CEFODIZIME, AN AMINOTHIAZOLYLCEPHALOSPORIN

II. COMPARATIVE STUDIES ON THE PHARMACOKINETIC BEHAVIOR IN LABORATORY ANIMALS

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The pharmacokinetic properties of cefodizime, a new aminothiazolyliminomethoxycephalosporin, were studied in laboratory animals and compared with the pharmacokinetics of another long-acting cephalosporin, ceftriaxone. Both cephalosporin derivatives showed high affinity (33~99%) for serum proteins. High and prolonged blood respectively serum levels of the antibiotics were achieved following subcutaneous and intravenous injection into mice, rats, rabbits, dogs and monkeys. Cefodizime elimination half-lives ranged from 1.17 hours in mice to 3.53 hours in rabbits compared to ceftriaxone half-lives ranging from 0.73 hour in mice to 7.31 hours in rabbits. The antibiotics were well distributed in the body and penetrated into tissues and body fluids to a high degree. Particularly high and prolonged levels were detected in the lungs, liver and kidneys of the experimental animals. Large amounts, approximately 35~55% of the given dose, were recovered in the urine of rabbits and dogs, while the recovery rate in the bile of rabbits was only 0.57% for cefodizime and 1.08% for ceftriaxone.

Despite the development of many new β-lactam antibiotics in the last few years, there has been great interest in novel compounds with improved antibacterial and pharmacokinetic properties. Recent studies in our and other laboratories demonstrated that cefodizime (HR 221) is a parenteral aminothiazolyliminomethoxycephalosporin with a broad spectrum of *in vitro* activity against Gram-positive and Gram-negative bacteria[1~5] and, moreover, very high and prolonged blood levels in laboratory animals. Therefore, we decided to compare the pharmacokinetic profile of cefodizime with that of another long-acting cephalosporin, ceftriaxone[6~8], and to determine the distribution of both these antibiotics in the tissues and body fluids of experimental animals.

Materials and Methods

Antibiotics

Cefodizime (HR 221) was synthesized at Hoechst AG Laboratories, Frankfurt, FRG. Ceftriaxone was a commercial preparation from Hoffmann-La Roche Laboratories, Basel, Switzerland.

Measurement of Protein Binding *In Vitro*

The test compounds were dissolved in phosphate buffer pH 6.88 and dialyzed against serum. Dialysis was performed in an equilibrium device (Dia-Norm, mfr: Bachofer, Reutlingen, FRG). Sartorius-ultrafilters with molecular weight cut-offs of 5,000 were used (mfr: Sartorius, Goettingen, FRG). The dialysis chambers were arranged in pairs, one being filled with 1.0 ml compound solution in phosphate buffer (pH 6.88, 0.1 M), the other with 1.0 ml serum. All concentrations of each compound were simultaneously checked in the same run of 4 hours of the machine at 37°C. The amount of nonbound substance in buffer was assayed microbiologically and the percentage of binding calculated.

Concentrations of Cefodizime and Ceftriaxone in Blood and Body Fluids

The cephalosporins were employed as solutions in sterile water. They were administered parenter-

ally in volumes of 0.1 ~ 1 ml/kg body weight.

Mice: Groups of twelve NMRI albino mice weighing 18 ~ 22 g were injected subcutaneously with 10 mg/kg cefodizime or ceftriaxone. At 10, 20, 30, 45, 60, 90, 120, 150, 180 and 240 minutes after dosing, 10 μl samples of blood were removed from a cut on the tip of the tail by means of capillary tubes (Wiretrol, mfr: Drummond, Broomall, USA). The blood samples were stored at 4°C until further processing.

Rats: Groups of nine Wistar rats, weighing 120 g, were dosed subcutaneously with 10 mg/kg cefodizime or ceftriaxone. Blood samples were taken as described above for mice.

Rabbits: Serum and bile level studies were performed in groups of four pentobarbital sodium-anaesthetised Himalayan rabbits (Nembutal, 40 mg/kg, Abbott GmbH, FRG) in which the common bile duct had been cannulated. The mean body weight of the animals was 2.3 kg. The rabbits were injected intravenously with 20 mg/kg cefodizime or ceftriaxone. Blood and bile samples were removed at 5, 10, 15, 20, 30, 45, 60, 90, 150, 180, 210 and 240 minutes after administration, and the blood was allowed to clot at room temperature. The serum was separated and stored at −20°C until further processing.

Dogs: Groups of six male beagle dogs weighing 18.5 ~ 24.5 kg were dosed intravenously with 4 mg/kg of one of the two drugs. Blood samples were withdrawn from the *Vena cephalica antebrachii* prior to injection and at 10, 20, 30, 45, 60, 90, 120, 150, 180, 240 and 360 minutes after injection. The serum was separated and stored at −20°C until being assayed for serum antibiotic levels. Urine was collected by catheterization of the bladder up to the sixth hour after injection.

Monkeys: Groups of six monkeys (species: *Macaca arctoides*) weighing 3 ~ 8 kg received intravenous injection of 10 mg/kg cefodizime or ceftriaxone. Blood was collected from an arm vein in 10 μl capillary tubes at 10, 20, 30, 45, 60, 90, 120, 150, 180, 240 and 360 minutes after dosing, anticoagulated with 38% sodium citrate solution and stored at 4°C until further processing.

Tissue Levels

Mice: 30 and 60 minutes after sc dosing with 50 mg/kg, groups of 10 NMRI mice were killed by exsanguination and the heart, lungs, kidneys and thigh muscle were removed. The various tissue samples from the individual animals were examined for their antibiotic concentration separately.

Rats: Groups of 6 female Wistar rats weighing 280 g were dosed sc with 20 mg/kg cefodizime or ceftriaxone. 30, 60 and 240 minutes thereafter, samples of heart, lungs, liver, kidneys and femoral muscle were taken and assayed for their antibiotic concentrations.

Rabbits: 4 male rabbits were dosed iv with 20 mg/kg cefodizime or ceftriaxone. Tissue samples were removed at 240 minutes after administration as described above for rats.

Samples of organs and tissues were weighed, homogenized with four times the weight of phosphate buffer (pH 6.0) in an Ultra-Turrax homogenizer (Janke & Kunkel KG, FRG) and centrifuged to separate the supernatant. All biological samples were stored at −20°C until being assayed.

Bioassay and Pharmacokinetic Analysis

Concentrations in blood, bile, urine and tissue samples were determined microbiologically in the agar diffusion test, using as the culture medium Mueller-Hinton broth with the addition of 1.9% agar and 10% sheep's blood. *Streptococcus pyogenes* A77 was used as the indicator organism. The standard solution and diluent were prepared with blood or serum for determining the blood/serum level, with phosphate buffer (pH 6.88, 0.1 M) for determining the urine and biliary levels, and with homogenates of untreated tissues and organs (tissue/buffer ratio 1: 5) for determining the concentrations in organs and tissues. The limits of detection for cefodizime were 0.1 μg/g in tissue homogenates and 1.6 μg/ml in serum, for ceftriaxone 0.1 mg/kg in tissue homogenates and 0.8 μg/ml in serum. The sequence of blood/serum concentrations was analyzed with the help of a computer programmed to calculate the regression curves by the method of least squares. Following subcutaneous injection of cefodizime and ceftriaxone into rodents, blood concentration-time data were fitted to a one-compartment model. The model equation was[9]:

$$C_t = C_0 \cdot [e^{-K_e \cdot (t-t_0)} - e^{-K_a \cdot (t-t_0)}]$$

The distribution of cefodizime and ceftriaxone in rabbits, dogs and monkeys after iv administration

followed an open two-compartment model. The model equation was[9]:

$$C_t = C_0 \cdot e^{-K_a \cdot t} + C_1 \cdot e^{-K_e \cdot t}$$

C_t is the concentration (μg/ml) at time t (hours), C_0 and C_1 are fictive concentrations (μg/ml) at t=0. K_a and K_e are rate constants for absorption and elimination phases. t_0 is the absorption delay (hours).

Further parameters derived from the equations were as follows: C_{max}, maximum blood concentration (μg/ml); t_{max}, time (hours) of C_{max}; $t_{1/2}$, elimination half-life (hours); V_d, the total apparent volume of distribution[1] and AUC, area under the concentration-time curve (μg·hours/ml) calculated by integrating C_t from zero to infinity with respect to time t. Tissue concentrations were calculated by regression analysis with the help of the standard curves in which the logarithms of concentrations were proportional to the areas of the inhibition zones. To differentiate between the extra- and intravascular concentrations of the antibiotics, the haemoglobin content of the individual tissue was determined and used as a parameter for the amount of blood in the tissue samples. The actual compound level (extravascular amount) in the tissues was calculated according to the following formula[10]:

$$C_C = C_T - \frac{HB_T \times C_B}{HB_B}$$

(C_C=compound concentration in the tissue (corrected), C_T=total antibiotic concentration in the tissue sample, C_B=antibiotic concentration in venous blood, HB_B=haemoglobin concentration in whole blood, HB_T=haemoglobin concentration in the tissue sample).

Results

Serum Protein Binding

As shown in Table 1, both compounds exhibited high affinity for serum proteins. In mouse, rat and dog serum, the level of binding of cefodizime (55~89%) was considerably higher than that of ceftriaxone (33~64%). In contrast, the binding of ceftriaxone to monkey and human serum was distinctly higher than that of cefodizime (82 and 89% as compared with 62 and 73%).

Concentrations in Blood and Body Fluids

Mice

Cefodizime and ceftriaxone, both showed extremely high and prolonged levels in murine blood. The mean blood levels of cefodizime and ceftriaxone reached peaks of 33.97±15.70 and 23.86±6.62 μg/ml, respectively within 19 minutes of subcutaneous injection of 10 mg/kg. High concentrations of 2.48±0.62 and 0.98±0.51 μg/ml could still be observed even four hours after dosing. The half-lives of cefodizime and ceftriaxone in mice were 70.2 and 43.8 minutes (Table 2). The areas under the concentration-time curves (AUC) were 42.85±8.54 and 25.98±7.98 μg·hours/ml respectively.

Rats

Fig. 1 demonstrates the outstandingly high blood levels in rats. As in the case of mice, cefodizime gave higher blood concentrations than ceftriaxone. Half an hour after subcutaneous injection of 10 mg/kg, the mean blood concentrations peaked at 21.66±6.57 and 17.39±3.15 μg/ml respectively. Afterwards, the levels fell progressively with elimination half-lives of approximately 3 and 2 hours. After 4 hours, the concentrations were still about 9.6±2.97 μg/ml for

Table 1. Binding of cefodizime and ceftriaxone to serum proteins (dialysis).

Serum protein	Binding ratio (%)	
	Cefodizime	Ceftriaxone
Mouse	76	52
Rat	89	64
Rabbit	99	98
Dog	55	33
Monkey	62	82
Human	73	89

Table 2. Pharmacokinetic parameters of cefodizime and ceftriaxone in experimental animals.

Species	Compound	Group size (No. of animals)	Dose (mg/kg)	A_0 (μg/ml)	V_d (liters)	t_{max} (hours)	C_{max} (μg/ml)	$t_{1/2}\beta$ (hours)	AUC (μg·hours/ml)
Mouse	Cefodizime	12	10 sc			0.32±0.07	33.97±15.70	1.17±0.34	42.85± 8.54
	Ceftriaxone	12	10 sc			0.31±0.10	23.86± 6.62	0.73±0.16	25.98± 7.98
Rat	Cefodizime	9	10 sc			0.45±0.15	21.66± 6.57	2.97±0.60	99.35± 26.46
	Ceftriaxone	9	10 sc			0.44±0.12	17.39± 3.15	1.87±0.85	56.85± 27.74
Rabbit	Cefodizime	4	20 iv	334.42±92.37	0.16±0.03			3.53±1.14	768.55±260.09
	Ceftriaxone	4	20 iv	157.37±27.06	0.31±0.03			7.31±1.35	917.64± 51.84
Dog	Cefodizime	4	4 iv	69.60±51.36	2.15±1.89			0.93±0.21	27.27± 11.17
	Ceftriaxone	4	4 iv	62.63±40.28	1.63±0.69			0.99±0.26	20.93± 4.10
Monkey	Cefodizime	6	10 iv	91.94±56.26	0.44±0.35			1.38±0.37	79.38± 17.38
	Ceftriaxone	6	10 iv	95.20±36.96	0.54±0.33			2.44±1.04	124.93± 34.74

A_0, fictive concentration at time t=0; V_d, apparent volume of distribution; C_{max}, maximum blood concentration; t_{max}, time of C_{max}; $t_{1/2}\beta$, elimination half-life; AUC, area under the concentration-time curve.

Fig. 1. Blood levels of cefodizime (●) and ceftriaxone (▲) in rats after a single subcutaneous dose of 10 mg/kg (mean of 9 animals).

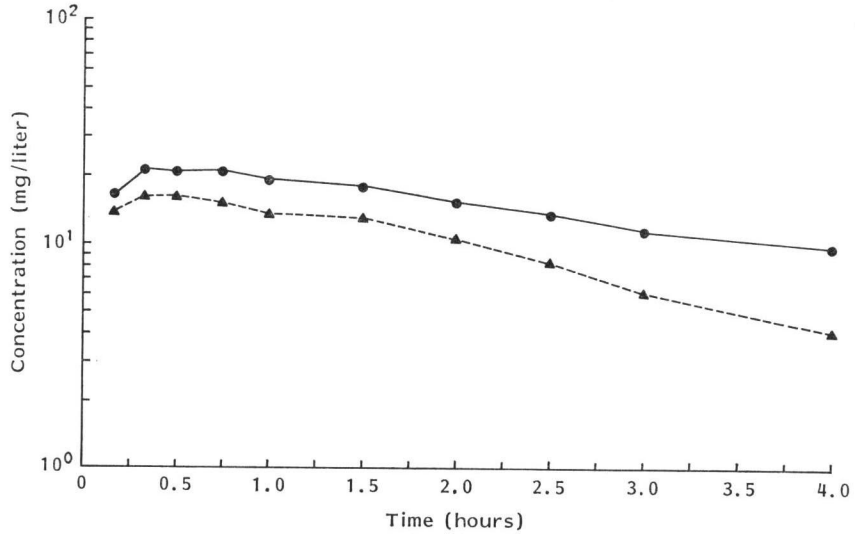
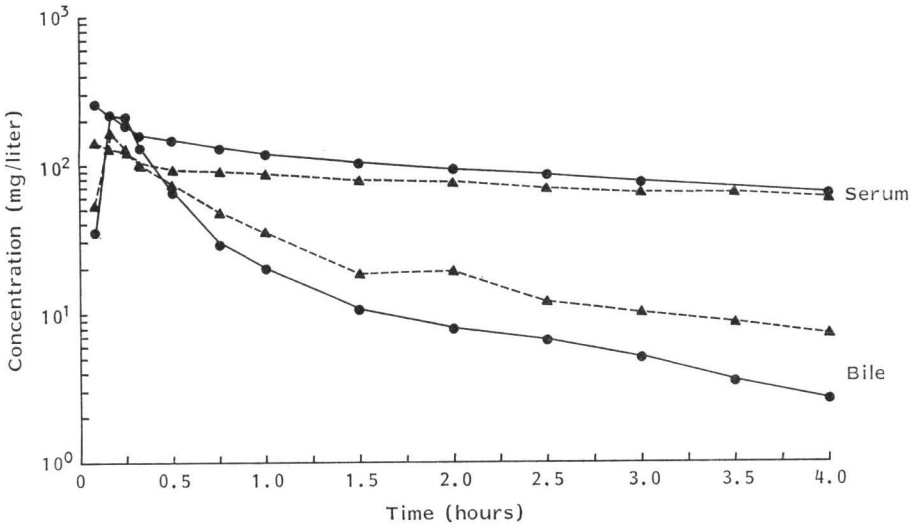


Fig. 2. Serum and bile levels of cefodizime (●) and ceftriaxone (▲) in rabbits after a single dose of 20 mg/kg (mean of 4 animals).



cefodizime and 4.1±2.10 μg/ml for ceftriaxone. The very high and prolonged blood levels could also be seen in the large AUC values calculated; 99.35±26.46 μg·hours/ml for cefodizime and 56.85± 27.74 μg·hours/ml for ceftriaxone (Table 2).

Rabbits

The results of the blood and bile level studies in anaesthetized rabbits receiving a single dose of 20 mg/kg iv are presented in Table 2 and Fig. 2. With cefodizime, a mean serum concentration of 259.56±55.98 μg/ml was obtained five minutes after injection, whereas ceftriaxone showed a significantly lower concentration of 144.03±18.19 μg/ml. The cefodizime concentrations, however, fell with a half-life of 3.53 hours, while the ceftriaxone levels, with a mean elimination half-life of 7.31 hours,

decreased distinctly more slowly. Four hours after dosing, the serum concentrations for the two compounds were almost the same (63.75±15.17 and 59.41±14.56 μg/ml). The longer serum elimination half-life of ceftriaxone is reflected in the larger AUC value (917.64±51.84 μg·hours/ml compared to cefodizime (768.55±260.09 μg·hours/ml).

Bile concentrations of both antibiotics peaked 10~20 minutes after injection. The peak mean concentration was 254.89±125.02 μg/ml for cefodizime and 169.38±28.12 μg/ml for ceftriaxone. Subsequently a rapid fall in bile levels was observed and four hours later, biliary concentrations of only 2.64±1.85 and 7.32±3.01 μg/ml respectively were found. For both cephalosporins the biliary elimination half-life (0.97 and 1.31 hours) was significantly shorter than that in the serum. Only 0.57±0.21% of the given cefodizime dose and 1.08±0.67% of the given ceftriaxone dose were recovered in the bile of rabbits. The urinary recovery, by contrast, was 36.04±16.7 (cefodizime) and 34.95±18.72% (ceftriaxone).

Dogs

After intravenous administration of 4 mg/kg to dogs, the mean serum levels and the pharmacokinetic parameters of the two β-lactam antibiotics were similar (Table 2). In spite of the low dose, serum concentrations of 0.2 μg/ml could still be found six hours after dosing. 46.21±22.86% of the cefodizime dose and 54.30±9.77% of the ceftriaxone dose were excreted *via* the urine within a six-hour period.

Monkeys

As observed in the other laboratory animals mentioned above, high peak levels were obtained and elimination was slow (Table 2 and Fig. 3). However, the pharmacokinetic data obtained for this species do not correspond to the trend seen in rodents, rabbits and dogs. After administration of a 10 mg/kg iv dose, significantly higher blood concentrations, a longer elimination half-life (2.44±1.04 vs. 1.38±0.37 hours) and a larger AUC (124.93±34.74 vs. 79.38±17.38 μg·hours/ml) were observed with ceftriaxone than with cefodizime.

Fig. 3. Blood levels of cefodizime (●) and ceftriaxone (▲) in monkeys (*Macaca arctoides*) after a single dose of 10 mg/kg iv (mean of 6 animals).

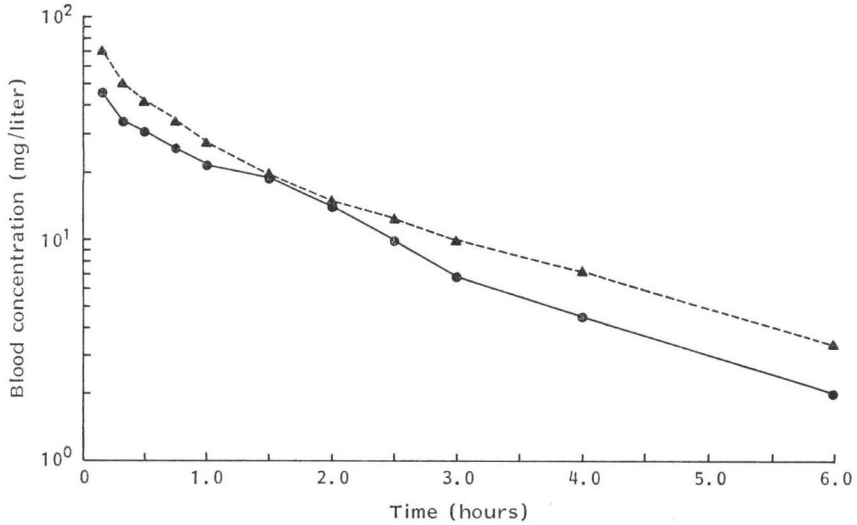
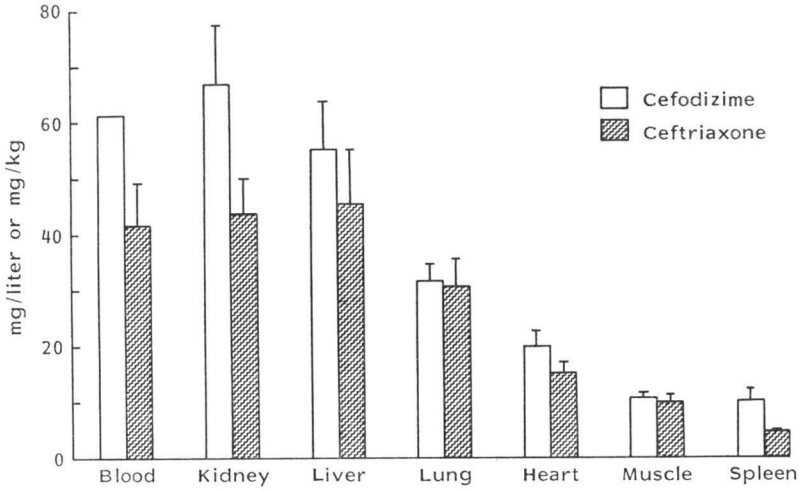


Table 3. Concentration of cefodizime and ceftriaxone in blood/serum and tissue of experimental animals.

Species	Compound	Time (hours)	Dose (mg/kg)	Concentrations in μg/ml or μg/g±SD						
				Serum/blood	Heart	Lung	Liver	Spleen	Thigh muscle	Kidney
Mouse (n=10)	Cefodizime	0.5	50 sc	61.42± 0.00	20.01±2.68	31.69± 3.04	55.17±8.60	10.10±2.23	10.72±1.01	66.77±10.74
		1.0	50 sc	56.16± 7.38	16.38±2.17	26.28± 3.46	23.66±4.38	5.11±0.73	6.87±1.08	61.29± 6.31
	Ceftriaxone	0.5	50 sc	41.59± 7.67	15.15±2.20	30.75± 4.89	45.41±9.75	4.74±0.48	9.91±1.35	43.80± 6.26
		1.0	50 sc	33.69± 6.83	10.24±2.19	15.09± 1.96	21.28±4.53	3.40±1.24	4.84±1.03	41.80± 8.02
Rat (n=6)	Cefodizime	0.5	20 sc	29.75±13.32	8.08±1.25	16.54± 1.86	5.71±2.07	5.88±1.15	3.34±0.94	15.41± 4.99
		1.0	20 sc	45.75± 5.62	11.87±1.47	26.33± 2.51	6.35±0.94	6.65±1.33	6.53±0.34	31.32±12.11
		4.0	20 sc	30.21± 8.00	6.57±2.23	17.73± 7.53	7.01±1.50	9.13±3.01	5.64±0.45	22.58± 4.69
	Ceftriaxone	0.5	20 sc	26.29± 6.48	6.02±0.64	10.92± 1.63	6.78±0.41	3.18±1.55	2.48±0.27	12.20± 1.56
		1.0	20 sc	36.94± 4.56	4.85±0.59	10.91± 2.61	7.34±0.83	3.37±1.04	3.14±0.58	13.76± 2.63
		4.0	20 sc	23.09± 6.17	4.94±1.42	6.98± 2.18	3.08±0.85	2.60±0.55	2.59±0.33	36.59± 7.34
Rabbit (n=4)	Cefodizime	4.0	20 iv	63.75±15.17	15.41±5.74	29.97± 7.65	6.49±1.73	N.D.	3.96±1.33	48.20±33.57
	Ceftriaxone	4.0	20 iv	59.41±14.56	4.84±2.51	21.56±10.40	2.95±1.20	N.D.	2.62±0.63	38.06±27.64

SD, standard deviation; N.D., not determined.

Fig. 4. Blood and tissue levels of cefodizime and ceftriaxone in mice, 0.5 hour after a single subcutaneous injection of 50 mg/kg (mean of 10 animals).



Tissue Levels

Cefodizime and ceftriaxone were well distributed in various tissues of the experimental animals. Tissue levels of both antibiotics at various times after administration are shown in Table 3 and Fig. 4. Comparably high and prolonged levels of the two cephalosporins were detected in the lungs, liver and kidneys of mice after 50 mg/kg sc, rats after 20 mg/kg sc and rabbits after 20 mg/kg iv. In some cases, the average concentrations in these organs exceeded those measured in blood or serum at the corresponding time. Concentrations many times the minimum inhibitory concentrations of both antibiotics for most pathogenic organisms were also found in the heart, thigh muscle and spleen. On subcutaneous or intravenous administration of 20 mg/kg cefodizime or ceftriaxone, average concentrations in the tissues of rats and rabbits, measured at the 4th-hour after injection, still ranged from 2.59 ± 0.33 μg/g in the thigh muscle of rats 48.20 ± 33.57 μg/g in the kidneys of rabbits. At all measuring times and in most cases, cefodizime showed somewhat higher tissue concentrations than ceftriaxone in the organs examined (Table 3).

Discussion

The results presented here demonstrate that cefodizime is a new cephalosporin derivative with an excellent pharmacokinetic profile in laboratory animals. Analogous to the presence of the 3-dihydro-hydroxy-methyl-oxo-as-triazinyl-thiomethyl moiety in ceftriaxone the carboxymethyl-methyl-thiazolyl-thiomethyl substituent in the 3-position on the cephalosporin nucleus in cefodizime resulted in favorable pharmacokinetic properties such as very high and prolonged blood levels, an exceptionally long elimination half-life and large areas under the concentration-time curves.

Our pharmacokinetic data on cefodizime and ceftriaxone support the supposition that the pharmacokinetics of β-lactam antibiotics depend, to a great extent, on the ratio of binding to serum proteins. The higher the level of binding, the higher the levels in the blood of the various animals tested. Thus, the pronounced affinity of cefodizime for serum proteins of rodents and dogs was coupled with a longer half-life and higher concentrations in these species, whereas ceftriaxone showed more favorable pharmacokinetics in monkeys.

As shown by preliminary clinical studies with cefodizime in human volunteers and numerous studies

with ceftriaxone, a correlation between protein binding and pharmacokinetic behavior can also be observed in humans. Ceftriaxone, the compound with the greater binding ratio to human serum proteins, also possesses a distinctly longer half-life in humans (8 hours[11] compared to 2.5~3.0 hours for cefodizime).[12]

Sustained high tissue concentrations after administration of both cephalosporins have been demonstrated in small animals, particularly in the lungs, liver and kidneys. This suggests that cefodizime, like ceftriaxone, shows excellent penetration from the blood stream into various tissues and body fluids. Excretion of cefodizime appears to take place mainly *via* the kidneys. Only a small amount of this antibiotic is recovered in the bile of animals.

These and other encouraging results in experimental studies of antibacterial and toxicological aspects have led to the initiation of clinical trials with cefodizime.

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