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Cephamycins, a New Family of β-Lactam Antibiotics. III. In Vitro Studies[1]

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Cephamycins A, B, and C are naturally produced cephalosporin-type antibiotics. Although A and B were found to be more active than C against gram-positive organisms, they were not so active against such strains as are cephalosporin C or the semisynthetic antibiotics cephaloridine and cephalothin. Against gram-negative organisms, cephamycin C was more active than A or B and, in general, was as active as the cephalosporins. In addition, cephamycin C was active in vitro against clinically isolated strains resistant to the cephalosporins, such as *Proteus*, *Providencia*, and *Escherichia coli*. The in vitro antibacterial activity of cephamycin C, cephalothin, and cephaloridine is primarily bactericidal. A 10,000-fold increase in inoculum of a strain of *Proteus mirabilis* resulted in 200-fold or greater increases in minimal inhibitory and minimal bactericidal end points of cephalothin and cephaloridine, but only 10- and 16-fold increases, respectively, for cephamycin C. After 15 passages through antibiotic-containing broths, during which time a culture of *E. coli* showed an increase in minimal inhibitory concentrations of streptomycin of >1,000-fold, end points for cephamycin C increased 4-fold, for cephalothin, 1.5- to 6-fold, and for cephaloridine, 128-fold.

Cephamycins A, B, and C are naturally produced cephalosporin-type agents whose isolation and biological characterization have been described by Stapley et al. (2). All three cephamycins have been shown to be resistant to cephalosporinase by Daoust et al. (*submitted for publication*). Studies of their chemical structure will be reported by Albers-Schonberg et al. (*manuscript in preparation*).

This paper describes in vitro tests that were used to examine some of the characteristics of the antibacterial activities of these agents. In vivo studies are described by Miller et al. (1).

MATERIALS AND METHODS

Cephamycin preparations of approximately 70% purity were studied. Commercial samples of penicillin (The Upjohn Co.), streptomycin (Merck & Co., Inc.), cephalothin (Keflin, Eli Lilly & Co.), and cephaloridine (Loridine, Eli Lilly & Co.), and a laboratory sample of cephalosporin C were used as control materials. The bacterial cultures used are maintained in the Merck Sharp & Dohme stock culture collection and their designated numbers have no significance outside this company.

[1] Presented in part at the 11th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlantic City, N.J., 19-22 October 1971.

Agar MIC. The compounds were incorporated in twofold dilutions either in nutrient agar (BBL) plus sterile horse serum (Bioquest) to a final concentration of 10% or in brain-heart infusion agar (Difco) plus 10% horse serum. The test organisms were grown overnight in nutrient broth (Difco) or brain-heart infusion (BBL), which for some organisms was supplemented with horse serum to 10%. The plates were seeded with approximately 10^4 or 10^5 organisms by means of a multiple inoculator (3). After 24 hr of incubation at 37 C the minimal inhibitory concentration (MIC) was read as the smallest concentration permitting no growth. In addition to the cephamycins, cephalothin, and cephaloridine, cephalosporin C was included in this test.

Speed of action of cephamycin C and the effect of whole blood on such activity. Test organisms were grown in brain-heart infusion broth for 2.5 hr (*Escherichia coli* 2017 and *Salmonella schottmuelleri* 3010) or 6 hr (*Proteus vulgaris* 1810) and were then diluted with brain-heart infusion broth or freshly drawn defibrinated rabbit blood to contain about 10^6 cells/ml. A 9-ml amount of such seeded fluid was added to 1 ml of antibiotic solution or water. The bacterial population in such mixtures was determined by plate counts made at various time intervals during the incubation at 37 C. Each test was run twice, and the results were averaged.

Activity of cephamycin C against cephalosporin-resistant cultures. MIC values in broth were de-

termined by use of 0.5-ml doubling drug dilutions seeded with 2.0 ml of brain heart infusion (BBL) containing approximately 10^4 organisms. Tubes were incubated at 37 C, and growth was recorded after 24 hr and again after 48 hr. All negative tubes were subcultured (0.2 ml into 9 ml of brain-heart infusion), and growth in such tubes was recorded after 72 hr at 37 C to determine the minimal bactericidal concentration (MBC). Included in this study were cephamycin C, cephalothin, cephaloridine, and streptomycin. The clinically isolated test organisms included five strains of *Proteus* and one of *E. coli*, chosen because they were resistant to cephalosporins.

Inoculum effect. The broth MIC test described above was set up so that replicate drug dilution series could be seeded with broths containing 10^3, 10^5, or 10^7 cells/ml. A clinically isolated strain of *P. mirabilis* was used against cephamycin C, cephalothin, and cephaloridine.

Serial transfer of E. coli through antibiotic-containing broth. The standard MIC test with seeded broth containing 10^7 cells/ml was used to determine the susceptibility of *E. coli* 2017 to cephamycin C, cephalothin, cephaloridine, and streptomycin. After the initial exposure, seeds of 10^6 to 10^7 cells/ml were prepared from that tube in the series which contained the highest concentration of antibiotic permitting maximal or almost maximal growth. MIC values were recorded at both 24 and 48 hr. Transfers into new drug series were made at 24 hr; subcultures for MBC values were made after the 48-hr reading. At the conclusion of the test, each exposed strain was tested against all four antibiotics in the standard MIC test, and the end points were compared with those of the parent strain recorded at the beginning of the test.

RESULTS

Agar dilution MIC. Table 1 lists the MIC values of cephamycins A, B, and C, cephalothin, cephaloridine, and cephalosporin C for 43 organisms representing 20 species of 15 genera of bacteria. It can be seen that, although cephamycins A and B were perhaps more active in vitro against gram-positive organisms than was cephamycin C, none of these was so active as cephalothin, cephaloridine, or cephalosporin C. Against the gram-negative organisms, none of the cephamycins or cephalosporins was active at these concentrations against the test strains of *Pseudomonas*, *Serratia*, and some *Aerobacter*. Against other gram-negative organisms, cephamycin C in general was not only more active than cephamycin A or B, but it was essentially as active as cephalothin. It is of interest that against many of these organisms cephamycin C was more active than the "natural" product cephalosporin C. Moreover, cephamycin C also was active against *P. morganii* strains that are resistant to the cephalosporins. Because cephamycin B activity was relatively low, this compound was not included in the remaining in vitro tests.

Speed of action and effect of blood. The antibacterial activity of cephamycin C was as effective in whole blood as it was in brain heart infusion broth. Figures 1 and 2 show that 100 μg of cephamycin C/ml of broth resulted in a decrease of 3 logs (99.9%) in the countable *E. coli* 2017 or *S. schottmuelleri* 3010 cells in less than 2 hr. A similar decrease was seen when the antibiotic and cells were suspended in rabbit blood. Figure 3 shows that against *P. vulgaris* 1810 25 μg of antibiotic/ml of broth produced a 3 log decrease in count in 2 hr; the antibiotic action in blood was a bit slower. Higher concentrations of cephamycin C were not tested.

Organisms isolated from plates used for counting the antibiotic-containing cultures, as well as from control cultures, were tested by the agar-disc method for their in vitro susceptibility to cephamycin C. Zones of inhibition for the antibiotic-exposed cultures indicated no loss of susceptibility to the antibiotic.

Activity of cephamycin C against cephalosporin-resistant cultures. The MIC and MBC end points of a clinically isolated cephalosporin-resistant

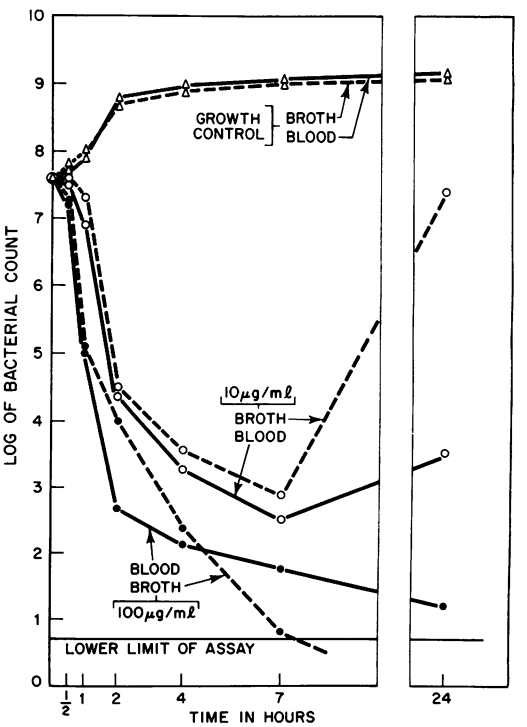

FIG. 1. *Effect of cephamycin C on a growing culture of Escherichia coli 2017.*

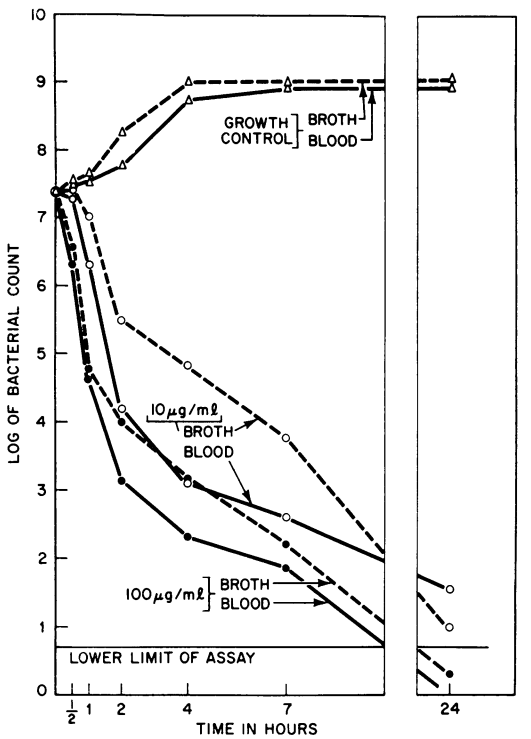

FIG. 2. *Effect of cephamycin C on a growing culture of Salmonella schottmuelleri 3010.*

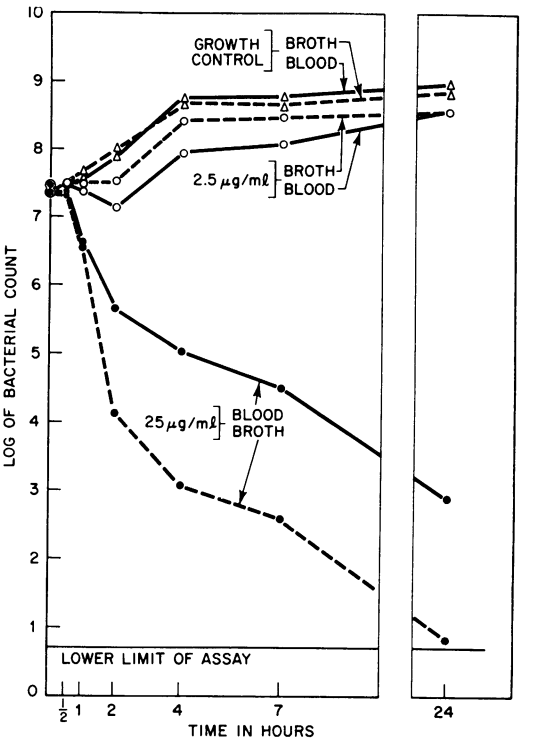

FIG. 3. *Effect of cephamycin C on a growing culture of Proteus vulgaris 1810.*

strain of *E. coli* and five such strains of *Proteus* are shown in Table 2. Included for comparison are end points of a clinically isolated cephalosporin-susceptible *Proteus* strain. Streptomycin was included in this test as a noncephalosporin type of bactericidal antibiotic.

The data show that these resistant *P. mirabilis* and *Proteus* sp. were less susceptible to cephaloridine than to cephalothin. Four of the five resistant *Proteus* cultures were susceptible to both cephamycin C and streptomycin. The fifth culture, *P. morganii* 3376, was resistant to streptomycin and was inhibited but not killed by high concentrations of cephamycin C. With this latter exception, a comparison of the MBC and MIC values for all of the *Proteus* and the *E. coli* cultures agree with the interpretation that all four antibiotics are bactericidal in action.

Inoculum effect. Increasing the inoculum of the clinically isolated *P. mirabilis* 3347 10,000-fold (from 10^3 to 10^7 cells/ml of broth) resulted in increases in the MIC or MBC of all of the antibiotics (Table 3). The cephamycin C increases were only 10- and 16-fold, whereas the MIC and MBC increased 200-fold or more for the cephalosporins.

Serial transfer of E. coli through antibiotic-containing broth. Table 4 lists the data obtained during 15 transfers of *E. coli* 2017 through broth containing cephamycin C, cephalothin, cephaloridine, or streptomycin. It can be seen that the MIC and MBC for streptomycin did increase, as expected, >1,000-fold. During this time, the MIC values for cephamycin C increased 4-fold, for cephalothin, 1.5- to 6-fold, and for cephaloridine, 128-fold.

To determine whether exposure to one antibiotic resulted in a change in susceptibility to the other antibiotics, the 15-times passaged cultures were used at about 10^7 cells/ml to determine the MIC and MBC of the other agents. Data in Table 5 show some indication of cross-resistance between cephamycin C and the cephalosporins, in that the cephamycin C-exposed cultures had cephalosporin MIC values in this test approximately eightfold those of the parent culture. The streptomycin-resistant culture was susceptible to cephamycin C and to both cephalosporins. The cephalothin-exposed culture was susceptible to all of the antibiotics, but the cephaloridine-exposed culture had a 32-fold decrease in susceptibility to

TABLE 1. *Agar dilution minimal inhibitory concentrations*

Test organism	MSD no.[a]	MIC (μg/mg of agar)					
		Cephamycins			Cephalothin	Cephaloridine	Cephalosporin C
		A	B[b]	C			
Aerobacter aerogenes	3148	200	>100	100	13	3	100
	3290[c]	>400	>100	>800	>100	>100	>100
	3309[c]	>400	>100	>800	>100	>100	>100
A. cloacae	2646[c]	>400	>100	>800	>100	>100	>100
Aerobacter-Klebsiella	3253[c]	400	>100	25	13	6	100
Escherichia coli	2017	400	>100	25	6	3	>100
	3296[c]	>400	>100	50	25	3	>100
	3349[c]	>400	>100	13	50	6	>100
Klebsiella pneumoniae	3083[c]	400	>100	6	13	13	50
	C-17	400	>100	25	25	6	100
Paracolobactrum arizonae	3271	400	>100	13	3	3	50
Pasteurella multocida	1590	3		6	0.2	0.4	3
Proteus mirabilis	3255[c]	13	>100	6	6	6	6
	3306[c]	0.8	>100	0.4	0.4	0.4	0.4
	3343[c]	6.0	>100	6	13	100	13
P. morganii	3202	>400	>100	13	>100	>100	>100
	3376[c]	>400	>100	13	>100	100	>100
P. vulgaris	1810	13	25	3	50		
	169	25	100	13	6		
Proteus sp.	191	>100	>100	50	>100		
	235	>100	>100	50	>100		
	343	>100	>100	50	>100		
	359	>100	>100	25	25		
Providencia	50	>100	>100	6	>100		
	946	>100	>100	6	>100		
Pseudomonas aeruginosa	3210	>400	>100	>800	>100	>100	>100
	3254[c]	>400		>800	>100	>100	>100
	3301	>400	>100	>800	>100	>100	>100
Salmonella pullorum	3198	100	>100	13	0.8	0.4	13
S. schottmuelleri	3010	400	>100	25	13	1.6	50
Serratia	3347[c]	>400	>100	>800	>100	>100	>100
Shigella	3304	400	>100	13	13	1.6	>100
Diplococcus pneumoniae	3377	100	25	200	0.2	0.5	50
Erysipelothrix rhusiopathiae	3162	100	>100	25	1.6	0.05	>100
Staphylococcus aureus	2949	200	>100	800	0.2	0.05	100
	3051[c]	400	>100	800	0.8	0.8	>100
	3089[c]	400	>100	800	0.8	0.8	>100
	3106[c]	400	>100	800	0.8	1.6	>100
	3147[c]	400	>100	800	0.4	1.6	>101
	3363[c]	400	>100	800	0.4	0.1	>100
Streptococcus agalactiae	1934	200	100	400	0.9		50
S. pyogenes	1685	100	100	200	0.9		50
	3009	100	>100	200	0.9		50

[a] Merck Sharp & Dohme stock culture collection number.

[b] Data for cephamycin B and all data for strains of *P. vulgaris* and *Providencia* were obtained in tests not run simultaneously with the one providing the remaining data.

[c] Known to produce penicillinase, cephalosporinase, or to have multiple antibiotic resistance.

cephalothin and an 8-fold decrease in susceptibility to cephamycin C.

DISCUSSION

Cephamycins A, B, and C are naturally produced antibiotic agents chemically related to the cephalosporins. The most active of these is cephamycin C. Although this antibiotic is not highly active against gram-positive bacteria, its activity against many gram-negative organisms is greater than that of the naturally produced cephalosporin C. The ability of cephamycin C to

resist degradation by certain cephalosporinases may account for its inhibition of *P. morganii* (*submitted for publication*), which is known to produce such an enzyme. Against the cephalosporin-susceptible gram-negative organisms, cephamycin C was essentially as active as the semisynthetic compounds cephalothin and cephaloridine.

TABLE 2. *In vitro activity of cephamycin C against some cephalosporin-resistant clinically isolated cultures*

Test organism	End point[a] (μg/ml)							
	Cephamycin C		Cephalothin		Cephaloridine		Streptomycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Proteus sp. 3348	8	47	63	63	750	1,000	66	66
P. mirabilis 3343	16	63	156	190	1,000	1,000	16	16
P. mirabilis 3347	47	63	63	63	1,000	1,000	16	31
P. morganii 3376	94	>1,000	4,000	>8,000	2,000	>8,000	>1,000	>1,000
P. morganii 3345	63	63	>1,000	>1,000	1,000	1,000	16	16
Escherichia coli 3349	16	16	110	140	140	297	14	14

[a] Minimal inhibitory concentrations (MIC) determined in brain-heart infusion broth containing 10^4 cells/ml. Minimal bactericidal concentrations (MBC) determined by subculture into broth. Figures given are averages of two tubes except that readings from four tubes were averaged for the *E. coli* data.

[b] Cephalosporin-susceptible.

TABLE 3. *In vitro inoculum effect*

Drug	*Proteus mirabilis* 3347 inoculum						Fold increase	
	4×10^7 cells		4×10^5 cells		4×10^3 cells			
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Cephamycin C	63[a]	500	23	63	6	31	10	16
Cephalothin	8,000	>8,000	94	312	31	31	260	>260
Cephaloridine	>8,000	>8,000	2,000	3,000	38	38	205	>205

[a] Micrograms per milliliter in brain-heart infusion broth.

TABLE 4. *Transfer of Escherichia coli 2017 through antibiotic-containing broth*[a]

Exposure no.	Cephamycin C		Cephalothin		Cephaloridine		Streptomycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	31	375	125	500	16	94	31	31
2	31	94[b]	31	141[b]	16	188[b]	47	47
3	31	63	63	375	13	31	63	63
4	63	63	94	250	16	375	188	188
5	63	63	125	500	47	281	125	125
6	63	94	94	500	63	1,000[b]	250	625[b]
7	63	156[b]	125	375	94	94	250	250
8	63	63	125	625	125	563[b]	250	375
9	94	94	125	1,000	375	>1,000	>2,000[b]	>2,000[b]
10	94	125	125	1,000	500	750	>8,000	>8,000
11	125	313[b]	188	1,000	1,000	1,000	>32,000	>32,000
12	125	125	94	1,000	1,000	1,000	>32,000	>32,000
13	125	313[b]	125	750	2,000	2,000	>32,000	>32,000
14	125	4,000[b]	125	1,000	2,000	8,000	>32,000	>32,000
15	125	125	188	1,000	2,000	>8,000	>32,000	>32,000

[a] Values are average of two tubes and are expressed in micrograms per milliliter of brain-heart broth. In all cases, inocula contained 10^6 to 10^7 cells/ml. MIC read after 48 hr and MBC read after 72 hr incubation at 37 C.

[b] Skipped tubes.

TABLE 5. *Cross-resistance of drug-exposed cultures of Escherichia coli 2017*[a]

Exposure of culture	Cephamycin C		Streptomycin		Cephalothin		Cephaloridine	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Unexposed	31	188	63	63	63	500	16	250
Exposed 15 times to cephamycin C	250	250	156[b]	156[b]	500	>2,000	125	125
Exposed 15 times to streptomycin	16	78	>32,000	>32,000	31	94	>3.9	23
Exposed 15 times to cephalothin	31	1,000	63	63	94	500	13	500
Exposed 15 times to cephaloridine	250	375	63	63	2,000	>2,000	2,000	>8,000

[a] Values are averages of two tubes and are expressed in micrograms per milliliter of brain-heart broth. MIC read about 48 hr, and MBC read about 72 hr; incubation at 37 C. In all cases, inocula contained 10^7 cells/ml.

[b] Skipped tube.

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