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In Vitro Activities of Five Oral Cephalosporins Against Aerobic Pathogenic Bacteria

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Cefaclor (Lilly 99638) and cefatrizine (BL-S640, SK&F 70771) are orally absorbed, broad-spectrum semisynthetic cephalosporins. They were compared in vitro with cephalexin, cephaloglycin, and cepharadine against a variety of aerobic pathogenic bacteria by an agar dilution procedure. Cefaclor and cefatrizine were found to be similar or superior to cephalexin, cephaloglycin, and cephradine in terms of activity against gram-positive cocci other than enterococci. Only cefatrizine demonstrated any potentially useful activity against some susceptible isolates of enterococci. Cefaclor and cefatrizine also were highly active, equally or more so than the other oral cephalosporins, against several gram-negative species including *Escherichia coli, Enterobacter aerogenes,* and *Klebsiella pneumoniae*. None of the cephalosporins were particularly active against *Enterobacter cloacae*. Both cefaclor and cefatrizine were active against *Proteus mirabilis*; cefatrizine was uniquely active against indole-positive *Proteus* species.

Cefaclor [3-chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid; Lilly 99638] and cefatrizine {7-D-α-amino-α-[4-hydroxylphenyl]-acetamido-[3-(1H-1,2,3-triazol-5-thio)-methyl]-3-cephem-4-carboxylic acid; BL-S640, SK&F 06771} are semisynthetic broad-spectrum cephalosporin antibiotics active against both gram-positive and gram-negative bacteria including penicillin-resistant organisms (1, 2, 6). Metabolic studies in animals have shown that cefaclor is readily absorbed from the gastrointestinal tract as intact drug (8). Cefatrizine also is absorbed from the gastrointestinal tract of rodents and is effective orally against experimental infections in mice (7). In vitro, cefatrizine is said to be more active than cephalothin or cephalexin against clinically important gram-negative and gram-positive bacteria not including *Haemophilus influenzae* and *Streptococcus pneumoniae* (10) but is less active than cephradine, cephalothin, and cefazolin against *Staphylococcus aureus* (9). Cefaclor recently has been reported to be more active in vitro than either cephalexin or cephradine against a large number of gram-positive and gram-negative pathogens but less resistant to staphylococcal penicillinase (2).

The purpose of this study was to compare the in vitro activity of cefaclor with that of cefatrizine and, further, to compare these two new oral cephalosporins with cephalexin, cephradine, and cephaloglycin, three oral cephalosporins now used clinically.

MATERIALS AND METHODS

Antibiotics. Cefaclor (Lilly 99638, lot S1-100-6C, activity of 961 μg/mg), cephaloglycin (lot 9NK09, activity of 890 μg/mg), and cephalexin monohydrate (lot S1-89-6B, activity of 940 μg/mg) were obtained from Lilly Research Laboratories. Cefatrizine (BL-S640PG, lot 75-F396, activity of 868 μg/mg) was obtained from Bristol Laboratories, and cephradine monohydrate (batch NN083NE, activity of 967 μg/mg) was obtained from the Squibb Institute for Medical Research. Solutions of cefatrizine, cephradine, and cephaloglycin were prepared in sterile distilled water. Cefaclor was dissolved in 0.1 M Sorenson buffer, pH 4.5, and cepahlexin was dissolved in 1.0% sorenson buffer, pH 6.0. All solutions were sterilized by membrane filtration.

Susceptibility testing. A modification of the standardized ICS agar dilution procedure was used (5). The test medium was Mueller-Hinton agar, which was supplemented with blood for use with fastidious organisms or with additional agar, for a total of 4%, for use with *Proteus* species. Test concentrations of drugs ranged from 128 to 0.063 μg/ml (actual adjusted activity). Plates containing cefatrizine, cephradine, or cephalexin were prepared 1 to 2 days in advance; plates containing cefaclor or cephaloglycin were prepared the same day as used. Inocula were prepared from overnight broth cultures grown in Mueller-Hinton broth. They were adjusted turbimetrically to contain approximately 10^8 cells per ml. Plates were inoculated using a Steers replicator (Melrose Machine Shop, Woodlyn, Pa.) and were incubated at 35°C for 24 h. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of drug inhibiting growth as indicated

by the presence of three discrete colonies or less or a barely visible haze at the inoculation site.

A total of 195 clinical isolates of gram-positive and gram-negative aerobic bacteria were tested. These included penicillin-susceptible *S. aureus* (25 isolates), *Streptococcus pyogenes* (20 isolates), *S. pneumoniae* (16 isolates), enterococci (25 isolates), *Escherichia coli* (25 isolates), *Enterobacter aerogenes* (11 isolates), *Enterobacter cloacae* (14 isolates), *Klebsiella pneumoniae* (21 isolates), *Proteus mirabilis* (24 isolates), and indole-positive *Proteus* species (14 isolates). Identifications of gram-positive organisms were based on microscopic morphology, macroscopic growth characteristics, bile solubility, coagulase activity, and growth in salt broth. Gram-negative organisms were identified by the API-20E system (Analytab Products, Inc.). *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 were included in all individual test runs.

Data analyses. Both geometric (G) and arithmetic means ($\bar{x}$) and standard deviations were determined for MIC responses against each species. Cumulative percentages of inhibitions were determined for each species. Geometric mean MIC values of cefaclor and cefatrizine were compared with each other and with those of cephalexin, cephaloglycin, and cephradine by Student's *t* test.

RESULTS

In vitro inhibitory activities of cefaclor, cefatrizine, cephalexin, cephaloglycin, and cephradine against gram-positive bacteria are presented, as cumulative percentages of inhibition, in Table 1 and against gram-negative organisms in Table 2.

Generally, cefatrizine and cefaclor were the most active of the five oral cephalosporins agains susceptible gram-positive cocci. Eighty percent of *S. aureus* isolates was inhibited by cefatrizine in a concentration of 1.0 μg/ml, whereas a similar concentration of cephradine inhibited only 56% of isolates tested. Cefaclor was significantly less active against *S. aureus* than either cefatrizine, cephalexin, or cephradine ($P < 0.01$, < 0.05, and < 0.02, respectively) but similar in activity to cephaloglycin.

Cefaclor and cefatrizine were the most active

TABLE 1. *In vitro activities of five oral cephalosporins against 86 isolates of gram-positive aerobic bacteria*[a]

Species (no. tested)	Antibiotic	G[b]	$\bar{x}$[c]	Concnt (μg/ml) and cumulative percentage of inhibition[d]								
				<0.5	1.0	2	4	8	16	32	64	≥128
S. aureus (25)	Cefaclor	2.17	2.76 ± 2.93		20	76	96	96	100			
	Cefatrizine	1.06	1.21 ± 0.73	12	80	96	100					
	Cephalexin	1.74	2.00 ± 1.41		32	92	96	100				
	Cephaloglycin	2.23	2.58 ± 1.52	4	12	72	96	100				
	Cephradine	1.52	1.84 ± 1.54		56	88	96	100				
S. pyogenes (20)	Cefaclor	0.11	0.20 ± 0.29	90	100							
	Cefatrizine	0.10	0.21 ± 0.34	85	100							
	Cephalexin	0.35	1.28 ± 2.11	70	75	80	95	100				
	Cephaloglycin	0.17	0.51 ± 0.96	80	90	95	100					
	Cephradine	0.19	0.63 ± 1.06	75	80	95	100					
S. pneumoniae (16)	Cefaclor	0.24	0.49 ± 0.96	88	94	94	100					
	Cefatrizine	0.22	0.53 ± 0.98	81	94	94	100					
	Cephalexin	0.87	1.64 ± 1.38	31	50	81	100					
	Cephaloglycin	0.32	0.59 ± 0.55	63	94	100						
	Cephradine	0.52	1.32 ± 2.05	56	75	88	94	100				
Enterococcus species (25)	Cefaclor	52.7	60.2 ± 13.9		4	4	4	4	4	8	100	
	Cefatrizine	40.0	50.4 ± 31.0				4	8	16	48	92	100
	Cephalexin	97.0	109 ± 36.0					4	4	8	24	100
	Cephaloglycin	99.7	106 ± 32.9							4	32	100
	Cephradine	57.3	65.4 ± 28.1				4	4	4	16	88	100

[a] As determined by a modification of the ICS agar dilution procedure with Mueller-Hinton agar and incubation at 35°C for 24 h.

[b] Geometric mean MIC, micrograms per milliliter.

[c] Plus or minus the standard deviation of the mean.

[d] Percentages rounded to nearest whole number.

TABLE 2. *In vitro activities of five oral cephalosporins against 109 isolates of gram-negative aerobic bacteria*[a]

Species (no. tested)	Antibiotic	G[b]	$\bar{x}$[c]	Concn. (μg/ml) and cumulative percentage inhibited[d]					
				<4	8	16	32	64	≥128
E. coli (25)	Cefaclor	1.94	2.56 ± 2.97	96	96	100			
	Cefatrizine	2.30	2.56 ± 1.44	96	100				
	Cephalexin	2.87	3.52 ± 3.12	88	96	100			
	Cephaloglycin	4.47	5.04 ± 2.95	76	96	100			
	Cephradine	10.8	11.8 ± 5.84		60	96	100		
E. aerogenes (11)	Cefaclor	26.5	50.7 ± 51.0	18	27	27	73	73	100
	Cefatrizine	26.5	45.3 ± 45.2	9	9	55	64	82	100
	Cephalexin	53.0	65.5 ± 43.2			9	46	73	100
	Cephaloglycin	28.2	57.3 ± 56.8	18	18	46	64	64	100
	Cephradine	56.4	68.4 ± 41.8			9	37	73	100
E. cloacae (14)	Cefaclor	86.1	98.3 ± 42.5				21	36	100
	Cefatrizine	67.3	84.6 ± 47.6			14	29	50	100
	Cephalexin	110.3	114 ± 27.3					21	100
	Cephaloglycin	95.1	112 ± 40.7			14	14	14	100
	Cephradine	58.0	70.9 ± 41.5			14	29	71	100
K. pneumoniae (21)	Cefaclor	0.82	5.00 ± 14.1	81	90	95	95	100	
	Cefatrizine	2.52	12.1 ± 28.3	67	76	86	95	95	100
	Cephalexin	6.35	11.3 ± 17.9	67	81	90	90	100	
	Cephaloglycin	7.74	22.9 ± 38.3	57	67	71	86	90	100
	Cephradine	9.43	17.1 ± 28.8	19	81	86	90	95	100
P. mirabilis (24)	Cefaclor	2.59	27.5 ± 52.7	79	79	79	79	79	100
	Cefatrizine	4.24	6.75 ± 8.66	79	83	92	100		
	Cephalexin	16.5	20.5 ± 23.3	4	8	92	96	96	100
	Cephaloglycin	5.82	6.33 ± 2.87	50	96	100			
	Cephradine	21.4	28.7 ± 23.1	4	4	42	96	96	100
Proteus species, indole positive (14)	Cefaclor	64.0	100 ± 47.7	14	14	14	14	29	100
	Cefatrizine	15.2	29.3 ± 42.2	21	21	86	86	86	100
	Cephalexin	90.5	107 ± 42.3			14	14	21	100
	Cephaloglycin	55.2	77.7 ± 49.3		14	21	29	57	100
	Cephradine	95.1	109 ± 39.8			7	14	21	100

[a] As determined by a modification of the ICS agar dilution procedure with Mueller-Hinton agar and incubation at 35°C for 24 h.

[b] Geometric mean MIC, micrograms per milliliter.

[c] Plus or minus the standard deviation of the mean.

[d] Percentages rounded to nearest whole number.

cephalosporins against *S. pyogenes*; all 20 isolates were inhibited by 1.0 μg of either drug per ml. Cephaloglycin was the next most active; in a concentration of 1.0 μg/ml, it inhibited 90% of isolates. Differences between cefaclor and cefatrizine and between cefatrizine and cephaloglycin were not significant. Cephalexin and cephradine were the least active against *S. pyogenes* and, based upon a comparison of G values, were approximately two to four times less active than either cefaclor or cefatrizine, although 4.0 μg of cephalexin and cephradine per ml inhibited 95 and 100% of isolates, respectively. Differences between cefaclor or cefatrizine and cephalexin and cephradine were significant ($P < 0.05$).

Cefaclor and cefatrizine were the most active cephalosporins against *S. pneumoniae*, both were over twofold more active than cephadrine and nearly fourfold more active than cephalexin. Differences between cefaclor or cefatrizine and cephalexin were highly significant (P

≤ 0.002). Differences between cefaclor or cefatrizine and cephradine also were significant ($P \leq 0.05$). Cephaloglycin was only slightly less active than either cefaclor or cefatrizine; 1.0 μg of either of the three drugs per ml was inhibitory for 94% of isolates. Similar concentrations of cephradine and cephalexin inhibited 75 and 50% of isolates, respectively.

None of the five cephalosporins, at concentrations of less than 8 μg/ml, demonstrated any remarkable activity against enterococci, although cefatrizine was the most active; 32 μg of cefatrizine per ml inhibited 48% of isolates. Cefaclor and cephradine were the next most active, inhibiting 100 and 88% of isolates, respectively, when used in a concentration of 64 μg/ml. Differences between cefaclor and cefatrizine were not significant ($P > 0.05$), but differences between cefatrizine and cephradine were ($P < 0.001$). Differences between cefaclor or cefatrizine and cephalexin or cephaloglycin were highly significant ($P < 0.001$).

Generally, cefaclor and cefatrizine were the most active cephalosporins against gram-negative aerobic species (Table 2). This was most pronounced in studies with *K. pneumoniae*. At a concentration of 1.0 μg/ml, cefatrizine and cefaclor inhibited 62 and 76%, respectively, of isolates tested. No inhibition was observed with cephalexin, cephaloglycin, or cephradine at this same concentration. Differences between cefaclor and cefatrizine and the other three compounds were significant ($P < 0.05$). Cefaclor and cefatrizine were the most active against *E. coli*, and cefaclor was 1.5 to 5.5 times more active than either cephalexin, cephaloglycin, or cephradine; differences between cefaclor and the latter drugs were significant ($P < 0.001$). Cefatrizine was two to five times more active than either cephaloglycin or cephradine ($P < 0.001$) but only slightly more active than cephalexin ($P = 0.02$).

Striking differences were observed in results with *E. aerogenes* and *E. cloacae*. Cefatrizine and cephaloglycin were the most active cephalosporins against *E. aerogenes*; in a concentration of 16 μg/ml, they inhibited 55 and 46%, respectively. In contrast, similar concentrations inhibited only 14% of isolates of *E. cloacae*. Both cefaclor and cefatrizine were twofold more active than either cephalexin or cephradine against *E. aerogenes* but only slightly more active than cephaloglycin. Only cephradine and cefatrizine demonstrated any degree of activity against *E. cloacae*, inhibiting 29% of isolates at 32 μg of either drug and 71 and 50%, respectively, at 64 μg of either drug per ml.

Two micrograms of cefaclor per ml was the most active cephalosporin against susceptible isolates of *P. mirabilis* with inhibition of 79% of isolates; however, five isolates were resistant. Both cefaclor and cefatrizine were fourfold or more active than either cephalexin or cephradine. Cephaloglycin was the third most active cephalosporin against *P. mirabilis*. Differences between cephaloglycin and cefaclor or cefatrizine were not significant, although cephaloglycin was twofold less active than cefaclor.

Cefatrizine was the most active drug against indole-positive *Proteus* species, being some fourfold more active than cefaclor and six times more active than the least active drugs, cephalexin and cephradine. The activity of cefaclor was significantly less than that of cefatrizine ($P < 0.001$) but did not differ significantly from those of cephalexin, cephaloglycin, and cephradine.

DISCUSSION

Both cefaclor and cefatrizine have been shown to be readily absorbed from the gastrointestinal tracts of experimental animals (1, 8). In humans, it has been stated that peak cefaclor plasma concentrations are obtained 30 to 45 min after oral administration; these ranged as high as 30 μg/ml, depending upon dose. Cefatrizine also is absorbed orally and is said to produce higher peak serum levels and to have a longer biological half-life than either cefazolin or cephalexin (1).

Our data show cefaclor to be comparable to cephradine and cephaloglycin in terms of in vitro activity against *S. pyogenes* and somewhat superior to cephalexin. Cefaclor also is comparable if not superior to cephalexin in terms of activity against *S. pyogenes* and *S. aureus*. Like cephalexin and cephaloglycin, cefaclor was devoid of clinically significant activity against enterococci.

In our study, cefaclor clearly was the most active drug against gram-negative bacteria including *K. pneumoniae*, *E. coli*, and *P. mirabilis*. Cefaclor also clearly was superior to cephalexin, cephaloglycin, and cephradine against these organisms as well as against *E. aerogenes* but not *E. cloacae*. These results agree with those of Bill and Washington (2).

Cefatrizine was comparable, if not identical, to cefaclor in terms of activity against *S. pyogenes* and *S. pneumoniae* and superior to cephalexin against *S. aureus*, *S. pyogenes*, *S. pneumoniae*, and enterococci. These latter results are comparable to those of both Del Busto et al. (4), obtained with the ICS agar dilution procedure, and Blackwell et al. (3), obtained with a broth dilution procedure. Most importantly, cefatrizine also was the only oral ceph-

alosporin with possible clinically significant activity against enterococci.

The activity of cefatrizine against gram-negative bacteria was, in most instances, comparable to that of cefaclor. As previously reported by Actor et al. (1) and Del Busto et al. (4), cefatrizine was, in our study, superior to cephalexin against *E. coli*, *K. pneumoniae*, and *P. mirabilis*. Our study also shows cefatrizine and cefaclor to be comparable against *E. coli* and *E. aerogenes* and superior to cephalexin, cephaloglycin, or cephradine. Cefatrizine was less active than cefaclor but again superior to cephalexin, cephaloglycin, or cephradine against *K. pneumoniae*. Cefatrizine also was highly active against indole-positive *Proteus* species.

In conclusion, our data indicate that the in vitro activities of cefaclor and cefatrizine are similar to each other and generally superior to those of cephalexin, cephaloglycin, or cephradine against clinical isolates of most gram-positive cocci, except enterococci, as well as against *E. coli*, *K. pneumoniae*, and *E. aerogenes*. Both cefaclor and cefatrizine have unique activities, cefaclor being uniquely active against *P. mirabilis* and cefatrizine against indole-positive *Proteus* species as well as against some enterococci.

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LITERATURE CITED

1. **Actor, P., J. V. Uri, L. Phillips, C. S. Sachs, J. R. Guarini, I. Zajac, D. A. Berges, G. L. Dunn, J. R. E. Hoover, and J. A. Weisback.** 1975. Laboratory studies with cefatrizine (SK&F 60771) a new broad-spectrum orally-active cephalosporin. J. Antibiot. 28:594–601.
2. **Bill, N. J., and J. A. Washington, II.** 1977. Comparison of in vitro activity of cephalexin, cephradine and cefaclor. Antimicrob. Agents Chemother. 11:470–474.
3. **Blackwell, C. C., E. H. Freimer, and G. C. Tuke.** 1976. In vitro evaluation of the new oral cephalosporin cefatrizine: comparison with other cephalosporins. Antimicrob. Agents Chemother. 10:288–292
4. **Del Busto, R., E. Haas, T. Madhavan, K. Burch, F. Cox, E. Fisher, E. Quinn, and R. Pohlod.** 1976. In vitro and clinical studies of cefatrizine, a new semisynthetic cephalosporin. Antimicrob. Agents Chemother. 9:397–405.
5. **Ericsson, H. M., and J. C. Sherris.** 1971. Antibiotic sensitivity testing. Report of an international collaborative study. Acta Pathol. Microbiol. Scand. Suppl. B 217:1–90.
6. **Leitner, F., R. E. Buck, M. Misiek, T. A. Pursiano, and K. E. Price.** 1975. BL-S640, a cephalosporin with a broad spectrum of antibacterial activity: properties in vitro. Antimicrob. Agents Chemother. 7:298–305.
7. **Leitner, F., D. R. Chisholm, Y. H. Tsai, G. E. Wright, R. G. Deregis, and K. E. Price.** 1975. BL-S640, a cephalosporin with a broad-spectrum of antibacterial activity: bioavailability and therapeutic properties in rodents. Antimicrob. Agents Chemother. 7:306–310.
8. **Sullivan, H. R., S. L. Due, D. L. K. Kau, J. F. Quay, and W. M. Miller.** 1976. Metabolism of [^{14}C]cefaclor, a cephalosporin antibiotic, in three species of laboratory animals. Antimicrob. Agents Chemother. 10:630–638.
9. **Vuye, A., J. Pijck, and H. Soep.** 1976. In vitro activity of BL-S640 against gram-negative bacilli and *Staphylococcus aureus* compared with activity of four other semisynthetic cephalosporins. Antimicrob. Agents Chemother. 9:422–432.
10. **Yourassowsky, E., E. Schoutens, and M. P. Vanderlinden.** 1975. Comparative inhibitory activity of BL-S640 and two other cephalosporins. J. Antibiot. 28:590–593.