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Biological Activity of Antibiotic G-418, a New *Micromonospora*-Produced Aminoglycoside with Activity Against Protozoa and Helminths

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On the basis of parallel in vitro studies with antibiotic G-418, gentamicin, neomycin, and kanamycin, antibiotic G-418 was found to be less potent than gentamicin but more active than either kanamycin or neomycin against most strains, with the exception of *Pseudomonas*, for which neomycin was more active than antibiotic G-418, and enterococci, for which antibiotic G-418 was more active than the other three antibiotics. Mouse protection tests indicated that antibiotic G-418 is approximately half as potent as gentamicin and its acute toxicity is one-half to one-third that of gentamicin.

Antibiotic G-418 is a novel aminoglycoside produced by a new species of *Micromonospora*, *M. rhodorangea*. Wagman and co-workers (1) have described the isolation and characterization of antibiotic G-418, as well as the taxonomy of the producing culture. Antibiotic G-418 is of interest not only because of its antibacterial activity but also because of its activity against a variety of protozoa and helminths. The antiparasitic activity of G-418 will be described elsewhere. This paper is concerned with the antibacterial aspects of antibiotic G-418.

MATERIALS AND METHODS

Bacterial strains used were from a standard collection of recent clinical isolates and represent a variety of gram-negative and gram-positive bacteria. In addition, a collection of 58 *Escherichia coli* strains, provided by Howard Bachmann, Schering Animal Health Division, had been isolated from a variety of diseases throughout the United States which were of importance to the Animal Health Division. Minimal inhibitory concentrations were done using an agar dilution procedure with two-fold dilutions based on 1 μg/ml. For these studies, Mueller-Hinton agar was used with an inoculum of 10^4 bacterial cells. Plates were inoculated using a Steers-Foltz replicating device. For all in vitro tests, gentamicin, neomycin, and kanamycin were included as reference antibiotics and were tested in parallel with antibiotic G-418. In vivo efficacy and toxicity tests were done in male CF1 mice weighing approximately 20 g each, as described earlier (2). For determination of efficacy, mean protective dose values in terms of milligrams per kilogram were determined on the basis of treatment groups of seven mice each at five to seven dose levels and a control group of 10 mice. The mice were given a single subcutaneous dose of drug 1 h after intraperitoneal infection with approximately 10^7 organisms per mouse. Infections routinely produced death in untreated infected controls in 18 to 24 h. Mean protective dose values were determined using probit procedures based on survivors 48 h after infection. All antibiotics were used as the sulfate salts; results are given in terms of base activity.

RESULTS AND DISCUSSION

Results of agar dilution minimal inhibitory concentration tests are shown in Table 1 for antibiotic G-418 in parallel with gentamicin, neomycin, and kanamycin. The results are presented in terms of cumulative percent inhibited by various concentrations for each of the bacterial species. For most of the strains tested, including the *E. coli* isolates from a variety of animal diseases, antibiotic G-418 was not as potent as gentamicin; it was, however, more active than either neomycin or kanamycin. Exceptions to this general pattern were noted with *Pseudomonas aeruginosa*. In Table 1 these are divided into 45 gentamicin-sensitive *Pseudomonas* strains, in addition to these strains plus 10 gentamicin-resistant strains. For both groups of *Pseudomonas*, neomycin was more active than antibiotic G-418. Another exception to the general trend was in the case of enterococci, for which antibiotic G-418 was more active than any of the other three antibiotics. Taking critical values for gentamicin of 4 or 8 μg/ml and for antibiotic G-418, neomycin, and kanamycin of 8 or 16 μg/ml, it can be determined that antibiotic G-418 is active against a similar or greater percent of strains of each of the species than either neomycin or kanamycin, with the exception of *Pseudomonas*

TABLE 1. *Comparative in vitro activity of gentamicin, neomycin, kanamycin and antibiotic G-418 by agar dilution tests*

Organism and antibiotic	No. of strains	Cumulative % inhibition (μg/ml)									
		≤.25	.5	1	2	4	8	16	32	64	>64
E. coli											
Gentamicin	9	22.2	66.7	100							
Neomycin	9		11.1	55.5	77.7	88.8	88.8	88.8	88.8	88.8	100
Kanamycin	9			22.2	66.7	88.8	88.8	88.8	88.8	88.8	100
G-418	9		11.1	33.3	77.7	88.8					100
E. coli (Animal Health isolates)											
Gentamicin	58	3.4	53.4	93.1	96.5	96.5	96.5	98.3	100		
Neomycin	58			43.1	70.6	72.4	72.4	72.4	74.1	82.7	100
Kanamycin	58				44.8	70.6	74.1	77.5	77.5	77.5	100
G-418	58			36.2	70.6	77.5	79.3	79.3	79.3	79.3	100
Enterobacter											
Gentamicin	9	22.2	77.7	88.8	100						
Neomycin	9			55.5							100
Kanamycin	9				55.5						100
G-418	9			44.4	55.5						100
Klebsiella											
Gentamicin	9	66.7	88.8						100		
Neomycin	9		22.2	55.5				66.7		77.7	100
Kanamycin	9		11.1	33.3	44.4	55.5				66.7	100
G-418	9			55.5	66.7			88.8	100		
P. aeruginosa (gentamicin sensitive)											
Gentamicin	45	4.4	24.4	55.5	80.0	100					
Neomycin	45		2.2	6.6	22.2	46.6	73.3	91.1	95.5	95.5	100
Kanamycin	45					2.2	2.2	17.1	31.1	73.3	100
G-418	45			2.2	4.4	17.7	31.1	44.4	77.7	91.1	100
P. aeruginosa (all strains)											
Gentamicin	55	3.6	20.0	45.4	65.4	81.8	81.8	87.3	89.0	98.2	100
Neomycin	55		1.8	5.4	18.2	40.0	61.8	76.4	81.8	87.3	100
Kanamycin	55					1.8	1.8	14.5	25.4	65.4	100
G-418	55			1.8	3.6	14.5	25.4	36.4	63.6	80.0	100
Salmonella											
Gentamicin	13	38.5	69.2	92.3	100						
Neomycin	13		7.7	76.9	84.6	100					
Kanamycin	13		7.7	7.7	53.8	84.6	100				
G-418	13		7.7	46.1	84.6	100					
Serratia											
Gentamicin	9	11.1	66.7	100							
Neomycin	18		5.5	77.7	83.3	94.4	94.4	94.4	94.4	100	
Kanamycin	18		5.5	5.5	77.7	88.8	94.4	94.4	94.4	94.4	100
G-418	18			55.5	83.3	94.4	100				
Staphylococcus											
Gentamicin	18	77.7	100								
Neomycin	8	75	75	87.5	87.5	100					
Kanamycin	8		25	75	75	100					
G-418	18		38.8	77.7	77.7	100					
Enterococcus											
Gentamicin	8						12.5	65.2	100		
Neomycin	8							12.5	12.5	75.0	100
Kanamycin	8					12.5	12.5	37.5	62.5	75.0	100
G-418	8						37.5	87.5	100		

TABLE 2. *Comparative in vivo activity of antibiotic G-418 and gentamicin using protection tests in mice*[a]

Infecting organism	Mean protective dose (mg/kg)		
	G-418	Gentamicin	Ratio G-418/gentamicin
E. coli			
619	3.8	0.8	4.8
887	6.2	2.4	2.6
Sc	3.4	0.9	3.7
13	18.0	13.0	1.4
5	2.2	2.0	1.1
15	3.4	2.8	1.2
3	1.8	1.8	1.0
10	5.0	2.4	2.1
3-1	4.3	2.7	1.6
10-1	2.5	2.8	0.9
Klebsiella pneumoniae			
63	2.1	1.9	1.1
70	0.8	0.6	1.3
70-1	1.9	2.1	0.9
Proteus vulgaris			
230	2.1	2.1	1.0
231	4.0	3.7	1.1
Proteus mirabilis Harding	2.0	2.0	1.0
Pseudomonas Coshon			
315	2.9	2.0	1.4
20	7.2	4.8	1.5
	0.8	0.3	2.7
Salmonella Sc	2.2	0.8	2.8

[a] Treatment was a single subcutaneous dose 1 h postinfection.

TABLE 3. *In vivo acute toxicity of gentamicin and antibiotic G-418 in mice*

Route	Mean lethal dose (mg/kg)	
	G-418	Gentamicin
Intravenous	120	75
Subcutaneous	1,050	440

for which neomycin was active against a greater number of strains.

Results of in vivo protection tests in mice done in parallel for antibiotic G-418 and gentamicin against a variety of gram-negative bacteria are shown in Table 2. Mean protective dose values, as well as the ratio of activity calculated for each infection by dividing the mean protective dose of antibiotic G-418 by that of gentamicin, are shown for both of the antibiotics. In these ratios, the higher the number, the less potent, relatively, was antibiotic G-418. The ratios range from 0.9 to 4.8 with an average slightly less than 2, indicating that in mouse protection tests it took approximately twice as much antibiotic G-418 as gentamicin to protect mice from death. Results of acute intravenous or subcutaneous toxicity tests in mice are shown in Table 3. Antibiotic G-418 was found to be approximately one-half to one-third as toxic as gentamicin.

In view of the good activity of antibiotic G-418, particularly against a group of 58 isolates from diseases of veterinary importance coupled with the antiprotozoal and antihelminthic activity reported elsewhere, antibiotic G-418 would seem to be of interest as a therapeutic antibiotic for animal health use. Initial studies with *Arizona* infection in poultry and *E. coli* in swine appear to be promising.

LITERATURE CITED

1. Wagman, G. H., R. T. Testa, J. A. Marquez, and M. J. Weinstein. 1974. Antibiotic G-418, a new *Micromonspora*-produced aminoglycoside with activity against protozoa and helminths: fermentation, isolation, and preliminary characterization. Antimicrob. Ag. Chemother. 6:144–149.
2. Waitz, J. A., E. L. Moss, Jr., C. G. Drube, and M. J. Weinstein. 1972. Comparative activity of sisomicin, gentamicin, kanamycin, and tobramycin. Antimicrob. Ag. Chemother. 2:431–437.