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In Vitro Studies with Sch 21420 and Sch 22591: Activity in Comparison with Six Other Aminoglycosides and Synergy with Penicillin Against Enterococci

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In vitro tests were performed with Sch 21420 and Sch 22591 to determine (i) their activity in comparison to six other aminoglycosides against 343 clinical isolates, and (ii) whether synergy with penicillin G could be demonstrated with enterococci. In broth dilution tests, Sch 22591 was more active than the seven other aminoglycosides against *Staphylococcus aureus, Enterobacteriaceae,* and most nonfermenting gram-negative bacilli. Sch 22591 was as active as tobramycin against *Pseudomonas aeruginosa.* The activity of Sch 21420 was comparable to gentamicin, sisomicin, netilmicin, and tobramycin but greater than amikacin or kanamycin against *S. aureus* and most genera of *Enterobacteriaceae.* Sch 21420, amikacin, and kanamycin were (i) more active than the other five aminoglycosides against *Proteus rettgeri* and *Providencia stuartii,* but (ii) less active than the other five aminoglycosides against *Neisseria gonorrhoeae,* enterococci, most nonfermenting gram-negative bacilli, *Proteus mirabilis,* and *Proteus morganii.* Studies on the bactericidal activity of Sch 22591 with penicillin indicated a synergistic interaction against enterococci, including strains highly resistant to streptomycin and kanamycin. This could be demonstrated with combinations containing 3.0 to 6.0 μg of Sch 22591 per ml and was comparable to that observed with penicillin/gentamicin. Penicillin plus Sch 21420 (25 μg/ml) also demonstrated synergy against enterococci, including strains highly resistant to streptomycin. However, synergy did not occur against strains highly resistant to kanamycin. These latter results were similar to those obtained in tests with penicillin/kanamycin.

Sch 22591 (5-episisomicin) and Sch 21420 [1-*N*-(*S*-3-amino-2-hydroxypropionyl)-gentamicin (GM)B] are two new semisynthetic aminoglycosides (7; T. L. Nagabhushan, A. B. Cooper, H. Tsai, and P. J. L. Daniels, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., Abstr. no. 249, 1977). Like many new aminoglycosides prepared by chemical modification or mutational biosynthesis (2, 3, 6), they were designed either to enhance potency of the parent compound and/or resist enzymatic inactivation. The purposes of this study were to (i) evaluate the in vitro activity of Sch 22591 and Sch 21420 against a large number of gram-positive and -negative clinical isolates and (ii) determine whether synergy of these two drugs with penicillin could be demonstrated against enterococci. For comparative purposes six other aminoglycosides were evaluated simultaneously: sisomicin (SIS; the parent compound of Sch 22591); netilmicin (NET; 1-*N*-ethyl-sisomicin); GM; tobramycin (TM); kanamycin (KM); and amikacin (AMK; 1-*N*-4-amino-2-hydroxybutyryl kanamycin A).

MATERIALS AND METHODS

Bacterial strains. Organisms used in this study were clinical isolates from St. Josephs Hospital and the Veterans Administration Hospital, Omaha, Neb. Penicillin-resistant *Neisseria gonorrhoeae* were from the Omaha-Douglas County Public Health Department, Omaha, Neb., and the Center for Disease Control, Atlanta, Ga.

Antibiotic solutions. Drug solutions were prepared (weight corrected for potency) on the day of use. Combinations of aminoglycoside and potassium penicillin G (PEN; E. R. Squibb & Sons) were prepared immediately before testing. Each of the following aminoglycosides was used as the sulfate: KM, AMK (Bristol Laboratories), GM, SIS, NET, Sch 21420, Sch 22591 (Schering Corp.), and TM (Eli Lilly & Co.).

Broth dilution susceptibility tests. Broth dilution susceptibility tests were performed on all isolates except *N. gonorrhoeae.* Serial twofold broth dilution

tests were performed in 3 ml of Mueller-Hinton broth (Baltimore Biological Laboratory [BBL]) and incubated for 18 to 24 h at 37°C in air. A bacterial test population of 10^4 colony-forming units (CFU) per ml was prepared from overnight broth cultures and used in each assay. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of drug inhibiting macroscopically detectable growth after incubation. Subcultures to blood agar were made by removing 0.01 ml from each clear tube. The minimal bactericidal concentration (MBC) was defined as the lowest concentration of drug preventing all growth on subculture.

Agar dilution susceptibility tests. Agar dilution susceptibility tests were performed with *N. gonorrhoeae*. Serial twofold agar dilution tests were performed with GC agar base (BBL) plus 1% Kellogg defined supplement (1). A standardized suspension of cells was prepared from an overnight culture on chocolate agar so that inoculation of each test plate with 0.01 ml (calibrated loop) yielded ca. 10^4 CFU/cm^2. The MIC was defined as the lowest concentration of each drug preventing all growth after 24 h of incubation at 37°C in 10% CO_2 in air.

Synergy studies with enterococci. Enterococci were designated highly streptomycin (SM) resistant if they were capable of growing in broth containing 2,000 μg of the drug per ml. Those not capable of growing in this concentration were designated SM susceptible. Methods for determining synergy were identical to those described previously (4). Mueller-Hinton broth (BBL), containing each drug alone or a combination of PEN and an aminoglycoside, was inoculated with cells (10^5 CFU/ml) from an overnight Mueller-Hinton broth culture and incubated at 37°C in air. At 6 and 24 h, a sample was removed, and the number of CFU per milliliter was determined by plate counts in brain heart infusion agar (Difco Laboratories). Penicillinase (BBL) was added to each sample containing PEN before counting. All results shown are averages of duplicate determinations. The concentration of each aminoglycoside used was below the MBC (determined by the standard dilution assay with an inoculum of 10^5 CFU/ml) for each strain. This was (i) 3.1 μg/ml for PEN (1 μg = 1.6 U), (ii) 25 μg/ml for KM and Sch 21420, (iii) 3.0 to 6.0 μg/ml for GM and Sch 22591, and (iv) 25 μg/ml for SM with all but highly resistant strains, for which 2,000 μg/ml was used. Synergy between PEN and the aminoglycoside was defined as (i) relative, if killing by the combination was at least 100-fold greater than that produced by the most effective drug alone, and (ii) complete, if complete killing of the inoculum occurred with the combination and no killing occurred with either drug alone (4). Results obtained after incubation of tests for 24 h were used to determine synergy.

RESULTS

In vitro comparison of Sch 21420 and Sch 22591 with six other aminoglycosides. The in vitro activity of Sch 21420 and Sch 22591 was determined against 343 clinical isolates of gram-positive and -negative bacteria and compared to GM, SIS, NET, TM, AMK, and KM. Results are shown in Table 1. In general, Sch 22591 was the most active agent of the eight aminoglycosides evaluated, whereas KM was the least active. None of the drugs was highly active against enterococci. In tests with *N. gonorrhoeae*, Sch 21420, AMK, and KM were two- to fourfold less active than the other five drugs. Results were similar when tests were performed against four penicillinase-producing strains.

Sch 22591 was the most active agent against the seven genera of *Enterobacteriaceae* tested. All 199 strains were susceptible to 6.2 μg of Sch 22591 per ml. The activity of Sch 21420 was generally less than Sch 22591. It was comparable to GM, SIS, NET, and TM and more active than AMK or KM against the majority of genera tested. However, Sch 21420, AMK, and KM were (i) more active than GM, SIS, NET, or TM against *Proteus rettgeri* and *Providencia stuartii* and (ii) less active than GM, SIS, NET, or TM against *Proteus mirabilis* and *Proteus morganii*. In tests with 40 *Pseudomonas aeruginosa*, Sch 22591 and TM were the two most active drugs, inhibiting and killing all strains at ≤6.2 μg/ml. The activity of Sch 21420 was somewhat less than GM or SIS but similar to NET and AMK. Against 39 other strains of nonfermenting gram-negative bacilli (including *Pseudomonas* sp., *Alkaligenes, Acinetobacter, Xanthomonas, Achromobacter, Flavobacterium,* and *Moraxella*), Sch 22591 was the most active agent, although these strains were generally less susceptible than *P. aeruginosa* to each of the eight aminoglycosides.

Synergy studies. The bactericidal activity of Sch 21420 and Sch 22591 alone and in combination with PEN was determined against 12 strains of enterococci. These included six strains that were highly resistant to SM. For comparative purposes, SM, KM, and GM were evaluated similarly.

Each of the five aminoglycosides displayed relative synergy with PEN (i.e., killing was at least 100-fold greater than either drug alone) against the six SM-"susceptible" strains (Table 2). Complete synergy (i.e., complete killing of the inoculum by the combination with no killing by either drug alone) was demonstrated between PEN and KM, GM, or Sch 22591 against each strain for which neither drug alone was bactericidal. The bactericidal activity of the aminoglycoside when tested alone precluded the demonstration of complete synergy against several strains.

Against enterococci highly resistant to SM, combinations of PEN plus SM (2,000 μg/ml)

TABLE 1. *Comparative in vitro activity of Sch 21420, Sch 22591, and six other aminoglycosides against 343 clinical isolates*

Organism (no. of strains)	Drug	MIC (μg/ml) for % of strains:			MBC (μg/ml) for % of strains:		
		50	75	100	50	75	100
Staphylococcus aureus (25)	Sch 21420	≤0.2	0.4	0.8	0.4	1.6	3.1
	Sch 22591	≤0.2	≤0.2	≤0.2	≤0.2	≤0.2	≤0.2
	GM	≤0.2	≤0.2	0.8	0.8	1.6	6.2
	SIS	≤0.2	0.4	0.8	0.4	0.4	0.8
	NET	0.4	0.4	0.8	0.4	0.8	0.8
	TM	≤0.2	≤0.2	6.2	0.4	0.8	>50.0
	AMK	0.8	1.6	3.1	3.1	6.2	>50.0
	KM	0.8	1.6	3.1	3.1	6.2	50.0
Enterococci (20)	Sch 21420	25.0	50.0	>50.0	>50.0	>50.0	>50.0
	Sch 22591	3.1	3.1	6.2	6.2	6.2	25.0
	GM	3.1	3.1	6.2	6.2	6.2	12.5
	SIS	3.1	3.1	6.2	6.2	6.2	12.5
	NET	3.1	3.1	6.2	12.5	12.5	50.0
	TM	6.2	6.2	12.5	12.5	12.5	25.0
	AMK	1.6	25.0	50.0	6.2	>50.0	>50.0
	KM	12.5	12.5	>50.0	25.0	50.0	>50.0
Neisseria gonorrhoeae (20)	Sch 21420	25.0	25.0	25.0	ND[a]		
	Sch 22591	3.1	6.2	12.5	ND		
	GM	6.2	6.2	12.5	ND		
	SIS	3.1	3.1	12.5	ND		
	NET	3.1	6.2	12.5	ND		
	TM	6.2	6.2	12.5	ND		
	AMK	25.0	25.0	50.0	ND		
	KM	12.5	12.5	25.0	ND		
Escherichia coli (40)	Sch 21420	0.4	0.8	6.2	0.8	1.6	6.2
	Sch 22591	0.4	0.4	1.6	0.4	0.8	6.2
	GM	0.8	1.6	6.2	1.6	3.1	6.2
	SIS	1.6	1.6	6.2	1.6	1.6	6.2
	NET	0.8	1.6	6.2	1.6	1.6	12.5
	TM	1.6	3.1	12.5	1.6	3.1	12.5
	AMK	3.1	6.2	12.5	6.2	12.5	25.0
	KM	6.2	12.5	>50.0	6.2	25.0	>50.0
Klebsiella sp. (20)	Sch 21420	≤0.2	0.4	0.8	0.4	0.4	0.8
	Sch 22591	≤0.2	0.4	0.8	≤0.2	0.4	1.6
	GM	0.4	0.4	1.6	0.4	0.4	1.6
	SIS	0.4	0.8	3.1	0.4	0.8	3.1
	NET	≤0.2	0.4	0.8	≤0.2	0.4	0.8
	TM	0.4	0.4	1.6	0.4	0.4	1.6
	AMK	0.8	1.6	6.2	0.8	1.6	6.2
	KM	1.6	3.1	>50.0	1.6	3.1	>50.0
Enterobacter sp.(40)	Sch 21420	0.4	0.8	1.6	0.4	0.8	1.6
	Sch 22591	≤0.2	0.4	0.8	0.4	0.4	1.6
	GM	0.4	0.8	1.6	0.8	0.8	1.6
	SIS	0.4	0.4	12.5	0.4	0.8	12.5
	NET	0.4	0.4	6.2	0.4	0.8	12.5
	TM	0.8	1.6	6.2	0.8	1.6	12.5
	AMK	1.6	1.6	6.2	1.6	3.1	50.0
	KM	1.6	3.1	>50.0	3.1	3.1	>50.0
Citrobacter sp. (15)	Sch 21420	0.4	0.8	3.1	0.4	0.8	50.0
	Sch 22591	≤0.2	0.4	1.6	≤0.2	0.4	3.1
	GM	0.8	1.6	6.2	0.8	1.6	6.2
	SIS	0.8	1.6	1.6	0.8	1.6	1.6
	NET	0.8	0.8	3.1	0.8	0.8	3.1
	TM	1.6	1.6	1.6	1.6	1.6	3.1
	AMK	3.1	3.1	6.2	3.1	3.1	6.2
	KM	6.2	12.5	25.0	6.2	25.0	>50.0
Serratia sp. (15)	Sch 21420	0.8	0.8	1.6	0.8	0.8	1.6
	Sch 22591	≤0.2	0.4	0.8	0.4	0.4	0.8
	GM	0.8	1.6	1.6	1.6	1.6	1.6
	SIS	0.8	1.6	3.2	1.6	3.1	6.2

TABLE 1 *continued*

Organism (no. of strains)	Drug	MIC (μg/ml) for % of strains:			MBC (μg/ml) for % of strains:		
		50	75	100	50	75	100
	NET	1.6	1.6	3.2	3.1	6.2	6.2
	TM	3.1	3.1	6.2	3.1	6.2	50.0
	AMK	3.1	3.1	6.2	6.2	6.2	12.5
	KM	3.1	3.1	6.2	6.2	6.2	12.5
Proteus mirabilis (20)	Sch 21420	3.1	6.2	12.5	6.2	6.2	25.0
	Sch 22591	0.8	0.8	3.1	0.8	1.6	3.1
	GM	1.6	1.6	3.1	1.6	3.1	6.2
	SIS	1.6	1.6	6.2	1.6	3.1	6.2
	NET	1.6	1.6	3.1	1.6	3.1	6.2
	TM	1.6	1.6	3.1	1.6	3.1	6.2
	AMK	6.2	6.2	12.5	6.2	12.5	25.0
	KM	3.1	6.2	25.0	6.2	12.5	25.0
Proteus morganii (29)	Sch 21420	0.8	1.6	3.1	1.6	3.1	6.2
	Sch 22591	0.4	0.4	0.8	0.4	0.8	3.1
	GM	0.8	0.8	0.8	0.8	0.8	3.1
	SIS	0.8	0.8	3.1	0.8	1.6	6.2
	NET	0.4	0.8	1.6	0.8	0.8	3.1
	TM	0.4	0.8	1.6	0.8	1.6	3.1
	AMK	1.6	3.1	6.2	3.1	3.1	6.2
	KM	3.1	3.1	6.2	3.1	6.2	12.5
Proteus vulgaris (6)	Sch 21420	1.6	1.6	6.2	1.6	1.6	6.2
	Sch 22591	0.4	0.4	0.8	0.4	0.8	1.6
	GM	0.8	1.6	1.6	1.6	3.1	6.2
	SIS	1.6	1.6	3.1	3.1	3.1	3.1
	NET	0.8	0.8	0.8	0.8	0.8	1.6
	TM	1.6	1.6	3.1	1.6	3.1	3.1
	AMK	3.1	3.1	6.2	3.1	6.2	6.2
	KM	3.1	6.2	6.2	3.1	6.2	12.5
Proteus rettgeri (6)	Sch 21420	0.4	0.8	3.1	0.4	3.1	3.1
	Sch 22591	≤0.2	0.8	0.8	≤0.2	0.8	0.8
	GM	1.6	3.1	6.2	1.6	6.2	12.5
	SIS	3.1	6.2	25.0	3.1	12.5	25.0
	NET	1.6	12.5	12.5	3.1	12.5	25.0
	TM	1.6	3.1	6.2	3.1	6.2	12.5
	AMK	0.8	1.6	1.6	0.8	0.8	1.6
	KM	0.8	3.1	3.1	3.1	3.1	3.1
Providencia stuartii (8)	Sch 21420	≤0.2	0.4	1.6	0.4	0.4	1.6
	Sch 22591	≤0.2	0.4	0.4	≤0.2	0.4	0.4
	GM	1.6	3.1	6.2	1.6	6.2	12.5
	SIS	1.6	1.6	6.2	3.1	6.2	6.2
	NET	3.1	3.1	12.5	3.1	6.2	>50.0
	TM	1.6	3.1	12.5	3.1	6.2	12.5
	AMK	0.8	3.1	3.1	1.6	3.1	12.5
	KM	0.8	1.6	3.1	1.6	3.1	3.1
Pseudomonas aeruginosa (40)	Sch 21420	0.4	0.8	12.5	0.8	1.6	50.0
	Sch 22591	≤0.2	≤0.2	0.8	0.4	0.4	6.2
	GM	0.4	0.8	3.1	0.8	1.6	12.5
	SIS	≤0.2	0.4	1.6	0.4	1.6	6.2
	NET	0.4	0.8	6.2	1.6	1.6	50.0
	TM	≤0.2	≤0.2	1.6	0.4	0.4	6.2
	AMK	0.4	0.8	12.5	1.6	1.6	50.0
Miscellaneous nonfermentors (39)	Sch 21420	1.6	12.5	>50.0	3.1	12.5	>50.0
	Sch 22591	0.8	3.1	>50.0	0.8	12.5	>50.0
	GM	3.1	25.0	>50.0	6.2	>50.0	>50.0
	SIS	0.8	12.5	>50.0	3.1	50.0	>50.0
	NET	3.1	50.0	>50.0	12.5	>50.0	>50.0
	TM	1.6	25.0	>50.0	1.6	50.0	>50.0
	AMK	3.1	12.5	>50.0	6.2	25.0	>50.0
	KM	25.0	50.0	>50.0	>50.0	>50.0	>50.0

[a] ND, Not done; susceptibility tests performed in agar.

demonstrated relative synergy against only two and complete synergy against none of the strains for which neither drug alone was bactericidal. Synergy could not be demonstrated at either level with combinations of PEN plus KM against two strains. One of these was moderately resistant to KM (MBC = 50 μg/ml), while the other was highly resistant (MBC = >10,000 μg/ml). Combinations of PEN plus Sch 21420 demonstrated relative synergy against all but the highly KM-resistant strain and complete synergy against one of four strains for which neither drug alone was bactericidal. Combinations of PEN plus GM or Sch 22591 demonstrated relative synergy against all six strains, including the highly KM-resistant strain, and complete synergy against three of four strains for which neither drug alone was bactericidal.

The kinetics of bacterial killing by the five aminoglycosides, alone and in combination with 3.1 μg of PEN per ml, against three strains is shown in Fig. 1 to 3. Against enterococcus 2, an SM-susceptible strain, none of the five aminoglycosides was completely bactericidal alone, while combinations containing PEN were completely bactericidal (Fig. 1). In tests with enterococcus 49, a strain highly resistant to SM and moderately resistant to KM, very little killing occurred with any aminoglycoside alone (Fig. 2). Combinations of PEN plus GM or Sch 22591 were rapidly and completely bactericidal, while combinations of PEN plus Sch 21420, KM, or

TABLE 2. *Synergy between PEN and five aminoglycosides against enterococci*

PEN (3.1 μg/ml) plus:	No. of tests with SM-susceptible strains showing:		No. of tests with highly SM-resistant strains showing:	
	Relative synergy[a]	Complete synergy[b]	Relative synergy	Complete synergy
SM[c]	6	4 (6)[d]	2	0 (5)[d]
KM (25 μg/ml)	6	6 (6)	4	3 (5)
Sch 21420 (25 μg/ml)	6	1 (2)	5	1 (4)
GM (3 to 6 μg/ml)	6	2 (2)	6	3 (4)
Sch 22591 (3 to 6 μg/ml)	6	2 (2)	6	3 (4)

[a] Killing by combination was at least 100-fold greater than the most effective drug alone.
[b] Complete killing of inoculum by combination; no killing by either drug alone.
[c] 25 μg/ml used in tests with SM-susceptible strains; 2,000 μg/ml used in tests with highly streptomycin-resistant strains.
[d] Numbers in parentheses represent number of strains for which neither drug alone was bactericidal.

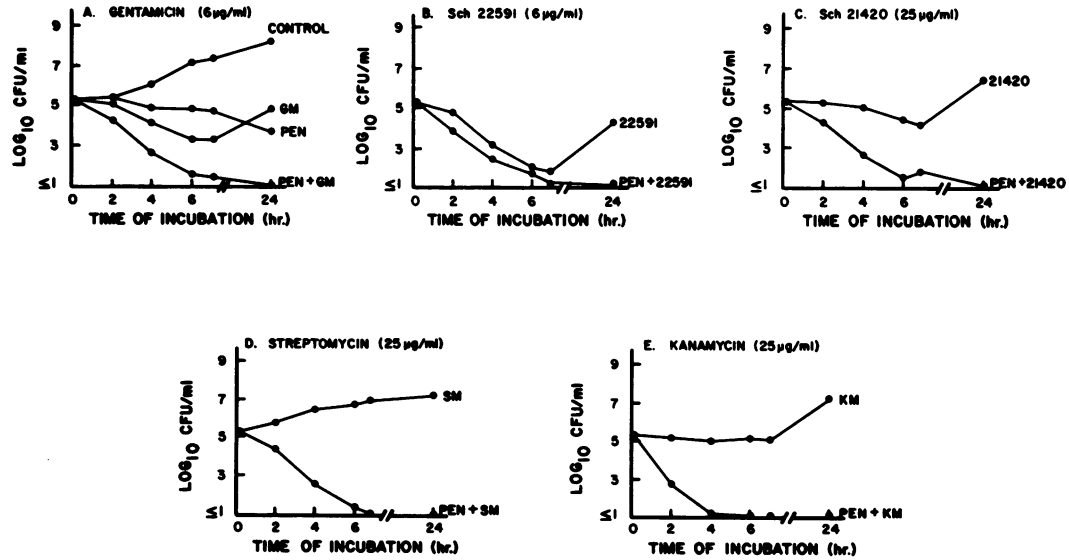

FIG. 1. *Bactericidal activity of five aminoglycosides, alone and in combination with PEN (3.1 μg/ml), against enterococcus 2, an SM-susceptible strain.*

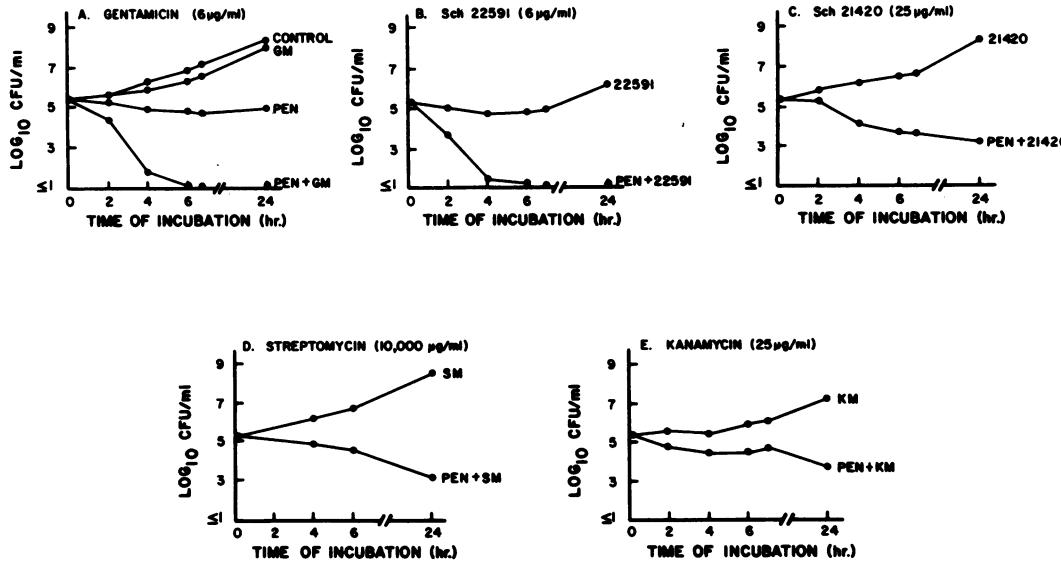

FIG. 2. *Bactericidal activity of five aminoglycosides, alone and in combination with PEN (3.1 µg/ml), against enterococcus 49, a highly SM-resistant, moderately KM-resistant strain.*

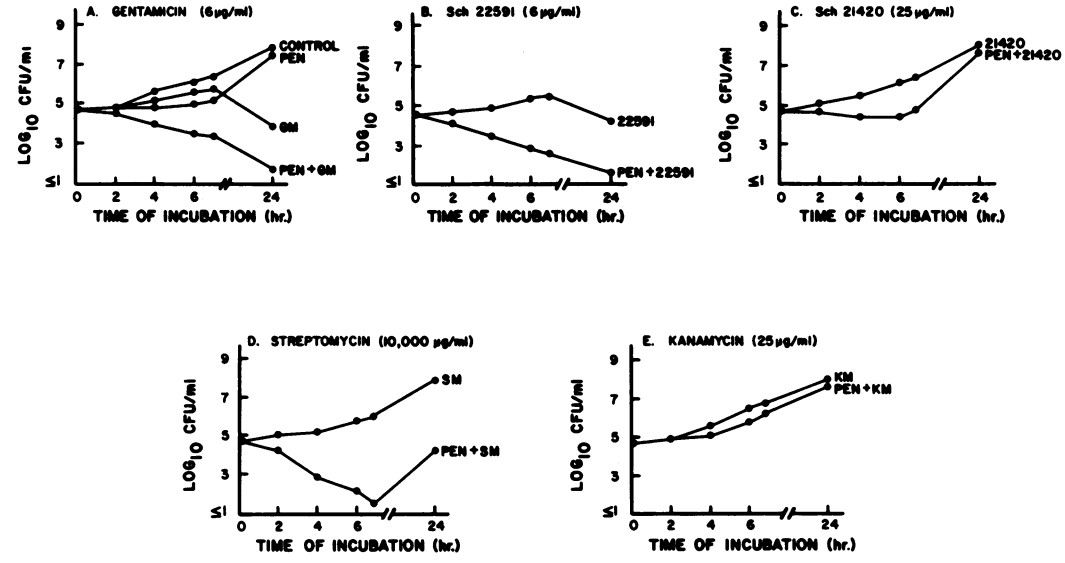

FIG. 3. *Bactericidal activity of five aminoglycosides, alone and in combination with PEN (3.1 µg/ml), against enterococcus 106, a strain highly resistant to SM and KM.*

SM were only partially bactericidal. In tests with enterococcus 106, a strain highly resistant to SM and KM, only combinations of PEN plus Sch 22591 or GM were effectively bactericidal after 24 h of incubation (Fig. 3).

DISCUSSION

Results of this investigation indicated that Sch 22591 (5-episisomicin) was the most active of the eight aminoglycosides evaluated. The increased potency of Sch 22591 in comparison to GM, TM, and AMK has been reported previously by Waitz et al. (7). Results of the current investigation were confirmatory and demonstrated increased activity of Sch 22591 over its parent compound, SIS, as well as NET (another SIS derivative), and KM. Also, the synergistic interaction between Sch 22591 and PEN was

found to be comparable to that observed with GM/PEN against enterococci, including strains highly resistant to SM and KM. This additional data, plus the observations of Waitz et al. that the multiple-dose toxicity of Sch 22591 is similar to that of GM (7), indicate Sch 22591 to be a highly potent broad-spectrum aminoglycoside warranting further investigation.

Results of tests with Sch 21420 [1-*N*-(*S*-3-amino-2-hydroxypropionyl)-GM B] reflected its "dual" chemical composition. Although a member of the GM family, it is structurally related to KM A because it possesses garosamine linked to the deoxystreptamine ring (Nagabhushan et al., Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 17th, New York, N.Y., Abstr. no. 249, 1977). This dual nature was reflected in a "GM-like" activity that was greater than AM or KM against a number of genera and a "KM-like" activity that was less than GM against other isolates including enterococci. Interestingly, *P. rettgeri* and *P. stuartii*, the only two species of *Enterobacteriaceae* uniformly more susceptible to AMK and KM than GM (5) were highly susceptible to Sch 21420—another "KM-like" activity. If future studies show the toxicity of Sch 21420 to be more similar to KM than GM, then certain in vitro advantages of this dual-nature aminoglycoside would warrant further investigation.

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