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In Vitro Activities of Ureidopenicillins Alone and in Combination with Amikacin and Three Cephalosporin Antibiotics

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The MIC and MBC activity of mezlocillin alone and in combination with two concentrations of ceftizoxime, moxalactam, and amikacin and a single concentration of cefoxitin was studied in a broth microdilution partial checkerboard against 472 strains of aerobic gram-negative and gram-positive bacteria. Azlocillin was tested alone and in the same combinations against *Pseudomonas aeruginosa*. Of the gram-negative bacilli tested, 38% were gentamicin resistant. Antagonism (less than or equal to a fourfold ureidopenicillin MIC increase) was observed frequently with combinations of ureidopenicillins plus cefoxitin and sporadically with ureidopenicillins plus ceftizoxime or moxalactam. Partial synergism (less than or equal to a fourfold ureidopenicillin MIC decrease) was evident with both combinations of ureidopenicillins plus amikacin and ureidopenicillins plus ceftizoxime or moxalactam, the percentage being dependent upon the individual species and combinations.

Combination antibiotic therapy has been used to broaden the antibacterial spectrum in the treatment of unidentified pathogens and possibly prevent or delay the development of resistant organisms (13, 20). Combinations of beta-lactam and aminoglycoside antibiotics have been advocated, but due to the potential oto- and renal toxicity of aminoglycosides and the expanded antibacterial spectrum of new cephalosporins and penicillins, combinations of two beta-lactam antibiotics have been proposed and used. The potential advantages of such combinations in addition to possible reduced renal toxicity include the following: (i) enhanced antipseudomonas and enterococcal activity by the addition of the penicillin to the cephalosporin, (ii) enhanced staphylococcal and *Enterobacteriaceae* activity by the addition of the cephalosporin to the penicillin, and (iii) possible synergistic activity against a wide range of potential pathogens. A major disadvantage to such combinations would be the occurrence of antagonism between the beta-lactam agents. Thus, we have examined the in vitro combination activities of mezlocillin with ceftizoxime, moxalactam, cefoxitin, and amikacin against gram-negative bacilli and gram-positive cocci and azlocillin in combination with the same agents against *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Antibiotics. The antibiotics utilized in this study were amikacin (Bristol Laboratories, Syracuse, N.Y.), azlocillin and mezlocillin (Miles Pharmaceuticals, West Haven, Conn.), cefoxitin (Merck Sharp & Dohme, West Point, Pa.), ceftizoxime (Smith Kline & French Laboratories, Philadelphia, Pa.), and moxalactam (Eli Lilly & Co., Indianapolis, Ind.).

Bacterial isolates. The bacteria were clinical isolates and included 104 strains of *P. aeruginosa*, 55 strains of *Escherichia coli*, 52 strains of *Klebsiella pneumoniae*, 50 strains of *Enterobacter aerogenes*, 50 strains of *Serratia marcescens*, 26 strains of *Proteus vulgaris*, 25 strains of *Morganella morganii*, 49 strains of *Streptococcus faecalis*, 51 strains of *Staphylococcus aureus*, and 10 strains of *Acinetobacter calcoaceticus* var. *anitratus*. Of the gram-negative isolates tested, 38% were resistant to gentamicin. Of the *P. aeruginosa* strains tested, 38% were resistant to gentamicin (MIC, >4 μg/ml), 22% were resistant to amikacin (MIC, >16 μg/ml), and 9% were resistant to tobramycin (MIC, >4 μg/ml). A total of 6% of *P. aeruginosa* strains were resistant to all three aminoglycosides. All isolates were kept on glass beads in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) plus 10% glycerol at −80°C until used (3).

Antibiotic susceptibility tests and synergy studies. Antibiotics were prepared in divalent cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) (1, 19). Calcium content (≥50 μg/ml) was confirmed on an ASTRA-8 (Beckman Instruments, Inc., Brea, Calif.) (17), and the magnesium concentration (≥25 μg/ml) was determined on an atomic absorption spectrophotometer (Instrumentation Laboratories, Inc. [model 253], Lexington, Mass.) (6). Broth microdilution synergy trays were prepared as partial checkerboards in plastic microdilution panels (Dynatech Laboratories, Inc., Alexandria, Va.) as follows: azlocillin or mezlocillin in serial twofold dilutions from 128 to 0.125 μg/ml was tested alone plus combined with two concentrations of ceftizoxime (1 and 64 μg/ml), moxalactam (1 and 64 μg/ml), and amikacin (2 and 16 μg/ml) and a single concentration of cefoxitin (8 μg/ml). Prepared panels were stored at −80°C.

The MICs of each agent against *E. coli* 25922, *S. aureus* 29213, and *S. faecalis* 29212 control strains (American Type Culture Collection, Rockville, Md.) were determined on the mezlocillin checkerboard, and *P. aeruginosa* 27853 was tested on both the azlocillin and mezlocillin checkerboards before and after the study commenced.

Panels containing mezlocillin were tested against all isolates, whereas those containing azlocillin were only tested against *P. aeruginosa*. Each isolate was inoculated into the panels at a final organism density of 5×10^5 to 1×10^6 CFU/ml, incubated overnight (18 to 20 h) at 35°C, and read as MICs.

MBC determination. The entire volume of the first three clear wells (0.1 ml) from each antibiotic combination and mezlocillin or azlocillin alone was subcultured as a single line onto separate 5% sheep blood agar plates. The subculture was allowed to dry onto the agar surface, then streaked in three directions, and incubated at 35°C overnight; the number of CFU was recorded (16).

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TABLE 1. In vitro activity of azlocillin and mezlocillin alone against gram-negative bacilli and gram-positive cocci

Organism	No. tested (all isolates)	Antibiotic	Concn (μg/ml)	
			MIC_{90}	MBC_{90}
Pseudomonas aeruginosa	104	Azlocillin	32	64
	104	Mezlocillin	128	128
Acinetobacter calcoaceticus var. *anitratus*	10	Mezlocillin	64	64
Escherichia coli	55	Mezlocillin	128	128
Klebsiella pneumoniae	52	Mezlocillin	>128	>128
Enterobacter aerogenes	50	Mezlocillin	64	64
Serratia marcescens	50	Mezlocillin	>128	>128
Proteus vulgaris	26	Mezlocillin	1	2
Morganella morganii	26	Mezlocillin	4	4
Staphylococcus aureus (penicillin resistant)	51	Mezlocillin	64	64
Streptococcus faecalis	49	Mezlocillin	2	2

The MBC was defined as the lowest antibiotic concentration (along with subsequent higher concentrations) at which 0.1% or less of the original inoculum survived after 18 to 20 h of incubation.

Combination interpretation. Antibiotic combination activity was interpreted as follows: partial synergism, a fourfold or greater decrease in the azlocillin or mezlocillin MIC; antagonism, a fourfold or greater increase in the azlocillin or mezlocillin MIC; indifference, the range of changes between synergism and antagonism; no interpretation, this determination was made (i) when any organism was susceptible to a concentration less than or equal to the concentration of the combination antibiotic or (ii) when any organism was resistant to the combination antibiotic concentration and at least a fourfold lowering in the ureidopenicillin MIC or MBC was not observed.

RESULTS

The azlocillin and mezlocillin concentrations at which 90% of strains were inhibited (MIC_{90}) and killed (MBC_{90}) are shown in Table 1. Ureidopenicillin activity in combination with ceftizoxime, moxalactam, and amikacin is shown in Table 2 (*P. aeruginosa*, *Enterobacteriaceae*, *S. aureus*, and *S. faecalis*). Data is expressed by percent partial synergism, indifference, and antagonism of the MIC for each combination and also by ureidopenicillin MIC_{90} and MBC_{90} alone compared with the combination values. Combinations with less than 10 evaluable isolates were not included in the tables.

Partial MIC synergism occurred with both ureidopenicillin-plus-amikacin and ureidopenicillin-plus-cephalosporin combinations, although percentages varied, depending upon the individual species and combination. Ten isolates of *A. calcoaceticus* var. *anitratus* produced 100% indifference with mezlocillin plus 1 μg of moxalactam per ml in data not shown in the tables. Among the *Enterobacteriaceae* (except *S. marcescens*), nearly all strains were inhibited by ≤1 μg of ceftizoxime and moxalactam per ml; thus, the few remaining strains were grouped together. The *Serratia* species consisted of a larger, more resistant species and was analyzed separately. In data not shown in the tables, the percent partial synergistic activity as measured by the MBC was approximately the same or was slightly lower compared with the MIC for both combinations of ureidopenicillins plus amikacin and ureidopenicillins plus cephalosporins.

We observed antagonism most often with the ureidopenicillin-and-cefoxitin combinations when tested against gram-negative bacilli, thus corroborating the method with published reports (8, 11, 15). Against *P. aeruginosa*, antagonism occurred with azlocillin plus cefoxitin (38%) and with mezlocillin plus cefoxitin (7%). Another 7% of azlocillin- and 21% of mezlocillin-plus-cefoxitin combinations were not interpretable for antagonism because of the test panel MIC range. Mezlocillin plus cefoxitin also produced antagonism when used against *S. marcescens* (16%) and *E. aerogenes* (6%). Another 60% of *S. marcescens* and 6% of *E. aerogenes* could not be interpreted for antagonism because of the test panel MIC range. No antagonism was found among gram-positive cocci. Sporadic antagonism was observed among a few isolated strains with ureidopenicillins combined with ceftizoxime or moxalactam as listed in Table 2.

DISCUSSION

The activity spectrum of azlocillin and mezlocillin is well known (2, 4, 14, 18). Ureidopenicillins may have enhanced activity when combined with other antibiotics producing synergistic effects (5, 10, 11, 21). Several methods are used to study antibiotic combinations in vitro and include killing curves, agar or broth dilution checkerboard susceptibility, and isobolograms. The methods differ by effects measured (bacterial killing rate, MIC, or MBC) and may not correlate with each other (12). We devised a partial broth microdilution checkerboard consisting of serial twofold ureidopenicillin dilutions and two constant (low and high) concentrations of combination antibiotics allowing many organisms and combinations to be analyzed, for partial synergism, indifference, or antagonism with MIC and MBC determinations.

We found significant antagonism among gram-negative bacilli with ureidopenicillins combined with cefoxitin with this method as expected (8, 11, 15). There was little in vitro evidence of antagonism between combinations of ureidopenicillins with moxalactam or ceftizoxime. There was no evidence of antagonism among gram-negative bacilli with amikacin combinations or among gram-positive cocci with any combination.

In vitro synergism has been demonstrated most often, although at variable rates, among gram-negative bacilli with combinations of ureidopenicillins and amikacin (5, 10); rare synergism and antagonism have been reported with ureidopenicillins combined with moxalactam or ceftizoxime (9, 11) among gram-negative bacilli. Our in vitro data suggest that ureidopenicillin plus moxalactam or ceftizoxime combinations are as likely as ureidopenicillins plus amikacin to demonstrate partial synergism against gram-negative and

gram-positive pathogens. This diversity among reports probably reflects the number of organisms studied, organism variability (including initial MICs), and the difference between agar and broth determinations.

MBC determinations are a measure of actual bactericidal activity. We found MBC partial synergism percentages to be less than or equal to the MIC percentages. Simple listing of combination percent partial synergism does not reflect combination ureidopenicillin MIC_{90} and MBC_{90} decreases, especially decreases to within the in vitro ureidopenicillin susceptibility range (≤64 μg/ml).

Of note were our results with mezlocillin alone against *S. faecalis*. We demonstrated equivalent MIC and MBC measurements which are in contrast to the report by Krogstad

TABLE 2. Activity of ureidopenicillins + combination antibiotics

Organism	Antibiotic(s)	No. tested[a]	Interaction (%)[b]			Concn (μg/ml)			
						Ureidopenicillin alone[a]		Ureidopenicillin in combination[a]	
			Partial synergism	Indifference	Antagonism	MIC_{90}	MBC_{90}	MIC_{90}	MBC_{90}
Pseudomonas aeruginosa	Azlocillin + 1 μg/ml ceftizoxime	104	1	98	1	32	64	32	64
	Azlocillin + 64 μg/ml ceftizoxime	44	41	59	0	64	128	64	128
	Azlocillin + 1 μg/ml moxalactam	104	1	96	3	32	64	32	64
	Azlocillin + 64 μg/ml moxalactam	11	82	18	0	>128	>128	64	64
	Azlocillin + 2 μg/ml amikacin	102	47	53	0	32	64	16	32
	Azlocillin + 16 μg/ml amikacin	23	78	22	0	64	64	16	16
	Mezlocillin + 1 μg/ml ceftizoxime	104	4	95	1	128	128	128	128
	Mezlocillin + 1 μg/ml moxalactam	104	1	98	1	128	128	128	128
	Mezlocillin + 64 μg/ml moxalactam	45	27	73	2	>128	>128	128	>128
	Mezlocillin + 2 μg/ml amikacin	102	45	55	0	128	128	32	64
	Mezlocillin + 16 μg/ml amikacin	26	87	13	0	>128	>128	16	16
Enterobacteriaceae (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Morganella morganii*)	Mezlocillin + 1 μg/ml ceftizoxime	14	57	36	7	128	>128	128	>128
	Mezlocillin + 1 μg/ml moxalactam	12	33	67	0	32	>128	32	128
	Mezlocillin + 2 μg/ml amikacin	23	83	17	0	>128	>128	16	16
Serratia marcescens	Mezlocillin + 1 μg/ml ceftizoxime	10[c]	80	20	0	>128	>128	64	128
	Mezlocillin + 1 μg/ml moxalactam	18[c]	72	28	0	>128	>128	64	>128
	Mezlocillin + 2 μg/ml amikacin	34[c]	97	3	0	>128	>128	64	>128
Staphylococcus aureus (penicillin resistant)	Mezlocillin + 1 μg/ml ceftizoxime	49	53	47	0	64	64	32	32
	Mezlocillin + 1 μg/ml moxalactam	51	80	20	0	64	64	4	4
Streptococcus faecalis	Mezlocillin + 1 μg/ml ceftizoxime	49	67	33	0	2	2	1	2
	Mezlocillin + 64 μg/ml ceftizoxime	47	94	6	0	2	2	0.5	0.5
	Mezlocillin + 1 μg/ml moxalactam	49	10	90	0	2	2	2	4
	Mezlocillin + 64 μg/ml moxalactam	49	47	53	0	2	2	2	2
	Mezlocillin + 2 μg/ml amikacin	49	2	98	0	2	2	2	2
	Mezlocillin + 16 μg/ml amikacin	49	8	92	0	2	2	2	2

[a] Number of strains tested. Numbers may be different from Table 1, as only isolates with MIC greater than the combination antibiotic concentration are included in Table 2.

[b] Interaction (%) based upon ureidopenicillin MIC values.

[c] Another 25% of isolates with no interpretation, mezlocillin MIC ≥128 μg/ml alone and in combination.

and Parquette (7), who found MBC/MIC ratios for cell wall active agents to be ≥32 when tested against enterococcus. Clinical experience for therapy of serious enterococcal infections, such as endocarditis, clearly indicates the need for combination chemotherapy, including a penicillin and an aminoglycoside, for this infection. In a more recent publication, however, Fass and Wright (2a) demonstrated mezlocillin alone to be equivalent to ampicillin plus gentamicin for therapy of *S. faecalis* endocarditis in rabbits. Additional in vitro and animal investigation of mezlocillin therapy for enterococcal infections may be useful.

The partial checkerboard method allows for studies of several organisms and many different antimicrobial combinations. Combination interactions can be evaluated from this abridged checkerboard version, and results concur with published data from the complete checkerboard method. Selected organisms from the abridged method could be further studied with the full two-antimicrobial checkerboard technique. The significance of the interactions should be further evaluated in animal and human studies to determine whether the ureidopenicillin-plus cephalosporin combination is as effective as the ureidopenicillin-plus-amikacin regimen.

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