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Effect of plasma lipid on pharmacokinetics of ciclosporin and its relationship with plasma prednisolone level in renal transplant patients

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Abstract

Ciclosporin (cyclosporine A, CyA) is a potent immunosuppressant used after organ transplantation. The pharmacokinetic properties of CyA vary widely and lipoproteins are the major complexing constituents for CyA in the plasma. Therefore, a change in lipoprotein level may influence the pharmacokinetic properties of CyA. Prednisolone (PSL) is concomitantly used with CyA as an immunosuppressant. After organ transplantation, hyperlipidaemia resulting from PSL therapy has been mostly observed and PSL increased the plasma lipoprotein level. Therefore, in this study, to obtain more useful information of the therapeutic drug monitoring (TDM) of CyA, the relationship between the plasma PSL level, plasma lipoprotein level and blood CyA level was investigated in detail. An open-label, non-randomized, retrospective study was performed. Data from 21 male and 11 female patients (age 11–65 years) who received a living-related renal transplantation from 2002 to 2004 were included. On postoperative days (PODs) 7, 14 and 28, the area under the plasma concentration–time curve until 9 h after 40 mg of PSL administration (AUC_{PSL40}^{0-9}) correlated well with total cholesterol (T-cho) ($r = 0.558$, 0.768, 0.660, all $P < 0.05$) and high-density lipoprotein (HDL) ($r = 0.688$, $P < 0.05$; 0.835, $P < 0.01$; 0.508, $P < 0.05$), and correlated negatively with very-low-density lipoprotein (VLDL) ($r = -0.486$, $P < 0.01$; -0.776, $P < 0.01$; -0.967, $P < 0.01$). In addition, AUC until 9 h after CyA administration (AUC_{CyA}^{0-9}) also correlated with T-cho ($r = 0.797$, $P < 0.01$; 0.577, $P < 0.05$; 0.901, $P < 0.01$), HDL ($r = 0.514$, $P < 0.05$; 0.614, $P < 0.05$; 0.893, $P < 0.01$) and low-density lipoprotein (LDL) ($r = 0.906$, $P < 0.01$; 0.573, $P < 0.05$; 0.537, $P < 0.05$), and there was a negative correlation with VLDL ($r = -0.480$, -0.630, -0.632, all $P < 0.05$). Moreover, AUC_{CyA}^{0-9} correlated well with AUC_{PSL40}^{0-9} ($r = 0.728$, $P < 0.01$; 0.482, $P < 0.05$; 0.688, $P < 0.05$); namely, it was considered that the variety of plasma PSL concentrations influenced the pharmacokinetic properties of CyA through the change in lipoprotein levels. These results suggested that monitoring of the biochemical parameters of the plasma lipid and plasma PSL level might be useful for the TDM of CyA.

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Introduction

Ciclosporin (cyclosporine A, CyA) has been established as a potent immunosuppressive agent that has been widely used for the prevention or treatment of graft rejection after organ transplantation. The large inter-individual variability in CyA pharmacokinetics, the change in the bioavailability with time after transplantation and the correlation between toxic and therapeutic effects with blood concentration led to the development of a strategic therapeutic drug monitoring (TDM) method that could optimize and individualize the immunosuppressive treatment (Kahan et al 2002). The trough blood level is widely used in clinical practice. In addition, the AUC could be closely related to clinical outcomes (Lindholm & Kahan 1993). Recently, a new approach, the blood CyA level at 2 h after dose (C2) monitoring, has been recommended (Levy 2001). However, in our institution, C2 monitoring of CyA is not suitable because the management of blood sampling time for TDM is insufficient.

In the blood, CyA is distributed in erythrocytes (70%) and plasma proteins (20%) (Lemaire & Tillement 1982), where lipoproteins are the major complexing constituents for CyA in the plasma (Lemaire & Tillement 1982; Neiderberger et al 1983). The binding of CyA to erythrocytes and lipoproteins seems to be a linear process (Lemaire & Tillement

1982). In addition, it was reported that increases in lipoprotein concentrations with age would result in a corresponding decrease in the free fraction of CyA, which would reduce the hepatic clearance of CyA (Yee et al 1987). Therefore, investigation of the association between the plasma lipoprotein level and the blood CyA level should give us useful information for the TDM of CyA.

Prednisolone (PSL) is a synthetic corticosteroid drug commonly used in the treatment of a large variety of diseases. In immunosuppressive therapy after renal transplantation, PSL is concomitantly used with CyA as an immunosuppressant. In renal transplant patients, hyperlipidaemia resulting from PSL therapy has been mostly observed (Casaretto et al 1974). In addition, it was reported that PSL increased the plasma lipoprotein level (Gokal et al 1979). Therefore, the plasma PSL level may be relevant to the plasma lipoprotein level. Consequently, not only the plasma lipoprotein level but also the plasma PSL level may give useful information for the TDM of CyA. In addition, because of the recovery of gastrointestinal function and increased intake of food with post-operation days (POD), a change in the biochemical parameters of plasma lipids is conceivable. Moreover, although the relationship of plasma PSL level and POD is unclear and has not been reported to the best of our knowledge, it is also conceivable that the clearance of CyA decreases by increasing the plasma protein-binding ratio because of the increase of plasma albumin level with the recovery of renal function. Therefore, the investigation of the change of these parameters with time after transplantation is also necessary for this study.

In this study, to obtain more information for the TDM of CyA, the relationships between the plasma PSL level, plasma lipoprotein level, blood CyA level and POD have been investigated in detail.

Materials and Methods

An open-label, non-randomized, retrospective study was performed. Data from 21 male and 11 female patients (age 11–65 years, mean weight 50.0 ± 9.7 (s.d.) kg) who received a living-related renal transplantation from 2002 to 2004 were included. The renal and liver functions and plasma protein levels in this population on PODs 7, 14 and 28 were as follows: aspartate aminotransferase (AST), 18.0 ± 5.6, 15.5 ± 5.6, 15.9 ± 7.8 IU L^{-1}; alanine aminotranserfase (ALT), 21.1 ± 19.9, 25.0 ± 22.4, 24.3 ± 22.6 IU L^{-1}; total protein (TP), 6.1 ± 0.5, 6.1 ± 0.6, 6.2 ± 1.0 g dL^{-1}; albumin (ALB), 3.5 ± 0.3, 3.7 ± 0.3, 4.0 ± 0.4 g dL^{-1}, blood urea nitrogen (BUN), 27.8 ± 6.9, 27.9 ± 12.0, 30.6 ± 19.4 mg dL^{-1}, serum creatinine (S-Cr), 1.3 ± 0.6, 1.2 ± 0.3, 1.2 ± 0.6 mg dL^{-1}. In all subjects, immunosuppressive therapy was performed in the same way. Main concomitant drugs for immunosuppressive therapy were nifedipine for renal hypertension and lafutidine for the prevention of steroid-induced gastric ulcer. Anti-hyperlipaemia drugs, including statins, were not used in our institution, because hyperlipaemia after transplantation was caused by corticosteroid medication and was reversible. In addition, there were no recipients that had diabetes and hyperlipidaemia before transplantation in this study.

Immunosuppressive regimen using CyA

The immunosuppressive regimen using CyA developed by the department of transplantation and regenerative surgery, Kyoto Prefectural University of Medicine, is described below.

For living-related renal transplantation, the initial dose of CyA (12 mg kg^{-1} daily) was administered orally for 2 days before transplantation. CyA (4 mg kg^{-1} daily) was administered by continuous intravenous infusion on the day of transplantation, followed by CyA (12 mg kg^{-1} daily) oral administration (after meals, twice daily) for 3 weeks. The dose of CyA was adjusted according to the blood trough level (TL_{CyA}). Target TL_{CyA} was 200–300 ng mL^{-1}. A dose of 500 mg of methylprednisolone was given on the day of transplantation, followed by 50 mg of PSL daily on days 0–3. Then, 40 mg of PSL daily was administered on days 4–11. After that, PSL was reduced each week (30, 25, 20, 15 and then 10 mg orally twice daily after meals). On day 21, azathioprine (1–1.5 mg kg^{-1} daily) or mycophenolate mofetil (20–25 mg kg^{-1} daily) was added to the regimen.

Pharmacokinetic study of CyA

The study population comprised 32 recent living-related renal transplant patients (21 male, 11 female; age 11–65 years) operated in Kyoto Prefectural University of Medicine.

To obtain the pharmacokinetic parameters (i.e. AUC), pharmacokinetic studies of CyA were performed on 2 days before transplantation and on days 7, 14 and 28 after transplantation. For this study, blood samples were obtained at times 0, 1, 2, 3, 4, 6 and 9 h after drug administration ($AUC_{CyA}{}^{0-9}$). Blood samples for trough blood level of CyA (TL_{CyA}) were obtained each day before dosing until day 14 after transplantation. Starting at day 14, blood samples were obtained every other day. The blood concentration of CyA was measured by fluorescence polarization immunoassay using an Abotto TDX system. The detection limit in this method was 25 ng mL^{-1}. Inter- and intra-assay reproducibility was 2.3–3.9 (C.V. %) according to the appended paper of the reagent package.

$AUC_{CyA}{}^{0-9}$ was calculated by the trapezoidal rule.

Pharmacokinetic study of PSL

Subjects participating in this study were 20 recent patients from which could be obtained a pair of CyA and PSL pharmacokinetic parameters (11 male, 9 female; age 11–66 years). The plasma PSL concentration was measured using high-performance liquid chromatography (HPLC) as described below. Plasma samples for the determination of the PSL concentration were collected at steady state before the oral dose of 40 mg daily on days 4–11 after transplantation. The mean PSL concentration of 3 samples (40 mg daily of PSL dose at steady state) obtained in each patient was as for the PSL trough level (TL_{PSL40}). To obtain the pharmacokinetic parameter AUC, multiple blood samplings were performed in the same way as for CyA on day 7 (40 mg daily of the PSL dose at the steady state) after transplantation ($AUC_{PSL40}{}^{0-9}$). These two parameters were considered to be

an index of the individual PSL clearance, because the bioavailability of PSL is almost 100% (Gambertoglio et al 1980; Frey & Frey 1990; Barth et al 1992).

Determination of the plasma concentration of PSL

The concentration of PSL was determined using the HPLC procedure. PSL and carbamazepine, used as internal standards, were obtained from Nacalai Tesque (Kyoto, Japan). Acetonitrile and methanol were of HPLC grade. All other reagents were of reagent grade. The preparation of plasma samples was based on liquid–liquid extraction with dichloromethane. The compounds were separated on a CN column using acetonitrile–methanol–water (66:17:17, v/v) as the mobile phase. UV detection was performed at a wavelength of 254 nm. The HPLC system (Shimadzu, Kyoto, Japan) consisted of an LC-10A liquid delivery module, an SPD-10A ultraviolet detector, a CTO-10A column oven and a Simpac CLC-CN column (150 mm × 6.0 mm i.d., 5 μm). Samples were injected with a SIL-10A automatic injector. The system was controlled with an SCL-10A system controller. The area under each peak was calibrated with a Shimadzu CR-8A data processor. The standard curves of PSL were linear over the range of 1–500 μg mL^{-1}. The C.V. values (%) of inter- and intra-assay reproducibility were under 10%.

Alteration of the biochemical parameters of plasma lipid and AUC_{CyA}^{0-9} with post-operation days (POD)

Investigation of the change of biochemical parameters of plasma lipid and AUC_{CyA}^{0-9} with time after transplantation was performed. In this study, the relationship between POD and AUC_{CyA}^{0-9} was investigated. Subjects in this study were the same patients as those in the CyA pharmacokinetic study. The relationship between POD and the biochemical parameter of plasma lipid was also investigated. Subjects in this study were same patients as those in the PSL pharmacokinetic study. The biochemical parameters of plasma lipid used in this study were total cholesterol (T-cho), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and total lipoprotein (Lipo). Theses parameters were obtained on days 0, 7, 14 and 28 after transplantation. Changes in these parameters, including AUC_{CyA}^{0-9}, on each POD were investigated using a repeated measures analysis of variance.

Relationship between the biochemical parameters of plasma lipid and plasma PSL level or blood CyA level

Subjects in this study were the same patients as in the PSL pharmacokinetic study. Correlations between the biochemical parameters of plasma lipid and AUC_{PSL40}^{0-9} or TL_{PSL40} were investigated on days 7, 14 and 28 after transplantation. In addition, correlations between the biochemical parameters of plasma lipid and AUC_{CyA}^{0-9} or TL_{CyA} were also investigated on days 7, 14 and 28 after transplantation. The biochemical parameters of plasma lipid used in this study were: T-cho, TG, HDL, LDL, VLDL and Lipo. In addition, correlations between AUC_{PSL40}^{0-9} or TL_{PSL40} and AUC_{CyA}^{0-9} or TL_{CyA} were investigated on days 7, 14 and 28 after transplantation.

To assess the relative effect of each variable on AUC_{CyA}, step-wise multiple linear regression was performed. As independent variables, HDL, LDL, VLDL, TL_{PSL40} and AUC_{PSL40}^{0-9} were used. Moreover, AUC_{CyA} before transplantation (AUC_{CyAint}), aspartate aminotransferase (AST), plasma creatinine (S-Cr), albumin (ALB), age and POD were used as factors that influenced the pharmacokinetic properties of CyA.

Generally, plasma lipid correlates with body weight. In this study, the renal transplant patients' body weights varied from 28 to 73.6 kg. Therefore, all parameters except AST, S-Cr, ALB and POD were corrected for the body weight (BW).

The study was approved by the Ethics Committee of Kyoto Prefectural University of Medicine, and informed consent was obtained from all subjects.

Results

Alteration of the biochemical parameters of plasma lipid and AUC_{CyA}^{0-9} with POD

Figure 1 shows the relationship between POD and AUC_{CyA}^{0-9}/dose/BW. Statistically significant differences were observed between each POD ($P < 0.01$, repeated measures analysis of variance). The AUC_{CyA}^{0-9}/dose/BW gradually increased, suggesting a decrease in CyA clearance with POD, and the correlation coefficient 0.451 ($P < 0.0001$) on day 28 was 1.6 times higher than that on day 7. Figure 2 shows the relationship between POD and T-cho/BW, TG/BW, HDL/BW, LDL/BW, VLDL/BW or Lipo/BW. No significant differences were observed in these parameters except for T-cho/BW between each POD ($P < 0.05$, repeated measures analysis of variance). However, a time-related increase with POD (not

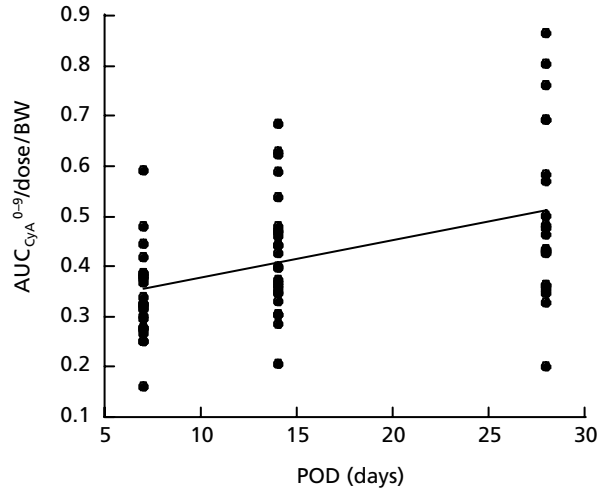

Figure 1 Relationship between POD and AUC_{CyA}^{0-9}/dose/BW. Each point shows the AUC_{CyA}^{0-9}/dose/BW values on days 7, 14 and 28. Statistical significant differences were observed between each POD (repeated measures analysis of variance, $P < 0.01$).

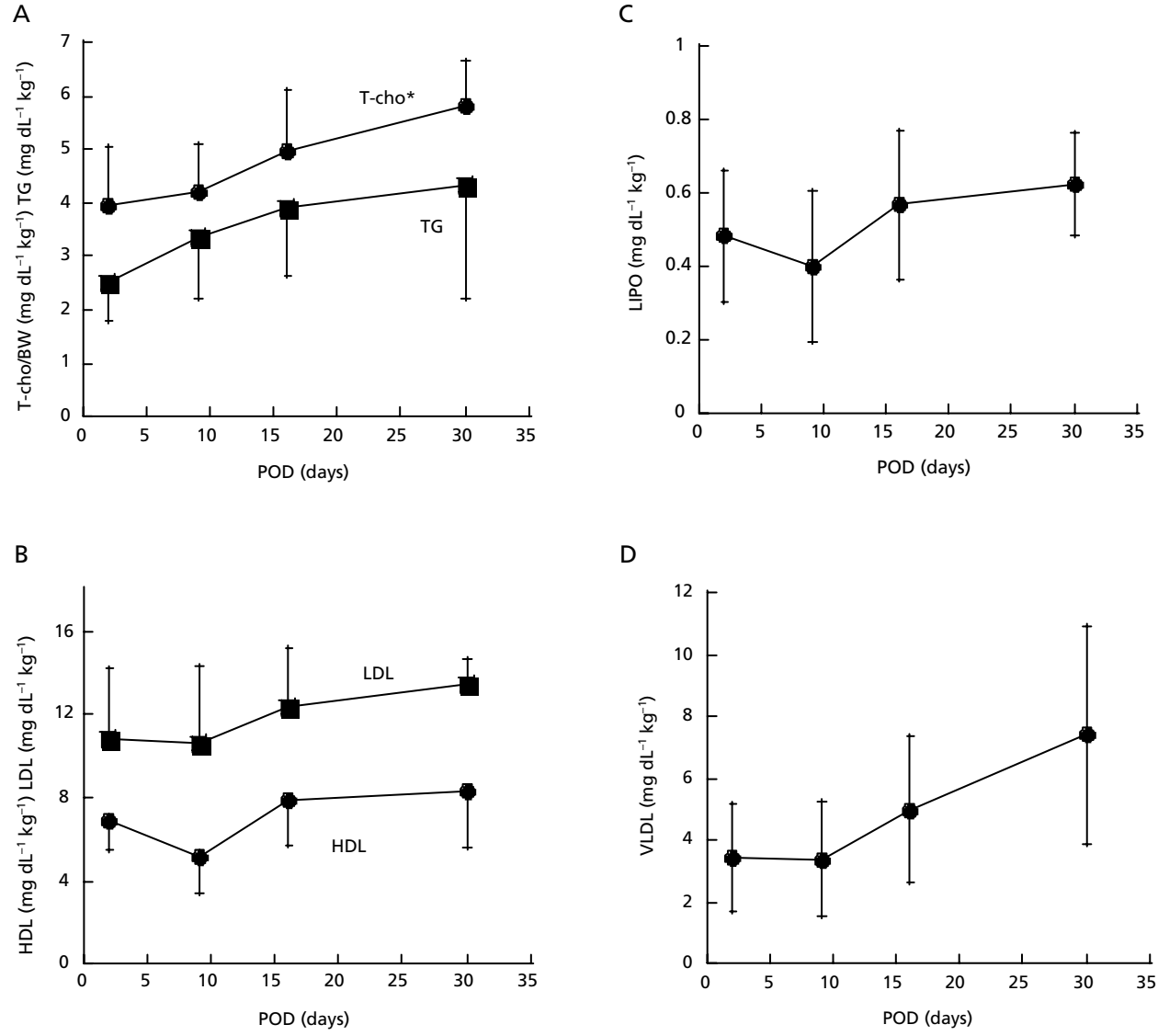

Figure 2 Relationship between POD and T-cho/BW (A), TG/BW (A), HDL/BW (B), LDL/BW (B), Lipo/BW (C) or VLDL/BW (D). Each point represents the mean ± s.d. Statistically significant differences were observed in T-cho/BW between each POD (repeated measures analysis of variance, $P < 0.05$).

significant) was observed in each plasma lipid (plasma lipids on day 28 were about 1.2–1.8 times those on day 7). A significant difference was observed in T-cho/BW between each POD ($P < 0.05$, repeated measures analysis of variance).

Mean $AUC_{CyA}{}^{0-9}$s on day 7, 14 and 28 were 6615.0 ± 1836.8, 6967.0 ± 1973.7 and 6313.0 ± 2126.0 ng h mL^{-1}, respectively. Mean C_{max}s were 1468.4 ± 454.0, 1531.0 ± 462.9 and 1544.2 ± 569.9 ng mL^{-1}, respectively. Mean T_{max}s were 2.4 ± 1.0, 2.6 ± 1.2 and 2.1 ± 1.1, respectively.

Relationship between the biochemical parameters of plasma lipid and plasma PSL level or blood CyA level

Table 1 shows the correlation coefficient of $AUC_{PSL40}{}^{0-9}$/BW or TL_{PSL40}/BW and T-cho/BW or TG/BW on days 7, 14 and 28. In each day, $AUC_{PSL40}{}^{0-9}$/BW well correlated with T-cho/BW (r = 0.558, 0.768, 0.660, all $P < 0.05$). Table 1 also shows the correlation coefficient of $AUC_{PSL40}{}^{0-9}$/BW or TL_{PSL40}/BW and HDL/BW, LDL/BW, VLDL/BW or Lipo/BW on days 7, 14 and 28. On each day, $AUC_{PSL40}{}^{0-9}$/BW correlated well with HDL/BW (r = 0.668, $P < 0.05$; 0.835, $P < 0.01$; 0.508, $P < 0.05$), and correlated negatively with VLDL/BW (r = −0.486, $P > 0.05$; −0.776, $P < 0.01$; −0.967, $P < 0.05$). The order of the tendency of correlation with VLDL was: day 28 (−0.967) > day14 (−0.776) > day7 (−0.486). The mean $AUC_{PSL40}{}^{0-9}$ was 1786.1 ± 618.6 ng h mL^{-1}, and the mean C_{max} and T_{max} was 390.1 ± 100.3 ng mL^{-1} and 3.0 ± 1.0 h, respectively.

Table 2 shows the correlation coefficient of $AUC_{CyA}{}^{0-9}$/dose/BW or TL_{CyA}/dose/BW and T-cho/BW or TG/BW on days 7, 14 and 28. On each day, $AUC_{CyA}{}^{0-9}$/dose/BW correlated well with T-cho/BW (r = 0.797, $P < 0.01$; 0.577, $P < 0.05$; 0.901, $P < 0.01$).

Table 1 Correlation coefficients between TL_{PSL40}/BW or AUC_{PSL40}^{0-9}/BW and various plasma lipids

	Correlation coefficient					
	T-cho/BW (mg dL^{-1} kg^{-1})	TG/BW (mg dL^{-1} kg^{-1})	HDL/BW (mg dL^{-1} kg^{-1})	LDL/BW (mg dL^{-1} kg^{-1})	VLDL/BW (mg dL^{-1} kg^{-1})	Lipoprotein/BW (mg dL^{-1} kg^{-1})
On day 7						
TL_{PSL40}/BW (ng mL kg^{-1})	–0.191	0.377	0.219	0.137	0.444	0.294
AUC_{PSL40}^{0-9}/BW (ng h mL^{-1} kg^{-1})	0.558*	–0.033	0.688*	0.946**	–0.486	0.879**
On day 14						
TL_{PSL40}/BW (ng mL kg^{-1})	0.478	–0.207	0.406	0.885	–0.593*	0.493*
AUC_{PSL40}^{0-9}/BW (ng h mL^{-1} kg^{-1})	0.768*	–0.075	0.835**	0.584*	–0.776**	0.395
On day 28						
TL_{PSL40}/BW (ng mL kg^{-1})	0.107	–0.420	0.608*	0.234	–0.951**	–0.504*
AUC_{PSL40}^{0-9}/BW (ng h mL^{-1} kg^{-1})	0.660*	–0.754**	0.508*	0.080	–0.967**	–0.601*

*$P < 0.05$; **$P < 0.01$.

Table 2 Correlation coefficients between TL_{CyA}/BW or AUC_{CyA}^{0-9}/BW and various serum lipids

	Correlation coefficient					
	T-cho/BW (mg dL^{-1} kg^{-1})	TG/BW (mg dL^{-1} kg^{-1})	HDL/BW (mg dL^{-1} kg^{-1})	LDL/BW (mg dL^{-1} kg^{-1})	VLDL/BW (mg dL^{-1} kg^{-1})	Lipoprotein/BW (mg dL^{-1} kg^{-1})
On day 7						
TL_{CyA}/dose/BW (ng mL kg^{-1})	0.129	0.026	0.870**	0.787**	–0.315	0.917**
AUC_{CyA}^{0-9}/dose/BW (ng h mL^{-1} kg^{-1})	0.797**	0.518*	0.514*	0.906**	–0.480*	0.798**
On day 14						
TL_{CyA}/dose/BW (ng mL kg^{-1})	0.252	–0.040	0.406	0.885**	–0.593*	0.404
AUC_{CyA}^{0-9}/dose/BW (ng h mL^{-1} kg^{-1})	0.577*	–0.282	0.614*	0.573*	–0.630*	0.324
On day 28						
TL_{CyA}/dose/BW (ng mL kg^{-1})	0.795**	–0.174	0.874**	0.512	–0.558*	0.024
AUC_{CyA}^{0-9}/dose/BW (ng h mL^{-1} kg^{-1})	0.901**	–0.528*	0.893**	0.537*	–0.632*	–0.024

*$P < 0.05$; **$P < 0.01$.

Table 2 also shows the correlation coefficient of AUC_{CyA}^{0-9}/dose/BW or TL_{CyA}/dose/BW and HDL/BW, LDL/BW, VLDL/BW or Lipo/BW on days 7, 14 and 28. For both AUC_{CyA}^{0-9}/dose/BW and TL_{CyA}/dose/BW, a positive correlation with HDL (r=0.514–0.893, $P<0.05$, except AUC_{CyA}^{0-9}/dose/BW on days 14 (r=0.406)) and LDL (r=0.537–0.906, $P<0.05$, except TL_{CyA}^{0-9}/dose/BW on days 28 (r=0.512)), and a negative correlation with VLDL (r=–0.480 to –0.632, $P<0.05$, except TL_{CyA}^{0-9}/dose/BW on days 7 (r=–0.315)) was recognized on each day. For HDL/BW and LDL/BW, about 1.1- to 2.8-fold increases with AUC_{CyA}^{0-9}/dose/BW or AUC_{PSL40}^{0-9}/BW were observed. On the other hand, for VLDL, about 2.8-fold decrease with AUC_{CyA}^{0-9}/dose/BW or AUC_{PSL40}^{0-9}/BW was observed.

Table 3 shows the correlation coefficient of AUC_{CyA}^{0-9}/dose/BW or TL_{CyA}/dose/BW and AUC_{PSL40}^{0-9}/BW or TL_{PSL40}/BW on days 7, 14 and 28. AUC_{PSL40}^{0-9}/BW was well correlated with TL_{CyA}/dose/BW or AUC_{CyA}^{0-9}/BW on each day (r=0.482–0.763, $P<0.05$) (Figure 3).

The result of the multiple linear regression, for which AUC_{CyA}^{0-9}/dose/BW was a dependent variable, is shown in Table 4. The eight variables (AUC_{CyAint}^{0-9}/dose/BW, AUC_{PSL40}/BW, T-cho/BW, TG/BW, VLDL/BW, HDL/BW, ALB and POD) were selected and entered in the regression equation. Adding more variables (S-Cr, AST, LDL/BW, TL_{PSL40}/BW and age) did not result in a significant ($P<0.01$) increase in the multiple correlation coefficient. The statistical

Table 3 Correlation coefficients between TL_{CyA}/dose/BW or AUC_{CyA}^{0-9}/dose/BW and TL_{PSL40}/BW or AUC_{PSL40}^{0-9}/BW

	Correlation coefficient	
	TL_{CyA}/dose/BW (ng mL^{-1} mg^{-1} kg^{-1})	AUC_{CyA}^{0-9}/dose/BW (ng h mL^{-1} mg^{-1} kg^{-1})
On day 7		
TL_{PSL40}/BW (ng mL^{-1} kg^{-1})	0.325	0.288
AUC_{PSL40}^{0-9}/BW (ng h mL^{-1} kg^{-1})	0.698**	0.728**
On day 14		
TL_{PSL40}/BW (ng mL^{-1} kg^{-1})	0.437	0.028
AUC_{PSL40}^{0-9}/BW (ng h mL^{-1} kg^{-1})	0.763**	0.482*
On day 28		
TL_{PSL40}/BW (ng mL^{-1} kg^{-1})	0.302	0.275
AUC_{PSL40}^{0-9}/BW (ng h mL^{-1} kg^{-1})	0.594*	0.688*

*$P < 0.05$; **$P < 0.01$.

significance for the regression of this predicted model was $P < 0.005$ (F-test). A regression plot (r = 0.875, $P < 0.001$) is shown in Figure 4.

Discussion

TDM is always needed for immunosuppressive therapy using CyA, because of its narrow therapeutic range of blood concentration and various pharmacokinetic properties. The systemic exposure is measured as AUC. Therefore, AUC was a good index for TDM, reflecting a pharmacological effect. In this study, as shown in Figure 1, the time-related change of AUC_{CyA}^{0-9}/dose/BW in the course of immunosuppressive therapy was recognized. The reason why the AUC_{CyA}^{0-9}/dose/BW gradually increased was considered to be as follows: CyA absorption increased with the recovery of gastrointestinal function; bile secretion increased with increased intake of food; the clearance of CyA decreased with increasing plasma protein-binding ratio because of the increasing plasma protein with the recovery of renal function. In addition, it was reported that the uptake of CyA into tissues was limited by its binding to plasma proteins (Lemaire et al 1988), and that increases in lipoprotein concentrations with age would result in a corresponding decrease in the free fraction, which would theoretically reduce the hepatic clearance of CyA (Yee et al 1987). Moreover, it was also reported that the cellular uptake of CyA was decreased by the increase of lipoprotein (Rifai et al 1996). Lemaire and co-workers considered that the clearance of CyA decreased due to the increasing of the lipoprotein level, as they are the major complexing constituents for CyA (Lemaire & Tillement 1982; Neiderberger et al 1983). Therefore, another reason for the change of AUC_{CyA}^{0-9}/dose/BW in the course of immunosuppressive therapy may be the increased T-cho/BW, TG/BW and Lipo/BW with POD as shown in Figure 2. Time-dependent change in CyA trough level was unclear in this study. However, AUC_{CyA}^{0-9} was well correlated with TL_{CyA} on PODs 7, 14 and 28 (r = 0.726, 0.622 and 0.700; all $P < 0.01$). Therefore, it was considered that TL_{CyA} also showed time-dependent change.

In the majority of renal transplant patients, the onset of hyperlipidaemia caused by corticosteroid immunosuppressive therapy has been observed (Casaretto et al 1974). AUC_{PSL40}^{0-9}/BW correlated well with T-cho/BW (Table 1). Therefore, it was suggested that the increase in plasma lipids was caused by PSL medication. Although hyperlipidaemia is also one of the adverse effects of CyA, the effect of PSL on plasma lipid levels may be higher than that of CyA because CyA exposure in all patients in this study was of the same degree as a result of the management of CyA target blood trough level. On the

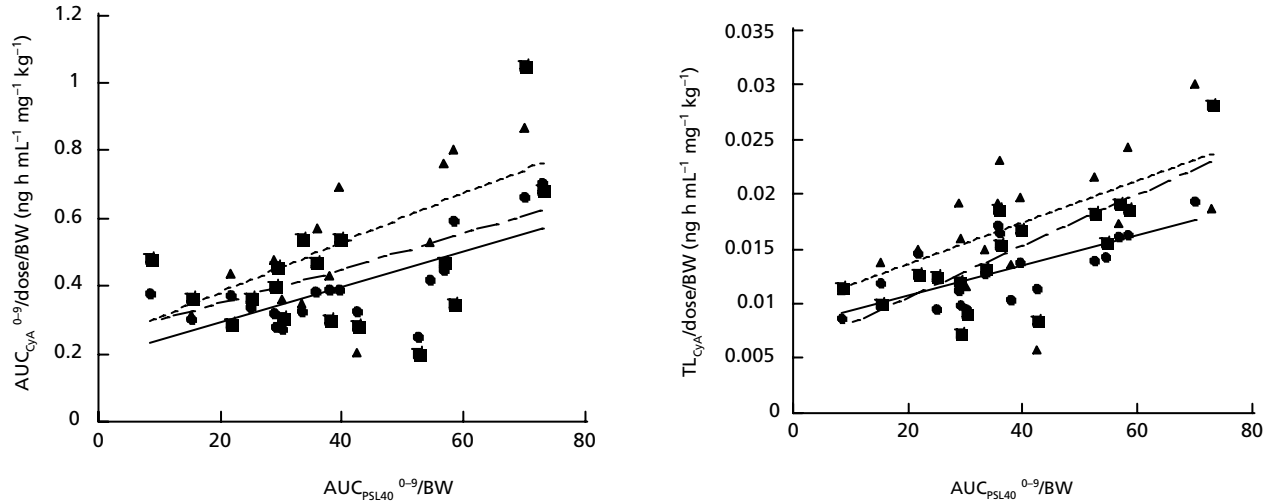

Figure 3 Relationship between AUC_{CyA}^{0-9}/dose/BW or TL_{CyA}/dose/BW and AUC_{PSL40}^{0-9}/BW; ●, day 7; ■, day 14; ▲, day 28. Correlation coefficients on days 7, 14 and 28 between AUC_{CyA}^{0-9}/dose/BW and AUC_{PSL40}^{0-9}/BW were 0.727, 0.482 and 0.702, respectively, and those between TL_{CyA}/dose/BW and AUC_{PSL40}^{0-9}/BW were 0.698, 0.763 and 0.594, respectively.

Table 4 Multiple linear regression

Dependent variables	Independent variables	Coefficient
AUC_{CyA}^{0-9}/dose/BW	AUC_{CyAint}^{0-9}/dose/BW	0.284
	AUC_{PSL40}/BW	0.020
	T-cho/BW	−0.130
	TG/BW	0.268
	VLDL/BW	−0.150
	LDL/BW	−0.076
	ALB	−0.070
	POD	0.012
	Intercept	−0.286

AUC_{CyAint}^{0-9} = AUC 0–9 h before transplantation. $P < 0.005$ (F-test).

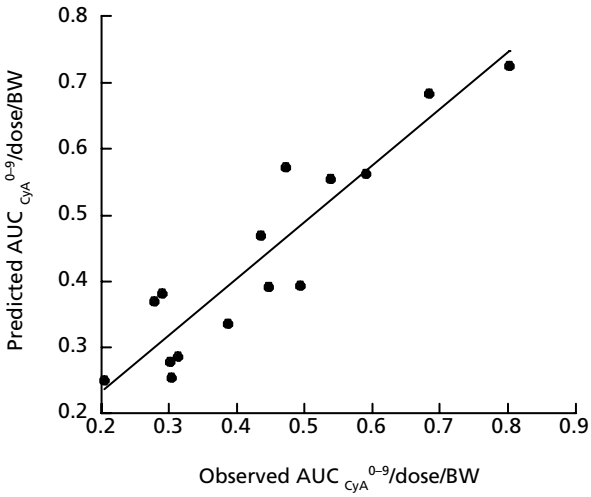

Figure 4 Correlation between actual and predicted AUC_{CyA}^{0-9}/dose/BW values using multiple regression equation (Table 4). Correlation coefficient was 0.875 ($P < 0.001$).

other hand, AUC_{PSL40}^{0-9}/BW correlated negatively with VLDL/BW and %VLDL. However, HDL and LDL correlated positively with AUC_{PSL40}^{0-9}/BW or TL_{PSL40}/BW (Table 1). It was reported that the activity of lipoprotein lipase, accelerating the hydrolysis of TG, was increased by the administration of corticosteroid (Hulsmann & Dubelaar 1986). Therefore, it was considered that one of the reasons for the decrease of the ratio of VLDL, which was rich in TG, was the high AUC_{PSL40}^{0-9}/BW value.

CyA binds strongly to lipoproteins, and the increase in the lipoprotein concentrations reduces the hepatic clearance of CyA (Yee et al 1987), as described above. It was reported that 7% of CyA was found in chylomicrons, 9% in VLDL, 28% in LDL, 39% in HDL and 12% in the non-lipoprotein protein fraction in healthy individuals. In addition, it was reported that the distribution varied from 12 to 19% in VLDL, 21 to 28% in LDL, 33 to 43% in HDL and 13 to 20% in the non-lipoprotein protein fraction in patients receiving CyA medication (Sgoutas et al 1986). On the other hand, Brunner et al (1991) performed a pharmacokinetic study, after a single intravenous infusion of CyA, in whole blood, plasma, HDL, LDL and VLDL lipoprotein fractions in bone marrow transplant patients, and reported that the dose-corrected AUC and elimination half-life of CyA from the VLDL fraction was significantly less than from the other fractions, suggesting that the binding affinity of CyA to VLDL was weaker. Therefore, it is suggested that the increase of CyA blood clearance was caused by an increase of the VLDL fraction in blood. In this study, VLDL negatively correlated with both AUC_{CyA}^{0-9}/dose/BW and TL_{CyA}/dose/BW, and HDL or LDL positively correlated with both AUC_{CyA}^{0-9}/dose/BW and TL_{CyA}/dose/BW. These results reflected that the increase in the VLDL fraction in the blood produced the increased CyA clearance, as described above. Moreover, AUC_{CyA}^{0-9}/dose/BW correlated with T-cho/BW (Table 2).

These results, in all points, suggested that the pharmacokinetic properties of CyA correlated with those of PSL through the plasma lipid at each POD. In this study, AUC_{CyA}^{0-9}/dose/BW or TL_{CyA}/dose/BW correlated well with AUC_{PSL40}^{0-9}/BW (Figure 3); namely, it was considered that the variety of plasma PSL concentrations influenced the pharmacokinetic properties of CyA through the change in lipoproteins. The result of the multiple linear regression, of which AUC_{CyA}^{0-9}/dose/BW was a dependent variable, reflected these results (Table 4). Moreover, the correlation coefficient for the actual and predicted values was 0.875 ($P < 0.001$) using this model (Figure 4), suggesting the possibility of clinical usefulness for the TDM of CyA. These results suggested that the monitoring of the biochemical parameters of plasma lipid and plasma PSL level should be useful for the TDM of CyA.

Conclusion

In this small, retrospective study, variation in plasma PSL concentrations influenced the pharmacokinetic properties of CyA through the change in lipoprotein levels. These results suggested that monitoring of the biochemical parameters of plasma lipid and plasma PSL level might be useful for the TDM of CyA. Although standards need to be established for measuring the plasma PSL level at an early stage after transplantation and plasma lipoprotein levels, results of this study may be able to contribute towards CyA TDM, such as minimizing the number of blood sampling and revising the dosage, using multiple regression equation.

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