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Report

Dose-Dependent Pharmacokinetics of a New Oral Cephalosporin, Cefixime, in the Dog

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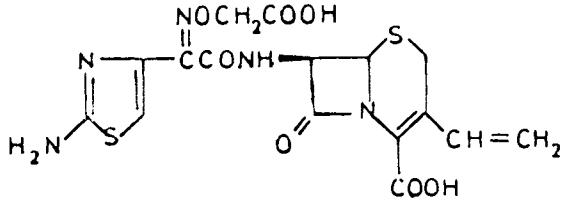
Cefixime (CL 284,635; FK 027) is a new third-generation oral cephalosporin. To study dose-dependent pharmacokinetics of cefixime in dogs, two balanced four-way crossover studies were conducted. In the first study, oral doses of 50, 100, and 200 mg/kg and an intravenous dose of 50 mg/kg cefixime were administered. In the second study, oral doses of 6.25, 12.5, and 25 mg/kg and an intravenous dose of 12.5 mg/kg cefixime were administered to the same dogs. A period of 1 month separated the two studies. When the two intravenous doses were compared (i.e., 12.5 and 50 mg/kg), a twofold increase in clearance and volume of distribution was observed after the higher dose. The oral systemic bioavailability in the dose range 6.25–50 mg/kg was 55%. It decreased to 44% at 100 mg/kg and 27% at 200 mg/kg. The average peak serum concentrations ranged from 15.8 μg/ml at 6.25 mg/kg to 119 μg/ml at 200 mg/kg. Within this concentration range, the fraction of free drug in serum (unbound to proteins) increased from 7 to 25%. This concentration-dependent protein binding was primarily responsible for changes in total clearance, volume of distribution, and bioavailability of the drug in dogs.

KEY WORDS: oral cephalosporin; cefixime; pharmacokinetics.

INTRODUCTION

Cefixime (CL 284,635; FK 027; (6R,7R)-7-[(Z)-2-(2-amino-4-thiazolyl)-2-(carboxymethoxyimino)acetamidol]-8-oxo-3-vinyl-5-thia-1-azabicyclo-(4,2,0)oct-2-ene-2-carboxylic acid; Scheme I) is a novel third-generation orally active cephalosporin undergoing advanced clinical trials for the treatment of urinary and respiratory tract infections. The drug is effective against a broad spectrum of gram-negative and gram-positive bacteria (1–3). Results from studies in dogs indicate that cefixime is eliminated mainly via glomerular filtration and is reabsorbed through the proximal tubules (4–6). Compared to other cephalosporins, cefixime has a longer half-life (3 hr), which is due to its high serum protein binding and tubular reabsorption (4,7). Following a 200-mg dose to humans, 27% of the administered dose of cefixime is excreted unchanged in the urine (8).

In the dog, cefixime exhibits a concentration-dependent protein binding, with a free fraction ranging from 7% at the low serum concentration range (0.5 to 30 μg/ml) to 45% at 300 μg/ml (5,9). The present study was conducted to investigate dose-proportionality and dose-dependent pharmacokinetics of cefixime after intravenous and oral administration to dogs. A wide range of doses was chosen to allow for the determination of the relationship among clearance, volume of distribution, absorption, protein binding, and serum concentrations of the drug.



MATERIALS AND METHODS

The study was divided into two phases. In the first phase, eight beagle dogs (average body weight, 8.29 ± 0.27 kg; range, 7.0–9.7 kg) received oral doses of 50, 100, and 200 mg/kg and an intravenous dose of 50 mg/kg cefixime (Medical Research Division, American Cyanamid Co., Pearl River, N.Y.) according to a four-way crossover balanced Latin square design with a 1-week washout period between each treatment. The second phase was conducted 1 month later in the same eight dogs at three oral doses of 6.25, 12.5, and 25 mg/kg and an intravenous dose of 12.5 mg/kg according to an identical study design. The drug was administered as a microcrystalline powder in a hard gelatin capsule. The iv solution was prepared by adding 0.37 g of sodium bicarbonate (per g of cefixime) to dissolve the drug in normal saline. For all doses, the dose was calculated as free acid. Food was withheld from 16 hr before to 6 hr after dosing. Water was available *ad libitum*. The dogs were

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Table I. Mean[a] Pharmacokinetic Parameters Obtained After Intravenous Administration of 12.5 and 50 mg/kg Cefixime in Eight Dogs

Pharmacokinetic parameter	Dose (mg/kg)	
	12.5	50
$t_{1/2}\beta$ (hr)	6.83 ± 0.69	6.92 ± 0.58
AUC (mg · hr/liter)	671 ± 108	1453 ± 253
CL (ml/min)	2.6 ± 0.53	4.85 ± 1.03*
$V\beta$ (liters/kg)	0.185 ± 0.021	0.354 ± 0.078*
V_{ss} (liters/kg)	0.161 ± 0.06	0.291 ± 0.034*
f_e (%)	44.3 ± 10.87[b]	49.5 ± 8.5
CL_r (ml/min)	1.2 ± 0.5	2.4 ± 0.73
MRT (hr)	8.66 ± 0.03	8.22 ± 1.13

[a] Each value represents the mean ± SD.
[b] Systemic urine collection was not performed in one dog; this value is therefore presented as a mean of seven dogs.
* Statistically significant difference between the two iv doses ($P < 0.01$).

placed in individual metabolic cages during the study. Serial blood samples (5 ml each) were taken by venapuncture (with no anesthesia) from the jugular vein at the following times: 0 (before dosing), 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 30, 36, and 48 hr after intravenous dosing. The same schedule was followed after oral dosing except the 0.083-hr sample was not taken. Blood samples were allowed to stand at room temperature for 1 hr and then serum was separated by centrifugation and stored at −20°C until analysis. Urine during the 0- to 24- and 24- to 48-hr intervals was collected in beakers surrounded by dry ice and a 10-ml aliquot stored at −20°C until analysis. Previous experiments have shown that cefixime is stable at −18°C for up to 6 months (10). The concentrations of cefixime in serum and urine were determined by specific, sensitive, and validated high-performance liquid chromatography (HPLC) assay procedures (10,11) which are modifications of published methods (12).

The elimination rate constant (β) was determined from the linear terminal slope of the logarithm of serum drug concentration (C_s) versus time (t) plot by least-squares regression analysis. The elimination half-life, $t_{1/2}\beta$, was calculated by dividing 0.693 by β. The volume of distribution at steady state (V_{ss}) was calculated by the following equation (13,14):

$$V_{ss} = \frac{\text{dose (AUMC)}}{(\text{AUC})^2}$$

where AUMC is the area under the first moment of the concentration–time curve. The area under the C_s versus t curve (AUC) was calculated by using the trapezoidal rule and extrapolated to infinity by dividing the last experimental serum concentration point by β (13). The peak serum concentration of the drug (C_{max}) and the time to reach C_{max} (t_{max}) were obtained by visual inspection of the data. The renal clearance (CL_r) after each administration was calculated from the ratio of X_u (total unchanged drug excreted in urine) and the corresponding AUC. The total-body clearance (CL) after intravenous administration was calculated by the ratio of the dose and AUC. The volume of distribution ($V\beta$) was calculated by dividing the clearance by β. The oral bioavailability was calculated by comparing oral to intravenous AUC (F) and X_u (F'), with appropriate normalization for the size of each dose. The mean residence time (MRT) was calculated from the quotient of the AUMC and AUC (15).

The difference between the MRT obtained after oral and that obtained after iv administration is equal to the mean absorption time (MAT). The absorption half-life ($t_{1/2}$ absorption) was calculated from the product of 0.693 and MAT (15). Statistical differences between the various parameters were evaluated by using the ANOVA method. The extraction ratio was calculated from the ratio of CL_r and the effective renal plasma flow value from the literature (16).

RESULTS AND DISCUSSION

Tables I and II summarize the mean pharmacokinetic parameters after intravenous and oral doses. Mean serum data are depicted in Figs. 1–3. The half-life values obtained after intravenous and oral administration for all doses were similar, with no statistically significant difference among the values.

As shown in Table I, renal clearance accounted for approximately 50% of the total body clearance. Data from the literature suggest that the mean glomerular filtration rate for a 10-kg dog is approximately 48 ml/min; the range is 22–60 ml/min (16). The mean renal clearance after the intravenous dose of 50 mg/kg was found to be 2.4 ± 0.73 ml/min, and

Table II. Mean Pharmacokinetic Parameters ± SD Obtained After Oral Administration of 6.25, 12.5, 25, 50, 100, and 200 mg/kg of Cefixime in Eight Dogs

Pharmacokinetic parameter	Dose (mg/kg)					
	6.25	12.5	25	50	100	200
$t_{1/2}\beta$ (hr)	6.77 ± 0.72	7.00 ± 0.86	6.98 ± 0.57	7.05 ± 0.94	7.15 ± 0.46	7.07 ± 0.73
AUC (mg · hr/liter)	205 ± 86	364 ± 100	612 ± 198	813 ± 145	1231 ± 213	1488 ± 233
F (%)	60.4 ± 23.3	55.5 ± 16.3	47.3 ± 17.3	55.8 ± 10.5	43.5 ± 8.6	26.5 ± 6.7
C_{max} (μg/ml)	15.8 ± 6.6	27.5 ± 8.3	45.6 ± 17.3	69.2 ± 11.2	91.7 ± 13.1	119.0 ± 12.9
t_{max} (hr)	2.9 ± 0.8	3.8 ± 1.9	2.9 ± 0.6	3.0 ± 0.53	3.1 ± 0.64	3.0 ± 0.76
F' (%)[b]	30.5 ± 15.3[a]	52.9 ± 22.8[a]	27.3 ± 13.4[a]	24.1 ± 5.0	22.0 ± 9.2	18.9 ± 12.9
CL_r (ml/min)	0.92 ± 0.45[a]	1.0 ± 0.40[a]	0.95 ± 0.24[a]	1.23 ± 0.5	1.20 ± 0.46	1.61 ± 0.45
MRT (hr)	10.7 ± 10	12.5 ± 1.8	11.7 ± 1.8	10.8 ± 1.2	12.4 ± 1.6	11.2 ± 0.5
$t_{1/2}$ absorption (hr)[c]	2.3 ± 1.5[c]	2.6 ± 1.7[c]	2.1 ± 1.2[c]	1.9 ± 0.7[d]	2.8 ± 1.5[d]	2.0 ± 0.7[d]

[a] Systemic urine collection could not be performed in one dog; the values presented are means of seven dogs.
[b] Oral bioavailability calculated from urine data (F').
[c] For the calculation of $t_{1/2}$ absorption, the MRT_{iv} obtained after the administration of 12.5 mg/kg iv was used.
[d] For the calculation of $t_{1/2}$ absorption, the MRT_{iv} obtained after the administration of 50 mg/kg iv was used.

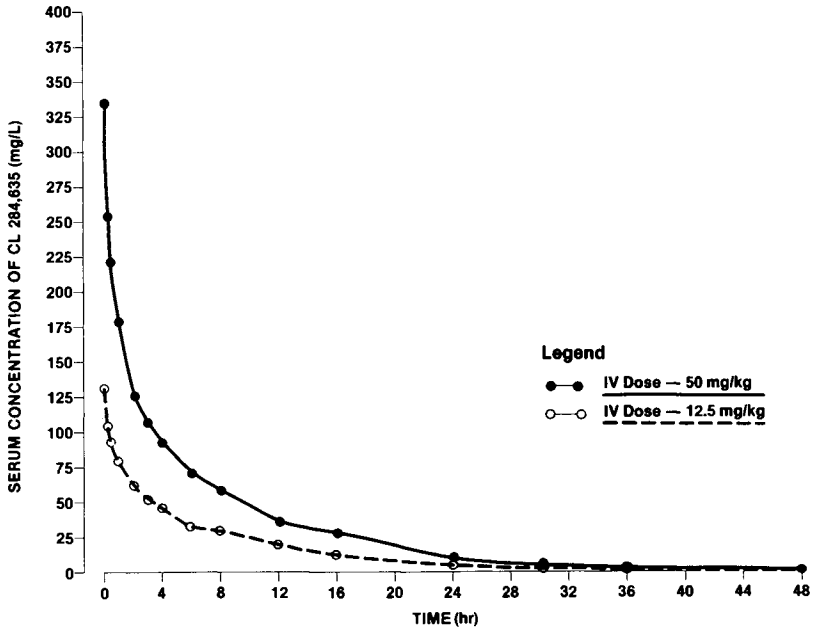

Fig. 1. Mean serum concentrations of cefixime (CL 284,635) after iv administration of 12.5 and 50 mg/kg in eight dogs.

that of the free drug about 24 ml/min. This low renal clearance value strongly suggests that in the dog cefixime undergoes net tubular reabsorption.

The effective renal plasma flow in the dog is about 300 ml/min (16). Cefixime, therefore, appears to have a very low kidney extraction ratio, about 1%. This means that the renal clearance of cefixime may not be affected by renal blood flow but may be limited by the degree of serum protein binding. In fact the protein binding did have an effect on the pharmacokinetics of cefixime. The total body clearance, renal clearance, and volume of distribution values after the intravenous dose of 12.5 mg/kg were only 50% of those obtained after the 50-mg/kg dose.

Within the 6.25- to 50-mg/kg dose range, the bioavailability of cefixime (F, calculated using AUC) was approximately 55% and was not affected by the dose. However, as the dose was increased from 50 to 200 mg/kg, the bioavailability dropped from 55 to 27%. The mean F values were 58.8, 47.3, and 26.5% after the 50-, 100-, and 200-mg/kg doses, respectively. The peak serum concentrations over the entire dose range did not increase proportionally to the dose. The mean t_{max} was 3 hr and was not affected by the

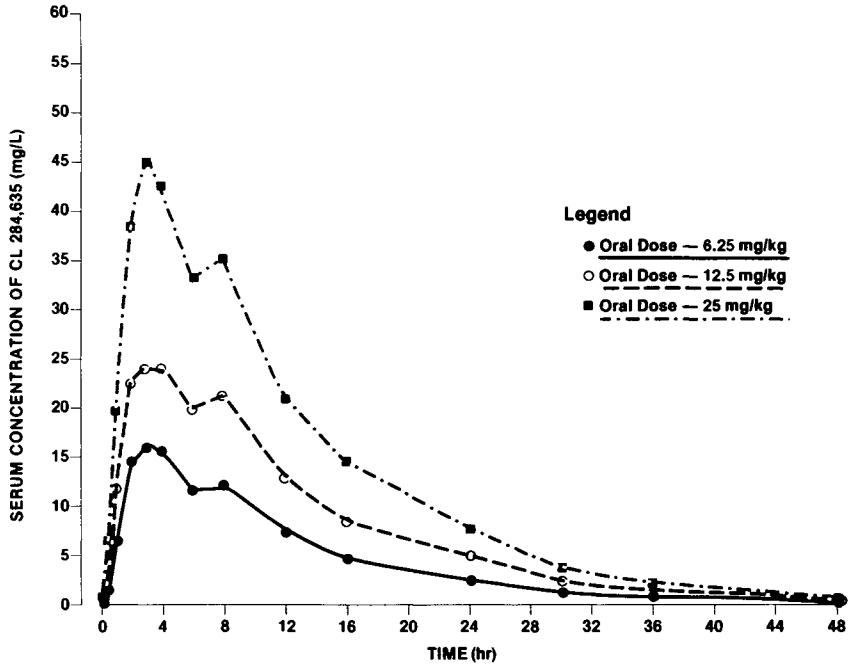

Fig. 2. Mean serum concentrations of cefixime (CL 284,635) after oral administration of 6.25, 12.5, and 25 mg/kg in eight dogs.

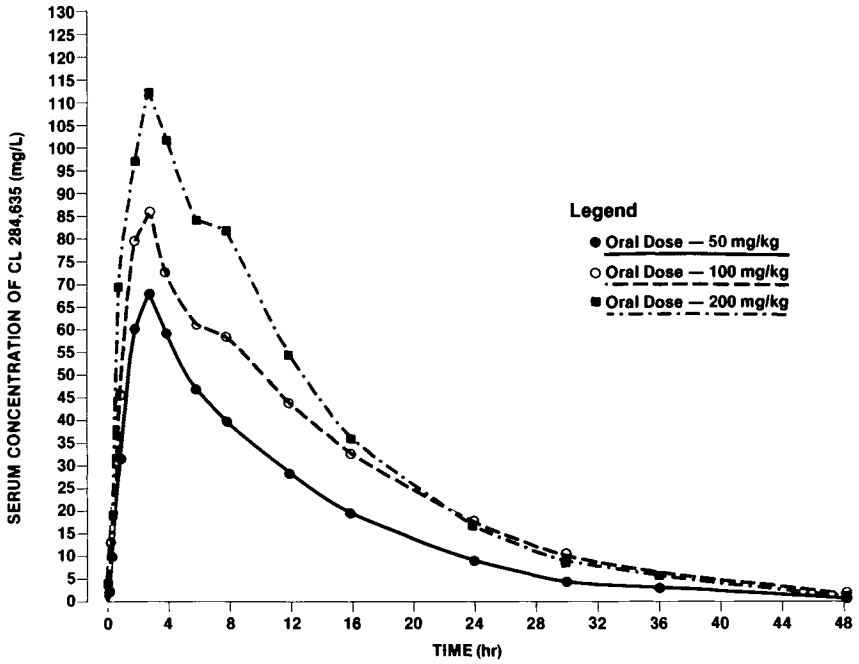

Fig. 3. Mean serum concentrations of cefixime (CL 284,635) after oral administration of 50, 100, and 200 mg/kg in eight dogs.

dose level. A discrepancy was noticed between the bioavailability value calculated using AUC data (F) and that calculated from data of unchanged drug excreted in urine (F'). Except for the oral dose of 12.5 mg/kg, the F' values were significantly lower than the corresponding F values. This discrepancy, as well as the decreases in F with the increase in the oral dose, can be explained by the concentration-dependent protein binding of cefixime. Additionally, F' values were less affected by the oral dose levels at the high dose range of 50 to 200 mg/kg.

The renal clearance values calculated after the oral doses of 6.25, 12.5, 25, 50, and 100 mg/kg were about half the renal clearance value obtained after the intravenous dose of 50 mg/kg and similar to the renal clearance value after the intravenous dose of 12.5 mg/kg. The individual renal clearance values in most of the dogs varied with the dose. These changes in the renal clearance would lead to the changes in the body clearance and thus cause a discrepancy between the bioavailability values of the drug obtained from serum (F) and those obtained from urine (F') data.

A protein binding study of cefixime showed that in dog serum, cefixime has a free fraction (f_u) value of 7.5 ± 0.5% within the serum concentration range of 0.5–30 μg/ml (5,9). A gradual increase in the f_u value occurs at higher serum concentrations (at >50 μg/ml), the value reaching as high as 45% at a concentration of approximately 300 μg/ml. This increase in f_u at higher doses (or concentrations) due to the concentration-dependent protein binding can result in an increased volume of distribution and total-body clearance as shown in this study. Since there was no change in $t_{1/2}\beta$ with dose, the data indicated that the increase in the volume of distribution and clearance was of similar magnitude.

The second peak observed in Fig. 2 is probably due to the existence of enterohepatic circulation of cefixime in the dog, particularly after digestion of a meal. Additionally the food eaten by the animals may have slowed the absorption of a small portion of the drug present in the gastrointestinal tract at that time.

In summary, cefixime was shown to have an absolute bioavailability of approximately 55% in the dog in the 6.25- to 50-mg/kg dose range. A decrease in oral bioavailability was observed when higher doses of 100 and 200 mg/kg were administered. The 6.25-mg/kg dose is a clinical relevant dose, whereas higher doses have been used in the safety evaluation studies. There was a significant increase in drug clearance and volume of distribution of cefixime at the higher intravenous dose due to the decrease in serum protein binding. This phenomenon, however, occurs at serum concentrations far exceeding (>50 times) those that would be observed after therapeutic doses of the drug in humans. Furthermore, cefixime is bound less to human serum proteins (65%) than to dog serum proteins (93%). It is therefore not expected that the decreased bioavailability of cefixime at higher oral doses observed in this study would have any clinical relevance.

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