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In Vitro and In Vivo Laboratory Evaluation of Cephaloglycin and Cephaloridine

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ABSTRACT

WICK, WARREN E. (The Lilly Research Laboratories, Indianapolis, Ind.), AND WILLIAM S. BONIECE. In vitro and in vivo laboratory evaluation of cephaloglycin and cephaloridine. Appl. Microbiol. **13**:248–253. 1965.—Two new antibiotics, structurally related to cephalothin, have been given the generic names cephaloglycin and cephaloridine. Cephaloglycin is the dipolar ion of 7-(D-α-aminophenylacetamido)-cephalosporanic acid. Cephaloridine is 7-[α-(2-thiophene)acetamido]-3-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine. These new compounds were evaluated simultaneously. The broad spectrum of activity observed in vitro and in vivo with both antibiotics, the good oral absorption obtained with cephaloglycin, and the stability of cephaloridine are emphasized. The data suggest that both antibiotics merit clinical trial in humans.

Two new antibiotics, structurally related to cephalothin (Chauvette et al., 1962; Boniece et al., 1962), have been given the generic names cephaloglycin and cephaloridine. Cephaloglycin is the dipolar ion of 7-(D-α-amino-phenylacetamido) cephalosporanic acid. This antibiotic is a white crystalline solid soluble at about 8 mg/ml in distilled water at 25 C. Cephaloridine is 7-[α-(2-thiophene) acetamido]-3-(1-pyridylmethy]-3-cephem-4-carboxylic acid betaine, also a white crystalline solid, but soluble at >100 mg/ml in water at room temperature. Structures of these new compounds and of cephalothin are compared in Fig. 1. The in vitro and in vivo biological activities of these new antibiotics were studied simultaneously. The broad spectrum of activity observed with both antibiotics, the good oral absorption obtained with cephaloglycin, and the stability of cephaloridine are emphasized. The data suggest that both antibiotics merit clinical trial in humans.

MATERIALS AND METHODS

Cultures. The bacteria used, both gram-positive and gram-negative, were different strains as determined by antigenic analysis, phage typing, or drug-sensitivity spectra.

Disc-plate sensitivity tests. Sensitivity tests were performed with Trypticase Soy Agar (BBL), except for *Haemophilus* strains for which Levinthal broth with 1.5% agar was used. Paper discs (6 mm) containing 30 μg of ampicillin, cephalothin, cephaloglycin, cephaloridine, chloramphenicol, or tetracycline were employed. 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.

Tube-dilution sensitivity tests. Trypticase Soy Broth (BBL) was the medium employed for all but the clostridia. For these, thioglycollate broth was utilized. For streptococci, pneumococci, and *Neisseria*, 5% defibrinated rabbit blood was added to the broth. The tubes were seeded from dilutions of standardized suspensions that gave inocula of 10^3 or 10^5 organisms per milliliter. In tests on staphylococci and gram-negative bacilli, minimal inhibitory concentration (MIC) end points were read after 12 and 24 hr of incubation at 37 C.

Stability studies. For determining the extent of degradation of the antibiotics in broth solutions, with and without sensitive bacteria, chromatography methods and viable-cell count studies similar to those reported by Wick (1964) were employed.

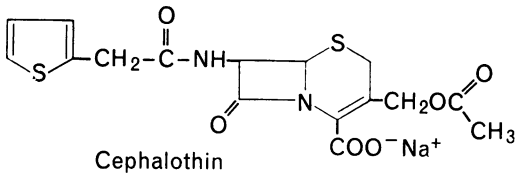
Bactericidal activity. Bactericidal activity was detected by subculturing serial dilution tubes with a loop to fresh antibiotic-free broth, and by determining changes in viable-cell counts during the incubation of tube-dilution tests.

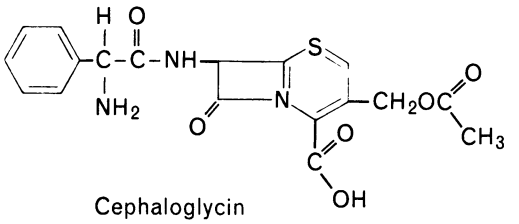
Penicillinase studies. Both *Bacillus cereus* and staphylococcal penicillinases were utilized. Phosphate buffer (*p*H 7.0) and enough of these enzymes to inactivate 100 units per ml of penicillin G in 1 hr at room temperature were employed. The concentration of the antibiotics exposed to the enzymes was 25 μg/ml. Degradation rates over the 24-hr period of observation were determined by biological assays.

Tests with serum. For determining the effect of serum on antibiotic activity, 25% of either human

or horse serum was incorporated in the broth or agar used in sensitivity tests.

Mouse blood level studies. To determine blood levels, a disc-plate assay with *Sarcina lutea* PC1-1001-FDA was utilized. After oral administration of antibiotic, the mice were bled at intervals from the orbital sinus. The blood was collected in heparinized hematocrit tubes. These were allowed to fill by capillary action. Paper discs (6 mm) were saturated with blood and immediately placed on seeded assay plates. This procedure permitted bleeding of the same mouse several times and eliminated any possibility of error due to time delay in collecting and assaying serum.





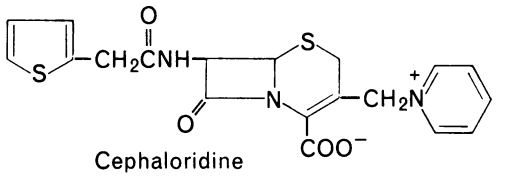


FIG. 1. *Structures of cephalothin, cephaloglycin, and cephaloridine.*

Experimental infections. Experimental infections were performed with groups of eight white mice (McAllister strain, 11 to 13 g). Decimal dilutions from fresh standardized suspensions were prepared in 5% hog gastric mucin and injected intraperitoneally. For streptococcal and pneumococcal infections, mucin was not necessary. Twofold dilutions of the antibiotics were administered orally or subcutaneously to five groups of mice at 1 and 5 hr after infection. The mice were observed for 7 days, and the ED_{50} and ID_{50} values were calculated by the method of Reed and Muench (1938). The ED_{50} is the twice-administered dose required to cure 50% of the infected mice.

RESULTS AND DISCUSSION

Cephaloglycin and cephaloridine both display a wide spectrum of activity in vitro (Tables 1, 2, and 3), comparable to that of cephalothin. However, against sensitive gram-positive bacteria, cephaloridine is the most active of the three structurally related antibiotics. Indeed, the activity of cephaloridine against these organisms approaches that of the penicillins.

As with cephalothin (Boniece et al., 1962), 30-μg discs of cephaloglycin and cephaloridine have been chosen for laboratory use in the clinical trials. In Table 4 are shown the results of the therapy of experimental infections in mice with organisms sensitive to 30-μg discs. Also given are the tube-dilution sensitivities of these organisms.

TABLE 1. *Sensitivity* of gram-negative bacteria to 30-μg discs of six antibiotics*

Organism	Total strains	No. sensitive to					
		Cephaloglycin	Cephaloridine	Cephalothin	Tetracycline	Chloramphenicol	Ampicillin
Escherichia coli	46	45	45	41	27	40	44
Proteus sp. (indole negative)	21	21	21	21	7	21	21
Proteus sp. (indole positive)	5	0	0	0	1	0	1
Pseudomonas sp.	13	0	1	0	8	2	2
Klebsiella-Aerobacter spp.	25	25	9	10	25	24	12
Salmonella sp.	16	16	16	16	4	16	16
Shigella sp.	11	11	11	11	9	11	10
Paracolobactrum sp.	4	3	0	1	2	4	2
Alcaligenes sp.	2	0	0	0	2	0	1
Haemophilus influenzae	4	4	4	2	4	4	4

* The organisms were judged sensitive whenever distinct zones of inhibition were observed, including those containing "satellite" colonies. Because of the relative instability of cephaloglycin, these were observed more frequently with discs containing this antibiotic.

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

Test organism	Strain	Cephaloglycin				Cephaloridine			
		10^3 organisms/ml		10^5 organisms/ml		10^3 organisms/ml		10^5 organisms/ml	
		12 hr	24 hr	12 hr	24 hr	12 hr	24 hr	12 hr	24 hr
Staphylococcus aureus	3055	0.78	6.25	1.56	>25	.007	.007	.015	0.015
	3123	0.78	3.12	1.56	>25	.007	.015	.015	0.015
	3129	3.12	6.25	3.12	12.5	.015	.015	.031	0.031
	S-112†	3.12	25	6.25	25	.015	.031	.031	0.062
	3074†	3.12	12.5	6.25	25	.015	.031	.031	0.062
	3125‡	3.12	12.5	6.25	25	.031	.062	.125	0.125
	H-43†	3.12	25	6.25	25	.031	.031	.031	0.062
	H-114†	3.12	12.5	6.25	25	.031	.031	.062	0.062
Streptococcus sp. (Group A)	C-203	ND	ND	ND	0.195	ND	ND	ND	0.0078
	12385	ND	ND	ND	0.195	ND	ND	ND	0.0078
Streptococcus sp. (Viridans group)	9961	ND	ND	ND	1.56	ND	ND	ND	0.015
	9943	ND	ND	ND	1.56	ND	ND	ND	0.062
Streptococcus sp. (Group D)	Salivarius	ND	ND	ND	0.195	ND	ND	ND	0.0078
	9960	ND	ND	ND	>25	ND	ND	ND	>2.0
Diplococcus pneumoniae	Type I	ND	ND	ND	0.39	ND	ND	ND	0.062
	Type II	ND	ND	ND	0.39	ND	ND	ND	0.062
	Type VII	ND	ND	ND	0.39	ND	ND	ND	0.031
	Type XIV	ND	ND	ND	0.39	ND	ND	ND	0.062
Clostridium tetani	OX	ND	ND	ND	0.62	ND	ND	ND	0.62
C. perfringens	PB6K	ND	ND	ND	2.5	ND	ND	ND	0.62
Corynebacterium diptheriae	gravis	ND	ND	ND	1.25	ND	ND	ND	0.15
	mitis	ND	ND	ND	1.25	ND	ND	ND	0.15
Neisseria gonorrhoeae	N-5	ND	ND	ND	1.25	ND	ND	ND	1.25
Neisseria meningitidis	Suederlin	ND	ND	ND	0.625	ND	ND	ND	1.25

* Results are expressed in micrograms per milliliter. ND = not done.
† Penicillin-resistant.
‡ Penicillin- and methicillin-resistant.

TABLE 3. *Tube-dilution sensitivity** *of representative gram-negative bacteria to cephaloglycin and cephaloridine*

Antibiotic	Organism	Minimal inhibitory concn (μg/ml)			
		With inoculum of 10^3/ml		With inoculum of 10^5/ml	
		12 hr	24 hr	12 hr	24 hr
Cephaloglycin	*Klebsiella-Aerobacter* sp. KA-14	1.56	6.25	3.12	12.5
	Escherichia coli EC-14	3.12	6.25	6.25	12.5
	Salmonella typhosa SA-12	<0.39	1.56	0.78	6.25
	Shigella flexneri SH-3	1.56	12.5	3.12	12.5
	Proteus sp. (indole-negative) PR-4	3.12	12.5	3.12	>25
Cephaloridine	*Klebsiella-Aerobacter* sp. KA-14	3.12	3.12	3.12	6.25
	E. coli EC-14	3.12	3.12	3.12	6.25
	S. typhosa SA-12	1.56	1.56	3.12	3.12
	S. flexneri SH-3	3.12	3.12	3.12	6.25
	Proteus sp. (indole negative) PR-4	6.25	6.25	6.25	6.25

* The bacteria were also sensitive to 30-μg discs of both antibiotics.

TABLE 4. *Activity of cephaloglycin and cephaloridine in the test tube and in experimental bacterial infections in mice with organisms sensitive to the 30-μg disc*

Antibiotic	Test	Test organism[a]							
		C-203	3055	Park I	KA-14	EC-14	SA-12	SH-3	PR-4
Cephaloglycin	12-hr MIC[b]	NR[c]	1.0	NR	3.12	6.25	0.78	3.12	3.12
	24-hr MIC[b]	0.195	4.0	0.39	6.25	12.5	6.25	12.5	>25
	Oral therapy	4.5/551	<2.6/74	29.2/623	15.8/371	13.7/4,690	15.2/46	15.5/316	41.5/1,480
	(ED_{50}/ID_{50})[d]		<2.6/7.4		<10.4/37	10.5/469		8.6/32	28/148
					<10.4/3.7	11.5/47			14.6/14.8
Cephaloridine	12-hr MIC[b]	NR	0.015	NR	3.12	3.12	3.12	3.12	6.25
	24-hr MIC[b]	0.0078	0.015	0.0625	6.25	6.25	3.12	6.25	6.25
	Oral therapy	<1.0/375	>41.5/275	5.8/1740	41.5/298	>166/1,000	119/1,000	36/16	140/10,000
	(ED_{50}/ID_{50})[d]		>41.5/27.5		28.7/29.8	>166/100	83/100		130/1,000
			6/2.7			>166/10			120/100
	Subcutaneous therapy	<0.026/375	<2.6/275	0.065/1740	5.2/298	7.6/1,000	36.7/10,000	<5.0/16	28.7/10,000
	(ED_{50}/ID_{50})[d]		2.6/27.5		<5.2/29.8	8.2/100	18.0/1,000		10.3/1,000
			1.8/2.7			2.8/10			14.4/100

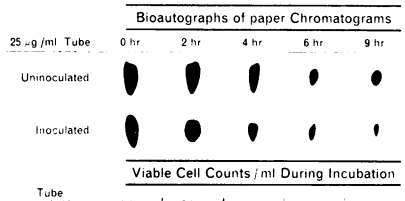
[a] Organisms as shown are *Streptococcus pyogenes* C-203, *Staphylococcus aureus* 3055, *Diplococcus pneumoniae* Park I, *Klebsiella-Aerobacter* sp. KA-14, *Escherichia coli* EC-14, *Salmonella typhosa* SA-12, *Shigella flexneri* SH-3, and *Proteus* sp. PR-4.

[b] Inoculum of 10^5 bacteria/ml. MIC expressed in micrograms per milliliter.

[c] NR = not read.

[d] ED_{50} value expressed as milligrams per kilogram × two treatments (1 and 5 hr postinfection). One ID_{50} = infecting dose of bacteria required to kill 50% of the mice.

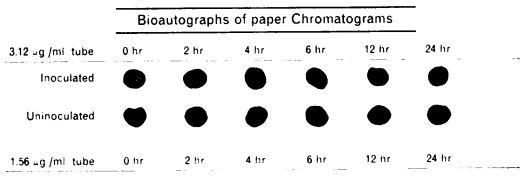
Because of the instability of cephaloglycin in broth solutions, tube-dilution sensitivity tests on staphylococci and gram-negative bacilli should be read after only 12 hr of incubation. This instability problem is illustrated in Fig. 2, in which is shown a diagrammatic representation of the bioautographs from chromatography studies done during a tube test. Also shown are the viable counts obtained from the tubes.



Viable Cell Counts / ml During Incubation

Tube (µg/ml)	0 hr	2 hr	4 hr	6 hr	9 hr	12 hr	% Kill
25	5.6×10^5	5.7×10^5	5.5×10^4	5.2×10^4	770	212	99
12.5	6.8×10^5	4.5×10^5	6.1×10^4	7.4×10^4	300	280	99
6.25	6.2×10^5	7.6×10^5	8.7×10^4	1.1×10^5	700	288	99
3.12	6.5×10^5	5.0×10^5	8.6×10^4	8.3×10^4	800	200	99
1.56	7.0×10^5	6.8×10^5	1.4×10^6	1.1×10^4	2.4×10^4	3.0×10^3	96
0.78	7.8×10^5	7.6×10^5	1.0×10^5	2.4×10^4	1.5×10^5	1.8×10^7	0
Inoculated Control	7.1×10^5	1.1×10^6	2.0×10^7	1.4×10^8	3.8×10^9	2.9×10^9	0

FIG. 2. *Diagrammatic representation of Bacillus subtilis bioautographs of paper chromatograms from cephaloglycin tubes. Also shown are the viable-cell counts obtained from the latter. The chromatograms were developed in 70% propanol. All inoculated tubes received 10^5 cells per milliliter of Salmonella typhosa. The minimal inhibitory concentration was 0.78 µg/ml at 12 hr. However, to demonstrate the rate of degradation, the chromatograms were developed from the 25 µg/ml tubes.*



Viable Cell Counts / ml During Incubation

Tube (µg/ml)	0 hr	2 hr	4 hr	6 hr	12 hr	24 hr	% Kill
25	4.0×10^5	2.4×10^3	200	116	28	8	99
12.5	5.6×10^5	1.0×10^4	700	220	64	4	99
6.25	3.7×10^5	1.1×10^5	2.0×10^3	400	64	12	99
3.12	3.9×10^5	5.0×10^5	8.0×10^3	3.3×10^4	1.1×10^3	28	99
1.56	3.7×10^5	4.9×10^5	2.0×10^5	1.3×10^5	1.6×10^5	2.0×10^6	0
.78	4.4×10^5	4.0×10^5	3.2×10^6	1.9×10^7	1.5×10^8	4.6×10^6	0
.39	4.0×10^5	5.5×10^5	9.5×10^6	1.4×10^8	9.9×10^8	1.0×10^9	0
Inoculated Control	3.5×10^5	4.0×10^5	1.0×10^7	1.9×10^8	8.6×10^8	9.0×10^8	0

FIG. 3. *Diagrammatic representation of Bacillus subtilis bioautographs of paper chromatograms from cephaloridine tubes. Also shown are the viable-cell counts obtained from the latter. The chromatograms were developed in 70% propanol. All inoculated tubes received 10^5 cells per milliliter of Salmonella typhosa. The minimal inhibitory concentration was 3.12 µg/ml at 24 hr.*

The stability of cephaloridine is demonstrated in Fig. 3, in which are shown the results of similar chromatography studies done during a tube test with this antibiotic. These data are in agreement with the observations on stability made by biological assays and by physicochemical methods. With cephaloridine, tube-dilution end points can be read after the usual overnight incubation.

The good oral absorption obtained with cephaloglycin is evident from the therapeutic response (Table 4) observed with this route of administration, and from the blood-level studies summarized in Fig. 4. When similar doses of cephaloglycin or penicillin V were given, blood levels of cephaloglycin were obtained that compare favorably with those observed with this oral penicillin.

Although cephaloridine has some degree of effectiveness in vivo (Table 4) against certain organisms after oral administration, the mouse blood-level studies (Fig. 4) suggest that the present form of this antibiotic is less readily absorbed than cephaloglycin or penicillin V.

It is evident from the data presented in Tables 5 and 6, and also those accompanying Figs. 2 and 3, that both antibiotics possess bactericidal activity.

Staphylococcal penicillinase had no effect on the rates of degradation of these antibiotics over the 24-hr period of observation. While cephaloglycin was unaffected by exposure to *B. cereus* penicillinase, this enzyme increased the rate of degradation of cephaloridine to the extent that only 34% remained after 6 hr. However, this

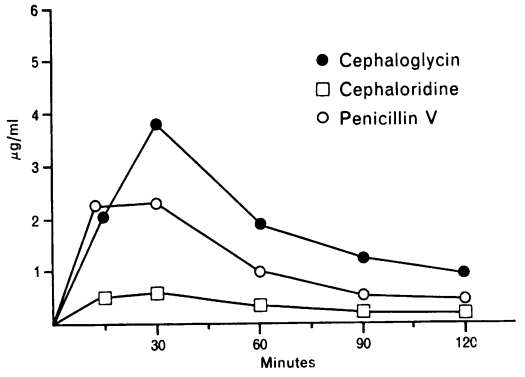


FIG. 4. *Mouse blood levels of cephaloglycin, cephaloridine, and penicillin V after oral administration of 20 mg/kg. Each point represents the average of four to six mice.*

TABLE 5. *Viable counts from tube-dilution sensitivity tests with cephaloglycin and cephaloridine on representative gram-negative bacteria**

Antibiotic	Tube	Viable-cell count/ml				
		KA-14	EC-14	SA-12	SH-3	PR-4
	μg/ml					
Cephaloglycin	25	700	152	110	240	100
	12.5	1,000	1,100	150	200	200
	6.25	3,700	3,100	120	250	2,700†
	3.12	10^6†	10^7†	2,100	10^4†	10^8
	1.56	10^8	10^9	10^4	10^8	10^9
	0.78	10^9	10^9	10^6†	10^9	10^9
	0.39	10^9	10^9	10^8	10^9	10^9
	Control	10^9	10^9	10^9	10^9	10^9
Cephaloridine	25	0	4	0	8	10^4
	12.5	4	4	4	12	10^4
	6.25	900†	12†	20	12†	10^6†
	3.12	10^9	10^8	28†	10^9	10^8
	1.56	10^9	10^9	10^8	10^9	10^9
	0.78	10^9	10^9	10^9	10^9	10^9
	0.39	10^9	10^9	10^9	10^9	10^9
	Control	10^9	10^9	10^9	10^9	10^9

* Inoculum of 10^5 bacteria per milliliter. The bacteria are *Klebsiella-Aerobacter* KA-14, *Escherichia coli* EC-14, *Salmonella typhosa* SA-12, *Shigella flexneri* SH-3, and *Proteus* sp. PR-4.

† Visual minimal inhibitory concentration. Cephaloglycin tubes were read and subcultured for counts after 12 hr, and cephaloridine after 24 hr.

TABLE 6. *Bacteriostatic and bactericidal concentrations of cephaloglycin and cephaloridine on Staphylococcus aureus*[a]

Antibiotic	Strain	Bacteriostatic and bactericidal concn (μg/ml)[b]	
		Bacteriostatic	Bactericidal
Cephaloglycin	3055	0.78	0.78
	3123	1.56	1.56
	3129	3.12	3.12
	S-112[c]	3.12	6.25
	3074[c]	3.12	12.5
	3125[d]	3.12	6.25
	H-43[c]	3.12	6.25
	H-114[c]	3.12	6.25
Cephaloridine	3055	0.015	0.031
	3123	0.031	0.031
	3129	0.031	0.062
	S-112[c]	0.062	0.062
	3074[c]	0.062	0.062
	3125[d]	0.12	0.25
	H-43[c]	0.062	0.062
	H-114[c]	0.062	0.062

[a] Inoculum of 10^3 bacteria per milliliter.

[b] Cephaloglycin tubes were read and loop subcultured to antibiotic-free broth after 12 hr, and cephaloridine after 24 hr.

[c] Penicillin-resistant.

[d] Penicillin- and methicillin-resistant.

inactivation of cephaloridine was much slower than that observed with penicillin G.

The incorporation of human or horse serum in test media had no significant effect on the outcome of sensitivity tests.

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