PHARMACOKINETICS OF FK482, A NEW ORALLY ACTIVE CEPHALOSPORIN, IN ANIMALS

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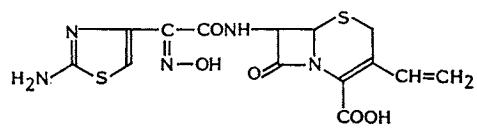
The pharmacokinetic profile of FK482 was studied in mice, rats, rabbits and dogs after oral dosing and compared with that of cefixime, cefaclor and cephalexin. Considerable differences in oral absorption of FK482 were observed among the animal species. Absolute bioavailabilities of FK482 were 12.6% in mice, 15.3% in rats, 32.3% in rabbits and 72.3% in dogs. In mice and rats, the absorption of FK482 was poor, and was the lowest of the reference antibiotics. FK482 was moderately well absorbed, with higher plasma levels than cefixime in rabbits and, like cefixime, gave higher plasma levels and a longer half-life than cefaclor or cephalexin in dogs. The increase in the area under the serum concentration time curve (AUC) of FK482 was strictly proportional to the increase in dose in the range of 2.5 to 40 mg/kg in rats and dogs, and 2.5 to 20 mg/kg in rabbits and the urinary recovery rates were almost constant. All tissue concentrations of FK482 in rats and rabbits were lower than those of the reference antibiotics and reflected its lower plasma concentrations in these animals. The urinary recovery rates of FK482 were 9.8% for mice, 15.5% for rats, 45.8% for rabbits and 47.1% for dogs. The biliary recovery rate of FK482 was low; 1.4% in rats and less than 0.1% in rabbits and dogs. No active metabolites were detected in the plasma, urine or bile samples from rats, rabbits or dogs. FK482 was mainly absorbed in the jejunum, and was inactivated in the large intestine. The serum-protein binding of FK482 was almost the same as that of cefixime: 60~77% for mouse, rabbit and human serum, and 90~93% for rat and dog serum.

We recently began developing FK482 (Fig. 1), a new orally active cephem antibiotic. Studies on the *in vitro* and *in vivo* antibacterial activities of FK482 showed that the drug was an orally active cephem antibiotic which offered some advantages over the commercially available cephalosporins in activity against Gram-positive and Gram-negative organisms[1,2]. In this report, the pharmacokinetic profiles of FK482 were studied in experimental animals.

Fig. 1. Chemical structure of FK482.



Materials and Methods

Antibiotics

The antibiotics used in this study were FK482 and cefixime (Fujisawa Research Laboratories, Osaka, Japan) and cefaclor and cephalexin (Eli Lilly and Company, Indianapolis, U.S.A.).

Animals

The following animals were used: 5 to 6-week-old male ICR-strain mice, 6-week-old male JCL: SD strain rats, male Japanese white rabbits (2.57 to 3.56 kg) and male beagle dogs (8.5 to 12.0 kg).

Dosing

For oral dosing, each of the antibiotics was suspended in 0.5% methyl cellulose solution. The animals, except mice, were starved overnight before dosing with 20 mg/kg, unless otherwise specified. For intravenous injection to evaluate the absolute bioavailability, FK482 was dissolved in 5% sodium bicarbonate solution, diluted with 0.9% saline and injected in doses of 20 mg/kg into the tail vein of mice and rats, the ear vein of rabbits and the antecubital vein of dogs.

Serum (Plasma) Sampling

Mice and rats were used in groups of ten for each sampling time of each drug. At specified times after dosing, the blood was obtained by heart puncture. Rabbits and dogs were used in groups of 4 to 8 for each drug. The blood samples were taken from the ear vein of rabbits and the antecubital vein of dogs at specified times. For FK482 and cefaclor, blood was collected into heparinized syringes. The resultant serum and plasma samples were frozen until assayed.

Pharmacokinetic Analysis

The pharmacokinetic parameters of FK482, cefixime, cefaclor and cephalexin after oral dosing in mice, rats, rabbits and dogs, and after intravenous dosing in mice and rats were estimated *via* a one-compartment open model using plasma (serum) concentration-time data. The model equations were:

$$C=\frac{K_a \cdot C_o}{K_a-K_e}(e^{-Ket}-e^{-Kat}) \quad \text{for oral dosing, and}$$

$$C=C_o \cdot e^{-Ket} \quad \text{for intravenous dosing,}$$

where C is the plasma (serum) concentration; K_a and K_e are first-order absorption and elimination rate constants, respectively; and C_o is the fictive plasma (serum) concentration at $t=0$. The plasma FK482 concentration-time data after intravenous administration in rabbits and dogs were analyzed by a two-compartment open model and NONLIN computer program. The model equation was:

$$C=Ae^{-\alpha t}+Be^{-\beta t}$$

where C is the plasma concentration; and α and β are the first-order rate constants in the distribution and elimination phases, respectively, and A and B are the coefficients of exponential term α and β, respectively.

Tissue Sampling

Rats and rabbits in groups of 3 or 4 were bled to death at specified intervals after oral dosing. The liver, kidney, lung, heart and spleen were removed, washed with 0.9% saline, and blotted with filter paper. The organs were homogenized with Polytron Homogenizer after addition of 2 ml of 1/15 M phosphate buffer (pH 7.0) per g of tissue. The homogenates were centrifuged at $10,000 \times g$ for 20 minutes to separate the supernatant.

Urinary Excretion

Groups of 10 mice and 9 to 10 rats were used for each drug. Urine samples were collected at 0 to 24 hours for mice and 0 to 3, 3 to 6 and 6 to 24 hours for rats after oral administration in a metabolism cage. Groups of 5 to 8 rabbits and 4 to 5 dogs were used for each drug. Urine was collected through a catheter at 0 to 3 and 3 to 6 hours, and in a metabolism cage at 6 to 24 hours.

Biliary Excretion

Groups of 5~10 rats were used for each drug. Rats were anesthetized with an intraperitoneal injection of 20 mg/kg of pentobarbital and confined in the supine position. After incising the abdomen, a polyethylene cannula was inserted into the bile duct and bile samples were collected at 0 to

3, 3 to 6 and 6 to 24 hours after oral administration of each drug. The biliary excretion of FK482 was also carried out in 3 rabbits and 3 dogs. Bile samples were collected by essentially the same procedure as in rats.

Absorption Site in the Gastrointestinal Tract of Rats

Male JCL: SD-strain rats were used after fasting for 24 hours and divided into 5 groups of 6 animals each. The rats were anesthetized by intraperitoneal injection of 20 mg/kg of pentobarbital sodium solution. The hair of the abdomen was removed and the operative site cleansed with 70% ethanol. After abdominal incision, both ends of the stomach, and a 10 cm-loop in length of the upper intestine, middle intestine, lower intestine or large intestine were ligated with silk. After 1 ml of FK482 solution (5 mg/ml) was injected into the each ligated gastrointestinal tract, the incision was closed with metal skin clips. The rats were fixed to collect the urine for 3 hours.

Stability in Gastrointestinal Contents of Rats

Male JCL: SD-strain rats were bled to death. The stomach, upper intestine, middle intestine, lower intestine, caecum and large intestine were removed, and the contents taken out and homogenized with a Polytron Homogenizer after adding 2.3 ml of distilled water to each g of contents. 1.0 ml of the homogenates was mixed with 1.0 ml of 200 μg/ml of FK482 solution and incubated at 37°C for 0, 2, 4, 6 and 24 hours. After incubation, 0.2 ml of the incubation mixture was added to 0.8 ml of ethanol and centrifuged to separate the supernatant. Residual FK482 concentration in the supernatant was determined by disc assay method.

Binding to Serum Protein

Serum of human, dog, rabbit, rat and mouse, adjusted to pH 7.4, were used. The degree of binding was determined by ultrafiltration. A 0.2-ml volume of drug solution in 1/15 M phosphate buffer (pH 7.0) was added to 1.8 ml of serum and incubated at 37°C for 20 minutes. This mixture was placed in a Visking tube (size: 8/32) and centrifuged at $1,000 \times g$ for 40 minutes to obtain the ultrafiltrate. The drug concentration in the filtrate was measured by bioassay. The degree of binding (% bound, P) was calculated from the following equation:

$$P = 100(1 - Cf/Ct)$$

Where Ct and Cf are total and unbound concentrations, respectively.

Identification of Active Metabolites in the Plasma, Urine and bile

Plasma samples were added to 2 volumes of ethanol, and the supernatant obtained by centrifugation was used as the test sample. Plasma, urine and bile samples of rats, rabbits and dogs were examined by TLC using a 5% KCl - dioxane - formic acid (5 : 1 : 1) solvent system and DC-Fertigplatten RP-8 F2545s Art. No. 15424 (Merck). Bioautography was then performed using *Providencia stuartii* ATCC 43665 and ATCC 43664 as the test organisms.

Microbiological Assay

FK482 concentrations were measured by the disc-plate diffusion method using *P. stuartii* ATCC 43665 as the test organism and Antibiotic medium No. 1 (Difco) as the test medium; FK482 concentrations under 4 μg/ml in the plasma were assayed in the same way using *P. stuartii* ATCC 43664 as the test organism in the same medium. Cefaclor and cephalexin concentrations were assayed in the same way using *Bacillus subtilis* ATCC 6633 as the test organism and sodium citrate agar (sodium citrate 1.0%, Polypeptone 0.5%, beef extract 0.3%, agar 1.0%) as the test medium. Cefixime concentrations were assayed in the same way using *Escherichia coli* ATCC 39188 as the test organism and nutrient agar (Difco) as the test medium. The diluents for the standard curves were prepared with serum (plasma) from the respective species of animals for determing the serum (plasma) levels, with 1/15 M phosphate buffer (pH 7.0) for determing the urinary, biliary and tissue levels and the concentrations in the ultrafiltrates for the protein binding study. The plates were incubated at 37°C for 18 hours, and the zones of inhibition were measured by TOSPIX computer (Toshiba), automated image analyzer.

Results

Plasma (Serum) Levels

The plasma (serum) levels and pharmacokinetic parameters of FK482 after single oral and intravenous doses of 20 mg/kg to mice, rats, rabbits and dogs are shown in Fig. 2 and Table 1, respec-

Fig. 2. Mean plasma levels of FK482 after single oral (A) and intravenous (B) doses of 20 mg/kg to various animals.

□ Mouse (n=10), △ rat (n=10), ○ rabbit (n=5), ● dog (n=5).
Vertical bars indicate the standard error.

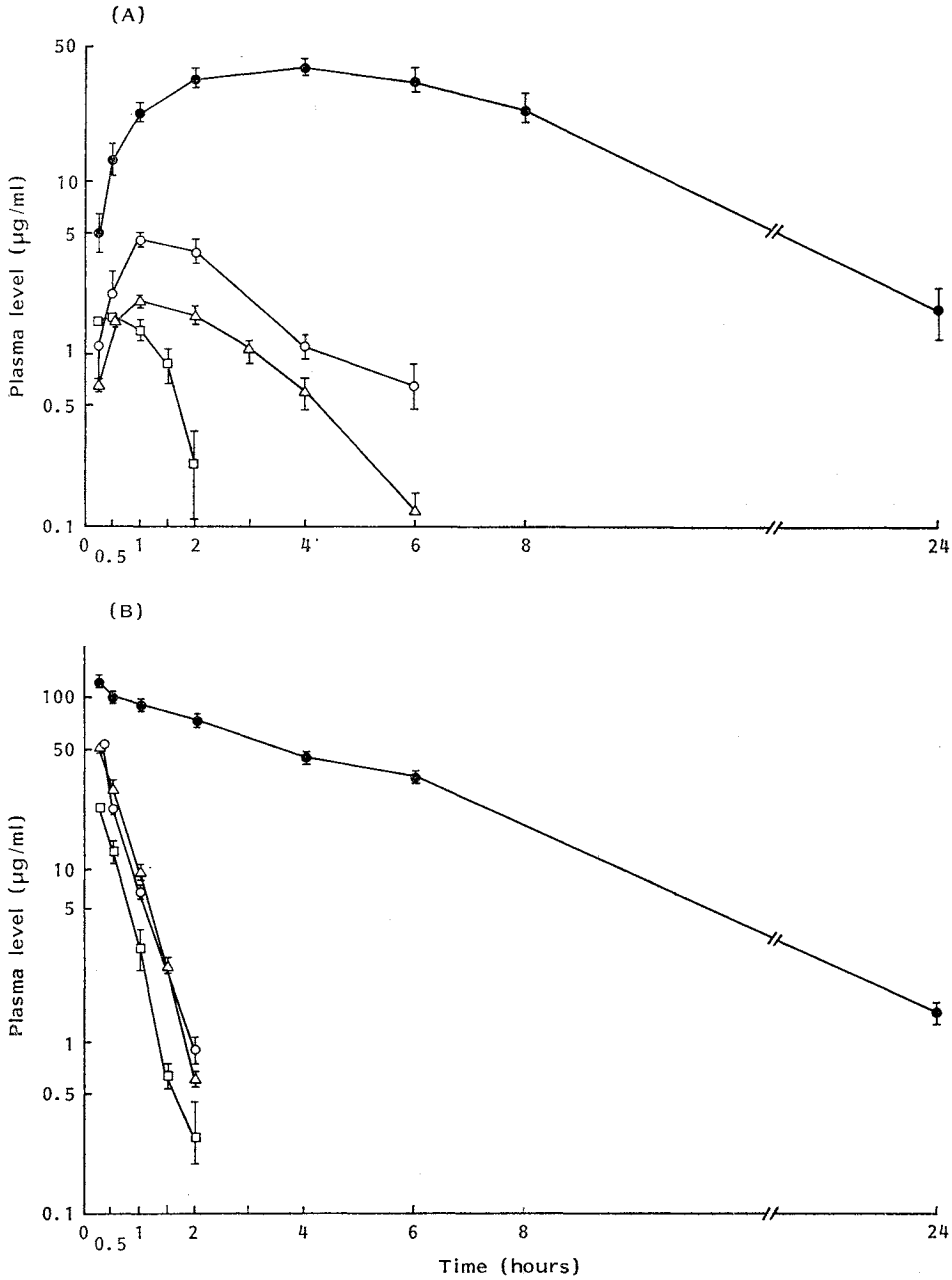


Table 1. Mean (±SE) pharmacokinetic parameters of FK482 after single oral and intravenous doses of 20 mg/kg to various animals.

Animal	*n*	Route	Cmax (μg/ml)	Tmax (hours)	$T_{1/2}$ (hours)	$AUC_{0\sim\infty}$ (μg·hour/ml)	Vc (ml/kg)	BA[a] (%)
Mouse	10	po[c]	1.58	0.51	0.40	2.2		12.6
		iv[c]	48.8[b]		0.27	17.5	446.6	
Rat	10	po[c]	2.10	1.18	0.76	6.1		15.3
		iv[c]	93.3[b]		0.28	39.9	203.5	
Rabbit	5	po[c]	4.83±0.23	1.16±0.18	1.05±0.11	13.6±0.80		32.3
		iv[d]	138.0±25.5[b]		0.37±0.02	42.1±2.94	161.7±23.8	
Dog	5	po[c]	43.1±1.9	3.28±0.56	3.39±0.12	413.9±52.2		72.3
		iv[d]	146.3±7.3[b]		3.87±0.09	572.5±32.9	130.8±6.6	

[a] Absolute bioavailability: (AUC(po)/AUC(iv)×100), [b] initial concentration at t=0, [c] pharmacokinetic parameters were estimated *via* a one-compartment open model, [d] pharmacokinetic parameters were estimated *via* a two-compartment open model.

Fig. 3. Comparison of plasma (serum) levels of FK482 and reference antibiotics after an oral dose of 20 mg/kg to rats (n=10) (A), rabbits (n=5~8) (B) and dogs (n=4~5) (C).

● FK482 (plasma), ○ cefixime (serum), △ cefaclor (plasma), □ cephalexin (serum).

Vertical bars indicate the standard error.

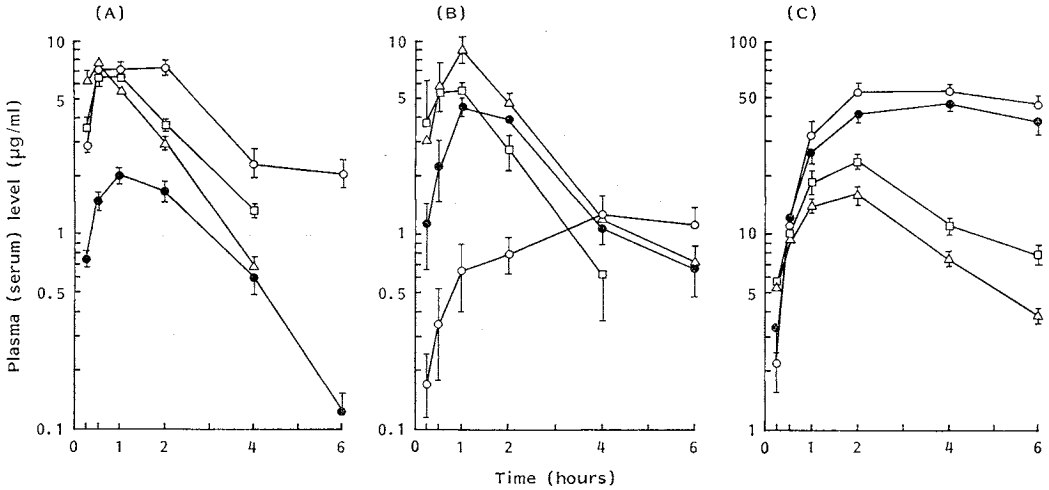


Table 2. Comparative pharmacokinetics of FK482 and reference antibiotics after an oral dose of 20 mg/kg to various animals.

Animal	Antibiotic	*n*	Cmax (μg/ml)	$T_{1/2}$ (hours)	$AUC_{0\sim\infty}$ (μg·hour/ml)
Rat	FK482	10	2.1	0.8	6.1
	Cefixime	10	8.0	2.1	30.8
	Cefaclor	10	7.4	1.0	13.9
	Cephalexin	10	6.7	1.2	16.5
Rabbit	FK482	5	4.8	1.1	13.6
	Cefixime	8	1.1	5.2	14.0
	Cefaclor	5	7.7	1.1	19.2
	Cephalexin	5	5.9	1.0	12.1
Dog	FK482	5	43.1	3.4	414
	Cefixime	5	54.7	5.8	669
	Cefaclor	4	15.2	1.4	63
	Cephalexin	5	20.4	1.7	100

Table 3. Mean (±SE) tissue levels of FK482 and reference antibiotics in rats and rabbits after an oral dose.

Tissue	Time (hours)	Tissue levels (μg/g(ml)) in rats[a]				Tissue levels (μg/g(ml)) in rabbits[b]		
		FK482	Cefixime	Cefaclor	Cephalexin	FK482	Cefaclor	Cephalexin
Liver	0.5	1.2±0.2	7.0±0.3	26.5±0.6	90.4±9.0	0.4±0.4	0.5±0.2	11.1±0.5
	1.0	1.4±0.3	10.1±1.8	19.8±0.6	80.1±9.9	2.8±0.7	2.6±1.7	13.5±1.6
	2.0	1.5±0.3	13.6±1.0	14.4±2.1	55.4±4.1	1.7±0.3	1.1±1.1	8.9±1.2
Kidney	0.5	15.6±1.0	11.4±1.0	56.4±6.0	137.7±22.9	22.5±9.7	67.7±14.9	132.0±13.5
	1.0	15.9±1.7	16.0±2.3	50.6±6.7	102.0±16.9	63.2±8.9	181.5±46.7	170.3±13.7
	2.0	16.1±2.5	23.6±4.6	32.0±1.8	67.3±2.2	28.5±4.4	69.9±6.1	97.1±18.9
Lung	0.5	1.1±0.1	3.8±0.3	7.2±0.9	9.8±1.5	0.8±0.4	2.5±0.5	3.0±0.6
	1.0	1.5±0.3	4.7±0.8	6.0±1.3	7.8±1.4	2.6±0.7	3.2±0.4	3.9±0.6
	2.0	1.1±0.1	10.8±2.7	4.1±0.6	5.3±0.3	1.6±0.1	1.3±0.0	2.3±0.2
Heart	0.5	0.7±0.1	4.2±1.0	3.4±0.6	5.3±0.9	0.6±0.6	1.8±0.3	2.8±0.3
	1.0	0.8±0.2	4.6±0.9	2.6±0.5	3.2±1.6	0.2±0.3	2.3±0.2	3.3±0.6
	2.0	0.8±0.1	7.0±1.9	1.9±0.4	1.6±0.8	0.5±0.4	1.2±0.0	<2.0
Spleen	0.5	0.4±0.0	1.2±0.1	6.8±1.0	7.7±1.4	0.6±0.3	1.0±0.2	2.2±0.1
	1.0	0.5±0.0	1.4±0.3	5.8±1.3	8.6±2.3	0.7±0.3	1.6±0.3	2.6±0.4
	2.0	0.4±0.0	3.2±0.68	3.7±0.5	5.5±0.4	0.4±0.4	0.6±0.0	2.2±0.0
Plasma	0.5	4.6±0.5	19.8±1.6	19.2±0.7	35.2±4.4	2.3±0.8	5.7±1.9	5.6±1.4
	1.0	4.9±0.6	33.4±1.7	9.2±0.4	26.3±2.1	4.6±0.4	8.9±1.3	5.6±0.7
	2.0	5.7±0.6	29.3±1.5	4.2±0.3	19.0±1.7	3.9±0.7	4.6±0.5	2.7±0.7

[a] Rats: 100 mg/kg, $n=3$, [b] rabbit: 20 mg/kg, $n=3\sim4$.

tively. When given orally, considerable differences in the plasma levels and half-lives of FK482 were observed among the animal species; the level was the highest and most prolonged in dogs and the lowest in mice. Absolute bioavailabilities of FK482 were 12.6% in mice, 15.3% in rats, 32.3% in rabbits and 72.3% in dogs. On the other hand, when given intravenously, there were no big differences in the plasma levels or half-lives among the animal species except dogs. When given intravenously FK482 in dogs, like oral dosing, the plasma level and half-life were the highest and most prolonged of the animals tested, and the plasma levels for 4 to 24 hours and half-life of FK482 given intravenously were similar to those given orally. The comparative plasma (serum) levels of FK482 and the reference drugs after an oral dose of 20 mg/kg to rats, rabbits and dogs are shown in Fig. 3 and the pharmacokinetics are listed in Table 2. The plasma level and AUC of FK482 in rats were lower than those of the reference drugs. In rabbits, the plasma level of FK482 was almost the same as that of cephalexin, lower than that of cefaclor, and higher than that of cefixime, and the AUC was almost the same as that of cephalexin and cefixime, and lower than that of cefaclor. In dogs, the plasma level of FK482, like cefixime, was higher and more prolonged than that of cefaclor or cephalexin. The AUC of FK482 was lower than that of cefixime, but much higher than that of cephalexin or cefaclor.

Tissue Distribution

The tissue concentrations of FK482 and the reference drugs were determined in rats and rabbits after a single oral dose of 100 and 20 mg/kg, respectively (Table 3). In rats, the tissue concentrations of FK482 in all the test organs, in proportion to its plasma levels, were lower than those of the reference drugs. In rabbits, the tissue levels of FK482 were relatively near to those of cefaclor except kidney, but lower than those of cephalexin.

Urinary and Biliary Excretion

The urinary and biliary excretion of FK482 after single oral and intravenous doses and the reference drugs after a single oral dose of 20 mg/kg in animals are shown in Table 4. Although there were scarcely any differences among the animal species in the urinary recovery rates of FK482 after intravenous dosing, those of FK482 after oral dosing differed considerably among the animal species; 9.8% in mice, 15.5% in rats, 45.8% in rabbits and 47.1% in dogs. The urinary recovery rates of oral FK482 in mice and rats were the lowest of the reference drug tested, but in rabbits and dogs were higher than that of cefixime or cefaclor, and lower than that of cephalexin. The biliary recovery rate of FK482 in rats was 1.4% of the given dose and was the lowest of the reference drugs. In rabbits

Table 4. Urinary and biliary excretion of FK482 and reference antibiotics after a single oral dose or intravenous dose of 20 mg/kg to various animals.

Animal	n	Mean (±SE) urinary (biliary) excretion in 24 hours (% of dose)				
		FK482		Cefixime (po)	Cefaclor (po)	Cephalexin (po)
		po	iv			
Mouse	10	9.8±1.2	76.1±1.3	13.0±1.1	68.5±3.7	65.2±1.9
Rat	9~10	15.5±0.2	80.0±3.4	29.5±2.2	46.8±3.7	80.9±2.1
	5~10	(1.4±0.3)	(3.4±0.9)	(12.1±1.0)	(7.3±0.5)	(10.1±0.5)
Rabbit	5~8	45.8±3.2	86.0±4.9	12.2±2.4	33.3±5.6	60.1±3.9
	3	(0.05±0.01)				
Dog	4~5	47.1±1.0	68.1±4.3	23.1±3.1	24.9±1.0	70.5±4.6
	3	(0.01±0.00)				

Fig. 4. Dose response of Cmax (A) and $AUC_{0\sim\infty}$ (B) of FK482 given orally to rats (△), rabbits (○) and dogs (●).

Vertical bars indicate the standard error.

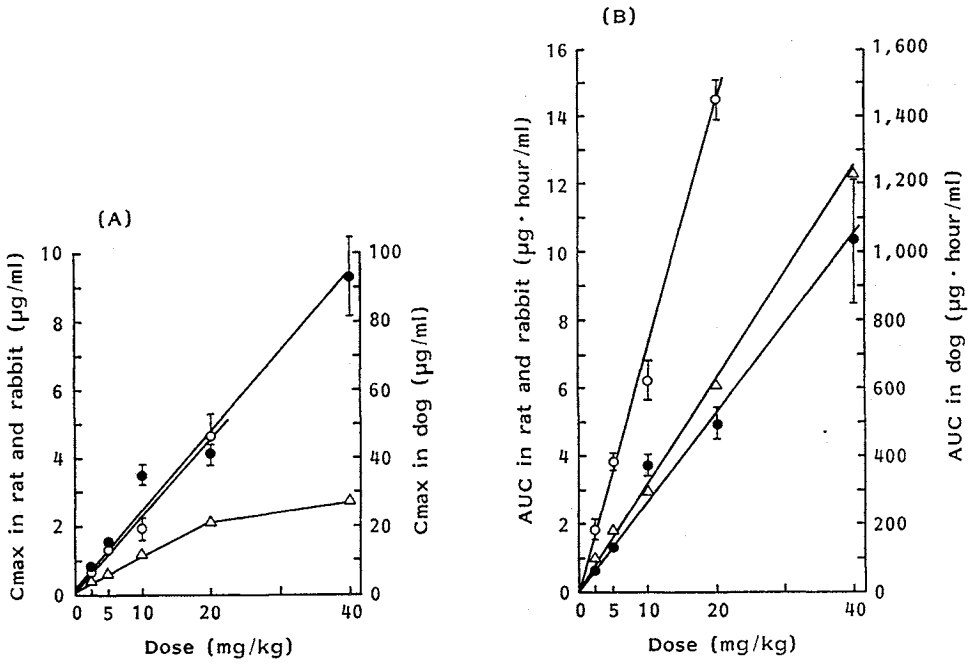
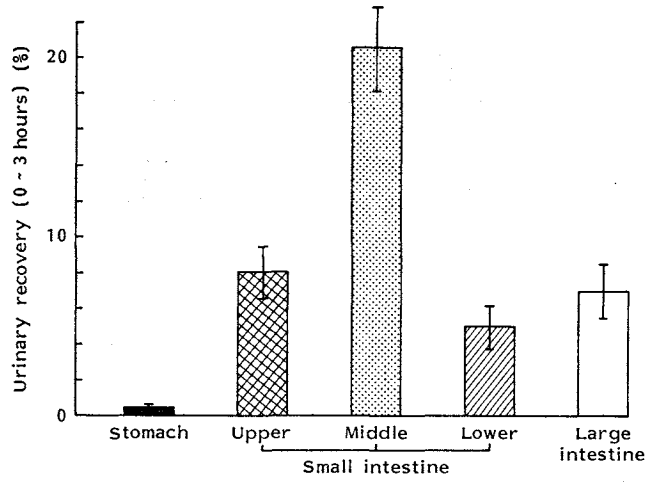


Fig. 5. Absorption site of FK482 in the gastrointestinal tracts of rats.

Vertical bars indicate the standard error.



and dogs, the recovery rates of FK482 were also low; 0.05% in rabbits and 0.01% in dogs.

Dose Response of Plasma Levels

Fig. 4 shows the dose proportionality of the peak plasma levels (Cmax) and AUCs. Although Cmax in rabbits and dogs was linearly related to the dose, Cmax in rats indicated non-linearity. The increase in AUCs was strictly dose-proportionate in the range of 2.5 to 40 mg/kg in rats and dogs,

Table 5. Serum-protein binding of FK482 and reference antibiotics.

Serum (90%)	Bound (%), Mean±SE			
	FK482	Cefixime	Cefaclor	Cephalexin
Human	73.1±2.2	70.2±0.4	39.7±0.3	28.0±3.4
Dog	92.8±0.2	92.8±0.8	21.7±2.3	17.8±4.1
Rabbit	60.2±1.6	51.9±2.7	23.1±2.1	5.7±1.0
Rat	89.6±0.6	85.8±1.0	43.8±2.3	17.2±1.0
Mouse	76.8±0.9	72.5±3.1	37.8±1.7	8.0±2.3

Drug concentration: 30 μg/ml.
Each value represents mean for 3 to 12 experiments.

and 2.5 to 20 mg/kg in rabbits. The urinary recovery rates were almost constant among these oral doses in the test animals.

Absorption Site in the Gastrointestinal Tract

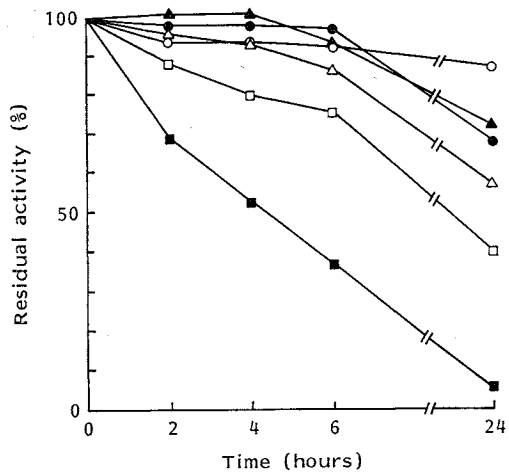
The absorption site of FK482 in the gastrointestinal tract of rats was determined by *in situ* loop method (Fig. 5). FK482 was mainly absorbed in the middle intestine, scarcely absorbed in the stomach.

Stability in Gastrointestinal Contents

The stability of FK482 in gastrointestinal contents of rats is shown in Fig. 6. FK482 was stable in the stomach and small intestine, however, in the large intestine it was almost all degraded after 24 hours.

Fig. 6. Stability of FK482 in gastrointestinal contents of rats.

▲ Stomach, ○ upper intestine, ● middle intestine, △ lower intestine, □ caecum, ■ large intestine.



Metabolites in Plasma, Urine and Bile Samples

No antimicrobial substances except FK482 were detected in the plasma, urine or bile samples of the test animals given FK482 orally.

Serum-protein Binding

The degree of serum-protein binding is shown in Table 5. The binding rates of FK482 to serum-protein of human and animals were almost the same as that of cefixime; 73% for human, 93% for dog, 60% for rabbit, 90% for rat and 77% for mouse.

Discussion

The pharmacokinetics of FK482 were examined in experimental animals and compared with those of cefixime, cefaclor and cephalexin. FK482 is a cephalosporanic acid derivatives having a hydroxyimino-aminothiazole side chain at the 7-position and a vinyl group at the 3-position. The only difference between FK482 and cefixime[8] in structure is at the 7-position where the drugs have hydroxyimino and a carboxymethoxyimino groups, respectively. Introduction of the hydroxyimino instead of the carboxymethoxyimino group at the 7-position of cefixime results in a significant enhancement of antibacterial activity against Gram-positive bacteria including Staphylococci[1]. In this pharmaco-

kinetic study, the plasma half-life of FK482 after oral dosing was distinctly shorter than that of cefixime[4,5], in spite of the similar relatively high protein binding of both agents. Further studies including mechanism of renal excretion and enterohepatic circulation are required to elucidate this reason. In addition, the pharmacokinetic profiles of FK482 typically differed among the species of animals used in this study; the plasma level and absolute bioavailability were highest and most prolonged in dogs of the animals tested, and those in mice were the lowest. Therefore, whether the pharmacokinetic profile of FK482 after oral dosing in human is similar to those in rabbits and dogs or mice and rats will be an important factor when extrapolating efficacy to humans. Acute and subacute toxicology studies and nephrotoxicity studies in animals have revealed that FK482 is very safe. Together with its excellent antibacterial activity[1,2], the above results suggest that FK482 merits further Phase 1 study.

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