Oral Absorption of FK506 in Rats

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The oral absorption of FK506 in solid dispersion formulation was studied in rats. The obtained area under the concentration versus time curve (AUC) increased in a nonlinear fashion with a small dose-dependent increase in the peak blood concentrations (C_{max}). The peak concentration time (T_{max}) was observed within 30 min after administration in all dosing groups (1–10 mg/kg) with or without feeding, whereas the oral absorption of FK506 was reduced to about 50% by gavage at a dose of 1 mg/kg. Participation of first-pass elimination was suggested by comparing the blood levels after infusion via the portal vein with those after infusion via the femoral vein. Further, in an *in vitro* stability study and an *in situ* loop absorption study, FK506 was fairly stable in the gastrointestinal juice and was absorbed predominantly from the upper part of the small intestine.

KEY WORDS: FK506; *in vitro* stability; oral absorption; gastrointestinal absorption site; first-pass elimination.

INTRODUCTION

FK506, a novel immunosuppressant, was isolated from *Streptomyces tukubaensis* as white crystalline powder (1). In the *in vitro* studies, FK506 was 100-fold more potent than cyclosporin (Cy A) (2). However, pharmacological effects were insufficient in *in vivo* studies because of poor bioavailability of the crystalline powder of FK506 when given via the oral route.

In order to improve the bioavailability, a number of dosage forms of FK506 were investigated. Among them, the solid dispersion formulation offered the best therapeutic advantage in rat skin allograft transplantation (3), and this formulation was developed as the oral dosage form of the compound. Clinical and preclinical studies have been performed using this formulation of FK506 (3–8). However, there has been no detailed study of the oral absorption of FK506 in the solid dispersion formulation.

In the present study, the oral absorption of FK506 in the solid dispersion formulation was investigated at three doses in rats under conventional fed conditions, as well as fasting, to assess the effect of feeding on the absorption of FK506. Although the bioavailability of this formulation was improved to almost the same level as that of FK506 dissolved in polyethylene glycol (5), even the dissolved FK506 still did not exhibit sufficient bioavailability (5,6). In order to clarify the reason for the poor bioavailability of orally administered FK506, the stability in gastrointestinal fluid, gastrointestinal absorption site, and first-pass elimination were investigated in rats given aqueous solutions of FK506.

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MATERIALS AND METHODS

Dosage Forms

FK506 in a solid dispersion formulation containing 20% active ingredient, 20% hydroxypropyl methylcellulose, 20% croscarmellose sodium, and 40% lactose was used for the oral absorption study (3). A formulation for injection containing 10 mg of FK506 in 1 mL of ethanol dissolving polyoxyethylated hydrogenated castor oil 60 (NIKKO Chemicals Co., Ltd. Tokyo) at 40% was used for the study of first-pass elimination. An aqueous solution solubilizing FK506 with polyoxyethylated hydrogenated castor oil 60 at a weight ratio of 1:50 was used for the gastrointestinal absorption site study and for the *in vitro* stability study.

Animals

Male Sprague–Dawley rats were used at the age of 7 weeks. Animals used for the gastrointestinal absorption site study, for the collection of gastrointestinal juice, and for the fasted group in the oral absorption study were deprived of food overnight; drinking water was readily accessible.

Oral Absorption

FK506 in the solid dispersion formulation suspended in distilled water was given orally at doses of 1, 3.2, or 10 mg/kg to the fed groups and at a dose of 1 mg/kg to the fasted group. At each sampling point, five animals were anesthetized with ethyl ether and blood samples were collected from the abdominal aorta and kept frozen at −20°C until assayed.

In Vitro Stability

Under ethyl ether anesthesia, the pylorus was ligated and 2 mL of distilled water was administered into the stomach. At 2 hr after administration of water, the stomach was excised and the gastric juice was collected. The supernatant obtained after centrifugation was used for the stability study. The small intestine was excised from the duodenum to the ileum and rinsed on the inside with 10 mL of pH 6.4 isotonic phosphate buffer. The rinse was centrifuged and the supernatant was used as intestinal juice. To 3.9 mL of gastric or intestinal juice placed in a 10-mL glass stoppered test tube, 0.1 mL of solubilized FK506 aqueous solution (1 mg/mL) was added. After agitation, the sample solutions were incubated in a water bath at 37 ± 0.1°C. At the appropriate time, 0.1 mL of the sample solution was collected and extracted with 4 mL of dichloromethane in the presence of 0.1 mL of saturated Na_2SO_4 aqueous solution. After centrifugation, 3 mL of the organic phase was evaporated to dryness. The residue was reconstituted with 150 μL of the mobile phase for the HPLC assay. For the pH stability study, buffer solutions were used instead of gastrointestinal juice and the sample solution was injected directly into the HPLC.

Gastrointestinal Absorption Site

The gastrointestinal region was exposed by a midline abdominal incision under ethyl ether anesthesia, and a 10-cm loop was prepared by double ligation at one of the following

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regions: duodenum (just below the pylorus with bile duct ligation), jejunum (10 cm below the pylorus), ileum (just above the cecum), and colon (just below the cecum). For the stomach, the cardiac and duodenum ends were ligated. Using a syringe, 0.5 mL of FK506 aqueous solution at a concentration of 100 μg/mL was injected into the loop. At 10, 20, and 30 min after dosing, 0.5 mL of blood sample was collected with a heparinized syringe from the jugular vein, and was kept frozen at −20°C until assayed. Immediately after the last blood sampling, animals were sacrificed and the loops were excised. The residual FK506 in the loops was recovered by rinsing with methanol to make 20 mL. The rinse was centrifuged and 1 mL of the supernatant was evaporated to dryness under nitrogen. The residue was reconstituted with 150 μL of the mobile phase of HPLC.

First-Pass Elimination

Under ethyl ether anesthesia, the portal or the femoral vein was cannulated with polyethylene tubing (Intramedic, PE-50, Clay Adams, Parsippany, NJ). A polyethylene cannula was also introduced in the femoral artery for blood collection. The formulation of FK506 for injection diluted with saline to give a concentration of 0.435 mg/mL was administered via the portal or the femoral cannula using an infusion pump at a flow rate of 0.02 mL/min for 30 min; the total dose of infused FK506 ranged from 0.996 to 1.03 mg/kg, depending on the body weight of the rats. Blood specimens were collected up to 2 hr and stored at −20°C until assayed.

Analysis of FK506

Since concentration-dependent changes of the blood/plasma distribution ratio of FK506 (5) have recently been reported, whole-blood levels were determined in this report to characterize the oral absorption of FK506 including dose dependency. After hemolysis with hypotonic pH 7 phosphate buffer, immunoreactive FK506 in whole blood was extracted with dichloromethane and assayed by the competitive enzyme immunoassay using mouse monoclonal antibody against FK506 (9).

FK506 measurements, except for the buffer solution in the stability study, were carried out by HPLC: Waters Model 6000A equipped with an autoinjector, Waters Wisp 710B, an ultraviolet detector, Waters Model 484 (Millipore, Bedford, MA), and a TSK-gel 120-OH column (5 μm, 25 cm × 4.6-mm i.d., Tosoh Co. Ltd., Tokyo). Thirty microliters of sample solution was injected into the HPLC using a mobile phase of normal hexane and ethanol (85:15, v/v) and a flow rate of 1 mL/min. The wavelength of the detector was 230 nm. FK506 in the buffer solution was assayed by HPLC using a reversed-phase column of TSK-gel ODS-120T (5 μm, 25 cm × 4.6-mm i.d., Tosoh Co. Ltd., Tokyo) and a mobile phase of tetrahydrofuran, methanol, and water at a volume ratio of 7.5:6:7.5.

RESULTS

Oral Absorption of FK506 in the Solid Dispersion Formulation

The mean blood levels of FK506 after oral administration in aqueous suspension of the solid dispersion formulation are shown in Fig. 1, and the pharmacokinetic parameters are listed in Table I. The AUC values increased in a nonlinear fashion with a small dose-dependent increase in C_{max}. A 30-fold increase was observed for the AUC values with increasing dose from 1 to 10 mg/kg, whereas the C_{max} values exhibited only a 4-fold increase within the same dose range. In the fasted group, the AUC value at 1 mg/kg was twice that obtained in the fed condition. A slightly higher C_{max} was observed in the fasted state, whereas there was no significant difference between the C_{max} values obtained in these two feeding conditions. For all dosing conditions, T_{max} was reached within 30 min after dosing.

In Vitro Stability

The results of the *in vitro* stability studies of FK506 in the pH range of 1 to 8 and in the gastrointestinal juice of rats are shown in Table II together with the pH values measured at the final sampling point. FK506 was stable over the pH range of 1 to 7 and in the gastric juice. However, a slight but significant decrease in residual percentage of FK506 was observed in the intestinal juice at the final sampling point. The degradation of FK506 in the intestinal juice might be caused by enzymic reactions, because the pH of the sample solution was maintained at around 6.4 with phosphate buffer.

Gastrointestinal Absorption Site

The gastrointestinal absorption site of FK506 was investigated by an *in situ* loop technique where a loop was made at one of five sites in the alimentary tract in rats. The blood levels of FK506 after instillation of the sample solution into the loop are shown in Fig. 2 as a function of time postdosing. The mean blood concentrations of FK506 given in the gastrointestinal regions, excepting the stomach, mainly peaked within 10 min after dosing, the first sampling point. The rank order of the absorption site by the observed mean blood levels of FK506 was jejunum ≥ duodenum > ileum ≥ colon > stomach. These results suggest that absorption of the dissolved FK506 is rapid and predominantly from the upper part of the small intestine.

Additionally, the residual amount of FK506 in the loops was determined. The percentage of recovered FK506 is shown in Table III. A fairly good correspondence was ob-

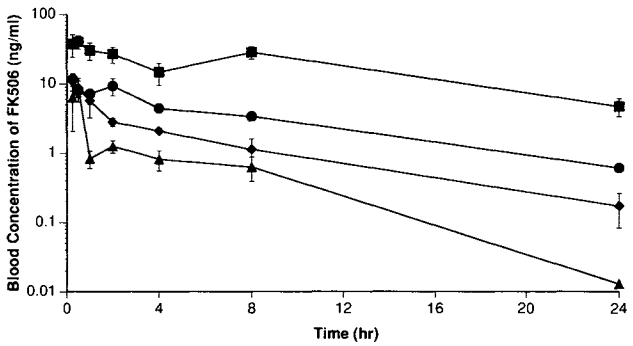

Fig. 1. Mean whole-blood concentrations of FK506 after oral administration in aqueous suspension of solid dispersion formulation. ■, 10 mg/kg (fed); ●, 3.2 mg/kg (fed); ▲, 1 mg/kg (fed); ◆, 1 mg/kg (fasted). Values are expressed as means ± SD of five animals. Minimum assay limit is 0.05 ng/mL.

Table I. Pharmacokinetic Parameters of FK506 After Oral Administration in an Aqueous Suspension of Solid Dispersion Formulation[a]

Feeding condition	Dose (mg/kg)	C_{max} (ng/mL)[b]	T_{max} (hr)	$AUC_{(0-24\ hr)}$ (ng · hr/mL)[c]
Fed	1	8.8 ± 4.9	0.5	16.2
	3.2	11.6 ± 5.3	0.25	76.9
	10	40.2 ± 19.4	0.5	450.2
Fasted	1	10.7 ± 6.3	0.25	33.2

[a] C_{max}, T_{max}, and AUC were calculated from the mean blood concentration-versus-time curve (n = 5).
[b] C_{max} values are listed together with standard deviations.
[c] AUC was calculated by the trapezoidal method.

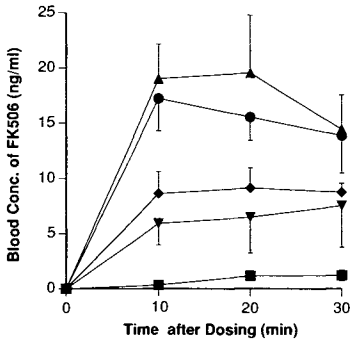

Fig. 2. Mean whole-blood concentrations of FK506 after administration of aqueous solution into loops. ▲, Jejunum; ●, duodenum; ◆, ileum; ▼, colon; ■, stomach. Values are expressed as means ± SD of three or four animals. Minimum assay limit is 0.05 ng/mL.

tained in the rank order of the gastrointestinal region between the blood levels and the percentage recoveries. Based on the residual amount in the loops, the extent of FK506 absorbed from the small intestine was estimated to be fairly large. However, FK506 might remain in the intestinal tissue or bind to the mucosal surface owing to its hydrophobicity; when using phosphate buffer as the rinsing medium, the recovery of FK506 was insufficient even immediately after instillation of the sample solution into the loops. The blood levels of FK506 infused into the ileum and colon loops were not significantly different, while a significant difference was observed in the residual amount of FK506 between these two loops. This phenomenon might be due to regional differences between the affinity of FK506 for the intestinal tissue and the mucosa.

First-Pass Elimination

The whole-blood levels of FK506 after starting infusion via the portal or the femoral vein are shown in Fig. 3. The blood levels of FK506 given via the femoral vein were significantly higher than those via the portal vein. The AUC values from 0 to 2 hr were 136 and 268 ng · hr/mL with standard deviations of 24 and 23 ng · hr/mL for administration via the portal and femoral vein, respectively.

DISCUSSION

To achieve sufficient bioavailability of FK506, a solid dispersion formulation using hydroxypropyl methylcellulose has been developed as the oral dosage form (3). This formulation exhibited rapid and sufficient drug release *in vitro* and provided much higher plasma levels of FK506 than the crystalline powder when given orally to dogs (3).

In the present study, dose dependency and effect of feeding were investigated in rats to characterize the oral absorption of FK506 in the solid dispersion formulation. The AUC values increased in a nonlinear fashion with small dose-dependent increases in C_{max} values. This was likely due to the slower absorption of FK506. The experiments were all performed under the same feeding conditions ruling out an effect of food. A larger amount of undissolved FK506 in the dosing sample could account for this result. In the oral absorption study using FK506 dissolved in polyethylene glycol, which was carried out in the fasted state, both AUC and C_{max} values exhibited a 20-fold increase over a range of 1 to 10 mg/kg (5).

On the basis of AUC values, the bioavailability of the solid dispersion formulation was improved to almost the same level as that of the polyethylene glycol solution of FK506 at the same dosing condition of 1 mg/kg in the fasted state (5). The AUC value of the solid dispersion formulation obtained in the nonfasted state was about 60% that of FK506 dissolved in polyethylene glycol at the dose of 10 mg/kg (5). Taking into account that the bioavailability of FK506 was reduced to about 50% by gavage, the solid dispersion formulation is considered to offer almost the same bioavailability of polyethylene glycol solution of FK506 with a small increase in C_{max} value even at the high dose of 10 mg/kg in the nonfasted state. This is in line with the high therapeutic

Table II. Stability of FK506 in Buffer Solutions and in Gastrointestinal Juice

	Residual %[a] at a final pH of						
	Buffer solution					Gastric juice	Intestinal juice
	1.07	3.93	5.91	6.98	8.01	1.42	6.42
1 hr[b]	—	—	—	—	—	98.2 ± 5.7	94.4 ± 5.4
4 hr	98.6 ± 0.2	101.5 ± 0.3	99.2 ± 0.5	101.4 ± 0.1	96.2 ± 1.9	99.6 ± 2.9	91.5 ± 2.4
24 hr	97.4 ± 1.1	98.6 ± 0.3	99.4 ± 0.4	101.0 ± 0.4	85.8 ± 1.7	—	—

[a] Values are expressed as means ± SD of two or three detections.
[b] Time after starting experiment.

Table III. Residual Amount of FK506 Recovered from *in Situ* Loops[a]

Site	% recovery
Stomach	88.7 ± 6.4
Duodenum	13.7 ± 5.0
Jejunum	13.8 ± 3.7
Ileum	26.5 ± 10.0
Colon	59.6 ± 14.8

[a] Values are expressed as means ± SD of three or four animals. Applied solution was recovered from loops at 30 min after dosing.

advantage obtained among the formulations tested in skin allograft transplantation in rats (3).

However, even dissolved FK506 offers a poor oral absorption efficiency (5,6). According to Iwasaki *et al.*, the bioavailability of FK506 in polyethylene glycol solution is about 10% at a dose of 1 mg/kg in fasted rats (5). Furthermore, in a clinical study using this formulation, the bioavailability was 27% when calculated from the plasma levels of FK506 at a dose of 0.15 mg/kg (7). The value was better than expected from the animal studies but still was not as good as desired. FK506 was found to be fairly stable in the gastrointestinal lumen considering the residence time before absorption, ruling out intestinal degradation as a factor in low bioavailability.

A likely explanation is the presence of an absorption window for FK506, which was suggested in the *in situ* loop absorption study. The presence of an absorption window was reported for Cy A in rats (10) and in healthy human volunteers (11). Another possible explanation is the participation of first-pass elimination in the absorption of FK506. A nonlinear increase in AUC with an increase in dose was observed in the present oral absorption study, while the AUC values increased in a linear fashion in fasted rats given polyethylene glycol solution orally (5). In order to clarify this hypothesis, the blood levels of FK506 infused via the portal vein were compared with those via the femoral vein. Under these conditions, about 50% of FK506 given via the portal vein underwent first-pass elimination before entering the systemic circulation. The discrepancy between the increasing AUC values in the studies using the solid dispersion formulation and polyethylene glycol solution of FK506 might be attributable to the difference in the extent of first-pass elimination affected by formulation and feeding.

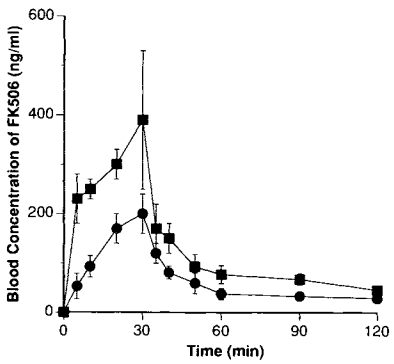

Fig. 3. Mean whole-blood concentrations of FK506 after starting infusion of FK506 aqueous solution via the portal and femoral vein. ■, Femoral vein; ●, portal vein. Values are expressed as means ± SD of five animals. FK506 equivalent to 1 mg/kg was infused for 30 min.

The oral absorption of Cy A is affected by food intake. A significant increase in C_{max} and AUC following coadministration of Cy A with food was observed in renal transplant patients (12) and healthy volunteers (13). The oral absorption of FK506 was also affected by food in rats. In contrast with Cy A, the bioavailability of FK506 was reduced by gavage. The increased bioavailability of Cy A seems to be related to food-induced change in physiological conditions such as splanchnic blood flow, plasma protein binding, and activity of drug metabolizing enzymes (14). Moreover, hepatobiliary secretion enhanced by food may help to dissolve the lipophilic drug (15). Such food-induced changes also seem to be applicable to the absorption of FK506. Whereas the absorption of FK506 was reduced by gavage, further investigation is needed to elucidate this phenomenon.

Thus FK506 possesses unfavorable properties to achieve sufficient bioavailability. Although the solid dispersion formulation does not offer optimal bioavailability, this formulation was selected as the best regimen, for clinical trials of FK506 in liver and kidney transplantation (7,8) and autoimmune diseases. FK506 oral absorption in these animal studies can serve as a guide to establish the optimum clinical regimen.

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