ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1984, p. 535–538
0066-4804/84/100535-04$02.00/0

Vol. 26, No. 4

Synergism at Clinically Attainable Concentrations of Aminoglycoside and β-Lactam Antibiotics

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We evaluated the in vitro synergistic activity at clinically attainable concentrations of combinations of aminoglycoside and β-lactam antibiotics against 30 gentamicin-resistant clinical isolates of gram-negative bacilli. All 56 pairs of 4 aminoglycosides and 14 β-lactams were evaluated. Combinations with amikacin demonstrated inhibitory synergistic activity in 29% of the assays, as compared with 22% for netilmicin ($P = 0.018$), 17% for gentamicin ($P < 0.001$), and 13% for tobramycin ($P < 0.001$). Among the β-lactams, combinations with cefoperazone, ceftriaxone, or cefpiramide (SM-1652) demonstrated inhibitory synergistic activity most often (39, 38, and 35% of the assays, respectively) and with ceforanide, cefsulodin, and imipenem least often (≤8% each). The most active combination was amikacin and ceftriaxone, with which 67% of the assays demonstrated inhibitory synergism. Isolates with high-level resistance to either antibiotic in a combination were unlikely to be inhibited synergistically by the combination. Further, combinations generally demonstrated little synergistic activity against isolates highly susceptible to β-lactams.

Antimicrobial synergism is said to occur when two or more antibiotics in combination exert an inhibitory effect on microorganisms that is greater than the additive effects of the individual antibiotics. Several animal (2, 3, 16, 18, 24) and human (1, 17) studies have demonstrated that certain antibiotic combinations are more effective than single antibiotics in eradicating serious infections and preserving life. Moreover, those combinations resulting in a successful therapeutic outcome are more likely to demonstrate in vitro synergism against infecting strains than less successful combinations (1, 9, 10, 14, 19, 25). Thus, it seems rational to use such in vitro data in selecting optimal combinations of antibiotics for the empirical therapy of serious bacterial infections. Most previous investigations of in vitro synergism have involved small numbers of different antibiotic combinations and bacterial species. In this study, we compared the in vitro synergistic activity of a large number of combinations of aminoglycoside and β-lactam antibiotics against a group of 30 gram-negative bacilli. Because synergism obtained at concentrations higher than those achieved with usual dosage regimens probably has little practical value, we expressed our results in terms of synergism obtained at clinically attainable concentrations of antibiotics.

(These studies were presented in part at the 13th International Congress of Chemotherapy, Vienna, Austria, 1983.)

MATERIALS AND METHODS

We selected for study 30 isolates of gentamicin-resistant (MIC, >8 μg/ml) gram-negative bacilli of several species from clinical specimens submitted to microbiology laboratories at the Seattle Veterans Administration Hospital and Harborview Medical Center in Seattle, Wash. Among the 30 isolates were (number of isolates in parentheses) species of *Achromobacter* (1), *Acinetobacter* (1), *Citrobacter* (2), *Enterobacter* (2), *Escherichia* (3), *Klebsiella* (5), *Providencia* (1), *Pseudomonas* (9), and *Serratia* (6). All isolates were identified by standard microbiological methods. We evaluated 4 aminoglycoside and 14 β-lactam antibiotics, including 8 cephalosporins and 6 non-cephalosporins. The antibiotics evaluated were amikacin, gentamicin, netilmicin, tobramycin, cefoperazone, ceforanide, cefotaxime, cefpiramide (SM-1652), cefsulodin, ceftazidime, ceftizoxime, ceftriaxone, imipenem, moxalactam, Sch 29482, apalcillin, mezlocillin, and piperacillin. Standard powders for these antibiotics were supplied by their manufacturers (see Acknowledgments).

Antimicrobial susceptibility testing was performed by the agar dilution method with Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) containing twofold increments of an antibiotic alone or in combination (aminoglycoside and β-lactam) in a "checkerboard" arrangement. Inocula of ca. 10^5 CFU in the log phase were applied to freshly prepared agar with the replicating device of Steers et al. (22). The range of drug concentrations evaluated for single-antibiotic (initial) MICs was 0.06 to 512 μg/ml; those for combination MICs were 0.06 to 32 μg/ml for aminoglycosides and 0.06 to 64 μg/ml for β-lactams. Plates were incubated for 18 h at 35°C in ambient air. The MIC of a single antibiotic or a combination of antibiotics was the lowest concentration producing inhibition of bacterial growth. MICs for control ATCC strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were determined each time a checkerboard assay was done. For each isolate, all 56 amino-glycoside–β-lactam pairs were evaluated for synergism (1,680 assays were done).

Synergism at clinically attainable concentrations was defined, for the purpose of this study, as the inhibition of growth at concentrations of antibiotics in combination that were no more than one-fourth the initial MIC of each drug and ≤16 μg/ml for amikacin, ≤8 μg/ml for netilmicin, ≤4 μg/ml for gentamicin and tobramycin, ≤8 μg/ml for cefotaxime, ceftizoxime, imipenem, and Sch 29482, ≤16 μg/ml for cefsulodin and ceftazidime, ≤64 μg/ml for cefpiramide and ceftriaxone, and ≤32 μg/ml for all other β-lactams. These concentration breakpoints were based on a review of the pharmacokinetic data in published articles and from pharmaceutical companies and represent levels generally attainable at the midpoint of dosing intervals at intervals and doses that one might use to treat serious infections. For

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comparison, some analyses were repeated with different criteria for clinically attainable concentrations. We excluded from synergism analyses assays involving isolates for which the initial MIC of either antibiotic was ≤0.125 μg/ml, as a fourfold reduction in MICs could not be assessed. Because of the limited range of concentrations used, we were not able to systematically evaluate our assays for the presence of antagonism.

Chi-square analyses were used to test for statistical significance.

RESULTS

Amikacin was the most active aminoglycoside against the 30 gram-negative isolates, having MICs of ≤16 μg/ml for 22 (73%) and ≤8 μg/ml for 19 (63%). The MICs of netilmicin, tobramycin, and gentamicin were ≤8 μg/ml for 18 (60%), 4 (13%), and 0 (0%) (per our selection criteria) isolates, respectively. The β-lactam antibiotics could be arbitrarily divided into three groups on the basis of their inhibitory activity. Ceftazidime, imipenem, and moxalactam were the most active, having MICs of ≤32 μg/ml for at least 90% of the isolates. Cefotaxime, ceftizoxime, ceftriaxone, and Sch 29482 were intermediate in activity, having MICs of ≤32 μg/ml for ca. two-thirds of the isolates. The MICs of the remaining seven antibiotics were this low for <50% of the isolates.

Of the 1,680 synergism assays done, 167 (10%) were excluded from the analyses because of the low initial MICs (≤0.125 μg/ml) of the β-lactams and, in two instances, because of technical problems with the assays. Of the 1,513 assays left, 306 (20%) showed synergism at clinically attainable concentrations. Amikacin was significantly more active than the other aminoglycosides in combination with β-lactams (Table 1). The difference between netilmicin and tobramycin was also statistically significant ($P < 0.001$). When clinically attainable concentrations were defined as ≤8 μg/ml for amikacin and ≤4 μg/ml for netilmicin, synergistic activity was demonstrated in 29% of the assays with amikacin (no change) and in 17% of the assays with netilmicin ($P < 0.001$). Cefoperazone, ceftriaxone, and cefpiramide in combination with aminoglycosides were the most active β-lactams in demonstrating synergism, whereas ceforanide, cefsulodin, and imipenem were the least active (Table 1). Certain β-lactams, particularly ceftazidime, imipenem, and Sch 29482, which were very active alone, showed relatively poor synergistic activity with aminoglycosides.

TABLE 1. Number of evaluable assays done and percentage of assays demonstrating inhibitory synergism at clinically attainable concentrations of antibiotics

Antibiotic	No. of assays	% of assays synergistic
β-Lactam + indicated aminoglycoside		
Amikacin	378	29
Gentamicin	379	17[a]
Netilmicin	379	22[b]
Tobramycin	377	13[a]
Aminoglycoside + indicated β-lactam		
Cefoperazone	120	39
Ceforanide	120	4
Cefotaxime	84	26
Cefpiramide	120	35
Cefsulodin	120	8
Ceftazidime	114	21
Ceftizoxime	80	23
Ceftriaxone	84	38
Imipenem	116	8
Moxalactam	76	29
Sch 29482	120	11
Apalcillin	120	19
Mezlocillin	119	19
Piperacillin	120	18

[a] $P < 0.001$ versus amikacin.
[b] $P = 0.018$ versus amikacin.

TABLE 2. Percentage of assays demonstrating inhibitory synergism with various antibiotic combinations and at clinically attainable aminoglycoside concentrations

β-Lactam	% of assays showing inhibitory synergism at indicated clinically attainable aminoglycoside concn (μg/ml)							
	Amikacin		Gentamicin		Netilmicin		Tobramycin	
	16	8	8	4	8	4	8	4
Cefoperazone	53	53	37	27	47	43	40	30
Ceforanide	7	7	7	3	7	7	0	0
Cefotaxime	38	38	24	19	29	19	29	19
Cefpiramide	43	43	40	40	30	27	37	27
Cefsulodin	7	7	3	3	7	3	10	7
Ceftazidime	29	29	24	17	31	28	7	7
Ceftizoxime	40	40	35	15	20	15	10	0
Ceftriaxone	67	67	29	24	43	38	24	19
Imipenem	14	14	10	7	3	0	7	7
Moxalactam	53	53	21	11	42	32	11	11
Sch 29482	23	23	0	0	13	13	10	7
Apalcillin	23	23	23	23	17	10	13	13
Mezlocillin	27	27	23	23	13	7	14	14
Piperacillin	13	13	27	23	20	10	23	17

Amikacin was the aminoglycoside most often synergistic with the majority of β-lactams, and amikacin with ceftriaxone was the most active combination tested, with synergism demonstrated in 67% of the assays (Table 2). Of the β-lactams, cefoperazone, cefpiramide, and ceftriaxone were the most consistently synergistic with the four aminoglycosides (Table 2). No combination with ceforanide, cefsulodin, or imipenem demonstrated synergistic activity in more than 14% of the assays. When, for comparison with results obtained with previously defined clinically attainable concentrations, we considered 32 μg/ml to be the clinically attainable concentration for all the β-lactams, the synergistic activity of cefotaxime was considerably higher with amikacin (71% of the assays) and netilmicin (43% of the assays), that of ceftizoxime was higher with amikacin (50% of the assays) and netilmicin (35% of the assays), and that of cefpiramide was lower with amikacin (27% of the assays), gentamicin (30% of the assays), and netilmicin (20% of the assays); most other differences were no more than 5%.

Synergistic activity at clinically attainable concentrations was associated with the initial MICs of antibiotics (Table 3). Assays with isolates for which the initial MICs of aminoglycosides were low showed more synergistic activity than assays with isolates for which the initial MICs of aminoglycosides were high. When the analyses were controlled for the initial MICs of aminoglycosides, no aminoglycoside was consistently more active synergistically than the other ami-

noglycosides among the different MIC categories (data not shown). There was also an association between synergistic activity and the initial MICs of β-lactams (Table 3). However, synergistic activity was lower when the initial β-lactam MICs were either low or high than when they were mid-range. The association of the initial MICs of β-lactams and those of aminoglycosides with synergistic activity appeared to be somewhat independent of each other (Table 3).

DISCUSSION

Combinations of aminoglycoside and β-lactam antibiotics are commonly used in clinical practice for the treatment of serious infections. Combination therapy results in a broader spectrum of coverage for the empirical therapy of presumed sepsis, may prevent or delay the development of resistance among organisms (8, 18), and may result in synergistic activity. The theoretical benefits of synergistic combinations are lower toxicity (3) and more rapid killing of bacteria (20). Because animal and human synergism data are sparse, in vitro data are often used to select optimal combinations for clinical use. Although the data are inconclusive, several studies have reported an association between the in vitro synergistic activity of combinations and the therapeutic outcome in both animals (2, 13, 21, 24) and humans (1, 9, 10, 14, 19, 25).

The findings in this study are similar to those of certain other investigators who have reported higher in vitro synergistic activity of amikacin than of other aminoglycosides (15, 23; H. Giamarellou, J. Bouzos, and Y. Tagari, Abstr. Int. Congr. Chemother. 13th, Vienna, Austria, part 48, p. 59–62, 1983) and higher activity of cefoperazone and lower activity of cefsulodin and imipenem than of other β-lactams (4; L. S. Young and D. Meyer-Dudnik, Abstr. Int. Congr. Chemother. 13th, Vienna, Austria, part 48, p. 40–43, 1983).

Some of the differences observed between the combinations evaluated in this study resulted from the various degrees of resistance of the strains we selected to the different antibiotics studied. The strong association between high initial MICs and low synergistic activity was not unexpected, given our requirement that MICs associated with synergism had to be clinically attainable. An association between antibiotic resistance and synergistic activity has been reported by others (5–7, 11, 12). However, the finding of an association between low β-lactam MICs and low synergistic activity has not, to our knowledge, been reported previously. Further studies must be done to determine the cause for and clinical significance of this phenomenon.

TABLE 3. Percentage of assays demonstrating inhibitory synergism by the initial MICs of aminoglycosides and β-lactams

Initial β-lactam MIC (μg/ml)[a]	Initial aminoglycoside MIC (μg/ml)[b]				
	≤8	16–32	64–128	≥256	Total
0.25–0.50	10 (58)	7 (55)	0 (6)	0 (16)	7 (135)
1–2	22 (64)	22 (54)	0 (21)	0 (21)	16 (160)
4–16	62 (92)	30 (130)	11 (53)	4 (79)	30 (354)
32–128	53 (85)	35 (116)	29 (69)	7 (58)	34 (328)
256	35 (26)	19 (26)	12 (26)	3 (38)	16 (116)
≥512	13 (168)	13 (107)	2 (63)	0 (82)	9 (420)
Total	31 (493)	24 (488)	13 (238)	3 (294)	

[a] MIC of an antibiotic tested alone.
[b] Numbers in parentheses represent numbers of assays performed.

The differences observed in synergistic activity between combinations were also influenced by our definition of clinically attainable antibiotic concentrations, as the pharmacokinetic properties of β-lactams may differ considerably and result in different attainable concentrations of these agents in serum. Although it is generally unclear how long the concentration of an agent in serum should optimally exceed the MIC for an infecting pathogen, we attempted to control for pharmacokinetic differences by requiring that combination MICs of a given β-lactam, to satisfy our definition of synergism, fall within concentrations attainable for ca. 50% of the dosing interval of that agent.

Although it remains unclear whether in vitro results can be reliably extrapolated for clinical use, in the absence of adequate clinical data it seems reasonable to use in vitro synergism data in selecting combinations for use in the empirical treatment of serious infections, when synergism is a desired goal of therapy. Our findings suggest that organisms with high-level resistance to both the aminoglycoside and β-lactam antibiotics alone are unlikely to be synergistically inhibited at clinically attainable concentrations of the antibiotics in combination. Further, combinations appear to be less active synergistically when the MIC of the β-lactam for the organism is low. Clinical trials must be done to properly evaluate these in vitro findings.

ACKNOWLEDGMENTS

We thank James J. Plorde for providing us with some of the clinical strains used in these assays, Delores Zeleznik for technical assistance, and Marsha Miller for secretarial assistance.

Amikacin and ceforanide and financial support for these studies were provided by Bristol Laboratories, Syracuse, N.Y. Additional drugs and support were provided as follows: cefsulodin, Abbott Laboratories, North Chicago, Ill.; ceftazidime, Glaxo Inc., Research Triangle Park, N.C.; cefotaxime, Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J.; ceftriaxone, Hoffmann-LaRoche Inc., Nutley, N.J.; piperacillin, Lederle Laboratories, Pearl River, N.Y.; tobramycin and moxalactam, Eli Lilly & Co., Indianapolis, Ind.; imipenem, Merck & Co. Inc., Rahway, N.J.; mezlocillin, Miles Pharmaceuticals, West Haven, Conn.; cefoperazone, Pfizer Inc., New York, N.Y.; gentamicin, netilmicin, and Sch 29482, Schering Corp., Bloomfield, N.J.; ceftizoxime, Smith Kline & French Laboratories, Philadelphia, Pa.; and apalcillin and cefpiramide (SM-1652), Wyeth Laboratories, Philadelphia, Pa.

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