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A Simple Process for the Experimental Induction of Resistance in *Schistosoma mansoni* to Antishistosomal Agents*

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Abstract: This study was carried out to identify the easiest process of inducing drug resistance in the parent generation of *S. mansoni* in mice after subjecting them to drug pressure. Adult albino mice were infected with *S. mansoni* cercariae and were given various subcurative doses of niridazole orally at 2 weeks, 4 weeks and 8 weeks (that is 14, 28 and 56 days) post-cercarial infection followed by the curative dose of niridazole for (250 mg kg^{-1} body weight for 5 days). We found that the adult worms (8 weeks old) were highly susceptible to niridazole treatment and did not survive the various subcurative treatment that was given. But when three consecutive courses of (100 mg kg^{-1} b.wt for 5 days) of niridazole was given to mice 14 and 28 days post cercarial infection, resistance (Tolerance) occurred with the recovery of 34.82 and 28.28% worms, respectively when curative treatment was given to mice. Mice with 2 week old infections were preferred and thus served as appropriate model for the induction of resistance to niridazole, while other antischistosomal agents may be tested using this method. The process was reproducible and has been valuable for experiments to study the biological characteristics of the drug resistant *S. mansoni*.

Key words: *S. mansoni*, antischistosomal agents, laboratory model, resistance, Nigeria

INTRODUCTION

The development of resistance to chemotherapeutic agents by *S. mansoni* has been reported both experimentally and in clinical samples from some endemic areas (WHO 1985; Bassily *et al*., 1978; Fallon and Doenhoff, 1994; Coles *et al*., 1987; Magdi *et al*., 1999; Cioli *et al*., 2004). The use of effective and well tolerated orally administered drugs used especially for mass chemotherapy campaigns for the control of morbidity in endemic areas thus indicates that worms with less susceptibility to the various drugs might increase and pose a threat to those living in endemic areas like Nigeria where Praziquantel and other agents have been extensively used, necessitating the development of new drugs. There is therefore a need to study the characteristics of these drug resistant parasites. Coles *et al*. (1986) had suggested a protocol for the laboratory induction of drug resistance in *S. mansoni* to the known chemotherapeutic agents already in used, but resistance was evaluated in the offspring generations of the worms.

Although praziquantel is the drug of choice in antischistosomal chemotherapy, we used niridazole which was readily available for this study, in order to understand the biological properties of resistant worms that arise due to resistance as a result of the massive control of schistosomiasis in our country. This chemotherapeutic agent has been used in mass chemotherapy campaigns in Nigeria (Federal

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Ministry of Health bulletin, 1992). The objective of this study was to examine the ease with which adult *S. mansoni* infections develop resistance to niridazole after drug pressure and to determine the stage of infection in which laboratory resistance could be induced in the parent generation.

MATERIALS AND METHODS

Source of Parasite Strain

Schistosoma mansoni cercariae were obtained from naturally infected snails (*Biomphalaria pfeifferi* collected from Jos, Plateau State of Nigeria. Snails were induced to shed cercariae in a beaker containing distilled water by light illumination for 1 and ½ h in the laboratory. The cercariae containing water distilled water was transferred into a clean beaker and the cercarial density was determined by counting the number of cercariae in 0.5 or 0.1 mL of the water containing the cercariae.

Source of Mice

Male and female adult white mice were collected from the animal house of the College of Medicine, UNILAG. They were fed with Pfizer mouse cubes and water *ad libitum* and the mice were maintained in cages. The protocol for this study was approved by the Academic board of studies at the College of Medicine, University of Lagos.

Infection of Mice

Infection of mice with cercariae obtained from snails was carried out as described by Moore *el al*. (1949). Briefly, the mice were stimulated to defecate in warm water (25°C) for 20 min after which they were individually transferred into glass jars which had perforated covers in which about 20 mL distilled water had been placed. In order to infect the mice between 180-200 cercariae were then transferred into each jar and the mice were allowed to paddle in the water for 1 and ½ h. At the end of the infection period, the mice were transferred back into their cages.

Induction of Niridazole Resistance in *S. mansoni* in Mice with Eight Week old Infections

Prior to the induction of drug resistance the minimum curative dose of niridazole on *S. mansoni* infections was determined and found to be equivalent to 250 mg kg^{-1} body weight of niridazole given orally to mice daily for 5 days (Lar and Oyerinde, 1996), to mice with 8 week old infections. To determine the ease of inducing resistance to niridazole in 8 week old worms, 150 mice grouped in 5 batches of 30 mice each were infected with cercariae 8 weeks earlier. The first 4 groups received the different concentrations of niridazole: 150; 125; 75.0 and 50 mg kg^{-1} b.wt, orally daily for 5 days. Up to 6 consecutive courses of treatment was administered. The 5th group served as the infected but untreated control for the experiment. There was 7 days rest for the mice between each course of treatment At the end of the 2nd, 3rd, 4th and 6th courses of treatment, three mice were sacrificed from each batch, in order to ascertain the state of the parasites. When worms were found, the curative dose was administered to three of the mice in order to ascertain the level of tolerance of the worms to the drug. The remaining surviving mice were then sacrificed 7 days (about 70-80 days post infection) after the last dose and worms were recovered and counted.

Determination of the Effect of Age of Parasite on the Induction of Niridazole Resistance in *S. mansoni* in Mice

In order to determine the effect of schistosome on the ease of developing resistance to niridazole, one treatment regime, which is 100 mg kg^{-1} body weight of niridazole, was used. Three consecutive courses of subcurative treatment were administered with 7 to 10 days rest between each course of

treatment. 4 groups of 30 of mice and each were infected with 180-200 cercariae. One group of mice was infected 2 weeks earlier, the second group was infected 4 weeks earlier and the third group 8 weeks earlier. The 4th group served as a parallel control of infected but untreated mice.

Fourteen days after the last course of treatment for each group, the curative treatment regime was administered to 12 mice in each of the groups. Surviving mice were then sacrificed 7 days post treatment and average counts were recorded. The percentage survival of the worms in the treated groups was calculated thus:

$$\frac{\text{Total worm count (Control)} - \text{Total worm count (Experimental)}}{\text{Total worms (control)}} \times 100$$

RESULTS

Worm recovery from mice that received various subcurative doses of niridazole show that adult (8 week old) *S. mansoni* failed to develop resistance. Table 1 shows the average number of worms per mouse recovered were comparable to the control mice which had also been given the curative treatment. This suggests that adult *S. mansoni* infections were highly susceptible to the various sub-lethal doses of niridazole. Worm counts decreased with increasing number of courses of treatment. Worms were killed even after the 2nd course of treatment and although there were surviving worms by the 6th course of treatment, it was evident that these were single male worms and had no significance to transmission.

The schistosomicidal action of the niridazole also was seen with the 4 week old groups confirming that the older worms were less suitable to select for resistance. Table 2 shows the relative susceptibilities of the worms in the different age groups to the curative treatment which was given after 14 days of the last sub curative treatment. 34.82% of the worms were recovered from the 2 week old groups of mice while 28.28 and 4% worm recovery was obtained for the 4 week old and 8 week old groups, respectively. Resistance (Tolerance) was developed in these worms since the worm recovery compared favorably to the non recovery of worms from control group I (these were mice that were infected but did not receive any subcurative treatment previously and were given the curative

Table 1: The average worm counts per mouse following administration of curative dose of niridazole to *S. mansoni* infected and treated mice

		Concentration of the subcurative doses of niridazole given to mice harboring 8 week old infection × 5 days in mg kg^{-1} body weight				
No. of successive subcurative niridazole treatment	*No. of mice used	Control	5	75	125	150
2	12	2	0	0	0	0
3	12	0	0	0	1	2
4	12	0	1	1	1	0
6	12	0	0	0	0	0

*There are on the average 12 surviving mice at the end of the experiments

Table 2: Percentage worm survival after administration of the curative dose (250 mg kg^{-1} daily for 5 days) following administration of 3 successive sub-curative treatment to different ages of *S. mansoni* infections in mice

Age of worms	Control 1	Control 2	2 week old	4 week old	8 week old
No. of courses of 100 mg kg^{-1} × 5 days	Nil	Nil	3	3	3
*No. of mice Sacrificed	10	10	9	10	10
Average No. of worms recovered	0	23	8	6	1
% worm survival as per control II	0	100	34.82	28.28	4

*Surviving worms

treatment subsequently). Control II which were mice that did not receive any treatment also served as the untreated control for the whole experiment. The worm distribution after curative treatment, revealed an average of one (1) male worm was recovered in the liver of mice in control (1) compared with 11.5 pairs of worms recovered from control (ii), however, paired worms were found in the mesenteries of the mice with the 2 week old and the 4 week old groups confirming resistance to the drug (data not show).

DISCUSSION

Schistosoma mansoni resistance to niridazole was induced when subcurative doses of niridazole was repeatedly administered to mice harboring 2 week old infections and to a lesser extent in mice harboring 4 week old infections. Resistance was measured by the percentage of worms that were exposed to subcurative dose of drug but had survived the curative dose (250 mg kg^{-1} body weight × 5 days) of niridazole which has been known to totally eliminate *S. mansoni* in mice. Resistance failed to develop when adult worms (8 weeks) were subjected to various doses of niridazole. Thus application of drug pressure to immature worms can easily select for resistance and provide the opportunity for the study of biological and genetic characteristics of drug resistant worms.

Lambert (1964) had failed to induce niridazole resistance in *S. mansoni* and we were unable to use the protocol for inducing resistance reported by Coles *et al*., (1986). Our observations suggest that niridazole resistance could be selected when worms are exposed to drug in their early stages of development. Jansma *et al*. (1977) suggested that the resistance of *S. mansoni* to hycanthone may have been initiated by the alteration within the worm oocytes since the drug has the ablitity to bind with the parasite DNA and parasite meiosis is known to occur just after the 4th week of development during which parasites had already been exposed to the drug in this present experiment. Kelly and Hall (1979) stated that contact with drug could stimulate extracellular DNA and the RNA to undergo change.

The application of drug pressure due to the administration of subcurative doses of drug with the time interval of 7-10 days between each course of treatment must have allowed the drug time to be adequately metabolized. Thus worms that are susceptible to the drug would have been gradually eliminated. The fact that a pre-existing population of niridazole-resistant *S. mansoni* could be present in this present experiment is not clear since field isolates of *S. mansoni* obtained from naturally occurring *B. pfeifferi* and infected to mice without prior exposure to drug were completely cured by the curative treatment that was obtained (Lar and Oyerinde, 1996).

We conclude that it is easier to use immature *S. mansoni* infections in mice to induce the development of drug resistance for the purpose of experimental studies, but there is the need to further determine whether treatment prior to infections would effect better induction of resistance.

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