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RESPONSE OF DRUG RESISTANT ISOLATES OF *SCHISTOSOMA MANSONI* TO ANTISCHISTOSOMAL AGENTS

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The susceptibility of four isolates of Schistosoma mansoni *(BH, MAP, MPR-1 and K) to four multiple doses of anti-schistosomal agents (hycanthone, niridazole, oxamniquine, and praziquantel) were evaluated in infected female Swiss albino mice. These schistosomal isolates had been maintained in the laboratory without further drug pressure for 20 to 30 generations. Multiple dosage regimens were used for each drug against each isolate of* S. mansoni *to generate* ED_{50} *(effective dose 50%) values. Results demonstrated that the K isolate is resistant to niridazole, the MPR-1 isolate to oxamniquine, and the MAP isolate to both hycanthone and oxamniquine. The BH isolate was susceptible to all drugs and was used as the reference isolate. All isolates were susceptible to praziquantel. The significance of the difference in response of the MPR-1 and MAP isolates is discussed. These results confirm the resistance of these isolates of* S. mansoni *to three schistosomicides and demonstrate that the resistance of these isolates are stable over long periods of time without exposure to drugs.*

Key words: *Schistosoma mansoni* – drug-resistance – praziquantel – hycanthone – oxamniquine – niridazole – susceptibility – schistosome isolates (BH, MAP, K and MPR-1)

During the past two decades several isolates of *Schistosoma mansoni* have been reported to be "resistant" to various antischistosomal drugs (Roger & Bueding, 1971; Katz et al., 1973; Jansma et al., 1977; Araujo et al., 1980; Pedro et al., 1980; Dias et al., 1982, 1988; Bruce et al., 1987; Coles & Bruce, 1987; Coles et al., 1987; Kohn et al., 1987; Yeang et al., 1987; Brindley et al., 1989). Isolates were from patients with uncured infections; derived by *in vivo* selection in mice; derived by *in vitro* selection or developed during long term maintenance involving passage through snail and mouse without any exposure to chemotherapeutic agents. Most of the laboratory studies conducted thus far have been carried out using hycanthone resistant isolates and were either molecular or biochemical in nature (Cioli & Mattoccia, 1984; Doong et al., 1987; Picaa Mattoccia et al., 1988, 1989; Brindley et al., 1989). Dias et al. (1988) carried out a single study in which the response of six isolates of *S. mansoni* to both clinically approved and experimental antischistosomal drugs was determined.

The present study was undertaken to ascertain the extent and stability of resistance and drug changes occuring over successive generations of schistosomal isolates without further drug pressure. For this purpose, dose-response curves for four antischistosomal drugs (niridazole, hycanthone, oxamniquine and praziquantel) against three drug-resistant and one-drug-susceptible isolate was determined. Dose response curves were used to calculate the ED_{50} value for each drug. In addition, the response between isolates to the same drug was compared as well as the most effective drug for use against each isolate.

MATERIALS AND METHODS

S. mansoni *isolates* – BH (Belo Horizonte, Minas Gerais, Brazil, reference isolate, sus-

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ceptible to current antischistosomal drugs). This isolate was obtained from an untreated patient in 1967 and has been maintained continuously in the laboratory by passage through *Biomphalaria glabrata* snails (Brazilian isolate) and outbred CD1 Swiss albino mice.

K (Kenyan, niridazole resistant isolate) – This isolate was isolated in the field in Kenya from an uncured patient and has been maintained in the laboratory with 30 passages through *B. sudanica* snails since 1984 (Kenyan isolate) and outbred CD1 Swiss albino mice without exposure no niridazole.

MAP (Minas Gerais, Brazil, oxamniquine and hycanthone resistant isolate) – This isolate was obtained from a patient in 1978 following unsuccessful treatment with hycanthone and then oxamniquine and has been maintained continuously in the laboratory with 24 passages through *B. glabrata* (Brazilian isolate) and outbred Swiss albino mice.

MPR-1 (Puerto Rican, oxamniquine resistant isolate) – This isolate was obtained from the field in 1953 and has been maintained continously in the laboratory with 25 passages since that time by passage through *B. glabrata* (Puerto Rican isolate) and outbred CD1 Swiss albino mice.

Each of these isolates are currently maintained at the Center for Tropical Diseases, University of Massachusetts at Lowell. The MAP and BH strains are also maintained at the Department of Parasitology, State University of Campinas.

These studies were carried out at the Center for Tropical Diseases, University of Massachusetts at Lowell.

Cultivation and maintenance of snail and schistosomal isolates – Details concerning the cultivation and maintenance of snail and schistosome isolates were those published by Liang et al. (1987).

Basically *B. glabrata* were exposed to 10 miracidia and examined for evidence of infection at 35 days after exposure. Cercariae collected from infected snails were used to expose mice (Liang et al., 1987).

Drugs – Dosage regimen for each drug (niridazole, hycanthone, oxamniquine and praziquantel) used to treat each isolate are shown in Tables I – V. Dosages used were designed to obtain data necessary to plot dose response curves. Preliminary estimates of dosage levels and treatment intervals were based on known efficacies of each drug from the literature. In some instances, with respect to a particular treatment schedule and isolate, an additional dosage was sometimes required in order to obtain more definitive results within the dose response curve for more precise estimates of ED_{50} values. Each drug was prepared as a 10% solution. Niridazole, oxamniquine and praziquantel were each administered per os using Tween Methyl Cellulose (TMCS) as the vehicle. Hycanthone was administered intramuscularly using sterile distilled water as the vehicle.

Efficacy studies – Female Swiss albino mice (Charles River Breeding Laboratory Wilmington, MA), weighing 17 to 19 grams each were provided with water and rodent chow (Ralston Purina, Richmond IN, U.S.A.) ad libitum. Each mouse was anesthetized with an injection of 10% sodium barbital (100 mg/kg), their abdomen shaved and wiped with aerated tap water and then exposed percutaneously to cercariae (Liang et al., 1987). Fifty days following exposure to cercariae, mice were divided into treatment groups of 10-12 mice each as shown in Table I. For each isolate a group of 20 mice were used as non-treated infection controls receiving only the corresponding drug vehicle.

Throughout the experiments, observations were made for obvious gross signs of drug toxicity such as weakness, lethargy, paralysis and death. At necropsy, (approximately two weeks after treatment), the mice were perfused (Radke et al., 1961) to recover schistosomes. Worms were sexed and counted and the livers and mesentery of the mice were removed and examined microscopically for presence of any remaining live or dead worms. The criteria used to determine drug efficacy was based on the number of worms recovered from control and treated animals. The therapeutic value of each drug was determined by use of the following equation (Kemp et al., 1956):

$$\frac{a - b}{a} \quad X \quad 100 = \text{efficacy}$$

where a = average number of live worms recovered from untreated animals and b = number of live worms from treated animals.

To determine the ED_{50} for each drug for each isolate, dose response curves were generated where response was plotted as % efficacy on the y-axis against dose (total mg/kg) on the x-axis. A curve was generated using a Cricket Graph Software Program (Cricket Software, Philadelphia, PA, U.S.A.). The ED_{50} values were obtained by observing the dose which intersected the response curve at 50% efficacy (Nies, 1990).

The Ed_{50} values obtained in mg/kg for each drug against each isolate of *S. mansoni* were converted to μmol/kg of drug using the following calculation (Byrne, 1989). ED_{50} (mg/kg) X 1m mole/molecular weight of drug (mg) X 1000 μmoles/m mole = ED_{50} (μmol/kg).

RESULTS

All mice of the four untreated infection control groups which survived the experimental period developed overt signs typical of experimental schistosomiasis. The mean number of worms recovered from each of the animals of the four control groups ranged from 60 to 72 per mouse (Tables I-IV).

The results obtained from groups of mice treated at 14 days following administration of various regimens of either niridazole, hycanthone, oxamniquine or praziquantel experimentally infected with various isolates of *S. mansoni* is presented in Tables I-IV.

The BH isolate (Table I) chosen as the drug sensitive reference isolate in this study was found to be susceptible (> 98% efficacy) to each of the four compounds used at a single dosage level of 100 mg/kg for hycanthone and oxamniquine and at dosages of 100 mg/kg/day for five days for niridazole and praziquantel. Efficacy of each drug decreased in a dose-dependent manner. The ED_{50} values for each of the four drugs are presented in Table V and show approximately similar potencies against this isolate.

The K isolate (Table II) was found to be susceptible (> 90% efficacy) to a single dose of 105 mg/kg for hycanthone, 100 mg/kg for oxamniquine and at a dose of 100 mg/kg/day given for five consecutive days for praziquantel. However it was insensitive to niridazole at 100 mg/kg/day given over a five day period. At 200 mg/kg/day for five days only 23% efficacy was obtained, while 400 mg/kg for five consecutive days was found to be toxic in the death of 70% of the animals. The ED_{50} values for the four

TABLE I

Antischistosomal activity of four drugs against the BH isolate of *Schistosoma mansoni* in mice

Experimental group	Drug dose (mg/kg/day x days)	No. of mice Treated	No. of mice Examined	No. of worms recovered Male	Female	Total	Range	Mean	SD ±	Efficacy (%)
Control[a]	–	–	18	610	559	1169	48-85	64.9	10.3	–
Niridazole	100 x 5	12	11	6	1	7	0-4	0.6	1.3	99.1
	50 x 5	12	10	223	83	306	20-53	30.6	9.2	52.9
	25 x 5	12	10	249	183	432	31-55	43.2	8.9	33.4
	10 x 5	10	10	295	231	526	47-59	52.6	4.7	19.0
Hycanthone	100 x 1	12	11	5	4	9	0-4	0.75	1.2	98.8
	84 x 1	10	10	234	170	404	36-47	40.4	3.4	37.8
	52 x 1	10	10	244	198	442	31-62	44.2	8.0	31.9
	26 x 1	10	10	279	248	527	43-60	52.7	6.3	15.8
Oxamniquine	100 x 1	12	10	1	0	1	0-1	0.1	0.3	99.8
	50 x 1	12	10	113	137	250	11-31	25.0	6.6	61.5
	25 x 1	12	10	175	176	351	27-43	35.1	5.8	45.9
	10 x 1	10	10	233	195	428	20-59	42.8	10.4	34.1
	5 x 1	10	10	268	228	496	46-55	49.6	3.1	23.6
Praziquantel	100 x 5	12	10	2	2	4	0-3	0.4	1.0	99.4
	50 x 5	12	10	132	114	246	17-35	24.6	5.5	62.1
	25 x 5	12	10	184	162	346	26-41	34.6	4.0	46.7
	10 x 5	10	10	270	268	538	40-68	53.8	10.8	17.1
	1 x 5	10	10	316	277	593	47-71	59.3	7.8	8.6

a: infected group not receiving drug treatment.

TABLE II

Antischistosomal activity of four drugs against the K isolate of *Schistosoma mansoni* in mice

Experimental group	Drug dose (mg/kg/day x days)	No. of mice Treated	Examined	No. of worms recovered Male	Female	Total	Range	Mean	SD ±	Efficacy (%)
Control[a]	–	–	15	534	397	931	39-85	62.1	13.3	–
Niridazole	400 x 5	10	3	62	8	70	20-28	23.3	4.2	38.8
	200 x 5	10	10	270	208	478	38-57	47.8	6.1	23.0
	100 x 5	10	10	318	239	557	47-76	55.7	8.5	10.3
Hycanthone	105 x 1	10	10	19	18	37	0-11	3.7	4.1	93.9
	90 x 1	10	9	204	116	320	31-42	35.6	3.5	35.6
	52 x 1	10	10	260	224	484	39-59	48.4	6.7	22.1
	26 x 1	10	10	329	261	590	34-74	59.0	14.5	5.0
Oxamniquine	100 x 1	12	11	22	24	46	0-11	4.1	3.8	93.2
	70 x 1	10	10	105	106	211	12-25	21.1	5.3	66.0
	50 x 1	10	10	313	162	475	35-62	47.5	7.5	23.5
	25 x 1	10	10	334	236	570	43-69	57.0	7.8	8.2
Praziquantel	100 x 5	12	12	6	5	11	0-4	0.9	1.4	98.6
	50 x 5	12	11	97	86	183	10-25	16.6	4.5	73.3
	25 x 5	12	10	235	172	407	30-56	40.7	7.4	34.5
	5 x 5	10	10	248	216	464	29-60	46.4	8.9	25.3

a: infected group not receiving drug treatment.

TABLE III

Antischistosomal activity of four drugs against the MAP isolate of *Schistosoma mansoni* in mice

Experimental group	Drug dose (mg/kg/day x days)	No. of mice Treated	Examined	No. of worms recovered Male	Female	Total	Range	Mean	SD ±	Efficacy (%)
Control[a]	–	–	19	707	657	1364	38-97	71.8	15.6	–
Niridazole	100 x 5	11	10	10	2	12	0-8	1.2	2.5	98.3
	75 x 5	12	11	241	109	350	21-41	31.8	6.6	55.7
	50 x 5	10	10	348	109	457	36-57	45.7	8.1	36.4
	25 x 5	10	10	389	224	613	48-73	61.3	9.4	14.6
Hycanthone	420 x 1	11	6	103	103	206	26-39	34.3	5.7	52.2
	210 x 1	11	9	265	211	476	44-62	52.9	6.9	26.3
	150 x 1	11	10	369	324	693	61-82	69.3	8.2	3.5
Oxamniquine	800 x 1	12	–	–	–	–	–	–	–	–
	600 x 1	12	5	72	74	146	21-37	29.2	7.3	59.3
	400 x 1	12	9	159	133	292	23-40	32.4	5.9	54.9
	200 x 1	11	10	233	188	421	20-65	42.1	13.2	41.4
	100 x 1	11	10	370	298	668	52-76	66.8	7.1	7.0
Praziquantel	100 x 5	10	10	2	2	4	0-3	0.4	1.0	99.4
	50 x 5	10	10	58	52	110	7-21	11.0	4.2	84.7
	25 x 5	10	9	113	107	220	19-30	24.4	3.7	66.0
	15 x 5	12	9	134	141	275	24-38	30.6	4.4	57.4
	5 x 5	11	10	248	212	460	32-54	46.0	6.2	35.9

a: infected group not receiving drug treatment.

drugs against this isolate of schistosome are shown in Table V. The ED_{50} values obtained for hycanthone, oxamniquine and praziquantel were similar. However, for niridazole the ED50 value was at least 10 fold higher for hycanthone than for the other three drugs (Table V).

TABLE IV

Antischistosomal activity of four drugs against the MRP isolate of *Schistosoma mansoni* in mice

Experimental group	Drug dose (mg/kg/day x days)	No. of mice Treated	No. of mice Examined	No. of worms recovered Male	Female	Total	Range	Mean	SD ±	Efficacy (%)
Control[a]	–	–	18	550	524	1074	34-88	59.7	14.6	–
Niridazole	100 x 5	10	10	21	4	25	0-7	2.5	2.4	95.8
	75 x 5	12	12	156	63	219	13-25	18.3	4.2	69.3
	50 x 5	10	9	116	132	248	14-32	27.6	5.5	53.8
	25 x 5	10	10	245	200	445	31-52	44.5	6.6	25.5
	10 x 5	12	11	335	287	622	44-66	56.5	6.2	5.4
Hycanthone	210 x 1	10	10	30	21	51	0-9	5.1	3.2	91.5
	105 x 1	12	12	35	22	57	0-15	4.8	5.3	92.0
	80 x 1	12	10	182	111	293	22-39	29.3	5.6	50.9
	52 x 1	10	10	173	185	358	24-51	35.8	10.2	40.0
	26 x 1	10	9	238	235	473	39-68	52.6	9.8	11.9
Oxamniquine	600 x 1	11	10	144	125	269	16-43	26.9	8.9	54.9
	400 x 1	11	9	165	145	310	25-41	34.4	5.8	42.4
	200 x 1	10	10	244	182	426	31-57	42.6	8.4	28.6
	100 x 1	9	9	334	193	527	50-64	58.6	11.3	1.8
Praziquantel	100 x 5	11	11	5	3	8	0-6	0.7	8.0	98.8
	50 x 5	10	10	96	106	202	16-28	20.2	4.3	66.2
	25 x 5	10	10	250	172	422	30-63	42.2	8.7	29.3
	15 x 5	10	10	270	239	509	42-68	50.9	8.3	14.7
	5 x 5	10	10	296	274	570	42-68	57.0	6.7	4.5

a: infected group not receiving drug treatment.

TABLE V

ED_{50} of antischistosomal agents against isolates of *Schistosoma mansoni* expressed as mg/kg and μmol/kg

Drugs	Schistosome isolates BH	K	MAP	MPR-1
Hycanthone	87[a]/244[b]	97/272	390/1094	80/224
Niridazole	46/217	504/2353	61/285	53/248
Oxamniquine	34/122	62/221	320/1146	512/1834
Praziquantel	42/134	36/114	13/42	46/149

a: values expressed as mg/kg body weight.
b: values expressed as μmol/kg body weight.

The efficacies of the four drugs against the MAP isolate of *S. mansoni* are presented in Table III. The isolate was sensitive (> 98% efficacy) to niridazole and praziquantel at a dose level of 100 mg/kg/day administered for five consecutive days. The isolate was insensitive to hycanthone and oxamniquine at a single dosage level of 150 mg/kg and 100 mg/kg respectively and at the highest tolerated doses the efficacy was only just over 50%. The ED_{50} values obtained for the four drugs against this isolate are also shown in Table V. The ED_{50} values obtained for hycanthone and oxamniquine are five – ten fold higher than those obtained for niridazole and praziquantel respectively.

The MPR-1 isolate was found to be susceptible (> 90% efficacy) to niridazole and praziquantel at a single dose of 100 mg/kg/day administrated for five consecutive days and a single dose of 105 or 210 mg/kg of hycanthone. For oxamniquine, the isolate was found to be insensitive at a single dose of 100 mg/kg. Increasing the dose to a level of 600 mg/kg only resulted in an efficacy of 54%. ED50 values obtained for oxamniquine were seven to ten fold higher than hycanthone, niridazole or praziquantel (Table V).

Susceptible isolates showed variable sensitivities to the four schistosomicides tested as indicated by differences in ED_{50} values. These values ranged from 42 in the MAP to 149 in MPR-1 isolate for praziquantel, 122 in the BH to 221 in the K isolate for oxamniquine, 217 for the BH to 285 for the MAP isolate for niridazole and 224 in the MPR-1 to 272 in the K isolate for hycanthone (Table V).

DISCUSSION

The results obtained in this study after treatment of four isolates of *S. mansoni* with four antischistosomal drugs confirm and extend previous observations concerning relative resistance or susceptibility of these isolates (Katz et al., 1973; Campos et al., 1976; Araújo et al., 1980; Pedro et al., 1980; Dias et al., 1982, 1988; Bruce et al., 1987).

The dosage range of each drug used to treat animals infected with each of the four isolates was chosen from reports in the literature (Bruce et al., 1987; Dias et al., 1988; Katz et al., 1991). These dosages were found to be effective against the sensitive isolates of *S. mansoni.* Maximum efficacy (> 90%) was also observed in this study using the previously effective doses of each antischistosomal drug, except in cases where a particular isolate was found to be resistant in which a five to ten fold or greater concentration of drug was required to obtain the ED_{50} value.

These ED_{50} values provide an accurate indication of the degree of susceptibility or resistance of these isolates to each drug. In addition, due to the large differences in molecular weights of the four used in this study, the ED_{50} values were converted to μmol/kg. This enabled the attainment of molecular concentration values which provide a more direct comparison of drug efficacy.

Differences observed in response of susceptible isolates to each of the anti-schistosomal agents could be indicative of the presence of low levels of resistance between the isolates or experimental "noise" in this biological model. Investigations involving *in vitro* or *in vivo* selection would be required to differentiate between the occurrence of worms with partial resistance, where as experimental variability (noise), if responsible for these differences could be determined by extending the studies reported herein.

All of the four isolates used in this study have been maintained at the Center for Tropical Diseases by continuous passage through snail and mouse host several years. The BH isolate was received from Brazil in 1984 and has maintained its susceptibility through 30 generations; the MPR-1 isolate was received in 1976 from the University of Michigan and was discovered to be resistant to oxamniquine in 1987 and has remained stable through 25 generations; the K isolate was received at this facility in 1984, found to be resistant to niridazole and remained stable through 30 generations; the MAP isolate was received at this facility from Brazil in 1986 and has remained stable through 24 generations.

The most interesting finding was the difference in response between the MAP and MPR-1 isolates. Both were resistant to hycanthone and oxamniquine. It is usual for cross resistance to occur between these two drugs (Coles & Bruce, 1991). It has been proposed that resistance to hycanthone and oxamniquine is due to loss of an enzyme that metabolises the two drugs to an active form that can bind to DNA (Pica-Mattoccia et al., 1988, 1989). The difference between the two isolates suggests that the mechanism of resistance is more complex than loss of a single enzyme and clearly warrants further investigation. Comparison of resistant (preferably homozygous) and susceptible isolates is the optimal way of determining the mechanism of action of antiparasitic drugs, assuming that resistance involves a change in drug receptors.

To this point in time, the use of chemotherapeutic agents has been fairly successful for the treatment of S. mansoni. However, with the use of any therapeutic agent, the development of resistant isolates of the organism from drug treated individuals is a risk. In this study we have demonstrated that drug resistance and susceptibility has remained stable in four isolates of *S. mansoni* maintained in the laboratory for 20 to 30 generations. The drug concentrations required to achieve ED_{50} values in resistant isolates were at least five fold greater than susceptible isolates. In each case, sensitivity and resistance determined in this study confirm previous findings with these isolates.

The results reported here should provide increased knowledge of schistosomal resistance and could be relevant to future development of chemotherapeutic agents.

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