PHARMACOKINETICS OF FK027 IN RATS AND DOGS

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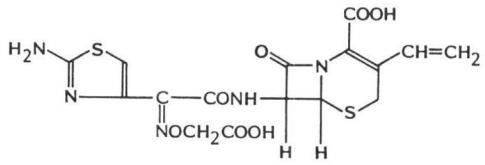
The pharmacokinetics of FK027, a new oral cephalosporin, were investigated in rats and dogs and compared with those of cefaclor, cephalexin and amoxicillin. Upon oral administration to either rats or dogs, FK027 produced higher and more sustained serum levels than the reference drugs, hence a longer half-life. After both oral and intravenous administration, the half-life of FK027 in dogs was approximately three fold that in rats. Although the concentrations of FK027 in rat kidney, liver and spleen were lower than those of cephalexin and amoxicillin, they were sustained similarly to the serum levels. The 24-hour urinary and biliary recovery rates of FK027 in rats after oral dosing with 100 mg/kg were 34.1 and 21.9%, respectively. The urinary excretion of FK027 was significantly lower than that of the reference drugs, however, the biliary excretion was higher. In dogs, 23.4 and 0.2% of the given dose of 40 mg/kg of FK027 was excreted in the 24-hour urine and bile, respectively. Bioavailability of FK027 after oral dosing was 38% in rats and 47% in dogs, as calculated from intravenous data. Binding of FK027 to serum protein in all species was the highest of the test drugs: 63% for human, 93% for dog, 61% for rat serum.

A number of new parenteral cephalosporins, with a broad spectrum and high potency, have been investigated over the last few years. However, the oral cephalosporins available are all cephalexin-type analogs. FK027 (Fig. 1), a new oral cephalosporin, was synthesized and evaluated in our laboratories. FK027 differs in structure from the commercially available products, and its antibacterial activity against the common Gram-negative bacteria is considerably more potent than that of other oral β-lactam antibiotics such as cephalexin, cefaclor and amoxicillin[1]. In this study, the pharmacokinetic profile of FK027 was investigated in rats and dogs, and compared with that of cefaclor, cephalexin and amoxicillin. Additionally, the serum-protein binding of the test compounds was investigated in five species.

Fig. 1. Chemical structure of FK027.



Materials and Methods

Antibiotics

The compounds used in this study were FK027 (Fujisawa Research Laboratories, Japan), cefaclor, cephalexin (Eli Lilly & Co., Indianapolis, Ind.) and amoxicillin (Beecham Research Laboratories, Betchworth, Surrey, England).

Animals

The following animals were used: 6-week-old male JCL: SD strain rats (weight range, 160~220 g) and male beagle dogs (9~16 kg).

Dosing

For oral dosing, each of the antibiotics was suspended in 0.5% methyl cellulose solution. The

animals were starved overnight before dosing with 100 mg/kg (10 ml/kg) in rats and 40 mg/kg (2 ml/kg) in dogs. For intravenous injection, FK027 was dissolved in 0.9% saline and injected in doses of 100 mg/kg (5 ml/kg) into the tail vein of rats and in doses of 40 mg/kg (0.5 ml/kg) into the antecubital vein of dogs.

Serum Sampling

Rats: Rats were used in groups of ten for each sampling of each drug. At specified times after dosing, the rats were killed in a dry-ice chamber, and the blood was collected by heart puncture, allowed to clot and centrifuged for 15 minutes. For cefaclor, plasma was used for assay since the compound is degraded during the treatment of blood samples[2]. Two ml of blood samples were placed in tubes with 0.05 ml of heparin sodium solution (1,000 units/ml) (Shimizu Pharmaceutical Co., Ltd.) for anticoagulation and centrifuged for 15 minutes. The serum or plasma was collected and frozen until assayed.

Dogs: Dogs were used in groups of five for each drug. Samples of blood were taken from the antecubital vein at various times and processed as above.

Pharmacokinetic Analysis

The mean serum FK027 concentration-time data after intravenous administration were analyzed by a two-compartment open model and NONLIN computer program[3]. The model equation was:

$$C = A\,e^{-\alpha t} + B\,e^{-\beta t}$$

where C is the mean serum 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 terms α and β, respectively. The pharmacokinetic parameters of FK027, cefaclor, cephalexin and amoxicillin after oral administration were estimated *via* a one-compartment open model using mean serum concentration-time data. The model equation was:

$$C = \frac{Ka \cdot D_0}{Ka - Ke}(e^{-Ket} - e^{-Kat})$$

where C is the mean serum concentration; Ka and Ke are first-order absorption and elimination rate constants, respectively; and D_0 is the fictive serum concentration at $t=0$.

Tissue Sampling

Rats in groups of three 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 for each group were pooled and homogenized with a Polytron Homogenizer after addition of 2 ml of 99% ethanol per g of tissue. The homogenates were centrifuged at 10,000 $\times g$ for 10 minutes to separate the supernatant. The sampling procedure was carried out three times, and the values were averaged.

Urinary Excretion

Rats: Groups of ten rats were used for the each drug. Urine samples were collected from rats confined to a metabolism stage at 0 to 3, 3 to 6 and 6 to 24 hours after intravenous administration and at 0 to 6 and 6 to 24 hours after oral administration.

Dogs: Groups of five dogs were used for each drug. Urine was collected at 0 to 3, 3 to 6 and 6 to 24 hours in a metabolism cage or through a catheter.

The samples were usually diluted 5-fold to 100-fold with 0.067 M phosphate buffer (pH 7.0) before bioassay. Urinary recovery was calculated from drug concentration and urine volume.

Biliary Excretion

Rats: Groups of ten rats were used for each drug. Rats were anesthetized with pentobarbital, 20 mg/kg intraperitoneally, and fixed in the supine position. After abdominal incision, a polyethylene cannula was inserted into the bile duct and bile samples were collected at specified intervals for a 24-hour period after oral administration of the test drugs.

Dogs: Biliary excretion of FK027 and cephalexin was carried out in four and three dogs, respectively. Bile samples were collected by essentially the same procedure as in rats.

The bile samples were diluted in the same way as the urine samples, and biliary recovery was cal-

culated from drug concentration and bile volume.

Binding to Serum Protein

Lyophilized human serum, "Consera" (Nissui, Tokyo), and serum of the dog, rabbit, rat and mouse, collected in the usual way were used. The degree of binding was determined by ultrafiltration. A 0.5-ml volume of drug solution in 0.067 M phosphate buffer (pH 7.0) was added to 4.5 ml of serum and incubated at 37°C for 30 minutes. This mixture was placed in a Visking tube (size: 8/32) and centrifuged at 1,000×*g* for 30 to 40 minutes to obtain the ultrafiltrate. The drug concentration in the filtrate was determined by bioassay. The degree of binding (% bound, *P*) was calculated from the following equation:

$$P=100\,(1-C_f/C_t)$$

where C_t and C_f are total and unbound concentrations, respectively.

Microbiological Assay

FK027 concentrations in the body fluids and tissue homogenates were measured by the disc-plate diffusion method using *Escherichia coli* ATCC 39188 as the test organism and nutrient agar (Difco) as the test medium. The reference drugs 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.

The diluent for standard curve were prepared with serum (plasma) from the respective species of animals for determining the serum (plasma) levels, with 0.067 M phosphate buffer (pH 7.0) for determining the urinary and biliary levels and the concentrations in the ultrafiltrates for the protein binding study, and with aqueous ethanol (ethanol - water, 2: 1) for determining the tissue levels. Standard curves were prepared for each assay run. The plates were incubated at 37°C for 18 to 20 hours, and the zones of inhibition were measured and compared with those attained with the standards.

Results

Serum Levels

The serum levels of FK027 in rats and dogs achieved after a single oral dose and compared with those of cefaclor, cephalexin and amoxicillin are shown in Fig. 2. FK027 had higher peaks and longer half-lives than the reference drugs. The mean serum levels of FK027 in rats peaked at 33.4 μg/ml, 1 hour after oral dosing with 100 mg/kg. The peak level was similar to that of cephalexin (32.9 μg/ml) but was higher than that of cefaclor (19.2 μg/ml) and amoxicillin (18.3 μg/ml). In dogs, oral dosing with FK027 (40 mg/kg) resulted in high and sustained serum levels which markedly exceeded those of the other drugs: 54.7 μg/ml at 4 hours and as high as 41.6 μg/ml at 8 hours. Though FK027 produced high and prolonged serum levels, the absorption was slow, and the time to reach the peak levels was delayed. The serum concentration data were analyzed using a one-compartment model. The observed serum concentrations were well described by this model. The pharmacokinetic parameters of the four test drugs are listed in Table 1. The biological half-life of FK027 and the area under the serum concentration curve (AUC) were the longest and highest of the antibiotics tested. As shown in Fig. 3, serum levels of FK027 after a single intravenous injection were compared with those after oral dosing. The ratio of AUC(oral)/AUC(iv) was 0.38 in rats and 0.47 in dogs. The pharmacokinetic parameters after intravenous injection are shown in Table 1.

Tissue Distribution

The tissue concentrations of FK027 and the reference drugs were determined in rats after a single oral dose of 100 mg/kg (Fig. 4). FK027 was well distributed to all the tissues tested. The tissue levels of FK027 2 hours after dosing were highest in the kidneys (23.6 μg/g), followed by the liver (13.6 μg/g),

Fig. 2. Mean serum (plasma) concentrations of FK027 and related antibiotics in rats and dogs after a single oral dose.

● FK027 (serum), ○ cefaclor (plasma), □ cephalexin (serum), △ amoxicillin (serum).
Vertical bars indicate the standard error. Rat: n=10, dog: n=5~10.

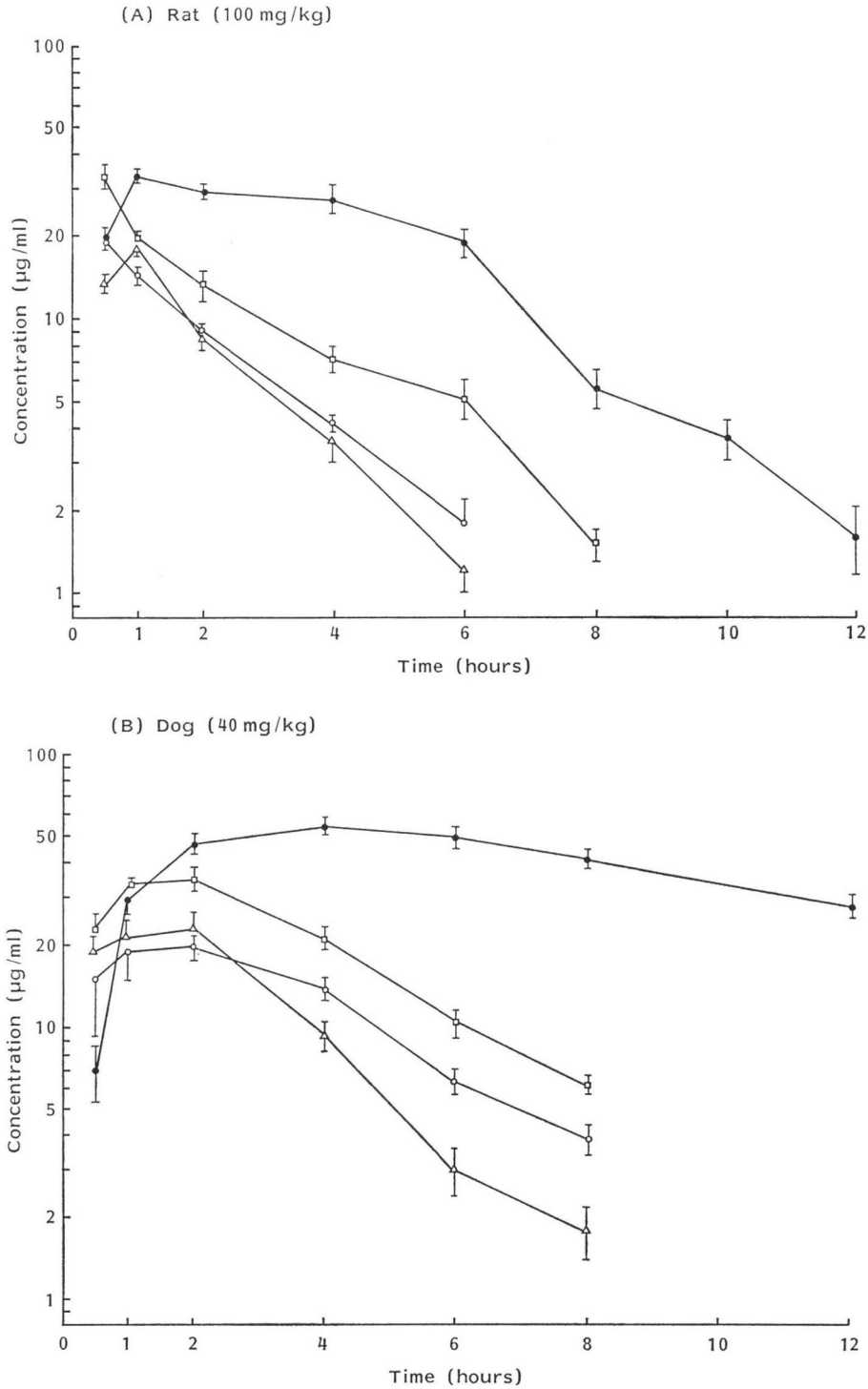


Table 1. Pharmacokinetic parameters of FK027 and related antibiotics in rats and dogs.

Species	Antibiotic	Route	A (μg/ml)	B (μg/ml)	α (hour^{-1})	β (hour^{-1})	Half-life (hours)	AUC (μg·hour/ml)	K_{12} (hour^{-1})	K_{21} (hour^{-1})	Ke (hour^{-1})	Ka (hour^{-1})	Peak concentration (μg/ml)	Peak concentration (hours)
Rat[a]	FK027	iv	265	164	2.36	0.41	1.68	510	0.78	1.16	0.84			
		po					2.29	192			0.30	0.80	32.0	1.96
	Cefaclor	po					1.64	48.3			0.42	10.0	17.7	0.46
	Cephalexin	po					1.67	83.3			0.41	23.7	32.1	0.17
	Amoxicillin	po					1.00	38.9			0.69	3.54	18.2	0.84
Dog[b]	FK027	iv	141	165	3.52	0.10	6.66	1,630	1.49	1.95	0.19			
		po					6.93	766			0.10	0.47	50.4	4.42
	Cefaclor	po					1.96	109			0.35	0.90	21.1	1.71
	Cephalexin	po					1.98	176			0.35	1.06	35.6	1.57
	Amoxicillin	po					1.47	88.9			0.47	1.20	22.9	1.28

[a] Rat: JCL: SD strain, 6-week-old male, dose: 100 mg/kg.

[b] Dog: Beagle dogs, male, 9.0~16.0 kg, dose: 40 mg/kg.

Fig. 3. Mean serum concentrations of FK027 in rats and dogs after a single oral dose or intravenous injection.

Points indicate observed serum concentrations; lines indicate regression serum concentration. Rat: n=5~10, dog: n=5~6.

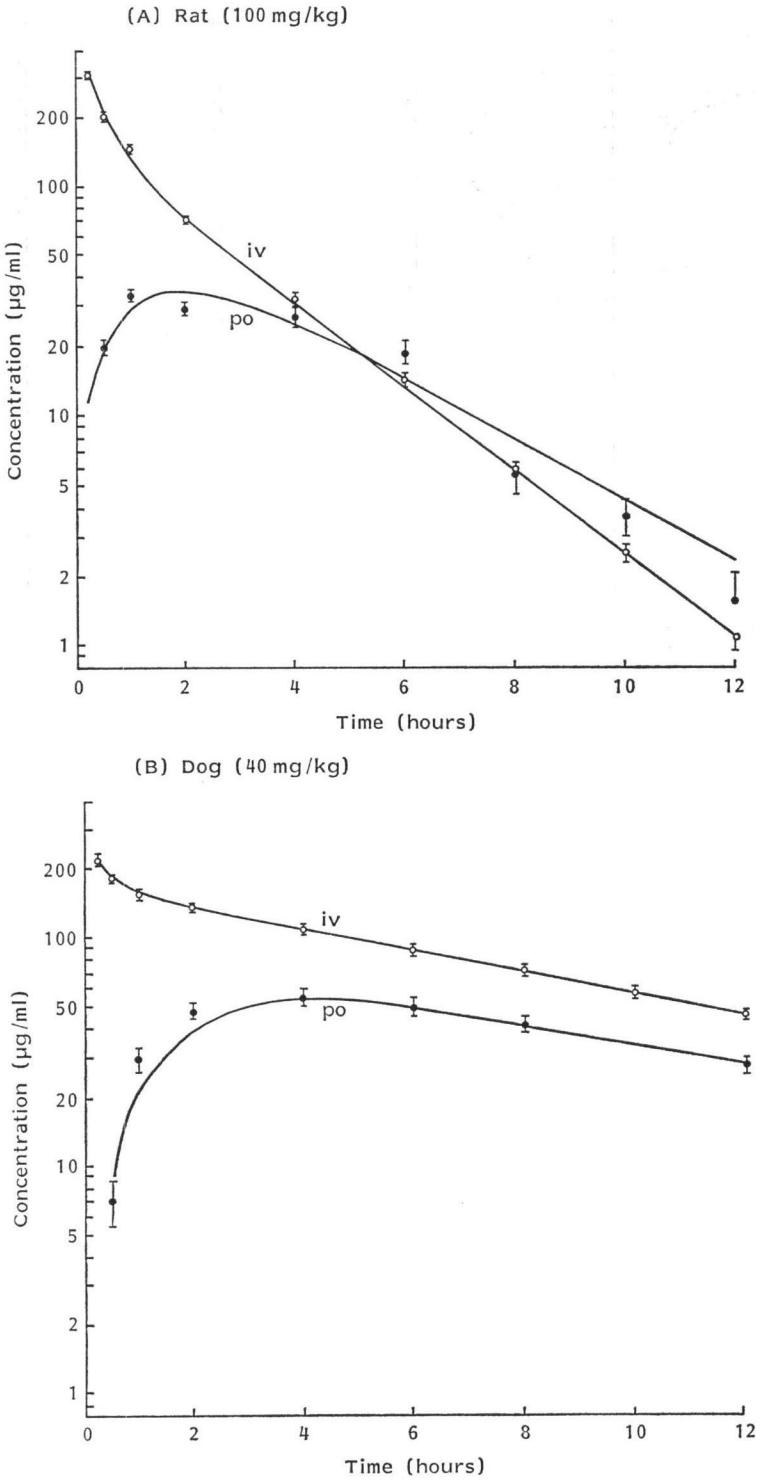


Fig. 4. Tissue distribution of FK027 and related antibiotics in rats after a single oral dose of 100 mg/kg. ● FK027, ○ cefaclor, □ cephalexin, △ amoxicillin. Rat: n=3×3.

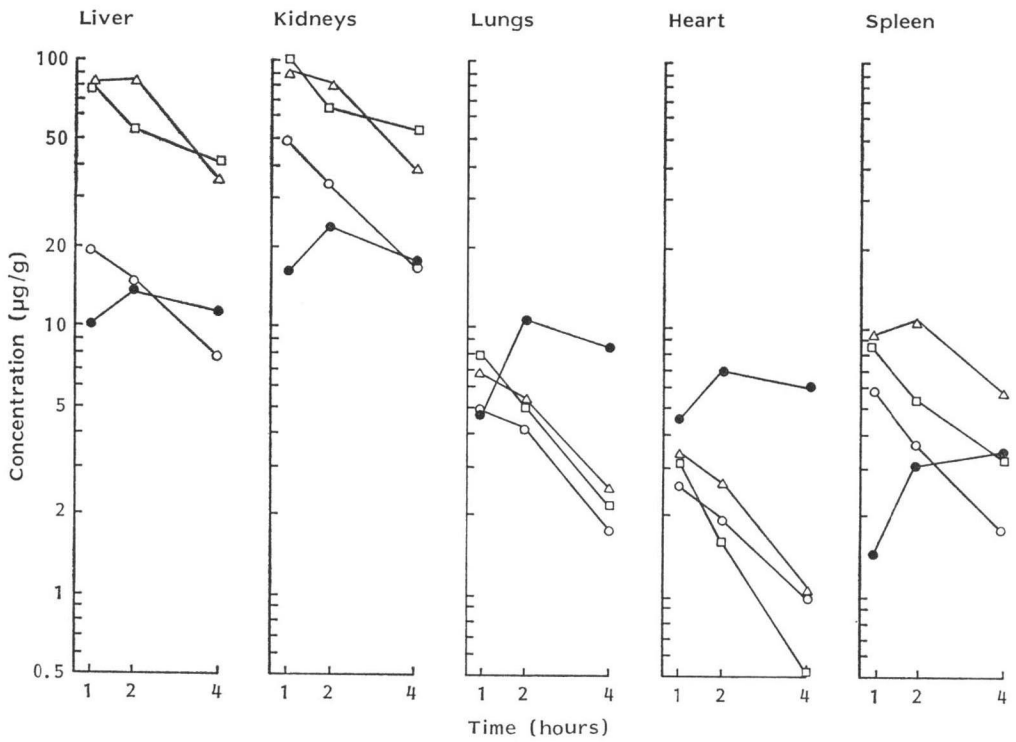


Table 2. Urinary recovery of FK027 and related antibiotics in rats after a single oral dose of 100 mg/kg. Rat: n=10.

Antibiotics	Recovery (% of dose), mean±S.E.						
	Urine			Bile			
	0~6 hours	6~24 hours	0~24 hours	0~3 hours	3~6 hours	6~24 hours	0~24 hours
FK027	23.7±1.1	10.4±2.5	34.1±2.7	6.3±1.2	10.0±2.4	5.5±0.4	21.9±2.7
Cefaclor	39.9±1.3	0.5±0.2	40.4±1.2	4.5±0.4	1.0±0.2	0.5±0.1	5.9±0.4
Cephalexin	73.4±2.3	3.9±0.7	77.3±2.5	4.0±0.7	5.6±2.1	3.7±0.9	13.3±2.7
Amoxicillin	45.6±2.3	3.0±0.4	48.6±2.3	3.4±0.9	0.9±0.2	0.5±0.1	4.7±1.1

lungs (10.8 μg/g), heart (7.03 μg/g) and spleen (3.17 μg/g). Unlike the reference drugs, the half-life of FK027 in the tissues was prolonged and the levels differed relatively little over the 1- to 4-hour period during which the measurements were made.

Urinary and Biliary Excretion

The 24-hour urinary recovery in rats after oral dosing with 100 mg/kg was 34.1% for FK027, 40.4% for cefaclor, 77.3% for cephalexin and 48.6% for amoxicillin; the recovery of FK027 was lower than that of the reference drugs (Table 2). The 24-hour biliary recovery of FK027 was 21.9%, twice as high as that of cephalexin (13.3%), and well in excess of that of cefaclor (5.9%) and amoxicillin (4.7%) (Table 2). The 24-hour urinary recovery of FK027 in rats after intravenous injection with 100 mg/kg was 74.5% (Table 4). In the dog experiments, the 24-hour urinary recovery of FK027 after oral dosing

Table 3. Urinary and biliary recovery of FK027 and related antibiotics in dogs after a single oral dose of 40 mg/kg.

Antibiotics	n	Recovery (% of dose), mean±S.E.			
		Urine			
		0~3 hours	3~6 hours	6~24 hours	0~24 hours
FK027	6	6.5±0.7	6.1±0.9	10.9±1.0	23.4±1.6
Cefaclor	5	24.8±3.1	15.5±1.7	2.6±0.7	42.8±3.1
Cephalexin	5	32.6±2.9	19.2±2.1	6.4±1.0	58.1±3.2
Amoxicillin	5	29.0±2.2	5.4±1.0	2.0±0.4	36.5±2.9

Antibiotics	n	Recovery (% of dose), mean ±S.E.			
		Bile			
		0~3 hours	3~6 hours	6~24 hours	0~24 hours
FK027	4	0.00±0.00	0.03±0.01	0.20±0.12	0.23±0.13
Cefaclor		NT	NT	NT	NT
Cephalexin	3	0.00±0.00	0.01±0.01	0.18±0.13	0.19±0.13
Amoxicillin		NT	NT	NT	NT

NT: Not tested.

Table 4. Urinary recovery of FK027 in rats and dogs after a single intravenous administration.

Animal	Dose (mg/kg)	n	Recovery (% of dose), mean±S.E.			
			0~3 hours	3~6 hours	6~24 hours	0~24 hours
Rat	100	10	64.5±2.49	5.97±1.12	3.98±0.87	74.5±2.14
Dog	40	5	40.1±2.85	13.0 ±1.01	19.2 ±1.90	72.3±4.10

Table 5. Protein binding of FK027 and related antibiotics.

Antibiotic	Protein binding (%) in				
	Human	Dog	Rabbit	Rat	Mouse
FK027	63	93	52	61	44
Cefaclor	47	22	23	41	34
Cephalexin	24	18	6	17	5
Amoxicillin	38	34	20	18	15

with 40 mg/kg was 23.4%, lower than that of the reference drugs (cefaclor: 42.8%, cephalexin: 58.1%, amoxicillin: 36.5%). The 24-hour biliary recoveries of FK027 and cephalexin were similar; only 0.2% of the given dose (40 mg/kg) was excreted (Table 3). The urinary recovery of FK027 after intravenous injection with 40 mg/kg was 72.3% (Table 4).

Serum-protein Binding

The degree of serum-protein binding is shown in Table 5. FK027 binding to serum proteins ranged from 44% for mouse serum to 93% for dog serum. The binding of FK027 to human serum protein was 63%, higher than that of cefaclor (47%), cephalexin (24%) and amoxicillin (38%).

Discussion

The pharmacokinetics of FK027 were examined in experimental animals, and these properties were compared with those of several other oral β-lactam antibiotics. After oral dosing, the serum half-life of FK027, *i.e.*, 2.3 hours in rats and 6.9 hours in dogs, was the longest of the antibiotics tested. The AUC was also the largest. Thus, the kinetic profile of FK027 was clearly distinct from the other oral β-lactams. Though the excretion of FK027 was lower than that of the reference drugs, serum levels were

high and prolonged. This may be explained in part by the relatively high degree of protein binding or the very effective tubular reabsorption of FK027[4]. The low rate of excretion of FK027 is due to the poor absorption from the intestine and not to degradation in the body after absorption, because FK027 is hardly metabolized, stable in the body fluids and well excreted in the urine after intravenous injection. The bioavailability of FK027 after oral dosing was calculated from the serum data after intravenous injection. The availability was higher than the urinary recovery rate, which may account for the enterohepatic circulation or dose-independent systemic clearance. Further studies are required to elucidate this point. As can be seen from tissue distribution studies, FK027 differed from the reference drugs, which achieved higher levels in the kidney and liver than in the other tissues. In contrast, the half-life of FK027 in the tissues was longer and similar to that in the serum. There was little difference among tissue levels. The serum levels of FK027 were clearly higher in dogs than in rats, which may be accounted for by species differences in protein binding and systemic clearance. FK027 was well excreted in the bile in rats, whereas the biliary excretion of both FK027 and cephalexin was lower in dogs. Although the biliary excretion of antibiotics in animals does not always parallel that in humans, the high excretion rates in rats suggest that FK027 would be excreted to some extent in the bile in humans.

The pharmacokinetics of antibiotics typically differ among the species of animals used in the study. The differences among animals and humans must be carefully considered on the basis of the results in animal studies when extrapolating efficacy in humans. The protective effect of FK027 against susceptible bacteria in mice was superior to that of the reference drugs, even though the peak serum levels of FK027 were lower than those of the reference drugs[1]. Peak serum levels of FK027 in volunteers after oral dosing were relatively low but long-lasting[5]. These data show that the kinetic profile of FK027 differs greatly from other oral cephalosporins, which have high peak levels and relatively short half-lives[6]. We previously reported that FR10612, an oral cephalosporin with a long half-life, was more potent in bactericidal activity than cephalexin in an *in vitro* kinetic model simulating serum levels[7]. In a comparative study with cefazolin, cefmetazole and cefoxitin, cefazolin had the longest half-life and the strongest bactericidal activity of the three drugs in the kinetic model[8]. These data suggest that a long serum half-life is an important factor in the bactericidal efficacy of cephalosporins. Therefore, FK027, which has a longer half-life and relatively low peak level, may be considered more potent in bactericidal activity than cephalexin-type drugs which have shorter half-lives and high peak levels. These favorable properties of FK027, together with its potent antibacterial activity, suggest that it will be effective in treating bacterial infections in humans.

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