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Cephamycins, a New Family of β-Lactam Antibiotics.

IV. In Vivo Studies[1]

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Cephamycin A was found to be more active in vivo than cephamycin B. In comparison with cephamycin C, cephamycin A was more active against gram-positive organisms but less active against gram-negative organisms. Given subcutaneously, cephamycin C had good in vivo gram-negative activity, comparing favorably with cephalothin and cephaloridine against cephalosporin-susceptible organisms. In general, against the gram-negative organisms, it was more active than cephalothin or cephalosporin C and about as active as cephaloridine. In addition, cephamycin C protected mice against β-lactamase-producing *Proteus* cultures, including clinically isolated strains. The compound is remarkably nontoxic. Cephamycin C was detected in the serum and recovered from the urine of treated mice to about the same extent as cephaloridine. Like cephaloridine and cephalosporin C, cephamycin C must be excreted mainly by glomerular filtration, because the use of probenecid did not enhance the therapeutic effectiveness nor concentrations of these agents in the sera of treated mice.

Cephamycins A, B, and C are cephalosporin-type agents whose isolation and biological characterization have been described by Stapley et al. (5) and whose chemistry will be described by Albers-Schonberg et al. (*manuscript in preparation*). In vitro tests showing the antibiotics to be primarily bactericidal in action and to have good antibacterial activity, with cephamycin C being especially active against gram-negative organisms, have been described by Miller et al. (3). The present paper reports in vivo tests of the antibacterial activity and pharmacological properties of cephamycins in mice.

MATERIALS AND METHODS

Cephamycin preparations of approximately 70% purity were studied. Commercial samples of cephalothin (Keflin, Eli Lilly & Co.) and cephaloridine (Loridine, Eli Lilly & Co.) were used as controls. A few tests included a laboratory sample of cephalosporin C. The antibiotics were used as water solutions. For experiments with probenecid, Benemid (Merck & Co., Inc.) was used as a suspension.

In vivo protection tests. As a standard procedure, Charles River CD-1 female mice (average weight, 18 to 20 g) were infected intraperitoneally with 3 to 50 times the number of organisms that should kill 50% of the infected, nontreated animals (LD_{50}). At the time of infection and again 6 hr later, therapy was given by the indicated routes. Five mice were used at each of the fourfold drug dilutions tested. Seven days after infection, the test was considered complete, and survival records of that day were used to calculate both the LD_{50} of that test and also the ED_{50}, i.e., the amount of antibiotic that should protect 50% of the infected, treated animals, by the method of Knudsen and Curtis (2).

Toxicity test. To determine that the preparations as used were not acutely toxic, the antibiotics were given to uninfected mice at the same levels and with the same dosage schedules and treatment routes used in the protection tests. In addition, for cephamycin C, each of five mice was given a single intraperitoneal dose of 200 mg (10 g/kg). Fourteen days later the animals were sacrificed and the organs were examined grossly.

Concentrations in the blood and urine. Mice selected to weigh 20 g each were given a single subcutaneous dose of the test antibiotic and also 0.5 ml of water by gavage. The animals, in groups of 5 or 10, were placed in metabolism cages without food or water. At stated intervals, a group of mice were bled from the heart, the pooled blood was allowed to clot at 5 C, and the serum was removed and frozen. Urine excreted during this time was collected in a tube suspended in an ice-water bath. It also was frozen. All such samples, as well as appropriate controls, were held in a frozen state until they could be assayed by the disc-plate methods for antibiotic content.

Effect of probenecid. To determine the effect of probenecid on the concentration of antibiotics in the

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serum and urine, mice were given a single subcutaneous dose of the antibiotic and an oral dose of 10 mg of probenecid or 0.5 ml water by gavage. At stated time intervals, mice were bled and urine samples were collected for assay as described above. To determine the effect of probenecid on the therapeutic effectiveness of the antibiotic, the oral doses of probenecid or water were given to infected mice at the time of each subcutaneous therapeutic treatment.

For these latter tests, 10 mice were used at each of the twofold drug dilutions tested.

RESULTS

In vivo protection. In vitro studies showed that cephamycin A had relatively low activity against both gram-positive and gram-negative organisms (3). The data in Table 1 show that this antibiotic has similar activity in vivo. It also is apparent that cephamycin A is not well absorbed from the gastrointestinal tract, precluding its use by the oral route.

Table 2 compares the activities of cephamycin A, B, and C given intraperitoneally against *Proteus vulgaris* 1810. It can be seen that cephamycin B had about one-tenth the activity of cephamycins A and C. Because of such relatively poor activity, this fraction was not included in the other in vivo tests.

Table 3 shows results from single tests comparing the subcutaneous ED_{50} of cephamycin C, cephalothin, or cephaloridine against seven gram-negative and three gram-positive organisms. Cephalosporin C was checked against three of these organisms. Against the gram-positive cultures (one species each of *Diplococcus*, *Staphylococcus*, and *Streptococcus*), cephamycin C showed the relatively poor activity that was anticipated from the in vitro spectrum. The cephalosporin C end point against *S. aureus* also agrees with the in vitro evidence of activity considerably less than that of cephalothin and cephaloridine (3).

TABLE 1. *Activity of cephamycin A against bacterial infections in mice*

Challenging organism	Route of therapy	ED_{50}[a] (μg)
Proteus vulgaris 1810	Intraperitoneal	33
	Subcutaneous	500
	Oral	12,100
P. mirabilis 3201	Intraperitoneal	200
	Oral	5,000
Salmonella schottmuelleri 3010	Intraperitoneal	419
	Subcutaneous	9,000
Escherichia coli 1939	Intraperitoneal	3,750
E. coli 2017	Intraperitoneal	1,330
Klebsiella pneumoniae B	Intraperitoneal	2,500
Proteus morganii 3202	Intraperitoneal	>4,000
S. gallinarum 3069	Intraperitoneal	1,670
S. pullorum 3198	Intraperitoneal	625
Diplococcus pneumoniae I37	Intraperitoneal	258
Staphylococcus aureus 2949	Intraperitoneal	927
Streptococcus pyogenes 3009	Intraperitoneal	625

[a] Given by the indicated route as two doses 0 and 6 hr after infection by the intraperitoneal route.

TABLE 2. *In vivo activity of cephamycins A, B, and C against Proteus vulgaris 1810*

Cephamycin	ED_{50}[a] (μg)	
	Test 1	Test 2
A	12	7
B	103	79
C[b]	19	

[a] Infection intraperitoneally with about 50 LD_{50} of *P. vulgaris* 1810 suspended in mucin. Therapy given intraperitoneally as two doses at the time of infection and again 6 hr later.

[b] Not tested simultaneously.

Against the cephalosporin-susceptible gram-negative organisms, cephamycin C compares very favorably with the other antibiotics tested, being, in general, more active than cephalothin or cephalosporin C and about as active as cephaloridine. In addition, cephamycin C was active against a β-lactamase-producing strain of *P. morganii* (*submitted for publication*) that is resistant to the cephalosporins.

To study further the activity of cephamycin C against cephalosporin-resistant organisms, five clinically isolated *Proteus* cultures, known to be resistant to cephalosporins, were used as infecting organisms in our in vivo test (Table 4). The clinical strain 3344 was known to be susceptible to cephalosporins, and its ED_{50} values are included for comparison with the others. Cephamycin C was active against all of the strains, even those highly resistant to the cephalosporins. Three of these cephalosporin-resistant cultures were shown to produce β-lactamases capable of degrading cephalothin more effectively than cephamycin C. These data reinforce those of the in vitro tests that showed cephamycin C to be active against β-lactamase-producing cephalosporin-resistant cultures.

Toxicity. Cephamycin C is remarkably nontoxic. Each of five 20-g mice was given a single intraperitoneal dose of 200 mg (10 g/kg), and all survived. When all five mice were sacrificed and autopsied 14 days after the injection, gross examination of the organs showed no evidence of toxicity.

Concentrations in the blood and urine and the effect of probenecid. Concentrations of cephamycin C and of cephaloridine in the serum, as well as their recovery in the urine, were very

TABLE 3. *In vivo activity of cephamycin C, cephalothin, cephaloridine, and cephalosporin C*

Organism	ED_{50} (μg)[a]			
	Cephamycin C	Cephalothin	Cephaloridine	Cephalosporin C
Aerobacter aerogenes 3148	207	207	76	
Escherichia coli 2017	250	2,000	125	2,060
Klebsiella pneumoniae 3068	414	2,420	134	
Proteus morganii 3202	500	>8,000	>8,000	>4,000
P. vulgaris 1810	125	151	151	
Salmonella schottmuelleri 3010	145	1,210	125	
S. typhimurium 2637	125	789	500	
Diplococcus pneumoniae I37	2,420	121	3	
Staphylococcus aureus 2949	>20,000	50	0.8	1,510
Streptococcus pyogenes 1685	8,540	31	0.5	

[a] Therapy given subcutaneously as two doses at the time of intraperitoneal infection and again 6 hr later.

TABLE 4. *In vivo activity against clinically isolated strains of Proteus*

Culture	ED_{50} (μg)[a]		
	Cephamycin C	Cephalothin	Cephaloridine
P. mirabilis 3344[b]	ca. 310	391	179
P. mirabilis 3343	350	2,575	3,270
P. mirabilis 3347[c]	186	721	3,020
Proteus sp. 3348	957	2,505	11,350
P. morganii 3376[c]	517	>20,000	10,000
P. morganii 3345[c]	273	>20,000	9,270

[a] Infection given intraperitoneally with 45 to 300 LD_{50} suspended in 5% hog gastric mucin. Therapy by the subcutaneous route as two doses 0 and 6 hr after infection.

[b] Cephalosporin-susceptible by agar diffusion disc test.

[c] Produces a β-lactamase more active against cephalothin than against cephamycin C.

TABLE 5. *Effect of probenecid on the concentration of antibiotics in the blood and their recovery from urine*

Antibiotic[a]	Concn in serum (μg/ml)[b]				Percent in urine[b]	
	0.5 hr	1 hr	2 hr	4 hr	0-2 hr	0-4 hr
Cephamycin C						
With saline	21.3	7.5	<2	<2	65	73
With probenecid	15	11	<2	<2	65	82
Cephalothin						
With saline	3.3	0.3	<0.1	<0.1	33	34
With probenecid	8.9	4.2	0.8	<0.1	32	44
Cephaloridine						
With saline	19.5	7.0	1.1	<0.1	67	87
With probenecid	20.8	4.7	1.7	<0.1	85	76
Cephalosporin C						
With saline	9.3	1.5	<2	<2	92	74
With probenecid	9.9	0.4	<2	<2	84	87

[a] Antibiotic given as a single subcutaneous dose of 20 mg/kg at 0 hr with saline or 10 mg of probenecid given as 0.5 ml by gavage at 0 hr.

[b] Because mice were sacrificed at each time interval, each figure represents a separate group of mice. However, the same group provided blood and urine samples for any given time

similar in mice given a single subcutaneous injection of 400 μg (20 mg/kg). Serum concentrations of cephalosporin C were intermediate between these and those of cephalothin. The relatively lower concentrations of cephalothin in such samples undoubtedly is influenced by the fact that this antibiotic is deacetylated in the body, and approximately 30% of an injected dose is excreted in this form (4).

Cephalothin is excreted mainly by renal tubular secretion and cephaloridine principally by glomerular filtration (1, 6). Probenecid blocks tubular but not glomerular excretion; therefore, its use with cephalothin results in an increase in the peak concentration and persistence of this antibiotic in the serum. Such a "probenecid effect" can be demonstrated in the mouse, as is shown by the data in Table 5. There was, as expected, no enhancement of cephaloridine concentrations when probenecid was used. Since cephamycin C and cephalosporin C values also were unaffected by probenecid, we conclude that these antibiotics, like cephaloridine, are excreted mainly by glomerular filtration. Treatment with probenecid had no effect on the overall recovery of any of these antibiotics in the 4-hr urine specimens.

Effect of probenecid on therapy. To determine whether the probenecid-induced increase in cephalothin concentration seen in the sera of mice was therapeutically useful, protection tests were performed. As can be seen in Table 6, the use of probenecid was accompanied by an approxi-

TABLE 6. *Effect of probenecid on therapeutic activity against Salmonella schottmuelleri*

Antibiotic	ED_{50} (μg)[a]	
	No probenecid	Probenecid
Cephamycin C.....	192 (5)[b]	195 (5)
Cephalothin.....	1,490 (6)	500 (6)
Cephaloridine.....	156 (2)	133 (2)

[a] Infection intraperitoneally with 10 to 40 LD_{50} of *S. schottmuelleri* 3010 in broth. Antibiotic therapy given subcutaneously as two doses 0 and 6 hr after infection. Probenecid (10 mg) or water given by gavage at 0 and 6 hr.

[b] Figures in parentheses indicate number of tests included in the geometric average.

mately threefold increase in the therapeutic activity of cephalothin, but, as anticipated, probenecid did not enhance the activity of either cephaloridine or cephamycin C. Nor, in another test, did probenecid enhance the therapeutic activity of cephalosporin C against *Escherichia coli*. These data show that the probenecid-enhanced concentration of cephalothin in the serum is therapeutically useful.

DISCUSSION

The cephamycins, naturally occurring antibiotics with a cephalosporin-type nucleus, were shown to have therapeutic activity in the mouse against both gram-positive and gram-negative organisms.

Of the three cephamycins, cephamycin C had the greatest overall activity. Given subcutaneously, cephamycin C protected mice against many gram-negative infections with a degree of activity better than that of cephalothin and approximately that of cephaloridine. In addition, its relative resistance to β-lactamase may be a factor in its ability to protect mice against enzyme-producing cultures resistant to therapy by the cephalosporins. Against the three gram-positive organisms studied, cephamycin C was less active than it was against gram-negative organisms.

Like several of the cephalosporins, cephamycin C is very nontoxic. Mice tolerated a single intraperitoneal injection of 10 g/kg. It was rapidly absorbed into the blood stream after subcutaneous injection, and was excreted in the urine. Its pharmacology in this respect resembles that of cephaloridine, known to be excreted mainly by way of the glomeruli rather than by tubular excretion. This is deduced from the fact that the use of probenecid, which inhibits tubular but not glomerular excretion, did not enhance either concentrations in the blood or the therapeutic activities of cephamycin C, cephalosporin C, or cephaloridine. These effects were seen when probenecid was given along with cephalothin, an agent known to be excreted mainly by way of the renal tubules.

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