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In Vitro and In Vivo Studies

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Cephalexin, a derivative of cephalosporin C, produces high serum and urine concentrations after oral administration. In addition, it shows good activity in vitro against most gram-positive and some gram-negative organisms. This study reports the in vitro *susceptibility to cephalexin of a series of clinical isolates, the serum levels and urine concentrations in human volunteers and the results of its use in infections due to susceptible organisms. Results of* in vitro *susceptibility testing reveal that cephalexin was effective against most strains of the gram-positive organisms tested — group A streptococci, pneumococci, and staphylococci — although the MIC's are higher than those found for cephaloridine and ampicillin, with the one exception being penicillinase producing staphylococcus against which ampicillin is ineffective. Cephalexin is effective against most strains of indole-negative proteus, Klebsiella and E. coli, but all strains of enterobacter show resistance. Forty-seven patients with a variety of clinical illnesses were treated with cephalexin. Five of 14 patients with urinary tract infections were cured and nine had a remission followed by a bacteriologic relapse. All patients with soft tissue infection were cured as were nine of 12 patients with pneumonia. Of the three patients with pneumonia who were not cured, one improved, while two were classified as treatment failures. Similarly, 11 of 12 patients with streptococcal pharyngitis had a clinical remission, although in three, the organism was isolated after therapy. One patient with an associated coagulase-positive staphylococci cultured from the pharynx failed to respond to treatment.*

Of the antibiotics derived from cephalosporin C, cephalothin and cephalordine have proven useful in the treatment of a variety of infections caused by most gram-positive and some gram-negative organisms. Both are poorly absorbed from the gastrointestinal tract and must be employed parenterally only. Another derivative of cephalosporin C, cephaloglycin, was absorbed when administered orally, but serum levels were inadequate for the treatment of most organisms which are susceptible *in vitro* to moderate levels of the drug, although urine levels were considerably higher and satisfactory results were obtained in the treatment of urinary tract infections due to susceptible organisms.[1-2] Recently, cephalexin (7[Dα = amino-αphenylacetamide]-3-methyl-cephem-4-carboxylic acid) was shown to produce high serum levels after oral administration. We, therefore undertook to study the *in vitro* susceptibility to cephalexin of a series of clinical isolates; the serum levels and urine concentrations in human volunteers; and, its efficacy in infections due to susceptible organisms. The result of these studies forms the basis for this report.

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Materials and Methods

The organisms used for susceptibility testing were isolated from patients at the Henry Ford Hospital and stored on

agar slants under sterile mineral oil. Minimal inhibitory concentration (MIC) for the 257 clinical isolates was determined in trypticase soy broth by the standard two-fold dilution method[3] using a 10^{-3} dilution of an 18-hour broth culture.

Serum levels and urinary excretion were determined in 10 healthy adult volunteers given 250 mg and 500 mg of cephalexin orally. Venous blood was drawn at 1, 2, 4, and 6 hours after ingestion of the cephalexin and all urine was collected over the six-hour period. The sera and urine levels were determined by the cup-plate method using *Sarcina lutea* as the test organism.[3]

Protein binding was determined by a parallel two-fold dilution method which has been described in a previous publication.[4]

Cephalexin was administered to 47 patients with a variety of infections caused by susceptible gram-positive and gram-negative organisms. Doses ranged from 1 to 2 grams per day in four equal doses and the treatment course ranged from 7 to 14 days.

Results

Ninety-one strains of E. coli were tested, and the results are listed in Figure 1. Ninety-three percent of strains of E. coli were susceptible to

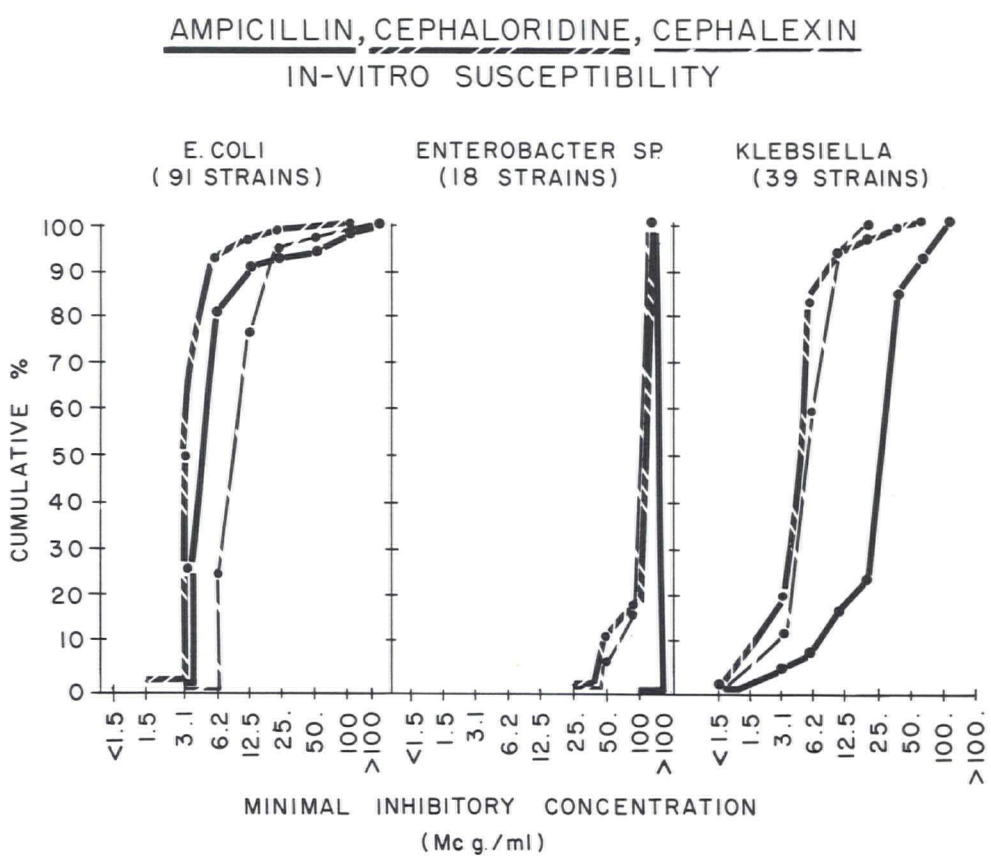

Figure 1

6.2 mcg or less of cephaloridine, 80% to 6.2 mcg or less of ampicillin, and, while only 28% were susceptible to 6.2 mcg or less of cephalexin, 95% were inhibited by 25 mcg or less. For cephaloridine and ampicillin at this level, the figures are 99% and 91% respectively. Thirty-nine strains of Klebsiella were tested and more than 90% were inhibited by 12.5 mcg or less of both cephalexin and cephaloridine while only 8% were susceptible to 12.5 mcg or less of ampicillin. In contrast, all 18 strains of Enterobacter studied were resistant to more than 25 mcg of all three agents.

The results with 35 strains of proteus species are shown in Figure 2. Twenty-three were indole negative and 12 indole positive. For the indole negative strains, ampicillin was most potent with 100% of strains inhibited by 6.2 mcg per ml. Cephaloridine inhibited all strains at a concentration of 12.5 mcg or less. Cephalexin showed comparable activity though 8% of strains required 50 mcg/ml for inhibition. All 12 strains of indole positive Proteus proved resistant to more than 50 mcg/ml of all three antibiotics.

The results for staphylococci are shown in Figure 3. All strains of non-penicillinase producing staphylococci were highly susceptible to ampicillin (0.2 mcg/ml or less) while none of the penicillinase producing strains were in-

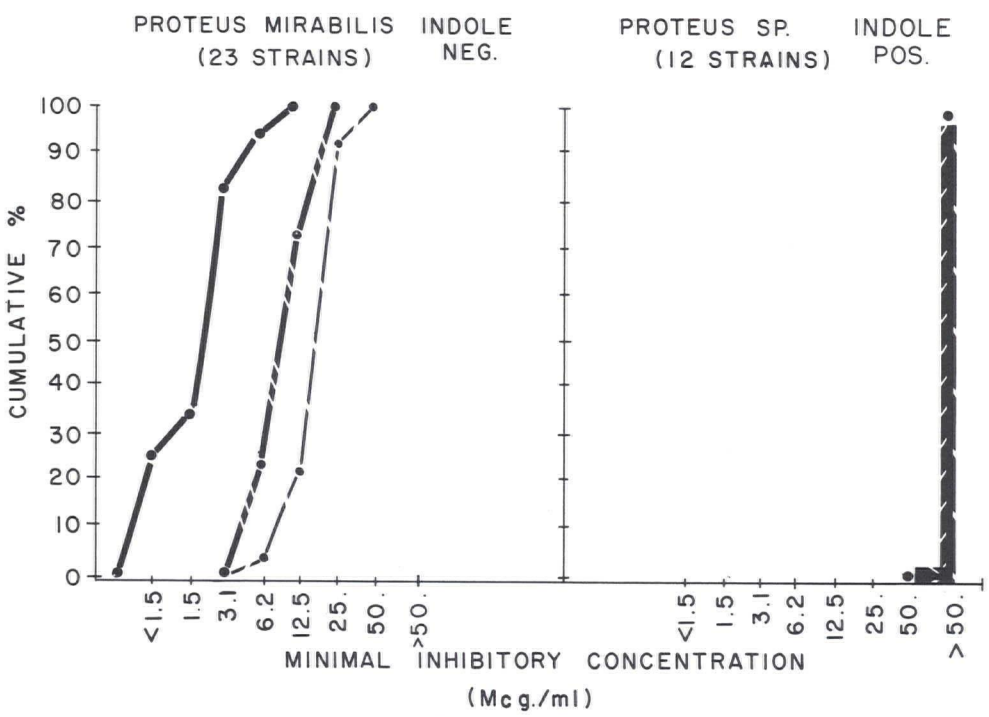

Figure 2

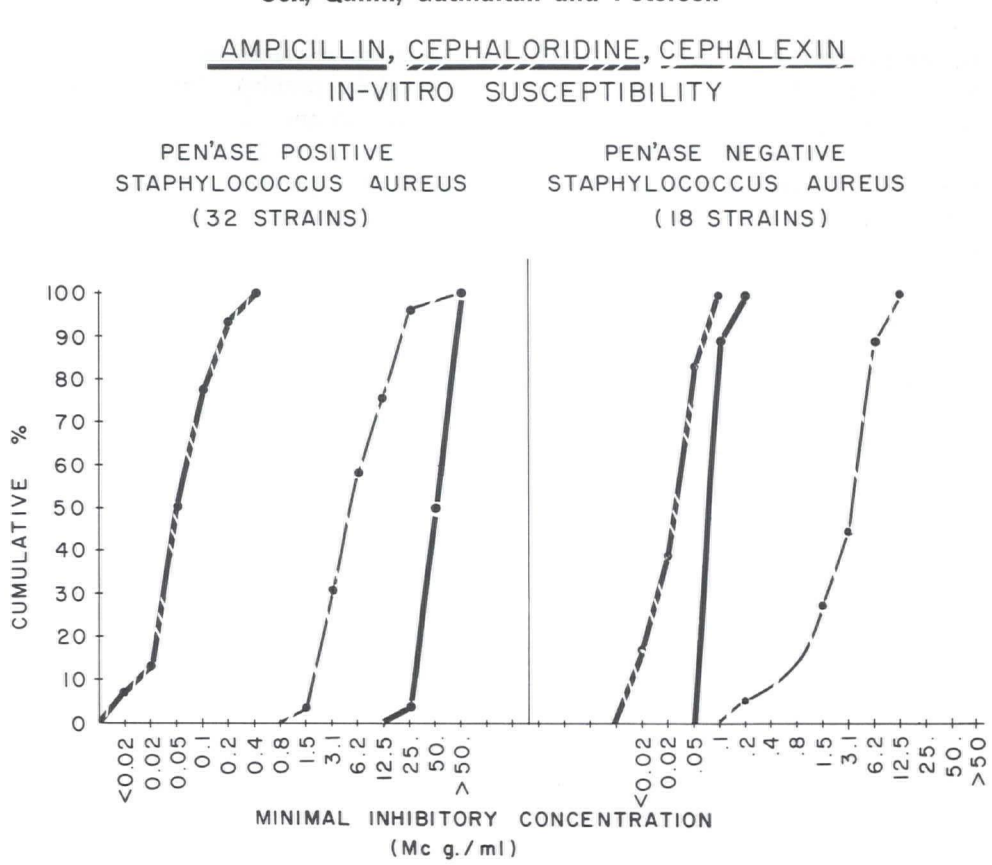

Figure 3

hibited by less than 25 mcg/ml. Both groups were inhibited by low levels of cephaloridine, but were less susceptible to cephalexin. For the penicillinase producing strains, the MICs ranged from 0.2 to 12.5 mcg/ml while of the non-penicillinase producing strains 75% were inhibited by 12.5 mcg/ml or less, 97% by 25 mcg/ml and 3% (one strain) was resistant to more than 50 mcg/ml.

Susceptibility of the other pathogenic gram-positive organisms is shown in Figure 4. Both the Pneumococcus and Group A streptococcus were inhibited by low levels of ampicillin and cephaloridine, while somewhat higher levels of cephalexin were required. Eighty percent of pneumococcal strains were sensitive to 1.5 mcg or less of cephalexin and all were inhibited by 6.2 mcg/ml. The sensitivities to cephalexin of the Group A streptococci fell between 0.1 and 1.5 mcg/ml.

Serum levels and urinary excretion of cephalexin were studied in normal adult volunteers. These were compared to the values obtained by Bunn and coworkers[5] for ampicillin and values for cephaloridine obtained previously in this laboratory.[4] The mean serum concentrations after a single oral dose of 250 mg of cephalexin are shown in Table I. Peak levels of cephalexin oc-

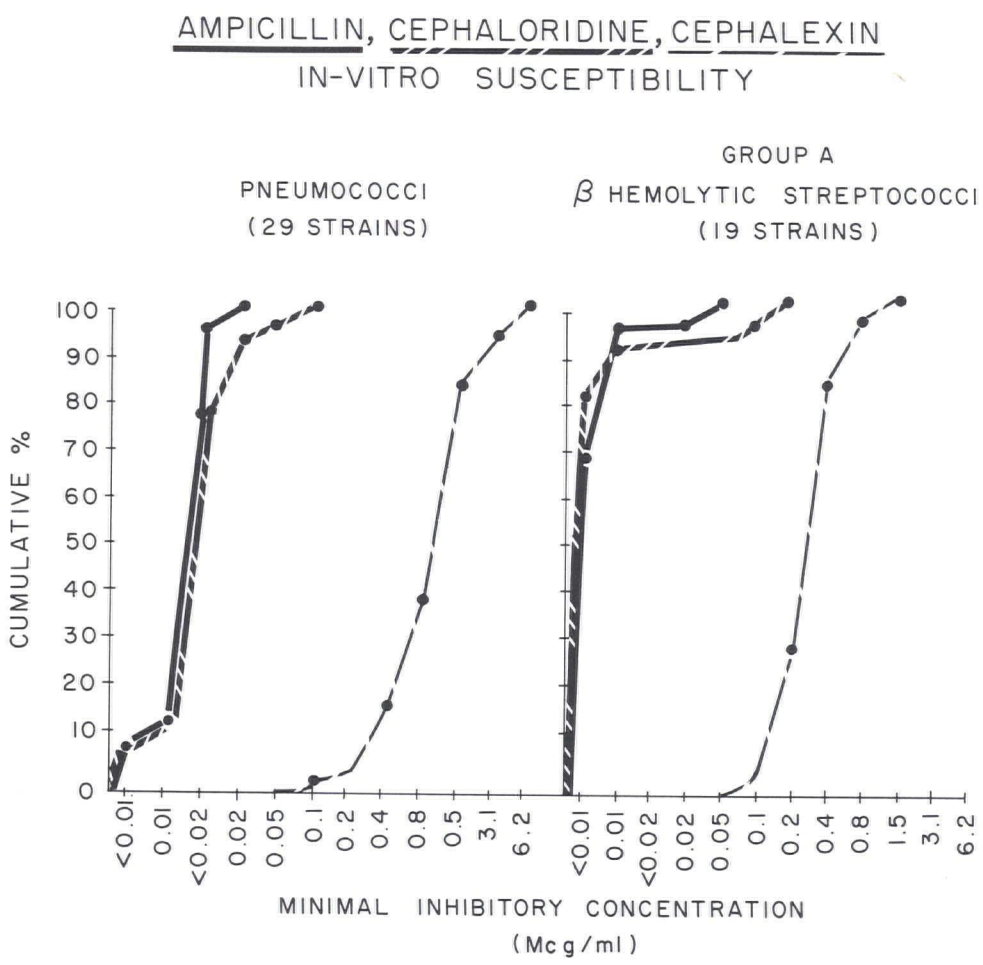

Figure 4

TABLE I. Serum Concentrations and Urinary Excretion After Single 250 mg. Dose

	Ampicillin* (Oral) mcg./ml.	Cephaloridine** (I.M.) mcg./ml.	Cephalexin (Oral) mcg./ml.
1/2	--	9.2	5.7
1 hr.	1.6	10.5	10.9
2 hr.	2.0	5.5	4.9
4 hr.	1.6	2.0	1.0
6 hr.	--	--	< 0.6
6 hr. Urinary Excretion (% of administered dose)	25.0%	60.4%	100.0%

* After Bunn (5)
** After Lane, et al (4)

curred at one hour and the levels at one and two hours are quite comparable to those observed with intramuscular cephaloridine. In contrast, the peak level of ampicillin was considerably lower, although at four hours similar levels were observed with all three. Urinary excretion amounted to 60% and 100% of the administered dose of cephaloridine and cephalexin respectively. Urinary excretion of ampicillin is 30%-40% of an administered dose.

In Table II, the results with the 500 mg dose are shown. All levels were higher, and again the relative relationship among the three antibiotics was maintained.

Protein binding of the three antibiotics was assayed by the method of determining the relative efficacy of each in broth medium as compared to serum. Ampicillin was 25% bound to serum protein, cephalexin 40%, and cephaloridine was not bound to any appreciable degree.

Clinical Studies

The efficacy of cephalexin was evaluated in 47 patients with a variety of clinical illnesses. Five patients were excluded from consideration because of incomplete data.

In Table III, the results of treatment in 14 patients with urinary tract infections who received two grams of cephalexin per day for seven days are shown. Five were cleared of bacteriuria, and their urine remained sterile over a six-week period. These are classified as cured. Nine of the patients experienced a satisfactory remission of their symptoms, while under treatment, and their urine was rendered free of bacteria. These nine patients showed bacteriological relapse, however, during the six-week followup period.

Five patients with soft tissue infection caused by Group A beta hemolytic streptococci, coagulase positive staphylococci, or both were treated with from 1-2 grams of cephalexin daily and all were cured, as noted in Table IV.

Table V depicts the results in 12 patients with pneumonia who were treated with two grams of cephalexin daily for seven days; nine were classi-

TABLE II. Serum Concentrations and Urinary Excretion After Single 500 mg. Dose

	Ampicillin* (Oral) mcg./ml.	Cephaloridine** (I.M.) mcg./ml.	Cephalexin (Oral) mcg./ml.
1/2 hr.	--	11.2	10.8
1 hr.	3.3	15.6	21.2
2 hr.	3.7	10.8	10.1
4 hr.	1.6	4.6	2.6
6 hr.	0.8	--	0.7
6 hr. Urinary Excretion (% of administered dose)	25.0%	68.5%	100.0%

* After Bunn (5)
** After Lane, et al (4)

TABLE III. Clinical Results of Patients Treated with Cephalexin

	Cured	Improved	Failed
Urinary Tract Infection			
Acute			
Klebsiella	1		
E. Coli	2*	2	
Proteus Mirabilis		1	
E. Coli and Proteus Mirabilis		1	
Chronic			
E. Coli	1	2	
Klebsiella		1	
Proteus Mirabilis and Enterococcus		1	
Asymptomatic			
E. Coli			1
Klebsiella and Enterococcus	1		

* One patient had initial IV Keflin

TABLE IV. Clinical Results of Patients Treated with Cephalexin

	Cured	Improved	Failed
Soft Tissue Infection			
Beta Hemolytic Streptococcus (Group A)	1		
Staphylococcus (Coagulase Positive)	2		
Beta Hemolytic Streptococcus (Group A) and Staphylococcus (Coagulase Positive)	2		

TABLE V. Clinical Results of Patients Treated with Cephalexin

	Cured	Improved	Failed
Pneumonia			
Pneumococcus	4	1	
with bacteremia	1		1
Hemophilus parainfluenza			1
E. Coli with bacteremia	1		
Undetermined	3		

TABLE VI. Clinical Results of Patients Treated with Cephalexin

	Cured	Improved	Failed
Pharyngitis			
Beta Hemolytic Streptococcus Group A	4		
Non Group A Plus Staphylococcus (Coagulase Positive)	4	1	1
Peritonsillar Abscess			
Beta Hemolytic Streptococcus (Group A)		1	
Acute Pharyngitis and Sinusitis			
Beta Hemolytic Streptococcus (Group A)		1	

fied as cured; one improved, with two failures. One patient with pneumococcal pneumonia improved clinically, but pneumococci of the same group were isolated from the sputum during and after the course of treatment. One patient with E. coli pneumonia and bacteremia was cured; however, another with pneumonia caused by H. influenzae failed.

Twelve patients with pharyngitis due to both Group A and nonGroup A beta hemolytic streptococci were treated with one gram of cephalexin for 10 days. It can be seen in Table VI that eight were cured and three had remission of symptoms, although the organism could still be cultured from the pharynx. One patient had an associated coagulase positive staphylococcal infection and failed to respond.

No major reactions to cephalexin were observed. One patient who had received ampicillin prior to the cephalexin experienced mild diarrhea, but was able to complete the course of cephalexin.

Discussion

The place of the currently available cephalosporin derivatives in the treatment of susceptible infections, particularly in patients who are allergic to penicillin, is clearly established. The use of cephalosporins has been limited by the necessity to employ them parenterally. Cephalexin administered orally was shown to produce serum levels that approached those attained with parenteral cephaloridine, while cephalexin's *in vitro* efficacy against the common gram-positive pathogens, such as Group A streptococci, pneumococci, and staphylococci, was less than cephaloridine on a mg for mg basis. Nonetheless, the serum level necessary for inhibitions of these organisms was well within the achievable range for cephalexin. For the susceptible gram-negative organisms, E. coli, Klebsiella, and Proteus mirabilis, cephalexin was almost as potent as cephaloridine. The high levels of urinary excretion of cephalexin suggested that it would be useful in the treatment of urinary tract infections caused by susceptible organisms. Lewison and co-workers[6] reported 23 patients with urinary tract infections treated with cephalexin; all became abacteriuric within 72 hours of initiation of treatment, and 10 remained free of infections for at least two months after treatment was discontinued. Our results are similar in the 14 patients with urinary tract infections. All became abacteriuric while on treatment and five remained clear for six weeks after cessation of cephalexin.

In our patients with pneumonia, three of 11 had a suboptimal result. This may be due to marginal serum drug levels. An increased dose of cephalexin might yield better results. The same may apply to four of 12 pharngidites, three exhibited a satisfactory clinical response, although the organism could still be cultured during treatment. In the fourth, a co-existent

staphylococcus and nonGroup A beta hemolytic streptococcus failed to respond. We suspect the dose of cephalexin was inadequate in this case, since higher serum levels are necessary to inhibit many strains of staphylococci.

In summary, cephalexin was shown to produce high serum and urine levels when administered by the oral route, was well tolerated, and was effective against infections caused by susceptible organisms.

Acknowledgement

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