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Studies on Effects of Drugs upon Protoscoleces of *Echinococcus granulosus in Vitro*

II Scolicidal Effect of Antibiotics, Antineoplastic and Other Agents against *Echinococcus granulosus in Vitro*[*1]

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Introduction

Several investigators are involved in studies on the chemotherapy of echinococcosis (*Echinococcus granulosus* and *E. multilocularis*). The problem remains unresolved. The present paper extends a previous study (Sakamoto and Gemmell; 1975) and reports on the *in vitro* activity of some antibiotic, antineoplastic, cytostatic and other agents against the protoscoleces of *E. granulosus*.

Materials and Methods

Methods in this *in vitro* screening test have previously been reported (Sakamoto and Gemmell; 1975). The 65 test compounds consisted of 20 antibiotics including 6 with antineoplastic activity, 8 cytostatic and 27 miscellaneous compounds as recorded in Table 1. The compounds were dissolved or suspended in propylene glycol at concentrations of 0.2, 2 and 20 mg per ml. The solution or suspension was added to the medium at the rate of 0.005 ml per ml to give 1, 10 and 100γ/ml concentration for each test compound. Each test was carried out in triplicate. The observations on the morphological characteristics of the protoscoleces were continued for up to 10 days.

Results

The duration of survival of the treated protoscoleces has been transformed into a survival percentage with respect to its control. The regression curves (time-mortality curves) were computed from the survival percentage over time. Where possible the time of exposure to achieve the 50 and 90 per cent mortality rates (LT_{50} and $_{90}$ for protoscoleces) was estimated from the quadratic equations of the regression curves. In cases where extremely high or low survival percentages occurred and these estimates could not be used, the lethal

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Table 1. Lethal times for 50 and 90 per cent of protoscoleces stored in a medium containing compounds at three concentrations

No.	DRUG	LT_{50} DOSE/ml			LT_{90} DOSE/ml		
		1γ	10γ	100γ	1γ	10γ	100γ
	ANTIBIOTICS	day	day	day	day	day	day
1	Citromycin chloride	55.21	44.33	–*[2]	–	–	60.73
2	Minimycin	81.05	14.65	9.65	–	18.65	13.37
3	Paromomycin	35.09	22.27	7.38	47.48	28.73	15.76
4	Aabomycin A	6.78	4.51	3.48	9.43	7.48	6.56
5	Aabomycin S	8.95	2.86	+++++*[2]	11.40	5.50	+++++
6	Tubercidin	–	–	5.61	–	–	–
7	Blasticidin-S-styreneoxide	9.79	5.09	4.14	13.62	8.36	6.70
8	Blasticidin-S-methylol	12.47	8.89	3.38	18.03	13.08	5.57
9	Blasticidin-S-benzylaminobenzen	21.09	13.10	10.18	27.40	16.63	13.31
10	Polyoxin	21.91	12.10	6.32	40.58	18.47	14.96
11	Homomycin	10.18	6.35	1.48	13.12	9.59	4.19
12	Kasugamycin	–	–	–	–	–	–
13	Colistin sulfate	–	–	6.44	–	–	9.61
14	Nigericin	+++++ (3.14)*[1]	+++++ (0.34)	+++++ (+++++)	+++++ (4.89)	+++++ (3.73)	+++++ (+++++)
	ANTINEOPLASTIC ANTIBIOTICS						
15	Cellocidin	–	9.15	1.80	–	11.86	4.29
16	Actynomycin D	31.62	20.93	19.917	45.58	29.11	28.05
17	Actidione	47.70	39.40	9.74	98.42	54.65	12.68
18	Toyomycin	11.15	7.96	0.37	13.40	10.09	3.56
19	Bleomycin hydrochloride	64.10	21.50	3.17	87.60	27.89	4.86
20	Mitomycin-C	74.19	24.70	8.01	101.78	31.92	9.69
	CYTOSTATIC COMPOUNDS						
21	Vincristine sulfate	–	43.84	14.97	–	59.46	19.25
22	Vinblastine sulfate	26.98	21.43	11.83	35.40	27.86	14.97

NO.	DRUG	LT_{50} DOSE/ml			LT_{90} DOSE/ml		
		1γ	10γ	100γ	1γ	10γ	100γ
23	5-Fluorouracil	–	47.86	16.38	–	65.62	20.71
24	6-Mercaptopurine	72.37	51.95	16.75	98.48	71.79	21.63
25	L-asparaginase	–	75.66	41.36	–	105.82	56.09
26	1, 4-Dimethane sulfonoxy butane	70.95	36.85	9.31	99.67	49.14	11.01
27	Nitromin	66.43	47.84	8.81	91.16	66.40	11.52
	MISCELLANEOUS COMPOUNDS						
28	Eglumine	49.34	14.39	11.99	66.11	18.65	16.84
29	6(2[(5-bromo-2-pyridyl) amino] vinyl)-1-ethyl-2-picolinium iodide	101.43	37.10	3.36	134.06	49.39	5.23
30	Pentachlorobenzylalcohol	46.19	36.80	26.40	62.14	49.65	34.44
31	Lauryl dimethyl benzyl ammonium saccharinate	35.81	25.62	3.22	48.42	35.07	5.17
32	Lauryl isoquinolium saccharinate	20.17	6.25	2.89	26.20	9.02	4.71
33	2, 3'-Ethylene bis (tetrahydro-4, 6-dimethyl-2H-1, 3, 5-thiodiazine-2-thione)	8.54	4.82	2.69	–	10.28	4.60
34	2, 3-Dicyano-1, 4-dithioanthraquinone	1.92	+++++	+++++	4.52	+++++	+++++
35	2, 4-Dichloro-6-(0-chloroanilino)-1, 3, 5-triazine	4.20	0.27	+++++	7.98	3.68	++++
36	N-trichloromethyl-thio-4-cyclohexene-1, 2-dicarooximide	2.27	+++++	+++++	4.57	+++++	+++++
37	Tetrachloroisophthalonitril	0.19	+++++	+++++	3.68	+++++	+++++
38	1, 2-Bis(3-ethoxycarbonyl-2-thioureido) benzene	15.30	7.10	3.43	24.83	9.15	5.56
39	Bis (dimethyl-dithio-carbamoyl) ethylene diamine	2.35	+++++	+++++	4.70	+++++	+++++
40	Methyl-1-(butyl-carbamoyl)-2-benzimidazole carbamate	15.57	11.42	5.26	27.87	21.20	8.99
41	Xexy p-hydroxy benzoate	13.35	3.53	+++++	17.69	7.52	+++++
42	N-vinyl phthalimide	31.30	22.01	4.01	45.09	31.66	7.05
43	3-Benzylideme amino-4-phenylthiazoline-2-thione	8.46	5.45	4.13	10.55	9.74	7.32
44	2, 6-Dichloro-3, 5-dicyano-4-phenyl pyridine	0.17	+++++	+++++	6.17	1.53	+++++
45	3-(3, 5-Dichlorophenyl)5-,5'-dimethyloxazoline dione-2, 4	27.41	19.27	3.74	43.44	27.05	11.36
46	N-(dichlorofluoromethylthio)-N-(dimethyl-sulfamoyl) aniline	8.10	+++++	+++++	10.71	0.88	+++++
47	N-(2-cyanoethyl) chloroacetamide	7.86	6.81	0.98	9.65	9.12	3.98

NO.	DRUG	LT_{50} DOSE/ml			LT_{90} DOSE/ml		
		1γ	10γ	100γ	1γ	10γ	100γ
48	N-tetrachloroethylthio-4-cyclohexene-1, 2-dicarboximide	11.28	+++++	+++++	15.843	+++++	+++++
49	1, 2-Bis(3-methoxycarbonyl-2-thioureido) benzene	171.54	21.36	8.61	282.31	33.96	21.34
50	Zinc-conjugated manganese ethylene bisdithiocarbamate	11.36	1.46	+++++	15.02	5.69	3.38
51	Sodium dihydroacetate	-	74.89	29.14	-	106.18	40.43
52	Dimethyl benzyl alkyl ammonium chloride	46.48	8.57	3.42	63.11	11.17	5.42
53	Piperonyl butoxide	71.39	21.07	5.09	94.25	28.85	8.11
54	Allethrin	18.78	16.32	6.18	24.22	22.96	8.99
55	N-ethyl chloroacetanilide	2.81	1.21	+++++	4.63	4.07	+++++
56	Zinc ethylene bisdithiocarbamate	18.67	15.17	12.93	-	30.28	22.70
57	Dizinc bis (dimethyldithiocarbamate) ethylene bis (dithiocarbamate)	2.85	+++++	+++++	4.66	+++++	+++++
58	Manganese ethylene bisdithiocarbamate	6.48	2.83	1.89	-	5.59	4.34
59	γ-Butyrolacetone	28.04	18.97	11.45	36.46	24.06	13.70
60	Phenazine-5-oxide	70.91	19.30	5.05	93.55	42.77	11.13
61	3-Hydroxy-5-methyl isoxazole	-	-	97.70	-	-	133.30
62	2-(1-Methyl heptyl)-4, 6-dinitrophenyl crotonate	7.66	4.00	+++++	10.62	6.54	0.98
63	N-butylmercurithiosalicylic acid n-butylester	2.62	+++++	+++++	5.26	+++++	+++++
64	O-o-dimethyl-S-2-(acetylamino) ethyldithiophosphate	33.59	29.50	3.08	46.12	40.91	4.90
65	Copper-8-quinolate	+++++ (6.24)	+++++ (0.53)	+++++ (+++++)	+++++ (17.69)	+++++ (7.52)	+++++ (+++++)

*1 Numbers parenthesized show the lethal times in medium with soidum carboxymethyl cellulose.

*2 In cases where quadratic equation could not be computed, estimates of the lethal times have been based on data obtained on the 10th day of incubation. These are expressed by 5 plus signs. Where survival percentages were greater than 10 per cent on the 10th day, the estimates of lethal times are indicated by dash.

exposure times were estimated from data obtained on the 10th day of incubation and are given in Table 1 by positive and negative symbols. Of the antibiotics, only nigericin (duamycin; polytherin A; Antibiotic X-464) showed strong scolicidal activity. The drug in concentration of 1 γ per ml in the medium with 0.5% propylene glycol killed all of protoscoleces within 48 hours. The scolicidal activity of the antineoplastic antibiotics and cytostatic compounds tested was generally low.

Of the miscellaneous compounds tested, only copper-8-quinolinolate showed strong activity and at 1 γ per ml the protoscoleces died within 48 hours. The order of other compounds showing activity was tetrachloroisophthalonitril, 2,6-dichloro-3,5-dicyano-4-phenyl pyridine, 2,3-dicyano-1,4-dithio-anthraquinone, bis(dimethyl-dithio-carbamyl)-ethylene diamine, n-trichloromethyl-thio-4-cyclohexene-1,2-dicarboximide, dizinc bis-(dimethyldithiocarbamate)ethylene bis(dithiocarbamate), n-butylmercurithiosalicylic acid n-butyl ester and n-ethyl chloroacetanilide.

The effects of propylene glycol and sodium carboxymethyl cellulose as additives in media were tested with nigericin and copper-8-quinolinolate. The scolicidal effect of these drugs suspended in the medium containing carboxymethyl cellulose was less than that when they were suspended in propylene glycol.

Discussion

With regard to the anthelmintic action of antibiotics, hygromycin[2] and destomycin[8,14] have been reported to be active against nematodes and tubercidin[4,5] has activity against *Schistosoma japonicum* and *S. mansoni*. Kelley et al.[7] reported the effect of hygromycin B for removing *Thysanosoma actinioides* from feedlot lambs. Demidov and Artamonova[1] tested 5 antibiotics in mice infected with *Hymenolepis nana*. They reported that monomycin reduced worm burdens by 88.6% and 97.8% with one and two treatments respectively. Salem and Al-Allaf[12] and Kanazawa[6] reported that paromomycin eliminated *Taenia saginata* and *Diphyllobothrium latum*, respectively. Except nigericin, the antibiotics tested in the present study showed low scolicidal activity. The LD_{50} of nigericin given intraperitoneally for mice was 10 mg per kg of body weight.

Hinz[3] and Pelster[11] observed that a cytostatic agent, cyclophosphamide showed activity against strobilocerci of *Hydatigera taeniaeformis* in mice. Lubinsky[9] also reported that cyclophosphamide and lucanthone inhibited the growth of secondary *E. multilocularis* in mice. Lubinsky et al.[10] observed inhibitory effects of the antineoplastic antibiotics, demecline and dactinomycin. In the present experiment, no obvious scolicidal effects by antineoplastic antibiotics and cytostatic compounds were observed. Nine of the 38 miscellaneous compounds tested revealed comparatively high scolicidal activity. Of these, copper-8-quinolinolate showed the strongest effect. The LD_{50} of this compound given orally to mice was determined to be 1000 mg per kg of body weight. When, however, the drug was given subcutaneously or intraperitoneally to mice, it was lethal at low dose rates.

Summary

The protoscoleces of *E. granulosus* were incubated for ten days in media to which a range of antibiotics, antineoplastic antibiotics, cytostatic and miscellaneous compounds were added at rates of 1, 10 and 100γ per ml with propylene glycol. The survival rates of the

treated and untreated protoscoleces were compared. Of the antibiotics tested, only nigericin showed strong activity. Of the antineoplastic antibiotics and cytostatic compounds tested, none showed strong activity. Of the miscellaneous compounds tested, copper-8-quinolinolate showed the greatest activity. The scolicidal effects of nigericin and copper-8-quinolinolate suspended in the medium with sodium carboxymethyl cellulose were less than when suspended in the medium with propylene glycol.

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