
30
0
0
10
20
30
40
Tacrolimus concentration
(ng/mL)
B
AUA0-24
(pmol*h/min/mg protein)
2000
1500
1000
500
0
20
40
60
80
CN0
(pmol/min/mg protein)