IN VITRO STUDIES OF CEPHANONE, A 3-HETEROCYCLIC-THIOMETHYL CEPHALOSPORIN DERIVATIVE

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The antibacterial spectrum of cephanone, a 3-heterocyclic thiomethyl cephalosporin derivative, is similar to that of cephalothin and cephalexin. Cephanone is less active against gram-positive organisms but is more active against *Escherichia coli* than cephalothin. Activity against *Klebsiella*, *Proteus mirabilis* and *Salmonella* is similar to cephalothin. Some *Enterobacter* strains are susceptible to 8 μg/ml or less. Cephanone is relatively resistant to hydrolysis by gram-negative beta-lactamases. It is not a competitive inhibitor of the hydrolysis of penicillins by beta-lactamases.

Cephanone 3-(5-methyl-1, 3, 4-thiadiazol-2-ylthiomethyl)-7-[2-(3-sydnone) acetamido]-3-cephem-4-carboxylic acid is a new cephalosporin derivative which differs significantly in structure from the cephalosporins available for clinical use[4]. Resistance of gram-negative organisms to beta-lactam antibiotics has been overcome with modifications of structure of penicillins but has not been very useful with cephalosporins. The presence of the thiomethyl side group suggested that this agent might be effective against certain gram-negative organisms resistant to cephalothin and cephalexin. In the case of cefazolin, which has been studied extensively in Japan, no marked *in vitro* improvement over cephalothin has been noted. We investigated the *in vitro* activity of cephanone with a particular interest to its stability against gram-negative beta-lactamases.

Materials and Methods

Cephanone was provided by Eli Lilly and Co. as a crystalline sodium salt which was prepared fresh daily in sodium phosphate buffer or medium. Cephalothin, cephaloridine and cephalexin were provided by Eli Lilly. Bacterial strains were clinical isolates from patients hospitalized at the Columbia-Presbyterian Medical Center, N.Y.C.

Susceptibility tests. The activity of cephanone was measured by a microtiter broth-dilution method. Serial two-fold dilutions were performed in brain-heart infusion broth (Difco) with an inoculum of 10^4 colony forming organisms (CFU) from an overnight culture. The first clear well was taken to be the minimum inhibitory concentration (MIC) well. Minimum bactericidal concentration (MBC) was determined by plating clear wells. Incubation was at 35°C.

Agar plate dilutions were performed with a 100-fold dilution of an overnight culture using a replica plating device. MUELLER-HINTON agar into which the antibiotics were incorporated was used.

Results

Ten strains each of *Streptococcus pyogenes*, *Diplococcus pneumoniae* and *Strep.*

viridans group were tested. All were susceptible to less than 0.3 μg/ml of cephanone. All strains were 2 to 4-fold less sensitive to cephanone than to cephalothin.

At 1 μg/ml 82% of *Staphylococcus aureus* strains were inhibited. The majority of enterococci were relatively resistant with the cephanone MIC 8~16 μg/ml. The few strains with a low MIC were *Strep. bovis* isolates (Table 1).

There is a wide spread of susceptibility of gram-negative organisms. Concentrations of 8 μg/ml inhibited 80% of *E. coli*, 83% of *Klebsiella*, 73% of *Salmonella*, 100% of *Shigella*, and 57% of *Proteus mirabilis*. The *Enterobacter* tested fell into two distinct groups. A small number were sensitive, 41%, and these were usually *E. aerogenes*, whereas, the rest were resistant often to more than 1,000 μg/ml. These were *E. cloacae*, *E. hafnia* and *E. liquifaciens*. Indole-positive *Proteus* species, *P. morganii*, *P. rettgeri* and *P. vulgaris*, were all highly resistant as were *Providencia*.

The majority of *Serratia*, 77%, were resistant to 64 μg/ml. Those strains which were susceptible were resistant to cephalexin, cephaloridine and cephalothin, but sensitive to carbenicillin. *Citrobacter* were moderately resistant showing a pattern of resistance similar to the resistance to cephalothin. *Pseudomonas* were uniformly

Table 1. *In vitro* activity of cephanone against gram-positive cocci

Cephanone (μg/ml)	*Staph. aureus*	*Staph. epid.*	*Enterococci*	*Strep. pyogenes*	*D. pneumoniae*	*Strep. viridans*
<0.12				1	2	1
0.12	3			7	6	2
0.25	8	1		2	2	4
0.5	3					
1.0	9	2				
2.0	5		2			
4.0		2	1			
8.0		2	12			
16			5			
32			2			
Total	28	7	22	10	10	7

* Determined by agar plate dilution

Table 2. *In vitro* activity of cephanone against gram-negative bacilli

Organism	No. of strains	Cephanone minimum inhibitory conc. (μg/ml)*											
		0.5	1	2	4	8	16	32	64	125	250	500	1,000	>1,000
E. coli	40	2	10	6	10	4	4		1	3				
Klebsiella	30	9	9	4	2	1		1	2	2				
Enterobacter	22		4	1	3	1					1	1	2	9
Proteus mirabilis	28		1	1	4	10	6	3	1	2				
Salmonella	11	4	2	1		1	1	2						
Shigella	10	2	3	4	1									
Citrobacter	10						2	3	2	3				
Serratia	22					1	1	1	2	2	5	6		4
Providence	11									1		3		7
Pseudomonas	10													10
Herellea	3					1					2			
P. morganii	14				1	1		1		1	6	1	1	2
P. vulgaris	14									4	4	2	2	2
P. rettgeri	7						1		1	2	1	1	1	

* Determined by micro-titer broth dilution

resistant to cephanone (Table 2).

Comparison of the activity of cephanone, cephalothin and cephalexin against *E. coli* and *Klebsiella* is shown in Table 3. Cephanone is more active than cephalothin or cephalexin against *E. coli*. The activity of cephanone and cephalothin is identical against *Klebsiella*. A few *E. aerogenes* strains were more susceptible to cephanone than to cephalothin and cephalexin, but there was no difference in susceptibility with *E. cloacae* strains. The inhibitory concentrations of cephanone, cephalexin and cephalothin were quite similar for *P. mirabilis* and indole-positive *Proteus* strains.

The effect of varying the inoculum size of *E. coli*, *Klebsiella* and *Staph. aureus* was tested. Only a two-fold increase in inhibitory concentration resulted when the inoculum was increased by 10^4 CFU. *Enterobacter* species, however, showed a marked inoculum effect. A strain that had a cephanone MIC of 32 μg/ml with 10^3 CFU had an MIC of >1,000 μg/ml at 10^7 CFU.

A number of media which differ in cation content were prepared at pH 7.4. The

Table 3. Comparison of *in vitro* activity of cephanone, cephalothin and cephalexin

Organism	Antibiotic	Minimum inhibitory concentration (μg/ml)*						
		0.8	1.6	3.2	6.25	12.5	25	50
E. coli	Cephanone	1	10	12		1		
	Cephalothin		1	3		10	9	9
	Cephalexin			1	7	12	4	4
Klebsiella	Cephanone	2	5	16	1	1		
	Cephalothin	3	5	16			1	
	Cephalexin			3	9	12	1	

* Determined by agar plate dilution

Table 4. Minimum inhibitory concentration of cephanone with different growth media

Organism	Minimal inhibitory concentration (μg/ml)*				
	Brain heart infusion	Columbia base broth	Nutrient broth	MUELLER-HINTON broth	Penassay broth, No. 3
E. coli 732	0.4	0.4	0.8	0.4	0.4
Klebsiella 1028	1.6	1.6	3.2	3.2	3.2
E. cloacae 1065	25	25	25	25	25
P. mirabilis 1069	6.4	3.2	0.8	3.2	3.2
Staph. aureus 932	0.4	0.4	0.2	0.2	0.2

* Determined by micro-titer broth dilution

Table 5. Hydrolysis of cephalosporin antibiotics by beta-lactamases

Beta-lactamase	Substrate hydrolyzed (μM/min.)*				
	Cephanone	Cephalothin	Cephaloridine	Cephalexin	Cephaloglycin
E. coli	1.7	1.8	7.0	0	1.9
Ps. aeruginosa	2.8	4.1	18.4	0.3	3.5
P. morganii	4.3	33.2	7.7	5.6	0.2
S. marcescens	5.1	15.5	7.0	5.0	3.7
S. typhimurium	1.8	2.3	12.0	0.2	—
B. cereus	1.5	0.67	12.3	0	1.7

* Hydrolysis of cephalosporins was determined by use of a spectrophotometric assay (1) utilizing enzymes purified by published procedures (2). The initial concentration of each substrate was 100 μM. Assays were run at 30°C in 0.05 M potassium phosphate, pH 7.0. Activity is expressed as μmoles hydrolyzed per minute.

cephanone MIC for a strain of *E. coli*, *Klebsiella*, *P. mirabilis*, *Staph. aureus* and *Enterobacter* was tested. Table 4 shows minor differences of one tube in different medium, but there was no consistent pattern. Addition of human serum (50 %) did not alter the MIC. In all cases, the MBC was identical with the MIC or only a single tube greater.

The effect of various β-lactamases upon cephanone was studied (Table 5). Cephanone was considerably more resistant to hydrolysis than was cephaloridine. But it was hydrolyzed by *E. coli* (Richmond, Type III)[3] enzymes at a rate comparable to the hydrolysis of cephalothin. Cephanone was hydrolyzed at a much slower rate than cephalothin by *P. morganii* and *Serratia* beta-lactamases. Cephaloglycin was the cephalosporin most resistant to hydrolysis, but cephanone show comparable resistance to a *Serratia* beta-lactamase.

Resistance to beta-lactamase activity does not seem to be related to the actual inhibitory concentration of the cephalosporin. For in the case of selected *Serratia* and *Enterobacter* strains the cephanone inhibitory concentration (16 μg/ml) was many fold below the MIC of cephaloglycin and cephalexin (500 μg/ml). Cephanone was a poor inducer of beta-lactamase activity in *Enterobacter* strains in comparison to cephalothin or cephaloridine. This may explain the activity against some of these organisms.

Cephanone does not act as a competitive inhibitor of the hydrolysis of penicillin or cephaloridine by *E. coli*, *P. morganii* or *E. aerogenes* beta-lactamases. It differs in this regard from cephalothin, cephalexin and cephaloglycin which are good competitive inhibitors of the hydrolysis of benzyl penicillin.

Discussion

Although cephanone is slightly more active against *E. coli* than are cephalothin and cephalexin, its activity against most other gram-negative organisms is similar to that of cephalothin. It is less active than cephalothin against gram-positive species.

The resistance of cephanone to hydrolysis by beta-lactamases is similar to that shown by cephalexin and cephaloglycin. But it is not possible to precisely correlate cephanone activity against gram-negative bacteria with its beta-lactamase resistance. Cephanone also has a poor affinity for beta-lactamases and does not act as an inhibitor of the hydrolysis of penicillin by either R-factor mediated or chromosomal beta-lactamases.

This compound although interesting fails to significantly broaden the current cephalosporin antibacterial spectrum.[5]

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