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RESISTANCE TO OXAMNIQUINE OF A *Schistosoma mansoni* STRAIN ISOLATED FROM PATIENT SUBMITTED TO REPEATED TREATMENTS

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SUMMARY

A strain of *Schistosoma mansoni* (R1) was isolated from patient previously submitted to four treatments with oxamniquine, and to another one with praziquantel. The results obtained with chemotherapeutic test, by using oxamniquine in mice infected with the strains R1 and LE (standard), showed an evident resistance to the drug in worms of the strain R1. Thus, at the dose of 250 mg/kg oxamniquine, all mice (17) infected with the LE strain did not show surviving worms, whereas 12 out of 17 mice infected with the R1 strain presented surviving worms. At the dose of 200 mg/kg, the LE strain showed recovery rates of 1.06% and 20.58%, whereas the R1 strain presented 18.57% and 61.14%, for male and female worms, respectively. At the dose of 100 mg/kg, the recovery of male worms was 2.6% for the LE strain, and 29.9% for the R1 strain. At the same dose, the recovery of females did not show statistically significant differences between the two strains (LE = 76.38%, R1 = 79.12%). Praziquantel showed similar antischistosomal activity against both studied strains, when administered at the dose of 500 mg/kg.

KEYWORDS: *Schistosoma mansoni*; drug resistance; oxamniquine; praziquantel.

INTRODUCTION

Resistance of human strains of *Schistosoma mansoni* to antischistosomal drugs has been described by several authors[1, 7, 8, 9, 13, 15, 16, 17, 21, 22, 26, 27, 33]. On the other hand, a large number of experiments have attained induction of resistance under laboratory conditions[4, 5, 6, 9, 18, 20, 25, 32]. The present study was undertaken to test a strain of *S. mansoni*, which was isolated from patient previously submitted to four treatments with oxamniquine and to one treatment with praziquantel. The patient showed no evidence of being reinfected, and treatments did not succeed.

MATERIAL AND METHODS

S. mansoni strains

The following strains were used in this work:

LE strain - This strain has been maintained through successive *Biomphalaria glabrata*-hamster (*Cricetus auratus*) passages in the laboratory of the Schistosomiasis Research Unit, UFMG - Brazil, for more than 30 years. It is the standard strain for antischistosomal drug experiments.

Isolated strain (designated R1) - This strain was isolated from faecal material obtained from a patient (G.M.C., 15 years old, male, white) living in Barão de Cocais, State of Minas Gerais, Brazil who had been submitted to four treatments with oxamniquine with conventional clinical doses, and one treatment with praziquantel. Viable eggs could be detected on posterior stool examinations. Nevertheless he informed that after the first and subsequent treatments he had not had any contact with water containing cercariae.

Isolation of this strain was obtained using a strain (BH) of *Biomphalaria glabrata* as follows: The faecal material, corresponding to the total amount of a dejection, was homogeneized with 0.85% saline, at 4° C. This suspension was filtrated through 4-time-folded surgical gauze, the filtrated material was resuspended in 0.85% saline at 4°C, and kept in the freezer for sedimentation. One hour later, the supernatant was put aside, and the sediment was seen in the bottom of the sedimentation flask. This sediment was transferred to two glass vessels (50 cm diameter, **15** cm high), and then some dechlorinated water (previously heated at 28° C) was added. One hundred B. glabrata snails (10 mm diameter)

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reared under laboratory conditions were placed into each of those glass vessels and exposed to miracidia overnight. The snails were then placed into appropriate aquaria at 28° C. Thirty days later, they were individually examined for cercariae, in small flasks (10 ml) containing infected water. The positive snails were maintained isolated in an aquarium placed in obscurity.ameter) reared under laboratory conditions were placed into each of those glass vessels and exposed to miracidia overnight. The snails were then placed into appropriate aquaria at 28° C. Thirty days later, they were individually examined for cercariae, in small flasks (10 ml) containing water. The positive snails were maintained isolated in an aquarium placed in obscurity.

Infection of mice

Albino Swiss mice without defined breed were used. Cercariae of both strains (LE and R1) were concentrated and counted. Fifty cercariae per animal were subcutaneously inoculated. Groups of mice infected with the standard strain LE and the strain R1 were submitted to chemotherapeutic treatment, on day 50 after infection.

1) Treatment with praziquantel (Cestox®)* - 500 gm/kg, and oxamniquine (Mansil®)** - 250 mg/kg

Fourteen mice infected with the strain R1 and other 14 infected with the strain LE were treated with praziquantel. In the same manner, 17 mice infected with the strain R1 and other 17 infected with the strain LE were treated with oxamniquine. Fifteen animals in each group, infected with the strains LE and R1 were kept as controls.

2) Treatment with oxamniquine (Mansilâ) - 200 and 100 mg/kg.**

Based on the results of the first experiment, two sub-doses of oxamniquine were chosen (200 and 100 mg/kg). Thus, 25 mice infected with the strain R1 and other 26 infected with the same strain were treated with 200 and 100 mg/kg, respectively. Simultaneously, 17 mice infected with the strain LE were treated with

* Cestox - Lab. Merck
** Mansil - Lab. Pfizer

TABLE 1

Recovery of male (m) and female (f) worms in mice infected with *Schistosoma mansoni* (LE strain), treated with praziquantel (500 mg/kg) and oxamniquine (250 mg/kg) on day 50 post-infection, and perfused on day 28 after treatment.

Mice	Control			250mg/Kg/oxamniquine			500mg/Kg/praziquantel		
N°	m	f	Total	m	f	Total	m	f	Total
1	8	3	11	0	0	0	0	0	0
2	13	12	25	0	0	0	0	0	0
3	11	9	20	0	0	0	0	0	0
4	11	12	23	0	0	0	0	0	0
5	21	13	34	0	0	0	1	0	1
6	14	14	28	0	0	0	0	0	0
7	24	12	36	0	0	0	0	0	0
8	6	3	9	0	0	0	0	0	0
9	10	4	14	0	0	0	0	0	0
10	6	3	9	0	0	0	0	0	0
11	13	4	17	0	0	0	0	0	0
12	16	13	29	0	0	0	1	0	1
13	13	8	21	0	0	0	0	0	0
14	7	2	9	0	0	0	0	0	0
15	23	14	37	0	0	0	-	-	-
16	-	-	-	0	0	0	-	-	-
17	-	-	-	0	0	0	-	-	-
Total	196	126	322	0	0	0	2	0	2
M±	13.1±	8.4±	21.5 ±	0	0	0	0.14±	0	0.14±
SD	5.8	4.72	9.92				0.36		0.36

TABLE 2

Recovery of male (m) and female (f) worms in mice infected with *Schistosoma mansoni* (R1 strain)., treated with praziquantel (500 mg/kg) and oxamniquine (250 mg/kg) on day 50 post-infection, and perfused on day 28 after treatment.

Mice	Control			250mg/Kg/oxamniquine			500mg/Kg/praziquantel		
N°	m	f	Total	m	f	Total	m	f	Total
1	13	6	19	0	1	1	1	0	1
2	18	9	27	0	1	1	0	0	0
3	9	6	15	0	0	0	0	0	0
4	15	12	27	1	0	1	0	0	0
5	1	3	4	1	0	1	0	0	0
6	10	4	14	0	0	0	0	0	0
7	6	0	6	1	1	2	0	2	2
8	4	3	7	4	0	4	0	0	0
9	5	4	9	0	0	0	0	0	0
10	8	5	13	1	0	1	0	0	0
11	13	7	20	2	0	2	0	1	1
12	8	9	17	2	0	2	0	0	0
13	10	10	20	0	0	0	0	1	1
14	16	8	24	1	1	1	0	0	0
15	5	2	7	1	0	1	-	-	-
16	-	-	-	3	0	3	-	-	-
17	-	-	-	0	0	0	-	-	-
Total	141	88	229	17	4	21	1	4	5
M ±	9.4±	5.9±	15.3 ±	1.0±	0.24±	1.21±	0.06±	0.29 ±	0.36±
SD	4.9	3.3	7.6	1.1	0.4	1.1	0.2	0.6	0.61

TABLE 3

Recovery of male (m) and female (f) worms in mice infected with *Schistosoma mansoni* (LE strain), treated with 200 and 100 mg/kg oxamniquine on day 50 post-infection, and perfused on day 30 after treatment.

Mice	Control			200mg/Kg/oxamniquine			100mg/Kg/oxamniquine		
N°	m	f	Total	m	f	Total	m	f	Total
1	17	1	18	0	0	0	0	1	1
2	24	5	29	0	0	0	0	5	5
3	18	3	21	0	0	0	1	4	5
4	20	2	22	0	1	1	0	11	
5	43	10	53	0	1	1	0	5	5
6	38	7	45	0	0	0	0	6	6
7	29	3	32	0	1	1	0	0	0
8	22	3	25	2	1	3	0	6	6
9	20	2	22	0	0	0	2	1	3
10	1	1	11	0	1	1	0	4	4
11	27	4	31	0	4	4	0	3	3
12	12	4	16	0	2	2	2	1	3
13	26	6	32	0	0	0	1	0	1
14	17	1	18	0	0	0	1	1	2
15	14	5	19	1	1	2	0	8	8
16	16	4	20	1	2	3	2	3	5
17	23	9	32	0	0	0	0	5	5
18	9	3	12	-	-	-	0	1	1
19	28	8	36	-	-	-	-	-	-
20	27	0	27	-	-	-	-	-	-
Total	440	80	520	4	14	18	9	55	64
M ± SD	21.0± 8.7	4.0 ± 2.8	26.0± 10.5	0.24± 0.6	0.82± 1.1	1.1± 1.3	0.50± 0.8	3.1± 2.4	3.6± 2.2

200 mg/kg oxamniquine, whereas other 18 were treated with 100 mg/kg oxamniquine. Twenty animals infected with each strain were maintained as controls.

Perfusion

Twenty eight or 30 days after treatment, the animals were perfused for worms, in accordance with the technique described by PELLEGRINO & SIQUEIRA[28]. The worms recovered were counted by means of a stereomicroscope and separated by sex.

Statistical analysis

The data obtained were analysed by Kruskal-Wallis non-parametric test[14].

RESULTS

In the first experiment, oxamniquine (250 mg/kg) and praziquantel (500 mg/kg) were administered to the animals infected with the strains R1 and LE. An equivalent activity of praziquantel could be detected for both strains, and the parasitism was practically eradicated. On the other hand, a statistically significant difference ($P < 0.001$) could be observed between the efficacy levels of oxamniquine for the strains LE and R1 (all mice infected with the strain LE were found to be cured, whereas 12 out of 17 mice infected with the strain R1 presented surviving worms) (Tables I and II).

The experiments that were designed to enhance the differences between the resistance levels previously observed showed a marked difference between the strains LE and R1, when oxamniquine was used at the doses of 200 mg/kg and 100 mg/kg (Tables III and IV)

Thus, taking into account the recovery rate in relation to the untreated controls, the strain R1 showed a worm recovery rate of 18.57% against 1.06% in the strain LE, for males and 61.14% against 20.58% in the strain LE, for females, when the dose of 200 mg/kg was employed. At the dose of 100 mg/kg, the worm recovery rate in the strain R1 was 29.9% against 2.26% in the strain LE, for males. As far as the recovery of female worms was concerned, at the same dose no statistically significant difference could be observed between the two strains studied (R1 = 79.12% and LE = 76.36%) (Table V). On the other hand, statistically significant difference could also be detected, when males and females were considered altogether (Table V).

DISCUSSION

Chemotherapeutic treatment against species of the genus *Schistosoma* has shown noteworthy advances in the last decades. In this way, some drugs with high therapeutic efficacy, low toxicity, administered by the oral route in a single dose, have rendered possible the mass treatment for the control of schistosomiasis, in various endemic countries. On the other hand, the generalized use of these drugs may have been the cause of the arising resistant strains, as a result of selective pressure. For the time being, oxamniquine and praziquantel are the most used drugs for the treatment of schistosomiasis, at both individual and mass treatment levels.

Oxamniquine was found to be efficient against *Schistosoma mansoni* alone, whereas praziquantel has shown good efficacy against all species of the genus *Schistosoma* infecting mankind. In Brazil, where only *S. mansoni* exists, millions of people have been submitted to treatment with oxamniquine in endemic areas; many have received more than one treatment, due either to reinfection or to treatment ineffectiveness. Thus, one may expect natural selection to play its role resulting in the forthcoming of resistant lineages. Regardless of this expectation, reports on isolation of *S. mansoni* strains resistant to oxamniquine are rare in Brazil. The first authors to report isolation of resistant strains in Brazil were Katz et al.[26]. They verified a slight resistance of a *S. mansoni* strain (isolated from two patients previously treated with Hycanthone and Niridazole) to oxamniquine. Later, various authors[1, 15, 16, 17, 19, 22, 27] have described new *S. mansoni* strains resistant to oxamniquine. As far as the reported strains are concerned, only the lineage MRP-1[19] has shown a resistance level similar to the strain R1, described in the present study. The percentage of surviving males (at the dose of 200 mg/kg) was 18.57% for the strain R1, and 44.36% for MRP-1 strain, while surviving female rate was 61.14% for the strain R1 and 34.7% for MRP-1 strain. This comparison shows a higher resistance of females and a lower resistance of males in the strain R1, in relation to MRP-1 strain.

TABLE 4

Recovery of male (m) and female (f) worms in mice infected with *Schistosoma mansoni* (R1 strain), treated with 200 and 100 mg/kg oxamniquine on day 50 post-infection, and perfused on day 30 after treatment.

Mice	Control			200mg/Kg/oxamniquine			100mg/Kg/oxamniquine		
N°	m	f	Total	m	f	Total	m	f	Total
1	37	7	44	14	10	24	14	14	28
2	28	9	37	3	1	4	6	8	14
3	26	16	42	5	5	10	7	5	12
4	12	2	14	4	5	9	10	5	15
5	13	5	18	3	6	9	14	8	22
6	35	10	45	9	8	17	4	2	6
7	38	11	49	5	4	9	8	8	16
8	15	4	19	1	5	6	14	10	24
9	20	7	27	9	5	14	8	6	14
10	16	7	23	1	1	2	3	4	7
11	20	6	26	10	4	14	16	8	24
12	23	6	29	4	3	7	7	7	14
13	25	5	30	1	1	2	9	6	15
14	24	5	29	7	5	12	6	6	12
15	28	8	36	2	4	6	13	4	17
16	34	11	45	10	13	23	4	3	7
17	44	7	51	3	0	3	6	4	10
18	10	1	11	4	3	7	4	3	7
19	40	5	45	2	4	6	6	4	10
20	16	8	24	1	4	5	4	4	8
21	-	-	-	6	1	7	4	6	10
22	-	-	-	1	2	3	1	1	2
23	-	-	-	0	0	0	1	2	3
24	-	-	-	4	3	7	4	4	8
25	-	-	-	8	10	18	6	3	9
26	-	-	-	-	-	-	12	9	21
Total	504	140	644	117	107	224	196	144	340
M±	25.2±	7.0 ±	32.2 ±	4.7±	4.3 ±	8.6 ±	7.4±	5.54±	12.9±
SD	10.1	3.4	12.1	3.6	3.2	5.6	4.2	2.9	6.7

TABLE 5

Recovery/reduction rates of the worm burden in *Schistosoma mansoni* strains (R1 and LE) after treatment with oxamniquine, at the dosages of 100 and 200mg/kg, in relation to the worm burden of untreated controls.

Sex of worms	100 mg/kg oxamniquine			200 mg/kg oxamniquine		
	R1 strain	LE strain	*P**	R1 strain	LE strain	*P**
m	29.9%	2.26%	<0.001	18.75%	1.06%	<0.001
f	79.12%	76.38%	NS	61.14%	20.58%	<0.05
m + f	37.71%	15.38%	<0.05	24.84%	4.95%	<0.001

m = male f = females NS = Not significant
Kruskal-Wallis test *

Recently, ARAÚJO et al.[2] have studied 10 *S. mansoni* strains isolated from patients indigenous to an endemic schistosome area (Itaguara, State of Bahia, Brazil), submitted to treatment with oxamniquine and later with praziquantel, both drugs being equally unsuccessful. Tests carried out in mice failed to detect resistance to oxamniquine in all strains studied. This last result shows that it is not easy to find resistant human strains.

Oxamniquine belongs to the group of "aminoalkyltoluenes", as well as hycanthone, and its mode of action is related to an anticolinergic effect which increases the parasite´s motility[23, 24], as well as to synthesis inhibition of nucleic acids[3, 30]. In the *S. mansoni* strains resistant to oxamniquine, the synthesis inhibition of nucleic acids after treatment is reversible, while in susceptible strains the inhibition is irreversible. The genetic mechanism linked to the acquisition of resistance against *S. mansoni* is probably due to a lack of a bioactivation process, perhaps owing to a specific enzyme that promotes the schistosomicide effect of the drug. An additional information originated from experimental genetic data suggests that a single enzymatic step is required for drug activation against schistosome, i.e., there is probably one single autosomal recessive gene involved in this process[10]. In this way PICA-MATTOCCIA et al[31], showed that a single gene is responsible for resistance against hycanthone and oxamniquine. This fact explains the findings of cross resistance related to those drugs in strains that have been initially isolated as resistant to only one of those two mentioned drugs CIOLI et al.[9] showed evidence that the related gene could be responsible for production of specific sulphotransferase, that may be the drug activating enzyme.

The evidence of *S. mansoni* strains resistant to conventional drugs stimulates and justifies the search for new compounds, which could alternatively be used. Thus, some compounds belonging to the series of 9-acridanone-hydrazone drugs have been already tested in primates[11, 12, 29, 34, 35, 36], and they were found to be a promising chemical group. Some of these compounds have shown excellent antischistosomal activity, both against adult worms and early developing forms of the parasite. This is the group of chemical compounds with the greatest potential use in the near future [9], but further studies on mutagenicity and carcinogenicity are needed.

Finally, the present study reports the finding of a *S. mansoni* strain highly resistant to oxamniquine. This resistance is particularly high in relation to male worms. Further studies are in progress aiming at identifying genetic markers related to this resistance, as well as a possible connection between those labels and the sex of the parasites.

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RESUMO

Resistência ao oxamniquine de uma cepa de *Schistosoma mansoni* isolada de paciente submetido a repetidos tratamentos

Uma cepa de *Schistosoma mansoni* (R1) foi isolada de paciente previamente submetido a quatro tratamentos com oxamniquine e a um outro com praziquantel. Os resultados obtidos com o teste quimioterapêutico, usando oxamniquine em camundongos infectados com as cepas R1 e LE (padrão) mostraram resistência evidente à droga em vermes de cepa R1. Assim, com a dose de 250 mg/kg de oxamniquine, todos os camundongos (17) dos 17 camundongos infectados com a cepa R1 apresentaram vermes sobreviventes. Com a dosagem de 200 mg/kg a cepa LE mostrou taxas de recuperação de 1,06 e 20,58% enquanto a cepa R1 apresentou 18,57 e 61,14% para os vermes machos e fêmeas, respectivamente. Com a dose de 100 mg/kg a recuperação de vermes machos foi de 2,6% para a Cepa LE e 29,9% para a R1. Com a mesma dosagem, a recuperação de fêmeas não mostrou diferenças estatisticamente significantes entre as duas espécies (LE = 76,38%, R1 = 79,12%). Praziquantel mostrou atividade-esquistossomicida semelhante contra ambas cepas estudadas quando administrado na dosagem de 500 mg/kg.

REFERENCES

1. ARAÚJO, N.; KATZ, N.; DIAS, E.P. & SOUZA, C.P. - Susceptibility to chemotherapeutic agents of strains of *Schistosoma mansoni* isolated from treated and untreated patients. **Amer. J. trop. Med. Hyg., 29**: 890-894, 1980.

2. ARAÚJO, N.; SOUZA, C.P.; PASSOS, L.K.J.et al. - Susceptibility to chemotherapeutic agents of *Schistosoma mansoni* isolates derived from patients treated with oxamniquine and praziquantel and not cured. In: INTERNATIONAL SYMPOSIUM ON SCHISTOSOMIASIS, 5., Salvador, Bahia, Brazil, 1995.

3. ARCHER, S.; EL HAMOULY, W.; SEYED-MOZAFFANI, A.et al. - Mode of action of the schistosomicide hycanthone: site of DNA alkylation. **Molec. Biochem. Parasit., 43**: 89-96, 1990.

4. BICKLE, Q.D. & ANDREWS, B.J. - Resistance following drug attenuation (Ro-11.3128 or oxamniquine) of early *Schistosoma mansoni* infections in mice. **Parasitology, 90**: 325-338, 1985.

5. BRINDLEY, P.J.; HEATH, S.; WATERS, S.A.; McCUTCHAN, T.F. & SHER, A. - Characterization of a programmed alteration in an 185 ribosomal gene that accompanies the experimental induction of drug resistance in *Schistosoma mansoni*. **Proc. nat. Acad. Sci. (Wash.), 88**: 7754-7758, 1991.

6. BRINDLEY, P.J.; LEWIS, F.A.; McCUTCHAN, T.F. & SHER, A. - A genomic change associated with development of resistance to hycanthone in *Schistosoma mansoni*. **Molec. Biochem. Parasit., 36**: 243-252, 1989.

7. CAMPOS, R.; MOREIRA, A.A.B.; SETTE, H.; CHAMONE, D.A. & SILVA, L.C. - Hycanthone resistance in a human strain of *Schistosoma mansoni*. **Trans. roy. Soc. trop. Med. Hyg., 70**: 261-262, 1976.

8. CIOLI, D; PICA-MATTOCCIA, L. & ARCHER, S. - Drug resistance in schistosomes. **Parasit. today, 9**: 162-166, 1993.

9. CIOLI, D.; PICA-MATTOCCIA, L. & ARCHER, S. -Antischistosomal drugs. Past, present... and future? **Pharmacol. Therap., 68**: 35-85, 1995.

10. CIOLI, D.; PICA-MATTOCCIA, L. & MORONI, R. - Hycanthone/oxamniquine resistance is controlled by a single autosomal recessive gene. **Exp. Parasit., 75**: 425-432, 1992.

11. COELHO, P.M.Z. & PEREIRA, L.H. - *Schistosoma mansoni*: preclinical studies with 9-Acridanone-hydrazones in *Cebus* monkeys experimentally infected. **Rev. Inst. Med. trop. S. Paulo, 33**: 50-57, 1991.

12. COELHO, P.M.Z.; PEREIRA, L.H. & MELLO, R.T. - Antischistosomal activity of acridanone-hydrazones in *Cebus* monkeys experimentally infected with the SJ strain of *Schistosoma mansoni*. **Rev. Soc. bras. Med. trop., 28**: 179-183, 1995.

13. COLES, G.C.; MUTAKI, W.T.; KINOTI, G.K.; BRUCE, J.J. & KATZ, N. - Tolerance of Kenyan *Schistosoma mansoni* to oxamniquine. **Trans. roy. Soc. trop. Med. Hyg., 81**: 782-785, 1987.

14. CONOVER, W.J. - **Practical nonparametric statistics**. New York, Wiley, 1980.

15. DIAS, L.C.S.; BRUCE, J.I. & COLES, G.C. - Variations in response of *Schistosoma mansoni* strains to schistosomicides. **Rev. Inst. Med. trop. S. Paulo, 30**: 81-85, 1988.

16. DIAS, L.C.S.; PEDRO, R.J. & DEBERLADINI, E.R. - Use of praziquantel in patients with schistosomiasis previously treated with oxamniquine and/or hycanthone: resistance of *Schistosoma mansoni* schistosomicidal agents. **Trans. roy. Soc. trop. Med. Hyg., 76**: 652—659, 1982.

17. DIAS, L.C.S.; PEDRO, R.J.; RIGO, E.; GOTO, M.M.F. & MAFRAQ, G.L. - Linhagem humana de *Schistosoma mansoni* resistente a esquistossomicidas. **Rev. Saúde públ. (S. Paulo), 12**: 110, 1978.

18. DOONG, Y.C.; WONG, L.J.; BRUCE, J.I. & WONG, S.S. - Enzymatic differences between hycanthone-resistant and sensitive strains of *Schistosoma mansoni*. **Comp. Biochem. Physiol., 87B**: 459-464, 1987.

19. DRESCHER, K.C.; ROGERS, E.J.; BRUCE, J.I. et al. - Response of drug resistant isolates of *Schistosoma mansoni* to antischistosomal agents. **Mem. Inst. Oswaldo Cruz, 88**: 89-95, 1993.

20. FALLON, P.G. & DOENHOFF, M.J. - Drug resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. **Amer. J. trop. Med. Hyg., 51**: 83-88, 1994.

21. FALLON, P.G.; STURROCK, R.F.; CAPRON, A.; NIANG, M. & DOENHOFF, M.J. - Diminished susceptibility to praziquantel in a Senegal isolate of *Schistosoma mansoni*. **Amer. J. trop. Med. Hyg., 53**: 61-62, 1995.

22. GUIMARÃES, R.X; TCHAKERIAN, A.; DIAS, L.C.S. et al. - Resistência ao hycanthone e oxamniquine em doentes com esquistossomose com a forma clínica hepato-intestinal. **Rev. Ass. méd. bras., 25**: 48-50, 1979.

23. HILLMAN, G.R. & SENFT, A.W. - Anticholinergic properties of the antischistosomal drug hycanthone. **Amer. J. trop. Med. Hyg., 24**: 827-834, 1975.

24. HILLMAN, G.R.; SENFT, A.W. & GIBLER, W.B. - The mode of action of hycanthone revised. **J. Parasit., 64**: 754-756, 1978.

25. JANSMA, W.B.; ROGERS, S.H.; LIU, C.L. & BUEDING, E. - Experimentally produced resistance of *Schistosoma mansoni* to hycanthone. **Amer. J. trop. Med. Hyg., 26**: 926-936, 1977.

26. KATZ, N.; DIAS, E.P.; ARAÚJO, N. & SOUZA, C.P. - Estudo de uma cepa humana de *Schistosoma mansoni* resistente a agentes esquistossomicidas. **Rev. Soc. bras. Med. trop., 6**: 381-387, 1973.

27. PEDRO, R.J.; DIAS, L.C.S.; AMATO-NETO, V. & CARVALHO, S.A. - Observations on the treatment of mansoni schistosomiasis with oxamniquine: efficacy in children and in persistent salmonellosis; resistance of a strain of *Schistosoma mansoni*; hepatotoxicity and neurological side effects. **Rev. Inst. Med. trop. S. Paulo, 22** (Supl. 4): 32-36, 1980.

28. PELLEGRINO, J. & SIQUEIRA, A.F. - Técnica de perfusão para colheita de *Schistosoma mansoni* de cobaias experimentalmente infestadas. **Rev. bras. Malar., 3**: 589-597, 1956.

29. PEREIRA, L.H.; COELHO, P.M.Z.; COSTA, J.O. & MELLO, R.T. - Activity of 9-Acridanone-hydrazone drugs detected as the pre-postural phase, in the experimental schistosomiasis mansoni. **Mem. Inst. Oswaldo Cruz, 90**: 425-428, 1995.

30. PICA-MATTOCCIA, L.; DIAS, L.C.S. & ARCHER, S. - Binding of oxamniquine to the DNA of schistosomes. **Trans. roy. Soc. trop. Med. Hyg., 83**: 89-96, 1989.

31. PICA-MATTOCCIA, L.; DIAS, L.C.S.; MORONI, R. & CIOLI, D. - *Schistosoma mansoni* genetic complementation analysis shows that two independ hycanthone/oxamniquine-resistant strains are mutated in the same gene. **Exp. Parasit., 77**: 445-449, 1993.

32. ROGERS, S.H. & BUEDING, E. - Hycanthone resistance: development in *Schistosoma mansoni*. **Science, 172**: 1057-1058, 1971.

33. STELMA, F.F.; TALLA, I.; POLMAN, K.B. et al. - Low efficacy and tolerance of praziquantel in a new intense focus of *Schistosoma mansoni*. **Amer. J. trop. Med. Hyg., 53**: 167-170, 1995.

34. STURROCK, R.F.; BAIN, J.; WEBBE, G.; DOENHOFF, M.J. & STOHLER, H. - Parasitological evaluation of curative and subcurative doses of 9-acridanone-hydrazone drugs against *Schistosoma mansoni* in baboons, and observations on the changes in serum levels of anti-egg antibodies detected by ELISA. **Trans. roy. Soc. trop. Med. Hyg., 81**: 188-192, 1987.

35. STURROCK, R.F.; OTIENO, M.; JAMES, E.R. & WEBBE, G. - A note on the efficacy of a new class of compounds, 9-acridanone-hydrazones, against *Schistosoma mansoni* in a primate - the baboon. **Trans. roy. Soc. trop. Med. Hyg., 79**: 129-131, 1985.

36. SULAIMAN, S.M.; ALI, H.M.; HOMEIDA, M.M.A. & BENNETT, J.L. - Efficacy of a new Hoffmann-La Roche compound (Ro-155458) against *Schistosoma mansoni* (Gezira strain, Sudan) in vervet monkeys (*Cercopithecus aethiops*). **Trop. Med. Parasit., 40**: 335-336, 1989.

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