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In Vitro Evaluation of Tobramycin, a New Aminoglycoside Antibiotic

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One hundred fifty-two strains of *Escherichia coli*, *Klebsiella-Enterobacter*, *Pseudomonas aeruginosa*, *Proteus* species, and *Staphylococcus aureus* were inhibited by 3.1 μg of tobramycin/ml in a broth-dilution method and showed zones of inhibition of 16 mm or more around a 10-μg tobramycin disc in the Kirby-Bauer method. Tobramycin was most active against *S. aureus*, 100% of strains being inhibited by 0.1 μg/ml. All strains of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and indole-positive *Proteus* species, and 80% of *Enterobacter* species were inhibited by 0.8 μg of tobramycin/ml, whereas only 48% of *P. mirabilis* strains were inhibited by this concentration. Tobramycin was approximately twice as active as gentamicin against *S. aureus*, four times as active against *P. aeruginosa*, slightly more active against *E. coli* and *Enterobacter* species, equally active against *P. mirabilis*, and slightly less active against *K. pneumoniae*. The minimal bactericidal concentrations of tobramycin and gentamicin were the same as or twice the minimal inhibitory concentrations for all strains except those of *P. aeruginosa*, against which greater concentrations of both gentamicin and tobramycin were required for bactericidal activity. Tobramycin sterilized cultures of *S. aureus*, *E. coli*, and *P. aeruginosa*, but the rate of bactericidal action was faster with a combination of tobramycin and carbenicillin than with either antibiotic alone in the same concentrations. Tobramycin retained potency in the presence of 200 to 600 μg of carbenicillin/ml for at least 6 hr of incubation at 37 C, but lost potency in the presence of 600 μg of carbenicillin/ml by 24 hr of incubation and in the presence of 800 μg/ml by 2 hr of incubation.

Tobramycin is a new aminoglycoside antibiotic for parenteral use. Preliminary studies suggested that tobramycin has nephrotoxic and ototoxic properties and an antibacterial spectrum similar to that of gentamicin (2). The present study was undertaken to assess the in vitro antibacterial activity of tobramycin in comparison with gentamicin.

MATERIALS AND METHODS

Antibacterial activity of tobramycin and comparison with gentamicin. The following microorganisms were isolated from patients: 34 strains of *Escherichia coli*; 27 strains of *Klebisella pneumoniae*; 25 strains each of *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*; 10 strains of *Enterobacter* species; and 6 strains of indole-positive *Proteus* species. The susceptibility of these bacteria to tobramycin and gentamicin was determined by an antibiotic-dilution method in Heart Infusion broth (Difco). The antibiotic was diluted in twofold steps in tubes containing 0.5 ml of broth. The bacterial inoculum for each tube was 0.5 ml of a 10^{-4} dilution of an 18-hr culture of each strain in Heart Infusion broth. The tubes were incubated at 37 C for 24 hr. The bacteriostatic end point was considered to be the minimal concentration of antibiotic which prevented turbidity. Broth from each tube without visible growth was streaked on the surface of Trypticase Soy Agar-blood plates with a 0.01-ml calibrated sterile platinum loop. After 24 hr of incubation at 37 C, the lowest concentration of antibiotic resulting in no growth on the plates was taken as the bactericidal end point.

Susceptibility to tobramycin was also determined by use of 10-μg discs in the Kirby-Bauer disc-diffusion method (1). Zones of inhibition of bacterial growth were measured and correlated with the minimal inhibitory concentrations as determined by the broth-dilution technique.

The effect of a larger inoculum size on the antibacterial activity of tobramycin was determined by comparing minimal inhibitory and bactericidal concentrations when inocula of 0.5 ml of 10^{-2} and 10^{-4} dilutions of 18-hr cultures of five strains each of *E. coli*, *P. mirabilis*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* were used in broth-dilution studies.

Tobramycin and carbenicillin in combination. The rate of bactericidal action of tobramycin alone,

carbenicillin alone, and tobramycin plus carbenicillin against one strain each of *E. coli*, *P. aeruginosa*, and *S. aureus* was determined by enumeration of viable bacteria remaining after addition of 10^4 bacteria to 10-ml volumes of Heart Infusion broth containing these agents. Samples were removed for quantitation of viable bacteria at 0.5, 1, 2, 4, 6, and 20 hr. Amounts of 1 and 0.1 ml of each sample and 0.1-ml amounts of 10-fold dilutions of each sample were plated on the surfaces of Trypticase Soy Agar plates. The colonies were counted after incubation of the plates for 18 hr at 37 C.

Against the strains of *E. coli* and *P. aeruginosa*, 50 μg of carbenicillin/ml and 1 μg of tobramycin/ml were tested alone and in combination; 25 μg of carbenicillin/ml and 0.5 μg of tobramycin/ml were similarly tested against the strain of *S. aureus*.

Assay for inactivation of tobramycin by carbenicillin. The effect of the presence of high concentrations of carbenicillin on the activity of tobramycin was determined with 10-ml solutions of 10 μg of tobramycin/ml alone and in combination with 800, 600, 400, or 200 μg of carbenicillin/ml in Heart Infusion broth. The solutions were incubated at 37 C, and 1-ml samples were removed at 1, 2, 4, 6, and 24 hr. The samples were assayed for tobramycin activity by the twofold dilution technique in Heart Infusion broth with the use of a strain of *K. pneumoniae* resistant to 800 μg of carbenicillin/ml. In this technique, the samples to be assayed and a known concentration of fresh tobramycin were each diluted in twofold steps in tubes containing 0.5 ml of broth. The bacterial inoculum for each tube was 0.5 ml of a 10^{-4} dilution in Heart Infusion broth of an 18-hr culture of the strain of *K. pneumoniae*. The tubes were incubated for 24 hr at 37 C. Tobramycin activity remaining in the assayed material was calculated by multiplying the reciprocal of the highest dilution that inhibited growth of the strain of *K. pneumoniae* by the minimal inhibitory concentration of fresh tobramycin.

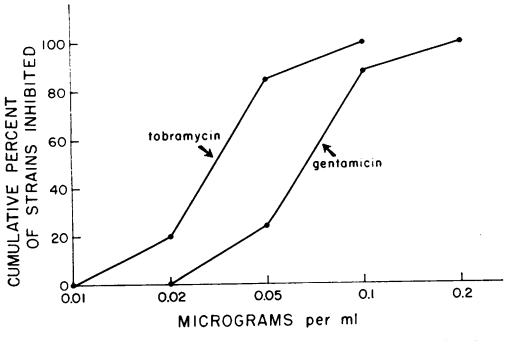

FIG. 1. *Minimal inhibitory concentrations of tobramycin and gentamicin for 25 strains of Staphylococcus aureus.*

RESULTS AND DISCUSSION

Antibacterial activity of tobramycin and comparison with gentamicin. Figure 1 shows the minimal inhibitory concentrations of tobramycin and gentamicin for 25 strains of *S. aureus*. Tobramycin was about twice as active as gentamicin against these strains. At a concentration of 0.05 μg/ml, tobramycin inhibited 84% of the strains, whereas only 24% were inhibited by the same concentration of gentamicin. All strains were inhibited by 0.1 μg of tobramycin/ml and 0.2 μg of gentamicin/ml.

Figure 2 shows the minimal inhibitory concentrations of tobramycin and gentamicin for 34 strains of *E. coli* and 25 strains of *P. mirabilis*. Tobramycin was slightly more active than gentamicin against *E. coli*; 42% of the strains were inhibited by 0.2 μg of gentamicin/ml, and 68% were inhibited by the same concentration of tobramycin. All strains of *E. coli* were inhibited by 0.8 μg of tobramycin/ml and 1.6 μg of gentamicin/ml. Tobramycin and gentamicin were about equally active against *P. mirabilis*; more than 95% of the strains were inhibited by either antibiotic in a concentration of 1.6 μg/ml. The strains of

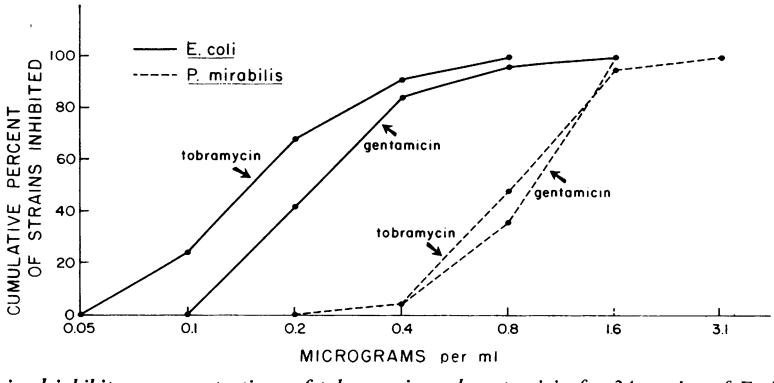

FIG. 2. *Minimal inhibitory concentrations of tobramycin and gentamicin for 34 strains of Escherichia coli and 25 strains of Proteus mirabilis.*

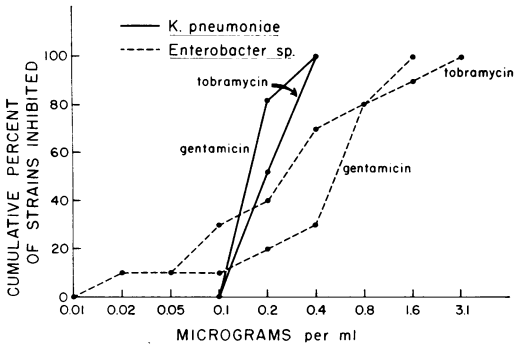

FIG. 3. *Minimal inhibitory concentrations of tobramycin and gentamicin for 27 strains of Klebsiella pneumoniae and 10 strains of Enterobacter species.*

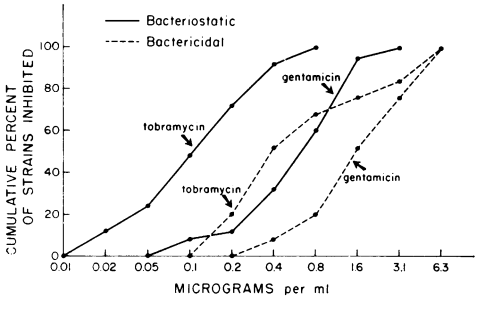

FIG. 4. *Minimal inhibitory and bactericidal concentrations of tobramycin and gentamicin for 25 strains of Pseudomonas aeruginosa.*

TABLE 1. *Effect of size of inoculum on MIC and MBC of tobramycin*[a]

Organism	Strain	Inoculum size[b]			
		10^{-4}		10^{-2}	
		MIC	MBC	MIC	MBC
Proteus mirabilis	1	0.8	0.8	6.3	6.3
	2	3.1	3.1	6.3	6.3
	3	1.6	3.1	6.3	25
	4	0.2	1.6	3.1	3.1
	5	0.8	0.8	6.3	25
Staphylococcus aureus	1	0.1	0.4	1.6	3.1
	2	0.1	0.4	0.8	1.6
	3	0.05	0.2	1.6	6.3
	4	0.05	0.1	3.1	6.3
	5	0.05	0.1	1.6	1.6

[a] MIC (minimal inhibitory concentrations) and MBC (minimal bactericidal concentrations) are expressed in micrograms per milliliter.

[b] Expressed as dilution of an 18-hr culture.

P. mirabilis were more resistant to tobramycin and gentamicin (about 40% of strains inhibited by either antibiotic in a concentration of 0.8 μg/ml) than strains of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* (most strains inhibited by 0.8 μg of gentamicin/ml and all all strains inhibited by 0.8 μg of tobramycin/ml).

Figure 3 shows the minimal inhibitory concentrations of tobramycin and gentamicin for 27 strains of *K. pneumoniae* and 10 strains of *Enterobacter* species. Gentamicin was slightly more active than tobramycin against *K. pneumoniae*; 52% of the strains were inhibited by 0.2 μg of tobramycin/ml, and 82% were inhibited by 0.2 μg of gentamicin/ml. All strains were inhibited by either antibiotic in a concentration of 0.4 μg/ml.

Tobramycin was slightly more active than gentamicin against strains of *Enterobacter* species; 70% of the strains were inhibited by 0.4 μg of tobramycin/ml, whereas only 30% were in-

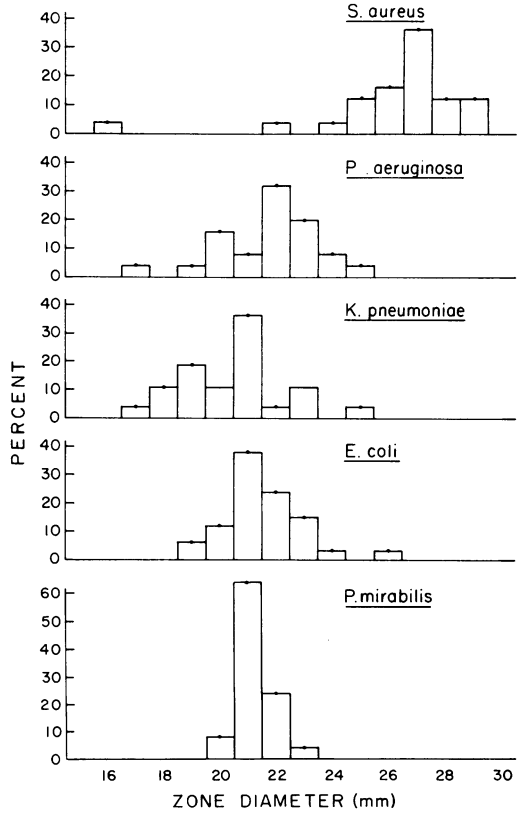

FIG. 5. *Sizes of zones of inhibition in the Kirby-Bauer disc-diffusion method shown as frequency distribution for Proteus mirabilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Staphylococcus aureus.*

hibited by the same concentration of gentamicin. All strains were inhibited by 1.6 μg of gentamicin/ml and 3.1 μg of tobramycin/ml.

Tobramycin was more active than gentamicin against indole-positive *Proteus* species; five of the six strains were inhibited by 0.4 μg of tobramycin/ml and three of the six strains were inhibited by the same concentration of gentamicin. All strains were inhibited by 0.8 μg of tobramycin/ml and 3.1 μg of gentamicin/ml.

The minimal bactericidal concentrations of both tobramycin and gentamicin were usually the same as or twice the minimal inhibitory concentrations for all strains tested, except for *P. aeruginosa*.

Figure 4 shows the minimal inhibitory and bactericidal concentrations of tobramycin and gentamicin for 25 strains of *P. aeruginosa*. Tobramycin was about four times as active as gentamicin against *P. aeruginosa*; 92% of the strains were inhibited by 0.4 μg of tobramycin/ml, whereas only 32% were inhibited by the same concentration of gentamicin. All strains were inhibited by 0.8 μg of tobramycin/ml and 3.1 μg of gentamicin/ml. The minimal bactericidal concentrations of gentamicin against *P. aeruginosa* were the same as, twice, or four times the minimal inhibitory concentrations for 96% of the strains. In contrast, the minimal bactericidal concentrations of tobramycin were the same as, twice, or four times the minimal inhibitory concentrations for only 60% of the strains; an additional 36% required 8 or 16 times the minimal inhibitory concentrations.

Zones of inhibition in the Kirby-Bauer disc-diffusion method for all organisms were 16 mm or more. All strains of bacteria tested by the disc-diffusion method were inhibited by 3.1 μg or less of tobramycin/ml as determined by the broth-dilution method. The frequency distributions of sizes of zones of inhibition are shown in Fig. 5 for *P. mirabilis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. More than 90% of strains of *P. mirabilis*, *E. coli*, and *P. aeruginosa* had zones of 19 to 24 mm. More than 90% of strains of *K. pneumoniae* had zones of 18 to 23 mm, and more than 90% of strains of *S. aureus* had zones of 24 to 29 mm.

An increase in inoculum from a 10^{-4} dilution of an overnight culture to a 10^{-2} dilution produced little change in the minimal inhibitory concentrations or minimal bactericidal concentrations of tobramycin for *E. coli*, *K. pneumoniae*, and *P. aeruginosa*; when the minimal inhibitory or bactericidal concentrations increased, it was usually by no more than twofold. In contrast, as shown in Table 1, with the increase in the size of the inoculum there was a 2- to 32-fold increase in the minimal inhibitory concentrations and minimal bactericidal concentrations for *P. mirabilis*, and a 4- to 64-fold increase for *S. aureus*.

Tobramycin and carbenicillin in combination. Tobramycin and carbenicillin alone were each bactericidal against strains of *E. coli*, *P. aeruginosa*, and *S. aureus*, and the rate and degree of bactericidal activity against these strains was increased by combination of tobramycin with carbenicillin. Tobramycin (1 μg/ml) combined with carbenicillin (50 μg/ml) sterilized a suspension of *P. aeruginosa* or *E. coli* within 6 hr of incubation, whereas neither antibiotic alone in the same concentration sterilized the broth within 6 hr. Tobramycin (0.5 μg/ml) combined with carbenicillin (25 μg/ml) rapidly reduced the titers and sterilized a suspension of *S. aureus* within 3 hr, whereas each antibiotic alone in the same concentration reduced the titers more slowly and required 4 hr for sterilization.

Assay for inactivation of tobramycin by carbenicillin. Solutions of 10 μg of tobramycin/ml alone and combined with either 200 or 400 μg of carbenicillin/ml showed no loss of tobramycin activity over a 24-hr period of incubation at 37 C. A solution of 10 μg of tobramycin/ml combined with 600 μg of carbenicillin/ml retained full tobramycin activity for at least 6 hr of incubation, but lost over 90% of activity by 24 hr. A solution of 10 μg of tobramycin/ml combined with 800 μg of carbenicillin/ml lost 50% of tobramycin activity by the second hour of incubation and over 90% of activity by 24 hr. These data for tobramycin are comparable to those reported for gentamicin (3).

Serum concentrations of carbenicillin greater than 400 μg/ml would seldom occur except (i) during brief periods after rapid intravenous administration in patients with normal renal function and (ii) in patients with renal failure given excessive doses.

These studies demonstrate that tobramycin has an antimicrobial spectrum very similar to that of gentamicin but is generally more active on a weight basis.

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