ANIMAL PHARMACOKINETICS OF FK037, A NOVEL PARENTERAL BROAD-SPECTRUM CEPHALOSPORIN

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(Received for publication May 25, 1992)

Single-dose pharmacokinetics of FK037 has been investigated in laboratory animals. After bolus intravenous dosing with 20 mg/kg, the elimination half-life of FK037 varied in the species; with values of 0.27, 0.30, 0.97, 1.29 and 1.76 hours in mice, rats, rabbits, dogs and monkeys, respectively. The volume of distribution ranged between 260 ml/kg in rats and 390 ml/kg in dogs. These parameters approximated those of ceftazidime and cefpirome used as reference drugs. The renal clearance of FK037 was almost equal to glomerular filtration rate (GFR) in rabbits. Probenecid did not affect the elimination half-life of FK037 and its clearance ratio to GFR. These findings suggest that FK037 is solely excreted by glomerular filtration. FK037 readily penetrated into the tissues and inflammatory exudate fluid in rats, and the tissue level was highest in the kidneys, and decreased in the following order; lungs > heart > liver > spleen. Penetration of FK037, cefpirome and ceftazidime into the cerebrospinal fluid were determined using induced staphylococcal meningitis in rabbits. The penetration percentage ranged from 14.2 to 16.0% for these drugs with no significant differences. The major route of excretion of FK037 was *via* the kidney, with more than 74% of the dose being excreted in the urine within 24 hours after dosing to each species. Biliary excretion was low, 0.79% in rats. Bioautograms showed only unchanged drug in the plasma, urine and bile. Serum protein binding was low (8.8 to 17.6%) in all the species studied.

FK037 is a novel parenteral cephalosporin derivative with an extremely broad antibacterial spectrum that includes strains of *Staphylococcus* and *Pseudomonas aeruginosa*[1,2]. It also has a potent activity against methicillin-resistant Staphylococci[3].

This study was performed to obtain fundamental pharmacokinetic data relating to FK037 in laboratory animals, prior to clinical trials.

Materials and Methods

Antibiotics

FK037 and cefpirome were synthesized as a sulfonated salt at the Research Laboratories of Fujisawa Pharmaceutical Co., Ltd. Ceftazidime was a commercial preparation from Glaxo Co. Cefazolin was

provided as a working standard in Fujisawa Pharmaceutical Co., Ltd. FK037 and cefpirome were dissolved in physiological saline after neutralization with 5% sodium bicarbonate. Ceftazidime and cefazolin were merely dissolved in physiological saline.

Test Animals

The following animals species were used: Mouse; 5~6 week-old male ICR strain, weight range, 22~33 g, rat; 6~7-week-old male JCL SD strain, weight range, 185~290 g, rabbit; male Japanese White, weight range, 1.9~3.3 kg, dog; male beagle, weight range, 10.0~12.0 kg and monkey; male Cynomolgus, weight range, 5.0~6.3 kg.

Plasma Concentrations

Antibiotics of 20 mg (potency)/kg were administered intravenously *via* the tail vein of mice and rats, the ear lobe vein of rabbits, cephalic vein of dogs and antecubital vein of monkeys. The dosing volumes were 10, 5, 1 and 0.5 ml/kg for mice, rats, monkeys and rabbits/dogs, respectively. In the dose-response study, FK037 was given intravenously at doses of 10, 20 and 40 mg/kg for both rats and dogs. Animals were used in groups of 3~5. Blood was sampled with a heparinized needle by cardiac puncture in mice and rats, *via* the ear lobe vein in rabbits, cephalic vein in dogs and antecubital vein in monkeys at fixed intervals after dosing. The plasma was separated from blood samples by conventional centrifugation.

In order to predict the renal excretion mechanisms of FK037, the effect of probenecid on the plasma concentrations of FK037 were studied in rabbits. FK037 was intravenously administered at a dose of 20 mg (potency)/kg to five male rabbits. Venous blood samples were collected in a same manner described above. One week later, the same experimental maneuvers were performed in the same animals, but probenecid (Sigma Chemical Co.), suspended in 0.5% methylcellulose solution, was orally administered at a dose of 200 mg/kg, 30 minutes before antibiotic injection.

Tissue Distribution

Rats were bled to death from the abdominal aorta under ether anesthesia at the assigned time after dosing with 20 mg/kg intravenously. The liver, kidneys, lungs, heart and spleen were immediately removed from each animal thereafter. Tissues were rinsed lightly in physiological saline, blotted on filter paper and weighed accordingly prior to homogenization with a polytron homogenizer in 2 ml of 1/15 M phosphate buffer (pH 7.0)/g of wet tissue weight. The homogenates were centrifuged at 10,000 × *g* for 5 minutes, and the resultant supernatants were used for assay.

Exudate Concentrations

After subcutaneous injection of 20 ml of air into the backs of rats, 1 ml of olive oil containing 1% croton oil was injected into the pouch of each animal to induce aseptic inflammation. For determination of exudate concentrations, rats in groups of 6~7 were used 6 days after the induction of granuloma pouch. Each antibiotic was given intravenously 20 mg/kg, and the exudate was collected at 0.5, 1, 2, 4 and 6 hours after dosing.

Penetration into the Cerebrospinal Fluid (CSF)

Staphylococcus aureus, a clinical isolate, was used to induce meningitis in rabbits. The organism was grown as a slant culture for 18 hours in Trypticase soy agar (BBL) at 37°C. The culture was then harvested in physiological saline to give $OD_{660\,nm}=1.6$. Rabbits were experimented in groups of 5. Under pentobarbital-anesthesia (15 mg/kg iv), 0.2 ml of the inoculum (10^7 cfu/rabbit) was slowly injected into the cisterna magna. The animals were allowed to recover consciousness prior returning to their cages. FK037, cefpirome or ceftazidime was injected intravenously *via* the ear vein at a single dose of 40 mg/kg 16 hours after infection. Samples of blood and CSF were taken at fixed intervals over a period of 6 hours from the ear lobe vein and the cisterna magna, respectively.

Urinary and Biliary Excretion

Mice and rats were used in groups of 9~10, and rabbits, dogs and monkeys in a group of 3~5. The animals were housed individually in a metabolism cage and urine 0~3, 3~6 and 6~24 hours after dosing

was collected from each animal. The spontaneous urine and the fluid for washing out the urinary bladder at the assigned intervals were combined in the case of rabbits and dogs. For biliary excretion, groups of 5~10 rats were anesthetized with pentobarbital 20 mg/kg intraperitoneally. After surgically exposing the abdomen, a polyethylene cannula was inserted in the common bile duct. On recovery from the anesthesia, the bile was collected from each rat at the following intervals of 0~3, 3~6 and 6~24 hours.

Renal Clearance in Rabbits

Four male rabbits were anesthetized with sodium pentobarbital (25 mg/kg) intravenously. The left ureter was exposed by a flank incision and catheterized with a polyethylene tube. Urine was collected through the cannula. Blood was collected from the right femoral vein, and the test compounds and solutions were injected or infused through the ear vein. After the operation, 100 mg of inulin per kg was injected intravenously as a priming dose. The sustaining solution (mannitol 15%, NaCl 0.9%, inulin 0.33%) was then infused at the rate of 0.3 ml/minute/kg. When urine flow had stabilized, FK037 injected intravenously at a priming dose of 6.75 mg/kg, and sustained by the infusion of the sustaining solution containing FK037 at the rate of 0.3 ml/minute/kg. FK037 was infused at the rate of 82.5 μg/minute/kg. Urine samples were collected during three successive 5-minute intervals, beginning 20 minutes after the start of FK037 infusion. Blood samples were taken at the midpoint of urine collections. After these procedures were completed, a single dose of 30 mg of probenecid per kg was administered intravenously, and urine and blood samples were collected again in manner similar to that described above.

Serum Protein Binding

Freshly separated sera of human, dog, rabbit, rat and mouse were used. The pH of sera was adjusted to 7.4 with 1 N HCl and 1 N NaOH just before use. FK037, cefpirome and ceftazidime were evaluated in this study. The degree of binding was determined by centrifugal ultrafiltration. Volumes of 0.02 ml of drug solution of 3,000 μg/ml in 1/15 M phosphate buffer (pH 7.0) was added to 1.98 ml of each serum, and incubated at 37°C for 20 minutes. Three samples were taken from each serum, and these samples were then placed in an Ultrafree C3LGC (Millipore) and centrifuged at 7,000 rpm (4,000 $\times g$) for 30 minutes by Centrifuge 5415 (Eppendrof) to obtain the ultrafiltrate. The antibiotic concentrations in the filtrates were bioassayed three times. Protein binding was calculated using the respective total concentration (Ct) and the concentration in the filtrate (unbound, Cf) according to the following formula:

$$\% \text{ Binding} = (1 - Cf/Ct) \times 100$$

TLC-Bioautography

For collection of the plasma, urine and bile, FK037 was given intravenously to animals at a dose of 20 mg/kg. Each plasma samples was diluted 2-fold with ethanol for deproteinization. The mixtures were then centrifuged and the resultant supernatants were applied to TLC. Urine and bile samples were applied to TLC after centrifugation. 20~30 μl of each biological specimen was spotted on a TLC plate (DC-Fertigplatten RP-8, F254s Art. 15424: Merck) and the plates were developed using a solvent consisting of 5% KCl-dioxane-formic acid (5:1:1). Bioautography was carried out using *Morganella morganii* IFO3848 as the test organism and Antibiotic Medium No. 1 (Difco) as the test medium. Inhibition zones for each plate were examined.

Analytical Procedure

Antibiotic concentrations were determined microbiologically by the agar diffusion method. A paper-disc of 8 mm in diameter (Advantec Toyo) was used for the assay. *Bacillus subtilis* ATCC 6633 was used as test organisms for the assays of FK037 and cefpirome. The assay media were sodium citrate agar (1.0 g sodium citrate, 0.5 g Polypepton, 0.3 g beef extract and 1.0 g agar dissolved in 100 ml distilled water). Ceftazidime concentrations were assayed in the same way using *Escherichia coli* ATCC 39188 as the test organism and nutrient agar (Difco) as the medium. All the antibiotics tested were detectable at 0.25 μg/ml in both plasma and buffer. The mean assay coefficient of variation for identical samples were slightly greater than 10% at the concentration range of 1 to 10 μg/ml, when *B. subtilis* and *E. coli* were used as the test organisms.

Inulin concentrations in plasma and urine were determined by the method of BROWN and NOLPH[4].

Pharmacokinetic Analyses of Plasma Concentrations

Mean plasma concentrations in mice and rats, and individual values for rabbits, dogs and monkeys were used for pharmacokinetic analyses after intravenous dosing. A one-compartment model was used for mice and rats, whereas a two-compartment model was employed for analytical studies in rabbits, dogs and monkeys with NONLIN computer program. In the studies of penetration into the exudate and CSF, the plasma, exudate and CSF concentrations were analyzed by a model independent method. The actual maximum concentration was designated as C_{max} and the time required to attain the maximum concentrations was represented as T_{max}. Elimination half-life was calculated by dividing In 2 by the regression slope which was estimated by the least squares method using individual points of data on the elimination phase expressed in logarithmic function. AUC values were determined by using the trapezoidal rule and extrapolation. The extent of penetration into CSF was estimated by expressing the AUC and C_{max} in CSF as a percentage of the AUC and C_{max} in plasma.

Results

Plasma Concentrations

Single-dose pharmacokinetics of FK037 were determined in laboratory animals after intravenous dosing, and compared with cefpirome and ceftazidime as reference drugs. FK037 showed favorable plasma concentrations in each species (Fig. 1). The elimination rate of FK037 varied according to the species; being fastest in mice, followed by rats, rabbits, dogs and monkeys in a descending order. Table 1 summarizes the mean plasma pharmacokinetic parameters. The half-life of FK037 was 0.27 hour in mice, 0.30 hour in rats, 0.97 hour in rabbits, 1.29 hours in dogs and 1.76 hours in monkeys. The increase in AUC was directly proportional to the size of the tested animal except for dogs. The volume of distribution ranged from 260 ml/kg in rats to 390 ml/kg in dogs. The pharmacokinetic parameters of FK037 were similar to those found for ceftazidime and cefpirome in all animals tested at a dose of 20 mg/kg.

Fig. 1. Mean plasma concentrations of FK037 in laboratory animals ($n=5$) after single intravenous dosing with 20 mg/kg.

□ Mouse, ■ rat, △ rabbit, ● dog, ○ monkey. Vertical bars indicate the standard error.

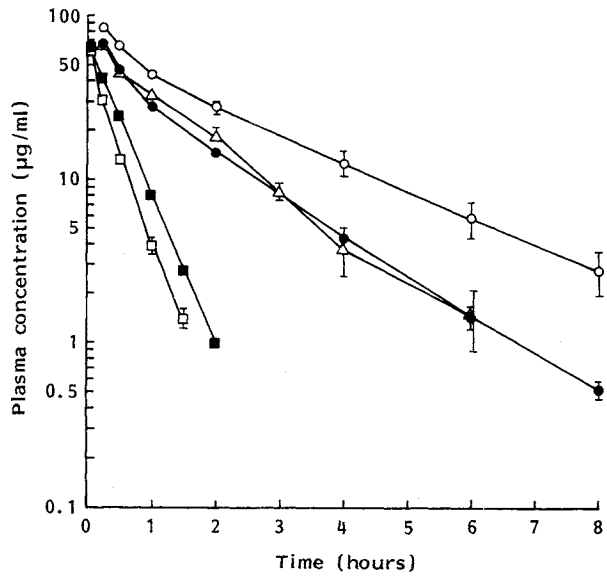


Table 1. Pharmacokinetic parameters of FK037, cefpirome and ceftazidime.

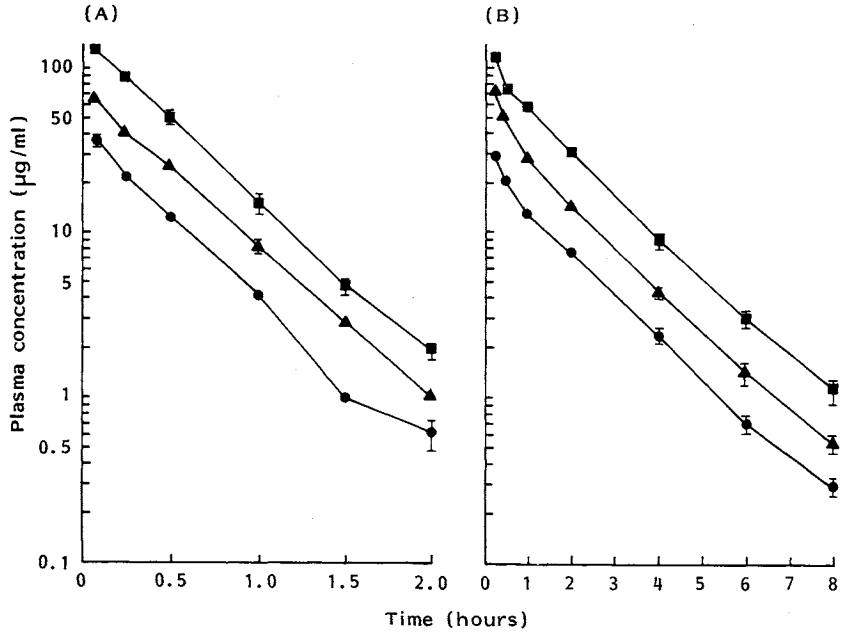
Animal	Antibiotic	Dose (mg/kg)	n	C_0 (μg/ml)	$T_{1/2}$-β (hours)	$AUC_{0\sim\infty}$ (μg·hours/ml)	Vd (ml/kg)	Cl_{plasma} (ml/minute/kg)
Mouse[a]	FK037	20	5	59.1	0.27	22.5	339	14.8
	Cefpirome	20	5	63.7	0.22	20.3	314	16.4
	Ceftazidime	20	5	78.1	0.21	23.7	256	14.1
Rat[a]	FK037	10	3	41.9	0.29	17.3	242	9.6
		20	5	76.9	0.30	33.2	260	10.0
		40	3	156	0.30	67.4	257	9.9
	Cefpirome	20	5	74.3	0.31	33.3	269	10.0
	Ceftazidime	20	5	72.5	0.26	34.6	215	9.6
Rabbit	FK037	20	5	75.9± 5.1	0.97±0.14	102.6± 6.3	269±21.6	3.3±0.20
	Cefpirome	20	5	87.6± 5.8	0.79±0.05	84.5± 3.6	269±17.3	4.0±0.17
	Ceftazidime	20	5	93.1± 7.1	0.73±0.05	72.6± 2.9	289±17.0	4.6±0.18
Dog	FK037	10	5	43.8± 1.4	1.23±0.05	47.5± 1.3	385±12.8	3.5±0.10
		20	5	119 ±23.3	1.29±0.09	97.1± 5.2	388±31.6	3.5±0.18
		40	5	131 ±17.0	1.35±0.17	190.3±11.1	414±54.7	3.6±0.19
	Cefpirome	20	5	87.4± 9.6	1.20±0.11	95.4± 5.0	371±47.6	3.5±0.18
	Ceftazidime	20	5	105 ±19.5	1.09±0.03	89.0± 3.8	353±19.5	3.8±0.15
Monkey	FK037	20	5	130 ± 5.6	1.76±0.21	177.9±16.8	283±11.8	1.9±0.18
	Cefpirome	20	5	161 ±20.5	1.32±0.08	144.4±15.5	268±15.8	2.4±0.23
	Ceftazidime	20	5	164 ±18.9	1.55±0.25	174.5±21.6	255±18.9	2.1±0.28

[a] Mean plasma concentrations were analysed. Values represent mean±standard error.

C_0: fictive plasma concentration at t=0, $T_{1/2}$-β: biological half-life, AUC: area under plasma concentration-time curve, Vd: distribution volume, Cl_{plasma}: Plasma clearance.

Fig. 2. Dose response of plasma concentrations of FK037 in rats ($n=3\sim5$) (A) and dogs ($n=5$) (B) after intravenous dosing.

● 10 mg/kg, ▲ 20 mg/kg, ■ 40 mg/kg. Vertical bars indicate standard error.



In the dose-response study, the plasma concentrations of FK037 in rats and dogs after intravenous dosing with 10, 20 and 40 mg/kg are shown in Fig. 2. The concentration showed good linearity, and the half-life and volume of distribution were virtually dose-independent across the dose range. These data indicate systemic exposure to the drug was proportional to the dose in these species.

The interaction between FK037 and probenecid was studied in rabbits after intravenous injection of 20 mg/kg with and without probenecid pretreatment. The pharmacokinetic parameters are listed in Table 2. A statistical analysis of the pharmacokinetic parameters was performed by using an analysis of variance. Small differences in the parameters did not test statistically significant.

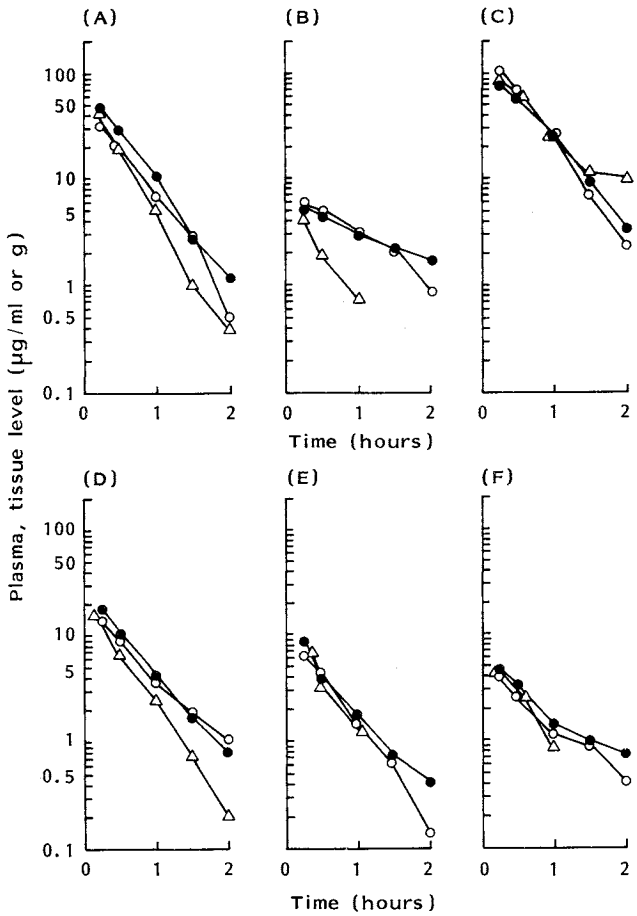
Table 2. Pharmacokinetic parameters of FK037 in rabbits after intravenous injection of 20 mg/kg with or without probenecid pretreatment.

Parameter	Without probenecid	With probenecid
C_0 (μg/ml)	139.2±14.4	153.4±20.2
α (hour^{-1})	7.13±4.46	5.15±1.54
β (hour^{-1})	0.72±0.06	0.69±0.05
$T_{1/2}$-β (hours)	0.96±0.07	1.01±0.08
$AUC_{0\sim\infty}$ (μg·hours/ml)	115.7±15.9	133.3±17.7
Vd (ml/kg)	145.0±15.2	132.2±17.5
Cl_{plasma} (ml/minute/kg)	2.93±0.41	2.53±0.31

α and β: the first-order rate constants in the distribution and elimination phase, respectively.

Fig. 3. Mean plasma (A), liver (B), kidney (C), lung (D), heart (E) and spleen (F) concentrations of FK037, cefpirome and ceftazidime in rats ($n=3$) after single intravenous dosing with 20 mg/kg.

● FK037, ○ cefpirome, △ ceftazidime.



Tissue Concentrations

Tissue concentrations of FK037 and the reference drugs were determined in rats. As shown in Fig. 3, FK037 was rapidly distributed into the tissues tested. The concentrations of FK037 at 15 minutes after dosing were the highest in the kidneys (74.7 μg/g); the organ responsible for elimination of the drug, followed by the plasma (46.8 μg/ml), lungs (17.7 μg/g), heart (8.6 μg/g), liver (5.1 μg/g) and spleen (4.5 μg/g). These values were similar to those of the reference drugs. The elimination rate of FK037 in the tissues, like cefpirome, was similar to that in the plasma except for the liver, in which the drug concentrations were sustained for a long time than in the plasma.

Penetration into the Exudate and CSF

Inflammatory exudate drug concentrations were investigated in rats with granuloma pouch after intravenous dosing with 20 mg/kg. The pharmacokinetic parameters of FK037, cefpirome and ceftazidime are summarized in Table 3. The exudate concentration of FK037 peaked at 6.35 μg/ml 1 hour after dosing. The elimination rate was slower in the exudate than in the plasma. There was no significant difference in C_{max} and AUC among FK037, cefpirome and ceftazidime. Penetration of FK037, cefpirome and ceftazidime into CSF was studied in rabbits with induced staphylococcal meningitis after intravenous dosing with 40 mg/kg. Mean plasma and CSF concentrations of FK037, cefpirome and ceftazidime are shown in Fig. 4, and the pharmacokinetic parameters are listed in Table 4. The peak CSF concentrations of FK037, cefpirome and ceftazidime were 8.78, 5.90 and 4.80 μg/ml, respectively. Corresponding peak plasma concentrations were 70.6, 72.6 and 70.7 μg/ml, respectively. CSF penetration of drug based on AUC values ranged from 14.2% to 16.0% with no statistical differences between the three drugs.

Excretion

The percentages of the dose excreted in each species are shown in Table 5. Urinary recovery of FK037 was 82.2% in mice within 6 hours, 86.5% in rats, 79.2% in rabbits, 78.9% in dogs and 73.8% in monkeys within 24 hours. These values indicated that FK037 was excreted mainly *via* the urine. In contrast, biliary excretion was negligible, 0.79% of the dose was excreted in the bile of rats within 24 hours. Large amounts of cefpirome and ceftazidime, similar to FK037, were excreted in the urine. All these three drugs manifested a similar excretion pattern.

Renal Clearance

Results of renal clearance experiments are shown in Table 6. The amount of FK037 excreted in the urine was almost equal to the amount estimated for glomerular filtration of FK037. The ratio of renal clearance of FK037 to that of inulin, *i.e.* GFR, was 0.93. Probenecid caused a significant decrease in the glomerular filtration and urinary excretion of FK037, which was concomitant with the insignificant decrease in GFR. The clearance ratio when probenecid was added was 0.95, not significant difference from the

Table 3. Pharmacokinetic parameters of FK037, ceftazidime and cefpirome in inflammatory exudate of rats after intravenous dosing with 20 mg/kg.

Antibiotic	*n*	C_{max} (μg/ml)	T_{max} (hours)	$T_{1/2}$ (hours)	$AUC_{0\sim\infty}$ (μg·hours/ml)
FK037	7	6.35±0.72	1.1±0.19	2.36±0.19	30.4±1.24
Cefpirome	6	6.43±0.41	1.1±0.20	2.56±0.33	30.3±2.40
Ceftazidime	6	6.71±0.74	1.2±0.14	3.04±0.24	33.9±2.16

Values represent mean±standard error.

Fig. 4. Plasma and CSF concentrations of FK037 (A), ceftazidime (B) and cefpirome (C) in rabbits ($n=5$) with staphylococcal meningitis after single intravenous dosing with 40 mg/kg.

○ Plasma, ● CSF. Vertical bars indicate standard deviation.

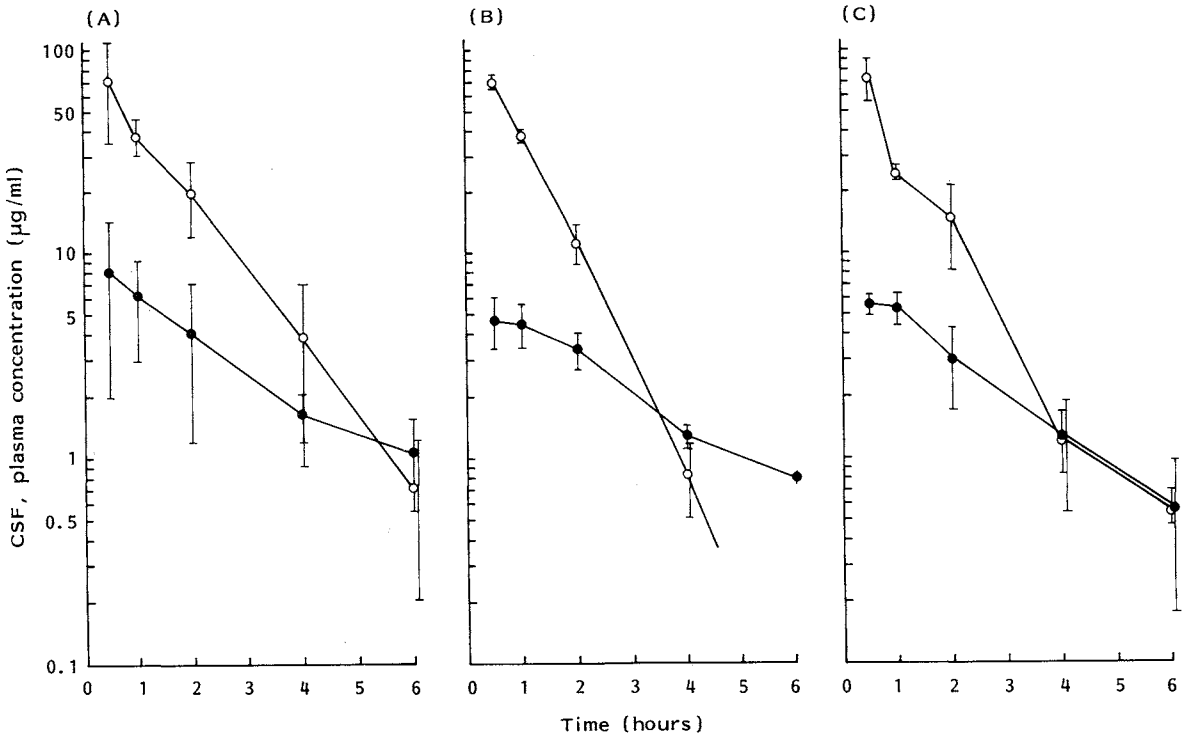


Table 4. Pharmacokinetic parameters of FK037, ceftazidime and cefpirome in rabbits with staphylococcal meningitis after intravenous dosing with 40 mg/kg.

Antibiotic	Specimen	C_{max} (μg/ml)	T_{max} (hours)	$T_{1/2}$ (hours)	$AUC_{0\sim6\,hours}$ (μg·hours/ml)	% Penetration	
						C_{max}	$AUC_{0\sim6\,hours}$
FK037	CSF	8.78± 5.65	0.7±0.3	2.28±0.73	19.0± 9.65	14.2±10.3	16.0± 6.5
	Plasma	70.6 ±35.4	0.5	0.81±0.10	119 ±32.3		
Ceftazidime	CSF	4.80± 1.21	0.7±0.3	1.96±0.28	14.0± 2.67	6.8± 1.6	14.2± 3.0
	Plasma	70.7 ± 4.74	0.5	0.54±0.11	99.3± 6.21		
Cefpirome	CSF	5.90± 0.32	0.8±0.3	1.85±0.49	14.1± 2.18	8.6± 2.3	14.9± 4.1
	Plasma	72.6 ±17.5	0.5	0.83±0.14	97.8±17.4		

Values represent mean±standard deviation ($n=5$).

Table 5. Urinary and biliary excretions of FK037, cefpirome and ceftazidime after intravenous dosing with 20 mg/kg.

	Animal	Antibiotic	Excretion rate (% of dose), mean±S.E.			
			0~3 hours	3~6 hours	6~24 hours	0~24 hours
Urine	Mouse	FK037				82.2 ±2.5[a]
		Cefpirome				88.5 ±2.5[a]
		Ceftazidime				81.3 ±1.8[a]
	Rat	FK037	83.8 ± 1.8	2.3 ±0.4	0.4 ±0.22	86.5 ±1.6
		Cefpirome	81.9 ± 2.1	1.2 ±0.2	0.1 ±0.01	83.2 ±2.1
		Ceftazidime	86.0 ± 1.6	1.4 ±0.8	0.2 ±0.01	87.6 ±2.0
	Rabbit	FK037	71.3 ± 2.7	7.5 ±1.3	0.4 ±0.1	79.2 ±3.2
		Cefpirome	79.9 ± 5.3	5.7 ±0.3	0.6 ±0.1	86.2 ±5.3
		Ceftazidime	77.2 ± 4.3	1.3 ±0.1	—[b]	78.6 ±4.4
	Dog	FK037	67.6 ± 3.0	8.3 ±0.7	3.0 ±1.0	78.9 ±2.5
		Cefpirome	76.0 ± 7.9	10.0 ±0.6	2.1 ±0.9	88.2 ±7.1
		Ceftazidime	81.4 ± 0.9	8.4 ±0.7	2.0 ±0.4	91.8 ±1.9
	Monkey	FK037	55.4 ±10.4	13.4 ±6.5	5.0 ±1.6	73.8 ±6.0
		Cefpirome	61.6 ± 5.9	11.1 ±3.0	2.3 ±0.6	75.0 ±2.8
		Ceftazidime	66.7 ± 6.8	5.2 ±2.8	4.2 ±1.8	76.1 ±6.3
Bile	Rat	FK037	0.63±0.05	0.14±0.01	0.03±0.01	0.79±0.05
		Cefpirome	1.49±0.13	0.10±0.03	0.01±0.01	1.60±0.13
		Ceftazidime	0.43±0.07	0.02±0.00	—[b]	0.45±0.07

[a] 0~6 hours; [b] not detected.

control value. The mean value of urinary pH was 7.73±0.12 and 7.80±0.14 during the control periods and probenecid pretreatment, respectively.

Antimicrobially Active Metabolite

The active metabolites of FK037 were examined by bioautography using the plasma, urine and bile of animals given the drug intravenously. Bioautograms showed only unchanged drug in all the specimens and active metabolite was not detected.

Serum Protein Binding

The degrees of serum protein binding of FK037, cefpirome and ceftazidime are indicated in Table 7. These drugs have a low affinity for serum proteins, the binding ratio of FK037 ranged from 8.8% for human to 17.6% for mouse. Cefpirome and ceftazidime also showed low binding rate with the same extent as FK037.

Table 6. Renal clearance of FK037 in rabbits[a].

Treatment	Rabbit No.	Plasma[b] concentration of FK037 (μg/ml)	GFR[c] (ml/minute)	FK037			Clearance ratio FK037/GFR
				Clearance (ml/minute)	Glomerular filtration (μg/minute)	Urinary excretion (μg/minute)	
Without probenecid (control)	1	22.9±1.8	4.46±0.49	4.61±1.32	94.2± 2.39	95.9±17.0	1.02±0.21
	2	36.3±2.5	3.53±0.12	2.67±0.21	110.2±13.6	83.3±12.8	0.76±0.04
	3	30.2±3.6	3.15±0.05	3.24±0.15	99.1± 6.96	101.9± 3.00	1.03±0.06
	4	47.4±2.1	2.49±1.07	2.21±1.03	117.5±48.3	103.6±45.2	0.91±0.20
	Mean	34.2±9.6	3.41±0.90	3.18±1.19	105.2±23.6	96.2±22.9	0.93±0.17
With probenecid	1	23.2±5.5	4.07±0.21	3.53±0.21	83.2± 8.46	71.9± 1.03	0.87±0.09
	2	34.0±1.7	3.19±0.21	2.50±0.39	97.6±10.1	76.1± 7.72	0.79±0.17
	3	34.4±1.8	2.52±0.09	2.78±0.45	88.6±11.2	96.7± 7.04	1.11±0.17
	4	39.0±1.7	1.65±1.02	1.66±0.90	59.4±31.1	60.2±26.8	1.04±0.11
	Mean	32.6±6.6	2.86±1.03	2.62±0.84	82.2±21.2*	76.2±18.4*	0.95±0.18

[a] Numbers represent mean±standard deviation ($n=3$).
[b] Total concentration.
[c] Glomerular filtration rate.
* Significantly different from the control value ($P<0.05$).

Table 7. Serum protein binding (*in vitro*).

Drug	Binding ratio (%)				
	Mouse	Rat	Rabbit	Dog	Human
FK037	17.6	15.4	10.7	11.0	8.8
Cefpirome	11.6	15.8	10.3	6.8	5.8
Ceftazidime	11.9	9.5	17.6	10.2	11.2

Method: Centrifugal ultrafiltration.
Drug concentration: 30 μg/ml.
Serum: 99% fresh serum.

Discussion

The pharmacokinetics of FK037 were studied in laboratory animals and compared with those of cefpirome and ceftazidime. FK037 exhibits favorable pharmacokinetic profiles that much resemble those of cefpirome and ceftazidime. The similality seems to be based on the chemical structure; these compounds have a quarternary salt of heteroaromatics at 3-position. As with most cephalosporins[5~7], plasma concentration, half-life and area under the plasma concentration-curve of FK037 increase proportionally to the size of animals except dogs. The results of excretion studies indicate that the kidney is the primary route of elimination of FK037 in animals tested. Therefore, we investgated the mechanism of renal excrtion of FK037 in rabbits. Probenecid, an inhibitor of renal tubular secretion of organic acid, did not modify the pharmacokinetic parameters of FK037. The renal clearance of FK037 was almost equal to the glomerular filtration rate (GFR), and its clearance ratio to GFR was not affected by probenecid. These results indicate that the renal excretion of FK037 takes place mostly through glomerular filtration and that there is little contribution from renal tubular secretion and reabsorption. Unlike many other cephalosporins[8~10], probenecid induced little change in the excretion of FK037. The results in this study differed from those obtained by CARBON *et al.*[11] in their study of the renal clearance of ceftazidime in rabbits. These authors reported that in acidic urine probenecid caused a transient increase in the excretion of ceftazidime based on the tubular secretion and a decrease in GFR. Though the decrease in GFR appeared in our experiment, we could not observed the tubular secretion of FK037 with or without probenecid. The difference might be due to the plasma concentration of probenecid; in our experiment the urine was collected at 30 minutes after probenecid added, whereas they did immidiately after probenecid injection.

Latamoxef has been demonstrated to be secreted through tubules in alkaline urine[10]. Thus the renal disposition of FK037 might differ from latamoxef.

The biliary excretions of FK037, cefpirome and ceftazidime were extremely low in rats as compared with those of ceftizoxime[5], cefotiam[5], cefamandole[5] and cefazolin[12]. However, therapeutically significant concentrations (about 8 μg/ml) were found at 0~3 hours after dosing.

FK037, cefpirome and ceftazidime penetrated into the tissues and exudate in rats and CSF in rabbits with same extent. The penetration of cephalosporin is throught to depend upon (i) the concentration gradient from plasma to tissues, (ii) degree of serum protein binding and (iii) lipid solubility. It was found that the cephalosporins used in this study depicted similar pharmacokinetic properties and serum protein binding. Similar penetration tendency for these compounds could be due to their similar pharmacokinetic properties. KOBAYASHI and HARUTA[13] have reported the penetration of various β-lactams into CSF of rabbit with meningitis and the penetration rate of ceftazidime is 16.2%, a percentage which is considered as one of the best exhibited by various β-lactams. At the dosage used in this study, CSF concentrations of FK037 compare favorably with the minimum inhibitory concentration of a majority of organisms that induced meningitis, such as *E. coli*, *Haemophilus influenzae*, *Neisseria menigitidis*, *Staphylococcus aureus* and *Streptococcus pneumoniae*[1]. These results suggest a high possibility for FK037 to manifest clinical effects in patients with bacterial meningitis.

In conclusion, the results of this study, together with its high antibacterial activity, suggest that FK037 is a promising candidate for clinical application.

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