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Cephalexin, a New Orally Absorbed Cephalosporin Antibiotic

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A new antibiotic, structurally related to cephaloglycin, has been assigned the generic name cephalexin, 7-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-5-carboxylic acid. In vitro antimicrobial activity of cephalexin does not equal that of cephaloglycin. However, excellent oral absorption and lack of serum binding of cephalexin compensates significantly for the lower in vitro activity. Exceptional efficacy against experimental bacterial infections in mice was obtained with cephalexin therapy as compared with cephaloglycin, tetracycline, and chloramphenicol. The data suggest that cephalexin merits clinical trial.

Cephalexin, 7-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid, is structurally related to cephaloglycin. This antibiotic is a white crystalline compound soluble at approximately 2 mg/ml in distilled water at 25 C. Structures for cephalexin and cephaloglycin are compared in Fig. 1. The present communication reports the results of the in vitro and in vivo laboratory evaluation of this new orally absorbed cephalosporin antibiotic.

MATERIALS AND METHODS

Test organisms. The bacteria used were strains of *Escherichia coli*, *Proteus* sp., *Salmonella* sp., *Shigella* sp., *Pseudomonas* sp., *Paracolobactrum* sp., *Alcaligenes* sp., *Streptococcus* sp., *Neisseria* sp., *Clostridium* sp., *Corynebacterium diphtheriae*, *Diplococcus pneumoniae*, *Staphylococcus aureus*, and members of the *Klebsiella-Aerobacter* group.

Susceptibility tests. Conventional disc-plate and tube dilution procedures were used to determine the sensitivity of the bacteria to the antibiotics examined. Trypticase Soy Agar (BBL) or Trypticase Soy Broth (BBL), with or without 5% defibrinated rabbit blood, was used for all organisms except the clostridia. Inocula of 10^6 bacteria/ml were employed in the tube tests, and plates for the disc-plate sensitivity procedure were prepared by swabbing from undiluted overnight broth cultures. For the clostridia, thioglycolate broth was utilized, and the tubes were inoculated with one drop from an overnight broth culture.

Stability studies. Stability of cephalexin in human sera or *p*H 7.0 phosphate buffer was determined by incubating solutions containing 10 μg of the antibiotic per ml at 4, 25, and 37 C. Samples were withdrawn at intervals and assayed immediately by disc-plate assays with *Sarcina lutea* PC1-1001-FDA.

Bactericidal activity. Bactericidal activity of cephalexin was detected by determining viable-cell counts after 24 hr of incubation of tube dilution tests.

Effect of human sera on antibacterial activity. Serum and buffer standard curves, from assays described above, were compared to estimate the percentage of antibacterial activity bound by human sera.

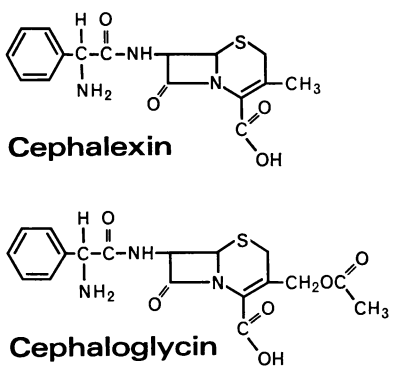

FIG. 1. *Structures for cephalexin and cephaloglycin.*

Mouse blood and urine levels. The previously described disc-plate assay was utilized to compare the blood and urine levels of cephalexin, cephaloglycin, and penicillin V, after oral administration of 20 mg of the antibiotics per kg. Blood was obtained from the orbital sinus with heparinized hematocrit tubes, and assayed immediately (2). Urine samples were diluted and assayed as they were collected.

Experimental infections. Groups of white mice (McAllister strain, 11 to 13 g) were treated orally at 1 and 5 hr after intraperitoneal bacterial challenge, and were observed for 7 days.

RESULTS AND DISCUSSION

The susceptibilities of a number of gram-negative organisms to 30-μg discs of cephalexin,

TABLE 1. *Susceptibility of gram-negative bacilli to 30-μg discs of five orally absorbed antibiotics*[a]

Bacterial species	Total strains	No. susceptible to				
		Cephalexin	Cephaloglycin	Tetracycline	Chloram-phenicol	Ampicillin
Proteus sp. (indole-negative)	21	21	21	7	21	21
Proteus sp. (indole-positive)	5	0	0	1	0	1
Escherichia coli	46	45	45	27	40	44
Pseudomonas sp.	13	0	0	8	2	2
Klebsiella-Aerobacter spp.	25	15	25	25	24	12
Salmonella sp.	16	16	16	4	16	16
Shigella sp.	11	11	11	9	11	10
Paracolobactrum sp.	2	2	3	2	4	2
Alcaligenes sp.	4	0	4	4	0	1
Total strains susceptible	143	110	125	87	118	109

[a] The bacteria were considered susceptible whenever distinct zones of inhibition were observed.

TABLE 2. *Activity of cephalexin by the disc-plate test, in the test tube, and in selected experimental mouse infections with gram-positive bacteria and Neisseria*

Bacteria	Strain	Zone diam (mm)		Tube MIC (μg/ml)	Mouse oral ED_{50}[a]
		30-μg disc	15-μg disc		
Staphylococcus aureus (penicillin-sensitive)	3055	28.5	26.5	3.12	1.15
	H290	29.0	27.4	6.25	ND[b]
	H563	30.2	28.2	6.25	ND
S. aureus (Penicillin-resistant)	H3074	26.8	24.8	6.25	3.7
	H232	18.6	17.1	3.12	ND
	H516	25.6	23.2	6.25	ND
S. aureus (Methicillin-resistant)	3125	9.8	8.0	50	ND
Streptococcus pyogenes (group A)	C203	32.0	29.6	0.5	1.8
	12385	26.0	25.1	0.39	ND
Streptococcus sp. (Viridans group)	9943	23.0	20.6	6.25	ND
	9961	26.0	24.9	6.25	ND
Streptococcus sp. (group D)	9960	13.0	0	200	ND
	sal	32.0	28.6	1.56	ND
Diplococcus pneumoniae	Type I	30.0	28.9	3.12	58.2
	Type II	40.0	36.6	3.12	ND
	Type IV	34.0	30.9	3.12	ND
	Type V	34.0	30.9	3.12	ND
Clostridium tetani	OX	ND	ND	0.62	ND
C. perfringens	PB6K	ND	ND	5.0	ND
Corynebacterium diphtheriae	gravis	ND	ND	1.25	ND
	mitis	ND	ND	1.25	ND
Neisseria gonorrhoeae	10150	ND	ND	0.312	ND
	N-5	ND	ND	1.25	ND
Neisseria meningitidis	OS	ND	ND	0.62	ND
	Suederlin	ND	ND	0.16	ND

[a] Expressed as milligrams per kilogram × two treatments (1 and 5 hr postinfection).
[b] Not done.

TABLE 3. *Activity of cephalexin by the disc-plate test, in the test tube, and in selected experimental infections in mice with gram-negative bacilli*

Bacteria	Strain	Zone diam (mm)		Tube dilution inhibitory concn. MIC (μg/ml)	Mouse therapy, oral ED_{50}[a]
		30-μg disc	15-μg disc		
Proteus sp. (indole-negative)	PR-4	16.6[b]	13.5[b]	50	22.1
	PR-6	18.5	14.1[b]	12.5	ND
	PR-13	16.9[b]	14.7[b]	100	ND
Proteus sp. (indole-positive)	PR-9	0	0	>100	>166
	PR-15	0	0	>100	>166
Salmonella newport	SA-6	18.8	16.8	12.5	ND
S. infantis	SA-8	18.9	17.0	6.25	ND
S. typhosa	SA-12	23.0	20.8	12.5	18.9
Shigella flexneri 1b	SH-2	19.2	16.2[b]	12.5	ND
S. flexneri 2b	SH-3	18.2	14.3[b]	6.25	<10
S. boydi 7	SH-9	21.1	18.7	12.5	ND
Klebsiella-Aerobacter spp.	KA-4	0	0	>100	ND
	KA-14	20.5	16.8	12.5	5.2
	KA-16	18.1	16.1[b]	12.5	ND
	KA-17	0	0	>100	166
	KA-18	18.0[b]	14.1[b]	25	ND
Escherichia coli	EC-6	16.5	13.9[b]	25	ND
	EC-14	17.3	15.4[b]	12.5	11.7
	EC-17	0	0	>100	>166
	EC-25	9.2[b]	0	>100	ND
	EC-38	16.2	15.2[b]	12.5	<10
Paracolobactrum sp.	PA-3	15.0	13.0[b]	25	ND

[a] Expressed as milligrams per kilogram × two treatments (1 and 5 hr postinfection). ND = not done.
[b] Zone of inhibition was of poor quality.

TABLE 4. *Stability of cephalexin in human sera and pH 7.0 phosphate buffer*[a]

Diluent	Sample time (hr)	Amt (μg/ml) of antibiotic remaining after incubation		
		At 4 C	At 25 C	At 37 C
100% serum	0	10.0	10.0	10.0
	4	9.0	9.5	9.0
	6	9.0	8.4	7.5
	24	9.3	6.8	4.5
	30	7.8	6.9	4.3
	48	8.5	5.1	2.5
*p*H 7.0 buffer	0	9.4	9.4	9.4
	4	10.0	8.9	9.1
	6	10.0	8.9	9.6
	24	10.0	8.5	5.0
	30	9.9	6.7	4.8
	48	9.1	5.7	2.8

[a] Initial concentration was 10 μg/ml.

cephaloglycin, tetracycline, chloramphenicol, and ampicillin are compared in Table 1.

Tube dilution end points and the results obtained with discs containing 30 or 15 μg of cephalexin, for both susceptible and resistant bacteria, are shown in Tables 2 and 3. Also included are ED_{50} values required for therapy of experimental mouse infections with selected bacteria. It is obvious from the disc-plate results in Table 2, against gram-positive cocci, that either a 30- or 15-μg disc accurately predicts tube dilution sensitivity or therapeutic efficacy in the experimental infections. However, examination of the zones of inhibition obtained with gram-negative bacteria (Table 3) revealed that the zones surrounding the 15-μg disc were of poor quality, when Trypticase Soy Agar was used. Therefore, as with cephalothin (1), a single 30-μg disc contained sufficient cephalexin to qualitatively detect all susceptible cultures, including the gram-negative bacilli. A study of the susceptibility of

TABLE 5. *Viable-cell counts from tube dilution sensitivity tests with cephalexin on staphylococci and gram-negative bacilli*[a]

Bacteria	Strain	Tube (μg/ml)				
		1.56	3.12	6.25	12.5	25.0
Staphylococcus aureus	3055	<10[b]	<10	<10	<10	<10
	3074[c]	10^9	<10^{4b}	<10	<10	<10
	3123	<10[b]	<10	<10	<10	<10
	3125[d]	10^9	10^9	10^9	10^{4b}	<10
	H43[c]	10^9	560[b]	<10	<10	<10
	H114[c]	10^9	2,100[b]	<10	<10	<10
	H232[c]	10^9	10^{4b}	2,000	<10	<10
	S112[c]	10^9	2,000[b]	<10	<10	<10
Klebsiella-Aerobacter spp.	KA-14	10^9	10^9	10^b	70	30
Escherichia coli	EC-14	10^9	10^9	<10^b	<10	<10
Salmonella typhosa	SA-12	10^9	1,100[b]	<10	<10	<10
Shigella flexneri 2b	SH-3	10^9	10^{5b}	<10	<10	<10
Proteus sp.	PR-4	10^9	10^9	10^9	20[b]	130

[a] Inocula of 10^3 bacteria per ml. Tubes were read and subcultured after 24 hr. To eliminate inhibition by residual antibiotic, a 1:10 dilution was made prior to plating. Counts are expressed as viable cells per milliliter. In control tubes with no antibiotic, the number of viable cells per ml was 10^9 for all organisms.

[b] Visual MIC.

[c] Penicillin-resistant.

[d] Methicillin-resistant.

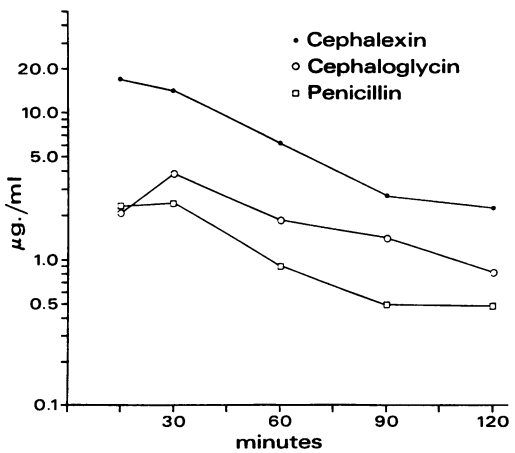

FIG. 2. *Mouse blood levels of cephalexin, cephaloglycin, and penicillin V after oral administration of 20 mg/kg. Each point represents the average of four mice.*

bacteria to cephalexin with the use of other culture media is now in progress.

The stability data for cephalexin at three temperatures are shown in Table 4. Because of the stability at 37 C, tube dilution mimimal inhibitory concentration (MIC) end points for cephalexin could be read after the usual overnight incubation.

It is evident from the data presented in Table 5 that cephalexin exhibits bactericidal activity. After 24 hr of incubation of tube dilution tests for these representative bacteria, viable-cell counts showed that MIC values were usually the bactericidal levels.

Standard curves obtained with *S. lutea*, by use of 6-mm discs saturated with solutions of cephalexin, in either human serum or *p*H 7.0 buffer were identical. Lack of difference in the two curves indicated no loss of antimicrobial activity of cephalexin in human serum. Under identical conditions, a 24% reduction of cephaloglycin activity was demonstrated.

Excellent oral absorption of cephalexin is evident from the mouse blood-level studies summarized in Fig. 2. Blood levels of cephalexin exceeded those obtained with similar doses of cephaloglycin and penicillin V. Urine concentrations of cephalexin, which were in excess of 2,500 μg/ml at 60, 90, and 120 min after oral administration, provided additional evidence for good oral absorption.

Activities of cephalexin, cephaloglycin, tetracycline, and chloramphenicol in the test tube and in experimental bacterial infections in mice are compared in Table 6. The amounts of cephalexin required for inhibition of the bacteria in vitro were higher than those for other antibiotics examined. However, the ED_{50} values obtained for cephalexin were equal to or lower than the values found for the other antibacterial agents evaluated.

TABLE 6. *Activities of four orally absorbed antibiotics in the test tube and in experimental bacterial infections in mice*[a]

Bacteria	Strain	Cephalexin		Cephaloglycin		Tetracycline		Chloramphenicol	
		MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}	MIC	ED_{50}
		μg/ml	mg/kg	μg/ml	mg/kg	μg/ml	mg/kg	μg/ml	mg/kg
Staphylococcus aureus	3055	3.12	1.15	1.56	1.7	0.78	12.6	12.5	51
S. aureus	3074	6.25	3.7	6.25	7.8	>100	>166	>100	>166
Streptococcus pyogenes	C203	0.5	1.8	0.39	3.6	0.2	6.1	3.12	26.2
Diplococcus pneumoniae	Type I	3.12	58.2	0.39	61.8	0.39	75.5	3.12	166
Proteus sp.	PR-4	50	22.1	6.25	18.4	50	89.0	25	11.2
Klebsiella-Aerobacter spp.	KA-14	12.5	5.2	6.25	14.7	1.56	19.6	3.12	16.2
Shigella flexneri 2b	SH-3	6.25	<10	3.12	23.2	0.78	26.5	0.78	14.5
Salmonella typhosa	SA-12	12.5	15.6	3.12	23.5	>12.5	>166	6.25	18.9
Escherichia coli	EC-14	12.5	11.7	1.56	23.3	1.56	47.8	6.25	27.0

[a] Minimal inhibitory concentrations were determined after 24 hr of incubation, except for cephaloglycin. For this antibiotic, end points for gram-negative bacilli and staphylococci were read after only 12 hr. The ED^{50} value is expressed as milligrams per kilogram in two oral treatments (1 and 5 hr post-infection).

This difference between in vitro and in vivo activity is probably attributable to the excellent oral absorption (Fig. 2) of cephalexin.

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