IN VITRO AND IN VIVO LABORATORY EVALUATION OF CEPHALOTHIN, A NEW BROAD SPECTRUM ANTIBIOTIC

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ABSTRACT

BONIECE, W. S. (Lilly Research Laboratories, Indianapolis, Indiana), W. E. WICK, D. H. HOLMES, AND C. E. REDMAN. In vitro and in vivo laboratory evaluation of cephalothin, a new broad-spectrum antibiotic. J. Bacteriol. **84**:1292–1296. 1962.—Cephalothin, a new cephalosporin antibiotic, is the sodium salt of 7-(thiophene-2-acetamido) cephalosporanic acid. The activity of this antibiotic against a variety of gram-positive and gram-negative bacteria is described, as determined by the tube-dilution and disc-plate methods. Correlations of the results of the therapy of experimental infections in mice with in vitro sensitivity to cephalothin are presented. The data from the gram-negative bacterial infection tests suggest the following relationship on a within strain basis: $ED_{50} = C_i LD_{50}^{b_i}$, where the subscript i stands for the various strains.

The sodium salt of 7-(thiophene-2-acetamido) cephalosporanic acid, which has been given the generic name cephalothin, is a new cephalosporin antibiotic reported by Chauvette et al., 1962. These investigators examined the structure and activity relationships of cephalosporin antibiotics, and compared these with those of their penicillin congeners. The present communication describes the activity of cephalothin when studied by the conventional tube-dilution method and by the disc-plate test commonly employed in hospital laboratories. Correlations of the results of the latter procedure with those obtained in experimental infections in mice are emphasized.

MATERIALS AND METHODS

New gram-negative clinical isolates. A large number of isolates were obtained from local hospital laboratories. They were carefully studied by the usual determinative procedures and classified as *Escherichia coli*, *Proteus* sp., *Pseudomonas* sp., *Alcaligenes* sp., *Salmonella* sp., *Paracolobactrum* sp., and species of the *Klebsiella-Aerobacter* genera. The latter cultures fitted the descriptions for both genera, and hence were grouped together.

In evaluating the activity of an antibiotic against any genus of microorganisms, it is important that the strains tested be, in fact, different. Hence, all these new clinical isolates were subjected to a battery of drug-sensitivity tests (disc-plate method), and only those which differed in sensitivity spectra were chosen for further study. These were then screened for their pathogenicity for mice, by the intraperitoneal route, when suspended in 5% hog gastric mucin.

Tube-dilution sensitivity tests. For the gram-negative bacteria listed in Table 1, Trypticase Soy Broth (BBL) and inocula of approximately 10^3 organisms per ml were employed. The gram-positive organisms and *Neisseria* tested are listed in Table 2. For the clostridia, thioglycolate broth was utilized, and the tubes were seeded with one drop from overnight broth cultures. For the remaining organisms, Trypticase Soy Broth and inocula of approximately 10^5 organisms per ml were employed. For the streptococci and pneumococci, 5% defibrinated rabbit blood was added to the broth. All end points were read after overnight incubation at 37 C.

Disc-plate sensitivity tests. For these, Trypticase Soy Agar (BBL) was the growth medium for all the organisms, except the last two genera listed in Table 3. Paper discs (6 mm), containing cephalothin concentrations of 1, 2.5, 5, 10, 30, and 50 μg, were employed. The tetracycline and chloramphenicol discs used were purchased from Difco Laboratories. The agar plates were seeded by swabbing from undiluted overnight broth cultures. The discs were placed on the seeded agar, and the plates were examined for zones of inhibition after overnight incubation at 37 C.

The *Haemophilus influenzae* strains were grown

on a modification of the fluid medium of Cohen and Wheeler (1946) with 1.5% agar.

The *Bordetella pertussis* strains were grown on charcoal agar (Ensminger, Culbertson, and Powell, 1953), and the sensitivity readings were made after 4 days of incubation.

Experimental infections. These tests were performed with groups of eight white mice (McAllister strain, 11 to 13 g). Of the gram-negative organisms, the clinical isolates pathogenic for mice were employed in therapy tests. From fresh standardized suspensions, decimal dilutions in 5% hog gastric mucin were prepared, and administered intraperitoneally. Twofold dilutions of the antibiotic were injected subcutaneously into five groups of mice at two or three different challenge levels. Two treatments were given, 1 and 5 hr after infection. The mice were observed for 7 days.

It was possible to employ quite large doses of cephalothin in the above tests because of its very low toxicity (Lee, Herr, and Anderson, *in preparation*). In fasted mice, the acute intravenous LD_{50} is greater than 4 g/kg.

In the experimental gram-positive infections, similar procedures were followed. However, the use of mucin was necessary only in the *Staphylococcus* tests.

The ED_{50} and LD_{50} values were calculated by the method of Reed and Muench (1938).

RESULTS AND DISCUSSION

The tube-dilution sensitivity data are presented in Tables 1 and 2. It should be noted that a relatively light inoculum is required for the detection of gram-negative bacilli sensitive to cephalothin.

The purpose of the study with the cephalothin discs of various concentrations was to select one

TABLE 1. *Tube-dilution* sensitivity of gram-negative bacteria to cephalothin*

Culture category	Total strains	Distribution of strains according to minimal inhibitory concn (μg/ml)					
		1.56	3.12	6.25	12.5	25	>25
Escherichia coli	40		2	8	20	5	5
Proteus sp.	18		1	6	4	1	6
Pseudomonas sp.	15					1	14
Klebsiella-Aerobacter spp.	23	1	4	2	1	5	10
Salmonella sp.	16	1	13	2			
Shigella sp.	11		3	4	4		
Paracolobactrum sp.	4					2	2
Alcaligenes sp.	2						2

* Inoculum of 10^3 organisms per ml.

TABLE 2. *Tube-dilution sensitivity of gram-positive bacteria and Neisseria to cephalothin*

Test organism	Strain	Minimal inhibitory concn
		μg/ml
Streptococcus pyogenes (group A)	C-203	0.1
	ATCC 10389	0.2
	ATCC 12344	0.2
	ATCC 12385	0.1
	ATCC 12961	0.1
Streptococcus sp. (Viridans group)	9962	0.39
	9943	0.39
	190B	0.39
	9961	0.39
Streptococcus sp. (group D)	sal	0.2
	Shrigley	25
	9960	>25
	9901	>25
	12253F	25
Diplococcus pneumoniae	Type I	0.25
	Type II	0.25
	Type VII	0.25
	Type XIV	0.5
Staphylococcus aureus†	S-112*	0.312
	H-228*	0.625
	H-232*	0.625
	H-290	0.312
	H-388*	0.312
	H-400*	0.312
	H-516*	0.312
	H-535	0.625
	H-541*	0.625
	H-563	0.312
	3055	0.312
Clostridium perfringens	BP6K	0.625
C. tetani	Harvard	0.078
Corynebacterium diphtheriae	*mitis gravis*	0.625
	intermedius	0.625
		0.156
Neisseria gonorrhoeae	N-2	1.25
N. meningitidis	ATCC 6253	0.625

* Penicillin resistant.

† Coagulase-positive hemolytic clinical isolates differing in phage lysis patterns.

TABLE 3. *Sensitivity of gram-negative bacteria to 30-μg discs of three antibiotics*

Culture category	Total strains	No. sensitive to:		
		Cephalothin	Tetracycline	Chloramphenicol
Escherichia coli	40	31	20	34
Proteus sp.	18	12	5	15
Pseudomonas sp.	15	0	7	4
Klebsiella-Aerobacter spp.	23	8	20	22
Salmonella sp.	16	16	4	16
Shigella sp.	11	11	9	11
Paracolobactrum sp.	4	1	2	2
Alcaligenes sp.	2	0	2	1
Haemophilus influenzae	16	5	11	14
Bordetella pertussis	15	13	14	13

TABLE 4. *Over-all sensitivity of 160 gram-negative clinical isolates to cephalothin discs of various concentrations*

Disc	Sensitive
μg	%
50	61.3
30	60.5
10	48.8
5	34.4
2.5	21.9
1	12.5

having enough antibiotic to detect all sensitive cultures, including the gram-negative bacilli. The latter (Table 1) are generally much less sensitive in the tube test than are other organisms (Table 2), with the exception of certain group D streptococci. The cephalothin disc data are summarized in Table 4. There is no significant difference in the results obtained with the 30- and 50-μg discs. However, there are statistically significant differences to be observed below the 30-μg level. Based on the results of this study, the 30-μg disc was chosen for laboratory use in the clinical trials.

The sensitivities of a number of gram-negative organisms to 30-μg discs of cephalothin, tetracycline, and chloramphenicol are compared in Table 3.

TABLE 5. *Activity of cephalothin, administered subcutaneously, in experimental gram-positive bacterial infections in mice*

Test organism	Strain	Challenge LD_{50}*	ED_{50} (mg/kg × 2)
Streptococcus pyogenes	C-203	40	1.55
	C-203	1,585	2.5
Diplococcus pneumoniae	Type I	10	6.7
	Type I	100	11.2
	Type II	19	3.08
	Type VII	10	1.3
	Type XIV	10	1.62
Staphylococcus aureus	3055	10	0.49
(penicillin-resistant)	3055	170	1.7
	3074	4	9.2

* One LD_{50} = challenge dose required to kill 50% of the mice.

The activity of cephalothin, administered subcutaneously, against experimental infections caused by gram-positive bacteria is shown in Table 5. The present form of this antibiotic is poorly absorbed orally, and must be given parenterally.

Examples of the activity of cephalothin in experimental gram-negative bacterial infections are illustrated in Fig. 1, 2, and 3. The data are plotted as shown because, on a within strain basis, the following relationship is suggested: $ED_{50} = C_i\ LD_{50}^{b_i}$ or, $\log ED_{50} = \log C_i + b_i \log LD_{50}$, where the subscript i stands for the various strains of organisms. Wherever three concurrently obtained ED_{50} values are plotted, it can be seen that the straight-line relationship predicted by the second equation holds. The mathematical relationship encountered here is surprising, and of considerable interest. Further investigation with other antibiotics to determine the general validity of the above relationship, and to arrive at some explanation for it, is planned.

There were 26 gram-negative cultures sensitive to the 30-μg disc, and pathogenic for mice. In every instance, the experimental infection responded to cephalothin therapy. Two cultures required a minimal inhibitory concentration of 25 μg/ml in the tube-dilution test. The remaining cultures were sensitive to 12.5 μg/ml or less.

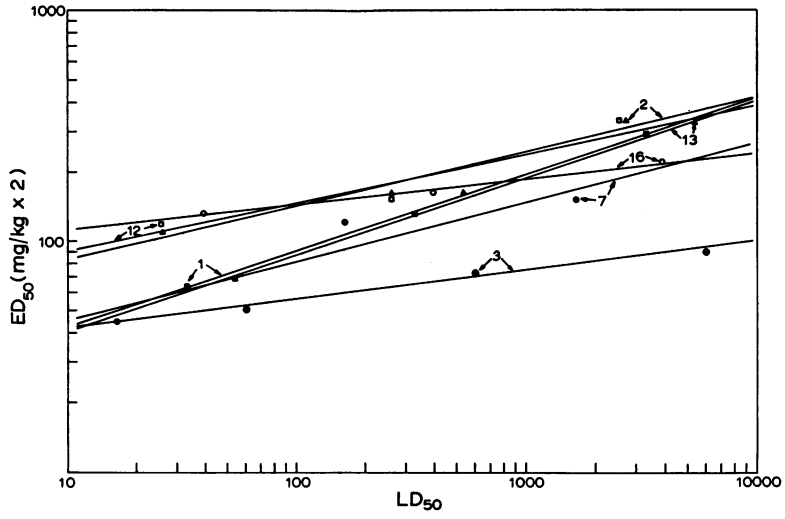

FIG. 1. *Activity of cephalothin, administered subcutaneously, in experimental Escherichia coli infections in mice. Numbers indicate strains. One* LD_{50} *= challenge dose required to kill 50% of the mice.*

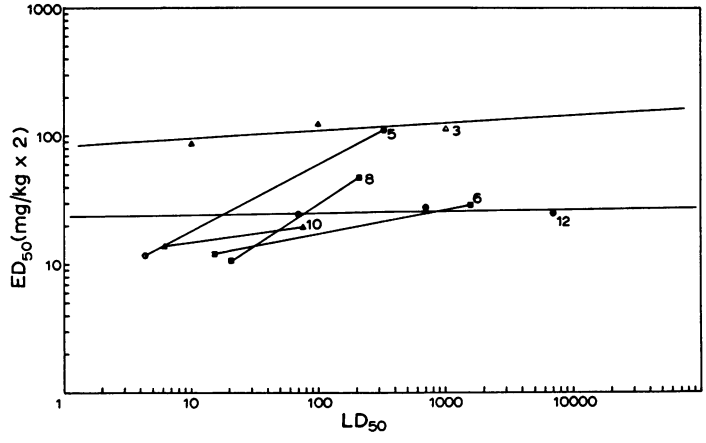

FIG. 2. *Activity of cephalothin, administered subcutaneously, in experimental Proteus infections in mice. Numbers indicate strains. One* LD_{50} *= challenge dose required to kill 50% of the mice.*

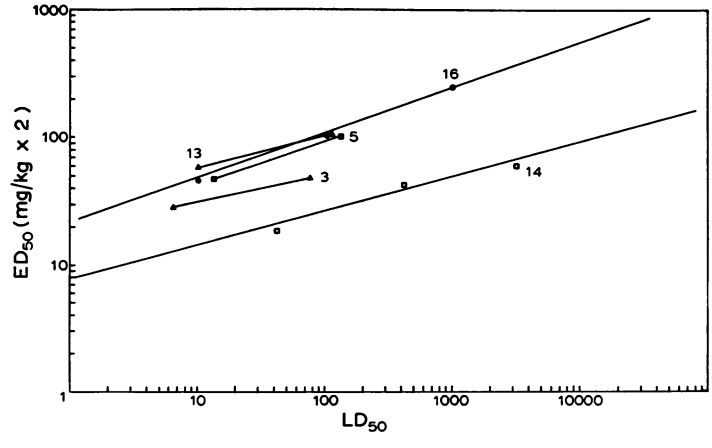

FIG. 3. *Activity of cephalothin, administered subcutaneously, in experimental Klebsiella-Aerobacter infections in mice. Numbers indicate strains. One* LD_{50} *= challenge dose required to kill 50% of the mice.*

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