Unpredictable Response of *Pseudomonas aeruginosa* to Synergistic Antibiotic Combinations In Vitro

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The combined activity of ticarcillin and each of three aminoglycosides, gentamicin, tobramycin, and amikacin, was studied with 45 clinical isolates of *Pseudomonas aeruginosa*. All strains individually were susceptible to each of these agents. Comparison of the results revealed highly variable interaction, both from strain to strain and from one aminoglycoside to another against the same strain. Against 11 strains, none of three antibiotic pairs tested was synergistic by conventional criteria; against the remaining 34, three, any two, or any one pair among those tested was synergistic. The minimal inhibitory concentrations of individual drugs gave no indication of the degree of synergism to be expected. We conclude that generalizations about the behavior of this organism vis-à-vis penicillin-aminoglycoside combinations in vitro are subject to many exceptions. Furthermore, no test using a particular antibiotic combination is reliable as a screen for other combinations. If this experience is duplicated in vivo, combination testing may be as important for the choice of combination therapy as individual susceptibility testing is for the choice of individual antibiotics.

Following the well-documented example of in vitro synergism between carbenicillin and gentamicin against *Pseudomonas aeruginosa* (4, 11), similar interaction was demonstrated between carbenicillin and tobramycin (1, 7) and between carbenicillin and amikacin (8, 9). The carbenicillin analog ticarcillin likewise acts synergistically with the newer aminoglycosides (5, 12). These findings suggest that synergism may be a predictable property of any combination of penicillin and aminoglycoside when an organism is susceptible to the individual drugs tested separately. Because of similarities in antibiotic spectrum and toxicity, the choice among the newer aminoglycosides and anti-pseudomonas penicillins is often dictated by individual bias, economic considerations, hospital policy, or the desire to hold a promising new agent in reserve. For these reasons, the choice of combination therapy is likely to be based on properties or availability of individual drugs rather than on examination of combinations as such.

To our knowledge, no systematic comparison of different aminoglycosides in combination with the same penicillin against individual strains of *P. aeruginosa* has been undertaken. The purpose of our study was to examine the extent to which three aminoglycosides (gentamicin, tobramycin, and amikacin) are interchangeable in combination with one penicillin (ticarcillin); what relationship exists between minimal inhib-

itory concentrations (MICs) of these aminoglycosides used alone and in combination with ticarcillin; and what generalizations about *P. aeruginosa* can be inferred from the study of a limited number of strains.

MATERIALS AND METHODS

Bacteria. Fifty strains of *P. aeruginosa* isolated from various clinical sources in 1976 and 1977 were used in this study. Identification was based on typical reaction on Sella medium, fluorescence, and growth at 42°C.

Antibiotics. Susceptibility-testing powders were obtained from the manufacturers, namely ticarcillin from Beecham Laboratories, Bristol, Tenn.; gentamicin from Schering Corporation, Bloomfield, N.J.; tobramycin from Ely Lilly & Co., Indianapolis, Ind.; and amikacin from Bristol Laboratories, Syracuse, N.Y.

Susceptibility to individual antibiotics. MICs of the four antibiotics were determined individually for each isolate of *P. aeruginosa*. From an initial concentration of 200 µg/ml, serial twofold dilutions of the drugs in 0.05-ml volumes of Trypticase soy broth were made in microtiter plates, using the Medimixer automatic diluter (Linbro Chemicals, New Haven, Conn.). Overnight growth of the organism in Trypticase soy broth was adjusted to a 0.5 MacFarland standard and further diluted 1:1,500 to give a concentration of approximately 10⁶ organisms per ml; 0.05 ml of this suspension was added to each well. The plates were incubated at 37°C in a moist chamber. The MIC was defined as the lowest concentration in which no turbidity was detected after overnight incubation. All

tests were performed in duplicate; in cases of disagreement, the lower value was recorded as the MIC.

Susceptibility to antibiotic pairs. From an initial concentration equal to twice the MIC, each member of the pair of antibiotics to be tested was diluted in eight parallel rows of wells, in the same microtiter plate, with ticarcillin from left to right and the aminoglycoside from top to bottom; the last well in each row received only diluent. To this panel of different antibiotic combinations an inoculum of the organism was added as above and, after overnight incubation, the pattern of inhibition was read. All tests were performed in duplicate.

RESULTS

Of the 50 strains tested, 5 were moderately resistant to gentamicin (MIC, 12.5 to 50 $\mu\text{g}/\text{ml}$); because this introduced an additional variable into the comparison of aminoglycosides, the 5 strains were excluded from the study. The remaining 45 strains were susceptible to all 4 agents, with MICs in ranges of 6.2 to 50 μg of ticarcillin, 0.8 to 6.2 μg of gentamicin, 0.4 to 6.2 μg of tobramycin, and 0.8 to 12.5 μg of amikacin per ml.

The patterns of antibiotic interaction varied considerably both from strain to strain and among the three aminoglycosides.

Figure 1 shows, for each strain, the MIC of each aminoglycoside used alone compared with its MIC in the presence of a subinhibitory concentration of ticarcillin. The concentration of ticarcillin selected for this comparison is one-half its MIC rather than a predetermined value, so that its contribution to the antibiotic pair is equivalent for each strain. Despite this equivalent contribution from ticarcillin, the effect on the MIC of the aminoglycoside is extraordinarily variable. For example, among the 16 strains inhibited by gentamicin alone at the same concentration, 3.1 $\mu\text{g}/\text{ml}$, the MICs of gentamicin in the presence of ticarcillin ranged 64-fold, from 0.025 to 1.6 $\mu\text{g}/\text{ml}$.

In comparing the three aminoglycosides, their patterns of interaction with ticarcillin appeared to vary independently of each other. Against any one strain, all three, any two, any one, or none of the aminoglycoside-ticarcillin pairs displayed synergism (defined as inhibition by a fourfold or greater reduction in concentration of each member of the pair compared with their individual MICs) (Table 1). By this conventional criterion, none of the 3 pairs would be considered synergistic against 11 of the 45 strains (24%). Among the remaining 34 strains, every possible pattern was seen. Thus, it is apparent that no matter which antibiotic pair was tested, the results could not be extrapolated to other pairs.

Even granting that the definition of synergism may be too rigid for a phenomenon of degree

rather than absolutes, the differences in degree are still sufficient to justify this conclusion. Some illustrative examples are given in Tables 2 and 3. In Table 2, the fractional inhibitory concentrations (FIC) and FIC indexes (10) of the three antibiotic pairs against selected bacterial strains are compared. The FIC index is a measure of synergism, and the contrasts among the antibiotic pairs are quite obvious. Table 3 compares, strain by strain, the effect of a constant concentration of ticarcillin (one-half the MIC) on the three aminoglycosides. It can be seen that the addition of ticarcillin so variably affects the aminoglycosides, even with respect to a single bacterial strain, that their rank order in mixture with ticarcillin may differ from that when tested alone.

DISCUSSION

In most of the literature on penicillin-aminoglycoside synergism against *P. aeruginosa*, the emphasis has been on the frequency with which this phenomenon can be demonstrated in vitro (1, 4, 5, 7-9, 11, 12) and its successful application in the therapy of pseudomonas infections in animals (2, 5) and humans (3, 6). The variability in response of bacterial strains is implicit in the published data but has not been stressed. Similarly, whereas the appearance of each new antipseudomonas agent has been followed by demonstration of synergistic interaction with others, no coordinated effort appears to have been directed specifically at comparing the effectiveness of synergistic pairs as distinct from the agents acting individually. The specific purpose of this study was to investigate the variability both among bacterial strains and among aminoglycosides.

The results presented here show that the be-

TABLE 1. Patterns of ticarcillin-aminoglycoside synergism against 45 strains of *P. aeruginosa*

No. of strains	Synergistic pairs ^a		
	TC-GM ^b	TC-TM	TC-AK
7	+	+	+
1	+	+	-
2	+	-	+
4	-	+	+
5	+	-	-
4	-	+	-
11	-	-	+
11	-	-	-

^a + and -, Presence and absence, respectively, of synergism, defined as inhibition by a pair in which the concentration of each member is one-fourth or less of its MIC when tested alone.

^b TC, Ticarcillin; GM, gentamicin; TM, tobramycin; AK, amikacin.

FIG. 1. Comparison of MICs of aminoglycosides when used alone and in the presence of ticarcillin. In all cases, the ticarcillin concentration is one-half its MIC. The aminoglycoside susceptibility of different strains (represented by dots) with the same MIC is affected very differently by equivalent concentrations of ticarcillin.

havior of any one strain of *P. aeruginosa* in response to antibiotic combinations cannot be predicted either from the behavior of others or even from their own behavior in response to single antibiotics. Furthermore, the substitution

of one aminoglycoside for another in combination with ticarcillin, even against strains susceptible to both individually, cannot be made on the assumption that they will display similar degrees of synergism.

TABLE 2. *Contrasting degrees of interaction between ticarcillin and different aminoglycosides against the same strains of P. aeruginosa*

Strain no.	Ticarcillin-aminoglycoside mixture ^a									
	TC-GM ^b			TC-TM			TC-AK			
	FIC ^c			FIC			FIC			
	TC	GM	FICI ^d	TC	TM	FICI	TC	AK	FICI	
6	0.500 (12.5/25)	0.250 (0.4/1.6)	0.750	0.125 (3.1/25)	0.250 (0.2/0.8)	0.375	0.125 (3.1/25)	0.250 (0.2/0.8)	0.375	
18	0.250 (3.1/12.5)	0.062 (0.1/1.6)	0.312	0.500 (6.2/12.5)	0.250 (0.2/0.8)	0.750	0.500 (6.2/12.5)	0.250 (1.6/6.4)	0.750	
21	0.500 (12.5/25)	0.125 (0.4/3.1)	0.625	0.500 (12.5/25)	0.250 (0.2/0.8)	0.750	0.125 (3.1/25)	0.250 (1.6/6.4)	0.375	
22	0.500 (12.5/25)	0.250 (0.8/3.1)	0.750	0.125 (3.1/25)	0.125 (0.4/3.1)	0.250	0.250 (6.2/25)	0.062 (0.4/6.4)	0.312	
31	0.250 (6.2/25)	0.125 (0.2/1.6)	0.375	0.500 (12.5/25)	0.125 (0.1/0.8)	0.625	0.500 (12.5/25)	0.250 (0.4/1.6)	0.750	

^a Data refer to the most synergistic mixture, i.e., that mixture for which the FIC index (see footnote d) has the lowest value.^b TC, Ticarcillin; GM, gentamicin; TM, tobramycin; AK, amikacin.^c FIC is equal to the ratio of concentration in the mixture to MIC when used alone (12), as shown in parentheses below the value.^d FIC_i, Fractional inhibitory concentration index, which is equal to the sum of FICs for the members of the mixture. For any synergistic mixture as defined in the text, the FIC_i is no greater than 0.500; the smaller the FIC_i, the greater the degree of synergism. In the table, the FIC_is of synergistic mixtures are boldface.

TABLE 3. *Disparate effects of a constant concentration of ticarcillin on MICs of different aminoglycosides against the same strains of P. aeruginosa*

Strain no.	MIC (μg/ml)						Concn. of TC (μg/ml) ^a
	AG ^b alone			AG in presence of TC			
	GM	TM	AK	GM	TM	AK	
1	3.1	1.6 ^{*c}	3.1	0.4	0.4	0.1 [*]	12.5
3	3.1	1.6 [*]	3.1	0.1 [*]	0.2	0.2	12.5
7	0.8 [*]	0.8 [*]	3.1	0.05 [*]	0.4	0.1	12.5
8	0.8 [*]	0.8 [*]	1.6	0.1	0.025 [*]	0.1	12.5
18	1.6	0.8 [*]	3.1	0.025 [*]	0.2	0.8	6.2
22	3.1 [*]	3.1 [*]	6.2	0.4	0.1 [*]	0.4	12.5
25	3.1	0.8 [*]	3.1	0.025 [*]	0.4	0.2	6.2
28	1.6	0.8 [*]	1.6	0.1	0.2	0.05 [*]	6.2
39	0.8 [*]	0.8 [*]	1.6	0.2	0.4	0.05 [*]	6.2
40	6.2	1.6 [*]	3.1	0.2	0.2	0.05 [*]	12.5
44	6.2	1.6 [*]	3.1	0.2	0.4	0.1 [*]	3.1
46	3.1	1.6 [*]	6.2	0.2 [*]	0.4	0.4	3.1
50	0.8 [*]	0.8 [*]	3.1	0.2	0.05 [*]	0.2	12.5

^a Equal to one-half of the MIC of ticarcillin when used alone for each strain.

^b AG, Aminoglycoside; TC, ticarcillin; GM, gentamicin; TM, tobramycin; AK, amikacin.

^c Asterisks designate the aminoglycoside(s) with the lowest MIC in each case and draw attention to the difference in rank order brought about by ticarcillin.

Whether these differences are clinically important cannot be predicted from in vitro studies alone. However, experimental in vivo studies to confirm contrasts such as shown in Tables 2 and 3 appear justified, particularly in view of the previously documented correlation between in vitro synergism and clinical effectiveness (5, 6). If the significance of these contrasts can be documented, it may eventually prove to be just as important to test antibiotic combinations as individual antibiotics in the clinical laboratory.

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LITERATURE CITED

- Anderson, E. L., P. K. Gramling, P. R. Vestal, and W. E. Farrar, Jr. 1975. Susceptibility of *Pseudomonas aeruginosa* to tobramycin or gentamicin alone and combined with carbenicillin. *Antimicrob. Agents Chemother.* 8:300-304.
- Andriole, V. T. 1971. Synergy of carbenicillin and gentamicin in experimental infection with *Pseudomonas*. *J. Infect. Dis.* 124:S64-S55.
- Bodey, G. P., R. Feld, and M. A. Burgess. 1976. Beta lactam antibiotics alone or in combination with gentamicin for therapy of gram-negative bacillary infections in neutropenic patients. *Am. J. Med. Sci.* 271:179-186.
- Brumfitt, W., A. Percival, and D. A. Leigh. 1967. Clinical and laboratory studies with carbenicillin. A new penicillin active against *Pseudomonas pyocyanea*. *Lancet* i:1289-1293.
- Comber, K. R., M. J. Basker, C. D. Osborne, and R. Sutherland. 1977. Synergy between ticarcillin and tobramycin against *Pseudomonas aeruginosa* and *Enterobacteriaceae* in vitro and in vivo. *Antimicrob. Agents Chemother.* 11:956-964.
- Klasteraky, J., R. Cappel, and D. Daneau. 1972. Clinical significance of in vitro synergism between antibiotics in gram-negative infections. *Antimicrob. Agents Chemother.* 2:470-475.
- Klasteraky, J., A. Henri, and L. Vandenborre. 1973. Antimicrobial activity of tobramycin and gentamicin used in combination with cephalothin and carbenicillin. *Am. J. Med. Sci.* 266:13-21.
- Kluge, R. M., H. C. Standiford, B. A. Tatem, V. M. Young, W. H. Greene, S. C. Schimpff, F. M. Calia, and R. B. Hornick. 1974. Comparative activity of tobramycin, amikacin, and gentamicin alone and with carbenicillin against *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 6:442-446.
- Marks, M. L., S. Hammerberg, G. Greenstone, and B. Silber. 1976. Activity of newer aminoglycosides and carbenicillin, alone and in combination, against gentamicin-resistant *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 10:399-401.
- Parsley, L., R. B. Provonchee, C. Glicksman, and S. H. Zinner. 1977. Synergistic activity of trimethoprim and amikacin against gram-negative bacilli. *Antimicrob. Agents Chemother.* 12:349-352.
- Sonne, M., and E. Jawetz. 1969. Combined action of carbenicillin and gentamicin on *Pseudomonas aeruginosa* in vitro. *Appl. Microbiol.* 17:893-896.
- Wald, E. L., H. C. Standiford, B. A. Tatem, F. M. Calia, and R. B. Hornick. 1975. BL-P1654, ticarcillin and carbenicillin: in vitro comparison alone and in combination with gentamicin against *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* 7:336-340.