Comparative Pharmacokinetics of Ceforanide (BL-S786R) and Cefazolin in Laboratory Animals and Humans

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Ceforanide (BL-S786R) is a new, broad-spectrum, parenteral cephalosporin. Pharmacokinetic properties were determined in rats (100 mg/kg), rabbits (30 mg/kg), dogs (25 mg/kg), and humans (2 g or 30 mg/kg) and compared with equivalent single doses of cefazolin. Plasma half-lives for ceforanide and cefazolin were 1.1 and 0.5 h in the rat, 5 and 0.3 h in the rabbit, 1 and 0.8 h in the dog, and 2.6 and 2 h in humans, respectively. The slower elimination of ceforanide, as reflected by longer plasma half-life, larger area under the curve, and peak plasma concentrations, was due to slower body and renal clearances. The apparent volumes of distribution of ceforanide and cefazolin were comparable. Rats, dogs, and humans excreted 80 to 100% of the ceforanide dose in the 0- to 24-h urine; rabbits excreted only 50%. Tubular secretion constituted 50% of ceforanide renal excretion in rabbits, dogs, and humans and 90% in rats; the remainder was excreted by glomerular filtration. There was no apparent correlation between the extent of tubular secretion and degree of plasma protein binding in different species. There was no significant pharmacokinetic interaction between ceforanide and amikacin in the rat. The slower elimination kinetics of ceforanide are indicative of the potential for a longer dosing interval and more effective antibiotic therapy as compared with available cephalosporins.

Ceforanide is a new, parenteral cephalosporin with a broad spectrum of antibiotic activity (7). Pfeffer et al. (M. Pfeffer, D. R. Van Harken, and R. C. Gaver, Int. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 157, 1978) determined the pharmacokinetics of ceforanide in humans at 0.5- to 2.0-g single and multiple intravenous and intramuscular doses. The apparent biological half-life of 3 h was longer than those of other cephalosporins, and there was no evidence of drug accumulation on multiple dosing. Intravenous administration of high doses of ceforanide to humans (4 g twice a day for 9.5 days) produced no obvious signs of nephrotoxicity and no change in a variety of renal function and clearance tests (p-amino-hippuric acid, endogenous creatinine) and renal-plasma pharmacokinetics (R. D. Smyth, M. Pfeffer, D. R. Van Harken, A. Glick, and G. H. Hottendorf, 18th ICAAC, abstr. no. 156). Ceforanide is not metabolized before elimination, and renal excretion is the major elimination route. The usual adult dose is 0.5 to 1.0 g administered twice daily. Since combination cephalosporin-aminoglycoside therapy is employed clinically, the potential for pharmacokinetic interaction of ceforanide and amikacin was also evaluated in rats. This paper reports the renal-plasma pharmacokinetics of ceforanide and cefazolin in laboratory animals (rats, rabbits, and dogs) and humans.

MATERIALS AND METHODS

Cephalosporins. Ceforanide (BL-S786R), the L-lysine salt of 7-(2-amino-methylphenylacetamido)-3-(1-carboxymethyltetrazol-5-yl-thiomethyl)-3-cephem-4-carboxylic acid, was synthesized at and supplied by Bristol Laboratories. Cefazolin, the sodium salt of 3-{[5-methyl-1,3,4-thiadiazol-2-yl)-thio]methyl} -7-[2-(1H-tetrazol-1-yl)-acetamido]-3-cephem-4-carboxylic acid, was used as the commercial product (Kefzol, E. Lilly Co.; Ancef, SmithKline Corp.) The dry powder of each antibiotic was reconstituted with sterile water to yield dosing solutions for parenteral administration.

Animal studies. The animals, doses, and routes of administration were as follows: rats (male, 250 g, Long Evans), 100 mg/kg, subcutaneous; rabbits, (male, 3 to 4 kg, New Zealand white), 30 mg/kg, intravenous bolus; dogs (male, 10 kg, beagle), 25 mg/kg, 30-min intravenous infusion. Heparinized blood samples for plasma preparation were collected at appropriate times in rats and rabbits. Blood samples, containing no anticoagulant, were collected in the dog study and prepared for serum. The rats and rabbits were housed in metabolic cages, and urine was collected in flasks placed in an ice bath. In the dog study, urine collection was accomplished by bladder catheterization.

Pharmacokinetics of ceforanide in the rats in the presence of amikacin. Subcutaneous doses of ceforanide (100 mg/kg) were administered to rats and followed promptly by a second subcutaneous injection of amikacin (25 mg/kg; Amikin supplied by Bristol Laboratories) at a separate site. Heparinized blood and urine samples were collected as before.

Human studies. There were seven subjects each

in the ceforanide and cefazolin groups. All subjects were healthy, adult male volunteers between the ages of 21 and 35 years, weighing 67 ± 10 kg with a mean surface area of 1.84 ± 0.11 m². None of the subjects received any other medication within 1 week before and during the study. Informed consent was obtained from all subjects. Physical examination, hemogram, prothrombin time, activated partial thromboplastin time, bleeding time, serum chemistries (SMA 12-60), and urinalysis were within normal range before admission to the study. Each subject received 2 g (30 mg/kg) of ceforanide or cefazolin by 30-min intravenous infusion. Heparinized blood and urine samples were collected at appropriate times.

Antibiotic assays. Plasma-serum and urine samples were stored at $-70°C$ before assay, usually within 2 weeks. The samples were assayed for ceforanide or cefazolin by standard cup-plate bioassay technique (6). The test organisms used in the bioassays were *Klebsiella pneumoniae* Bristol A9977 (a mutant strain of *K. pneumoniae* A9977) for ceforanide and *Bacillus subtilis* ATCC 6633 for cefazolin. Amikacin, when present in the ceforanide-treated biological samples, was inactivated by cellulose phosphate (11). The assay results were reported as antibiotic-free acid equivalent concentrations. Amikacin concentrations in rat plasma and urine samples were measured by cup-plate assay with the test organism *B. subtilis* ATCC 6633. β-Lactamase was used to inactivate ceforanide, when present (11). The amikacin assay results were reported as antibiotic-free base equivalent concentrations.

Bioautographic analysis for antibiotically active urinary metabolites of ceforanide. Urine samples collected from rats, rabbits, and humans receiving ceforanide were diluted with 0.1 M potassium phosphate buffer (pH 7) and spotted on 1.2- by 57-cm strips of Whatman no. 1 filter paper. The strips were developed in the descending mode using *n*-butanol–glacial acetic acid–water, 60:15:25 (vol/vol/vol), as the solvent. The strips were air dried and overlaid on seed agar inoculated with *B. subtilis* ATCC 6633 and incubated at 28°C. Zones of inhibition were visualized by spraying with 1.2% triphenyl tetrazolium chloride in 1.25% dextrose solution. The R_f value of ceforanide in this system was 0.3.

Plasma protein binding. Ceforanide or cefazolin was added to fresh, pooled, heparinized 90% plasma at concentrations of 25, 50, and 100 $\mu g/ml$ and incubated at 37°C for 15 min with gentle shaking. Replicate solutions were prepared in aqueous pH 7.4 buffer containing 0.05 M NaCl and 0.10 M *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (Calbiochem). These samples were subjected to centrifugal ultrafiltration (10) with DF50A Centriflo membrane cones (Amicon). The drug concentrations in the filtrates were determined by the same cup-plate assay techniques used for plasma and urine analyses. The concentration in the plasma filtrate as a percentage of the total concentration in the unfiltered plasma sample was subtracted from 100% to determine the percentage bound to plasma protein. Ceforanide and cefazolin did not bind to the ultrafiltration membrane. Plasma protein binding was constant over a range of 25 to 100 μg of cephalosporin per ml.

Pharmacokinetic analysis. The comparative pharmacokinetic parameters employed were as follows. C_{max} is the observed peak plasma concentration. T_{max} is the time at which C_{max} occurred. $t_{1/2}$ is the plasma half-life, calculated as $t_{1/2} = (\ln2)/\beta$, where β, the apparent elimination rate constant, is the terminal slope of the least-squares linear regression line through a plot of the natural logarithm of plasma-serum concentration (lnC) versus time (t).

AUC is the area under the C versus t curve from zero to infinite time, calculated by the trapezoidal rule. Cl_p is the body-plasma clearance, calculated as Cl_p = dose/AUC. V_d is the apparent volume of distribution, calculated as $V_d = Cl_p \div \beta$. Cl_r is the renal clearance, calculated as $Cl_r = Fe/$AUC, where Fe is the total amount of drug excreted in the urine.

The value for drug clearance by glomerular filtration (Cl_g) corrected for plasma protein binding was calculated by Cl_g = GFR$(1 - p)$, where GFR is the glomerular filtration rate and p is the fraction of antibiotic bound to plasma protein. The GFR was determined in human studies (116 ± 22 ml/min per 1.73 m²) and estimated (1) in animal studies at 9.4, 4.0, and 3.0 ml/min per kg for rat, dog, and rabbit, respectively. The drug clearance due to tubular secretion (Cl_t) could be found by subtracting Cl_g from total clearance, assuming no significant tubular reabsorption: $Cl_t = Cl_r - Cl_g$. The percentage of contribution of glomerular filtration to drug renal clearance is $(Cl_g/Cl_r) \times 100$ and of tubular secretion is $(Cl_t/Cl_r) \times 100$. The percentages of dose recovered in the urine were calculated from the urinary excretion data. K_a, the absorption rate constant of ceforanide or cefazolin in the rat after subcutaneous doses, was calculated by the Wagner-Nelson method (4).

RESULTS AND DISCUSSION

The plasma concentrations of ceforanide and cefazolin are presented in Fig. 1 (rats), 2 (rabbits), 3 (dogs), and 4 (humans). The calculated pharmacokinetic parameters of ceforanide are compared with those obtained after equivalent single doses of cefazolin (Tables 1 and 2).

Comparative plasma and renal pharmacokinetics of the two cephalosporins indicated that cefazolin was eliminated more rapidly than ceforanide. The plasma half-lives ($t_{1/2}$) of ceforanide were longer than the cefazolin $t_{1/2}$. The $t_{1/2}$'s were 1.1 and 0.5 h in the rat, 5 and 0.3 h in the rabbit, 1 and 0.8 h in the dog, 2.6 and 2 h in humans for ceforanide and cefazolin, respectively. At similar doses, ceforanide had larger AUCs. The cefazolin AUCs were 4% (rabbits), 57% (dogs), 65% (rats), and 79% (humans) of the ceforanide AUCs.

The ceforanide peak plasma concentrations (C_{max}) were generally higher. The C_{max}'s for ceforanide and cefazolin were 369 and 230 $\mu g/ml$ in the rabbit and 46.5 and 31.2 $\mu g/ml$ in the dog, respectively. C_{max}'s were of comparable magnitude in humans (about 250 $\mu g/ml$). In rats, however, cefazolin (235 $\mu g/ml$) had a considerably higher C_{max} than ceforanide (169 $\mu g/ml$). This difference was probably due to slower absorption
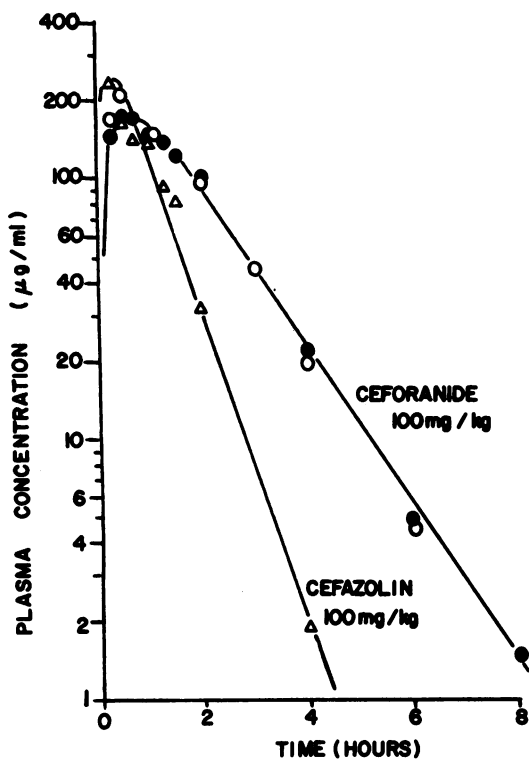
FIG. 1. *Plasma concentrations of cefazolin (△) and ceforanide (●) and of ceforanide administered concomitantly with amikacin (○) in rats after subcutaneous doses. Each data point represents the mean of four plasma samples.*
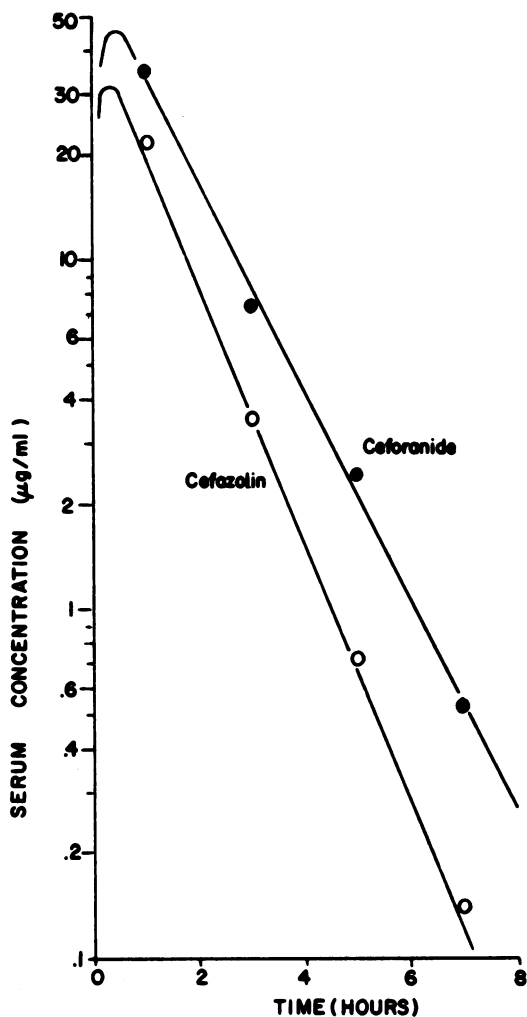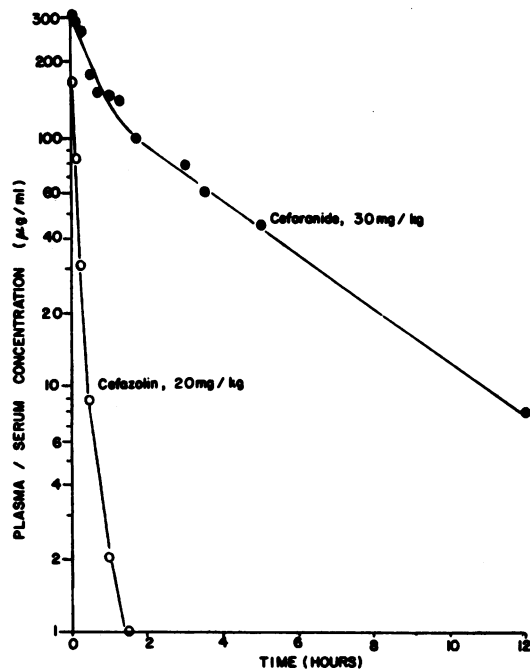

FIG. 3. *Serum concentration-time profiles of ceforanide (●) and cefazolin (○) in dogs after intravenous infusion (25 mg/kg). Each data point represents the mean of three sera.*

of ceforanide after subcutaneous administration, as reflected by absorption rate constants (k_a) of 1.5 h^{-1} for ceforanide and 2.0 h^{-1} for cefazolin.

The slower elimination kinetics of ceforanide, as reflected by longer $t_{1/2}$, larger AUC, and generally higher C_{max}, were attributed to slower plasma (Cl_p) and renal (Cl_r) clearances than cefazolin. The ceforanide Cl_p's were 8% (rabbits), 56% (rats), 63% (dogs), and 78% (human) of cefazolin. The differences in the Cl_r's were of

FIG. 2. *Plasma-serum concentration-time profiles of ceforanide (●) and cefazolin (○) in rabbits after intravenous bolus doses. Each ceforanide data point represents the mean of three plasma samples. The cefazolin serum data are abstracted from reference 5.*
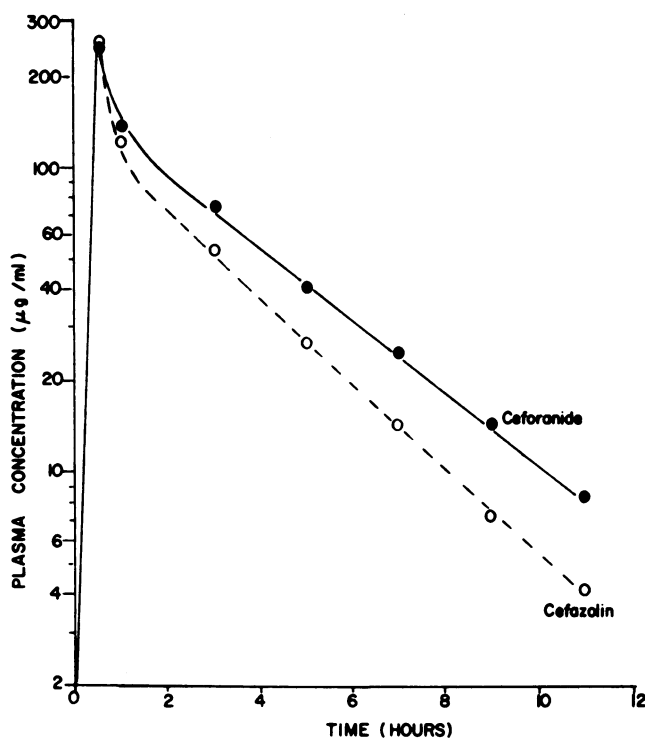
FIG. 4. *Plasma concentration-time profiles of ceforanide (●) and cefazolin (○) in human volunteers after intravenous infusion (2 g or 30 mg/kg). Each ceforanide-cefazolin data point represents the mean of seven plasma samples.*

TABLE 1. *Comparative plasma pharmacokinetics of ceforanide and cefazolin in laboratory animals and humans[a]*

Species	k_a (h^{-1})		C_{max} (µg/ml)		T_{max} (h)		PLasma half-life ($t^{1/2}$, h)		AUC (µg·h/ml)		Cl_p (ml/min per kg)		V_d (liters/kg)	
	CN[b]	CZ[b]	CN	CZ	CN	CZ	CN	CZ	CN	CZ	CN	CZ	CN	CZ
Rat	1.5	2.0	169	235	0.5	0.3	1.1	0.5	416	269	5.4	9.7	0.5	0.4
Rabbit			369	230			5.0	0.3	1,440	59	0.4	5.3	0.2	0.1
Dog			46.5[c]	31.2[c]			1.0	0.8	72	41	6.5	10.4	0.5	0.7
Human			246[c]	256[c]			2.6	2.0	707	559	0.7	0.9	0.2	0.2

[a] Coefficient of variation was ≤10% for all parameters.

[b] CN, Ceforanide; CZ, cefazolin. The various dosages of antibiotics for different species are reported in the text, except for cefazolin given to rabbits at 20 mg/kg, the data for which were calculated from the serum level reported elsewhere (9).

[c] Peak plasma concentration at the end of a 30-min intravenous infusion.

similar magnitude. Although comparative clinical trials are necessary for conclusive assessment of the therapeutic advantages of ceforanide, the generally higher C_{max} and AUC (3) and slower elimination (5) of this cephalosporin could result in a more effective therapy.

Rats, dogs, and humans excreted 80 to 90% of the ceforanide and cefazolin doses as intact drug in the 24-h urine. Rabbits, however, excreted 50% of the ceforanide dose and 90% of the cefazolin dose in the same period. Bioautographic analysis of the urine indicated that, similar to rats and humans, there were no antibiotically active metabolites present in the rabbit. The lower urinary recovery of ceforanide in the rabbit could be due to urinary excretion of antibiotically inactive metabolites, biliary excretion or incomplete urine collection as a result of the long half-life. Urinary excretion appeared to be a major route of elimination of both compounds in all species, with the possible exception of ceforanide in the rabbit.

TABLE 2. *Comparative renal pharmacokinetics of ceforanide and cefazolin in laboratory animals and humans[a]*

Species	Cl_r (ml/min per kg)		% by tubular secretion		% by glomular filtration		Renal excretion (% dose)		Collection time (h)
	CN	CZ	CN	CZ	CN	CZ	CN	CZ	
Rat	4.4	5.6	87	90	13	10	80	80	0–48
Rabbit	0.2	4.7	46	97	54	3	50	90	0–24
Dog	5.0	5.9	56	64	44	36	80	80	0–8
Human	0.7	0.9	46	82	55	18	90	90	0–24

[a] See footnotes to Table 1.

Tubular secretion constituted about 50% of the ceforanide renal excretion in the rabbits, dogs, and humans and about 90% in rats; the remainder was excreted by glomerular filtration. Cefazolin was excreted primarily by tubular secretion (82 to 97%) in all species, except the dog (64%). For both antibiotics, there was no apparent correlation between the extent of tubular secretion and degree of plasma binding and distribution.

The apparent volumes of distribution of ceforanide and cefazolin were comparable. Ceforanide and cefazolin had V_d of 0.5 and 0.4 in rats, 0.5, and 0.7 liters/kg in dogs, and 0.2 liters/kg in humans, respectively. The similarity suggests comparable tissue protein binding and distribution.

In the cephalosporin-aminoglycoside interaction study, the amikacin and ceforanide doses were the same as that used by Barza et al. (2). With the coadministration of amikacin (Fig. 1), the ceforanide half-life (1.0 h), AUC (413 µg·h/ml), 0- to 24-h urinary excretion (60 to 100%), and therefore Cl_p and Cl_r were similar to the data obtained in rats which received ceforanide alone. In addition, the plasma concentration-versus-time profile of amikacin administered in combination with ceforanide was similar to that after amikacin alone, indicating a complete lack of pharmacokinetic interaction. This lack of pharmacokinetic interaction in the rat suggests that concomitant ceforanide-amikacin therapy could be safely evaluated in the clinic (8).

ACKNOWLEDGMENTS

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