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# VARIATION IN RESPONSE OF SCHISTOSOMA MANSONI STRAINS TO SCHISTOSOMICIDES (1)

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## SUMMARY

The BH strain of **Schistosoma mansoni** was found to be highly susceptible to hycanthone (1 x 80 mg/kg), oxamniquine (1 x 100 mg/kg) niridazole (5 x 100 mg/kg), praziquantel (1 x 100 mg/kg), oltipraz (5 x 125 mg/kg) and amoscanate (1 x 300 mg/kg) and is therefore a good reference strain for chemotherapeutic trials. By contrast, the MPR-1 strain was shown to have developed resistance to oxamniquine without ever having been dosed with oxamniquine. Other oxamniquine/hycanthone resistant strains were shown to have maintained their resistance and a strain believed to be partially resistant to oltipraz was evaluated.

**KEY WORDS: Schistosoma mansoni;** Experimental chemotherapy — resistance to drugs.

## INTRODUCTION

It is probable that resistance to schistosomicides arises by selection of small numbers of worms that are naturally tolerant to the drug(s). This can explain how **JANSMA** et al.[11] were able to select for hycanthone resistance in **Schistosoma mansoni** in the laboratory. However, selection for hycanthone resistance is not always successful demonstrating that variation between different worm populations occur[18]. It is important to know the susceptibility of laboratory and experimental strains of **S. mansoni** as reference material for future research into drug resistance[2].

The present study reports the response of six strains of **S. mansoni** to clinically approved and experimental schistosomicides.

## MATERIAL AND METHODS

**Schistosoma mansoni** strains:

**BH (Belo Horizonte, Minas Gerais, Brazil).**

This strain was isolated from an untreated patient in 1967 and has been maintained continuously in the laboratory by passage through albino **Biomphalaria glabrata** (Brazilian strain) and Swiss albino mice.

**MAP (Minas Gerais, Brazil; oxamniquine and hycanthone resistant).**

The MAP strain was obtained from a patient in 1978[9] following unsuccessful treatment with

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hycanthone and then oxamniquine. This strain has been maintained continuously in the laboratory by passage through albino **Biomphalaria glabrata** (Brazilian strain) and Swiss albino mice.

**MPR-1 (University of Michigan — Puerto Rico).**

This strain has been maintained continuously in the laboratory since 1953 by passage through **Biomphalaria glabrata** (Puerto Rican strain) and $CD_1$ Swiss albino mice.

**H-30 (Johns Hopkins, Puerto Rico; hycanthone resistant).**

This strain was derived by **in vivo** selection[11] in mice treated with hycanthone in 1976 and has been maintained continuously in the laboratory by passage through **Biomphalaria glabrata** (Puerto Rican strain) and $CD_1$ Swiss albino mice.

**MPROXR (University of Massachusetts, Puerto Rico; oxamniquine resistant).**

This strain was derived by **in vitro** selection of the MPR-1 strain[5] and has been maintained continuously in the laboratory by passage through **Biomphalaria glabrata** (Puerto Rican strain) and $CD_1$ Swiss albino mice since its selection in 1985.

**MPROL (University of Massachusetts, Puerto Rico).**

This strain was derived by **in vitro** selection of the MPR-1 strain[5] with oltipraz and has been maintained continuously in the laboratory by passage through **Biomphalaria glabrata** (Puerto Rican strain) and $CD^1$ Swiss albino mice since its selection in 1985.

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

The work was carried out in the Center for Tropical Diseases, University of Lowell.

## EXPERIMENTAL DESIGN

The procedures used for cultivation and maintenance of snail and schistotosome parasite were those published by **BRUCE & RADKE**[1] and **LIANG**[13].

**Biomphalaria 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[14]. Mice were each exposed to 80 ± 10 cercariae by the tail immersion method[1, 13]. Exposed mice were checked for infection by direct smear examination[12].

Fifty days after exposure, groups of 15 mice were treated with hycanthone (single intramuscular dose of 80 mg/kg), oxamniquine (single oral dose of 100 mg/kg), amoscanate (single oral dose of 300 mg/kg), niridazole (100 mg/kg per day x 5, per os), oltipraz (300 mg/kg per day X 1 or 125 mg/kg per day X 5 per os) and praziquantel (100 mg/kg per day X 5 per os). Groups of mice which were exposed to exact numbers of cercariae as the corresponding treated groups, received no drug, but were dosed with an equal amount of the vehicle used for preparation of the drugs. Drugs were prepared and administered in 1% Cremophor in 25% glycerol[7] except for hycanthone which was prepared in sterile distilled water.

Mice were sacrificed at 14 days after therapy except for those receiving 300 mg/kg of oltipraz which were killed at 56 days after treatment[6]. The mice were necropsied and worms collected by use of the perf-o-suction technique[17]. Following perfusion the intestines and livers were removed and examined for presence of living or dead schistosomes remaining in veins after perfusion. Fragments of the small intestine crushed between a slide and coverslip were used to record changes in the oogram[16] pattern. The oogram pattern was considered altered when one or more stages of immature eggs were found to be absent[16].

The therapeutic value of each drug was determined on the basis of average worm burdens, presence or absense of changes in the oogram

pattern, distribution of worms (%), and percentage of dead worms in the liver.

The average worm burden and the distribution of worms (%) includes dead and live worms.

$$\%\text{ of dead worms in the liver} = \frac{\text{No. of dead worms in liver}}{\text{No. of dead \& live worms in liver}} \times 100$$

## RESULTS

The data obtained at 14 days following administration of hycanthone, oxamniquine, amoscanate, niridazole, oltipraz or praziquantel to mice experimentally infected with one of the strains of **S. mansoni** used in this study is shown in Tables 1 and 2.

TABLE 1

The activity of Some Schistosomicides Against a Brazilian strain (BH) of **Schistosoma mansoni**

| Drug | Dose (mg/kg) | Number of Animals | | Mean Worm Burden | Distribution of Worms (%) | | Dead Worms in Liver (%) | Oogram Changes (%) |
|---|---|---|---|---|---|---|---|---|
| | | Treated | Examined | | Liver | Mesenteric Veins | | |
| Hycanthone | 1 X 80 | 15 | 9 | 36.2 | 98.1 | 1.9 | 97.2 | 100.0 |
| Oxamniquine | 1 X 100 | 15 | 14 | 33.3 | 94.8 | 5.1 | 96.4 | 100.0 |
| Niridazole | 5 X 100 | 15 | 13 | 37.6 | 96.0 | 4.0 | 93.8 | 92.3 |
| Praziquantel | 5 X 100 | 15 | 11 | 29.2 | 100.0 | 0.0 | 100.0 | 100.0 |
| Oltipraz | 5 X 125 | 15 | 12 | 28.4 | 100.0 | 0.0 | 100.0 | 100.0 |
| Amoscanate | 1 X 300 | 15 | 13 | 35.8 | 94.8 | 5.1 | 99.3 | 100.0 |
| Control | — | 15 | 13 | 52.1 | 22.8 | 77.2 | 0.0 | 0.0 |

All animals were sacrificed at 14 days after dosing.

TABLE 2

The Activity of some Schistosomicides Against 5 Strains of **Schistosoma mansoni**

| Strain | Drug | Dose (mg/kg) | Number of Animals | | Mean Worm Burden | Distribution of Worms (%) | | Dead Worms in Liver (%) | Oogram Changes (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | Treated | Examined | | Liver | Mesenteric Veins | | |
| MAP | Hycanthone | 1 X 80 | 15 | 10 | 37.6 | 9.7 | 90.3 | 46.7 | 0.0 |
| | Oxamniquine | 1 X 100 | 15 | 10 | 39.6 | 15.6 | 84.3 | 0.0 | 0.0 |
| | Control | — | 15 | 13 | 48.0 | 23.6 | 76.4 | 0.0 | 0.0 |
| MPR-1 | Oxamniquine | 1 X 100 | 15 | 13 | 21.1 | 29.0 | 70.9 | 0.0 | 0.0 |
| | Praziquantel | 5 X 100 | 15 | 14 | 17.0 | 100.0 | 0.0 | 80.0 | 100.0 |
| | Control | — | 15 | 12 | 22.2 | 17.2 | 82.8 | 0.0 | 0.0 |
| H-30 | Hycanthone | 1 X 80 | 15 | 15 | 28.2 | 22.5 | 77.5 | 1.5 | 6.7 |
| | Control | — | 15 | 12 | 30.0 | 23.4 | 76.5 | 0.0 | 0.0 |
| MPROXR | Oxamniquine | 1 X 100 | 15 | 13 | 24.5 | 5.0 | 95.0 | 33.3 | 0.0 |
| | Control | — | 15 | 14 | 26.6 | 14.3 | 85.7 | 0.0 | 0.0 |
| MPROL | Oltipraz | 5 X 125 | 15 | 15 | 9.0 | 23.8 | 76.2 | 93.0 | 70.0 |
| | Oltipraz | 1 X 300* | 15 | 15 | 2.1 | 100.0 | 0.0 | 56.2 | 100.0 |
| | Control | — | 15 | 15 | 25.8 | 18.6 | 81.4 | 0.0 | 0.0 |

*Sacrificed on day 56 after dosing; all others sacrificed at 14 days after dosing.

The BH strain (Table 1) was found to be susceptible to all of the antischistosomal agents used in this study. Nearly all worms found were observed to be dead in the liver as compared to the control (untreated) mice where all worms found were alive. Oogram changes showed nearly maximal alteration as compared to control (untreated) animals which showed no changes.

The MPR-1 strain (Table 2) was found to be resistant to oxamniquine but susceptible to praziquantel. Oogram changes showed maximal alteration for praziquantel treated mice as opposed to no observed alterations for the oxamniquine treated and control (untreated) mice.

The MAP strain was found to be still resistant to hycanthone and oxamniquine, as previously reported, with no observed alterations in oogram patterns for treated or control (untreated) mice.

Resistance of the H-30 strain to hycanthone was sustained, as previously reported, with very slight alteration in oogram patterns observed for the treated mice and none for control mice.

Following treatment with oxamniquine, the MPROXR strain was found to be very resistant; its stability being maintained with no oogram changes observed in either the treated or in control (untreated) mice as previously reported.

The MPROL strain was observed to be resistant to oral treatment with 125 mg/kg of oltipraz per day X 5 and at a regimen of 300 mg/kg X 1 day it was apparently more resistant as judged by migration of worms from mesenteries to liver and by oogram changes.

## DISCUSSION

The data showing that the BH strain is susceptible to hycanthone, oxamniquine, niridazole, amoscanate, oltipraz and praziquantel is very important. It is essential that a strain known to be susceptible to the currently used antischistosomal drugs is available for evaluating novel compounds as well as for comparing resistant and susceptible strains. Because of widespread use of schistosomicides, it cannot be assumed that fresh field isolates from infected persons will all be fully susceptible to the currently available antischistosomal drugs.

The data obtained which indicated that the MPR-1 strain is resistant to oxamniquine was unexpected especially since this strain was isolated in the field (Dr. Henry van der Schalie obtained the MPR-1 strain from Dr. Paul Thompson of the Park Davis Pharmaceutical Company in 1953) before hycanthone or oxamniquine were introduced clinically for use in the field. Apparently, resistance has developed as passage through mice and snails occurred without the strain ever having encountered the drug oxamniquine. Alterations of isoenzyme patterns have been reported following passage of schistosome strains in mice[4, 15] and the present results indicate that changes in susceptibility to a particular drug could also conceivably occur, although when and how rapidly it occurred is not known.

**CIOLI** et al.[3] have suggested that hycanthone resistance is associated with loss of an enzyme that activates the drug. Unless the presence of the enzyme confers an advantage to the worm in the mouse, a situation not suggested by experience with the MPR-1 strain, resistance would be expected to be stable. **DIAS** et al.[10] and **DIAS & OLIVIER**[8] reported that resistance has remained relatively stable from 1 to 5 and the 14 th generations of the MAP strain, and the present data confirms this at the 24th passage. The data also confirms the resistance of the worms derived by **in vitro** selection of the MPR-1 strain with oxamniquine. The situation with the MPROL strain is not clear. It appears that this strain may have a slight degree of resistance.

With suitable modifications, it should be possible to use the method of **in vitro** selection[5] to look for a small percentage of worms tolerant to a schistosomicide, and therefore predict whether resistance might develop on prolonged routine passage of the strain in the laboratory.

## RESUMO

**Suscetibilidade de cepas de** Schistosoma mansoni **a drogas esquistossomicidas**

A cepa BH de **S. mansoni** foi suscetível ao hycanthone (1 X 80 mg/kg), oxamniquine (1 X 100 mg/kg), niridazole (5 X 100 mg/kg), praziquantel (1 X 100 mg/kg), oltipraz (5 X 125 mg/kg) e amoscanato (1 X 300 mg/kg). Assim, essa cepa do trematódeo é importante como referência nos estudos de quimioterapia experimental. Por outro lado, a cepa MPR-1 apresentou resistência ao oxamniquine e/ou hycanthone. Foi possível constatar em uma cepa resistência parcial ao oltipraz.

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