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Pharmacokinetics of Ceftizoxime in Animals after Parenteral Dosing

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The pharmacokinetic profile of ceftizoxime was studied in mice, rats, dogs, and monkeys given the drug in a single parenteral dose. The serum data after an intravenous injection were analyzed by the two-compartment open model. Cefotiam, cefmetazole, cefotaxime, and cefamandole were used as reference drugs. High concentrations of ceftizoxime were attained in the sera of all test animals and in the tissues of rats after parenteral dosing. The serum concentrations of ceftizoxime were higher than those of the other antibiotics in large animals (dogs and monkeys), but were lower in small animals (mice and rats). About 80% of ceftizoxime was excreted unchanged in the 24-h urine of all species tested. The biliary excretion of ceftizoxime was low: 3.7% in rats and 0.59% in dogs. However, therapeutically significant concentrations of ceftizoxime were found in the bile of dogs. Ceftizoxime was stable in biological fluids such as serum, urine, and tissue homogenates, but cefotaxime was unstable in rat tissue homogenates. Binding of ceftizoxime to serum protein in all species was the lowest of all the antibiotics: 31% for humans, 17% for dogs, and 32% for rats.

Ceftizoxime, a new parenteral cephalosporin derivative, is more active against various gram-negative bacilli, including the opportunistic pathogens such as *Enterobacter, Citrobacter* spp., and *Serratia marcescens*, than such cephalosporins as cefotiam, cefuroxime, cefotaxime, and cefmetazole (5). In the present study the pharmacokinetics, metabolism in animals, binding to serum protein, and stability of ceftizoxime were evaluated. Cefotiam, cefamandole, cefotaxime, and cefmetazole, all recently developed cephalosporins, were used as reference drugs.

MATERIALS AND METHODS

Antibiotics. The compounds used in this study included ceftizoxime (FK 749, Fujisawa, Japan), cefotiam (SCE 963, Takeda, Japan), cefamandole (Eli Lilly & Co., Indianapolis, Ind.), cefmetazole (CS 1170, Sankyo, Japan), and cefotaxime (HR 756, Hoechst-Roussel, West Germany).

Animals. The animals used in this study included 6-week-old male JCL:ICR strain mice, 6-week-old male JCL:SD strain rats, 7.5- to 15.0-kg male beagle dogs, and 5.8- to 9.1-kg male rhesus monkeys.

Assay procedures. Drug concentrations were measured by the disk-plate diffusion technique using *Bacillus subtilis* ATCC 6633 as the test organism and sodium citrate agar (sodium citrate, 1.0%; agar, 1.0%; polypeptone, 0.5%, and beef extract, 0.3%) as the test medium. The plates were incubated at 37°C for 18 to 20 h, and zones of inhibition were measured and compared with similarly prepared standards. Analysis by high-performance liquid chromatography was performed on a model ALC 202 chromatograph equipped with a model U6K injector and a μ-Bondapak C_{18} column (30.5 cm by 4.0 mm) (Waters Assoc., Milford, Mass.). The mobile phase consisted of 8% methanol in 4% aqueous acetic acid solution. The flow rate was maintained at 1.8 ml/min at a pressure of 1,400 to 1,500 lb/in^2. The detector monitored absorption at 254 nm. recorded at 0.1 to 0.05 absorbance unit full scale.

Dosing. The antibiotics for injection were dissolved in 0.9% saline. The drugs were given in a dose of 20 mg/kg to all test animals. The volumes were: 0.25 ml per animal by the intravenous (i.v.) and subcutaneous routes to mice; 5 ml/kg of body weight by the intramuscular (i.m.) and i.v. routes to rats; and 0.5 ml/kg of body weight by the i.m. and i.v. routes to dogs and monkeys.

Serum sampling. Blood samples were collected: (i) from the carotid artery of mice at specified intervals after injection; (ii) by heart puncture from rats; (iii) from the antecubital veins of dogs and monkeys. The antibiotic concentrations in the serum were bioassayed using standard solutions prepared with control serum of the respective species of animals.

Pharmacokinetic analysis. The serum concentration-time data were fitted to a two-compartment open model (4) using Marquardt's nonlinear least-squares regression analysis (6) with the aid of a FACOM 230/38 digital computer (Fujitsu Co., Ltd., Japan).

Tissue sampling. Groups of three rats each were exsanguinated at intervals after drug dosing. The liver, kidney, lung, and heart were removed, washed with 0.9% saline, and blotted with filter paper. These organs for each rat 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 × g for 10 min. The drug concentrations in each supernatant were bioassayed using standard solutions prepared with aqueous ethanol solution

(ethanol-water, 2:1). The same procedure was carried out three times, and the values were averaged.

Urinary excretion. Urine samples were collected at specified intervals for a 24-h period after i.m. dosing from rats fixed to a metabolism stage and from dogs and monkeys in a metabolism cage or through a urinary catheter. Urinary recoveries were calculated by concentration of drug and volume of each urine sample. Standard solutions for bioassay were prepared in 0.067 M phosphate buffer (pH 7.0).

Biliary excretion. Rats in groups of 10 were anesthetized with pentobarbital, 20 mg/kg intraperitoneally. They were then fixed in a supine position, and a polyethylene cannula was inserted into each bile duct. Bile samples were collected at 0 to 3, 3 to 6, and 6 to 24 h after dosing with the test drugs. Bile samples were collected by essentially the same procedure from dogs at 0.5, 1, 2, 3, 4, 5, 6, and 0 to 24 h after i.v. injection. The drug concentrations in the bile were bioassayed against standard solutions prepared in 0.067 M phosphate buffer (pH 7.0).

Identification of metabolites in urine and bile. Urine samples of rats, dogs, and monkeys and bile samples of rats were examined by thin-layer chromatography (8) using an *n*-butanol–acetic acid–water (4:1:2) solvent system and Eastman Chromagram Sheet no. 6064. Bioautography was then performed using *B. subtilis* ATCC 6633 and *Escherichia coli* NIHJ JC-2 as the test organisms. High-performance liquid chromatography was performed under the conditions described above.

Binding to serum protein. A 0.5-ml volume of an antibiotic solution (300 μg/ml) in 0.067 M phosphate buffer (pH 7.0) was added to 4.5 ml of fresh serum and incubated at 37°C for 1 h. This mixture was placed in a Visking tube (size 8/32) and centrifuged at 1,000 × *g* for 30 to 40 min to obtain the ultrafiltrate. The drug concentration in the filtrate was bioassayed using standard solutions prepared with 0.067 M phosphate buffer (pH 7.0). The degree of binding of the antibiotics was calculated in a conventional manner.

Stability in biological fluids and buffers. The stability of the test drugs in biological fluids and buffers was determined at 37°C. The stability of ceftizoxime, cefotiam, and cefotaxime in three different fluids, i.e., 90% human serum and 27% rat liver and kidney homogenates was determined at an initial concentration of 50 or 100 μg/ml. The mixtures were incubated at 37°C, sampled at set intervals, and immediately chilled to 0°C. The drug concentrations were determined by high-performance liquid chromatography or bioassay after deproteinizing by adding 2 ml of ethanol to 1 ml of the reaction mixtures.

RESULTS

Serum concentration. The serum concentrations of ceftizoxime in different species of animals are shown in Fig. 1 to 3.

Ceftizoxime showed favorable serum levels in each species of animal. The elimination rate of ceftizoxime from the serum differed according to species, and was the fastest in mice, followed in descending order by rats, monkeys, and dogs. The drug was undetectable in the sera of mice 1.5 h after a single subcutaneous or i.v. dose of 20 mg/kg, but was present in amounts of 1.4 and 1.7 μg/ml in the sera of dogs 6 h after the same single i.v. and i.m. doses, respectively.

The serum concentration data in all animals after a single i.v. dose were analyzed using a first-order two-compartment open model. The observed serum concentrations in each species of animal were well described by this model (Fig. 1). The pharmacokinetic parameters are shown in Table 1. The biological half life ($T_{1/2}\beta$) of ceftizoxime was 0.267 h in mice, 0.333 h in rats, 0.738 h in monkeys, and 1.06 h in dogs. When elimination of ceftizoxime from the serum was compared among the four species tested, the elimination was faster in the small animals (mice and rats) than in the large animals (dogs and monkeys).

The comparative findings on serum concentrations of ceftizoxime, cefotiam, cefamandole, cefotaxime, and cefmetazole in mice, rats, dogs, and monkeys are summarized in Fig. 2 and 3. The serum concentrations of ceftizoxime were lower in the small animals but higher in the large animals than those of the other drugs. In dogs the serum concentration of ceftizoxime was the highest of all the antibiotics tested. In monkeys the serum concentration of ceftizoxime was also the highest of the test drugs except that of cefmetazole at 0.5 h.

Tissue concentration. The tissue concentrations of ceftizoxime and the related antibiotics were determined in rats 15 and 30 min after a single i.m. dose of 20 mg/kg (Table 2). Ceftizoxime was well distributed to all the tissues tested. The tissue concentrations 15 min after injection were the highest in the kidneys (68.6 μg/g), followed by the liver (12.6 μg/g), the lungs (7.0 μg/g), and the heart (3.2 μg/g). The tissue concentrations of ceftizoxime were higher than those of cefotiam except in the liver. Although the serum concentration of cefotaxime was the highest in rats, the liver and kidney concentrations of cefotaxime in this species were the lowest of the four drugs. The kidney and liver concentrations of cefamandole were higher than those of ceftizoxime. The liver concentration of cefmetazole was the highest of all the test drugs.

Urinary excretion. Urinary excretion of ceftizoxime was determined in rats (Fig. 4) and in dogs and monkeys (Fig. 5) after a single i.m. dose of 20 mg/kg. The 24-h urinary recovery in rats was 80.1% for ceftizoxime, 55.6% for cefotiam, 58.7% for cefamandole, 52.7% for cefotaxime, and 9.3% for cefmetazole. In dogs, the 24-h urinary recovery of ceftizoxime was 93.8% and was the highest of all the antibiotics tested. In monkeys, 71.5% of the given dose was excreted in the 4-h urine and 80.3% in the 24-h urine. The

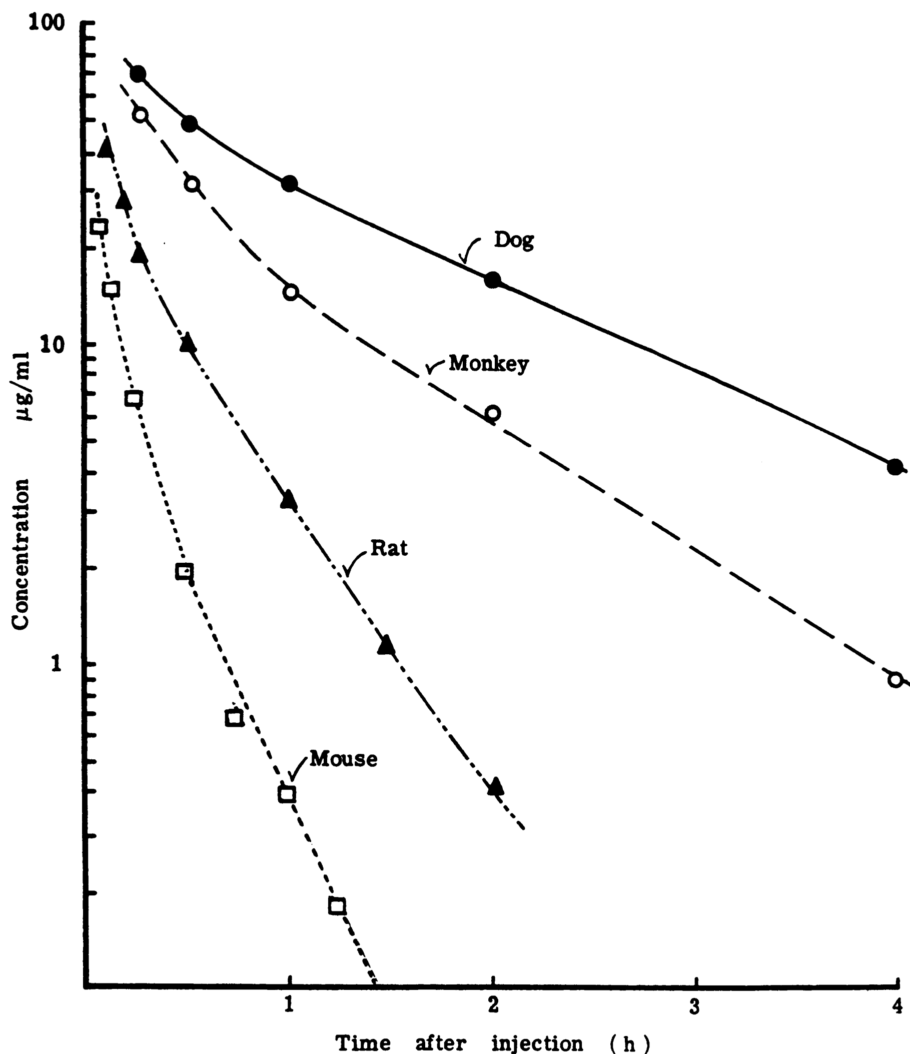

FIG. 1. *Regression serum concentration of ceftizoxime in different species of animals after a single i.v. injection by two-compartment model. Dose: 20 mg/kg. Points indicate observed serum concentration; lines indicate regression serum concentration.*

24-h urinary excretion was 51.3% for cefotiam, 78.0% for cefamandole, 68.9% for cefotaxime, and 80.2% for cefmetazole. Ceftizoxime was mainly excreted in the urine: more than 80% of the given drug appeared in the 24-h urine in all species tested.

Biliary excretion. The 24-h biliary recovery in rats was 3.7% for ceftizoxime, 35.7% for cefotiam, 24.6% for cefamandole, 1.0% for cefotaxime, and 79.1% for cefmetazole (Fig. 4). The biliary excretion of ceftizoxime was low in rats. Total recovery in the bile and urine of the antibiotics except cefotaxime was more than 80% for 24 h (Fig. 4), whereas that of cefotaxime was 53.7% of the given dose and was the lowest of the antibiotics tested. In dogs, the biliary excretion of ceftizoxime was also low, 0.59% in the 24-h bile. However, therapeutically significant concentrations (179 µg/ml at 1 h, 148 µg/ml at 2 h, and 13.0 µg/ml at 5 h) were found in the bile after a single i.v. dose of 20 mg/kg (Fig. 6).

Metabolites in urine and bile samples. No antimicrobial substances except ceftizoxime itself were detected in the urine samples by thin-layer chromatography–bioautography. An antimicrobial substance different from ceftizoxime, however, was found in the bile of rats when *E. coli* NIHJ JC-2 was used as the test organism. The R_f values were 0.30 for the metabolite and 0.63 for ceftizoxime. The inhibition zone of this

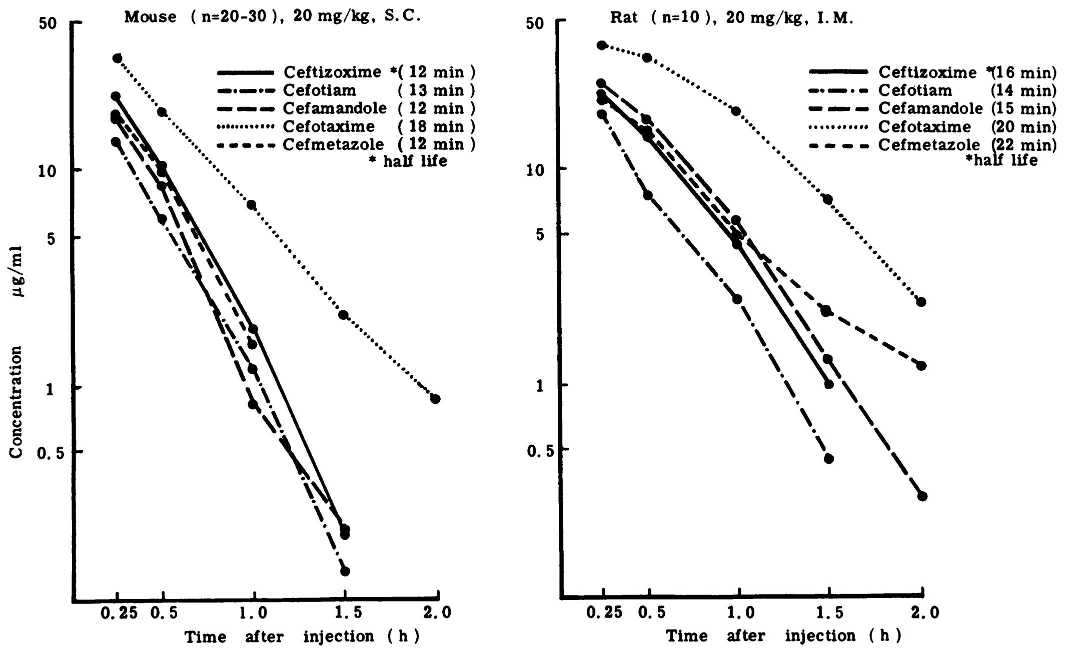

FIG. 2. *Serum concentration of ceftizoxime and related antibiotics in mice and rats.*

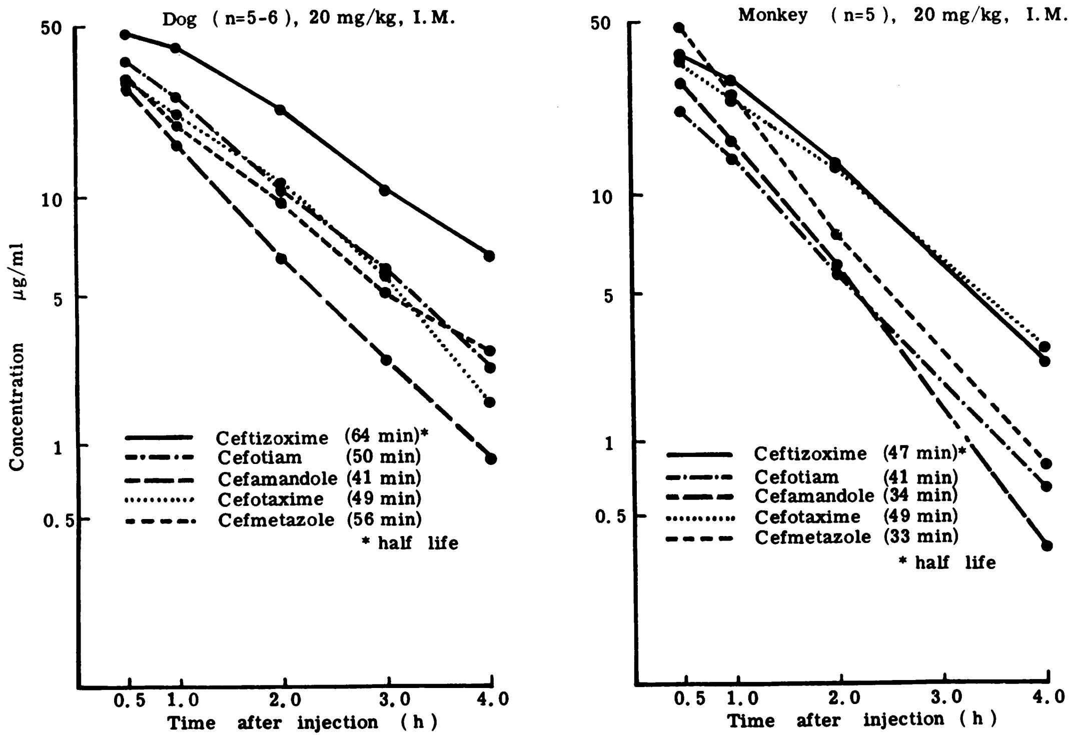

FIG. 3. *Serum concentration of ceftizoxime and related antibiotics in dogs and monkeys.*

unknown metabolite in the rat bile was considerably smaller than that of ceftizoxime. These results were verified by high-performance liquid chromatography. The peak of this unknown substance was identical to that of the unknown metabolite found by thin-layer chromatography-bioautography.

Serum protein binding. The degree of se-

TABLE 1. *Pharmacokinetic parameters of ceftizoxime in animals after a single i.v. injection of 20 mg/kg*

Parameter (unit)	Animal			
	Mouse	Rat	Dog	Monkey
A (μg/ml)	27.6	48.6	61.9	78.0
α (h^{-1})	7.44	11.0	4.57	5.09
($T_{1/2}\alpha$[h])	(0.093)	(0.063)	(0.152)	(0.136)
B (μg/ml)	4.52	26.8	58.1	38.4
β (h^{-1})	2.60	2.08	0.653	0.939
($T_{1/2}\beta$[h])	(0.267)	(0.333)	(1.06)	(0.738)
K_{12} (h^{-1})	0.863	3.47	1.50	1.65
K_{21} (h^{-1})	3.28	5.25	2.55	2.31
K_e (h^{-1})	5.90	4.38	1.17	2.07
V_c (ml/kg)	624	265	167	172
V_t (ml/kg)	164	175	98.3	123
CL-body (ml/h per kg)	3,670	1,160	195	356
AUC (μg·h/ml)	5.45	17.3	100	56.2

TABLE 2. *Tissue distribution of ceftizoxime and related antibiotics in rats after a single i.m. injection*[a]

Tissue	Time (min)	Antibiotic, mean ± SE (μg/g or ml)				
		Ceftizoxime	Cefotiam	Cefamandole	Cefotaxime	Cefmetazole
Liver	15	12.6 ± 0.8	19.2 ± 5.6	20.4 ± 2.1	4.4 ± 0.9	73.2 ± 9.1
	30	7.7 ± 1.2	15.3 ± 4.2	13.4 ± 1.2	4.9 ± 0.6	53.5 ± 3.0
Kidney	15	68.6 ± 1.2	55.1 ± 5.4	85.5 ± 9.9	18.9 ± 0.9	45.7 ± 3.3
	30	43.6 ± 6.5	36.6 ± 5.1	47.6 ± 2.9	15.5 ± 1.0	35.5 ± 1.7
Lung	15	7.0 ± 0.3	2.8 ± 0.3	5.0 ± 0.2	7.7 ± 1.7	5.0 ± 0.2
	30	6.4 ± 1.4	1.8 ± 0.1	4.1 ± 0.6	5.5 ± 0.4	3.7 ± 0.4
Heart	15	3.2 ± 0.3	1.4 ± 0.2	3.0 ± 0.4	3.7 ± 0.2	3.0 ± 0.3
	30	2.7 ± 0.4	0.6 ± 0.3	2.4 ± 0.2	4.0 ± 0.3	2.1 ± 1.1
Serum	15	22.4 ± 1.5	18.1 ± 0.9	26.4 ± 0.8	37.9 ± 2.5	21.5 ± 0.5
	30	14.0 ± 0.7	7.6 ± 0.6	16.9 ± 0.9	33.3 ± 1.2	14.5 ± 1.0

[a] Rats: JCL:SD strain, 6-week-old males, $n = 3 \times 3$. Dose: 20 mg/kg. SE, Standard error.

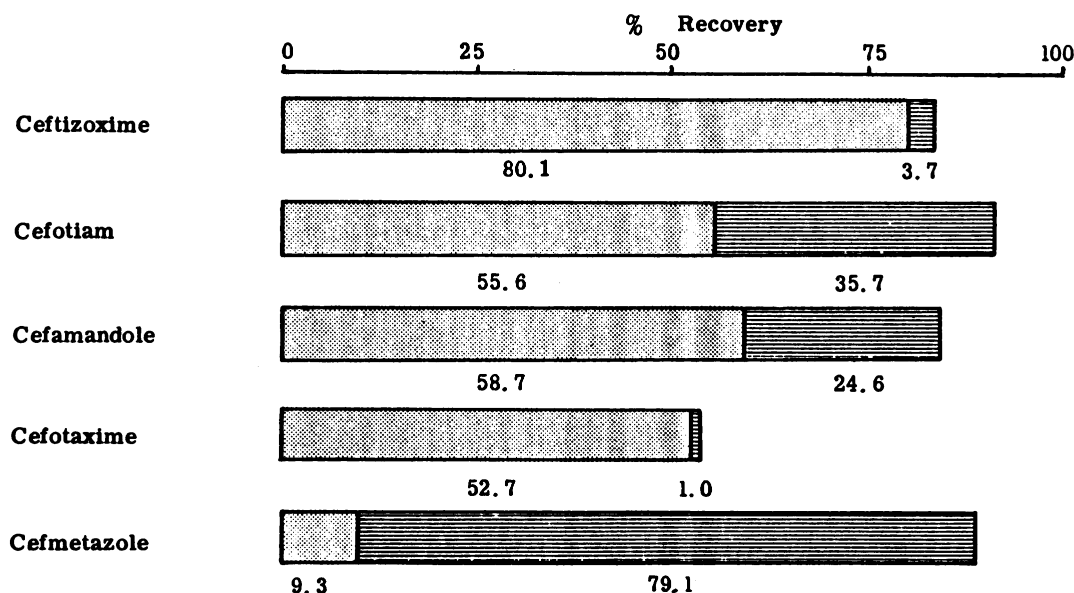

FIG. 4. *Urinary and biliary recovery of ceftizoxime and related antibiotics in rats after a single i.m. injection. Rats: JCL:SD strain, 6-week-old males, n = 10. Dose: 20 mg/kg. Stippled bar, Urine; striped bar, bile. Total recovery: ceftizoxime, 83.8%; cefotiam, 91.3%; cefamandole, 83.3%; cefotaxime, 53.7%; cefmetazole, 88.4%.*

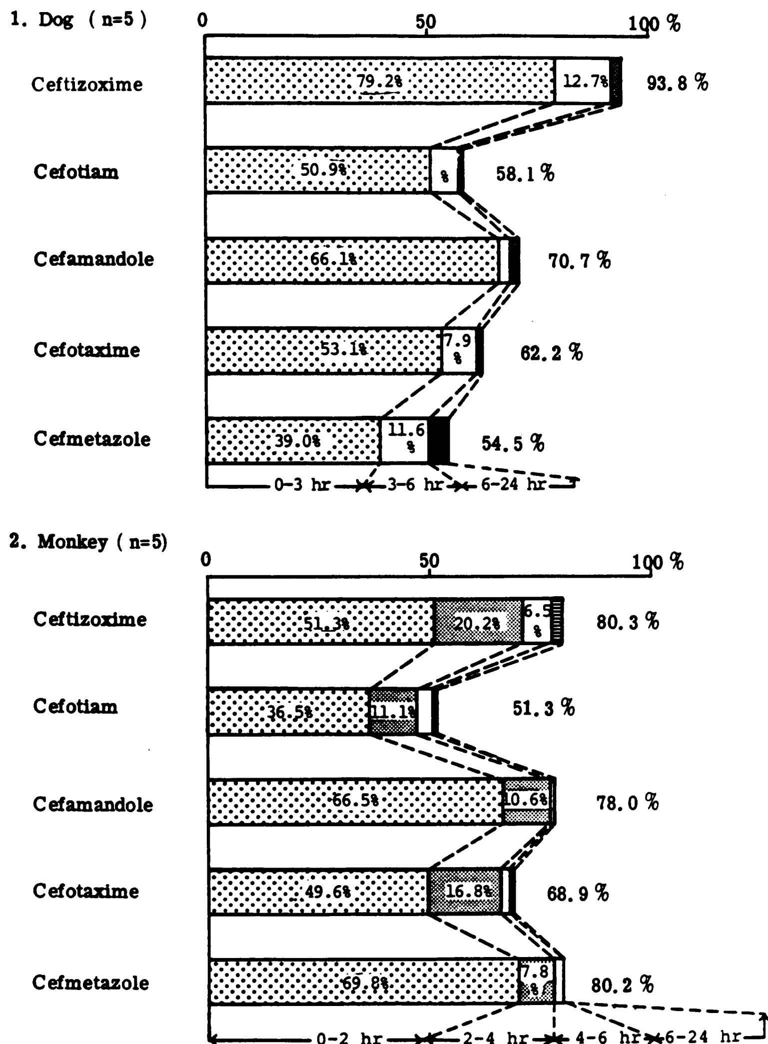

FIG. 5. *Urinary recovery of ceftizoxime and related antibiotics in dogs and monkeys after a single i.m. injection of 20 mg/kg.*

rum protein binding is shown in Table 3. Binding of ceftizoxime to human serum protein was similar to that of cefotaxime, but lower than those of cefotiam, cefamandole, and cefmetazole. The serum protein binding of ceftizoxime was also the lowest of the test drugs (Table 3).

Stability in biological fluids. Ceftizoxime was more stable than cefotiam and cefotaxime in human serum and urine and rat tissue homogenate at 37°C. The half-life was 93 to 170 h for ceftizoxime, 16 to 30 h for cefotiam, and 55 to 59 h for cefotaxime in the test samples. Cefotaxime was very unstable in rat tissue homogenate; the half-life was 0.1 h for cefotaxime, 16 h for cefotiam, and 120 to 170 h for ceftizoxime. Ceftizoxime was the most stable of the three antibiotics tested at pH 5.0, 7.0, and 9.2. The half-life at pH 7.0 was 148 h for ceftizoxime, 44 h for cefotaxime, and 17 h for cefotiam.

DISCUSSION

The pharmacokinetic profile of ceftizoxime in animals was examined in these experiments. The serum concentrations of ceftizoxime were clearly higher in the large animals, but were lower in the small animals than those of the other test antibiotics. Such low serum concentrations of ceftizoxime in the small animals are believed to be due to the faster renal clearance of this antibiotic. In contrast to the low serum concentrations, tissue concentrations of ceftizoxime in rats were considerably higher. Conversely, the liver

and kidney concentrations of cefotaxime were about half those of ceftizoxime, though the serum concentrations of cefotaxime were twice those of ceftizoxime. The high tissue concentrations of ceftizoxime in rats reflect the stability of this agent in kidney and liver homogenates.

These results suggest that the desacetyl metabolite of cefotaxime is formed in the body, as is the case with cephalothin (2), cephapirin (1), and cephacetrile (7). A very small amount of antimicrobially active metabolite of ceftizoxime was detected in rat bile by thin-layer chromatography-bioautography and high-performance liquid chromatography. No metabolite, however, was found in any human bile samples obtained from a recent clinical study (unpublished data on phase I study). Further evaluations on metabolite formation in rat bile will be reported in another paper.

Although biliary excretion of antibiotics in rats does not always parallel that in humans, cefmetazole, cefotiam, and cefamandole were well excreted in the bile. Conversely, ceftizoxime and cefotaxime were significantly lower in biliary excretion than the other antibiotics. However, the high biliary concentration of ceftizoxime in dogs suggests that similarly high concentrations might be attained in humans. Protein binding of ceftizoxime was clearly lower than that of cefazolin (9) and ceftezole (10), and was similar to that of cefuroxime (3). This low serum protein

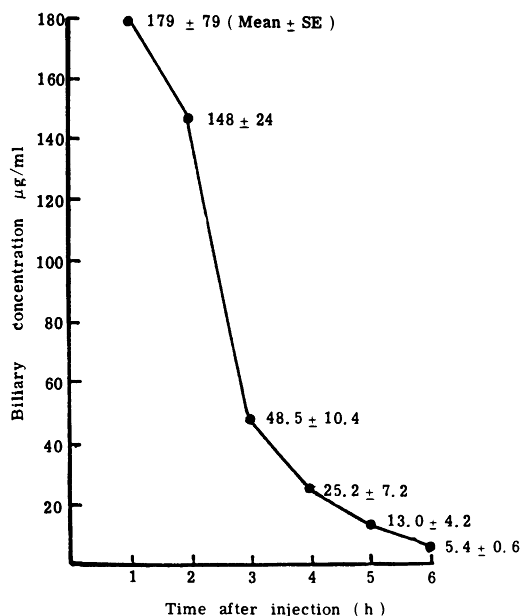

FIG. 6. *Biliary concentration of ceftizoxime in dogs after i.v. injection of 20 mg/kg. Dogs: beagle, male, 11.0 to 11.5 kg, n = 4.*

TABLE 3. *Protein binding of ceftizoxime and related antibiotics*

Antibiotic	Protein binding (%)[a] in:				
	Humans	Dogs	Rabbits	Rats	Mice
Ceftizoxime	31	17	25	32	13
Cefotiam	53	17	57	42	23
Cefamandole	75	28	80	83	23
Cefotaxime	37	31	71	84	27
Cefmetazole	66	26	53	36	28

[a] At 90% serum and 30 μg of antibiotic per ml.

binding of ceftizoxime may favorably affect the distribution of the drug to the tissues and body fluids.

Data on the protective effect of antibiotics in experimental infections in animals are useful for estimating therapeutic efficacy of antibiotics in humans. However, the pharmacokinetic profiles of drugs usually differ among species of animals, as shown in the present study. Because of this, the differences in pharmacokinetic profile of antibiotics among animals and humans must be carefully considered when efficacy in humans is estimated on the basis of results in animal studies. In our previous studies (5), the protective effect in mice was compared with that of other antibiotics including cefotaxime. The protective effect of ceftizoxime was not inferior to that of cefotaxime; however, the serum levels of ceftizoxime were about half those of cefotaxime.

It is of interest that the species of animal that most closely resembles humans in pharmacokinetic profile of ceftizoxime is the monkey (unpublished data on phase I study).

In conclusion, the results of this study suggest that ceftizoxime with its excellent antibacterial activity has high promise for clinical application.

LITERATURE CITED

1. **Cabana, B. E., D. R. Van Harken, and G. H. Hottendor.** 1976. Comparative pharmacokinetics and metabolism of cephapirin in laboratory animals and humans. Antimicrob. Agents Chemother. **10**:307–317.
2. **Cooper, M. J., M. W. Anders, and B. L. Mirken.** 1973. Ion pair extraction and high speed liquid chromatography of cephalothin and desacetylcephalothin in human serum and urine. Am. Soc. Pharmacol. Exp. Therap. **1**: 659–662.
3. **Foord, R. D.** 1976. Cefuroxime: human pharmacokinetics. Antimicrob. Agents Chemother. **9**:741–747.
4. **Gibaldi, M., and D. Perrier.** 1975. Two compartment model, p. 48–86. *In* J. Swarbrick (ed.), Drugs and pharmaceutical sciences, vol. 1: Pharmacokinetics. Marcel Dekker, Inc., New York.
5. **Kamimura, T., Y. Matsumoto, N. Okada, Y. Mine, M. Nishida, S. Goto, and S. Kuwahara.** 1979. Ceftizoxime (FK 749); a new parenteral cephalosporin: in vitro and in vivo antibacterial activities. Antimicrob. Agents Chemother. **16**:540–548.
6. **Marquardt, D. W.** 1963. An algorithm for least-squares

estimation of nonlinear parameters. J. Soc. Indust. Appl. Math. **11**:431-441.

7. **Mashimo, K., K. Fukaya, O. Kunii, and M. Suzuki.** 1976. Studies on cephacetrile. Chemotherapy (Tokyo) **24**:112-117.
8. **Murakawa, T., Y. Wakai, M. Nishida, R. Fujii, M. Konno, K. Okada, S. Goto, and S. Kuwahara.** 1970. Chromatographic assay of mixed penicillins, ampicillin and cloxacillin, in body fluids. J. Antibiot. **23**:250-251.
9. **Nishida, M., T. Matsubara, T. Murakawa, Y. Mine, and Y. Yokota.** 1970. Cefazolin, a new semisynthetic cephalosporin antibiotic. II. In vitro and in vivo antimicrobial activities. J. Antibiot **23**:137-148.
10. **Nishida, M., T. Murakawa, T. Kamimura, N. Okada, H. Sakamoto, S. Fukada, S. Nakamoto, Y. Yokota, and K. Miki.** 1976. In vitro and in vivo evaluation of ceftezole, a new cephalosporin derivative. Antimicrob. Agents Chemother. **10**:1-13.