Study of *Schistosoma mansoni* Isolates from Patients with Failure of Treatment with Oxamniquine

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After three successive treatments with oxamniquine the continuing elimination of Schistosoma mansoni *eggs was observed in patients, who came from various regions of Brazil, with different clinical forms of schistosomiasis. The objective of the present study was to determine the experimental behaviour of five different* S. mansoni *isolates in Swiss Webster mice that were submitted to treatment with the same drug. The experimental group with failure of treatment showed higher mean number of surviving male worms when it was compared to the group without failure of treatment. These date suggest the possibility of resistance to oxamniquine.*

Key words: *Schistosoma mansoni* - patients - oxamniquine - failure of treatment

Foster et al. (1971 a,b) and Pellegrino and Katz (1972), reported promising schistosomicidal activity for oxamniquine. Pica-Mattoccia and Cioli (1985), emphasized that a single oral dose is usually effective, immediate toxicity is low and general side effects are weak or absent. Its biochemical mechanisms is related to an anticholinergic effect which increases the parasite's motility (Hillman & Senft 1975), as well as to synthesis inhibition of nucleic acids (Pica-Mattoccia et al. 1989). Bina (1977, 1995), in endemic areas of Bahia, stressed the effects of specific treatment, mainly with oxamniquine, plus environmental modification involving the prevention and reversibility of the hepatosplenic form of schistosomiasis. Andrade (1998) presented important new data on this subject. In Brazil, Katz (1980) revealed a cure index with the drug of 80 to 98% for adults and 60 to 90% for children. Lambertucci et al. (1990) obtained a lower cure index of children treated in the acute phase (45%). Foster and Cheetam (1973) reported the possibility of a variation in the response to the drug according to the geographic origin of the parasite. Clarke et al.

(1976) detected a lower cure index in some African countries in which oxamniquine has been used at much higher doses. Coles et al. (1987) described tolerance by Kenyan isolates of *Schistosoma mansoni* treated with this drug.

Pioneering clinical studies in Brazil (Katz et al. 1973) have approached the topic of resistance of *S. mansoni* isolates to oxamniquine. Jansma et al. (1977) induced resistance experimentally. Araujo et al. (1980) compared several *S. mansoni* isolates from patients in the State of Minas Gerais and detected variations in the response to the drug. Dias et al. (1982) refered to the use of praziquantel in patients with oxamniquine and hycanthone resistant *S. mansoni*. According to Cioli et al. (1989) genetic crosses between phenotypically resistant and sensitive schistosomes demonstrated that resistance to hycanthone and oxamniquine behaves like a resistant trait, thus suggesting that resistance is due to the lack of a bioactivation process, maybe owing to a specific enzyma that promotes the schistosomicide effect of the drug (Coelho et al. 1997). Doenhoff and Bain (1978) showed the involvement of immune mechanisms in drug induced killing of adult worms (antimonial). Doenhoff et al. (1987) concluded that the duration of infection and infection intensities affect the outcome of chemotherapy. Also, Doenhoff (1987) indicated that immune effector mechanisms can enhance the activity of schistosomicidal drugs. Praziquantel, oxamniquine, hycanthone and antimony were shown to be less effective against *S. mansoni* infection in mice immunosuppressed by T cell deprivation than against comparable infec-

tion in normal mice. The results obtained for Drescher et al. (1993) confirmed the resistance of *S. mansoni* isolates to niridazole, oxamniquine and hycanthone, although all isolates were susceptible to praziquantel. Coelho et al. (1997) isolated from patient treated four times with oxamniquine a resistant strain of *S . mansoni* (R1). Coelho et al. (1998) showed that resistance recorded could not be detected in schistosomules at the skin and pulmonary phases, but in females, at the sexual maturation phase.

Variation in response to the drug was also observed at the outpatient clinic of the Serviço de Doenças Infecciosas e Parasitárias (Conceição et al. 1995, 1997) and motivated the present experimental study examining isolates of the parasite that did not respond to oxamniquine.

PATIENTS AND METHODS

In relation to the clinical form recorded for 954 *S. mansoni* infected patients, 572 (59.9%) presented the schistosomiasis infection form (Type I), 298 (31.2%) the hepatointestinal form (Type II), and 68 (7.1%) the hepatosplenic form (Type III); one case progressed to schistosomotic pulmonary hypertension and another developed infantilism (deficient somatic and sexual development). Sixteen others cases (1.6%) presented the toxemic form of the disease.

Fecal examination was performed by the method of Kato (1960) modified by Katz et al. (1972). The clinical forms of schistosomiasis were determined according to the classification of Pessoa and Barros (1953), modified by Barbosa (1966). Based on a random selection the patients were treated with a single oral dose of oxamniquine (15 mg/kg), or praziquantel (40 mg/kg).

Five *S. mansoni* strains were isolated from patients who continued to eliminate parasite eggs after three consecutive treatments with oxamniquine and did not present any evidence of reinfection. All patients reported chronical schistosomiasis and there was no clinical data or serological tests that had shown immunologically impairment. It was not possible to obtain isolates in cases in which there was a diagnosis of viable eggs by the rectal biopsy. The patients selected (Table I) delivered containers with their feces to the Hospital Universitário Clementino Fraga Filho, and the material was immediately transported to the Laboratory of the Department of Tropical Medicine, Oswaldo Cruz Institute, Rio de Janeiro, Brazil. The isolates were maintained in sucessive generations in mice and snails until the begining of the experimental treatment.

Each *S. mansoni* isolate was obtained by sucessive sedimentation of fecal material and there-

after placed in a Chaia bottle for miracidium hatching (Chaia 1956). Forty *Biomphalaria glabrata* snails were individually infected with five miracidia from each of the five strains. The mollusks were from the rural area of Capitão Andrade, Minas Gerais, and had been reared under laboratory conditions since 1979. After 30 days, the mollusks were examined to determine the rates of infection. A pool of mollusks presenting cercaria emergence was formed. Fifty cercariae were quantified for infection by the percutaneous (caudal) route of each of 60 male albino mice aged 21 days and weighing 11 to 16 grams (Conceição & Coura 1986). Thirty of the 60 mice infected with *S. mansoni* from each of the five patients who presented "failure of treatment" to oxamniquine (denoted isolates Hospital Universitário 1, 2, 3, 4 and 5) were treated with oxamniquine at a dose of 100 mg/kg body weight on the 50th day post-infection using a tube adapted in the laboratory. On the 14th day post-treatment the animals were submitted to perfusion of the portal system and the worms were counted in the liver and mesentery using the technique of Radke et al. (1961), in order to determine the number of live worms recovered. The remaining 30 animals (out of a total of 60) were not treated with oxamniquine and were used as controls (isolates HU 1c, 2c, 3c, 4c and 5c). The therapeutic value of the drug was evaluated using the equation of Kemp et al. (1956): $\frac{a - b}{a} \times 100$ = efficacy where a: mean number of live worms recovered from *untreated* animals, and b: mean number of live worms recovered from treated animals.

TABLE I

Distribution of patients with and without failure of treatment to oxamniquine according age, sex, areas of origin and clinical form

Patients identification	Age (years)	Sex (F/M)	Areas of origin	Clinical form (Type)
With failure				
R. S.	35	F	Minas Gerais	II
E.M.S.	26	F	Minas Gerais	II
M.J.B.	37	F	Minas Gerais	I
J.V.	14	M	Bahia	I
V.M.	22	M	Paraíba	I
Without failure				
M.R.S.	28	F	Bahia	II
T.R.	24	F	Bahia	I
L.C.D.	13	M	Minas Gerais	III
D.E.	31	M	Minas Gerais	II
O.N.	34	M	Paraíba	I

Type I: schistosomiasis infection; Type II: hepatointestinal; Type III: hepatosplenic; F: female; M: male

We also selected five *S. mansoni* isolates from patients who *responded* to oxamniquine. The eggs for hatching of miracidia were obtained before treatment. Sixty mice were infected with each isolate. Thirty animals (denoted isolates HU 6, 7, 8, 9 and 10) were submitted to treatment with oxamniquine (100 mg/kg body weight by the oral route) on the 50th day after infection. On the 14th day post-infection, the animals were submitted to perfusion of the portal system and the worms were counted in the liver and mesentery by the technique of Radke et al. (1961). The 30 remaining mice out of a total of 60 were not treated and formed the control group (Hospital Universitário 6c, 7c, 8c, 9c, and 10c).

Statistical analysis were performed: (1) by the Analysis of Variance of Kruskal-Wallis to compare the mean number of recovered worms among the four groups: (a) experimental group-treated mice-isolates with failure of treatment; (b) experimental group without failure of treatment; (c) control group with failure of treatment; (d) control without failure of treatment. The test of Multiple Comparisons based on the statistic of Kruskal-Wallis aiming determine which inter group differences; (2) Wilcoxon Signed Rank test to compare the mean number of male and female worms separately for each group.

The p-value was significant at the 0,05 level. Statistical analysis were performed using Statistical Applications Software (SAS, Cary, NC, USA).

RESULTS

Among the patients treated with oxamniquine, 10.3% continued to eliminate *S. mansoni* eggs even after being submitted to three consecutive treatments with the drug, as determined by the method of Kato modified by Katz et al. (1972) and/or by rectal biopsy.

In this study comparing the possibility of cure of schistosomiasis infection with oxamniquine and praziquantel, no case of a *S. mansoni* isolate with resistance to praziquantel was observed. All patients responded to this medication, however, Ismail et al. (1995) revealed praziquantel resistance in Egypt. The side effects of both drugs occurred at low percentages and were similar.

The five *S. mansoni* isolates obtained from patients with *lack of response* to oxamniquine behaved in mice as indicated in Table II.

The isolates, denoted HU 1, 2, 3, 4 and 5, were maintained in 30 mice which were treated with oxamniquine. Acidental death of some mice occurred at the time of introduction of the esophageal tube used for treatment. These isolates were compared to those maintained in untreated animals (denoted isolates Hospital Universitário 1c, 2c, 3c, 4c and 5c). Mean (+ SD) recovery of live worms from treated animals ranged from 4 + 0.4 to 6 + 1.9. In the control group of untreated animals, the recovery of live worms ranged from 24 + 8.3 to 30 + 9.4. The efficacy of treatment with oxamniquine ranged from 72.4% to 83.3% (Table II).

Table III summarizes the results obtained with the five *S. mansoni* isolates from patients who *responded* to treatment with oxamniquine in mice submitted to treatment with the same drug (Experimental Group, Isolates Hospital Universitário 6, 7, 8, 9 and 10). These same isolates were maintained in mice not treated with the drug (Control Group, denoted Isolates HU 6c, 7c, 8c, 9c and 10c). The mean (+ SD) number of live worms recovered from the Study Group ranged from 2 + 02 to 3 + 0.6 and that recovered from the Control Group ranged from 29 + 6.9 to 30 + 8.3. The efficacy of treatment with oxamniquine in this group, in which the isolates originated from patients who respond to the drug, ranged from 88.3% to 92.8%.

In the Table IV is shown the mean number of male, female and total worms from patients with failure and without failure of treatment with oxamniquine, and the equivalent data from the

TABLE II

Efficacy of oxamniquine in isolates of *Schistosoma mansoni* from patients with failure of treatment to the drug, after perfusion in treated and untreated Swiss Webster mice

Experimental group (treated mice)				Control (untreated mice)				
Isolates HU	No. of mice	No. of worms live recoverd	SD	Isolates HU	No. of mice	No. of worms live recovered	SD	Efficacy
1	30	5	± 1.1	1c	30	30	± 9.2	83.3
2	28	6	± 1.9	2c	29	30	± 9.4	80.0
3	29	8	± 0.8	3c	30	29	± 8.9	72.4
4	28	6	± 1.4	4c	28	26	± 8.7	76.9
5	29	4	± 0.4	5c	30	24	± 8.3	83.3

SD: standard deviation; HU: Hospital Universitário Clementino Fraga Filho

TABLE III

Efficacy of oxamniquine in isolates of *Schistosoma mansoni*, from patients without failure of treatment to the drug, after perfusion in treated and untreated Swiss Webster mice

Experimental group (treated mice)				Control (untreated mice)				
Isolates HU	No. of mice	No. of worms live recoverd	SD	Isolates HU	No. of mice	No. of worms live recoverd	SD	Efficacy
6	26	2	± 0.3	6c	29	27,9	± 8.3	92.8
7	24	2	± 0.2	7c	30	27	± 8.1	92.6
8	28	3	± 0.5	8c	29	26	± 7.9	88.5
9	28	2	± 0.3	9c	29	25	± 6.9	92.2
10	29	3	± 0,4	10c	30	24	± 8.3	88.3

SD: standard deviation; HU: Hospital Universitário Clementino Fraga Filho

TABLE IV

Comparative study of the mean number of male and female *Schistosoma mansoni*, from patients with failure and without failure of treatment with oxamniquine, after perfusion in treated and untreated Swiss Webster mice

Sex of worm	Experimental group (treated mice)		Control group (untreated mice)	
	With failure (n = 144) Mean SD	Without failure (n = 136) Mean SD	With failure (n= 146) Mean SD	Without failure (n= 145) Mean SD
Male	3.09 ± 1.30	1.35 ± 0.90	14.28 ± 3.33	13.94 ± 3.58
Female	2.72 ± 1.30	1.28 ± 0.92	13.94 ± 3.24	13.96 ± 3.91
Total	5.81 ± 1.83	2.63 ± 1.40	28.22 ± 5.652	7.91 ± 6.85

SD: standard deviation

control groups with and without failure. There was a significant difference in the number of male worms detected in the four groups (p = 0.0001). The experimental groups were significantly different and showed differences in relation to the control groups. The control groups did not reveal any significant difference. The same was observed between females and total worms.

A comparison of the mean number of males and female in the groups with failure of treatment, revealed a significative difference (p = 0.001), with a higher mean number of male worms recovered than female worms. However, there was no difference: (a) in the experimental group without failure (p = 0.58); (b) in the control group with failure (p = 0.21); or (c) in the control group without failure (p = 0.77).

DISCUSSION

When the experimental results concerning the efficacy of treatment with oxamniquine against *S. mansoni* isolates obtained from patients who did not respond to the drug were compared, a variation from 72.4% to 83.3% was observed, indicating that the isolates were susceptible to the drug (mean efficacy of 79.7%). The present results obtained for animals inoculated with *S. mansoni* isolates from patients with failure to oxamniquine did not confirm the probable resistance observed by Katz et al. (1973) using a human strain of the parasite resistant to oxamniquine and to other schistosomicidal agents. Araújo et al. (1980), Dias et al. (1978, 1982), Meang et al. (1987), Bruce et al. (1987), emphasized similar results with Brazilian isolates from Minas Gerais. Kinoti (1987) reported variations in the suscetibility of *S. mansoni* to oxamniquine within certain areas of Brazil and Kenya. In our study we recovered five *S. mansoni* isolates from patients from Minas Gerais, Bahia and Paraíba who did not respond to the drug. It was not possible to obtain isolates from the same area, because in these cases the viable eggs were diagnosed by rectal biopsy. Kaye, quoted by Foster (1987) did not suggest differences in the pharmacokinetics of the drug to explain the variation in susceptibility, in a study with patients from Tanzania, South Africa and Brazil since he found similar concentrations of the drug in all blood samples.

The five *S. mansoni* isolates selected from patients who did not respond to the drug showed an efficacy of experimental treatment of approximately 80% after a single dose of 100 mg/kg body

weight. The mean number of male worms recovered from the experimental group with failure of treatment with oxamniquine was higher than in the experimental group without failure of treatment. The analysis into the groups with failure of treatment revealed higher number of males surviving in the isolate HU 3, which denotes a possible resistance to the drug in that isolate corroborating the previous results of Coelho et al. (1997). The study is being continued in order to determine the genomic denomination of the DNA of these *S. mansoni* isolates and to compare it with that of parasite isolates recovered from patients who responded to treatment with oxamniquine. We intend to characterize the parasite strains at the molecular biochemical level to clarify the factor(s) responsible for the absence of cure in patients infected with *Schistosoma* even after repeated treatment with oxamniquine.

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