Tobramycin (Nebramycin Factor 6): In Vitro Activity Against *Pseudomonas aeruginosa*[1]

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Tobramycin (factor 6 of the nebramycin complex) is a new aminoglycoside antibiotic isolated from *Streptomyces tenebrarius* which is active against *S. aureus*, *Enterobacteriaceae*, and *Pseudomonas aeruginosa*. Susceptibility to tobramycin of 96 strains of *P. aeruginosa*, including 45 recent isolates from blood, was studied by using agar and broth dilution methods. The minimum inhibitory concentration (MIC) for 83 of 96 strains was 3.12 μg/ml or lower in Mueller Hinton agar; MIC values were two to eight times lower in Mueller Hinton broth tests. Agar dilution MIC values were generally lower than those obtained in parallel tests with gentamicin. Killing curves obtained from serial sampling of broth cultures showed a 100- to 10,000-fold decline in viability of log-phase organisms within 30 min of exposure to the drug. Two-dimensional agar dilution tests with carbenicillin and tobramycin with 79 strains showed additive or synergistic effects; no antagonism was documented. Seventy-eight of 79 strains were inhibited by a combination of 50 μg of carbenicillin per ml and 1.56 μg of tobramycin per ml, blood levels which seem attainable in man. Tobramycin appears to be a potent, rapidly bactericidal antibiotic against *P. aeruginosa* and merits clinical evaluation.

The search for antimicrobial agents with significant activity against gram-negative bacilli has received impetus because such organisms are now the commonest cause of serious nosocomial infections in large hospitals (1), in patients with neoplasms (2), burns (16), and organ transplants (20). Of the gram-negative bacillary organisms, *Pseudomonas aeruginosa* has been the most resistant to treatment.

Antibiotics currently available for treatment of *P. aeruginosa* infections include the polymyxins (26), the aminoglycoside gentamicin (5, 15–17, 28), and carbenicillin (4, 6, 22). However, the dosage of gentamicin for use in systemic infections may be limited by ototoxicity or renal toxicity. Carbenicillin has been used in combination with gentamicin in an effort to increase therapeutic effectiveness, to prevent development of resistant organisms, and to achieve safe inhibitory serum levels of both drugs (21). With the exception of one report of antagonism (18), in vitro studies have consistently shown carbenicillin and gentamicin to act synergistically against many strains of *P. aeruginosa* (8, 19, 21, 23).

Tobramycin (nebramycin factor 6) is a stable, water-soluble aminoglycoside antibiotic isolated from the nebramycin complex of antibacterial substances produced by *Streptomyces tenebrarius* (11, 24, 27, 29). (Factor 6 of the nebramycin complex was originally given the generic name of ebbramycin in 1970. Subsequently, its official generic designation was changed to tobramycin.) Previous studies have shown a broad spectrum of activity in vitro against *Staphylococcus aureus* and a wide variety of gram-negative bacilli including *P. aeruginosa* (3, 29).

The present study was undertaken to determine (i) the in vitro susceptibility of recent clinical isolates of *P. aeruginosa* to tobramycin; (ii) the nature and rapidity of its antibacterial effect; (iii) the degree of correlation of cross-sensitivity and cross-resistance to gentamicin; and (iv) the effect of combining carbenicillin with tobramycin against *P. aeruginosa*.

MATERIALS AND METHODS

Antibiotics. Tobramycin was supplied as a dry powder (compound 818-100-B-218-1) and later as a solution (compound 47663) in a concentration of 1,000 μg/ml by Warren Wick and Henry Black of Eli Lilly and Co. (Indianapolis, Ind.) and stored at
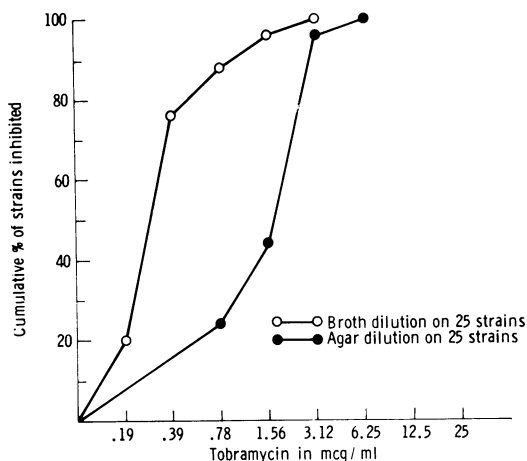
FIG. 1. *Comparison of Mueller Hinton agar and broth dilution MIC values for 25 strains.*

4 C. The dry powder was diluted with sterile saline to 1,000 µg/ml and either incorporated immediately into test media or stored at −65 C until further dilution. Disodium carbenicillin (775.2 µg/mg free acid activity was supplied by Christopher Demos of Beecham Pharmaceuticals (Clifton, N.J.) as a dry powder, stored at 4 C, and freshly diluted with sterile phosphate saline buffered to pH 7.4. Gentamicin, supplied as a dry powder by George Arcieri of Schering Corp. (Bloomfield, N.J.), was stored at 4 C and diluted with sterile saline.

In vitro tests. Ninety-six clinical isolates of *P. aeruginosa* recovered in the microbiology laboratory of Memorial Sloan-Kettering Cancer Center during 1969–1970, including 45 blood culture isolates, were identified by the method of Cowan and Steel (7). These isolates were cultured on Mueller Hinton agar (Difco) overnight at 37 C and then inoculated into Mueller Hinton broth (BBL) and incubated for 4 to 6 hr at 37 C to visible turbidity corresponding to approximately 5×10^7 organisms per ml. By using the inoculum-replicating device of Steers et al. (25), approximately 10^5 organisms of each strain were delivered onto Mueller Hinton agar plates containing twofold increments of tobramycin (0.39, 0.78, 1.56, 3.12, 6.25, 12.5, and 25 µg/ml) alone or carbenicillin (6.25, 12.5, 25, 50, 100, 250 µg/ml) alone. In two-dimensional tests, plates containing all possible combinations of the above concentrations of the two antibiotics were used. Minimal inhibitory concentrations (MIC) were recorded after 18 hr of incubation at 37 C as the lowest level of drug concentration to inhibit growth completely. Control plates without antibiotics were also inoculated to confirm viability of each tested strain. MIC values for each strain were recorded for single drugs, and the effect of two drug combinations (addition, synergism, or antagonism) were expressed by constructing isobolograms after the methods of Loewe (14) and Lacey (13). From the latter data, geometric mean MIC values were calculated for strains inhibited either additively or synergistically by combination of tobramycin and carbenicillin.

Broth MIC values were determined for 25 strains by inoculating approximately 10^8 and 10^6 organisms from overnight broth cultures into Mueller Hinton broth containing tobramycin and recording the lowest concentration at which no turbidity was detectable after 24 hr. Minimal bactericidal concentrations (MBC) were determined by subculturing from broth tubes showing no turbidity onto Mueller Hinton agar and were recorded as that concentration associated with no growth on agar.

Kinetics of bactericidal action. To determine the rapidity of killing, approximately 10^6 organisms of four strains in the early logarithmic phase of growth were inoculated into control tubes and Mueller Hinton broth containing tobramycin at a concentration equivalent to the MBC. Tubes containing antibiotic and bacteria were tumbled at 37 C on a Lab Tek aliquot mixer, and 0.005-ml samples were withdrawn at 0, 30, 60, 120, and 240 min, diluted in 1 ml of Mueller Hinton broth and incorporated into pour plates. Pour plates were made with agar at 55 C and were counted to determine residual viability after 24 hr of incubation at 37 C. The original broth samples were examined at 24 hr for turbidity.

RESULTS

Effect of tobramycin on P. aeruginosa. Figure 1 compares the agar and broth MIC values of tobramycin for the same 25 strains. Agar dilution MIC values were two to eight times higher than broth MIC levels. Table 1 summarizes the geometric mean MIC and MBC values, the standard deviations of these for the 25 strains, and the effect of inoculum size on MIC. In 13 strains tested with 10^8 and 10^6 organisms, ten showed an MIC two to eight times higher when the higher inoculum was used. MBC values were two to eight times higher than broth MIC for the same strain in 24 of 25 strains tested.

Rate of bactericidal effect of tobramycin. Killing curves for four strains showed a mean decrease in viability of 2.5 logs in 30 min and complete killing by 2 hr as evidenced by no growth on pour plates and clear broth tubes (Fig. 2). Control cultures incubated and tumbled without tobramycin showed a minimum increase of 100-fold in 2 hr.

TABLE 1. *Inhibition of P. aeruginosa in broth: comparison of inoculum size*

No. of strains	Inhibition with inoculum of		
	10^8 (MIC)[a]	10^6 (MIC)[a]	10^6 (MBC)[b]
13	1.18 ± 0.18	0.54 ± 0.19	1.56 ± 0.19
25		0.47 ± 0.18	1.44 ± 0.19

[a] Values expressed as minimum inhibitory concentration ± standard deviation.

[b] Values expressed as minimum bactericidal concentration ± standard deviation.
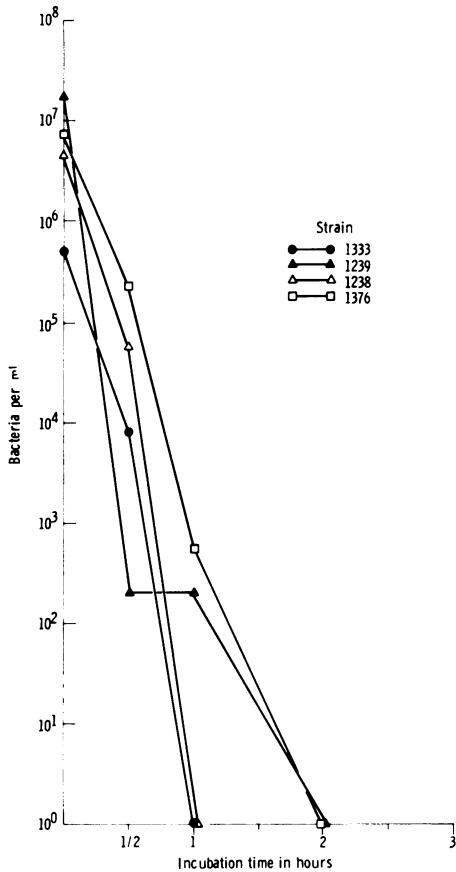
FIG. 2. *Growth curves of four strains of P. aeruginosa in tobramycin. Control increased by two logs.*

Tobramycin versus gentamicin. Figure 3 compares the agar dilution MIC obtained for tobramycin and gentamicin. For this group of 96 strains tested, agar dilution MIC for *P. aeruginosa* of tobramycin were lower than with gentamicin. Generally the difference was twofold, but a notable exception was one strain (S.S. no. 1456) with an MIC for tobramycin of 0.78 μg/ml in agar and no inhibition by 25 μg/ml of gentamicin in agar as well as broth dilution tests. Of 30 strains inhibited in agar by 3.12 μg/ml or greater of tobramycin, three were more sensitive to gentamicin on a weight basis. Of 29 strains inhibited in agar by 6.25 μg/ml or greater of gentamicin, 26 of 29 were inhibited by 3.12 μg/ml or less of tobramycin. In eight of 96 strains, there was wide discrepancy between the MIC values of gentamicin and tobramycin, with neither drug being consistently the more potent; in this group the MIC values varied by four or more dilutions.

Effect of combining tobramycin with carbenicillin. Analysis of the isobolograms for tobramycin

and carbenicillin showed 64 strains which were additively inhibited with a straight isobole, and eight strains which were synergistically inhibited with a concave isobole. No isoboles were convex, suggesting a lack of antagonistic effect between the two antibiotics. The geometric mean MIC values of these 72 strains which were tested in two-dimensional agar dilution tests could be divided into two groups, and these findings are illustrated in Fig. 4. Seven additional strains had no end points for synergism because of extreme sensitivity to either tobramycin or carbenicillin. Whereas their combined effects could have been assessed at higher dilutions, such concentrations would be greatly exceeded by doses which have been attained in human serum. Seventy-eight of 79 strains were sensitive to a combination of 50
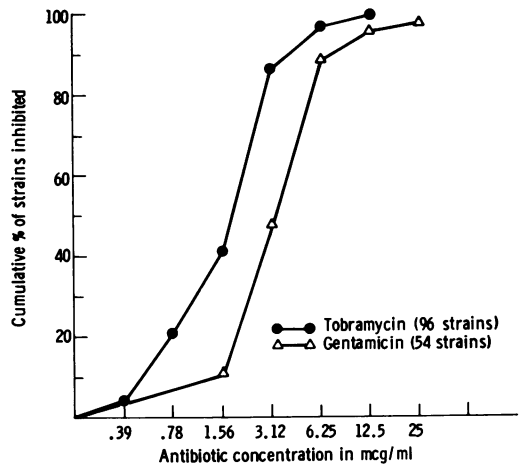

FIG. 3. *Comparison of P. aeruginosa susceptibility to tobramycin and gentamicin by agar dilution (Mueller Hinton) test.*
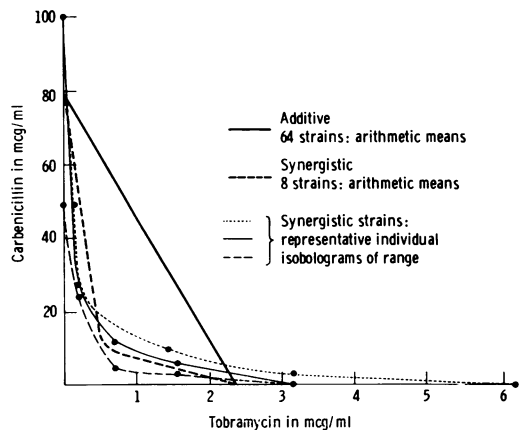

FIG. 4. *Isobolograms of 72 strains of P. aeruginosa with carbenicillin plus tobramycin.*

μg of carbenicillin per ml and 1.56 μg of tobramycin per ml.

DISCUSSION

Tobramycin appears to be effective in vitro against *P. aeruginosa* with 95% of strains tested showing a broth MIC of 1.56 μg/ml or less and 87% showing an agar dilution MIC of 3.12 μg/ml or less. The greater sensitivity to the drug in Mueller Hinton broth media as compared to Mueller Hinton agar has been described for gentamicin, another aminoglycoside antibiotic, and this phenomenon has been related to higher concentrations of magnesium ion in the agar (9). This may also apply to susceptibility testing with tobramycin and needs further evaluation. In other respects, tobramycin is comparable to gentamicin, the antibiotic which it most closely resembles. Like gentamicin, its effects include rapid bactericidal activity (10), and in vitro tests show the moderate effect of inoculum on broth MIC values (12).

With 78 of 79 strains inhibited by 50 μg of carbenicillin per ml and 1.56 μg of tobramycin per ml, it seems that the additive antibacterial effects and lack of antagonism may be more important than the in vitro synergism demonstrated against 8 of 79 isolates. This blood level of carbenicillin is easily attained clinically (22), and a blood level of 2.09 μg/ml and urinary concentration of 21.6 μg/ml were found after intramuscular administration of 50 mg of tobramycin in 10 patients (3). Only a single strain showed resistance to gentamicin at 25 μg/ml and showed inhibition with carbenicillin only at 250 μg/ml, but its susceptibility to tobramycin at 0.78 μg/ml is notable. Although most strains showed greater susceptibility to tobramycin than gentamicin, the marked variations in MIC values observed with strains indicate that exact correlations cannot be made.

The greater potency by weight of tobramycin, as compared to gentamicin, against *P. aeruginosa* suggests potential clinical benefits. However, its pharmacology and toxicity must be evaluated further before any real comparisons between the two drugs can be made.

Development of resistance to gentamicin with increased clinical usage does not appear to be a problem now (L. S. Young, J. Infec. Dis., *in press*), but it does appear that further studies to evaluate degree of multiple antibiotic resistance, including tobramycin, will be indicated.

The susceptibility of virtually all of our recently isolated strains of *P. aeruginosa* to concentrations of tobramycin alone or in combination with carbenicillin at levels attainable in vivo indicate that clinical evaluation is merited.

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