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Comparison of In Vitro Antibacterial Activity of Three Oral Cephalosporins: Cefaclor, Cephalexin, and Cephradine

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Cefaclor, a new oral cephalosporin, was compared in vitro with cephalexin and cephradine against 233 organisms. Evaluations were performed in Mueller-Hinton and nutrient broth and agar using two inoculum sizes. In agar, cefaclor had greater antibacterial activity than either cephalexin or cephradine against isolates of *Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Klebsiella pneumoniae,* and *Salmonella typhi*. All three drugs were relatively inactive against isolates of enterococci, *Enterobacter* species, and indole-positive *Proteus*. Cefaclor, however, did exhibit the greatest activity of the three antibiotics against these organisms. Although there was wide variability with respect to test parameters, the broth results generally paralleled the agar results. In nutrient broth a clear separation of the results with these three cephalosporins was seen with *K. pneumoniae, E. coli,* and *S. typhi*. Cefaclor was the most active, cephalexin had intermediate activity, and cephradine was the least active. From the data obtained in this in vitro study, it can be concluded that cefaclor, which has a substituted chloro group attached to the molecule, had increased antibacterial activity over cephalexin and cephradine. Comparative clinical trials with cefaclor will determine whether the differences outlined above are of clinical significance.

Cefaclor, 3-chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid, is a new orally absorbed semisynthetic cephalosporin. This drug is similar to cephalexin and cephradine in both structure and reported blood levels attained after administration of comparable dosages (Lilly Research Laboratories, Indianapolis, Ind.; Smith Kline & French Laboratories, Philadelphia, Pa.) (2). However, by substitution of a chloro group for the methyl group present in both cephradine and cephalexin molecules, cefaclor may be more active than other cephalosporin antibiotics.

This study was designed to compare the in vitro antibacterial activity of cefaclor, cephradine, and cephalexin against certain selected bacterial species.

MATERIALS AND METHODS

Broth and agar dilution methods were used to determine minimum inhibitory concentrations (MIC) of the three antibiotics by methods previously described from this laboratory (3, 5). Mueller-Hinton broth (MHB, Difco), Mueller-Hinton agar (MHA), nutrient broth (NB, Difco), and nutrient agar (NA) were used. Minimum bactericidal concentrations (MBC) were defined as the lowest concentration of antibiotic that yielded 10 viable colonies or less when 0.005 ml was subcultured onto a blood agar plate containing no antibiotic. Agar dilution studies were performed by using a Steers replicator device (4), and MICs were defined as the lowest concentration of drug that yielded three viable colonies or less after incubation at 37°C for 18 h. A total of 233 isolates of various microorganisms were studied including isolates of *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Staphylococcus aureus, Salmonella typhi,* enterococci, and *Enterobacter* species representing 10 strains of *E. aerogenes,* 8 of *E. cloacae,* 4 of *E. hafniae,* 4 of *E. liquefaciens,* and 4 of *E. agglomerans*. Indole-positive *Proteus* species were also tested, including 10 strains of *P. vulgaris,* 8 of *P. morganii,* and 7 of *P. rettgeri*. Inoculum sizes of 10^5 and 10^7 organisms per ml were used for testing gram-negative organisms, and 10^4 and 10^6 organisms per ml were used for *S. aureus*. A laboratory reference strain of *E. coli* inhibited by 1.0 μg of cefaclor per ml, 7.5 μg of cephradine per ml, and 5.0 μg of cephalexin per ml was used as a control throughout the experiments.

RESULTS

Agar dilutions. The cumulative percentages of isolates of *E. coli, S. typhi, K. pneumoniae, P. mirabilis, S. aureus, Enterobacter* species, enterococci, and indole-positive *Proteus* inhibited by increasing concentrations of cefaclor, cephradine, and cephalexin in agar dilution testing are shown in Tables 1 and 2. Cefaclor

TABLE 1. *Agar dilution MICs for cefaclor, cephradine, and cephalexin obtained using two inoculum sizes of bacterial cells and MHA*

Organism (no.)	Inoculum size (organisms/ml)	Median MIC	Cumulative percentage of strains susceptible with MIC (μg/ml) of:												
			≤0.5	1.0	2.5	5.0	7.5	10	12.5	15	20	25	50	100	>100
E. coli (30)															
CC[a]	10^7	2.5	0	40	100										
	10^5	1.0	17	97	100										
CD	10^7	7.5				0	53	77	97	97	100				
	10^5	7.5			0	20	73	97	97	100					
CL	10^7	7.5			0	27	80	97	97	100					
	10^5	5.0			0	80	97	100							
S. aureus (30)															
CC	10^6	2.5	0	10	77	100									
	10^4	1.0	30	57	100										
CD	10^6	5.0		0	30	93	100								
	10^4	5.0		0	47	100									
CL	10^6	5.0		0	30	93	100								
	10^4	2.5		0	60	100									
K. pneumoniae (30)															
CC	10^7	1.0	10	87	93	100									
	10^5	0.75	50	93	100										
CD	10^7	7.5			0	7	67	80	87	90	90	93	100		
	10^5	5.0			0	57	83	90	90	90	97	97	100		
CL	10^7	5.0		0	7	83	90	90	97	100					
	10^5	5.0		0	43	90	100								
S. typhi (30)															
CC	10^7	1.0	0	97	97	97	97	100							
	10^5	1.0	7	97	97	100									
CD	10^7	2.5		0	80	90	93	93	97	97	97	97	100		
	10^5	2.5	0	7	83	93	97	97	97	97	97	97	100		
CL	10^7	2.5	0	7	90	93	97	97	97	97	97	97	100		
	10^5	2.5	0	17	93	97	97	97	97	97	97	97	100		
P. mirabilis (30)															
CC	10^7	2.5	4	35	92	96	100								
	10^5	1.0	8	85	92	100									
CD	10^7	15.0			0	4	11	26	44	74	93	93	100		
	10^5	10.0			0	4	15	56	93	93	93	93	100		
CL	10^7	7.5			0	22	67	93	93	100					
	10^5	7.5			0	44	89	93	93	100					
Enterococci (27)															
CC	10^7	100.0										0	36	100	
	10^5	50.0										0	92	100	
CD	10^7	>100.0											0	26	100
	10^5	100.0								0	4	4	4	81	100
CL	10^7	>100.0												0	100
	10^5	>100.0									0	4	4	4	100
Enterobacter (30)															
CC	10^7	100.0		0	3	10	10	13	13	20	20	33	40	63	100
	10^5	20.0	0	10	17	30	37	37	43	47	63	67	77	87	100
CD	10^7	100.0					0	10	13	13	13	20	30	50	100
	10^5	50.0				0	13	13	13	17	23	37	57	63	100
CL	10^7	>100.0			0	3	7	7	10	10	13	13	30	47	100
	10^5	50.0			0	10	13	13	13	17	23	33	50	60	100

TABLE 1—*Continued*

Organism (no.)	Inoculum size (organisms/ml)	Median MIC	Cumulative percentage of strains susceptible with MIC (μg/ml) of:												
			≤0.5	1.0	2.5	5.0	7.5	10	12.5	15	20	25	50	100	>100
Indole-positive *Proteus* (25)															
CC	10^7	50.0	0	14	19	24	28	38	38	38	38	38	62	71	100
	10^5	20.0	10	19	33	38	38	38	38	38	52	67	67	86	100
CD	10^7	>100.0	0	4	4	8	8	8	12	16	16	16	10	18	100
	10^5	>100.0	0	4	4	8	8	8	16	16	16	24	32	44	100
CL	10^7	>100.0	0	4	4	8	16	16	16	16	16	16	20	40	100
	10^5	100.0	0	4	8	8	16	16	16	16	16	24	40	52	100

[a] CC, Cefaclor; CD, cephradine; CL, cephalexin.

was more active than cephradine and cephalexin against all tested bacteria. For example, 2.5 μg of cefaclor per ml inhibited 100% of the isolates of *E. coli* in both media. At that same concentration, no strains were inhibited by cephradine or cephalexin in MHA or NA.

The median MICs of the various antibiotics for each organism with respect to agar, inoculum size, and antibiotic are also summarized in Tables 1 and 2. As can be seen, considerable variability exists depending upon the test procedure used. The difference in antibiotic can best be demonstrated with results found when testing *P. mirabilis* isolates. The cefaclor median MIC in MHA was 2.5 μg/ml with the higher inoculum and 1.0 μg/ml with the lower inoculum. On the other hand, cephradine had median MIC values of 15 and 10 μg/ml, respectively. With the smaller inoculum size, cefaclor exhibited a 10-fold increase in antibacterial activity when compared with cephradine. Similarly, cefaclor was seven times as active as cephalexin under these testing conditions. Various differences can also be seen with respect to the media used in testing. In general, cefaclor appeared consistently more active in NA, whereas both cephradine and cephalexin showed variation with respect to organism and medium. For example, *S. typhi* was shown to be inhibited by cefaclor to a greater extent in NA, whereas with cephradine and cephalexin, *S. typhi* appeared to be inhibited to a greater extent in MHA. Median MICs of cephradine in NA for *Klebsiella* were more than twice those in MHA, whereas those for *P. mirabilis* were higher in MHA than in NA. In agar dilution studies cephradine was the least active of these cephalosporins. Table 3 takes into consideration reported obtainable blood levels and mean MICs of each antibiotic and expresses this relationship in the form of an inhibitory index. The inhibitory index is a ratio of the mean peak serum level and the mean MIC and may present a more accurate picture of potential efficacy for the drugs studied. For organisms usually susceptible to cephalosporins, cefaclor demonstrated more antibacterial activity than either cephradine or cephalexin.

Broth dilutions. MICs and MBCs were determined in MHB and NB for 5 to 15 isolates each of *S. aureus, Klebsiella, Enterobacter, E. coli, P. mirabilis,* and *S. typhi.* All drugs had less antibacterial activity in broth dilution testing than they did in agar and demonstrated a much greater inoculum effect. Results varied widely and inconsistently among the three antibiotics and between the two broth media used. Cefaclor was more active against isolates of *E. coli* in NB, whereas cephradine and cephalexin exhibited their greatest activity against this organism in MHB. On the other hand, when testing isolates of Klebsiella, cefaclor showed more antibacterial activity in MHB than it did in NB. For *P. mirabilis,* MICs for all three drugs were consistently lower in NB, but a wider disparity (up to a 7-tube difference) between MIC and MBC also was seen in NB compared with MHB.

In MHB, *E. coli* results exemplified the marked differences obtained with high and low inocula. With 10^5 organisms per ml of inoculum, all three antibiotics inhibited and killed 100% of the strains at a concentration ≤20 μg of antibiotic per ml, whereas with the higher inoculum, only cefaclor exhibited any activity at 20 μg of antibiotic per ml, inhibiting 20% of those *E. coli* isolates. In general, cefaclor had the lowest MICs and MBCs of the three antibiotics tested in MHB. An exception was *S. aureus,* where cephradine was the most active.

In NB a clear separation of the results with these three cephalosporins was seen especially with *Klebsiella, E. coli,* and *S. typhi* isolates. Cefaclor was the most active, cephalexin had intermediate activity, and cephradine was least active. For other organisms the trend was the

TABLE 2. *Agar dilution MICs for cefaclor, cephradine, and cephalexin obtained using two inoculum sizes of bacterial cells and nutrient agar*

Organism (no.)	Inoculum size (organisms/ml)	Median MIC	Cumulative percentage of strains susceptible with MIC (μg/ml) of:												
			≤0.5	1.0	2.5	5.0	7.5	10	12.5	15	20	25	50	100	>100
E. coli (30)															
CC[a]	10^7	1.0	0	83	100										
	10^5	1.0	0	100											
CD	10^7	15.0						0	30	70	100				
	10^5	12.5					0	23	73	100					
CL	10^7	7.5				0	73	97	97	100					
	10^5	7.5			0	37	97	97	100						
S. aureus (30)															
CC	10^6	≤0.5	100												
	10^4	≤0.5	100												
CD	10^6	2.5	0	20	97	100									
	10^4	1.0	10	80	100										
CL	10^6	2.5	0	7	97	100									
	10^4	1.0	0	60	100										
K. pneumoniae (30)															
CC	10^7	1.0	0	93	100										
	10^5	1.0	0	97	100										
CD	10^7	20.0							0	3	67	97	100		
	10^5	20.0					0	3	10	33	90	100			
CL	10^7	10.0				0	3	80	93	100					
	10^5	10.0				0	27	93	97	100					
S. typhi (30)															
CC	10^7	≤0.5	80	97	100										
	10^5	≤0.5	93	97	100										
CD	10^7	7.5			0	37	97	97	97	97	97	97	100		
	10^5	5.0			0	63	97	97	97	97	97	97	100		
CL	10^7	2.5		0	60	97	97	97	97	97	97	97	100		
	10^5	2.5		0	90	97	97	97	97	97	97	100			
P. mirabilis (30)															
CC	10^7	≤0.5	85	96	100										
	10^5	≤0.5	92	100											
CD	10^7	7.5		,	0	15	63	85	93	96	96	96	100		
	10^5	5.0		0	7	56	93	96	96	96	96	100			
CL	10^7	5.0		0	4	70	93	96	96	96	100				
	10^5	5.0		0	15	93	96	96	96	96	100				
Enterococci (27)															
CC	10^7	15.0							0	68	92	92	100		
	10^5	15.0					0	8	8	96	96	96	100	96	100
CD	10^7	50.0							0	4	7	15	96	100	
	10^5	25.0				0	4	4	4	4	19	70	96	100	
CL	10^7	50.0								0	4	4	89	96	100
	10^5	50.0						0	4	4	4	7	96	96	100
Enterobacter (30)															
CC	10^7	100.0		0	7	7	7	10	10	13	13	13	47	77	100
	10^5	20.0	0	7	11	27	27	30	30	43	53	57	77	83	100
CD	10^7	>100.0								0	3	7	17	40	100
	10^5	>100.0								0	10	13	20	43	100
CL	10^7	>100.0				0	7	7	10	10	10	13	20	40	100
	10^5	100.0				0	10	13	17	17	17	17	27	53	100

TABLE 2—*Continued*

Organism (no.)	Inoculum size (organisms/ml)	Median MIC	Cumulative percentage of strains susceptible with MIC (μg/ml) of:											
			≤0.5	1.0	2.5	5.0	7.5	10	12.5	15	20	25	50	100	>100
Indole-positive *Proteus* (25)															
CC	10^7	7.5	14	19	24	43	52	52	57	57	57	57	76	100	
	10^5	5.0	19	27	48	52	52	62	62	62	76	81	95	100	
CD	10^7	>100.0	0	4	4	8	16	20	20	24	28	32	36	40	100
	10^5	>100.0	0	8	8	8	20	24	28	28	32	36	44	44	100
CL	10^7	100.0	0	4	8	16	16	16	16	16	32	40	40	52	100
	10^5	100.0	8	8	8	16	16	16	28	28	40	40	44	56	100

[a] CC, Cefaclor; CD, cephradine; CL, cephalexin.

TABLE 3. *Inhibitory indexes of three cephalosporins based on mean MIC for each organism and peak drug concentration in serum*

Organism (no.)	Antibiotic	Mean MIC	Inhibitory index[a]	
			250 mg[b]	500 mg
E. coli (30)	Cefaclor	0.97	6.70	10.42
	Cephradine	7.83	1.65	2.45
	Cephalexin	5.58	1.51	3.37
S. aureus (30)	Cefaclor	1.50	4.33	7.00
	Cephradine	3.83	3.37	5.01
	Cephalexin	3.50	2.40	5.37
K. pneumoniae (30)	Cefaclor	0.85	7.65	12.35
	Cephradine	8.50	1.52	2.26
	Cephalexin	3.92	2.15	4.80
S. typhi (30)	Cefaclor	1.12	5.80	9.38
	Cephradine	4.65	2.77	4.13
	Cephalexin	3.92	2.15	4.80
P. mirabilis (30)	Cefaclor	1.20	5.42	8.75
	Cephradine	12.00	1.08	1.60
	Cephalexin	6.50	1.29	2.89
Enterobacter (30)	Cefaclor	34.36	0.19	0.30
	Cephradine	59.50	0.22	0.32
	Cephalexin	63.42	0.13	0.29
Enterococci (27)	Cefaclor	54.00	0.12	0.19
	Cephradine	97.04	0.13	0.20
	Cephalexin	97.22	0.09	0.19
Indole-positive *Proteus* (25)	Cefaclor	40.20	0.16	0.26
	Cephradine	75.24	0.17	0.26
	Cephalexin	70.74	0.12	0.27

[a] Ratio between the mean peak serum levels and the MIC of the isolates susceptible in MHA using an inoculum of 10^5 organisms per ml.

[b] Peak serum levels (μg/ml) for the 250-mg dosage are: cefaclor 6.5; cephradine, 12.9; and cephalexin, 8.4; for the 500-mg dosage: cefaclor 10.5; cephradine 19.2; and cephalexin 18.8.

same but the differences were not as clear-cut, especially with the higher inoculum. With the higher inoculum, considerable differences between MICs and MBCs were found. For example 2.5 μg of cefaclor per ml inhibited all strains of *P. mirabilis*, but a concentration of 2.5 μg of cefaclor per ml killed only 20% of these same strains. In NB, cephradine was not

more active than cefaclor against isolates of *S. aureus*, as was seen when tested in MHB.

Broth dilution studies were not performed with isolates of enterococci and indole-positive *Proteus*.

DISCUSSION

It would appear from the studies detailed earlier that cefaclor has more in vitro antibacterial activity than cephalexin and cephradine. Presumably this is because of the substituted chloro group attached to the molecule.

Although this study limits itself to eight microbial species commonly responsible for infections, other organisms have been reported to be quite susceptible to cefaclor. Cefaclor has been reported to have a two- to eightfold increase in activity over cephalexin against isolates of *S. pneumoniae* and *Haemophilus influenzae* (D. A. Preston, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 16th, Chicago, Abstr. no. 352, 1976). Other comparative results, similar to those in this study, have also been reported showing cefaclor to have an increase in activity over cephalexin or cephradine (1). In addition, cefaclor was shown to be substantially more active than the other two drugs against gonococci, meningococci, *Citrobacter diversus*, and shigellae (1). In studies not included in this report, we found the drug to be inactive against isolates of *P. aeruginosa*.

Throughout this study there were demonstrated differences with respect to variables used in testing. It is not at all certain that the tests actually indicate whether or not these results can be accurately extrapolated to clinical practice. These testing variables include such factors as method of testing, type of media, and inoculum size. For example, MICs of all three antibiotics were much lower when tested in agar than in broth. Also, inoculum size in broth appeared to exert a much greater effect than it did on agar. In most cases, it was also shown that antibacterial activity was greater in nutrient media than in Mueller-Hinton media.

From the results obtained in this in vitro study using these organisms, it can be concluded that cefaclor appears to have considerably greater activity than the other two commercially available oral cephalosporin antibiotics studied. Only comparative clinical trials will show whether the differences in in vitro activity shown here among these three cephalosporins are of clinical significance.

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