Evaluation of Pharmacokinetic Interaction between Cyclosporin A and Probucol in Rats

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Purpose. The purpose of this study was to clarify the mechanism of pharmacokinetic interaction between cyclosporin A and probucol in clinical cases.

Methods. The whole blood concentration of cyclosporin A was measured after oral administration of cyclosporin A with or without probucol in rats. Cyclosporin A was administered as three types of solutions: the contents of the conventional formulation (Sandimmun® capsule) diluted with corn oil and the contents of the new microemulsion preconcentrate formulation (Neoral® capsule) diluted with saline or corn oil. The solubility of cyclosporin A and another lipophilic agent tacrolimus in water with or without probucol was also measured.

Results. The area under the blood concentration-time curve (AUC) after the administration of Sandimmun® (corn oil) and Neoral® (corn oil) was significantly decreased to 26% and 41% of the control by coadministration of probucol. However in the case of Neoral® (saline), it was unchanged. The terminal elimination rate constant was not affected by probucol in any type of cyclosporin A solution. The solubility of cyclosporin A or tacrolimus in water dropped to 49% or 16% of the respective control in the presence of probucol.

Conclusion. The interaction between cyclosporin A and probucol is caused by the decreased absorption of cyclosporin A partly based on the lowered solubility in the presence of probucol.

KEY WORDS: cyclosporin A; probucol; drug interaction; pharmacokinetics.

INTRODUCTION

Cyclosporin A is an immunosuppressive agent widely used for organ transplantation or autoimmune diseases. The introduction of this drug in 1983 revolutionized immunosuppressive therapy. The conventional formulation of cyclosporin A (Sandimmun®) shows large intraindividual as well as interindividual variability in absorption because of its oily nature (1). Studies have shown that the absorption of cyclosporin A (Sandimmun®) is affected by bile flow, food intake, and gastrointestinal motility (2–4). Although the addition of Sandimmun® to immunosuppressive therapy improved graft survival rates and quality of life in transplant patients (5), it became evident that a more predictable formulation was needed. Neoral® was approved as a new formulation of cyclosporin A with microemulsion preconcentrate containing a surfactant, lipophilic and hydrophilic solvents and ethanol. Neoral® exhibits a better bioavailability and a smaller pharmacokinetic variability than Sandimmun® (6,7).

Cyclosporin A causes significant adverse reactions including metabolic disturbances, nephropathy, hypertension, and liver injury (8,9). Hyperlipidemia is another adverse reaction of cyclosporin A. Moreover in some autoimmune diseases, patients suffer from hyperlipidemia. In such cases, when dietary management fails to control hyperlipidemia, drug therapy should be considered. Probucol is an antilipemic agent used for patients with serious hyperlipidemia. Sundararajan *et al.* (10) reported that when probucol was coadministered with cyclosporin A in heart transplant patients, the whole blood concentration of cyclosporin A decreased to 76% of the value without probucol. Gallego *et al.* (11) also reported that withdrawal of probucol increased the trough concentration of cyclosporin A in renal transplant patients treated with cyclosporin A. We recently reported the interaction of cyclosporin A and probucol in patients with nephrotic syndrome (12). When probucol was coadministered with Sandimmun®, the trough blood concentration/dose ratio of cyclosporin A decreased to 50% of the value without probucol (12). A decrease in the oral bioavailability of cyclosporin A (Sandimmun®) in rats on coadministration of probucol has also been shown (13). However, the precise mechanism of the interaction between cyclosporin A and probucol has not been clarified. For Neoral®, whether interaction with probucol occurs has not been reported. Since Neoral® capsules are used more frequently than Sandimmun® capsules, whether interaction occurs between Neoral® and probucol should be clarified for the benefit of patients on cyclosporin A therapy.

In this study, we investigated the effect of probucol on the pharmacokinetics of cyclosporin A (Sandimmun® and Neoral®) in rats to clarify the interactive mechanisms. Our results suggest that the pharmacokinetic interaction between cyclosporin A and probucol is due to reduced absorption of cyclosporin A, which is at least partly caused by a decrease in the solubility of cyclosporin A in the presence of probucol. In the case of Neoral®, the pharmacokinetics of cyclosporin A is affected little by probucol when it is administered without oil.

MATERIALS AND METHODS

Materials

Sandimmun® capsules, Neoral® capsules and cyclosporin A were obtained from Novartis Pharma KK (Tokyo, Japan). Probucol was purchased from Wako Pure Chemical Co. (Osaka, Japan). Carboxymethyl cellulose sodium salt was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Tacrolimus was a gift from Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan). All other chemicals used were of the highest purity available.

Animals

Male Wistar rats weighing 230 to 260 g were used. Rats were fasted overnight before cyclosporin A administration and for a further 24 h, but given free access to water. Blood samples were collected from the tail vein under light ether anesthesia. The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University. The experimental protocol was approved by the

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Animal Research Committee, Graduate School of Medicine, Kyoto University (Med Kyo 01200).

Pharmacokinetic Studies in Rats

Cyclosporin A was administered as three types of solutions. The content of Sandimmun® capsules was diluted in corn oil to 5 mg/ml. The content of Neoral® capsules was diluted in corn oil or saline to 5 mg/ml. Probucol (40 mg) was suspended in 2 ml of 0.5% carboxymethyl cellulose sodium salt (CMC-Na) solution. The dosage of probucol was referred from a previous report (13). Rats were at first given probucol (40 mg/rat) or vehicle (0.5% CMC-Na solution) by gastric gavage, and after 10 min cyclosporin A (5 mg/kg) was administered. Rats treated with vehicle served as a control. Blood samples were collected from the tail vein at 1, 2, 4, 6, 8, 10, 12, and 24 h after the administration of cyclosporin A by gastric gavage. Samples (150–200 μl) were taken into EDTA anticoagulant tubes.

Solubility Studies *in Vitro*

Cyclosporin A was applied as three types of solutions, which were the contents of Sandimmun® capsules diluted in corn oil and the content of Neoral® capsules diluted in saline or corn oil to 5 mg/ml. The solutions (0.25 ml) were added to 5 ml of 0.5% CMC-Na solution in the absence or presence of 40 mg of probucol. In another experiment, an excess amount of cyclosporin A (1 mg) or tacrolimus (1 mg) was added to the 0.5% CMC-Na solution (5 ml) in the absence or presence of 100 mg of probucol. The solution was shaked well for 3 min on a vortex mixer, and centrifuged at 30,000 rpm for 10 min. The supernatant was diluted with saline or a 1:1 mixture of methanol and saline for cyclosporin A or tacrolimus and filtered through a microfilter (pore size 0.45 μm, Cosmonice Filter W, Japan Millipore Inc., Japan).

Analytical Methods

The concentration of cyclosporin A was measured using a monoclonal antibody fluorescence polarization immunoassay technique (TDx system, Dainabot Co. Ltd., Tokyo, Japan). The range of this system is 25 to 1500 ng/ml for a whole blood sample. Samples were diluted to an appropriate concentration before analysis with whole blood obtained from normal male Wistar rats. The tacrolimus concentration was measured with a semiautomated microparticle enzyme immunoassay (IMx system, Dainabot Co. Ltd.).

Pharmacokinetic Analysis

The pharmacokinetic parameters of cyclosporin A were calculated with a non-compartmental analysis based on the statistical moment theory. The area under the whole blood concentration-time curve (AUC) after oral administration was calculated using the linear trapezoidal rule and extrapolated to infinity by adding the ratio of the cyclosporin A concentration at the last sampling time (C_{24}) to the terminal elimination rate constant (k_β). The mean residence time (MRT) was determined by using the relationship of AUMC/AUC, where AUMC is the area under the first moment curve (whole blood concentration × time vs. time). The highest observable concentration and associated time point were defined as maximum concentration (C_{max}) and corresponding time (T_{max}), respectively.

Statistical Analysis

Values are expressed as the means ± standard error of the mean (SE) for *n* experiments. If the variances of groups were similar, the statistical significance of differences between mean values was calculated using the non paired *t*-test, otherwise the Mann-Whitney *U*-test was applied. Differences were considered significant at $p < 0.05$.

RESULTS

Effect of Probucol on the Whole Blood Concentration of Cyclosporin A in Rats

We first examined the effect of probucol on whole blood concentration of cyclosporin A. When Sandimmun® was administered, the whole blood concentration of cyclosporin A was significantly decreased by coadministration of probucol (Fig. 1), but in the case of Neoral® (saline), it was not affected by probucol (Fig. 2, A). On the other hand, the whole blood concentration of cyclosporin A after the administration of Neoral® (corn oil) was significantly decreased by coadministration of probucol (Fig. 2B).

Pharmacokinetic parameters of cyclosporin A (Sandimmun®) after oral administration are summarized in Table I. Those of Neoral® after oral administration are summarized in Table II. When rats were administered Sandimmun® or Neoral® (corn oil), the AUC of cyclosporin A in the presence of probucol was decreased to 26% or 41% of the respective control value. However when Neoral® (saline) was administered, no parameter was significantly changed by the coadministration of probucol. The values of MRT and k_β were not affected by probucol in any type of cyclosporin A solution.

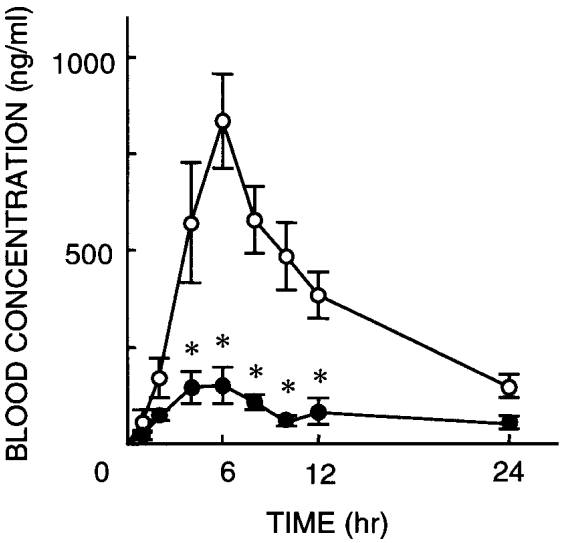

Fig. 1. Effect of probucol on the whole blood concentration of cyclosporin A after oral administration of Sandimmun® diluted with corn oil in rats. Cyclosporin A was orally administered at a dose of 5 mg/kg 10 minutes after oral administration of vehicle (○) or probucol (●) (40 mg/rat). Blood samples were collected at specified times after injection. Each point represents the mean ± SE of four rats. *$p < 0.05$, significantly different from control.

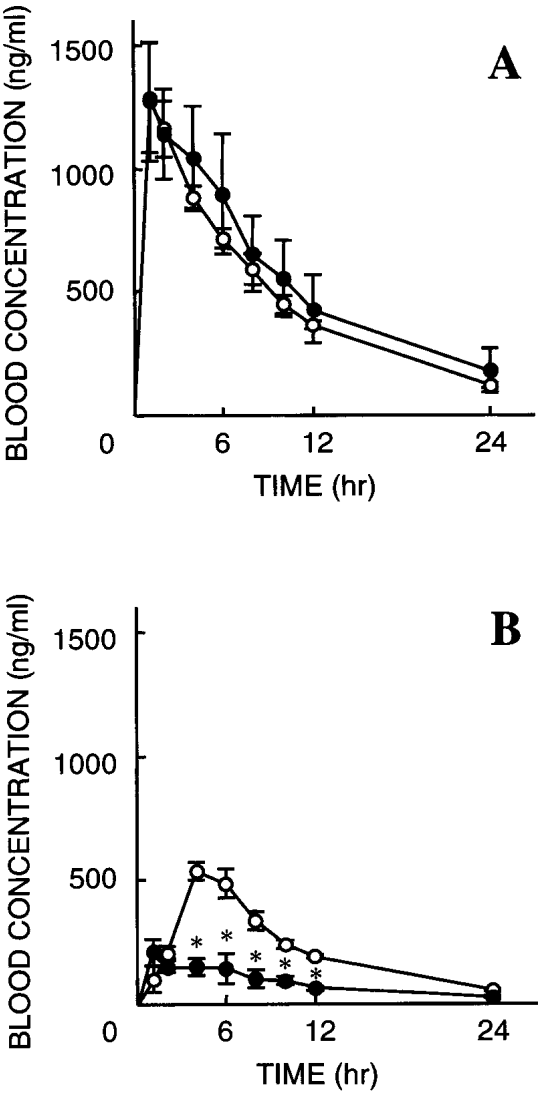

Fig. 2. Effect of probucol on the whole blood concentration of cyclosporin A after oral administration of Neoral® (diluted with saline) (A) or Neoral® (diluted with corn oil) (B). Cyclosporin A was administered at a dose of 5 mg/kg 10 min after oral administration of vehicle (○) or probucol (●) (40 mg/rat). Blood samples were collected at specified times after administration. Each point represents the mean ± SE of four to five rats. *$p < 0.05$, significantly different from control.

The value of C_{max} decreased significantly to 21% (Sandimmun®) and 38% (Neoral® in corn oil) in the presence of probucol. The value of C_{24} after the administration of Sandimmun® was reduced significantly to 37% by probucol, and it was also reduced by probucol after the administration of Neoral® (corn oil), although not significantly.

Effect of Probucol on the Solubilities of Cyclosporin A in Different Types of Formulations *in Vitro*

From the results obtained from experiments in rats, we hypothesized that the interaction between cyclosporin A and probucol was caused by the decrease in the intestinal absorption of cyclosporin A resulting from the lowered solubility of cyclosporin A in the presence of probucol. Then we examined the effect of probucol on the solubility of two types of Neoral® solutions *in vitro*. When Neoral® (saline) was added, the concentration of cyclosporin A in the filtrate was not changed by probucol (Fig. 3A). However when Neoral® (corn oil) was added, it decreased to 65% of the control by probucol (Fig. 3B). In the case of Sandimmun®, we could not measure the cyclosporin A concentration because of the detection limitation.

Table I. Pharmacokinetic Parameters of Cyclosporin A (Sandimmun®) after Oral Administration in Rats

Parameters	Control	Probucol
AUC (mg h/l)	10.6 ± 1.6	2.77 ± 0.93[a]
MRT (h)	14.3 ± 1.4	17.8 ± 3.9
k_β (h^{-1})	0.092 ± 0.011	0.102 ± 0.032
C_{max} (ng/ml)	863 ± 123	183 ± 41[a]
T_{max} (h)	6.0 ± 0.5	4.0 ± 0.8
C_{24} (ng/ml)	149 ± 29	54.4 ± 16.4[a]

Note: Cyclosporin A (Sandimmun®) diluted with corn oil was orally administered at a dose of 5 mg/kg 10 min after oral administration of vehicle or probucol (40 mg/rat).
Each value represents the mean ± S.E. of four rats.
[a] $P < 0.05$, significantly different from control.

Effect of Probucol on the Solubilities of Cyclosporin A and Another Lipophilic Agent Tacrolimus *in Vitro*

When probucol was present, the concentration of cyclosporin A in filtrate decreased to 49% of the control value (Fig. 4A). Because the solubility of cyclosporin A was decreased by probucol, we considered that the solubility of tacrolimus, which is also known to be a lipophilic agent, might be lowered by probucol. The solubility of tacrolimus decreased to 16% of the control in the presence of probucol (Fig. 4B).

DISCUSSION

Although there have been some clinical reports about interaction between cyclosporin A and probucol (10,11,12), the precise mechanism of this interaction has yet to be elucidated. Therefore we investigated the effect of probucol on the pharmacokinetics of cyclosporin A in rats to clarify the mechanism.

In the present experiment, the AUC and C_{max} of cyclosporin A (Sandimmun®) in rats decreased to 26% and 21% of the respective control after a single oral administration of probucol. In the case of Neoral® (saline), the blood concentration of cyclosporin A was not changed by probucol. The results obtained on Neoral® (saline) suggested that probucol did not affect the pharmacokinetics of cyclosporin A after absorption from the intestine. Sugimoto *et al.* (13) reported that the blood concentration of cyclosporin A after intravenous administration in rats was not affected by probucol, which is consistent with our results. The result that k_β after oral administration of Sandimmun® was not changed by probucol also indicated that probucol did not affect the elimination of cyclosporin A. These findings strongly suggested that probucol interacted with cyclosporin A during the absorption process. On the other hand, when Neoral® was administered after dilution in corn oil, the AUC and C_{max} of cyclosporin A decreased to 41% and 38% of the respective control in the

Table II. Pharmacokinetic Parameters of Cyclosporin A (Neoral®) After Oral Administration in Rats

Parameters	Saline		Corn oil	
	Control	Probucol	Control	Probucol
AUC (mg h/l)	12.8 ± 0.3	15.0 ± 5.2	5.88 ± 0.80	2.41 ± 0.44[a]
MRT (h)	10.1 ± 0.5	9.84 ± 1.03	10.7 ± 1.2	12.5 ± 2.1
k_β (h^{-1})	0.106 ± 0.004	0.113 ± 0.004	0.122 ± 0.016	0.095 ± 0.013
C_{max} (ng/ml)	1368 ± 164	1409 ± 222	557 ± 69	212 ± 52[a]
T_{max} (h)	1.4 ± 0.3	2.8 ± 1.1	3.0 ± 0.6	1.0 ± 0.0[a]
C_{24} (ng/ml)	119 ± 13	179 ± 90	56.9 ± 16.7	28.8 ± 5.6

Note: Cyclosporin A (Neoral®) diluted with saline or corn oil was orally administered at a dose of 5 mg/kg 10 min after oral administration of vehicle or probucol (40 mg/rat).
Each value represents the mean ± S.E. of four to five rats.
[a] $P < 0.05$, significantly different from control.

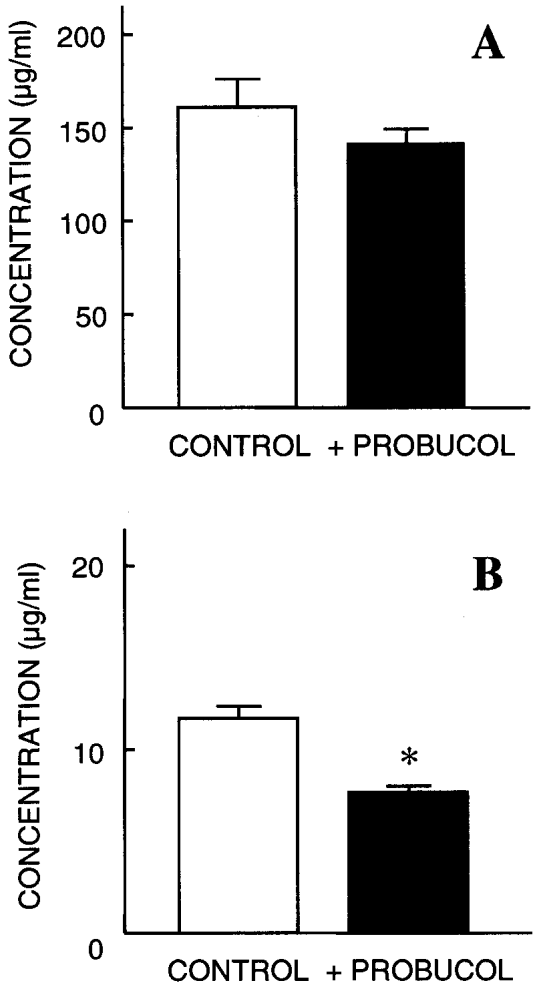

Fig. 3. Effect of probucol on the solubility of cylcosporin A *in vitro* when Neoral® (diluted with saline) (A) or Neoral® (diluted with corn oil) (B) were used. Neoral® (5 mg/ml, 0.25 ml) was added to 5 ml of 0.5% CMC-Na solution in the absence (open column) or presence (closed column) of 40 mg probucol. Each column represents the mean ± SE of three independent experiments. *p < 0.05, significantly different from control.

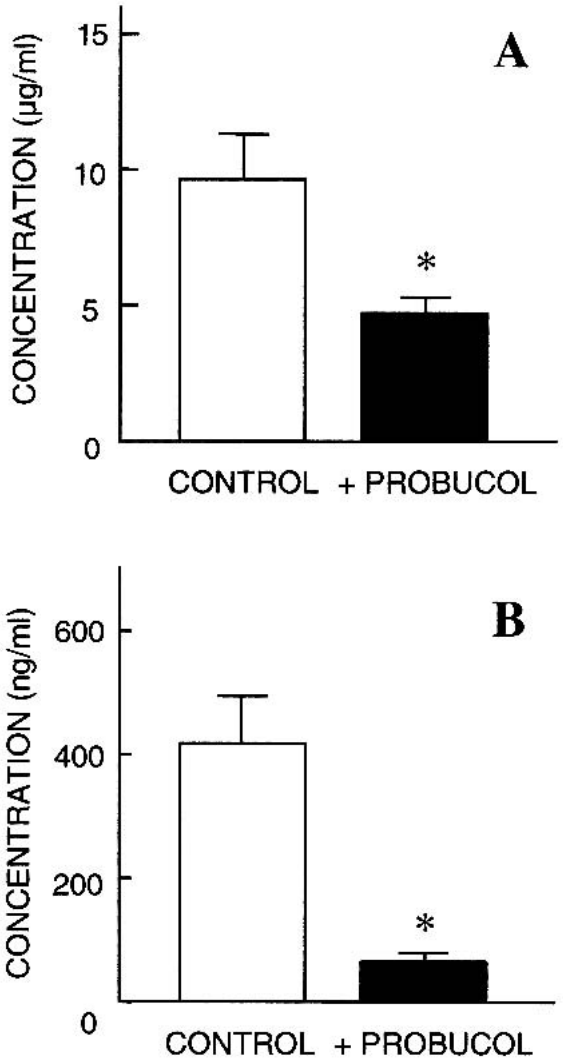

Fig. 4. Effect of probucol on the solubility of cylcosporin A (A) or tacrolimus (B) *in vitro*. Cyclosporin A (1 mg) or tacrolimus (1 mg) was added to 5 ml of 0.5% CMC-Na solution in the absence (open column) or presence (closed column) of 20 mg/ml probucol. Each column represents the mean ± SE of three to five independent experiments. *p < 0.05, significantly different from control.

presence of probucol. The reasons for these results are that Neoral® could not form a microemulsion when diluted in corn oil because it can do so only when mixed in water, and that the absorption of cyclosporin A was reduced by probucol.

Cyclosporin A is incompletely absorbed from the gastrointestinal tract, and absorption takes place predominantly in the small intestine (14). The absorption of cyclosporin A is low and influenced by many factors, e.g., food, vehicle, type of transplant or amount of bile in the gut (15). One reason for this variability is the lipophilicity of cyclosporin A, and the lack of dispersion of this drug is also one cause of incomplete absorption (16). Because of its lipophilicity, cyclosporin A distributes across most of the biologic membranes and tissues. Low-density lipoprotein (LDL) is reported as a carrier vehicle for cyclosporin A, and may facilitate its entrance into cells via the LDL receptor (17). Cyclosporin A is known to be a substrate of P-glycoprotein (18,19), and is mainly metabolized by the cytochrome P450 IIIA4 (CYP3A4) in the liver and the intestine (19,20). Lown *et al.* reported that intestinal P-glycoprotein as well as CYP3A4 in the liver and intestinal membrane accounted for the interpatient variability in blood cyclosporin A concentration (21). To date, several explanations for the decrease in the oral bioavailability of cyclosporin A on coadministration of probucol have been proposed (11,13): a decrease in serum cholesterol caused by probucol, an increase of first-pass metabolism of cyclosporin A following induction of CYP3A4 by probucol, and a decrease of absorption caused by activating P-glycoprotein in the presence of probucol, or by competition with probucol in bile acid. However there is no direct evidence that probucol induces CYP3A4, or activates P-glycoprotein.

Both cyclosporin A and probucol are highly lipophilic compounds. Probucol is known to be hardly absorbed from the gastrointestine and be excreted into bile (22). Therefore, it is possible that probucol remains in the intestine for a long time because of the entero-hepatic circulation, and continues to interact with cyclosporin A there. Since lipophilic agents are hardly soluble in water, the passage of unstirred water layer in the gastrointestine is a rate-limiting step in absorption. We considered that when the solubility of cyclosporin A in aqueous gastrointestinal fluid is inhibited by probucol, the absorption of cyclosporin A decreases. The different results obtained on administration of Neoral® (saline) and Neoral® (corn oil) support this. To confirm the hypothesis, we examined the effect of probucol on the solubility of cyclosporin A when Neoral® (saline) and Neoral® (corn oil) were used *in vitro*. In the case of Neoral® (corn oil), probucol was shown to significantly decrease the concentration of cyclosporin A in water phase. However in the case of Neoral® (saline), it was not changed. Moreover, the solubility of cyclosporin A powder also decreased in the presence of probucol. So it was made clear that the interaction between cyclosporin A and probucol is at least partly caused by inhibition of the solubility of cyclosporin A in aqueous gastrointestinal fluid by probucol. Although there is no report of interaction between probucol and any other drug, we considered that probucol might interact with other lipophilic agents as well as cyclosporin A. Tacrolimus is a potent immunosuppressive agent widely used for organ transplantation and known to be a highly lipophilic compound (23). We then examined the effect of probucol on the solubility of tacrolimus in water. The solubility of tacrolimus in water was also significantly decreased in the presence of probucol. Considering these results obtained from *in vitro* experiments, probucol might reduce the absorption of other highly lipophilic drugs.

There are some reports about interaction between cyclosporin A (Sandimmun®) and a high-fat meal (3,24). In these reports, a fat-rich meal increases the oral bioavailability of cyclosporin A (Sandimmun®) in healthy volunteers because of the stimulated bile flow. A standard diet may also play a role in the appearance of a second serum peak concentration of cyclosporin A after oral administration (1). The plasma probucol level is also reported to increase with the intake of fat-rich foods (25). For Neoral®, the interaction with fat-rich meals is not well reported. However Mueller *et al.* (24) found that a fat-rich meal moderately decreased the peak concentration of cyclosporin A after Neoral® administration. From the results of this experiment (Fig. 2B), when Neoral® is given with a high-fat meal, it is possible that the bioavailability of cyclosporin A decreases because a microemulsion could not form, and that the interaction with probucol occurred. Furthermore, in patients treated with Neoral®, the degree of decrease in the blood concentration of cyclosporin A caused by probucol might be attributed to the lipid content of their diet. Further clinical studies on the interaction between Neoral® and probucol are needed.

In conclusion, the interaction between cyclosporin A and probucol at least partly originated from the decreased solubility of cyclosporin A in the presence of probucol during the intestinal absorption. The present findings suggest that in the case of Neoral®, the pharmacokinetics of cyclosporin A is affected little by probucol when it is administered without oil.

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