In Vitro Activity of Tobramycin and Gentamicin

MICHAEL R. BRITT, RICHARD A. GARIBALDI, JAMES N. WILFERT, AND CHARLES B. SMITH

Division of Infectious Diseases, Department of Internal Medicine, University of Utah College of Medicine, Salt Lake City, Utah 84112

Received for publication 2 June 1972

The in vitro antimicrobial activity of tobramycin and gentamicin was compared against 362 bacterial isolates. The minimal inhibitory concentration (MIC) of tobramycin was fourfold less than the MIC of gentamicin against most of 119 *Pseudomonas* organisms. Gentamicin and tobramycin had similar in vitro activity against *Enterobacteriaceae* and *Staphylococcus aureus*. *Proteus rettgeri* were commonly resistant to both tobramycin and gentamicin. The 10- μ g tobramycin disc separated resistant (MIC ≥ 5 μ g/ml) and susceptible (MIC < 5 μ g/ml) organisms in 359 of 362 tested. In disc diffusion testing, the tobramycin and gentamicin zone diameters were found to vary significantly with concentrations of magnesium ions in the media employed. The MIC of tobramycin varied with the size of the inoculum, and tobramycin was most effective at a neutral pH.

Tobramycin, a new aminoglycoside antibiotic separated from the nebramycin antibiotic complex, has been found to have greater in vitro activity than gentamicin against *Pseudomonas* species, as well as a wide spectrum of activity against the *Enterobacteriaceae* and *Staphylococcus aureus* (3, 5, 6, 10, 13, 14, 18). The clinical importance of *Pseudomonas* infections in the hospital setting (1, 11) and reports of gentamicin-resistant strains of *Pseudomonas* (16) and other *Enterobacteriaceae* (20) prompted this comparative study of the activity of tobramycin and gentamicin against gram-negative organisms and *S. aureus*.

MATERIALS AND METHODS

Organisms. *Pseudomonas*, *Proteus*, *Escherichia coli*, *Klebsiella-Enterobacter*, and staphylococcal organisms tested in this study were isolated from patients hospitalized between August 1969 and October 1971 at the University of Utah Medical Center, Salt Lake Veterans, or Latter-day Saints Hospitals. These organisms were isolated from blood, urine, sputum, or wound sources, from patients considered to have clinically significant infections. Half of the *Serratia marcescens* organisms tested were from the type strain collection of W. H. Ewing of Atlanta, Ga., and the remainder were from clinical sources.

Antibiotics. Tobramycin (Eli Lilly & Co.) was supplied in 2-ml vials containing 1,000 μ g/ml. This was diluted with distilled water to a working solution of 800 μ g/ml on the day of the test. Unused portions were stored at -4 C until the next test. Stock solutions containing 800 μ g of gentamicin/ml were prepared in sterile distilled water from gentamicin sulfate powder (Schering Corp.). These were stored at -4 C and thawed immediately before each test.

MIC. Minimal inhibitory concentrations (MIC)

were determined by the replica-plate method of Steers, Foltz, and Graves (17). Mueller-Hinton agar was used in all MIC testing. This agar was prepared in our laboratory by combining 99.15% Mueller-Hinton broth (Difco) with 0.85% Ionagar No. 2 (Colab Laboratories) and is referred to hereafter as MHB+A. A 10^{-4} dilution of an overnight (18 to 24 hr) culture in Mueller-Hinton broth was used as the inoculum.

Medium pH was adjusted by addition of 0.1 N HCl or 0.1 N NaHCO₃ and tested with the agar at 54 C.

Disc diffusion testing. The disc diffusion susceptibility testing for each organism was carried out on the same day as the MIC testing on MHB+A media by the method described by Bauer et al. (2). Gentamicin and tobramycin discs containing 10 μ g of antibiotic and 50- μ g carbenicillin discs were used. To evaluate the effect of media on the size of disc zones, 29 organisms were tested simultaneously on MHB+A and commercially prepared Mueller-Hinton agars (BBL and Difco, hereafter called MHA-BBL and MHA-D). Magnesium and calcium concentrations of these media were determined with an atomic absorption spectrophotometer.

RESULTS

In vitro susceptibility of various microorganisms to tobramycin and gentamicin. The relative susceptibility of various microorganisms to tobramycin is shown in Fig. 1. *S. aureus* was the most susceptible of all of the bacteria tested; the MIC for all strains was less than 0.075 μ g/ml. The *Escherichia coli*, *Pseudomonas*, and *Klebsiella-Enterobacter* strains, and the indole-positive strains of *Proteus* except *P. rettgeri*, had comparable susceptibility: the MIC for 85 to 95% of all of these organisms

FIG. 1. Susceptibility of 362 organisms to tobramycin as determined by agar dilution technique. Parentheses indicate number of strains tested.

tested was less than 0.65 µg/ml. *P. mirabilis* and *S. marcescens* tended to be more resistant, but 95% of strains were still susceptible to 2.5 µg or less per ml. The most resistant species was *P. rettgeri*; 75% (12 of 16) of strains required 5 µg or more per ml for inhibition.

Pseudomonas species were consistently more susceptible to tobramycin than to gentamicin (Fig. 2). The tobramycin geometric mean MIC for 119 strains was 0.52 ± 0.42 µg/ml, whereas the mean MIC of gentamicin was 2.26 ± 1.90 µg/ml. Four times as much gentamicin as tobramycin was required for inhibition of 86% of the *Pseudomonas* strains tested. Four *Pseudomonas* strains (3%) were resistant to gentamicin (MIC > 5 µg/ml), and the MIC for 19 strains (15.9%) was 5 µg/ml. Tobramycin MIC values paralleled gentamicin MIC values; that is, strains most resistant to one antibiotic tended also to be resistant to the other. One strain isolated from a chronically infected skin ulcer was highly resistant to both tobramycin (MIC, 500 µg/ml) and gentamicin (MIC > 3,000 µg/ml). The patient had not received gentamicin or tobramycin prior to isolation of this organism.

In contrast to *Pseudomonas* strains, *S. marcescens* was generally more susceptible to gentamicin (mean MIC, 0.34 ± 0.14 µg/ml) than to tobramycin (mean MIC, 2.25 ± 5.02 µg/ml; Fig. 2). No consistent differences in the susceptibility to tobramycin and gentamicin were seen for *E. coli*, *Klebsiella-Enterobacter*, *Proteus* species (indole-positive and indole-negative strains), and *S. aureus*.

Comparison of MIC with disc diffusion susceptibility tests. Zones of inhibition around 10-µg tobramycin discs for each strain of bacteria were determined with the same overnight culture used for the MIC testing (Fig. 3). There was a sufficient range of MIC values among the *Proteus* species to permit separation of resistant and susceptible strains at a MIC of 5 µg/ml by using the 10-µg disc and a zone diameter of 16 mm. Nearly all (11 of 12) strains of *Proteus* for which the MIC was 5 µg/ml or greater had zones of inhibition of less than 16 mm. A 16-mm zone of inhibition separated susceptible (MIC < 5 µg/ml) from resistant organisms (MIC ≥ 5 µg/ml) in 359 of 362 instances.

Effect of media on zone diameter. The zone

FIG. 2. Comparative susceptibility of 119 *Pseudomonas* and 23 *S. marcescens* strains to tobramycin and gentamicin. Each dot represents one isolate.

FIG. 3. Correlation between inhibition zone diameter around 10- μ g tobramycin disc tested on Mueller-Hinton broth plus Ionagar and MIC of tobramycin determined by replica-plate method.

diameter of 16 mm is applicable only to assays of disc diffusion susceptibility performed on media consisting of MHB+A as prepared in our laboratory. A consistent difference in the zone diameters for tobramycin and gentamicin was observed for the 27 strains of assorted gram-negative bacteria simultaneously tested on three different Mueller-Hinton agars (Table 1). Zones of inhibition determined on MHB+A were consistently (26 of 29) and significantly ($P < 0.005$)

larger than zones determined on the two commercially prepared Mueller-Hinton agars. The two commercially available media showed no significant differences in zone size when compared with each other.

The magnesium concentrations of the various media were as follows: MHB+A, 0.54 mg/100 ml; MHA-BBL, 1.07 mg/100 ml; and MHA-D, 1.37 mg/100 ml. The calcium ion concentration of MHB+A was 1.1 mg/100 ml, that of

TABLE 1. Comparison of disc diffusion zone diameters on different Mueller-Hinton agars (29 organisms)

Medium ^b	Tobramycin disc, 10 µg/ml		Gentamicin disc, 10 µg/ml		Carbenicillin disc, 50 µg/ml	
	Mean zone diam (mm)	Mean of difference between zones	Mean zone diam (mm)	Mean of difference between zones	Mean zone diam (mm)	Mean of difference between zones
MHB + A.....	22.41	3.48 ± 3.56	22.68	4.04 ± 2.40	21.37	-1.4 ± 2.52
MHA-D.....	18.93	<i>P</i> < 0.005 ^c	18.93	<i>P</i> < 0.005	22.89	NS
MHB + A.....	22.41	2.66 ± 1.80	22.68	3.22 ± 2.0	21.37	-1.44 ± 4.03
MHA-BBL.....	19.79	<i>P</i> < 0.005	19.65	<i>P</i> < 0.005	22.65	NS
MHA-D.....	18.93	0.59 ± 2.01	18.93	0.81 ± 1.40	22.89	-0.22 ± 2.97
MHA-BBL.....	19.79	NS	19.65	<i>P</i> < 0.01	22.65	NS

^a Seventeen *E. coli*, five *Enterobacter*, two *Klebsiella*, four *Pseudomonas*, and one *Staphylococcus aureus* strains.

^b MHB + A: Mueller-Hinton broth (Difco), 99.15% plus 0.85% Ionagar No. 2 (Colab). MHA-D: Mueller-Hinton agar (Difco). MHA-BBL: Mueller-Hinton agar (BBL).

^c *P* value by analysis of variance. NS: not significant.

MHA-BBL was 0.63 mg/100 ml, and that of MHA-D was 2.3 mg/100 ml. The zones of inhibition for tobramycin and gentamicin were largest on the media containing the lowest magnesium ion concentration, MHB + A. In contrast, calcium ion concentrations in the media tested did not appear to correlate with observed variations of zone diameters.

No differences in zone diameters were seen around carbenicillin discs tested simultaneously on the three media.

Effect of inoculum size and medium pH on tobramycin MIC. The tobramycin MIC values were determined for selected organisms with a 10⁻⁴ dilution, a 10⁻² dilution, and the undiluted overnight culture as inoculum (Fig. 4).

When the inoculum size was increased from a 10⁻⁴ to a 10⁻² dilution of an overnight culture, 11 of 12 strains showed a twofold increase in the tobramycin MIC. When the inoculum was increased to an undiluted overnight culture, 10 of 12 strains showed an additional twofold or greater increase in MIC. When the undiluted overnight culture and the 10⁻⁴ dilution were compared, 11 of 12 strains showed an eightfold or greater increase in MIC. Thus, the tobramycin MIC was proportional to the inoculum size.

The MIC values for 15 strains of bacteria were compared on MHB + A at pH 5.5, 7.0, and 8.3 with a 10⁻⁴ dilution as inoculum (Fig. 5). At the acid pH, the tobramycin MIC values were twofold greater for all strains tested than at neutral pH. At alkaline pH, the tobramycin MIC was twofold higher for all five *Pseudomonas* species tested, two of three *Proteus* strains, and two of four *E. coli* strains. The remaining three strains showed no differences. These data suggest that tobramycin was most effective at a neutral pH.

DISCUSSION

These in vitro studies demonstrated that tobramycin was consistently more active than gentamicin against *Pseudomonas* species. The relative susceptibility of *Pseudomonas* to tobramycin and gentamicin paralleled each other, but the MIC for most *Pseudomonas* strains was consistently fourfold lower for tobramycin than for gentamicin. A single strain highly resistant to gentamicin was also resistant to tobramycin. Against three *Pseudomonas* strains for which the MIC of gentamicin was 10 µg/ml, the MIC of tobramycin was less than 5 µg/ml. Our findings are consistent with earlier published reports comparing these antibiotics (3-6, 10, 14, 18).

We chose the 5-µg level as the cut-off point for susceptibility to gentamicin because most reported studies of the clinical pharmacology of gentamicin indicate that the usual intramuscular dose of 1.5 mg/kg gives a mean peak concentration in serum of 5 to 7 µg/ml (8, 9, 19). Among recent clinical isolates of *Pseudomonas* in our institutions, 22% showed a gentamicin MIC in the 5 to 10 µg/ml range, a level we would consider relatively resistant. In contrast, the MIC of tobramycin for the majority of these *Pseudomonas* strains was below 1.25 µg/ml. The susceptibility threshold for tobramycin was originally proposed by Preston and Wick (14) to be 8 µg/ml; however, Shadomy and Kirchoff (15) argued for a threshold of 1.56 to 3.13 µg/ml based on initial human studies by Eli Lilly & Co. In either case, the MIC of tobramycin for most strains of *Pseudomonas* in our study was below the suggested thresholds, suggesting that the increased in vitro activity of tobramycin over gentamicin against *Pseudomonas* might have clinical significance, providing the two drugs prove to have similar toxicity.

FIG. 4. Effect of inoculum size on MIC of tobramycin for 12 isolates (2 *E. coli*, 4 *Pseudomonas*, 3 *Proteus*, 2 *Klebsiella-Enterobacter*, and 1 *S. aureus*).

The antimicrobial activity of tobramycin against *Enterobacteriaceae* and *S. aureus* is similar to that of gentamicin. This finding would suggest that tobramycin could be utilized in place of gentamicin in patients with suspected gram-negative sepsis. Only in the rare clinical situation of *S. marcescens* infection do our in vitro data suggest a disadvantage in using tobramycin in place of gentamicin.

The diameter of the tobramycin and gentamicin zone of inhibition was found to vary significantly with the media employed. The size of the zone around gentamicin discs has been shown to vary with the concentration in the media of calcium and magnesium ions (7). The variation of the magnesium ion concentration had the greatest effect on the gentamicin MIC values and zone diameters (7, 12). In the present study, the larger tobramycin and gentamicin zone diameters were found on the media which had approximately

FIG. 5. Effect of pH on MIC of tobramycin for 15 isolates (4 *E. coli*, 5 *Pseudomonas*, 3 *Proteus*, 3 *Klebsiella-Enterobacter*).

half the concentration of magnesium ions. This variation in the zone diameter has application to the usual clinical practice of determining susceptibility and resistance by predetermined zone diameters. The size of the zone used to distinguish tobramycin resistance and susceptibility must not only specify the media but also the magnesium and calcium ion concentration.

ACKNOWLEDGMENTS

We thank Robert Barrett and Barbara Saxon for technical assistance.

This investigation was supported by a grant from Eli Lilly & Co. and by Public Health Service Training Grant 5 T01 AI00039.

LITERATURE CITED

1. Adler, J. L., J. P. Burke, and M. Finland. 1971. Infection and antibiotic usage at Boston City Hospital, January 1970. *Arch. Intern. Med.* 127:460-465.
2. Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Amer. J. Clin. Pathol.* 45:493-496.
3. Black, H. R., and R. S. Griffith. 1971. Preliminary studies with nebramycin factor 6. *Antimicrob. Ag. Chemother.* 1970, p. 314-321.
4. Brusch, J. L., M. Barza, M. G. Bergeron, and L. Weinstein.

1972. Cross-resistance of *Pseudomonas* to gentamicin and tobramycin. *Antimicrob. Ag. Chemother.* 1:280-281.
5. Del Bene, V. E., and W. E. Farrar, Jr. 1972. Tobramycin: in vitro activity and comparison with kanamycin and gentamicin. *Antimicrob. Ag. Chemother.* 1:340-342.
6. Dienstag, J., and H. C. Neu. 1972. In vitro studies of tobramycin, an aminoglycoside antibiotic. *Antimicrob. Ag. Chemother.* 1:41-45.
7. Gilbert, D. N., E. Kutscher, P. Ireland, J. A. Barnett, and J. P. Sanford. 1971. Effect of the concentrations of magnesium and calcium on the *in vitro* susceptibility of *Pseudomonas aeruginosa* to gentamicin. *J. Infect. Dis.* 124(Suppl.):S37-S45.
8. Jackson, G. G., and G. Arcieri. 1971. Ototoxicity of gentamicin in man: A survey and controlled analysis of clinical experience in the United States. *J. Infect. Dis.* 124(Suppl.):S130-S137.
9. Jackson, G. G. 1967. Laboratory and clinical investigation of gentamicin. *Int. Symp. on Gentamicin*. Ist, p. 62-74 and 172-176. Schwabe, Basel.
10. Levison, M. E., R. Knight, and D. Kaye. 1972. In vitro evaluation of tobramycin, a new aminoglycoside antibiotic. *Antimicrob. Ag. Chemother.* 1:381-384.
11. McNamara, M. J., M. C. Hill, B. P. Low, and E. B. Tucker. 1967. A study of the bacteriologic patterns of hospital infections. *Ann. Intern. Med.* 66:480-488.
12. Medeiros, A. A., T. F. O'Brien, W. E. C. Wacker, and N. F. Yulug. 1971. Effect of salt concentration on the apparent *in vitro* susceptibility of *Pseudomonas* and other gram negative bacilli to gentamicin. *J. Infect. Dis.* 124(Suppl.):S59-S64.
13. Meyer, R. D., L. S. Young, and D. Armstrong. 1971. Tobramycin (nebramycin factor 6): in vitro activity against *Pseudomonas aeruginosa*. *Appl. Microbiol.* 22:1147-1151.
14. Preston, D. A., and W. E. Wick. 1971. Preclinical assessment of the antibacterial activity of nebramycin factor 6. *Antimicrob. Ag. Chemother.* 1970, p. 322-327.
15. Shadomy, S., and C. Kirchoff. 1972. In vitro susceptibility testing with tobramycin. *Antimicrob. Ag. Chemother.* 1:412-416.
16. Shulman, J. A., P. M. Terry, and C. E. Hough. 1971. Colonization with gentamicin-resistant *Pseudomonas aeruginosa*, pyocine type 5, in a burn unit. *J. Infect. Dis.* 124(Suppl.):S18-S23.
17. Steers, E., E. L. Foltz, and B. S. Graves. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot. Chemother.* 9:307-311.
18. Traub, W. H., and E. A. Raymond. 1972. Evaluation of the in vitro activity of tobramycin as compared with that of gentamicin sulfate. *Appl. Microbiol.* 23:4-7.
19. Winters, R. E., K. D. Litwack, and W. L. Hewitt. 1971. Relation between dose and levels of gentamicin in blood. *J. Infect. Dis.* 124(Suppl.):S91-S95.
20. Witchitz, J. L., and Y. A. Chabbert. 1971. High level transferrable resistance to gentamicin. *J. Antibiot. (Tokyo)* 24:137-139.