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In Vitro Antimicrobial Activity and Human Pharmacology of Cephalexin, a New Orally Absorbed Cephalosporin C Antibiotic

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Concentrations of cephalexin (an orally absorbed derivative of cephalosporin C) in serum and urine were determined in normal volunteers and patients. The in vitro antibacterial activity was also studied. All strains of group A β-hemolytic streptococci and *Diplococcus pneumoniae* were inhibited by 3.1 μg/ml. Of the *Staphylococcus aureus* strains, 88% were inhibited by 6.3 μg/ml, and 12.5 μg/ml was inhibitory for all *S. aureus*, 80% of *Escherichia coli*, 72% of *Klebsiella-Aerobacter*, and 56% of *Proteus mirabilis* strains. About 90 to 96% of *E. coli*, *Klebsiella-Aerobacter*, and *P. mirabilis* strains were inhibited by 25 μg of cephalexin per ml. *Pseudomonas* and indole-positive *Proteus* strains proved to be quite resistant to cephalexin. Cephalexin was well absorbed after oral administration. A peak serum concentration of cephalexin of at least 5 μg/ml was achieved in each volunteer with 250 and 500-mg doses. A mean peak serum concentration of 7.7 μg/ml was achieved with 250-mg doses; 12.3 μg/ml was achieved with 500-mg doses of antibiotic. Food did not interfere with absorption. Probenecid enhanced both the peak serum concentration and the duration of antibiotic activity in the serum. Over 90% of the administered dose was excreted in the urine within 6 hr. The mean peak serum concentration of cephalexin after an oral dose of 500 mg was adequate to inhibit all group A streptococci, *D. pneumoniae*, and *S. aureus*, 85% of *E. coli*, and about 40 to 75% of *Klebsiella-Aerobacter* and *P. mirabilis* strains. Levels of cephalexin in urine were adequate to inhibit over 90% of *E. coli* and *P. mirabilis* and 80 to 96% of *Klebsiella-Aerobacter* strains.

Cephalothin and cephaloridine, the two commercially available derivatives of cephalosporin C, are poorly absorbed when administered orally and must therefore be given parenterally. Cephaloglycin (3), the first derivative of cephalosporin C developed for oral use, is incompletely absorbed, and the serum and urine concentrations achieved are relatively low (1).

Recently, cephalexin [7-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid], another derivative of cephalosporin C, has been synthesized and has been shown to be more completely absorbed than cephaloglycin after oral administration (2).

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

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MATERIALS AND METHODS

Subjects. Serum levels of cephalexin were determined in crossover studies in five normal male and two normal female volunteers, 20 to 30 years of age. Each subject received a single oral dose of each of the following: 250 and 500 mg of cephalexin after an overnight fast, 500 mg of cephalexin within 0.5 hr after a standard breakfast (juice, two eggs, bacon, toast, and coffee), and 500 mg of cephalexin preceded by 0.5 g of probenecid, 7 hr and 1 hr before. There was at least a 2-day interval between doses of cephalexin. Cephalexin was provided by Eli Lilly & Co. (Indianapolis, Ind.) in 250-mg capsules. Blood was obtained 1, 2, 4, and 6 hr after administration of cephalexin. Each subject voided before taking cephalexin, and urine voided during the subsequent 6 hr was collected.

Patients receiving 500 mg of cephalexin every 6 hr in the fasting state as therapy for urinary tract infection were also studied.

Blood urea nitrogen levels were normal in all volunteers and patients, except for patients 2 and 6, who had blood urea nitrogens of 22 mg/100 ml.

Assay method. Serum was separated and stored at −20 C until the time of assay; urine was stored at −20 C until the time of assay. Assays were usually performed within 1 week.

The serum and urine levels of cephalexin were determined by the agar-diffusion method of Wick by utilizing paper discs (3). A 24-hr culture of *Sarcina lutea* (ATCC 9341) was washed from the surface of a Trypticase Soy Agar slant with Nutrient Broth (BBL) adjusted to *p*H 6.6. This suspension was adjusted to 80% light transmittance at 660 nm in a Coleman Junior spectrophotometer 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 made with 10 ml per plate were allowed to harden. Sterile paper discs (Sensi-Disc, BBL) were submerged in known concentrations of cephalexin freshly prepared in pooled human serum. Discs were also submerged in sera from the subjects and patients. The discs were placed on the pour plates and incubated at 26 C for 24 hr. A temperature of 26 C was used, as suggested by Eli Lilly & Co.; zones of inhibition were clearer at 26 C than at 37 C. Each known and unknown serum was plated in quadruplicate. A known serum was plated on each pour plate to measure uniformity of the plates. Zones of inhibition were determined with an Antibiotic Zone Reader (Fisher Scientific Co., Union, N.J.), and a standard curve was constructed from the known concentrations of cephalexin. Concentrations of cephalexin in the subjects and patients were determined from this curve. As the standard curve was accurate only up to 12.5 μg/ml, sera containing higher concentrations were diluted 1:2 or 1:4 in pooled human serum before determination of levels. Urines were diluted 1:100 and 1:1,000 in nutrient broth before determination of cephalexin concentrations, and the standard curve was constructed from known concentrations of cephalexin in nutrient broth.

In vitro activity of cephalexin. The following strains of bacteria were isolated from patients: 25 strains each of *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, and *Klebsiella-Aerobacter* (22 *K. pneumoniae* and 3 *Enterobacter aerogenes*), and 10 strains each of *Proteus* species other than *P. mirabilis* (*P. vulgaris*, *P. rettgeri*, and *P. morganii*), *Pseudomonas*, *Diplococcus pneumoniae*, and group A β-hemolytic streptococci (*Streptococcus pyogenes*). The susceptibility of these bacteria to cephalexin was determined by an antibiotic-dilution method in Trypticase Soy Broth (*p*H 7.3) and in nutrient broth (*p*H 6.6). Sensitivity studies on *D. pneumoniae* and group A streptococci were done in Trypticase Soy Broth with 2% sheep blood. Cephalexin was diluted in twofold steps in tubes containing 0.5 ml of broth. The bacterial inoculum for each tube was 0.5 ml of a 10^{-4} dilution of an 18-hr culture of each strain in the appropriate broth. The tubes were incubated at 37 C for 18 hr. The bacteriostatic end point was considered to be the minimal concentration of antibiotic that prevented turbidity. About 0.005 ml from each tube without visible growth was then streaked on Trypticase Soy Agar-blood plates with a sterile platinum loop. After 24 hr of incubation at 37 C, the lowest concentration of antibiotic resulting in no growth on the plates was taken as the bactericidal end point.

RESULTS

Susceptibility of bacteria to cephalexin. Figure 1 shows the minimal inhibitory concentrations of cephalexin in Tryptocase Soy Broth for strains of group A β-hemolytic streptococci, *D. pneumoniae*, *S. aureus*, *E. coli*, *P. mirabilis*, and *Klebsiella-Aerobacter*. All of the strains of group A β-hemolytic streptococci and *D. pneumoniae* were inhibited by 3.1 μg/ml. Of the *S. aureus* strains, 88% were inhibited by 6.3 μg/ml; 12.5 μg/ml was inhibitory for all *S. aureus*, 80% of *E. coli*, 72% of *Klebsiella-Aerobacter*, and 56% of *P. mirabilis* strains. About 90 to 96% of *E. coli*, *Klebsiella-Aerobacter*, and *P. mirabilis* strains were inhibited by 25 μg of cephalexin per ml. All 10 strains of *Pseudomonas* species and 9 of 10 indole-positive *Proteus* strains were resistant to 50 μg of cephalexin per ml. The remaining indole-positive *Proteus* strain was inhibited by

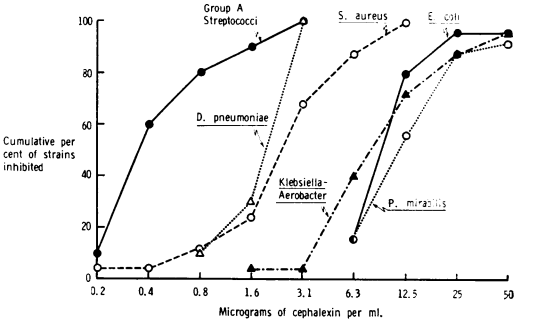

FIG. 1. *Cumulative percentage of bacteria inhibited by cephalexin in Trypticase Soy Broth (10 strains of group A streptococci, 10 strains of D. pneumoniae, and 25 strains each of S. aureus, E. coli, P. mirabilis, and Klebsiella-Aerobacter).*

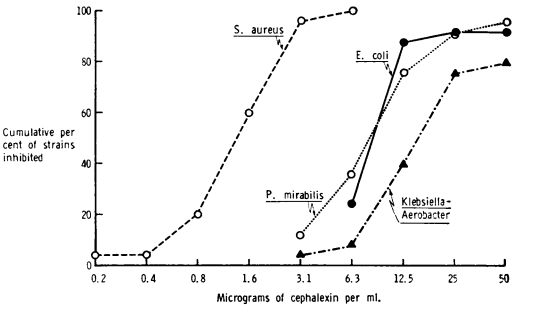

FIG. 2. *Cumulative percentage of bacteria inhibited by cephalexin in nutrient broth (25 strains each of S. aureus, E. coli, P. mirabilis, and Klebsiella-Aerobacter).*

25 μg of cephalexin per ml. The *Enterobacter* strains were more resistant to cephalexin than the *K. pneumoniae* strains; two of the three *Enterobacter* strains were resistant to 50 μg of cephalexin per ml. The minimal bactericidal concentrations of cephalexin were usually the same as, or twice, the minimal inhibitory concentrations for all strains tested.

The minimal inhibitory concentrations of cephalexin in nutrient broth against *S. aureus*, *E. coli*, *P. mirabilis*, and *Klebsiella-Aerobacter* strains are shown in Fig. 2. Cephalexin in nutrient broth appeared to be slightly more active against *S. aureus* and *P. mirabilis* strains and slightly less active against *Klebsiella-Aerobacter* strains than cephalexin in Trypticase Soy Broth. There was no difference in activity with *E. coli* strains.

Serum and urine concentrations of cephalexin. The serum and urine concentrations of cephalexin achieved in the seven volunteers are listed in Table 1. The mean concentrations of cephalexin

TABLE 1. *Serum and urine concentrations of cephalexin in seven normal subjects*

Subject	Serum concn (μg/ml)				Urine concn (6-hr collection)	Total excreted in 6 hr
	1 Hr after dose	2 Hr after dose	4 Hr after dose	6 Hr after dose		
					μg/ml	*mg*
250-Mg dose of cephalexin in fasting state						
1	6.4	3.1	0.8	0.2	1,100	264
2	5.7	5.2	1.5	0.2	570	256
3	5.2	5.1	0.4	0.2	1,300	247
4	10.8	4.9	1.4	0.2	540	275
5	11.0	4.1	0.8	0.1	270	205
6	6.8	2.8	0.4	0.0	830	274
7	1.7	8.2	1.9	0.3	1,200	252
Mean	6.8	4.8	1.0	0.2	830	253
500-Mg dose of cephalexin in fasting state						
1	15.2	8.2	1.5	0.4	1,500	450
2	11.7	7.8	1.5	0.4	1,150	472
3	11.1	7.3	0.7	0.2	1,000	500
4	12.4	11.3	3.1	0.9	530	447
5	15.6	8.2	1.6	0.5	1,000	513
6	11.8	8.9	3.0	0.3	1,300	663
7	8.3	5.7	0.5	0.0	1,200	372
Mean	12.3	8.2	1.7	0.4	1,097	488
500-Mg dose of cephalexin after standard breakfast						
1	11.5	8.1	2.9	0.9	2,000	700
2	0.0	5.0	1.9	0.6	2,100	483
3	0.8	5.4	2.0	0.3	670	302
4	15.6	10.1	1.8	0.5	1,200	360
5	17.0	9.1	2.9	0.9	670	504
6	16.6	10.5	3.0	0.0	880	484
7	15.3	7.1	1.7	0.3	1,600	560
Mean	11.0	7.9	2.3	0.5	1,303	485
500-Mg dose of cephalexin preceded by 0.5-g doses of probenecid 7 and 1 hr before						
1	29.5	16.3	7.6	2.9	1,700	595
2	15.1	8.5	3.9	2.0	810	413
3	6.2	6.0	2.8	0.8	2,300	345
4	16.4	19.0	7.6	4.6	480	365
5	18.4	15.6	7.0	2.9	540	540
6	24.0	16.7	7.8	4.2	880	510
7	26.0	14.5	11.1	5.7	1,000	500
Mean	19.4	13.8	6.8	3.3	1,101	467

at each time interval after ingestion of the different doses are shown in Fig. 3.

As shown in Table 1 and Fig. 3, the peak serum concentration of cephalexin was usually achieved 1 hr after administration of the antibiotic. A peak concentration of at least 5.0 μg/ml was reached in each volunteer on each antibiotic regimen. A mean peak serum concentration of 7.7 μg/ml was obtained on 250-mg doses of cephalexin, 12.3 μg/ml on 500-mg doses taken with or without food, and 19.7 μg/ml on 500-mg doses taken with probenecid.

Mean serum levels were higher at all time intervals after the administration of 500-mg doses of cephalexin than after 250-mg doses. Serum concentrations were higher and more sustained after ingestion of 500 mg of cephalexin in conjunction with probenecid than with doses of 500 mg in the fasting state or 500 mg after breakfast. The significant differences ($P < 0.05$ by the t-test of significance for paired observations) were: (i) 1 and 2 hr after the administration of cephalexin, serum concentrations were significantly higher in subjects receiving 500 mg in the fasting state than in those receiving 250 mg; (ii) 4 and 6 hr after the administration of cephalexin, serum concentrations were significantly higher in subjects receiving 500 mg with food than in those receiving 250 mg; and (iii) at all times studied, serum concentrations were significantly higher after 500 mg of cephalexin with probenecid than with any of the other regimens. Serum levels attained with 500-mg doses taken with food did not differ significantly ($P > 0.05$) from levels attained with 500 mg taken in the fasting state at any of the times tested.

All volunteers had urine concentrations of at least 270 μg/ml after 250 mg of cephalexin, and at least 480 μg/ml after 500 mg (Table 1). The mean urinary recovery of the antibiotic exceeded 90% with each dose regimen.

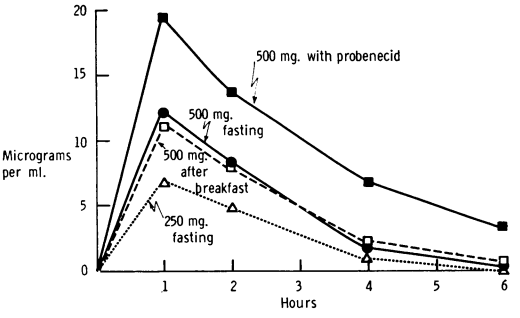

FIG. 3. *Mean serum concentrations of cephalexin (μg/ml) in seven volunteers who had received 250 and 500 mg in the fasting state, 500 mg after breakfast, and 500 mg preceded by 0.5-g doses of probenecid, 7 and 1 hr-before.*

TABLE 2. *Serum and urine concentrations of cephalexin in six patients receiving 500 mg four times a day*

Patient	Serum concn (μg/ml)					Urine concn (6 hr collection)
	1 Hr after dose	2 Hr after dose	3 Hr after dose	4 Hr after dose	6 Hr after dose	
						μg/ml
1[a]	11.4	5.6		1.2	0.2	390
2[b]	33.5	24.6		11.1	4.7	1,200
3[b]			10.9			450
4[b]	15.4	8.5		5.4		450
5[b]	14.4					1,600
6[a]		19.0		10.9	6.2	680

[a] Concentrations determined after first dose of cephalexin.

[b] Concentrations determined after 2 to 14 days of therapy.

Table 2 shows the serum and urine concentrations of cephalexin in six patients who were receiving 500 mg of cephalexin every 6 hr. All patients achieved a peak cephalexin serum concentration of at least 10.9 μg/ml and a urine concentration in a 6-hr collection of at least 390 μg/ml.

DISCUSSION

The serum concentrations of cephalexin in the present study are lower than those previously reported by the Lilly Laboratory for Clinical Research (*Cephalexin. An Investigational New Drug*). At 1 hr after 250 and 500 mg of cephalexin, the mean serum concentrations in the present study were about 75% of the concentrations reported by the Lilly Laboratory. The reason for these differences is not apparent. It is probably not related to deterioration of cephalexin in the samples, as the half-life of cephalexin in serum is over 90 days at −20 C (information provided by Lilly Laboratory for Clinical Research). In the present study, specimens were stored at −20 C and usually assayed within 1 week.

Although the standard breakfast did not appreciably lower the mean serum concentrations achieved, ingestion of food markedly interfered with absorption in two volunteers (2 and 3) and resulted in a delay in the peak serum concentration until 2 hr. The Lilly Laboratory for Clinical Research (*Cephalexin. An Investigational New Drug*) reported that ingestion of food decreases serum concentrations and delays peak serum concentrations more than was found in

the present study. The reason for these differences in results is unknown.

The highest serum concentrations achieved in this study were in two patients who had decreased renal function and in volunteers given probenecid. In both of these situations, the renal clearance of cephalexin was probably diminished.

The peak serum concentrations of cephalexin achieved in the present study were about 10-fold higher and the urinary recovery was about 9-fold higher than was achieved with cephaloglycin in a previous study (1). However, cephalexin is about one-fourth to one-half as active in vitro as cephaloglycin against *S. aureus*, *E. coli*, *Klebsiella-Aerobacter*, and *P. mirabilis* in nutrient broth with a *p*H of 6.6. (An acid medium must be used for cephaloglycin.) The excellent absorption of cephalexin after oral administration with 10-fold higher peak serum levels and less loss of activity in human serum (2) are probably more than sufficient to compensate for the lower in vitro activity.

The 500-mg dose of cephalexin given to volunteers and patients produced mean peak serum levels of antibiotic which were sufficient to inhibit all group A streptococci, *D. pneumoniae*, and *S. aureus*, about 85% of *E. coli*, and about 40 to 75% of *Klebsiella-Aerobacter* and *P. mirabilis* strains, depending on the medium used. Urine levels of cephalexin were high enough to inhibit more than 90% of *E. coli*, *P. mirabilis*, and *Klebsiella-Aerobacter* strains (with the exception of only 80% of *Klebsiella-Aerobacter* strains in nutrient broth).

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