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Activities of Various β-Lactams and Aminoglycosides, Alone and in Combination, Against Isolates of *Pseudomonas aeruginosa* from Patients with Cystic Fibrosis

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The inhibitory and bactericidal activities of carbenicillin, ticarcillin, moxalactam, cefoperazone, azlocillin, piperacillin, ceftazidime, and three aminoglycosides, alone and in various combinations, were determined against 60 isolates of *Pseudomonas aeruginosa* from the sputum of patients with cystic fibrosis. Ceftazidime was the most active β-lactam, with minimum inhibitory and bactericidal concentrations for 90% of isolates of 4 μg/ml. Moxalactam was the least active of the new β-lactams, with activity equivalent to that of carbenicillin; each had a minimum inhibitory concentration for 90% of isolates of 64 μg/ml and a minimum bactericidal concentration for 90% of isolates of 128 μg/ml. All combinations of an aminoglycoside plus a β-lactam showed favorable inhibitory effects. Combinations of β-lactams showed mostly addition or indifference. Although little antagonism was seen with combinations of β-lactams or with aminoglycoside–β-lactam combinations, no consistent advantage of β-lactam combinations was demonstrated in vitro. These results suggest several single drugs and combinations that merit clinical evaluation in cystic fibrosis patients with *Pseudomonas* pulmonary infections.

Of the recently introduced β-lactam derivatives, azlocillin, cefoperazone, ceftazidime, moxalactam, and piperacillin have a higher order of activity than their predecessors against not only the *Enterobacteriaceae* but also against *Pseudomonas aeruginosa* (2, 10, 13, 15, 18). Thus, they might be useful in the treatment of *P. aeruginosa* pulmonary infections which are currently difficult to manage in patients with cystic fibrosis (CF). This possibility led to the current study of the activities of these new β-lactams, alone and in combination with each other or gentamicin, tobramycin, and amikacin, against 60 isolates of *P. aeruginosa* from the sputum of patients with CF.

MATERIALS AND METHODS

A total of 60 isolates of *P. aeruginosa*, including mucoid and nonmucoid strains, was identified by standard microbiological methods. The organisms were then stored on tryptic soy agar slants and were checked for purity and subcultured monthly.

Moxalactam (Eli Lilly & Co.), piperacillin (Lederle Laboratories), carbenicillin (Roerig-Pfizer Co.), azlocillin (Delbay Research Corp.), ticarcillin (Beecham Laboratories), cefoperazone (Pfizer Inc.), ceftazidime (Glaxo Research Group), gentamicin (Schering Corp.), tobramycin (Eli Lilly), and amikacin (Bristol Laboratories) were furnished as dry powders. Stock solutions were prepared from these powders and were used immediately or stored at −70°C for no longer than 2 weeks.

Minimum inhibitory concentrations (MICs) were determined by microbroth dilution, and combination studies were done by microbroth checkerboard methods. Mueller-Hinton broth was used for all determinations. Mueller-Hinton broth was adjusted to optimal calcium and magnesium concentrations and a pH of 7.2 to 7.4 (6). The preparation and inoculation of microbroth plates were accomplished with the MIC-2000 system (Dynatech Corp.). Plates were used immediately or stored at −70°C for no longer than 2 weeks. Microbroth plates received an inoculum consisting of a 10^{-3} dilution of a log-phase culture adjusted to the density of a 0.5 McFarland standard. Colony counts verified this inoculum to be between 8×10^4 and 2.5×10^5 colony-forming units per ml. Plates were incubated for 18 h at 35°C in ambient air. The MIC was defined as the lowest concentration of a drug that yielded no visible growth; the minimum bactericidal concentration (MBC) was defined as the lowest concentration of a drug which yielded no growth after a subculture of 1.5 μl from each well of the microtiter plate onto Mueller-Hinton agar and overnight incubation at 35°C. Control organisms (*Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) were included in all sets of inoculations, and the MICs were consistent within one twofold dilution of established values. Combination effects were defined as follows: synergy, at least a fourfold reduction in the MICs of

TABLE 1. Susceptibilities of 60 *P. aeruginosa* isolates from CF patients to various antimicrobial agents (microbroth dilution method)[a]

Antibiotic	Range		MIC_{50}[b]	MBC_{50}[b]	MIC_{90}[b]	MBC_{90}[b]
	MIC	MBC				
Ceftazidime	0.5–4	0.5–8	1	1	4	4
Cefoperazone	0.5–64	0.5–128	4	4	16	16
Moxalactam	4–128	4–256	16	32	64	128
Azlocillin	1–128	2–256	8	32	32	128
Piperacillin	1–128	1–256	4	8	32	128
Ticarcillin	2–64	2–256	16	32	32	128
Carbenicillin	4–128	8–256	16	64	64	128
Gentamicin	0.5–32	0.5–64	4	4	16	16
Tobramycin	0.5–16	0.5–32	2	2	4	4
Amikacin	0.5–64	0.5–64	8	8	16	32

[a] All results are given in micrograms per milliliter.
[b] Concentrations at which 50 or 90% of the organisms were inhibited (MIC) or killed (MBC).

both antibiotics; addition, a twofold reduction in the MIC of either or both antibiotics; indifference, no change in the MICs of the antibiotics; and antagonism, a fourfold increase in the MICs of either or both antibiotics. Significance was determined by the chi-square test (16).

RESULTS

The comparative activities of 10 single drugs against the 60 *P. aeruginosa* CF sputum isolates are summarized in Table 1. On a weight basis, ceftazidime was the most active antimicrobial agent tested, with a median MIC and MBC of 1 μg/ml; all strains were inhibited by 4 μg/ml and killed by 8 μg/ml. Moxalactam was the least active of the newer β-lactams, with a median MIC of 16 μg/ml and a median MBC of 32 μg/ml. All strains were inhibited by 128 μg of moxalactam per ml, and 2 of 60 strains were not killed by 256 μg/ml. Carbenicillin was very similar to moxalactam by all of these parameters. Bactericidal and inhibitory endpoints were similar at the inocula (10^5 colony-forming units per ml) tested for cefoperazone, piperacillin, ceftazidime, moxalactam, ticarcillin, and the aminoglycosides. Some divergence was noted for azlocillin and carbenicillin; however, cases in which the MBC was more than four times the MIC were rare. No significant differences in the activity of any drug were seen between mucoid and nonmucoid strains. Single-drug and combination data obtained from agar dilution testing performed at the same time as the microbroth testing were not significantly different from the data reported here.

The results of aminoglycoside–β-lactam combinations are shown in Table 2. The combination of azlocillin plus amikacin was most often synergistic (65%), however, all combinations of a β-lactam plus an aminoglycoside showed favorable inhibitory effects (synergy or addition) against a majority of isolates. The lowest percentage was seen with ceftazidime plus tobramycin (65%), and the highest was seen with azlocillin plus tobramycin (88%). Antagonism was rarely seen with any aminoglycoside–β-lactam combination; the highest was 3.3% for cefoperazone plus amikacin. Chi-square analysis showed no significant differences in the antibacterial effects of any combination of an aminoglycoside plus a β-lactam.

When β-lactams were combined (Table 3), the results were less favorable. For all combinations, the most common results were addition or indifference. Synergy ranged from 1.7% for piperacillin plus moxalactam and piperacillin plus ceftazidime to 15% for piperacillin plus cefoperazone and azlocillin plus ceftazidime. Chi-square analysis revealed no significant differences between any two combinations of β-lactam antibiotics. Antagonism was also rare; the highest was 6.7% for moxalactam plus ticarcillin.

DISCUSSION

No single drug or combination has consistently eradicated *P. aeruginosa* from the sputum of patients with CF, despite marked in vitro activity. This may be due in part to the low and erratic penetration of antibiotics into the sputum of these patients (7). In a recent report, the tobramycin dose was adjusted to achieve specific serum and sputum concentrations, and a considerable bacteriological response was noted in 74% of patients with CF (H. R. Rabin, L. E. Bryan, and F. L. Harley, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 509, 1980).

The antibacterial activities of drugs are often compared on a weight basis or in terms of achievable serum concentrations. In view of the aforementioned facts, it might be appropriate to consider the activities of drugs against *P. aeruginosa* with respect to concentrations that might

TABLE 2. Percentage of 60 isolates of *P. aeruginosa* inhibited by combinations of aminoglycoside and β-lactam drugs (checkerboard microbroth dilution method)

Combination		% (no.) of 60 isolates with the following effect:			
Aminoglycoside	β-lactam	Synergy	Addition	Indifference	Antagonism
Amikacin	Ticarcillin	46.7 (28)	38.3 (23)	15.0 (9)	0
	Moxalactam	51.7 (31)	21.7 (13)	25 (15)	1.7 (1)
	Cefoperazone	45 (27)	25 (15)	26.7 (16)	3.3 (2)
	Azlocillin	65 (39)	20 (12)	13.3 (8)	1.7 (1)
	Piperacillin	53.3 (32)	33.3 (20)	13.3 (8)	0
	Ceftazidime	26.7 (16)	43.3 (26)	30.0 (18)	0
Tobramycin	Ticarcillin	38.3 (23)	31.7 (19)	30 (18)	0
	Moxalactam	41.7 (25)	28.3 (17)	30 (18)	0
	Cefoperazone	50 (30)	26.7 (16)	21.7 (13)	1.7 (1)
	Azlocillin	58.3 (35)	30 (18)	11.7 (7)	0
	Piperacillin	60 (36)	23.3 (14)	16.7 (10)	0
	Ceftazidime	23.3 (14)	41.7 (25)	35.0 (21)	0
Gentamicin	Ticarcillin	48.3 (29)	33.3 (20)	18.3 (11)	0
	Moxalactam	43.3 (26)	30 (18)	26.7 (16)	0
	Cefoperazone	51.7 (31)	30 (18)	18.3 (11)	0
	Azlocillin	60 (36)	18.3 (11)	20 (12)	1.7 (1)
	Piperacillin	48.3 (29)	26.7 (16)	23.3 (14)	1.7 (1)
	Ceftazidime	25 (15)	43.3 (26)	31.7 (19)	0

be achieved in the bronchial secretions of patients with CF. Unfortunately, there are few data available to make this comparison meaningful for all of the drugs tested in this study. We have reviewed the literature and included in Table 4 references to studies in which the antibiotics in our study were quantitated in the sputum of patients with chronic bronchitis or CF. We then made the assumption that these drugs might be active if they were present in sputum in concentrations of at least four times the MIC, an assumption that is used successfully for the prediction of serum antibacterial effects in humans with systemic infection. Based on the MICs reported here, the percentage of isolates of *P. aeruginosa* which could be expected to be inhibited by concentrations of antibiotics achievable in the sputum of patients with CF are shown in Table 4. Although this type of analysis is highly speculative, especially considering the poor penetration of antibiotics into sputum and bronchial secretions, it provides another ratio-

TABLE 3. Percentage of 60 isolates of *P. aeruginosa* inhibited by combinations of β-lactam drugs (checkerboard microbroth dilution method)

Combination	% (no.) of 60 isolates with the following effect:			
	Synergy	Addition	Indifference	Antagonism
Moxalactam plus:				
Carbenicillin	11.7 (7)	40 (24)	43.3 (26)	5 (3)
Ticarcillin	10 (6)	36.7 (22)	46.7 (28)	6.7 (4)
Azlocillin	10 (6)	28.3 (17)	60 (36)	1.7 (1)
Piperacillin	1.7 (1)	15.0 (9)	83.3 (50)	0
Ceftazidime	8.3 (5)	55 (33)	36.7 (22)	0
Cefoperazone plus:				
Carbenicillin	13.3 (8)	36.7 (22)	50 (30)	0
Ticarcillin	11.7 (7)	33.3 (20)	53.3 (32)	1.7 (1)
Azlocillin	10 (6)	26.7 (16)	61.7 (37)	1.7 (1)
Piperacillin	15 (9)	23.3 (14)	58.3 (35)	3.3 (2)
Ceftazidime	8.3 (5)	55 (33)	36.7 (22)	0
Ceftazidime plus:				
Carbenicillin	10 (6)	53.3 (32)	36.7 (22)	0
Ticarcillin	8.3 (5)	50 (30)	41.7 (25)	0
Piperacillin	1.7 (1)	16.7 (10)	81.7 (49)	0
Azlocillin	15 (9)	46.7 (28)	36.7 (22)	1.7 (1)

TABLE 4. Activity of single antibiotics against 60 *P. aeruginosa* isolates from CF patients[a]

Antibiotic	One-fourth of the expected peak drug concn in sputum (μg/ml)	% of isolates inhibited at expected sputum concn	References used to estimate expected drug concn in sputum
Azlocillin	60	95	17
Cefoperazone	15	88	4, 8[b]
Carbenicillin	20	52	5,[b] 7[b]
Ticarcillin	3	2	5,[b] 12[b]
Tobramycin	1.5	20	1, 9[b]
Amikacin	1.5	8	3
Gentamicin	0.5	7	11

[a] Antibiotics are ranked in terms of the percentage of isolates inhibited by one-fourth of the expected drug concentrations.

[b] Refers to studies of antibiotic concentrations in the sputum of CF patients.

nale for the selection of antibiotic regimens in the treatment of *Pseudomonas* pulmonary infections in patients with CF.

Because of the problem of aminoglycoside toxicity and the possibility that the new β-lactam drugs might bind to different bacterial proteins and act synergistically, combinations of β-lactam drugs were also tested. However, little synergy was revealed, and no combination was significantly better than any other.

Antagonism, defined as a fourfold increase in the MIC of either drug, was rare, with most combinations showing none. Antagonism between moxalactam and piperacillin has been reported, with a twofold increase in the MIC of either drug as the criterion (D. F. Welch, D. N. Wright, M. I. Marks, and J. M. Matsen, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 614, 1980). We also noted some twofold increases in the MIC of piperacillin when certain concentrations of piperacillin and moxalactam were combined. These specific concentrations were deemed not likely to occur simultaneously in vivo and thus probably not clinically significant.

The MICs of the β-lactam drugs that we tested were 4 to 16 times lower than those recently reported by Prince and Neu against *P. aeruginosa* from CF patients (14). Prince and Neu used an agar dilution technique which, in our experience, gives slightly higher results than does the microbroth method. It is also possible that some of these isolates were from patients who had received these antibiotics for the treatment of pulmonary infections. Our results for azlocillin and amikacin alone and in combination are similar to those reported by Zinner et al. against 39 strains of *P. aeruginosa* whose origin was not specified (19).

We acknowledge the limitations of in vitro antibacterial studies for predicting clinical outcomes. Nevertheless, we believe that appropriate regimens for clinical evaluations of antibiotic therapy of pulmonary infections in CF patients should be selected in part by correlations of single and combination antibacterial activities with selected pharmacokinetic data.

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