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A NOVEL PROTOCOL FOR INDUCTION OF PRAZIQUANTEL RESISTANCE IN *Schistosoma mansoni* IN MICE

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ABSTRACT

The control of *Schistosomamansoni* infection by mass chemotherapy that is being attempted in the absence of effective drug regulation will ultimately create a major socio-economic and public health problem. The aim of the present study was to see how rapidly resistance to PZQ could be induced in *S. mansoni*, by giving sub-curative doses of the drug to mice infected with this parasite. Eighty mice, in eight groups of 10 mice each, were each infected with 150 *S. mansoni* cercariae, maintained until their parasites were adult worms, and then either left untreated (one group of negative controls) or given PZQ in various doses and regimens. Some treatments led to infections that were at least partially resistant to a normally effective treatment (i.e. a single dose of praziquantel at 40mg/kg). Three treatments at 8mg/kg produced infections that were more resistant to the drug than those produced using five doses at the same level ($p<0.05$). Given the current dependence on PZQ for the treatment of human schistosomiasis in endemic areas, including regions where drug control and regulation are poor, it is likely that *S. mansoni* isolates in the field will be subject to curative doses similar to those used in the present study. Therefore, careful surveillance to detect resistance to PZQ in the field is advocated.

Keywords: Induction, praziquantel, resistance, *Schistosoma mansoni*

INTRODUCTION

The treatment of schistosomiasis caused by *S. mansoni* has been transformed by the introduction of praziquantel (PZQ), which has a broad spectrum of activity against all the species of schistosomes pathogenic to humans (Andrews 1981; Kokaliaris et al. 2022). The main risk associated with extensive reliance on one, well-tolerated, orally administrable drug which is highly effective and safe to use as a single dose (Davis *et al*. 1979) is the development of resistance. Such resistance is more likely to occur as unregulated use of the drug, often in sub-curative doses, becomes more frequent.

The earliest suspicion of resistance developing in schistosomes was reported by Davis (1966), who observed that patients infected with *Schistosoma haematobium* who had been treated with niridazole for the second time were less likely to be cured than those who had taken the drug for the very first time. Since then, the resistance of human strains of *S. mansoni* to various schistosomicidal drugs has been documented, largely as treatment failures (Cioli *et al*. 1995; Fallon *et al*. 1995; Stelma *et al*. 1995).

The development of resistance to PZQ by *S. mansoni* might be anticipated in countries where the drug has been used aggressively for more than twenty years (Ismail *et al*. 1999; Muthoni *et al*. 2018). As yet, however, there appears to be no conclusive evidence of resistance to PZQ alone occurring in the field (Katz *et al*. 1991; WHO 1999). The expected appearance of PZQ-resistant *S. mansoni* in a country like Nigeria, where control of schistosomiasis mansoni by mass chemotherapy is being attempted in the absence of effective drug regulation (Mafe and Olawuyi 1998; Anon. 1999; Oyeyemi et al, 2018) will ultimately create a major socio-economic and public health problem. The aim of the present study was to see how rapidly resistance to PZQ could be induced in *S. mansoni*, by giving sub-curative doses of the drug to mice infected with this parasite.

ANIMALS AND METHODS

Mice

Six-week old albino mice, each weighing between 20 and 25g were used. They were purchased from the Animal Center of the College of Medicine of the University of Lagos, housed in polycarbonate cages with sawdust bedding, fed on a conventional pelleted diet and supplied with clean tap water *ad libitum*.

Snails and Parasites

The cercariae used were of Nigerian *S. mansoni* and were obtained from experimentally infected *Biomphalaria pfeifferi*.

Infection and Induction of Resistance

Eighty mice, in eight groups of 10 mice each, were each infected with 150 *S. mansoni* cercariae (Webbe and James 1971), maintained until their parasites were adult worms, and then left untreated (one group of negative controls) or given PZQ in various doses and regimens. As positive controls, the mice in one group were each given only a single (curative) dose of 40 mg PZQ/kg on day 53 post-infection and sacrificed on day 63. The mice in another three groups were each given three low (sub-curative) doses of 2 mg/kg in one group, 4 mg/kg in another, and 8 mg/kg in the third on days 37, 49 and 56 post-infection and then the normal curative dose (40 mg/kg) on day 63 before being sacrificed on day 73. The mice in the remaining three groups were each treated five times (on day 37, 49, 58, 67 and 76 post-infection) with sub-curative doses (again of 2, 4 or 8 mg/kg) and then given the normal curative dose on the day 80 before being sacrificed on day 90. The ten (10) negative-control mice were sacrificed on day 73 or 90 post-infection. Immediately after being sacrificed, all the mice were checked for adult *S. mansoni* in their mesenteries and liver, using the method of Wilson and Coulson (1986). Unpaired worms in the liver and mesenteries of each mouse were counted.

Statistical Analysis

The percentage reductions in worm burdens in the liver or mesenteries, compared with those recovered from the infected but untreated controls, were then calculated as follows:

$$100 - (100\ t/c)$$

Where:

C = mean number of worms in the infected, untreated mice sacrificed on day 73 (for comparisons with the mice given three low doses) or 90 (for comparisons with the mice given five low doses)

T = mean number in the mice in the test group.

A percentage reduction that is less than that seen in the positive controls (i.e. the infected mice treated only once, with 40 mg PZQ/kg) was considered indicative of resistance to the drug. Mean worm recoveries were compared using Student's t-tests.

RESULTS

Treatment of Mice With Three Low Doses

The number of worms recovered from the three groups of mice each treated with three low doses of PZQ is summarized in Table 1. There was evidence of resistance in all three groups (i.e. the mean number of worms recovered/mouse in each were higher than in the mice given a single treatment of 40mg PZQ/kg).

Mesenteric Worms

A significantly higher number of mesenteric worms were recovered from mice given three doses at 2 mg/kg than from mice given three doses at 4 or 8 mg/kg ($t = 10.461$; degrees of freedom (df) = 18; $p<0.05$). The other two higher doses yielded similar worm recoveries ($t = 0.5015$; $df = 18$; $p>0.05$).

Liver Worm

The number of worms recovered from the liver of mice given three doses at 4 mg/kg was similar to number of worms recovered from the liver of mice given three doses at 8 mg/kg ($t = 0.5015$; $df = 18$; $p>0.05$. Comparatively, the number of worms recovered from the liver of mice given three doses at 2 mg/kg was relatively low ($p<0.05$ for each).

Total Worms

In terms of the percentage reductions in the total number of worms recovered/mouse, three doses at 2 or 4 mg/kg appeared to induce similar levels of resistance, which were much less than that induced by three doses at 8mg/kg.

TABLE 1: The mean recoveries of adult *Schistosoma mansoni* after the administration of a normal curative dose (i.e. 40 mg/kg) of praziquantel (PZQ) to mice previously exposed to three lower doses of the drug

		Mean worm recoveries (worm/mouse) from the:												
		Mesenteries				Liver				Liver and mesenteries				
Nos. of mice	Treatment	Unpaired males	Unpaired females	Worm pairs	Total	Unpaired males	Unpaired females	Worm pairs	Total	Unpaired males	Unpaired females	Worm pairs	Total	% reduction in recovery
5	None	6.7	6.7	10.3	**34.0**	53.0	20.7	2.3	**78.3**	60.0	27.3	12.7	**112.3**	-
10	One dose at 40 mg/kg	2.7	0.3	1.7	**6.4**	1.7	0.7	2.0	**6.4**	4.3	1.0	3.7	**12.8**	88.6
10	Three doses at 2 mg/kg and then one at 40 mg/kg	2.7	12.7	6.3	**28.0**	3.0	9.7	1.3	**15.7**	5.7	22.3	7.7	**43.7**	61.1
10	Three doses at 4 mg/kg and then one at 40 mg/kg	1.3	3.3	4.3	**13.2**	1.0	29.0	0.7	**31.4**	2.3	32.3	5.0	**44.6**	60.3
10	Three doses at 8 mg/kg and then one at 40 mg/kg	7.3	11.3	3.7	**26.0**	7.3	54.0	2.0	**65.3**	14.7	65.3	5.7	**91.3**	18.7

Treatment of Mice With Five Low Doses

The number of worms recovered from the three groups of mice each treated with five low doses of PZQ is summarized in Table 2. The mesenteric-worm recoveries were relatively high for the mice given doses at 2 mg/kg (as with the three-dose regimens) and 8 mg/kg. Liver-worm recoveries, however, were always less than those from the control mice only given a single dose of 40 mg PZQ/kg. In terms of total number of worms, only five doses at 8 mg/kg apparently induced resistance to PZQ. Curiously, however, three doses at the highest dosage tested (8mg/kg) appeared to induce greater resistance than five doses at the same dosage (Table 1 and 2 and Figure 1).

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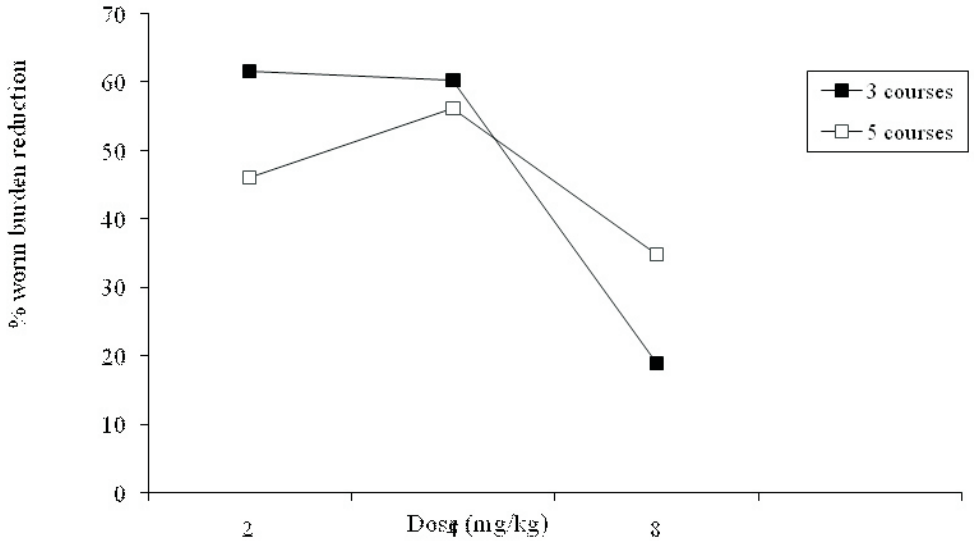
TABLE 2:The mean recoveries of adult *Schistosoma mansoni* after the administration of a normal curative dose (i.e. 40 mg/kg) of praziquantel (PZQ) to mice previously *exposed to five lower doses of the drug*

		Mean worm recoveries (worm/mouse) from the:												
		Mesenteries				*Liver*				*Liver and mesenteries*				
No. of mice	*Treatment*	*Unpaired males*	*Unpaired females*	*Worm pairs*	***Total***	*Unpaired males*	*Unpaired females*	*Worm pairs*	***Total***	*Unpaired males*	*Unpaired females*	*Worm pairs*	***Total***	*% reduction in recovery*
5	*None*	*9.7*	*9.70*	*16.7*	***52.8***	*17.0*	*2.7*	*5.0*	***29.7***	*26.7*	*12.3*	*21.7*	***82.5***	*-*
10	*One dose at 40 mg/kg*	*16.0*	*0*	*5.7*	***27.4***	*18.7*	*0*	*0.7*	***20.1***	*34.7*	*0*	*6.3*	***47.5***	*42.4*
10	*Five doses at 2 mg/kg and then one at 40 mg/kg*	*15.0*	*0*	*7.0*	***29.0***	*14.3*	*0.3*	*0.3*	***15.2***	*29.3*	*0.3*	*7.3*	***44.2***	*46.2*
10	*Five doses at 4 mg/kg and then one at 40 mg/kg*	*12.7*	*0*	*2.7*	***18.1***	*16.0*	*0*	*1.0*	***18.0***	*28.7*	*0*	*3.7*	***36.1***	*56.2*
10	*Five doses at 8 mg/kg and then one at 40 mg/kg*	*17.7*	*0*	*9.7*	***37.1***	*13.3*	*0.7*	*1.3*	***16.6***	*31.0*	*0.7*	*11.0*	***53.7***	*34.9*

Figure 1: The reductions in worm burdens observed after mice infected with adult *Schistosoma mansoni* had been treated with three (□) or Five (■) low doses of praziquantel before being given a normally curative dose (40 mg/kg) of the same drug



DISCUSSION

The present results clearly demonstrate the successful induction of PZQ resistance using repeated (three-dose) administration of sub-curative doses (8 mg/kg) of PZQ to mice with mature (37-day-old) *S. mansoni* infections. The administrations of three or five sub-curative doses appear to induce resistance. Fallon and Doenhoff (1994) also induced PZQ resistance in *S. mansoni* infections in mice but only by administering sub-curative doses over several worm generations. It seems likely that development of resistance depends not just on the size of each dose and the numbers of doses given but also on the length of the between-dose intervals. Such intervals presumably have to be long enough to allow one dose of the drug to be adequately metabolized by the worms before another dose is administered.

The use of a single susceptible host (such as the mouse) and a single indicator of the level of drug resistance (parasite mortality after a normal curative dose) appear adequate for the detection of any PZQ resistance arising from sub-curative multiple chemotherapy of schistosomiasis mansoni. Given the current dependence on PZQ for the treatment of human schistosomiasis in endemic areas, including regions where drug control and regulation are poor, it seems likely that *S. mansoni* isolates in the field will be subject to curative doses similar to those used in the present study. Careful surveillance to detect resistance to PZQ in the field, as advocated by Fallon *et al.* (1996), therefore appears very necessary. In addition, efforts should be made towards discovering effective additional or alternative therapeutic strategies to combat PZQ-resistant schistosomes, whenever and wherever they may occur in the future.

CONFLICT OF INTEREST

I declare that I have no conflict of interest. The author does not also have any relevant financial or non-financial interests to disclose.

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