APPLIED MICROBIOLOGY, July 1968, p. 1006-1010

Vol. 16, No. 7
Printed in U.S.A.

In Vitro Antimicrobial Activity and Human Pharmacology of Cephaloglycin

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Received for publication 23 February 1968

Serum and urine concentrations of cephaloglycin (an orally absorbed derivative of cephalosporin C) were determined in normal volunteers and in patients. The in vitro activity of cephaloglycin was also studied. All strains of group A streptococci (*Streptococcus pyogenes*) and *Diplococcus pneumoniae* were inhibited by 0.4 μg of cephaloglycin per ml. Eighty per cent of the *Staphylococcus aureus* strains and about 50% of the *Escherichia coli* and *Proteus mirabilis* strains were inhibited by 1.6 μg of cephaloglycin per ml. *Klebsiella-Aerobacter* species were more resistant to cephaloglycin and 12.5 μg per ml was required to inhibit 70% of these strains. When single doses of 250, 500, or 1,000 mg of cephaloglycin were administered to fasting volunteers, a peak serum concentration of at least 0.5 μg per ml was achieved. A full breakfast did not interfere with absorption of cephaloglycin. Probenecid enhanced both the peak serum concentration and the duration of antibiotic activity in the serum. Serum concentrations of cephaloglycin were even higher in patients who were receiving repeated doses. The peak serum concentrations of cephaloglycin in all volunteers and patients were adequate to inhibit all strains of group A streptococci and *D. pneumoniae*. Many of the peak serum concentrations were adequate to inhibit some strains of *S. aureus*, *E. coli*, and *P. mirabilis*. Urine levels of cephaloglycin were high enough in all volunteers and patients to inhibit more than 90% of the *E. coli* and *P. mirabilis* strains and over 70% of the strains of *Klebsiella-Aerobacter*.

Cephalothin, a derivative of cephalosporin C, is poorly absorbed when administered orally and therefore must be used parenterally. Recently, a new derivative of cephalosporin C, cephaloglycin [7 - (D - α - aminophenylacetamido) - cephalosporanic acid] has been synthesized. In contrast to the other cephalosporins, cephaloglycin is well absorbed after oral administration. Furthermore, this drug has an antibacterial spectrum comparable to that of cephalothin (1, 2).

The present study was undertaken to determine serum and urine concentrations of cephaloglycin after oral administration to volunteer subjects and patients. The in vitro antibacterial activity of cephaloglycin was also evaluated.

MATERIALS AND METHODS

Subjects. Serum levels of cephaloglycin were determined in cross-over studies in five normal male volunteers, 25 to 30 years of age. Each subject received a single oral dose of each of the following: 250, 500, and 1,000 mg of cephaloglycin after an overnight fast; 500 mg of cephaloglycin after a standard breakfast (juice, two eggs, bacon, toast, and coffee); and 500 mg of cephaloglycin preceded by 0.5-g doses of probenecid, 7 hr and 1 hr before. All subjects were given the same regimen on the same day. There was at least a 3-day interval between doses of cephaloglycin. Cephaloglycin was provided by Eli Lilly & Co. (Indianapolis, Ind.) as cephaloglycin dihydrate in 250-mg capsules. Blood was obtained 1, 2, 4, and 6 hr after administration of cephaloglycin. Each subject voided before taking cephaloglycin, and urine was then collected at the end of the following 6-hr period.

Patients receiving cephaloglycin (500 mg every 6 hr in the fasting state) as therapy for urinary tract infection were also studied. Blood and urine specimens were collected on the 2nd to 20th days of therapy after the first dose in the morning.

Assay method. Serum was separated and stored at −20 C until the time of assay. Urine was filtered through a 0.45-μ filter (Millipore Corp., Bedford, Mass.) and stored at −20 C until the time of assay.

The serum and urine levels of cephaloglycin were determined by the agar diffusion method of Wick utilizing paper discs (2). This method was found to yield highly reproducible results. A 24-hr culture of *Sarcina lutea* (ATCC 9341) was washed from the sur-

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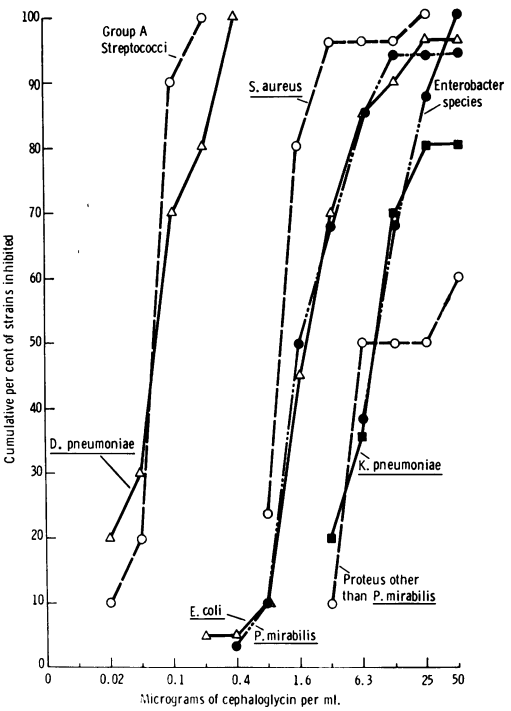

FIG. 1. *Cumulative per cent of bacteria inhibited by cephaloglycin (10 strains of group A streptococci, 10 strains of Diplococcus pneumoniae, 25 strains of Staphylococcus aureus, 41 strains of Escherichia coli, 37 strains of Proteus mirabilis, 24 strains of Klebsiella pneumoniae, 9 strains of Enterobacter species, and 10 strains of Proteus species other than P. mirabilis).*

face of a Trypticase Soy Agar slant with Nutrient Broth (BBL) adjusted to *p*H 6.6. This suspension was adjusted to 80% light transmission at 660 mμ in a Coleman Junior spectrophotometer (Coleman Instruments, Inc., Maywood, Ill.) by adding nutrient broth. A volume of 1.25 ml of the suspension was then mixed with 100 ml of melted Nutrient Agar (BBL) which had been adjusted to *p*H 6.6. Pour plates were made with 10 ml of this mixture for each plate and were allowed to harden. Sterile paper discs (Sensi-Disc, BBL) were submerged in known concentrations of cephaloglycin in pooled human sera, and discs were also submerged in sera from the subjects and patients. The discs were placed on the pour plates and incubated at 37 C for 24 hr. Each known and unknown serum was plated in quadruplicate. Zones of inhibition were determined with the Fisher-Lilly Antibiotic Zone Reader (Fisher Scientific Co., Union, N.J.), and a standard curve was constructed from the known concentrations of cephaloglycin. Concentrations of cephaloglycin in the subjects and patients were determined from this curve. As the standard curve was only accurate up to 2.5 μg/ml, sera from the subjects or patients containing higher concentrations were diluted 1:2 or 1:3 in pooled human sera before determination of levels. Urine samples were diluted 1:10, 1:100, and 1:1,000 in nutrient broth before determination of cephaloglycin concentrations, and the standard curve was constructed from known concentrations of cephaloglycin in nutrient broth.

In vitro activity of cephaloglycin. The following bacteria were isolated from urine of patients with urinary tract infection: 41 strains of *Escherichia coli*, 33 strains of *Klebsiella-Aerobacter* [24 *K. pneumoniae*, 8 *Enterobacter aerogenes* (*A. aerogenes*), and 1 *E. cloacae* (*A. cloacae*)], 37 strains of *Proteus mirabilis*, 10 strains of *Proteus* species other than *P. mirabilis* (5 *P. morganii*, 4 *P. vulgaris*, and 1 *P. rettgeri*), and 10 strains of *Pseudomonas* species. Ten strains each of group A β-hemolytic streptococci (*Streptococcus pyogenes*) and *Diplococcus pneumoniae* were isolated from sputum or throat cultures of patients. Twenty-five strains of *Straphylococcus aureus* were isolated from wounds or blood of patients.

The susceptibility of these bacteria to cephaloglycin was determined by an antibiotic-dilution method in nutrient broth at a *p*H of 6.6. Cephaloglycin was diluted in twofold steps in tubes containing 0.5 ml of nutrient broth. The bacterial inoculum for each tube was 0.5 ml of a 10^{-4} dilution of an 18-hr nutrient broth culture of each strain. The tubes were incubated at 37 C for 18 hr. The bacteriostatic endpoint was considered to be the minimal concentration of antibiotic that prevented turbidity. About 0.005 ml was then removed from each tube without visible growth with a sterile platinum loop and was streaked on Trypticase Soy Agar blood plates. After 24 hr of incubation at 37 C, the plates were examined and the lowest concentration of antibiotic resulting in no growth was taken as the bactericidal endpoint. Sensitivity studies on *D. pneumoniae* were done in Trypticase soy broth with 2% sheep blood.

RESULTS

Susceptibility of bacteria to cephaloglycin. Figure 1 shows the minimal inhibitory concentrations of cephaloglycin for strains of group A streptococci, *D. pneumoniae*, *S. aureus*, *E. coli*, P. *mirabilis*, *Proteus* species other than *P. mirabilis*, and *Klebsiella-Aerobacter* (subdivided into *K. pneumoniae* and *Enterobacter* species). All of the strains of group A streptococci and *D. pneumoniae* were inhibited by 0.4 μg of cephaloglycin per ml. Eighty per cent of the *S. aureus* strains and about 50% of the *E. coli* and *P. mirabilis* strains were inhibited by 1.6 μg of cephaloglycin per ml. Over 95% of the *S. aureus* strains and about 70% of the *E. coli* and *P. mirabilis* strains were inhibited by 3.1 μg of cephaloglycin per ml. *Klebsiella-Aerobacter* species and *Proteus* species other than *P. mirabilis* were more resistant to cephaloglycin and 12.5 μg/ml was required to inhibit most of the strains. Cephaloglycin was about equally active against strains of *K. pneumoniae* and *Enterobacter* species. All strains of *Pseudomonas* were resistant to 50 μg of cephaloglycin per ml.

TABLE 1. *Serum and urine concentrations of cephaloglycin in five normal subjects*[a]

Expt	Subject	Serum concn (μg/ml)				Urine concn in 6-hr collection (μg/ml)	Total cephaloglycin in 6-hr urine collection (mg)
		1 hr[a]	2 hr	4 hr	6 hr		
I[b]	1	0.63	0.87	0.90	0.73	225	90
	2	1.00	0.60	0.55	0.29	269	54
	3	0.38	0.54	0.37	0.00	100	32
	4	1.00	2.00	0.47	0.13	160	32
	5	0.50	2.20	0.70	0.00	150	30
	Mean	0.70	1.24	0.60	0.23	181	47.6
II[c]	1	0.88	1.35	1.30	0.35	54	16
	2	0.13	1.20	0.76	0.58	275	58
	3	0.46	0.75	0.51	0.00	110	22
	4	0.10	0.78	0.83	0.19	140	28
	5	0.12	0.70	0.62	0.17	360	90
	Mean	0.34	0.96	0.80	0.26	188	42.8
III[d]	1	1.40	1.95	1.30	0.18	150	59
	2	0.94	0.65	0.55	0.32	220	42
	3	1.12	1.30	0.42	0.00	150	35
	4	1.46	2.21	0.62	0.35	400	70
	5	0.96	1.40	0.80	0.17	300	105
	Mean	1.18	1.50	0.74	0.20	244	62.2
IV[e]	1	0.00	0.8	1.75	0.71	60	18
	2	0.00	0.90	0.70	0.50	140	25
	3	1.70	1.80	0.66	0.13	119	87
	4	2.00	3.60	2.00	0.24	150	24
	5	0.00	1.40	1.37	0.31	136	34
	Mean	0.74	1.70	1.30	0.38	121	37.6
V[f]	1	1.40	2.60	2.70	0.72	50	19
	2	0.70	1.30	1.13	0.93	120	36
	3	1.23	1.90	1.20	0.70	132	61
	4	0.14	0.95	1.35	0.87	69	31
	5	1.28	1.61	0.68	0.45	106	51
	Mean	0.95	1.67	1.41	0.73	95	39.6

[a] After administration of cephaloglycin.
[b] A 250-mg dose of cephaloglycin was given to subjects in the fasting state.
[c] A 500-mg dose of cephaloglycin was given to subjects in the fasting state.
[d] A 1,000-mg dose of cephaloglycin was given to subjects in the fasting state.
[e] A 500-mg dose of cephaloglycin was given to subjects after breakfast.
[f] A 500-mg dose of cephaloglycin, preceded by 0.5-g doses of probenecid 7 hr and 1 hr before, was given to subjects.

The minimal bactericidal concentrations of cephaloglycin for group A streptococci, *D. pneumoniae*, or *S. aureus* were usually the same as, or twice, the minimal inhibitory concentrations. The minimal bactericidal concentrations of cephaloglycin for the gram-negative bacilli were usually four or more times the minimal inhibitory concentrations.

Serum and urine concentrations of cephaloglycin. The serum and urine concentrations of cephaloglycin achieved in the five volunteers are listed in Table 1. The mean serum concentrations of cephaloglycin for these five subjects, at each time interval after ingestion of the different doses, are shown in Fig. 2.

As shown in Table 1 and Fig. 2, the peak serum concentration of cephaloglycin was usually reached 2 hr after the administration of the antibiotic. A peak serum concentration of at least 0.5 μg/ml was achieved in each volunteer with each of the cephaloglycin regimens studied. The mean peak serum concentrations were at least 0.96 μg/ml for each dose.

Mean serum levels were higher after the administration of 1,000-mg doses of cephaloglycin than after 500- or 250-mg doses in the fasting state. However, mean serum levels were higher with 250-mg doses than with 500-mg doses in the fasting state. Serum concentrations were higher and more sustained after ingestion of 500 mg of cephaloglycin following breakfast or in conjunction with probenecid than with doses of 250, 500, or 1,000 mg in the fasting state. The significant differences were: (i) 1 hr after the administration of cephaloglycin, serum levels were significantly higher in subjects receiving 1,000 mg of cephaloglycin than in those receiving 500 or 250 mg in the fasting state, and levels were higher in subjects receiving 500 mg of cephaloglycin with probenecid than in those receiving 500 mg of cephaloglycin in the fasting state ($P < 0.05$ by the t test of significance for paired observations); (ii) 2 hr after the administration of cephaloglycin, serum concentrations were higher in the subjects receiving probenecid than in subjects receiving 500 mg of cephaloglycin in the fasting state ($P < 0.05$); (iii) 4 hr after the administration of cephaloglycin, serum concentrations were higher in the groups receiving probenecid or 500 mg of cephaloglycin after breakfast than in the group receiving 250 mg of cephaloglycin in the fasting state ($P < 0.05$); (iv) and 6 hr after the administration of cephaloglycin, the group receiving probenecid had higher serum concentrations than any of the other four groups ($P < 0.05$). There were no other significant differences between serum concentrations achieved with different regimens at the same time after ingestion of cephaloglycin.

As shown in Table 1, all of the volunteers had urine concentrations of at least 50 μg/ml after each of the doses of cephaloglycin. It is noteworthy that urine concentrations of cephaloglycin tended to be lower when probenecid was taken in conjunction with cephaloglycin.

Table 2 shows serum levels of cephaloglycin in 16 patients who were receiving 500 mg of cephalo-

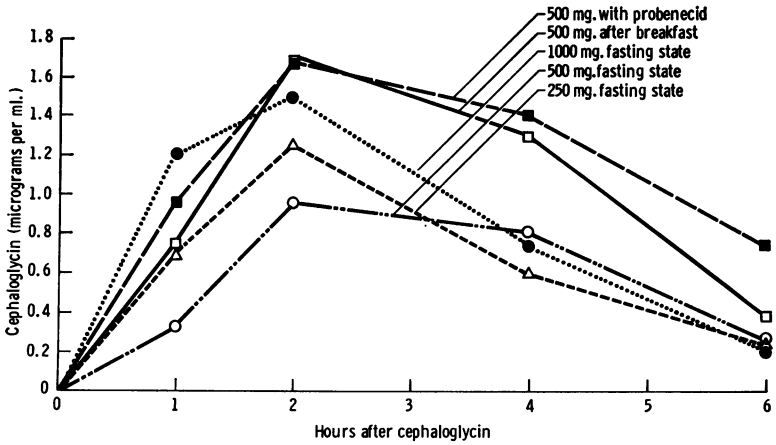

FIG. 2. *Mean serum concentrations of cephaloglycin (μg per ml) in five volunteers who had received cephaloglycin doses of 250, 500, and 1,000 mg in the fasting state; 500 mg after breakfast; and 500 mg preceded by 0.5-g doses of probenecid 7 hr and 1 hr before.*

TABLE 2. *Serum and urine concentrations of cephaloglycin in 18 patients*[a]

Patient	Serum concn (hr after dose)					Urine concn (hr after dose)		
	1	2	3	4	6	2	4	6
1			2			50		
2	4.8	6			2			443
3		4.8		4.0	2.9			
4		2.6		1.3	0.6		30	
5	2.2	1.1	1.1	0.56				
6		1.5		0.85	0.25		165	
7		3.9	4.2	2.1			100	
8	0.5	0.6		1.3			100	
9		1.3			0.75			305
10			0.88					140
11		1.05	0.74					18
12	1.2					100		
13		0.80						680
14		1.1						
15		1.2		0.58				
16			0.9					
17[b]				5.0	2.6			
18[b]	2.6	3.2	10.0					

[a] Concentrations in μg/ml.
[b] Patients 17 and 18 received 0.5 mg of probenecid every 6 hr.

glycin every 6 hr as therapy for urinary tract infection. An additional two patients (numbers 17 and 18) also received 500 mg of probenecid every 6 hr. All of the patients had peak cephaloglycin serum concentrations of at least 0.8 μg/ml, and 15 of the 18 had peak concentrations of 1 μg/ml or more. The two patients receiving probenecid achieved peak serum cephaloglycin levels of at least 5 μg/ml. Urine concentrations of cephaloglycin were determined in 11 of the 18 patients at 2, 4, or 6 hr after administration of cephaloglycin; these concentrations ranged from 18 to 680 μg/ml.

DISCUSSION

Cephaloglycin is unstable at an alkaline *p*H (information provided by Lilly Laboratory for Clinical Research). The rate of degradation of cephaloglycin in Trypticase soy broth (*p*H 7.3) is 14.0% per hour. In contrast, the rate of degradation of cephaloglycin in nutrient broth is only 3.5% per hour (information provided by Lilly Laboratory for Clinical Research). Therefore, in the present studies, nutrient broth was used for all in vitro antibiotic sensitivity studies except for studies with *D. pneumoniae*. (Trypticase soy broth was used to determine the sensitivity of *D. pneumoniae* to cephaloglycin as nutrient broth did not support the growth of these microorganisms.) The fact that Trypticase soy broth was used in studies with *D. pneumoniae* and that cephaloglycin is unstable in Trypticase soy broth might explain the finding of slightly greater resistance of *D. pneumoniae* to cephaloglycin than group A streptococci.

The relative instability of cephaloglycin in nutrient broth (only about 35% remains after 18 hr of incubation; information provided by Lilly Laboratory for Clinical Research) may in part explain the differences observed between minimal inhibitory concentrations of cephaloglycin and minimal bactericidal concentrations.

In the present study, all of the volunteers and patients absorbed cephaloglycin and achieved measurable serum and urine concentrations of this antibiotic. It is not clear why the peak serum levels achieved with ingestion of 500 mg of cephaloglycin in the fasting state were somewhat lower than with 250 mg in the fasting state or with 500 mg after a meal. (These differences were not significant.) It is clear, however, that in these

studies food did not interfere with absorption of cephaloglycin. Probenecid enhanced both the peak serum concentration and the duration of antibiotic activity in the serum. The higher peak serum concentration observed in the patients than in the volunteers may have been due in part to the fact that the patients received repeated doses of cephaloglycin, whereas the volunteers received single doses.

All of the doses given to volunteers and patients produced serum concentrations of cephaloglycin which were high enough to inhibit all group A streptococci and *D. pneumoniae*. Many of the peak serum concentrations were adequate to inhibit some strains of *S. aureus*, *E. coli*, and *P. mirabilis*. With the addition of probenecid, most volunteers and patients achieved serum levels of cephaloglycin high enough to inhibit 80% of the *S. aureus* strains and about 50% of the *E. coli* and *P. mirabilis* strains. Urine levels of cephaloglycin were high enough in all volunteers and patients to inhibit more than 90% of the *E. coli* and *P. mirabilis* strains and over 70% of the strains of *K. pneumoniae* and *Enterobacter* species.

ACKNOWLEDGMENTS

This investigation was supported by a grant from Eli Lilly & Co., the Health Research Council of the City of New York under contract U-1107, and by Public Health Service grants AI 07581, AI 05940, and HE 03479 and training grant T1 AI 255.

Judith Gips and Natalie Seecof rendered valuable technical assistance.

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