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Prediction of systemic exposure to cyclosporine in Japanese pediatric patients

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ABSTRACT

The monitoring of the blood concentration at 2 hours (C_2) after oral administration of a cyclosporine (CsA) microemulsion was reconfirmed to be useful for prediction of systemic exposure, the area under the blood concentration-time curve from 0 to 4 hours (AUC_{0-4}), in Japanese patients, consisting of 33 children aged 5-15 years and 19 young adults aged 16-27 years, with a greater correlation for C_2 ($r = 0.927$) than the trough concentration ($r = 0.488$). The dose-normalized AUC_{0-4} was independent of gender or indications for CsA, while it depended on body size, *i.e.*, age ($p = 0.065$) and total body weight ($p = 0.026$). *MDR1* C3435T had a weak, but insignificant, effect ($p = 0.072$); it was about 22-31% lower in the patients with TT^{3435}. Co-administration of a steroid and further treatment with nifedipine had a more intensive effect ($p = 0.018$); co-administration resulted in a 51% increase in the dose-normalized AUC_{0-4}. A strong effect was also observed for serum total cholesterol level ($p = 0.001$). Collectively, the discrepancies in the results on *MDR1* C3435T among investigators might be due to variability in age/total body weight, co-administration drugs or serum lipid level.

Keywords: Cyclosporine (CsA); C_2 monitoring; Pediatric patients; MDR1

Introduction

Cyclosporine (INN, ciclosporin; CsA) has been an essential drug for immunosuppressive therapy for more than 20 years, but it is only recently that a patient management strategy to ensure greater safety and efficacy has come to be proposed (Fahr 1993; Levy 2001; Wong 2001; Cole et al. 2002; Levy et al. 2002; Monaco 2002; Nashan et al. 2002; Citterio 2004). This is primarily due to the development of the oral formulation of a CsA microemulsion, Neoral®, and the accumulation of clinical studies on CsA pharmacokinetics and clinical outcomes in adult renal and liver transplant recipients. CsA is a drug with a low therapeutic index, requiring individualization and continuous adjustment of the dosage regimen based on therapeutic drug monitoring. Conventional monitoring of the trough level (C_0) of the blood concentration has been used to predict systemic exposure to CsA, usually assessed based on the area under the blood concentration-time curve (AUC), after oral administration. Recently, it has become evident that the AUC is indeed an appropriate index of the immunosuppressive effect and toxicity of CsA, however, it is little reflected by C_0. Considerable intra- and inter-patient variability in exposure to CsA is found during the initial period after oral administration, suggesting the monitoring of AUC during the initial 4 hours (AUC_{0-4}) to be useful for patient

management, and later, the monitoring of the blood concentration at 2 hours post-dose (C_2) as a surrogate marker based on clinical results showing AUC_{0-4} to be correlated with C_2. In 2002, the Consensus on Neoral C_2: Expert Review in Transplantation (CONCERT) conference was convened to undertake a multidisciplinary review of pharmacokinetic and clinical data on CsA microemulsions, and an international consensus on a patient management strategy with the oral administration of a CsA microemulsion and C_2 monitoring was achieved (Cole et al. 2002; Levy et al. 2002; Monaco 2002; Nashan et al. 2002).

This strategy has resulted from over 20 years of investigation, however, most of the data are on adult patients, and relatively little information is available on pediatric patients. The applicability of C_2 monitoring to pediatric patients is still being debated given the age-specific pharmacokinetic properties of CsA (del Mar Fernández de Gatta et al. 2002; Dunn 2003). Thus, the present study was conducted 1) to reconfirm the usefulness of C_2 monitoring to predict the systemic exposure to CsA in pediatric patients, and 2) to identify the factors affecting the systemic exposure to CsA. The factors analyzed herein included pre-existing conditions, such as the gender, age, total body weight, indications for CsA, and *MDR1* genotype of the patients as well as day-to-day variable conditions, such as the co-administration of a steroid and further

treatment with nifedipine and the results of clinical laboratory tests on the day of investigation. *MDR1* encodes the multi-drug resistant transporter MDR1/P-glycoprotein (Sakaeda et al. 2003; 2004; 2005; Okamura et al. 2004). CsA is a typical substrate of MDR1, and MDR1 in the villus epithelium of the small intestine is considered to play a role in limiting the intestinal absorption of CsA (Lown et al. 1997; Sakaeda et al. 2003; 2004; 2005; Okamura et al. 2004).

Materials and methods

Patients

Fifty-two Japanese patients, 32 boys and 20 girls, visiting the Department of Pediatrics, Kobe University Hospital, Kobe, Japan were enrolled in this study. Age (±standard deviation (SD)) was 14.1±5.2 years, and the patients were classified into 2 subpopulations: 33 children aged 5-15 years and 19 young adults aged 16-27 years. Total body weight (±SD) was 42.5±12.9 kg (19.0-74.5 kg). The patients were maintained in a stable condition by a twice daily oral dosing of the CsA microemulsion (Neoral®; Novartis Pharma, USA), and the indications for CsA included post-renal transplantation (n=19), nephrotic syndrome (n=25) and systemic lupus erythematosus (SLE; n=8). The maintenance dose (±SD) was 3.36±0.94 mg/kg/day (1.34-6.00 mg/kg/day). A steroid, *i.e.*, prednisolone or methylprednisolone, was co-administered to 39 of 52 patients, and nifedipine was additionally administered to 11 of 39 patients, and their maintenance dose (± SD) was 0.63±0.57 mg/kg/day (0.11-1.75 mg/kg/day), 0.22±0.12 mg/kg/day (0.08-0.60 mg/kg/day) and 0.70±0.33 mg/kg/day (0.40-1.33 mg/kg/day), respectively. No serious renal impairment was confirmed by periodic pathohistological examinations. The aims of the study were fully explained to every subject and/or their parents, and written informed consent was obtained. The

protocol was approved by the Institutional Review Board of Kobe University Hospital, Kobe University, Japan.

Assessment of systemic exposure to CsA after oral administration, and its predictability by C_2 monitoring

After confirmation that the patient was in a steady-state based on continuous C_0 monitoring, the pharmacokinetic profile was evaluated. On the day of the investigation, whole blood samples were obtained from the peripheral vein in EDTA-containing polyethylene tubes prior to and at 1, 2, 3 and 4 hours after oral administration to assess C_0, C_1, C_2, C_3, and C_4, respectively. The samples were stored at -20°C until the analysis. CsA concentrations were measured by monoclonal fluorescence polarization immunoassay using the TDx operation system (Abbott Laboratory, North Chicago, IL, USA) according to the manufacturer's manual, and the measurement method was validated in terms of precision and accuracy, and also day-to-day variation. The systemic exposure to CsA was assessed using $AUC_{0\text{-}4}$, which was calculated by the linear trapezoidal rule using the pharmacokinetic software package WinNonlin^{TM} Ver. 4.0 (Pharsight Co., Mountain View, CA, USA). The correlation between $AUC_{0\text{-}4}$ and either C_0, C_1, C_2, C_3 or C_4 was tested using Pearson's

correlation test, and *p* values of less than 0.05 were considered significant. The correlation was also evaluated after stratification based on the gender, age, indications for CsA, and *MDR1* genotypes of the patients, as well as co-administration of a steroid and further treatment with nifedipine.

Assessment of factors affecting systemic exposure to CsA

The analysis was conducted after exclusion of the data on 4 slow absorbers, defined herein as patients with a t_{max}, time to maximum blood concentration (C_{max}), of 4 hours or more (see Figure 1), since the values of AUC_{0-4} were expected to be underestimated. There was considerable variation in the maintenance dose of CsA, thus the analysis was performed after dose-normalization of AUC_{0-4} at a dose of 3 mg/kg/day according to the equation: $AUC_{0-4,corr.} = AUC_{0-4}$ x [3 (mg/kg/day) / dose (mg/kg/day)]. The factors analyzed in terms of association with $AUC_{0-4,corr.}$ included gender (31 male, 17 female), age (29 children, 19 young adults), total body weight (20.0-74.5 kg), indications for CsA (16 post-renal transplantation, 24 nephrotic syndrome, 8 SLE), and *MDR1* genotype. The effects of the co-administration of a steroid and further treatment with nifedipine (12 control, 25 a steroid, 11 both a steroid and nifedipine), and serum alanine aminotransferase (ALT, 12-33 IU/L), serum aspartate

aminotransferase (AST, 5-80 IU/L), serum albumin level (Alb, 2.5-4.6 g/dL), serum creatinine level (Scr, 0.24-2.17 mg/dL), hematocrit (Ht, 26.0-45.5%) and serum total cholesterol level (T-Chol, 120-588 mg/dL) on the day of investigation were also evaluated. The *MDR1* genetic polymorphisms examined herein were T-129C (36 TT, 12 CT, 0 CC), C1236T (5 CC, 22 CT, 21 TT), G2677A,T (10 GG, 7 GA, 16 GT, 0 AA, 7 AT, 8 TT) and C3435T (13 CC, 27 CT, 8 TT). The comparisons of $AUC_{0\text{-}4,corr.}$ between and among groups were performed using the unpaired Student t-test and ANOVA/Tukey-Kramer test, respectively, provided that the variances of the groups were similar as assessed by the F-test. If this was not the case, a Mann-Whitney U-test or a Kruskal-Wallis analysis/Mann-Whitney U-test was applied, respectively. *p* values of less than 0.05 (two-tailed) were considered significant. For age, total body weight and the results of clinical laboratory tests, dependencies were analyzed using Pearson's correlation test.

MDR1 T-129C, C1236T, G2677A,T and C3435T genotyping

Genomic DNA was extracted from 0.5 mL of whole blood using a DNA extractor WB Kit (Wako Pure Chemical Industries Ltd., Osaka, Japan) as described previously (Sakaeda et al. 2001; Nakamura et al. 2002; Horinouchi et al. 2002; Morita et al. 2003).

The genotypes of T-129C, G2677A,T and C3435T of the *MDR1* gene were determined by polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) analysis as described before (Sakaeda et al. 2001; Nakamura et al. 2002; Horinouchi et al. 2002; Morita et al. 2003) and confirmed by direct sequencing. Herein, C1236T was also determined by direct sequencing according to the report by Kim *et al.* (2001).

Results

Systemic exposure to CsA after oral administration, and its prediction by C_2 monitoring

Figure 1 shows the blood concentration-time profiles of CsA up to 4 hours after oral administration of the CsA microemulsion in 52 Japanese patients. There was considerable variation in the blood concentration-time profiles, and the t_{max} was 1 hour (n = 28), 2 hours (n = 13), 3 hours (n = 7) and 4 hours (n = 4). The ratio of slow absorbers, defined as a patient with a t_{max} of 4 hours or more, was 4/52 (7.7%). Their maintenance dose (±SD) was 3.74±0.75 mg/kg/day, which was not different from that in other patients. The slow absorbers consisted of 1 boy and 3 girls. At an average age (±SD) of 11.5±4.0 years, they were slightly younger than the others. Total body weight (±SD) was 32.3±11.8 kg, also down slightly, but the slow absorbers were not characterized by the other factors assessed herein, including indications for CsA, *MDR1* genotypes at 4 positions, co-administered drugs and the values of ALT, AST, Alb, T-Chol, Scr and Ht.

Figure 2 shows the relationship between $AUC_{0\text{-}4}$ and C_0 or C_2 in 52 Japanese patients. A statistically significant correlation was observed for both, but the coefficient was higher for C_2 (r = 0.927) than C_0 (r = 0.488). Exclusion of the 4 slow

absorbers resulted in a higher correlation coefficient for C_0 (r = 0.590), but no for C_2 (r = 0.917). Table 1 lists the association of age with the coefficients of the correlation between $AUC_{0\text{-}4}$ and C_0 to C_4, where the patients were classified into 2 subpopulations of 33 children and 19 young adults. $AUC_{0\text{-}4}$ values were better correlated with the values of C_0 to C_4 for children than young adults, but the best surrogate marker was C_2 for both (r = 0.945 and 0.854, respectively). Interestingly, C_0 was correlated with $AUC_{0\text{-}4}$ in children (r = 0.551), whereas no correlation was found for young adults (r = 0.237). The correlation between $AUC_{0\text{-}4}$ and C_2 was independent of gender, indications for CsA, *MDR1* genotypes, and co-administration of a steroid and further treatment with nifedipine.

Factors affecting systemic exposure to CsA

Table 2 summarizes the results of statistical analyses for the association of various factors with $AUC_{0\text{-}4,corr.}$. The average values of $AUC_{0\text{-}4,corr.}$ in each group are listed in Table 3. No effect of gender on $AUC_{0\text{-}4,corr.}$ was observed (p = 0.508). The values of $AUC_{0\text{-}4,corr.}$ tended to be lower in younger patients, although the analysis was not sufficiently powered to reach statistical significance (p = 0.065), and the value for children, 1711±718 ng*h/mL, was about 10% less than that for young adults, 1915±552

ng*h/mL. The $AUC_{0\text{-}4,corr.}$ depended on total body weight (p = 0.026), with heavier patients having greater values, but was independent of indications for CsA (p = 0.948). Among the 4 genotypes of *MDR1*, C3435T had the greatest effect on $AUC_{0\text{-}4,corr.}$, but the difference was not statistically significant (p = 0.072). The average values were 1738±590 ng*h/mL, 1948±678 ng*h/mL and 1351±536 ng*h/mL for the patients with CC^{3435}, CT^{3435} and TT^{3435}, respectively; 22-31% lower in those with TT^{3435} than the others. Comparisons among the patients with GG^{2677}/CC^{3435}, GT^{2677}/CT^{3435} and TT^{2677}/TT^{3435} revealed no advantage over the stratification based on only C3435T (data not shown).

There was an intensive effect of the co-administration of a steroid and further treatment with nifedipine (p = 0.018). The co-administration with a steroid resulted in about a 1.2-fold increase in $AUC_{0\text{-}4,corr.}$ to 1758±537 ng*h/mL from that in the patients without a steroid or nifedipine treatment (1468±743 ng*h/mL), and further treatment with nifedipine resulted in a 51% increase, to 2221±639 ng*h/mL. The values of AST, Alb, Scr and Ht had no effect on $AUC_{0\text{-}4,corr.}$, while T-Chol had a substantial effect (p = 0.001), with higher values associated with a higher $AUC_{0\text{-}4,corr.}$. ALT values also gave a significant correlation (p = 0.007), although those values were all within its normal range.

Discussion

The CONCERT conference in 2002 was held to compare conventional C_0 monitoring with alternatively proposed C_2 monitoring and to reach an agreement on target values of C_2 (Cole et al. 2002; Levy et al. 2002; Monaco 2002; Nashan et al. 2002). A range of clinical investigations have confirmed that C_2 monitoring with the use of a CsA microemulsion, Neoral®, is superior for the prevention of acute rejection in de novo renal and liver transplant recipients without serious detrimental effects on renal function, and also in maintenance through the avoidance of unexpected over- or under-exposure to CsA. In 2003, a report of MO2ART (monitoring of 2-hours absorption in renal transplantation), the first prospective, multicenter trial of C_2 monitoring in de novo renal recipients receiving CsA microemulsions, was published and target values of C_2 monitoring were presented (Thervet et al. 2003).

The data with which this conclusion was made are those on adult patients. Since there are two unique features to consider regarding the pharmacokinetics of CsA in pediatric patients; 1) the bioavailability of CsA correlates with age, being lower in younger patients, and 2) pediatric patients have a higher rate of metabolism (del Mar Fernández de Gatta et al. 2002; Dunn 2003), the guidelines established in adult patients may not apply to pediatric patients. Here, the age-dependent pharmacokinetics of

CsA after oral administration of a CsA microemulsion was evaluated to reconfirm the usefulness of C_2 monitoring in pediatric patients in terms of predictability of systemic exposure to CsA. There were 4 slow absorbers (7.7%), patients with a t_{max} of 4 hours or more (Figure 1), for which C_2 monitoring would result in an over-dose of CsA (Cole et al. 2002; Levy et al. 2002; Monaco 2002; Nashan et al. 2002; Nozu et al. 2005). It is noted that the concentrations were determined up to 4 hours after administration, and these 4 patients might be low absorbers, not be slow absorbers. All belonged to the children's group, aged 5-15 years. Although further investigation is needed, the delay of the peak time is expected to be due to immature gastrointestinal function, including defective bile acid secretion, or overexpression of metabolizing enzymes or efflux transporters in the intestine and/or liver. Despite this issue, it has been found that $AUC_{0\text{-}4}$ values were better correlated with the C_0 to C_4 for the children than young adults aged 16-27 years, but the best surrogate marker was C_2 for both groups (Figure 2, Table 1). In Figure 2, the data of 4 slow absorbers were all located in left-lower part, suggesting the underestimation of $AUC_{0\text{-}4}$, and the data on these patients were excluded in the latter assessment.

This study was conducted also to identify the factors affecting systemic exposure to CsA, and for it, the $AUC_{0\text{-}4}$ values were normalized at a dose of 3 mg/kg/day as

$AUC_{0\text{-}4,corr.}$ after exclusion of the data on the 4 slow absorbers. As shown in Tables 2 and 3, the $AUC_{0\text{-}4,corr.}$ was independent of gender or indications for CsA, but depended on body size, *i.e.*, it tended to be higher in older patients ($p = 0.065$), and in the patients with a higher total body weight ($p = 0.026$). This is explained by age-dependent metabolic ability via the CYP3A family (del Mar Fernández de Gatta et al. 2002; Dunn 2003).

MDR1 T-129C, C1236T, and G2677A,T had no effect on the $AUC_{0\text{-}4,corr.}$, but C3435T had a weak, though insignificant, effect ($p = 0.072$). As the pharmacokinetics of CsA is regulated by MDR1 and the CYP3A family (Lown et al. 1997; del Mar Fernández de Gatta et al. 2002; Kelly and Kahan 2002; Ponticelli 2005), the genetic effects of these proteins are often investigated in terms of the steady-state pharmacokinetics of CsA. Most research has focused on C3435T for *MDR1*, due to its association with the expression of MDR1 in the tissues (Sakaeda et al. 2003; 2004; 2005; Okamura et al. 2004), and on *CYP3A4*1B* or *CYP3A5*3*, due to its association with a higher level of CYP3A4 (Amirimani et al. 1999) and a deficiency of protein (Hustert et al. 2001; Kuehl et al. 2001), respectively. The first report was presented by von Ahsen *et al.*, who indicated that the maintenance dose or dose-adjusted C_0 of CsA was independent of either *MDR1* C3435T or *CYP3A4* genotype in renal transplant

recipients (2001). Several other investigators indicated no effect of *MDR1* T-129C, C1236T, G2677T or C3435T on the dose-adjusted concentrations of CsA (Mai et al. 2003; Kuzuya et al. 2003; Hesselink et al. 2003; Haufroid et al. 2004). Hesselink *et al.* reported no effect of *CYP3A4*1B* and *CYP3A5*1* on the dose-adjusted C_0 of CsA (2003), but Haufroid *et al.* suggested that values were higher for *CYP3A5*3/*3* than **1/*3* (2004). In contrast, Yates *et al.* reported that the dose-adjusted concentration of CsA was higher in renal transplant recipients with CC^{3435} than CT^{3435} or TT^{3435} (2003). Bonhomme-Faivre *et al*. also suggested that the dose-adjusted concentration of CsA was higher, whereas a lower maintenance dose was needed, in T^{3435}-allele carriers among liver-transplant recipients (2004). Anglicheau *et al.* suggested that dose-adjusted concentrations of CsA were independent of C3435T, but were higher in T^{1236}-allele carriers in renal transplant recipients (2004). As listed above, there are still considerable discrepancies in the results on C3435T, suggesting that MDR1 has no or only slight effects on CsA pharmacokinetics after oral administration (Sakaeda et al. 2003; 2004; 2005; Okamura et al. 2004). In this study, several factors proved to have a stronger effect on CsA pharmacokinetics than MDR1 C3435T genotype, also supporting the speculation mentioned above.

In this study, it has been elucidated that co-administration of a steroid and further

treatment with nifedipine results in a higher $AUC_{0\text{-}4,corr.}$ ($p = 0.018$). Orphan nuclear receptors have been recognized as key molecules of drug-induced changes in both metabolism and transporting mechanisms, including CYP3A4 and MDR1 (Pascussi et al. 2003; Staudinger et al. 2003; Wang and LeCluyse 2003). Co-administration of a steroid is expected to reduce the systemic exposure to CsA, but it was not supported in this study (Tables 2 and 3). The maintenance dose of prednisolone or methylprednisolone were 0.63±0.57 mg/kg/day and 0.22±0.12 mg/kg/day, respectively, and these concentrations might be insufficient for induction of CYP3A4 or MDR1 to give a decrease of systemic exposure to CsA. Nifedipine is well-known to be a typical substrate for CYP3A4 (Galetin et al. 2003), but CsA pharmacokinetics is not affected to a clinically significant extent in adult patients (Legault et al. 1993; Crocker et al. 1994). In this study, co-administration of nifedipine resulted in an increase of CsA exposure, and this is specific for pediatric patients (Crocker et al. 1994). The values are higher for patients with a higher T-Chol ($p = 0.001$), and this can be explained simply by the lipophilicity of CsA resulting in condensation in the lipid components in circulating blood, as confirmed by animal experiments (Nakamura et al. 2001). Statically significant correlation ($p = 0.007$) was also detected for ALT among the results of clinical laboratory tests, but the values of ALT were all within its normal

range of our hospital of 8-34 IU/L, suggesting no conclusive information on the association of hepatic hunction with CsA systemic exposure. As shown in Tables 2 and 3, these effects were greater than those of *MDR1* genotype, and therefore the discrepancies in the results on C3435T among the investigators might be, in part, due to variability in age/total body weight, type of co-administered drug or lipid status of the patients.

Multivariate analysis was performed to a regression function to estimate the AUC_{0-4} value and/or the optimized dose to give a constant AUC_{0-4}. Inclusion of concentration data as variants gave a higher predictability, but it was impossible to elucidate the relationship with the factors affecting it. In contrast, their exclusion resulted in larger coefficient of variation each for parameters. These results strongly suggested extensively large intra- and inter-individual variation in CsA pharmacokinetics, being consistent with difficulties in model establishment for population pharmacokinetics.

In summary, C_2 monitoring re-proved useful to predict systemic exposure to CsA, AUC_{0-4} even in pediatric patients. The dose-normalized AUC_{0-4} was independent of gender or indications for CsA, while it depended on body size, *i.e.*, age and total body weight. *MDR1* C3435T, but not T-129C, C1236T or G2677A,T, had a weak, but

insignificant, effect, *i.e.*, it was about 22-31% lower in the patients with TT^{3435} than the others. Co-administration of a steroid and further treatment with nifedipine had a more intensive effect, *i.e.*, co-administration resulted in a 51% increase in the dose-normalized AUC_{0-4}. A strong effect was observed for the value of T-Chol, but not AST, Alb, Scr or Ht. The discrepancies in the results on *MDR1* C3435T among investigators might be due to variability in these factors.

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Legends for figures

Figure 1. Blood concentration-time profiles after oral administration of the cyclosporine microemulsion (Neoral®) in 52 Japanese patients. The subjects were consisting of 29 children and 19 young adults. There were 4 slow absorbers (7.7%).

Figure 2. Relationship between AUC_{0-4} and C_0 (A) or C_2 (B) in 52 Japanese patients. The subjects were consisting of 29 children and 19 young adults. A higher correlation coefficient was obtained for C_2 ($r = 0.927$, $p < 0.05$) than C_0 ($r=0.488$, $p <0.05$). The exclusion of 4 slow absorbers resulted in a higher correlation coefficient for C_0 ($r = 0.590$), but not for C_2 ($r = 0.917$).

Table 1. Coefficients of the correlation (r) between AUC_{0-4} and C_0-C_4 of cyclosporine in 52 Japanese patients.

	Age	N	C_0	C_1	C_2	C_3	C_4
Children	5-15	33 (29)	0.551 * (0.718 *)	0.818 * (0.793 *)	0.945 * (0.937 *)	0.812 * (0.809 *)	0.641 * (0.816 *)
Young adults	16-27	19	0.237	0.584 *	0.854 *	0.638 *	0.727 *
Total		52 (48)	0.488 * (0.590 *)	0.766 * (0.737 *)	0.927 * (0.917 *)	0.755 * (0.754 *)	0.640 * (0.791 *)

*: $p < 0.05$. The patients were classified into 2 subpopulations; children aged 5-15 years and young adults aged 16-27 years. Correlation coefficients after the exclusion of 4 slow absorbers, all belonging to the children's group, are in parentheses.

Table 2. Association of various factors with systemic exposure to cyclosporine, $AUC_{0\text{-}4,corr.}$ in 48 Japanese patients.

	Unpaired Student's t-test	ANOVA/ Tukey-Kramer test	Pearson's correlation Test
Gender	p = 0.508	—	—
Age [a)]	p = 0.300	—	r = 0.268, p = 0.065
Total body weight	—	—	r = 0.321, p = 0.026
Indications for CsA	—	p = 0.948	—
MDR1 T-129C [b)]	p = 0.414	—	—
C1236T	—	p = 0.478	—
G2677A,T [c)]	—	p = 0.150	—
C3435T	—	p = 0.072	—
Co-administration [d)]	—	p = 0.018	—
ALT	—	—	r = −0.386, p = 0.007
AST	—	—	r = 0.006, p = 0.966
Alb	—	—	r = −0.221, p = 0.131
Scr	—	—	r = 0.183, p = 0.214
Ht	—	—	r = −0.012, p = 0.935
T-chol	—	—	r = 0.458, p = 0.001

The analysis was performed after exclusion of the data on 4 slow absorbers, and dose-normalization of AUC_{0-4} at a dose of 3 mg/kg/day according to the equation: $AUC_{0-4,corr.} = AUC_{0-4}$ x [3 (mg/kg/day) / dose (mg/kg/day)].

a) The effect of age was analyzed after the stratification of patients into 2 subpopulations; children aged 5-15 years and young adults aged 16-27 years, or using Pearson's correlation test.

b) There was no patient with CC^{-129}, and the data on TT^{-129} and TC^{-129} was compared.

c) The effect of *MDR1* G2677A,T was analyzed among the patients with GG, GA + GT and AA + AT + TT.

d) A comparison was performed among the patients treated without either a steroid or nifedipine, with a steroid, and with both a steroid and nifedipine. All patients treated with nifedipine were administered a steroid.

Table 3. Effects of age, *MDR1* C3435T and co-administration of a steroid and further treatment with nifedipine on systemic exposure to cyclosporine, $AUC_{0\text{-}4,corr.}$ in 48 Japanese patients.

		N	Dose mg/kg/day	$AUC_{0\text{-}4}$ ng*h/mL	$AUC_{0\text{-}4,corr.}$ ng*h/mL
Total		48	3.33 ± 0.95	1909 ± 708	1792 ± 659
Age	Children (5-15 y)	29	3.45 ± 1.04	1894 ± 820	1711 ± 718
	Young adults (16-27 y)	19	3.14 ± 0.80	1932 ± 513	1915 ± 552
MDR1 C3435T	CC	13	3.22 ± 1.10	1831 ± 818	1738 ± 590
	CT	27	3.35 ± 1.01	2060 ± 649	1948 ± 678
	TT	8	3.42 ± 0.48	1526 ± 623	1351 ± 536
Co-administration	None	12	3.02 ± 0.82	1349 ± 515	1468 ± 743
	Steroid	25	3.47 ± 0.89	1979 ± 587	1758 ± 537
	Steroid + Nifedipine	11	3.33 ± 1.22	2367 ± 787	2221 ± 639

The values are the mean ± SD. The analysis was performed after exclusion of the data on 4 slow absorbers, and dose-normalization of $AUC_{0\text{-}4}$ at a dose of 3 mg/kg/day according to the equation: $AUC_{0\text{-}4,corr.} = AUC_{0\text{-}4}$ x [3 (mg/kg/day) / dose (mg/kg/day)]. The factors analyzed in terms of association with $AUC_{0\text{-}4,corr.}$ included gender, age, total body weight,

indications for CsA and *MDR1* T-129C, C1236T, G2677A,T and C3435T genotype, as well as co-administration of a steroid and further treatment with nifedipine and the values of ALT (IU/L), AST (IU/L), Alb (g/dL), Ht (%), Scr (mg/dL) and T-Chol (mg/dL) on the day of investigation. No factors other than age, *MDR1* C3435T and co-administration were demonstrated to have an effect on $AUC_{0\text{-}4,corr.}$.

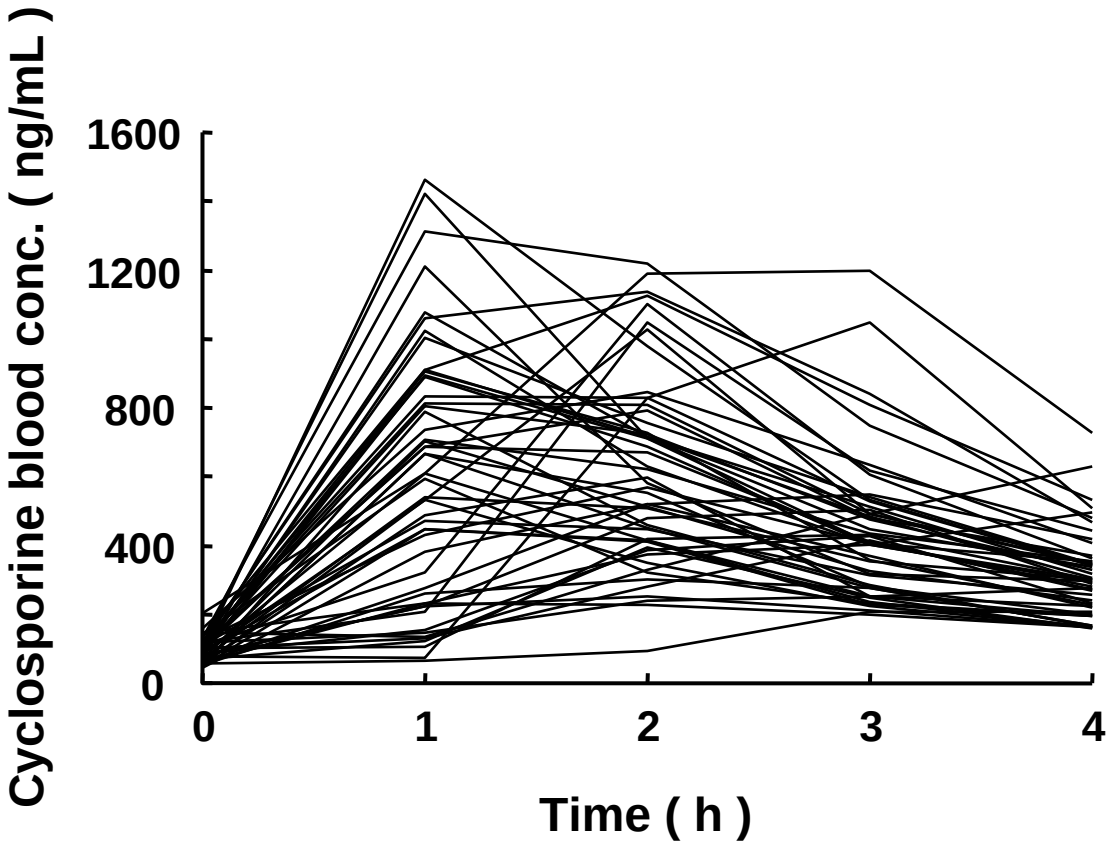

Fig 1. T. Sakaeda et al.

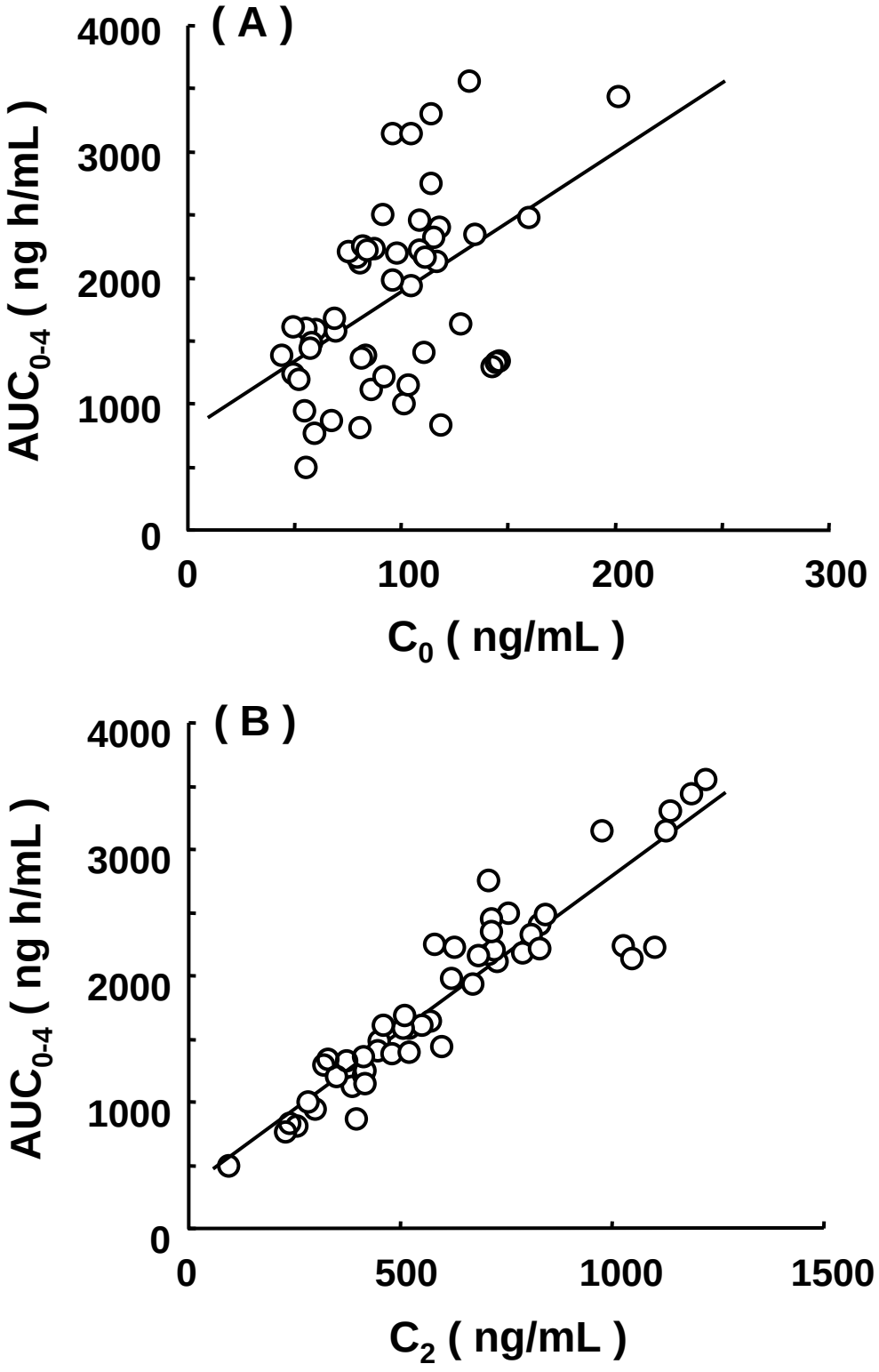

Fig 2. T. Sakaeda et al.