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In Vitro Activities of Moxalactam and Cefotaxime Against Aerobic Gram-Negative Bacilli

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The in vitro activities of two new beta-lactam antibiotics, moxalactam disodium (LY 127935) and cefotaxime (HR-756), were compared with cefoxitin, cefamandole, cefuroxime, cephalothin, and, in some instances, carbenicillin, gentamicin, and amikacin against aerobic gram-negative bacilli. Test isolates included normally cephalosporin-resistant members of the *Enterobacteriaceae* and *Pseudomonas* spp. and a variety of nonfermentative or oxidase-positive bacteria. Both moxalactam and cefotaxime demonstrated impressive in vitro activities against both groups of microorganisms. The two new drugs were clearly more active than any of the other beta-lactam antibiotics against species of *Escherichia, Citrobacter, Enterobacter, Klebsiella, Proteus, Providencia, Pseudomonas,* and *Serratia.* An additive or synergistic effect could also be demonstrated with the majority of *Pseudomonas* and *Serratia* isolates when either moxalactam or cefotaxime was combined with amikacin.

Moxalactam disodium (LY 127935) and cefotaxime (HR-756) are two new semisynthetic beta-lactam antibiotics. Moxalactam is structurally unique among beta-lactam compounds in that an oxygen molecule has been substituted for sulfur in the cephem nucleus (1). Thus, the compound is properly termed a 1-oxa-beta-lactam antibiotic. Cefotaxime, on the other hand, is a highly beta-lactamase-resistant semisynthetic cephalosporin (3). Initial reports (1, 4–6) indicate that both compounds possess an extremely broad spectrum, which includes aerobic gram-positive cocci, *Enterobacteriaceae, Pseudomonas aeruginosa, Haemophilus influenzae,* and certain anaerobes. The present study compares the activities of both moxalactam and cefotaxime with other currently available antibiotics against normally cephalosporin-resistant members of the *Enterobacteriaceae, Pseudomonadaceae,* and a variety of glucose nonfermentative or oxidase-positive fermentative bacilli.

MATERIALS AND METHODS

Antibiotics. Moxalactam disodium, cefamandole, and cephalothin were furnished by Lilly Research Laboratories, cefotaxime was provided by Hoechst-Roussel Pharmaceuticals, and cefoxitin was supplied by Merck Sharp & Dohme. Carbenicillin was obtained from Roerig, Division of Pfizer Inc., cefuroxime came from Glaxo Laboratories, gentamicin was from Schering Corp., and amikacin was provided by Bristol Laboratories.

All antibiotics were supplied as dry powders which were used to prepare antibiotic stock solutions.

Test organisms. Bacterial test strains represented clinical isolates from the Microbial Pathology Laboratories of the Bexar County Hospital District and the Audie Murphy Veterans Administration Hospital. *Enterobacteriaceae* organisms were identified by the API Profile Recognition System (Analytab Products, Inc.) and additional conventional methods when required. Nonenteric bacteria were identified by the methods and media described by Weaver (7).

Antibiotic susceptibility tests. Agar dilution susceptibility tests were performed by the World Health Organization international collaborative study method (2) with Mueller-Hinton agar (Difco Laboratories) and an inoculum of 10^4 microorganisms applied with a Steers replicator. After 24 h of incubation at 35°C, minimal inhibitory concentrations (MICs) were defined as the least concentration of antimicrobial agents which prevented visible growth or allowed growth of no more than three colonies.

Synergy determinations. The ability of moxalactam or cefotaxime to act synergistically with amikacin was determined by agar dilution checkerboard isobolograms (8). Synergy was defined as a fourfold decrease in the MICs of both of the antimicrobial agents when tested in combination. An additive effect resulted when at least a twofold decrease in the MICs occurred as a result of the combination.

RESULTS

Table 1 shows the results of susceptibility testing of normally cephalosporin-resistant species of the *Enterobacteriaceae.* Moxalactam and cefotaxime showed marked in vitro activity against nine isolates of *Proteus morganii* (MICs $\leq$ 0.06 and 0.125 μg/ml, respectively). Cefamandole was the next most active agent, followed by

TABLE 1. *Comparative activities of six beta-lactam antibiotics against selected Enterobacteriaceae*

Organism (no. of isolates)	Antibiotic	MIC_{50}[a] (μg/ml)	MIC_{90}[a] (μg/ml)	MIC range (μg/ml)
Proteus morganii (9)	Moxalactam	0.125	0.125	0.125
	Cefotaxime	<0.06	<0.06	<0.06
	Cefoxitin	8	8	2–16
	Cefamandole	2	2	0.5–4
	Cefuroxime	16	32	1–32
	Cephalothin	>128	>128	4–>128
P. rettgeri (7)	Moxalactam	<0.06	0.25	<0.06–0.5
	Cefotaxime	<0.06	1	<0.06–1
	Cefoxitin	8	>128	1–>128
	Cefamandole	4	32	0.25–64
	Cefuroxime	8	32	0.5–128
	Cephalothin	>128	>128	32–>128
Citrobacter freundii (10)	Moxalactam	0.125	0.25	<0.06–0.5
	Cefotaxime	0.125	0.5	0.06–1
	Cefoxitin	64	>128	2–>128
	Cefamandole	2	64	0.5–64
	Cefuroxime	4	16	1–32
	Cephalothin	64	>128	4–>128
Enterobacter aerogenes (10)	Moxalactam	0.125	0.5	<0.06–0.5
	Cefotaxime	0.125	4	<0.06–4
	Cefoxitin	>128	>128	2–>128
	Cefamandole	1	32	1–64
	Cefuroxime	4	64	2–64
	Cephalothin	>128	>128	2–>128
E. cloacae (15)	Moxalactam	0.125	0.25	<0.06–4
	Cefotaxime	0.125	2	<0.06–4
	Cefoxitin	>128	>128	>128
	Cefamandole	2	>128	1–>128
	Cefuroxime	4	128	16–>128
	Cephalothin	>128	>128	>128
Serratia marcescens (12)	Moxalactam	0.5	4	0.125–4
	Cefotaxime	0.25	4	0.125–4
	Cefoxitin	16	64	4–128
	Cefamandole	128	>128	8–>128
	Cefuroxime	128	>128	32–>128
	Cephalothin	>128	>128	>128

[a] MIC_{50} and MIC_{90}, MICs at which 50 and 90% of the organisms were inhibited, respectively.

cefoxitin and cefuroxime. Similar results were observed with seven isolates of *Proteus rettgeri*. All isolates were inhibited by 1 μg or less of moxalactam or cefotaxime per ml. Cefamandole was again the next most active of the remaining drugs. Cefoxitin and cefuroxime were less active, although 50% of the isolates were inhibited by drug concentrations achievable in serum.

Moxalactam and cefotaxime were the most active agents tested against 10 isolates of *Citrobacter freundii* (MICs ≦ 1 μg/ml). Cefamandole and cefuroxime were the next most active agents, followed by cefoxitin and cephalothin. Moxalactam and cefotaxime were essentially equivalent in activity against *Enterobacter aerogenes* and *Enterobacter cloacae* (MICs ≦ 4 μg/ml). Cefamandole and cefuroxime were the next most active against *Enterobacter* spp., whereas cefoxitin and cephalothin failed to demonstrate inhibition of these isolates. Moxalactam and cefotaxime were the most active agents tested against 12 isolates of gentamicin-susceptible *Serratia marcescens*, which were not effectively inhibited by the remaining beta-lactam drugs.

Results of testing selected gentamicin-resistant *Enterobacteriaceae* are shown in Table 2. Eleven isolates of *Escherichia coli* were inhibited by 0.125 μg of either moxalactam or cefotaxime per ml. The remaining beta-lactam antibiotics and amikacin demonstrated rela-

TABLE 2. *Comparative activities of newer beta-lactam antibiotics and aminoglycosides against gentamicin-resistant Enterobacteriaceae*

Organism (no. of isolates)	Antibiotic	MIC_{50}[a] (μg/ml)	MIC_{90}[a] (μg/ml)	MIC range (μg/ml)
Escherichia coli (11)	Moxalactam	<0.06	0.125	<0.06–0.125
	Cefotaxime	<0.06	0.125	<0.06–0.125
	Cefoxitin	2	4	2–32
	Cefamandole	8	8	1–32
	Cefuroxime	2	4	2–8
	Cephalothin	16	16	4–32
	Gentamicin	32	128	8–128
	Amikacin	4	64	1–64
Klebsiella pneumoniae (14)	Moxalactam	0.125	0.125	<0.06–0.5
	Cefotaxime	<0.06	<0.06	<0.06–0.5
	Cefoxitin	4	4	2–32
	Cefamandole	8	8	1–32
	Cefuroxime	2	4	2–32
	Cephalothin	8	16	4–64
	Gentamicin	32	64	16–128
	Amikacin	1	2	1–4
Serratia marcescens (22)	Moxalactam	8	16	0.25–32
	Cefotaxime	8	16	0.25–32
	Cefoxitin	>128	>128	32–>128
	Cefamandole	>128	>128	>128
	Cefuroxime	>128	>128	64–>128
	Cephalothin	>128	>128	>128
	Gentamicin	128	128	32–>128
	Amikacin	8	8	0.5–16

[a] See Table 1, footnote *a*.

TABLE 3. *Comparative activities of seven antibiotics against Pseudomonas spp.*

Organism (no. of isolates)	Antibiotic	MIC_{50}[a] (μg/ml)	MIC_{90}[a] μg/ml)	MIC range (μg/ml)
Pseudomonas aeruginosa (53)	Moxalactam	16	32	2–>128
	Cefotaxime	16	32	2–>128
	Cefoxitin	>128	>128	>128
	Cefamandole	>128	>128	>128
	Cefuroxime	>128	>128	>128
	Cephalothin	>128	>128	>128
	Carbenicillin	64	64	16–256
	Gentamicin	2	8	0.5–8
	Amikacin	2	4	0.5–8
P. maltophilia (12)	Moxalactam	>128	>128	128–>128
	Cefotaxime	32	64	16–64
	Cefoxitin	>128	>128	>128
	Cefamandole	>128	>128	>128
	Cefuroxime	>128	>128	>128
	Cephalothin	>128	>128	>128
	Carbenicillin	256	512	32–512
P. putida (10)	Moxalactam	64	128	32–128
	Cefotaxime	16	64	16–64
	Cefoxitin	>128	>128	>128
	Cefamandole	>128	>128	>128
	Cefuroxime	>128	>128	>128
	Cephalothin	>128	>128	>128
	Carbenicillin	512	>512	256–>512
P. putrefaciens (4)	Moxalactam	1	2	1–2
	Cefotaxime	0.125	2	0.125–2
	Cefoxitin	4	4	4
	Cefamandole	32	32	16–32
	Cefuroxime	2	4	2–4
	Cephalothin	>128	>128	>128
	Carbenicillin	128	256	32–256

[a] See Table 1, footnote *a*.

tively good action against these isolates. However, four of these isolates were found to be amikacin resistant. Fourteen gentamicin-resistant isolates of *Klebsiella pneumoniae* were inhibited by 0.5 μg or less of either moxalactam or cefotaxime per ml. The remaining beta-lactam compounds and amikacin showed moderate inhibition of these isolates. Moxalactam and cefotaxime were equivalent to each other by being the most active compounds tested against 22 multiply resistant isolates of *S. marcescens*. All *Serratia* isolates were amikacin susceptible but were not inhibited by any of the other beta-lactam antibiotics. Three gentamicin-resistant *Providencia stuartii* and one *C. freundii* isolate (not shown in Table 2) were susceptible to 1 μg or less of either moxalactam or cefotaxime per ml.

Table 3 shows results of testing species of *Pseudomonas* against these same compounds plus carbenicillin. Both moxalactam and cefotaxime demonstrated in vitro inhibition of *P. aeruginosa*, in contrast to the other cephalosporin or cephamycin class of antibiotics, which failed to show inhibition. Cefotaxime proved slightly superior to moxalactam against *Pseudomonas maltophilia*, *Pseudomonas putida*, and *Pseudomonas putrefaciens*, none of which was effectively inhibited by the other beta-lactam drugs.

Table 4 lists results obtained when other commonly isolated glucose-nonfermentative gram-

TABLE 4. *Comparative activities of seven beta-lactam antibiotics against frequently isolated nonfermentative organisms*

Organism (no. of isolates)	Antibiotic	MIC_{50}[a] (μg/ml)	MIC_{90}[a] (μg/ml)	MIC range (μg/ml)
Acinetobacter anitratus (22)	Moxalactam	32	64	4–128
	Cefotaxime	16	32	2–64
	Cefoxitin	64	128	4–>128
	Cefamandole	64	128	16–>128
	Cefuroxime	32	64	4–128
	Cephalothin	>128	>128	64–>128
	Carbenicillin	16	32	2–64
A. lwoffi (15)	Moxalactam	4	8	1–64
	Cefotaxime	1	2	<0.06–8
	Cefoxitin	4	8	0.125–16
	Cefamandole	8	8	<0.06–16
	Cefuroxime	1	2	0.06–8
	Cephalothin	16	16	1–64
	Carbenicillin	1	8	0.25–16
Comamonas terrigena (5)	Moxalactam	0.5	1	0.25–1
	Cefotaxime	2	4	0.5–4
	Cefoxitin	1	1	0.5–1
	Cefamandole	32	128	1–128
	Cefuroxime	32	64	8–64
	Cephalothin	>128	>128	2–>128
	Carbenicillin	256	512	4–512
Achromobacter xylosoxidans (4)	Moxalactam	4	64	4–64
	Cefotaxime	64	128	64–128
	Cefoxitin	128	>128	64–>128
	Cefamandole	>128	>128	>128
	Cefuroxime	>128	>128	>128
	Cephalothin	>128	>128	64–>128
	Carbenicillin	8	>512	8–>512
Moraxella spp. (4)	Moxalactam	<0.06	0.06	<0.06–0.06
	Cefotaxime	<0.06	0.125	<0.06–0.125
	Cefoxitin	0.25	0.5	<0.06–0.5
	Cefamandole	0.125	0.5	<0.06–0.5
	Cefuroxime	0.125	2	<0.06–2
	Cephalothin	0.06	1	<0.06–1
	Carbenicillin	<0.06	0.25	<0.06–0.25

[a] See Table 1, footnote *a*.

negative bacilli were examined. Cefotaxime was slightly superior to moxalactam against the *Acinetobacter* spp., whereas moxalactam demonstrated greater activity than did cefotaxime against *Comamonas terrigena* and *Achromobacter xylosoxidans*. The *Moraxella* spp. were exquisitely susceptible to all of the drugs tested.

When glucose-fermentative, oxidase-positive gram-negative bacilli were tested (Table 5), all isolates were uniformly susceptible to the new compounds and the currently available antibiotics. Exceptions were observed in the relative insusceptibility of *Aeromonas hydrophila* to cephalothin and carbenicillin and the relative resistance of the *Vibrio* spp. to carbenicillin.

Table 6 summarizes susceptibility testing of a variety of less commonly isolated miscellaneous nonenteric gram-negative bacilli. Susceptibility to moxalactam and cefotaxime varied somewhat among these species. Both drugs were ineffective against *Pseudomonas fluorescens* and *Flavobacterium* sp. (group IIb). Cefotaxime showed greater inhibition of *Pseudomonas diminuta*, *Pseudomonas stutzeri*, *Pseudomonas cepacia*, and CDC Ve-2, whereas moxalactam was more active against *Alcaligenes odorans*, *Bordetella bronchiseptica*, and CDC IVC-2 isolates.

Checkerboard tests for synergy were performed with moxalactam and cefotaxime plus amikacin against 22 gentamicin-resistant *Serratia* isolates. Synergy was demonstrated against 18 of 22 isolates with cefotaxime plus amikacin and against 15 of 22 with moxalactam and amikacin. When agar dilution checkerboard tests for synergy were performed with 48 isolates of *P. aeruginosa* (including 16 gentamicin-resistant strains), synergy was observed with 8 of 48 isolates with moxalactam plus amikacin, and an additive effect was demonstrated with 27 of the remaining 40 isolates. The combination of cefotaxime plus amikacin was synergistic against 5 of 48 isolates of *P. aeruginosa*, whereas an additive effect occurred with 27 of the 43 remaining strains.

DISCUSSION

In this study, both moxalactam and cefotaxime showed marked in vitro activity against many cephalosporin-resistant gram-negative bacilli, including *Pseudomonas* spp. Currently available "newer" cephalosporin or cephamycin antibiotics, such as cefamandole, cefoxitin, and cefuroxime, are not uniformly active against cephalothin-resistant *Enterobacteriaceae* and generally are inactive against pseudomonads (4, 5). Thus, cefotaxime appears to be the forerunner of a "third generation" of cephalosporins with extreme gram-negative beta-lactamase resistance and therefore unprecedented activity against many aerobic gram-negative bacilli (1, 4, 6). Although moxalactam contains a modified cephem nucleus, it is not properly classified as

TABLE 5. *Comparative activities of seven beta-lactam antibiotics against oxidase-positive fermentative bacilli*

Organism (no. of isolates)	Antibiotic	MIC_{50}[a] (μg/ml)	MIC_{90}[a] (μg/ml)	MIC range (μg/ml)
Pasteurella spp. (5)	Moxalactam	<0.06	0.06	<0.06–0.06
	Cefotaxime	<0.06	0.06	<0.06–0.06
	Cefoxitin	0.25	0.5	<0.06–0.5
	Cefamandole	<0.06	<0.06	
	Cefuroxime	<0.06	0.06	<0.06–0.06
	Cephalothin	<0.06	0.125	<0.06–0.125
	Carbenicillin	0.25	512	0.125–512
Aeromonas hydrophila (8)	Moxalactam	<0.06	0.06	<0.06–0.5
	Cefotaxime	<0.06	0.125	<0.06–1
	Cefoxitin	4	64	0.5–>128
	Cefamandole	0.5	16	0.25–16
	Cefuroxime	0.25	2	0.125–8
	Cephalothin	32	>128	1–>128
	Carbenicillin	128	512	32–>512
Vibrio spp. (4)	Moxalactam	<0.06	0.25	<0.06–0.25
	Cefotaxime	<0.06	0.125	<0.06–0.125
	Cefoxitin	4	8	0.25–8
	Cefamandole	1	2	0.5–2
	Cefuroxime	0.25	8	0.25–8
	Cephalothin	1	4	1–4
	Carbenicillin	128	512	32–512

[a] See Table 1, footnote *a*.

TABLE 6. *Comparative MIC ranges of seven beta-lactam antibiotics against less frequently isolated aerobic gram-negative bacilli*

Organisms (no. of isolates)	MIC range (μg/ml) of following antibiotic:						
	Moxalactam	Cefotaxime	Cefoxitin	Cefamandole	Cefuroxime	Cephalothin	Carbenicillin
Alcaligenes odorans (1)	<0.06	0.5	1	0.25	32	1	8
Bordetella bronchiseptica (2)	1	64	128->128	16	>128	8	16
CDC EF-4 (3)	0.125-0.25	0.25	8	4-8	32	64	0.5-1
CDC Ve-2 (1)	16	8	>128	>128	64	>128	64
CDC II-F (4)	0.5-16	0.125-16	0.25-1	0.5-8	1-64	0.125-16	0.25-4
CDC IVC-2 (1)	16	128	64	>128	64	>128	>512
CDC IVe (3)	0.06	<0.06-0.5	0.5-1	0.25-0.5	1-2	0.25-0.5	<0.06-0.125
Flavobacterium sp. (1)	32	64	16	>128	>128	>128	>512
Plesiomonas shigelloides (2)	<0.06	<0.06	1-2	<0.06	<0.06-0.06	0.5	16-32
Pseudomonas cepacia (1)	2	1	16	32	16	64	8
P. denitrificans (1)	0.5	0.5	4	8	16	32	2
P. diminuta (1)	64	4	32	64	32	32	64
P. fluorescens (3)	128->128	16-64	>128	>128	>128	>128	32->512
P. pseudoalcaligenes (2)	<0.06-0.06	0.125-0.25	0.5	0.25-0.5	2	1	1
P. stutzeri (1)	8	4	16	64	128	>128	16

a cephalosporin, but rather as a new 1-oxa-beta-lactam antibiotic.

In our study, both of these new compounds showed striking in vitro activity against the *Enterobacteriaceae* and good activity against *P. aeruginosa*. Moxalactam and cefotaxime were shown to have similar activities against most of the organisms included in this study. However, several interesting differences occurred in which one of the two agents appeared markedly more active than the other. Cefotaxime seemed superior against *P. maltophilia*, *P. putida*, *P. diminuta*, *Acinetobacter lwoffi*, and *P. fluorescens*. Moxalactam appeared somewhat more active against *A. xylosoxidans*, *C. terrigena*, *A. odorans*, *B. bronchiseptica*, and CDC IVC-2.

It is noteworthy that both moxalactam and cefotaxime were comparable to or more active than carbenicillin against pseudomonads and other nonfermentative organisms. The action of these new compounds against 22 isolates of multiply resistant *S. marcescens* was particularly encouraging. The ability of both antibiotics to act synergistically with amikacin against the majority of *Serratia* isolates and to produce at least an additive effect against most *P. aeruginosa* isolates further amplifies the potential usefulness of these new beta-lactam antibiotics.

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