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Antibacterial Activity of Cefamandole, a New Cephalosporin Antibiotic, Compared with that of Cephaloridine, Cephalothin, and Cephalexin

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The in vitro antibacterial activity of cefamandole, a new cephalosporin antibiotic, was compared with that of cephaloridine, cephalothin, and cephalexin against 1,213 strains of gram-positive and gram-negative bacteria recently isolated from clinical sources. The decreasing order of activity of the four agents against gram-positive cocci was cephaloridine, cephalothin, cefamandole, and cephalexin. However, cefamandole was the most active of the four against *Haemophilus* species and gram-negative bacilli susceptible to cephalosporins. It was also active against many strains resistant to the other cephalosporins, such as *Enterobacter* species and indole-positive *Proteus* species, but there was a marked inoculum effect with all of these organisms, and minimal bactericidal concentrations were usually considerably higher than minimal inhibitory concentrations. Cefamandole, like other cephalosporins, had no useful activity against *Pseudomonas* species.

Cefamandole, 7-D-mandelamido-3-(1-methyl-1H -tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid, sodium salt, is a new semisynthetic, parenteral cephalosporin antibiotic with a broad antibacterial spectrum. The purpose of this study was to compare its antibacterial activity with that of cephaloridine, cephalothin, and cephalexin (Fig. 1) against a variety of bacteria from clinical sources.

MATERIALS AND METHODS

Freshly isolated strains of bacteria were obtained from the Clinical Laboratory in St. Thomas' Hospital. These consisted of *Staphylococcus aureus* (25 penicillin-susceptible, 50 penicillin-resistant and methicillin-susceptible, and 28 methicillin-resistant); coagulase-negative staphylococci (93); *Streptococcus faecalis* (79); alpha-hemolytic streptococci (89); *Streptococcus pneumoniae* (94); beta-hemolytic streptococci, Lancefield groups A (32), B (33), C (16), F (3), and G (13); *Neisseria gonorrhoeae* (181); *Haemophilus influenzae* (74); *Haemophilus parainfluenzae* (14); *Escherichia coli* (33); *Citrobacter koseri* (24); *Klebsiella aerogenes* (49); *K. ozoenae* (15); *Proteus mirabilis* (28); *Citrobacter freundii* (30); *Enterobacter aerogenes* (8); *E. cloacae* (41); *Hafnia alvei* (3); *Serratia marcescens* (13); *Proteus vulgaris* (26); *P. morganii* (27); *P. rettgeri* (15); *Providencia stuartii* (8); *Providencia alcalifaciens* (10); *Acinetobacter anitratus* (5); *A. lwoffi* (11); *Pseudomonas aeruginosa* (10); *P. thomasii* (11); *P. cepacia* (10); and *P. maltophilia* (12).

Inocula for susceptibility testing were prepared by overnight culture in nutrient broth (Southern Group Laboratories) supplemented with 10% lysed horse blood for investigations on *N. gonorrhoeae* and *Haemophilus* species. This culture was either diluted so that a multiple inoculator used with solid media delivered approximately 10^4 organisms in 0.003 ml, or so that 0.02 ml contained approximately 10^4 organisms for the inoculation of liquid media. Inocula of 10^6, 10^7, and 10^8 organisms were also prepared for investigation of the inoculum effect.

Minimal inhibitory concentrations (MIC) were determined on solid medium (Oxoid DST Agar, CM 261) containing doubling dilutions of each antibiotic, incubated aerobically at 37 C for 18 h. The MIC was defined as the lowest concentration of antibiotic on which there was no visible growth.

Minimal bactericidal concentrations (MBC) were determined in 1.5% glucose indicator broth (Oxoid Nutrient Broth No. 2, CM 67) containing doubling dilutions of antibiotic in a volume of 2 ml. After overnight aerobic incubation at 37 C, the broth was subcultured by use of a standard loop (approximately 0.005 ml) on antibiotic-free blood agar (Oxoid blood agar base No. 2, CM 271 plus 4% defibrinated horse blood). The MBC was defined as the lowest concentration that yielded no growth on subculture. However, for staphylococci it was defined as the lowest concentration that killed at least 99% of the inoculum.

RESULTS

Table 1 shows cumulative percentages of *S. aureus* strains susceptible to increasing concentrations of each antibiotic. Results for penicillin-susceptible strains, penicillin-resistant and methicillin-susceptible strains, and methicillin-resistant strains are shown separately, as are results for large and small inocula for each antibiotic. It should be noted that only small numbers of penicillin-susceptible staphylococci were tested with the larger inoculum, accounting for the limited distribution of susceptibilities.

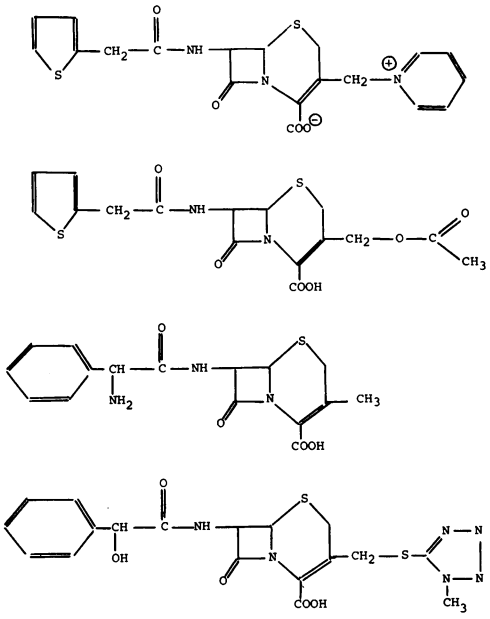

FIG. 1. *Chemical structures of (top to bottom): cephaloridine, cephalothin, cephalexin, and cefamandole.*

Table 2 lists results for alpha- and beta-hemolytic streptococci, *S. pneumoniae*, and *N. gonorrhoeae* (cefamandole only), and Table 3 gives results for coagulase-negative staphylococci, *S. faecalis*, *H. influenzae*, and *H. parainfluenzae*. Results for the last two organisms are combined, as the distribution of susceptibility is similar.

In Table 4, results are given in three groups. Organisms in the first group (*E. coli* etc.) are fundamentally susceptible to cephalosporin C, although a few resistant strains of *E. coli* and *K. aerogenes* are included. All of the organisms in the second group (*C. freundii* etc.) are intrinsically resistant to cephalosporin C, as are those in the third group of nonfermenting gram-negative bacilli.

Table 5 shows the effect of increasing the inoculum on the MIC of cefamandole for 25 selected gram-negative bacilli. Determination of the end point was often made difficult by growth of large numbers of tiny colonies at higher concentrations of cefamandole when the inoculum was very heavy.

TABLE 1. *Activity of cephaloridine, cephalexin, cephalothin, and cefamandole against Staphylococcus aureus*[a]

MIC (μg/ml)	Penicillin-susceptible								Penicillin-resistant methicillin-susceptible								Methicillin-resistant							
	C'dine		C'xin		C'thin		C'dole		C'dine		C'xin		C'thin		C'dole		C'dine		C'xin		C'thin		C'dole	
	10^{4}[b]	10^{7}	10^{4}	10^{7}	10^{4}	10^{7}	10^{4}	10^{7}	10^{4}	10^{7}	10^{4}	10^{7}	10^{4}	10^{7}	10^{4}	10^{7}	10^{4}	10^{7}	10^{4}	10^{7}	10^{4}	10^{7}	10^{4}	10^{7}
0.02	52	20	—	—	8	—	—	—	4	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—
0.04	100	100	—	—	60	60	4	—	40	—	—	—	22	7	—	—	—	—	—	—	—	—	—	—
0.09	—	—	—	—	92	100	4	—	88	—	—	—	78	10	—	—	—	—	—	—	—	—	—	—
0.19	—	—	—	—	92	—	60	100	100	5	—	—	100	38	6	2	—	—	—	—	—	—	—	—
0.39	—	—	8	—	100	—	88	—	—	14	2	—	—	76	88	19	—	—	—	—	11	—	14	—
0.78	—	—	56	—	—	—	96	—	—	45	20	2	—	88	100	64	32	—	—	—	79	—	14	—
1.56	—	—	92	100	—	—	100	—	—	62	86	24	—	100	—	95	93	—	—	—	100	—	57	8
3.12	—	—	96	—	—	—	—	—	—	71	100	62	—	—	—	100	100	—	—	—	—	27	96	15
6.25	—	—	100	—	—	—	—	—	—	83	—	78	—	—	—	—	—	4	7	—	—	66	100	70
12.5	—	—	—	—	—	—	—	—	—	95	—	90	—	—	—	—	—	15	21	—	—	92	—	100
25.0	—	—	—	—	—	—	—	—	—	100	—	100	—	—	—	—	—	100	100	100	—	100	—	—
No. of strains	25	5	25	5	25	5	25	5	50	42	50	42	50	42	50	42	28	26	28	26	28	26	28	26

[a] Results are expressed as cumulative percentages of strains susceptible to the amount of antibiotic shown in the MIC column.

[b] Inoculum, expressed as total number of viable organisms inoculated.

TABLE 2. *Activity of cephaloridine, cephalexin, cephalothin, and cefamandole against streptococci and gonococci*[a]

MIC (μg/ml)	Alpha-hemolytic streptococci				*S. pneumoniae*				Beta-hemolytic streptococci				*N. gonorrhoeae* (C'dole)
	C'dine	C'xin	C'thin	C'dole	C'dine	C'xin	C'thin	C'dole	C'dine	C'xin	C'thin	C'dole	
0.002	1	—	—	—	—	—	—	—	—	—	—	—	—
0.005	2	—	—	—	17	—	13	—	—	—	—	—	40
0.01	11	—	—	—	51	—	21	—	—	—	—	—	58
0.02	48	—	1	—	95	—	29	1	84	1	33	14	64
0.04	79	—	14	—	100	—	51	3	87	6	70	88	70
0.09	95	—	50	18	—	—	96	30	88	18	89	95	77
0.19	99	—	72	42	—	—	100	72	88	27	92	100	83
0.39	100	—	92	76	—	—	—	97	90	58	93	—	92
0.68	—	7	98	91	—	19	—	98	90	65	94	—	98
1.56	—	27	99	94	—	74	—	98	95	83	94	—	100
3.12	—	100	100	100	—	100	—	100	95	92	95	—	—
6.25	—	—	—	—	—	—	—	—	95	93	95	—	—
12.5	—	—	—	—	—	—	—	—	97	94	97	—	—
25.0	—	—	—	—	—	—	—	—	100	100	100	—	—
No. of strains	89	89	89	33	94	94	94	69	97	97	97	97	181

[a] Results are expressed as cumulative percentages of strains susceptible to the amount of antibiotic shown in the MIC column. The inoculum was 10^4 organisms in all cases.

TABLE 3. *Activity of cephaloridine, cephalexin, cephalothin, and cefamandole against coagulase-negative staphylococci, Streptococcus faecalis, and Haemophilus species*[a]

MIC (μg/ml)	Coagulase-negative staphylococci				*S. faecalis*				*Haemophilus* species			
	C'dine	C'xin	C'thin	C'dole	C'dine	C'xin	C'thin	C'dole	C'dine	C'xin	C'thin	C'dole
0.02	47	1	16	1	—	—	—	—	5	—	—	2
0.04	80	5	36	6	—	—	—	—	5	—	3	3
0.09	87	9	56	29	—	—	—	—	6	1	6	9
0.19	95	11	72	60	—	—	—	—	7	1	9	29
0.39	97	16	87	72	—	—	—	—	8	2	12	62
0.78	97	39	97	94	—	—	—	—	9	2	27	81
1.56	99	58	99	97	—	—	—	—	20	7	45	93
3.12	100	73	99	98	5	—	—	—	49	14	81	100
6.25	—	90	100	99	73	—	25	—	84	43	93	—
12.5	—	96	—	100	100	—	94	15	98	79	98	—
25.0	—	100	—	—	—	1	100	89	100	100	100	—
50.0	—	—	—	—	—	37	—	100	—	—	—	—
100	—	—	—	—	—	100	—	—	—	—	—	—
No. of strains	93	93	93	93	79	79	79	79	88	88	88	88

[a] Results are expressed as cumulative percentages of strains susceptible to the amount of antibiotic shown in the MIC column. The inoculum was 10^4 organisms in all cases.

Table 6 shows the MBC of cefamandole for the same 25 gram-negative bacilli. MBC values were from 2 to 80 times the MIC values, and, although organisms with a low MIC usually had a low MBC, it was not possible to predict results.

DISCUSSION

Our comparison of the antibacterial activity of the new antibiotic cefamandole with that of the three cephalosporin antibiotics currently available for clinical use in Great Britain, cephaloridine, cephalothin, and cephalexin, shows certain advantages of cefamandole, almost entirely due to its activity against gram-negative bacilli.

Most gram-positive cocci, including penicillin-susceptible and -resistant *S. aureus* strains, are clearly less susceptible to cefamandole than to cephaloridine or cephalothin, but more sus-

TABLE 4. *Activity of cephaloridine, cephalexin, cephalothin, and cefamandole against gram-negative bacilli*[a]

MIC (μg/ml)	Group 1: *E. coli, C. koseri, K. aerogenes, K. ozoenae, P. mirabilis*				Group 2: *C. freundii, E. aerogenes, E. cloacae, H. alvei, S. marcescens, P. vulgaris, P. morganii, P. rettgeri, Providencia*				Group 3: *A. anitratus, A. lwoffi, P. aeruginosa, P. thomasii, P. cepacia, P. maltophilia*			
	C'dine	C'xin	C'thin	C'dole	C'dine	C'xin	C'thin	C'dole	C'dine	C'xin	C'thin	C'dole
0.3	—	—	—	16	—	—	—	9	—	—	—	—
0.6	1	—	7	55	—	—	1	25	—	10	—	—
1.2	7	3	14	74	1	1	1	41	—	19	—	2
2.5	38	11	37	88	2	2	2	55	2	22	—	2
5.0	64	55	58	94	3	2	2	64	4	22	—	4
10.0	87	84	89	95	4	7	7	72	7	25	5	12
25.0	94	96	94	96	11	22	19	78	13	27	25	42
50.0	95	98	97	98	14	33	26	84	37	37	36	68
100	95	98	97	100	20	41	32	92	51	56	46	73
>100	100	100	100	100	100	100	100	100	100	100	100	100
No. of strains	149	149	149	149	181	181	181	181	59	59	59	59

[a] Results are expressed as cumulative percentages of strains susceptible to the amount of antibiotic shown in the MIC column. The inoculum was 10^4 organisms in all cases.

TABLE 5. *Effect of increasing inocula of 25 strains of gram-negative bacilli*[a] *on the MIC of cefamandole*

MIC (μg/ml) with inoculum of		
10^4 cells	10^6 cells	10^8 cells
0.3	0.3-1.25	0.6-10
0.6	1.25-50	25-500
1.25	2.5-5.0	100-500
2.5	5-125	10-500
5.0	25	100-500
10	100-250	250->500
25	100	500
100	>500	>500

[a] *Escherichia coli* (3), *Citrobacter freundii* (2), *C. koseri* (1), *Klebsiella aerogenes* (7), *K. ozoenae* (1), *Hafnia alvei* (1), *Enterobacter aerogenes* (2), *E. cloacae* (7), *Serratia marcescens* (2).

TABLE 6. *MIC and MBC of cefamandole for 25 selected gram-negative bacilli*

MIC (μg/ml)	No. of strains	MBC (μg/ml)	Ratio of MBC to MIC
0.3	6	0.6, 0.6, 1.25, 2.5, 2.5, 5	2-16
0.6	3	2.5, 5, 25	4-32
1.25	3	2.5, 10, 100	2-80
2.5	6	5, 10, 25, 50, 100, 100	2-40
5.0	2	100, 100	20
10	3	25, 100, 500	2.5-50
25	1	100	4
100	1	>500	>5

[a] Organisms as listed in Table 5.

ceptible than to cephalexin. However, on the basis of the relatively small inoculum effect with penicillinase-producing staphylococci, cefamandole may be more stable to staphylococcal penicillinase than the other cephalosporins. As with other cephalosporins, methicillin-resistant staphylococci are less susceptible to cefamandole than are methicillin-susceptible staphylococci. The MBC of cefamandole was usually about 10 times the MIC for penicillinase-producing staphylococci, but was more nearly equal to the MIC for penicillin-susceptible strains.

Among other gram-positive cocci examined, cefamandole is inferior to cephaloridine and cephalothin in its inhibition of alpha-hemolytic streptococci, pneumococci, and almost all strains of beta-hemolytic streptococci. The exceptions were a few strains belonging to Lancefield's groups other than A that were relatively resistant to other cephalosporins, but susceptible to cefamandole. None of the antibiotics had high activity against *S. faecalis*.

The only gram-negative cocci examined were 181 strains of *N. gonorrhoeae*. Most of these were very susceptible to cefamandole, but comparisons with other cephalosporins were not made. It was known, however, that the MIC of penicillin for 32% of these strains was greater than 0.06 μg/ml.

Cefamandole has several interesting features in its activity against a variety of gram-negative

organisms. First, it is considerably more active than the other three cephalosporins against *Haemophilus* species, with MIC values comparable to those of ampicillin (2). This pattern is maintained for all of the gram-negative bacilli intrinsically susceptible to cephalosporin C (group 1 in Table 4), and it is therefore the most active of the four cephalosporins examined against *E. coli*, *C. koseri*, *K. aerogenes*, *K. ozoenae*, and *P. mirabilis*. More than 90% of strains of this group of organisms are inhibited by 5 μg or less of cefamandole/ml, whereas only 55 to 60% were as susceptible to the other cephalosporins. This is a particularly important finding, since 30% of the strains of *E. coli* were resistant to cephaloridine, and against half of these the MIC of cefamandole was 5 μg or less/ml.

The second important property of cefamandole is its activity against *Enterobacteriaceae* intrinsically resistant to cephalosporins, those organisms in group 2 in Table 4. More than 60% of these organisms are inhibited by 5 μg of cefamandole/ml, whereas only 2 to 3% are equally susceptible to the other cephalosporins. Among the strains in this group for which the MIC of cefamandole was higher are the 13 strains of *Serratia* examined, few in number because they have been rare in most British hospitals. The only *Enterobacteriaceae* consistently resistant to cefamandole, as to other cephalosporins, are *Proteus vulgaris* and *Providencia stuartii*. *Providencia alcalifaciens* strains, on the other hand, were very susceptible to cefamandole.

Caution is necessary in the interpretation of these promising results for *Enterobacteriaceae*. Not only is there often a striking inoculum effect among organisms with moderate or high MIC values, with only partial inhibition of large inocula by high concentrations of cefamandole, but MBC values are usually considerably higher than the MIC values. The inoculum effect has been noted with other cephalosporins (1).

Cefamandole, like the other cephalosporins, appears to have no useful action on most of the organisms in group 3 (Table 4), although both *Acinetobacter* species were highly varied in susceptibility to all four agents. The only striking finding in this group is the high activity of cephalexin against *Pseudomonas thomasii*, an organism related to *P. cepacia* (3).

This work has shown that cefamandole has distinct advantages over other cephalosporins in its broader spectrum and higher intrinsic activity in vitro against *Enterobacteriaceae* and *Haemophilus* species. If pharmacokinetic and toxicological studies give favorable results, it would seem reasonable to undertake clinical trials with this agent.

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