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Cephanone: In Vitro Antibacterial Activity and Pharmacology in Normal Human Volunteers

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Cephanone, a new 3-heterocyclic-thiomethyl cephalosporin antibiotic, was found to have an antibacterial spectrum similar to that of cephalothin. The compound was active in vitro against a variety of gram-positive and gram-negative bacteria. All strains of *Staphylococcus aureus* tested were inhibited by concentrations of 6.2 μg or less of cephanone per ml. Beta-hemolytic group A streptococci and pneumococci were exquisitely sensitive. Among strains of *Escherichia coli* and *Klebsiella* sp., 83 and 82%, respectively, were inhibited by 3.1 μg or less of cephanone per ml. Excellent serum concentrations of the antibiotic were obtained after parenteral administration. Peak concentrations of 38 and 81.2 μg/ml were achieved in the serum after intramuscular and intravenous doses of 1 g of cephanone, respectively. The serum concentrations of cephanone fell gradually during the 12 hr after administration. Very high concentrations of cephanone were found in the urine.

Cephanone, 3-(5-methyl-1,3,4 thiadiazol-2-ylthiomethyl)-7-[2-(3-sydnone)acetamido]-3-cephem-4-carboxylic acid sodium salt (Fig. 1), is a new semisynthetic cephalosporin derivative with a broad antibacterial spectrum similar to that of cephalothin (8). Preliminary data indicated that high concentrations are achieved in the serum after intramuscular administration and that these concentrations fall gradually over a 12-hr period (8). Initial toxicity studies in animals have not revealed renal tubular damage as described with cephaloridine (compound 49544, Preliminary Report, Eli Lilly & Co. Research Laboratories, Indianapolis, Ind., 1971).

The purpose of this study was to investigate the in vitro antibacterial spectrum and pharmacological behavior of this new antibiotic.

MATERIALS AND METHODS

Freshly isolated strains of bacteria from clinical specimens were obtained from the diagnostic bacteriology laboratory of The Mount Sinai Hospital. A 0.5-ml portion of a 10^{-3} dilution of overnight growth of the organisms was used as an inoculum. Heart infusion broth (Difco) was used as growth medium for all organisms.

Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of drug were determined with a standard twofold dilution method by using heart infusion broth. The MIC was defined as the lowest concentration of antibiotic in which no growth could be seen after incubation for 18 hr at 37 C. All tubes were incubated at 37 C, except cultures of *Streptococcus pyogenes*, *S. virdans*, and *Diplococcus pneumoniae* which were placed in a carbon dioxide incubator at 37 C. After 18 hr of incubation, the cultures were examined for visible growth. The MBC (except for staphylococci) was defined as the lowest concentration of antibiotic from which wire-loop subcultures onto agar plates showed no growth at 18 hr. Trypticase Soy Agar (TSA; BBL) was used for all cultures, except those of *S. pyogenes*, *S. viridans*, and *D. pneumoniae*, which were streaked on sheep blood-agar plates and incubated at 37 C in a carbon dioxide incubator. The MBC for staphylococci was defined as <10 colonies per streak on the 18-hr agar plates. When subculturing the clear tubes with wire loops onto TSA plates to determine the MBC for staphylococci, a small number of organisms were often found to be present in the subcultures (1). Since the original inoculum was 10^8 organisms/ml, the finding of 10 colonies or less would suggest that 99% of the introduced organisms were killed. We therefore define the MBC for staphylococci as 99% killing of the inoculum (1). *Staphylococcus aureus* was defined as Pen-S (susceptible), Pen-MR (moderately resistant), or Pen-R (resistant) according to the method of Bauer et al. (3).

Drug concentrations were determined in the serum and urine of 10 healthy volunteers (7 males and 3 females) aged 21 to 30 years. The 10 volunteers received 1 g of cephanone diluted in 2 ml of saline intramuscularly. One week later, five of the volunteers (two males, three females) received 500 mg of cephanone diluted in 500 ml of dextrose and water infused over a 30-min period; the remaining five volunteers (males) received 1 g of cephanone in the same manner. Serum samples were obtained prior to injection and at 0.25, 0.5, 1, 2, 4, 6, 8, and 12 hr after beginning the injec-

tion. Urine specimens were collected during the first 8 hr and from 8 to 24 hr after administration of cephanone. All volunteers were fasting and had voided just before the injections. The volunteers had normal hemograms, blood urea nitrogen, serum creatinine, bilirubin, serum glutamic oxalacetic acid transaminase, serum glutamic pyruvic acid transaminase, alkaline phosphatase, albumin, and globulin values before and after the study. The volunteers remained sedentary during the study period. Blood specimens were centrifuged; sera and filtered urine specimens were frozen and stored at −70 C. The cup-plate assay with *Bacillus subtilis* spores was employed to determine cephanone concentrations (4).

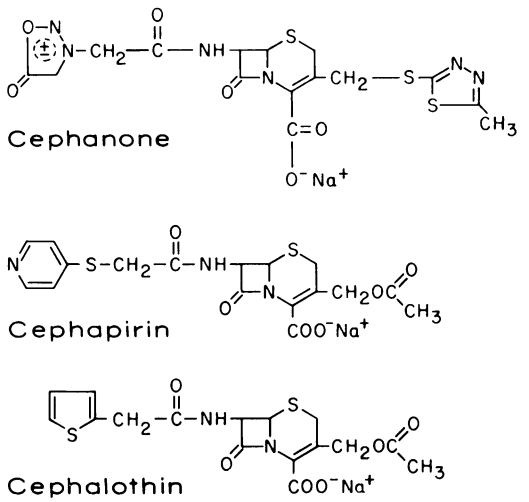

FIG. 1. *Chemical structures of cephanone, cephapirin, and cephalothin.*

RESULTS

Bacterial susceptibility. All strains of *S. aureus* were inhibited by 6.2 μg or less of cephanone per ml; 97% (70 of 72) of the strains were inhibited by 3.1 μg or less per ml (Table 1). Whereas all 19 Pen-S strains of *S. aureus* were inhibited by 3.1 μg or less per ml, 92% (23 of 25) of Pen-R strains were inhibited at this concentration. Wick and Preston (8) found methicillin-resistant *S. aureus* strains also to be resistant to cephanone. Beta-hemolytic streptococci and pneumococci were exquisitely sensitive; all strains were inhibited by concentrations of 0.18 μg or less/ml. Strains of *S. viridans* were inhibited at a level of 0.09 μg/ml. Enterococci were inhibited at much higher concentrations; 57% (8 of 14) were inhibited at 25 μg/ml and the remainder at 50 μg/ml (Table 1).

Gram-negative organisms showed variable susceptibility to cephanone (Table 2). Concentrations of 3.1 μg or less per ml inhibited 83% (19 of 23) of strains of *Escherichia coli*. Strains of *Proteus mirabilis* were only somewhat less susceptible; 72% (21 of 29) were inhibited by 6.2 μg or less per ml. Three indole-positive strains of *Proteus* sp. tested were inhibited by concentrations greater than 25 μg/ml. Concentrations of 3.1 μg or less per ml inhibited 82% (22 of 27) of strains of *Klebsiella* sp. The susceptibility of *Enterobacter aerogenes* strains was the most variable. The MIC ranged from 1.5 to >50 μg/ml, with 9 of 18 strains susceptible to 12.5 μg or less/ml, whereas the remaining 9 strains needed ≥50 μg/

TABLE 1. *In vitro activity of cephanone against gram-positive cocci*[a]

Cephanone (μg/ml)	Pen-S *S. aureus*		Pen-R *S. aureus*		Pen-MR *S. aureus*		*S. viridans*		*S. pyogenes*		*D. pneumoniae*		Enterococci	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<0.022	—	—	—	—	—	—	—	—	—	—	7	1	—	—
0.022	1	—	—	—	—	—	—	—	—	—	—	—	—	—
0.045	—	—	—	—	—	—	—	—	—	—	3	7	—	—
0.09	—	—	—	—	1	—	3	—	12	6	1	3	—	—
0.18	1	—	—	—	6	—	—	—	1	1	1	1	—	—
0.37	12	2	16	1	18	3	—	—	—	2	—	—	—	—
0.75	3	5	4	—	3	—	—	1	—	—	—	—	—	—
1.5	1	2	3	3	—	6	—	—	—	—	—	—	—	—
3.1	1	—	—	6	—	1	—	1	—	1	—	—	—	—
>6.2	—	2	2	4	—	2	—	1	—	—	—	—	—	—
6.2	—	—	—	—	—	—	—	—	—	3	—	—	—	—
12.5	—	—	—	4	—	7	—	—	—	—	—	—	—	—
25	—	8	—	7	—	9	—	—	—	—	—	—	8	2
>25	—	—	—	—	—	—	—	—	—	—	—	—	6	12
Total	19		25		28		3		13		12		14	

[a] Results show the number of strains for which each concentration was the MIC (minimal inhibitory concentration) or the MBC (minimal bactericidal concentration).

TABLE 2. *In vitro activity of cephanone against gram-negative bacilli*[a]

Organism	No. of strains	Cephanone concn (μg/ml)																	
		0.75		1.5		3.1		6.2		>6.2		12.5		25		50		>50	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	23	8	1	8	7	3	2	1	4	—	—	1	4	—	1	2	4	—	—
Klebsiella	27	3	1	13	9	6	8	—	2	—	—	2	2	—	—	—	2	3	3
Enterobacter aerogenes	18	—	—	5	2	1	3	2	1	—	—	1	1	—	1	—	—	9	10
Proteus (indole-negative)	29	—	—	1	—	3	1	17	3	—	—	5	9	2	7	—	—	1	9
Proteus (indole-positive)	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	3	3
Serratia	2	1	—	1	—	—	1	—	—	—	—	—	—	—	—	—	1	—	—
Salmonella	5	—	—	2	—	3	3	—	—	—	2	—	—	—	—	—	—	—	—
Shigella	6	3	1	1	—	—	—	1	1	1	4	—	—	—	—	—	—	—	—
Erwinia	2	1	1	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Herellea	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
Pseudomonas	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10	10

[a] Results show the number of strains for which each concentration was the MIC (minimal inhibitory concentration) or the MBC (minimal bactericidal concentration).

ml for inhibition. *Pseudomonas* strains were all highly resistant to cephanone.

Effect of inoculum size. The effect of inoculum size on MIC and MBC values for five strains of *E. coli* and *S. aureus* are shown in Table 3. With the gram-negative organisms tested, an increase in inoculum size of 10^4 organisms/ml increased the MIC fourfold in one strain and twofold in three strains. A 10^4 organisms/ml increase in inoculum size of *S. aureus* increased the MIC twofold in four of the five strains.

Effect of human serum. A comparison of the MIC and MBC values in 100% human serum and in heart infusion broth for five strains of Pen-R *S. aureus* is shown in Table 4. Serum did not alter the MIC of cephanone for these strains but did increase the MBC. Wick and Preston (8) reported that the serum binding of cephanone was intermediate between that of cephalothin and cephaloridine and that it increased as the concentration of cephanone decreased.

Serum concentrations. The average drug concentration in serum 0.5 hr after the intramuscular administration of 1 g of cephanone was 38 μg/ml (Table 5). Peak concentrations (55.6 μg/ml) were attained at 1 hr and were maintained during the 2nd hr (56.6 μg/ml). The concentrations decreased gradually to 31 μg/ml at 4 hr and 22.1 μg/ml at the 6th hr. At 12 hr, the compound was still present in the serum at an average concentration of 5.3 μg/ml (Table 5). The half-life ($t_{1/2}$) was 174 min, compared to 47 minutes for cephapirin (2).

Higher serum concentrations were attained after intravenous administration of 1 g of cephanone (Fig. 2). At 0.25 hr after injection, the average concentration in serum was 81.2 μg/ml; at 0.5 hr, the average was 67.1 μg/ml; at 1 hr, 63.8 μg/ml; at 2 hr, 38.2 μg/ml; at 4 hr, 22.2 μg/ml; at 6 hr, 13.8 μg/ml; and at 8 hr, 8 μg/ml. No drug was detectable in the 12-hr specimen (Table 5). The $t_{1/2}$ was 126 min, compared to 21 min for cephapirin (2).

A dose of 500 mg was given intravenously to five volunteers. The average peak concentration in serum, attained at 0.25 hr, was 38.5 μg/ml. At 0.5 hr, the average concentration was 31.4 μg/ml; at 1 hr, 26.3 μg/ml; and at 2 hr, 20.3 μg/ml. The serum concentrations declined to 7.1 μg/ml at 6 hr and to 4.6 μg/ml at 8 hr. No drug was found in the 12-hr specimen (Table 5).

Urine concentrations. The total amounts of antibiotic recovered in the urine and the concentrations achieved are given in Table 6. After a 1-g dose of cephanone intravenously, 96% was excreted within the first 8 hr. Only 35% was excreted during this same period after a 500-mg

TABLE 3. *Effect of inoculum size on minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of cephanone for Escherichia coli and Staphylococcus aureus*

Organism and strain no.	Inoculum size					
	10^{-3}		10^{-5}		10^{-7}	
	MIC	MBC	MIC	MBC	MIC	MBC
E. coli						
1	6[a]	>6	1.5	1.5	1.5	1.5
2	6	>6	3	3	3	3
3	1.5	1.5	0.75	0.75	0.75	0.75
4	0.75	0.75	0.75	0.75	0.75	0.75
5	1.5	6	0.75	0.75	0.75	0.75
S. aureus						
1	0.38	0.38	0.19	0.19	0.19	0.19
2	0.38	1.5	0.19	>1.5	0.19	0.19
3	0.38	0.38	0.19	0.38	<0.09	0.19
4	0.38	>1.5	0.19	1.5	0.19	0.19
5	0.38	>1.5	0.38	0.38	0.19	0.19

[a] Values expressed in micrograms of cephanone per milliliter.

TABLE 4. *Comparison of the effect of 100% human serum on minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of cephanone for Staphylococcus aureus*

Strain	Cephanone (μg/ml)			
	MIC		MBC	
	Serum	HIB[a]	Serum	HIB
1	0.38	0.38	3	0.38
2	0.38	0.38	>3	1.5
3	0.38	0.38	>3	0.38
4	0.38	0.38	3	>1.5
5	0.38	0.38	>3	>1.5

[a] Heart infusion broth.

intravenous dose. Whereas the total amount of drug was excreted during 24 hr after a 1-g dose, only 38% was found in the urine after a 500-mg injection. When 1 g was administered intramuscularly, 498 mg (50%) was excreted during the first 8 hr, and 102 mg (10%) was excreted during the 18- to 24-hr period.

Very high concentrations of cephanone were found in the urine. The average concentrations of cephanone found during the 8-hr period after injection of 1 g intramuscularly and intravenously were 1,719 and 5,025 μg/ml, respectively.

TABLE 5. *Concentrations of cephanone in serum after intravenous (iv) and intramuscular (im) administration*

Time after drug administration (hr)	Avg serum concn (μg/ml)		
	500 mg iv	1 g iv	1 g im
0.25	38.5 ± 4.6	81.2 ± 10.8	—
0.5	31.4 ± 3.2	67.1 ± 12.2	38.1 ± 18.9
1	26.3 ± 6.1	63.8 ± 7	55.6 ± 19
2	20.3 ± 3.7	38.2 ± 4.2	56.6 ± 10.2
4	9.7 ± 1.9	22.2 ± 6.2	31.1 ± 4.6
6	7.1 ± 1.3	13.8 ± 2.17	22.1 ± 3.7
8	4.6 ± 0.39	8 ± 2.1	—
12	0	0	5.3 ± 1.6

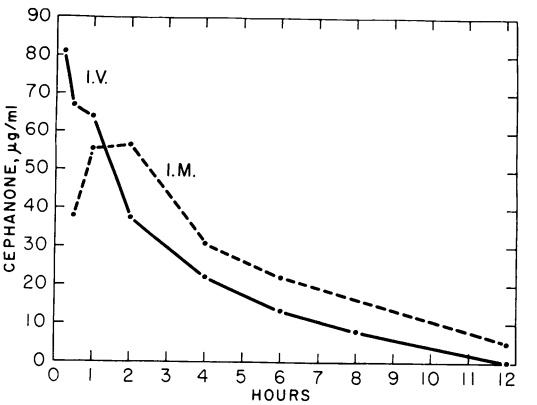

FIG. 2. *Concentrations of cephanone achieved in serum after 1-g doses of cephanone administered intravenously (I.V.) and intramuscularly (I.M.).*

DISCUSSION

Cephanone is a cephalosporin derivative with a broad antibacterial spectrum similar to that of cephalothin (7), cephaloridine (5), and cephapirin (1). Gram-positive cocci, with the exception of the enterococci, were very susceptible to cephanone. Many gram-negative species, including *E. coli*, *Klebsiella*, *Proteus mirabilis*, and *Salmonella*, were susceptible to this compound. Among the strains of *E. aerogenes* tested, 50% were susceptible, although most of these strains were resistant to cephalothin. Indole-positive strains of *Proteus* and *Pseudomonas* strains were not inhibited by cephanone. Cephanone produces very high concentrations in serum when given parenterally. After administration of a 1-g dose intramuscularly, the serum concentration attained at 1 hr (55 μg/ml) was approximately three times the levels obtained with cephapirin (2) and cephalothin (6). These high concentrations were sustained, and at 6 hr a concentration of 22 μg/ml was found compared with very slight

TABLE 6. *Urine recovery of cephanone after intravenous (iv) and intramuscular (im) administration*

Dose and route	Time after drug administration				Total recovery	
	0–8 hr		8–24 hr			
	Avg total amt (mg)	Avg concn (μg/ml)	Avg total amt (mg)	Avg concn (μg/ml)	Amt (mg)	Percentage of dose
500 mg iv	170.7 ± 97.4	294 ± 231.4	18.3 ± 14.6	27 ± 14.7	189	38
1 g iv	958 ± 378	5,025 ± 3,169	91.3 ± 18	212 ± 53.1	1,049.3	100
1 g im	497.5 ± 144	1,718.9 ± 1,140	102.2 ± 106	214.7 ± 131	599.7	60

amounts of cephapirin (2) and no cephalothin (6). Even at 12 hr, the concentration in serum (5.3 μg/ml) exceeded the MIC for 97% of all staphylococci, 100% of beta-hemolytic group A streptococci and pneumococci, 83% of *E. coli* strains, 33% of *E. aerogenes* strains, and 82% of *Klebsiella* strains. After 1-g intravenous doses, the cephanone concentrations in serum were quite high, reaching a peak of 81 μg/ml at 0.25 hr. At 1 hr, the concentration of 64 μg/ml achieved was approximately 10 times that of cephapirin (2). At 8 hr, the concentration of cephanone obtained in serum was higher than the MIC for all gram-positive organisms tested except enterococci. The concentrations in serum achieved after parenteral administration are compared with those reported for cephalothin and cephapirin in Table 7.

The concentrations of cephanone obtained in the urine were quite high after both intramuscular and intravenous injections, and many cephanone-resistant organisms would be inhibited at these concentrations. Thus, the drug may prove useful in the treatment of urinary tract infections. The drug was tolerated well by the volunteers with no untoward side effects. There were no changes in the hematologic, hepatic, and renal function tests measured.

The very high and prolonged concentrations in serum observed after both intravenous and intramuscular administration of cephanone, as compared with cephalothin and cephapirin, would appear to be great advantages in therapeutic use. Cephanone seems to merit further clinical evaluation.

TABLE 7. *Comparison of drug concentrations in serum after parenteral administration of 1 g of cephanone, cephapirin, and cephalothin*

Route of administration	Time after administration (hr)	Concn in serum (μg/ml)		
		Cephanone	Cephapirin	Cephalothin
Intramuscular	0.5	38	24	22.8
	1	55	19	14.7
	2	56	6.3	7.3
	4	31	1.2	0.4
	6	22	0.04	0
	8	—	—	—
	12	5.3	—	—
Intravenous	0.25	81	72.6	—
	0.5	67	23	—
	1	64	6	2.5–40
	2	38	1.7	—
	4	22	0.2	2.5–10
	6	14	0	0.6–1.25
	8	8	—	—
	12	0	—	—

[a] Data for cephapirin are from Axelrod et al. (2); those for cephalothin are from Klein et al. (6).

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