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Antibiotic G-418, a New Micromonospora-Produced Aminoglycoside with Activity Against Protozoa and Helminths: Antiparasitic Activity

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Antibiotic G-418 was shown to be superior to paromomycin and metronidazole in cecal amoebiasis. Of particular interest was the high degree of activity seen with a relatively short treatment at low levels. Although the antibiotic was trichomonacidal in vitro at low levels, in vivo results indicated that at levels tested the antibiotic did not always affect cures. The antibiotic appears to have promise as an anticestode agent, being more active than paromomycin against *Hymenolepis nana* and active as a single oral dose at low levels against *Taenia* spp.

Antibiotic G-418 is a new aminoglycoside antibiotic produced by a new species of *Micromonospora*. *Micromonospora rhodorangea*. Details of the isolation, fermentation, and taxonomy of the producing organism have been previously described (14), as have aspects of its antibacterial activity (16). This report describes the in vitro and in vivo antiparasitic activity of G-418 against *Trichomonas vaginalis*, *Entamoeba histolytica*, *Hymenolepis nana*, *Syphacia obvelata*, and *Taenia* spp.

MATERIALS AND METHODS

Antibiotic G-418 was used as the water-soluble sulfate salt. All results are expressed in terms of base activity as determined by microbiological assay. The strain of *T. vaginalis* used was the Kupferberg strain, originally obtained through the courtesy of Frans Goble, Ciba, and maintained by passage in simplified Trypticase serum medium containing 5% horse serum. In vitro studies with *T. vaginalis* were done in this system. Drug-containing media in a volume of 10 ml, as well as controls, were inoculated with *T. vaginalis* (1.2×10^3 organisms/ml) and incubated at 37 C. Drug effects were determined by counting the number of motile trichomonads after 24 and 48 h of incubation. Culture (0.02 ml) was dispensed under a 22-mm^2 cover slip, and the average number of motile trichomonads per low-power field was determined by counting at least 10 low-power fields. The entire cover slip was searched for motile trichomonads before samples were concluded to be negative. Untreated cultures show approximately a 20- to 30-fold multiplication during the test.

The strain of *E. histolytica* used for in vitro tests was the JH strain obtained through the courtesy of Nathan Entner, New York University Medical Center. *E. histolytica* was maintained in the modified Shaffer-Frye medium (10), as a monoaxenic culture with *Bacteriodes symbiosis*, horse serum, and penicillin, and the in vitro tests employed this system. Tubes containing 10 ml of media, inoculated with 10^4 amoebae were incubated at 37 C in an anaerobic incubator (10% CO_2:90% N_2). Tubes were read microscopically every 24 h for a total of 96 h. The number of amoebae growing in each of the tubes was graded from 0 to 4 as follows: 0, no amoebae visible, <500 amoebae/tube; 1, amoebae visible only in the pellicle, about 2,500 amoebae/tube; 2, amoebae visible in the pellicle and a few in adjacent areas, about 15,000 amoebae/tube; 3, amoebae visible in the pellicle with many in the adjacent areas and a few along the test tube wall, about 60,000/tube; 4, amoebae visible in the pellicle and adjacent areas, with many on the test tube wall, about 250,000 amoebae/tube.

Male CF1 mice weighing 20 to 25 g each were used for in vivo evaluation of *T. vaginalis*. Abscesses were produced by the subcutaneous injection of approximately 4×10^6 trichomonads in a volume of 1 ml. Control animals showed palpable lesions from day 3 or 4 which frequently perforated after day 7. Treatment was given orally, by gavage, starting 4 h after infection and continued daily for 3 days. Mice were sacrificed on day 7, and lesions were graded from 0 to 4 based upon increasing severity. Lesion contents in all mice with 0 to 1 scores were examined microscopically and cultured to see if the mice were actually cured.

The strain of *E. histolytica* used for in vivo tests was the J-190 (Center for Disease Control) strain obtained from Francis Gregory, Wyeth Laboratories, and was maintained in rats and in modified Boeck-Drbholav medium (13). Chronic cecal amoebiasis was produced in 50- to 70-g Royal Hart male rats. The test procedure was a modification of one previously described (18). Rats were infected intracecally following laparotomy, using cecal contents and scrapings from previously infected control rats. Treatment was oral starting the day after infection and lasted up to 6 days. The day after the end of treatment, all animals were sacrificed, and cecae were removed, placed in petri

dishes, and split open. The cecal wall was examined grossly for morphological changes and scrapings were made and examined for amoebae. Cecal contents were also examined for the presence of amoebae. In each animal, the relative number of amoebae seen in the cecal contents and cecal wall scrapings was recorded, as well as the presence of morphological changes in the cecal wall. A modified average degree of infection, based upon gross observations of morphological changes in the cecae and microscopy observations of the number of amoebae found, was recorded on a scale from 0 to 4, 0 representing a substantially normal cecum.

For anthelmintic testing male Royal Hart mice, weighing 18 to 20 g and having a natural infection of *S. obvelata*, were experimentally infected with approximately 200 *H. nana* eggs. Two weeks later, mice were given drug in the diet for 5 days, or given the drugs orally by gavage. All animals were fasted from the afternoon of the last day of treatment until autopsy the following morning. The criterion used for efficacy evaluation was the number of worms remaining in the animals at autopsy. The number of *H. nana* was determined after pressing the small intestine between two glass plates. Individual worm counts were recorded for *H. nana*. *S. obvelata* were counted after mincing the cecae in petri dishes containing 5 ml of a 0.86% saline solution. The Mann-Whitney U test was used to determine if treated groups differed significantly (α = 0.05) from control groups as to worm burdens.

Mongrel dogs and cats naturally infected with *Taenia* spp. were used for further anthelmintic studies. Stoll egg counts and zinc-sulfate flotations were done for 2 weeks before and after treatment, after which time the animals were sacrificed and the intestines were examined for the presence of worms.

RESULTS

Antibiotic G-418 was completely trichomonacidal in vitro at levels of 10 μg or greater per ml. The in vivo antitrichomonal activity of G-418 compared to metronidazole is shown in Table 1. All control mice had average lesion scores of 4.0, and all yielded positive microscopy examinations or culture results 1 week after lesion induction. Metronidazole, at 50 mg (for 3 days) or greater per kg per day, produced negative lesion scores and negative cultures. In mice treated with G-418, variable results were obtained, although reductions in the average lesion scores were seen.

The in vitro antiamoebic activity of G-418 compared to paromomycin is shown in Table 2. It can be seen that G-418 compared very favorably with paromomycin. Both were active at 1 μg/ml after 96 h. Several in vivo experiments compared G-418, paromomycin, and metronidazole in induced cecal amoebiasis in weanling rats. Treatment was orally, once a day up to 6 days, and the results are shown in Table 3. The pooled controls represent 44 rats, 40 of which were positive for amoebae based on microscopy examinations at the time of sacrifice. Twenty of the 44 also showed morphological changes in the cecae as represented by characteristic lesions and/or thickening of the cecal wall. Infected but untreated controls generally had an average degree of infection ranging from 2.0 to 4.0. In Table 3, the controls averaged 2.2 with 17 rats showing thickened walls and seven showing lesions. Treatment with antibiotic G-418 resulted in cures in all rats (53) except one, treated for 6 days with levels of 3.5 mg or higher per kg, and all rats (12) except one, treated for 3 days with 6.5 mg or higher per kg. In the two rats that were not cured, one had only a few amoebae (6-day treatment) and the other had

TABLE 1. *In vivo activity of antibiotic G-418 and metronidazole against subcutaneous T. vaginalis lesions in mice (treatment for 3 days)*

Agent	No. of mice	Dose (mg/kg/day)	Route	Average lesion score	Deaths	Suppression of lesion score (%)	Culture results (positive/total)
Test 1							
G-418	7	150	Oral	0	2	100	4/5
	7	200	Oral	0	1	100	5/6
Pooled controls	9			4.0	0	0	9/9
Test 2							
G-418	7	25	Oral	0.8	0	79	7/7
	7	50	Oral	0	0	100	7/7
	7	100	Oral	0	0	100	7/7
	7	150	Oral	1.0	1	75	6/6
	7	200	Oral	0.6	0	85	7/7
Metronidazole	7	50	Oral	0	0	100	0/7
	7	100	Oral	0	0	100	0/7
	7	200	Oral	0	0	100	0/7
Pooled controls	10			4.0	0	0	10/10

TABLE 2. *In vitro activity of antibiotic G-418 and paromomycin against E. histolytica strain JH*

Concn (μg/ml)	No. of tubes	Average relative number of amoebae at various incubation times (h)[a]							
		Antibiotic G-418				Paromomycin			
		24	48	72	96	24	48	72	96
50	2	1	1	0	0				
40	2	1	1	0	0				
20	2	1	1	0	0				
18	4	1	1	0	0				
15	2	1	1	0	0				
12	2	1	1	0	0	2	1	0	0
10	2	1	1	0	0	1	1	0	0
8	4	1.5	1	0	0	2	1	0	0
5	2	2	1	1	0	2	1	1	0
3	4	2.5	1.5	1	0	2	2	1	0
1	2	3	2	1	1	2	2	1	1
0	8	2.5	3.5	3.5	4	2.5	3.5	3.5	4

[a] O, No amoebae visible, <500/tube; 1, amoebae in pellicle, 2,500/tube; 2, amoebae in pellicle and adjacent region, 15,000/tube; 3, amoebae in pellicle and higher up tube wall, 60,000/tube; 4, many amoebae in pellicle and high on tube wall, 250,000/tube.

TABLE 3. *Oral activity of antibiotic G-418 and reference substances against experimental cecal E. histolytica infections in rats*

Preparation	Oral dose (mg/kg-(day)	Days dosed	Parasitological cure (negative/total)	ADI[a]
Antibiotic G-418	25.0	6	19/19	0
	12.5	6	24/24	0
	10.0	3	7/7	0
	10.0	1	4/7	0.7
	6.5	6	4/4	0
	6.5	3	4/5	0.4
	6.5	1	2/5	1.0
	3.5	6	5/6	0.2
	3.5	3	0/7	1.7
Paromomycin	25.0	6	6/7	0
	12.0	6	4/8	0.8
	10.0	3	2/6	1.0
	10.0	1	0/7	2.1
	6.5	6	2/4	1.3
	6.5	3	1/5	1.0
	6.5	1	0/4	1.0
	3.5	6	1/6	1.5
	3.5	3	1/7	1.9
Metronidazole	25.0	6	1/5	0.8
	13.0	6	2/6	0.8
	6.5	6	1/4	0.8
	6.5	3	0/3	1.7
	6.5	1	0/4	1.5
Sham-dosed controls (water)		6	4/44	2.2

[a] ADI, Average degree of infection; based on morphological changes and numbers of amoebae found.

numerous amoebae (3-day treatment). In neither case was any thickening of the cecal wall or lesions seen. In the rats treated with metronidazole, although no lesions were seen at any levels, three had thickened cecal walls and 18 were positive for amoebae. In rats treated with paromomycin at 3.5 mg/kg for 6 days, two had lesions whereas four had thickened cecal walls; at 6.5 mg/kg for 3 days, three had thickened cecal walls whereas at higher levels only amoebae were seen in 11 of 25 rats.

The results of anthelmintic tests with antibiotic G-418 are shown in Table 4. These indicate 100% activity against both induced tapeworm (*H. nana*) infections and natural pinworm (*S. obvelata*) infections, at diet levels as low as 0.06% (74 mg/kg per day) for 5 days, although by gavage the antibiotic does not appear to be as effective. Recent investigations have shown that the antibiotic is 100% effective in cats and dogs against natural *Taenia* infections at levels as low as 50 mg/kg in a single oral dose.

DISCUSSION

Some aminoglycoside antibiotics are known to be effective in amoebiasis and trichomoniasis, and against tapeworms. Neomycin was found to be only amoebistatic at high concentrations in vitro (1,000 μg/ml) and was also effective in rats only at high doses (13). Kanamycin under comparable conditions was amoebicicdal at 125 μg/ml (12). Streptomycin had very little antiamoebic activity in vitro; however, in vivo it was effective in rats at high doses (8, 13). Gentamicin also had very little in vitro antiamoebic activity (1). Paromomycin, on the other hand, has been shown to have excellent antiamoebic activity both in vitro (3.9 to 10 μg/ml) (3, 12) and in vivo (rats, 22 to 44 mg/kg per day for 4 days by gavage) (12). Metronida-

TABLE 4. *Activity of antibiotic G-418 against helminths in mice, dogs, and cats*

Animal	No. of animals	Drug in diet			Gavage		Helminths				
							H. nana		*S. obvelata*		*Taenia* spp.
		%	No. of days	MKD[a]	No. of days	MKD[a]	% of mice negative	MWB[b]	% of mice negative	MWB[b]	% negative
Mice	7	0.0625	5	74			100	0	100	0	
Mice	7				1	100	0	3.6[c]	43	4.6	
Mice	7				1	50	0	6.0	43	3.3	
Mice	7				3	25	14	6.0	71	0.9[c]	
Mice	7				2	25	14	5.4	43	3.4	
Mice	7				1	25	0	7.1	43	5.7	
Pooled controls	30						4	6.4	5	13.0	
Dogs	1				1	200					100[d]
	1				3	100					100[d]
	1				1	100					100[d]
	2				1	50					100[d]
Cat	1				1	50					100

[a] MKD, Milligrams per kilograms per day.
[b] MWB, Mean worm burden.
[c] Statistically significant.
[d] Plus reduction in hookworm and *Trichuris* egg burdens.

zole is now considered to be a drug of choice in amoebiasis since it is active against all stages of the disease (9). This is in spite of the fact that the minimal inhibitory concentration in vitro was found to be 25 μg/ml, and the median effective dose in rats was found to be 30 mg/kg for 3 days (5). The results of these studies showed that antibiotic G-418 was as effective as paromomycin in vitro and considerably more effective in vivo than both paromomycin and metronidazole in this model of cecal amoebiasis. Of particular interest is the high degree of activity seen with a relatively short (3 day) treatment at low levels, 6 to 10 mg/kg per day.

Against *T. vaginalis*, streptomycin has been shown to be active in vitro only at high levels (17); neomycin had either no activity (17), moderate activity (11), or high activity (7); and paromomycin had moderate activity in vitro and in vivo (12). Metronidazole in vitro was active at levels of 0.8 (2) to 2.5 μg/ml (6) and in vivo in subcutaneous infections in mice from 4.5 mg/kg by gavage for 5 days to 40 mg/kg for 4 days (5). Although antibiotic G-418 was trichomonacidal in vitro, most of the mice yielded positive cultures after in vivo trials, indicating a lack of cure with the antibiotic presumably due to low oral absorption.

Paromomycin has been shown to have slight activity against *H. nana* at levels down to 0.125% in the diet and at 2 g/kg per day by gavage; however, good activity was seen against *Hydatigera taeniaeformis* in cats in single doses of 250 mg/kg or in 5 daily doses of 50 mg/kg (15). Antibiotic G-418 appears to show promise as an anticestode agent. It is more active than paromomycin against *H. nana* and is active in single oral doses against *Taenia* spp. in dogs and cats. These studies are continuing.

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