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Sisomicin: Evaluation In Vitro and Comparison with Gentamicin and Tobramycin

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Sisomicin is a new antibiotic produced by *Micromonospora inyoensis*. The in vitro activities of sisomicin, gentamicin, and tobramycin, three similar aminoglycosides, were determined against 228 clinical isolates representing 10 genera of common pathogens. No difference was noted in the activities of these antimicrobial agents when assayed by a standard broth dilution technique against *Klebsiella*, *Enterobacter*, *Escherichia*, *Salmonella*, *Citrobacter*, enterococci, or *Staphylococcus aureus*. Sisomicin was significantly more active than tobramycin against *Serratia* and indole-positive *Proteus* strains. Sisomicin was significantly more active than gentamicin against indole-negative *Proteus* strains and slightly more active against indole-positive *Proteus* strains. Tobramycin was more active than sisomicin or gentamicin against *Pseudomonas* and indole-negative *Proteus* strains. Gram-negative bacilli resistant to one of the three antimicrobial agents were not necessarily resistant to either of the other two. Activity of sisomicin was independent of the susceptibility or resistance of these isolates to nine other antimicrobial agents as assayed by the Bauer-Kirby technique. The presence of 50% human serum did not antagonize the in vitro activity of sisomicin against gram-negative isolates. Because sisomicin showed certain advantages over gentamicin or tobramycin in vitro, further investigation of this new antimicrobial agent is warranted.

Sisomicin is a new aminoglycoside antibiotic produced by *Micromonospora inyoensis*. Preliminary studies have shown sisomicin (antibiotic 6640) to be similar to gentamicin in both chemical structure and antibacterial spectrum. Its activity in vitro is relatively unaffected by the presence of horse serum (11, 12). This study was designed (i) to determine the in vitro activity of sisomicin against a large number of recent clinical isolates, (ii) to compare this activity with that of gentamicin and tobramycin, two very similar antimicrobial agents, (iii) to determine the extent of cross-resistance among these three antimicrobial agents, and (iv) to determine the effect of human serum on the in vitro activity of sisomicin.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains were recent clinical isolates obtained from the Clinical Microbiology Laboratories of the Shands Teaching Hospital and the Veterans Administration Hospital, Gainesville, Fla.

Speciation of indole-positive Proteus. Indole-positive *Proteus* strains were speciated on the basis of H_2S production and of maltose and inositol fermentation (1).

Susceptibility testing methods. Minimal inhibitory concentrations (MIC) were determined by a serial twofold dilution technique in brain heart infusion broth (Difco). Approximately 2×10^4 to 4×10^4 colony-forming units (CFU)/ml were incubated, and tubes were examined for turbidity after incubation for 18 hr at 37 C in air. The MIC was defined as the lowest concentration of drug that prevented visible growth. Organisms inhibited by sisomicin, gentamicin, or tobramycin at a concentration of 6.25 μg or less per ml were considered susceptible; those inhibited by concentrations greater than 6.25 μg/ml were designated resistant. This concentration was chosen because it represents a level that may possibly be achievable in human serum without significant risk of toxicity. *Proteus* strains inhibited by 12.5 μg or less of kanamycin or carbenicillin/ml were considered susceptible; those inhibited by concentrations greater than 12.5 μg/ml were designated resistant. This concentration was chosen because it represents the maximal con-

centration achieved in human serum at dosages recommended for treatment of *Proteus* infections (8-10).

Disc susceptibility tests were performed by the method of Bauer et al. (2).

To determine the effect of serum on the in vitro activity of sisomicin, the MIC was determined as described above in 50% (by volume) heat-inactivated pooled human serum in brain heart infusion broth. This serum contained 8.6×10^{-2} M (8.6 mg/100 ml) calcium and 2.2×10^{-2} M (2.2 mg/100 ml) magnesium.

RESULTS

In vitro activity of sisomicin and comparative results. No difference was noted in the in vitro activities of sisomicin, gentamicin, and tobramycin when assayed by a standard broth dilution technique against seven genera of common pathogens. Most strains of *Klebsiella*, *Enterobacter*, *Salmonella*, *Citrobacter*, *Escherichia*, and *Staphylococcus aureus* were inhibited by each of the three drugs at a concentration of 6.25 μg or less per ml. None of 20 enterococcal isolates was inhibited by any of the three drugs at 6.25 μg/ml (Table 1). Against 13 strains of *Serratia*, both sisomicin and gentamicin were significantly more active than tobramycin (Fig. 1). Tobramycin was more active than gentamicin or sisomicin against 19 indole-negative *Proteus* strains (Fig. 2) and against 20 strains of *Pseudomonas* (Fig. 3).

Sisomicin was somewhat more active than gentamicin or tobramycin against 50 indole-positive *Proteus* strains (Fig. 4). However, at 6.25 μg/ml, none of these three antimicrobial agents inhibited more than 82% of the strains tested. Therefore, these indole-positive *Proteus* strains were separated by species, the MIC of kanamycin and carbenicillin was determined, and activities of all five drugs were compared for the individual species. Although only six strains of *P. vulgaris* were tested, results suggested that sisomicin and gentamicin were the most active against this species.

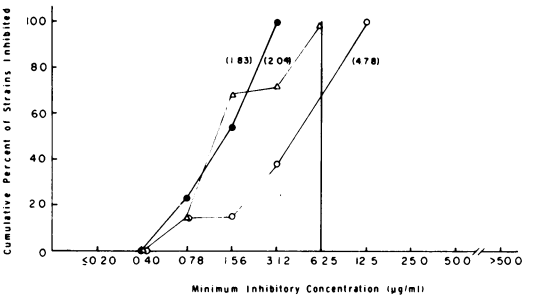

FIG. 1. *Activities of sisomicin, gentamicin, and tobramycin against 13 Serratia strains. Number in parentheses denotes geometric mean of minimal inhibitory concentrations. Percentage of strains inhibited by 6.25 μg/ml was 100% for sisomicin (●), 100% for gentamicin (Δ), and 69% for tobramycin (O).*

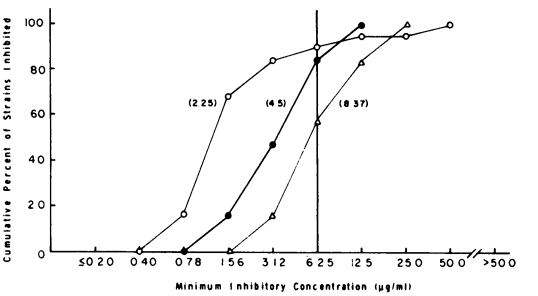

FIG. 2. *Activities of sisomicin, gentamicin, and tobramycin against 19 indole-negative Proteus strains. Number in parentheses denotes geometric mean of minimal inhibitory concentrations. Percentage of strains inhibited by 6.25 μg/ml was 84% for sisomicin (●), 58% for gentamicin (Δ), and 90% for tobramycin (O).*

TABLE 1. *Comparative activities of sisomicin (SS), gentamicin (GM), and tobramycin (TM) against various clinical isolates*

Organism	No. of strains tested	Minimal inhibitory concn (μg/ml)						Percentage of strains inhibited by 6.25 μg/ml		
		Geometric mean			Range					
		SS	GM	TM	SS	GM	TM	SS	GM	TM
Klebsiella	10	1.18	0.80	0.96	0.78-1.56	0.04-1.56	0.78-1.56	100	100	100
Salmonella	18	1.62	1.34	1.82	0.78-6.25	0.40-6.25	0.40-3.12	100	100	100
Citrobacter	17	1.38	2.24	1.33	0.78-6.25	1.56-6.25	0.78-3.12	100	100	100
Escherichia	21	3.45	3.56	5.12	0.78-25.0	1.56-50.0	1.56-25.0	90	90	86
Enterobacter ...	14	1.29	1.56	1.22	0.40-50.0	0.78-3.12	≤0.20-50.0	93	100	93
Enterococci	20	23.3	25.0	27.7	12.5->50.0	12.5->50.0	12.5->50.0	0	0	0
Staphylococcus aureus	24	0.83	0.74	0.57	≤0.20-3.12	≤0.20-3.12	≤0.20-3.12	100	100	100

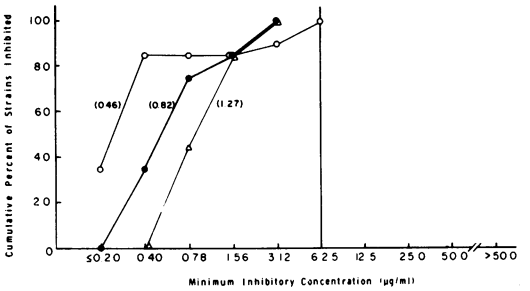

FIG. 3. *Activities of sisomicin, gentamicin, and tobramycin against 20 Pseudomonas strains. Number in parentheses denotes geometric mean of minimal inhibitory concentrations. Percentage of strains inhibited by 6.25 μg/ml was 100% for sisomicin (●), gentamicin (Δ), and tobramycin (O).*

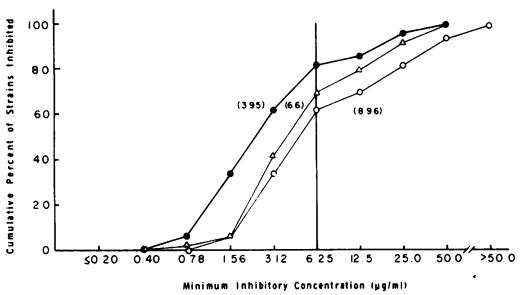

FIG. 4. *Activities of sisomicin, gentamicin, and tobramycin against 50 indole-positive Proteus strains. Number in parentheses denotes geometric mean of minimal inhibitory concentrations. Percentage of strains inhibited by 6.25 μg/ml was 82% for sisomicin (●), 70% for gentamicin (Δ), and 62% for tobramycin (O).*

The geometric mean MIC was 2.78 μg/ml for sisomicin, 3.93 μg/ml for gentamicin, 8.83 μg/ml for tobramycin, 10.5 μg/ml for kanamycin, and 25.0 μg/ml for carbenicillin. Against 28 *P. morganii* strains, all five drugs showed similar activity, with at least 90% of strains susceptible to each drug. Mean MIC values were 2.26 μg/ml for sisomicin, 4.20 μg/ml for gentamicin, 4.20 μg/ml for tobramycin, 9.52 μg/ml for kanamycin, and 0.93 μg/ml for carbenicillin. Against 16 *P. rettgeri* strains, kanamycin and carbenicillin were highly active, whereas the other three drugs were weakly active (Fig. 5). When the indole-positive *Proteus* strains were considered as a single group, results indicated that kanamycin and carbenicillin were the most active of the five antimicrobial agents tested. However, examination of the results for the individual species included in the indole-positive *Proteus* group suggested that kanamycin and carbenicillin were not always the most active drugs.

Cross-resistance to sisomicin, gentamicin, and tobramycin. Activity of sisomicin was independent of the susceptibility or resistance of all isolates to nine other antimicrobial agents as assayed by the Kirby-Bauer technique. The nine drugs tested were ampicillin, carbenicillin, cephalothin, penicillin, chloramphenicol, kanamycin, streptomycin, polymyxin B, and tetracycline.

As noted above, organisms not inhibited by 6.25 μg or less of sisomicin, gentamicin, or tobramycin per ml were considered resistant. Only fourfold or greater differences in the MIC of the three drugs were considered significant.

Of the 184 strains of gram-negative bacilli included in the study, two strains of *Escherichia* and seven indole-positive *Proteus* strains were resistant to all three antimicrobial agents (Table 2). The following seven strains were resistant to only two of the three drugs: one indole-negative and four indole-positive *Proteus* strains resistant to gentamicin and tobramycin, and one *Enterobacter* strain and one indole-positive *Proteus* strain resistant to sisomicin and tobramycin (Table 3). Resistance to only one of the three drugs was displayed by one indole-negative *Proteus* strain resistant to sisomicin only, by five indole-negative *Proteus* strains resistant to gentamicin only, and by three *Serratia* strains and three indole-positive *Proteus* strains resistant to tobramycin only (Table 4). These results showed that cross-resistance was not complete, at least among 19 clinical isolates representing three genera of gram-negative bacilli.

Effect of human serum on activity of sisomicin. It has been shown that calcium and

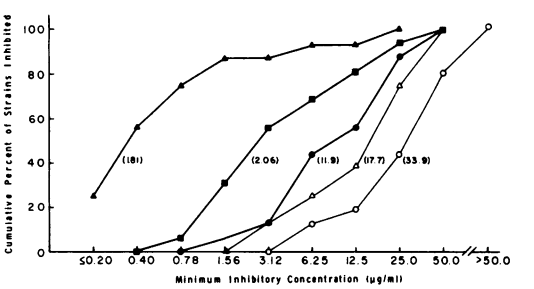

FIG. 5. *Activities of five antimicrobial agents against 16 strains of Proteus rettgeri. Number in parentheses denotes geometric mean of minimal inhibitory concentrations. Percentage of strains inhibited by 6.25 μg/ml was 44% for sisomicin (●), 25% for gentamicin (Δ), and 13% for tobramycin (O). Percentage of strains inhibited by 12.5 μg/ml was 94% for kanamycin (■) and 93% for carbenicillin (▲).*

TABLE 2. *Minimal inhibitory concentrations for gram-negative bacilli resistant[a] to all three aminoglycosides*

Organism	Minimal inhibitory concn (μg/ml)		
	Sisomicin	Gentamicin	Tobramycin
Escherichia	25	50	25
Escherichia	25	25	12.5
P. rettgeri	25	25	50
P. rettgeri	25	50	>50
P. rettgeri	25	25	25
P. rettgeri	50	25	>50
P. rettgeri	25	50	50
P. rettgeri	12.5	25	25
P. rettgeri	25	25	25

[a] Minimal inhibitory concentration >6.25 μg/ml.

TABLE 3. *Minimal inhibitory concentrations for gram-negative bacilli resistant[a] to two of three aminoglycosides*

Organism	Minimal inhibitory concn (μg/ml)		
	Sisomicin	Gentamicin	Tobramycin
P. mirabilis	6.25	25	50
P. rettgeri	6.25	50	>50
P. rettgeri	3.12	50	12.5
P. rettgeri	6.25	25	50
P. rettgeri	1.56	12.5	25
Enterobacter	50	1.56	50
P. rettgeri	50	6.25	50

[a] Minimal inhibitory concentration > 6.25 μg/ml.

human serum under certain conditions may antagonize the in vitro activities of gentamicin and tobramycin (6, 7; V. Zimelis and G. G. Jackson, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 122, 1972). Therefore, the effect of 50% human serum (in brain heart infusion broth) on the activity of sisomicin was determined with five *Pseudomonas* strains and six other gram-negative bacilli. With only one of the five *Pseudomonas* strains tested was a significant increase (fourfold) in MIC noted in the presence of human serum; with the other four *Pseudomonas* strains, no significant change in MIC was observed. Of six other gram-negative bacilli tested, no effect was seen with one strain, a fourfold decrease in the MIC was noted with three, and an eightfold decrease in the MIC was noted with the remaining two strains (Table 5). These results indicated that human serum in general did not antagonize the activity of sisomicin against *Pseudomonas* and increased its activity against other gram-negative bacilli.

DISCUSSION

The in vitro activity of sisomicin was found to be similar to that of both gentamicin and tobramycin. However, several differences were noted. Sisomicin was significantly more active than tobramycin against *Serratia* and indole-positive *Proteus* strains, and significantly more active than gentamicin against indole-negative *Proteus* strains. Both sisomicin and gentamicin were less active than tobramycin against indole-negative *Proteus* strains and *Pseudomonas* strains. The results indicated that the spectrum and degree of activity of sisomicin is more similar to that of gentamicin than that of tobramycin.

The in vitro activity of five antimicrobial agents was determined against 50 indole-positive *Proteus* strains. When considered as

TABLE 4. *Minimal inhibitory concentrations for gram-negative bacilli resistant[a] to one of three aminoglycosides*

Organism	Minimal inhibitory concn (μg/ml)		
	Sisomicin	Gentamicin	Tobramycin
P. mirabilis	12.5	3.12	1.56
P. mirabilis	1.56	25	0.78
P. mirabilis	3.12	12.5	1.56
P. mirabilis	3.12	12.5	0.78
P. mirabilis	6.25	25	0.78
P. mirabilis	3.12	12.5	1.56
Serratia	3.12	1.56	12.5
Serratia	3.12	1.56	12.5
Serratia	1.56	1.56	12.5
P. vulgaris	1.56	3.12	25
P. rettgeri	6.25	3.12	50
P. rettgeri	6.25	3.12	50

[a] Minimal inhibitory concentration >6.25 μg/ml.

TABLE 5. *Effect of heat-inactivated human serum on sisomicin activity*

Organism	Minimal inhibitory concn (μg/ml)	
	BHIB[a]	50% serum-BHIB
Pseudomonas 1	3.12	3.12
Pseudomonas 2	0.78	1.56
Pseudomonas 3	0.78	3.12
Pseudomonas 4	0.78	0.78
Pseudomonas 5	1.56	1.56
Enterobacter	1.56	0.2
Citrobacter	1.56	0.4
Escherichia	6.25	0.78
Proteus	6.25	1.56
Serratia	0.78	0.2
Salmonella	1.56	0.78

[a] Brain heart infusion broth.

a single group, results indicated that kanamycin and carbenicillin were the most active of the five drugs. However, examination of results for the individual species included in the indole-positive *Proteus* group revealed that (i) sisomicin and gentamicin were the most active drugs against *P. vulgaris*, (ii) kanamycin and carbenicillin were the most active drugs against *P. rettgeri*, and (iii) all five drugs were highly active against *P. morganii*. Therefore, generalizations regarding the activity of these antimicrobial agents should not be made from results for the indole-positive *Proteus* group as a whole, but rather from results for the individual species.

The extent of cross-resistance of organisms to gentamicin and tobramycin has been controversial (3-5). This study considered the extent of cross-resistance of clinical isolates to sisomicin, gentamicin, and tobramycin. In this study, 13 gram-negative bacilli were resistant to either gentamicin or tobramycin but not to both. Ten gram-negative bacilli were resistant to either sisomicin or gentamicin but not to both. Furthermore, 13 gram-negative bacilli were resistant to sisomicin or tobramycin but not to both. It therefore appears from this study that gram-negative clinical isolates resistant to one of the three drugs are not necessarily resistant to either of the other two.

We found that 50% heat-inactivated human serum had little effect on the in vitro activity of sisomicin against *Pseudomonas* and no antagonistic effect against other gram-negative bacilli. These observations are in accord with those of Waitz et al. (11), but in contrast to those of Zimelis and Jackson (V. Zimelis and G. G. Jackson, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 122, 1972). These differences, however, may only reflect variations in methods and in calcium and magnesium concnetrations studied. Results of this study suggest that the concentrations of calcium and magnesium that are normally present in human serum may not antagonize the in vitro activity of sisomicin.

Waitz et al. (11) observed only a minor effect of 50% horse serum on the in vitro activity of gentamicin. Davis and Iannetta, on the other hand, observed a marked antagonistic effect of serum and calcium on the in vitro activity of gentamicin (6) and tobramycin (7) against *Pseudomonas*. It should be pointed out that lower concentrations of serum and calcium were used in these latter studies. Therefore, a comparison of the effect of serum on sisomicin, gentamicin, and tobramycin cannot be made.

Results of the study indicated: (i) that against certain bacterial species sisomicin has greater activity in vitro than gentamicin or tobramycin, (ii) that gram-negative bacilli resistant to either gentamicin or tobramycin or to both are not necessarily resistant to sisomicin, and (iii) that human serum does not have an antagonistic effect on the in vitro activity of sisomicin. In view of these potential advantages, further investigation of this new antimicrobial agent is warranted.

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