Rev. Inst. Med. trop. São Paulo
35(6):557-561, novembro-dezembro, 1993

SCHISTOSOMA MANSONI: EVALUATION OF THE ACTIVITY OF OXAMNIQUINE ON SCHISTOSOMULES, AT 24 HOURS AFTER INFECTION

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SUMMARY

Mice transcutaneously infected with about 400 cercariae were submitted to treatment with oxamniquine (400 mg/kg), 24 hours after infection. The recovery of schistosomules, at 4, 24, 48 and 72 hours and 35 days after treatment, showed the activity of the drug on the parasites, thus practically preventing their migration from the skin to the lungs. Worm recovery performed in the lungs (96 hours after treatment) showed recovery means of 0.6 worms/mouse in the treated group and 53.8 in the control group (untreated). The perfusion of the portal system carried out at 35 days after treatment clearly showed the elimination of all the parasites in the treated group, whereas a recovery mean of 144.7 worms/mouse was detected in the control group (untreated).

These findings confirm the efficacy of oxamniquine at the skin phase of infection, and also show similarity with the immunization method that uses irradiated cercariae. The practical application of these findings in the medical clinic is discussed too.

KEYWORDS: *Schistosoma mansoni*; Oxamniquine; Schistosomules; Migration; Chemotherapeutic treatment.

INTRODUCTION

Oxamniquine (6-hydroximethyl-2-isopropylamino-methyl-7-nitro-1,2,3,4-tetrahydroquinoline) is the most utilized drug for chemotherapeutic treatment of schistosomiasis mansoni.

In Brazil, thousands of people living in endemic areas were treated through governmental programmes aiming at diminishing the prevalence and morbidity of the disease (KATZ, 1980).

The pioneer works on the schistosomicidal activity of the drug were carried out by FOSTER & CHEETHAM, 1973; FOSTER et al., 1973 and FOSTER, 1973 in experiments with laboratory animals. KATZ et al. (1973) were the first ones to show the effectiveness of the drug in patients with *schistosomiasis mansoni*.

FOSTER's work (1973) shows a marked schistosomicidal activity on 1-day infections (reduction of 97% of the worm burden), when mice were treated with a dosage of 50 mg/kg, intramuscular route.

Other works confirmed the activity of the drug at the early phases of infection (PEREIRA et al., 1975; PELLEGRINO et al., 1977). On the other hand, COELHO et al. (1991) show the presence of protective immunity against reinfection with cercariae, in mice previously infected with cercariae and treated with 400 mg/kg oxamniquine at the early moments of infection. The authors raised the hypothesis that the drug might kill immature parasites at the skin level, similarly to the immunization system that uses 400-500 irradiated cercariae (40-60 Krad), which almost all would die at the

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skin (BICKLE et al., 1979; MANGOLD & DEAN, 1984; CARDOSO & COELHO, 1989a, 1989b). This fact, that induces to a significant resistance against reinfection with normal cercariae, was first demonstrated by VILLELLA et al., 1961; PERLOWAGORA-SZUMLEWICZ & OLIVIER, 1963; RADKE & SADUN, 1963, and later confirmed by several other authors, as described in a comprehensive review by DEAN (1983).

The present study aims at determining the capacity of migration (skin-lungs-portal system) of parasites in mice, after treatment with 400 mg/kg oxamniquine, one day after transcutaneous infection with 400 cercariae.

MATERIAL AND METHODS

Infection of mice - Albino outbred Swiss mice were infected with 400 *Schistosoma mansoni* cercariae (LE strain). This strain was isolated from a patient in Belo Horizonte, Brazil, and kept at the laboratories of the Schistosomiasis Research Unit, Federal University of Minas Gerais, for more than 30 years. The infection was performed by sprinkling 0.5 ml of a suspension, containing about 400 cercariae/0.5ml, on the abdominal skin. In general terms, the technique followed the method described by BARBOSA et al. (1978).

Following a 24-hour period, a group of infected mice was treated with 400 mg/kg oxamniquine (salt in acqueous suspension), single dose, by oral route. At 4-24-48-72-hour intervals after treatment, the treated mice and their respective controls (untreated) were killed by cervical fracture. The abdominal skin, used as site of infection, was removed and submitted to the technique described by BARBOSA et al. (1978) for schistosomule recovery, as follows: the skin area where the cercariae penetrated was removed and chopped into small fragments, by means of sharp pointed scissors, and placed in a small Petri dish containing 0.2 ml of Hanks' balanced salt solution (HBSS) with pH from 7.2 to 7.6. The moistened fragments were then transferred to a 2 cm diameter cylindrical vessel closed at its lower end by a stainless steel screen of mesh 0.09 mm and supported by three long plastic legs, 1 cm long. This vessel was put into a 50 ml beaker and heparinized HBSS with pH 7.2 to 7.6 at 37° C was poured in up to the screen level so that it moistened the fragments of screen tissue. The young parasites migrated from the tissue to the warm HBSS and could be collected after a four-hour incubation. Ninety six hours after treatment (5 days after infection), the treated mice and their respective controls (untreated) were killed by cervical fracture, the lungs being removed for worm recovery according to the method already alluded to (BARBOSA et al., 1978).

Thirty-five days after treatment, the treated animals and their respective controls were sacrificed, and the portal-mesenteric system was perfused for worm recovery, in general terms according with the technique described by PELLEGRINO & SIQUEIRA (1956).

The statistical analysis of the data was undertaken by using the STUDENT's t test, a minimum significance level of $p < 0.05$ being observed (SNEDECOR & COCHRAN, 1971).

RESULTS

Table 1 shows that, 4 and 24 hours after treatment, schistosomules connected with the treated group could be recovered from the skin in significantly small numbers. Conversely, after 48 and 72 hours after treatment,

TABLE 1

Recovery of *Schistosoma mansoni* worms in mice treated with 400 mg/kg oxamniquine (single dose, oral route) 24 hours after transcutaneous infection with 400 cercariae, and sacrificed at 4, 24, 48, 72, 96 hours and 35 days after treatment.

Time after treatment	Site of recovery	Number of animals Treated T Controls C	Mean ± Standard deviation	Analysis of variance
4h	skin	T (8)	19.9 ± 8.6	$p < 0.02$
		C (6)	38.3 ± 17.6	
24h	skin	T (8)	29.6 ± 9.1	$p < 0.003$
		C (6)	63.0 ± 23.6	
48h	skin	T (8)	13.3 ± 10.8	ns
		C (8)	16.4 ± 6.1	
72h	skin	T (8)	2.6 ± 2.2	ns
		C (8)	0.6 ± 1.8	
96h	lung	T (8)	0.6 ± 1.1	$p < 0.0001$
		C (8)	53.8 ± 18.9	
35 days	portal system	T (20)	0.0 ± 0.0	$p < 0.0001$
		C (22)	144.7 ± 26.6	

ns = Not significant

no statistically significant differences between the recovery means for schistosomules in the skin (treated and control groups) could be observed.

Ninety-six hours after treatment, practically no immature worms in the lungs of treated mice could be recovered (m=0.6), when compared with the high recovery mean detected in the control (untreated) animals (m=53.8) (Table 1).

Thirty-five days after treatment, the total elimination of parasites in the treated group became evident, in contrast with the high recovery mean (m=144.7) recorded for the control (untreated) group (Table 1).

DISCUSSION

The results show that the activity of oxamniquine on the parasites at the skin, 24 hours after infection, is already evident 4 hours after treatment, since the method used for schistosomule recovery, as prescribed by BARBOSA et al. (1978), is a process of the parasites active migration from skin fragments to Hanks' solution heated at 37° C. Moreover, this method is based on the parasites positive thermotropism. Thus, the small number of parasites recovered by using the above mentioned method, within a period of 4 and 24 hours after treatment (28 and 48 hours after infection), shows that most worms in the treated group are not able to migrate from the skin fragments to the heated Hanks' solution. In the period of 48 and 72 hours after treatment (72 and 96 hours after infection), when a considerable number of schistosomules has migrated from the skin to the lungs, the method earlier mentioned is not capable of detecting statistically significant differences between the schistosomule recovery means of treated and untreated (control) groups. Thus, we can infer that schistosomules from the treated group have less capacity to migrate to the lungs, dying or remaining damaged at the skin. The result obtained in relation to the recovery of immature worms in the lungs (96 hours after treatment) does support our view, since a significantly lower number of immature worms in the treated group can be seen reaching the lungs (worm mean = 0.6 against 53.8 in the untreated group). It is worth noting that the 5th day after infection was found to be the peak period for worm recovery in the lungs, in experimental infections in mice (BARBOSA et al., 1978).

Perfusion carried out at 35 days after treatment (36 days post-infection) clearly shows the effectiveness of the schistosomicidal activity of oxamniquine, at the dosage of 400 mg/kg, this dose being able to kill all the parasites that infected the mouse before they reach the portal system, 24 hours before treatment.

COELHO et al. (1991) showed that oxamniquine, at the dose of 400 mg/kg, when administered 24 hours after transcutaneous infection with about 350 cercariae, was able to induce a significant resistance in the treated mouse against reinfection with cercariae. The authors raised the hypothesis that the drug (at the dose of 400 mg/kg) could provoke the death of the parasites at the skin level. The skin is a very important site connected with immunoprotective mechanisms in the schistosomiasis mansoni, being also the first barrier that the parasite must overcome. Some authors believe that the skin would constitute the most important obstacle for the continuation of the evolutive cycle in the host with acquired immunity. The mechanisms involved in the process of the parasites' elimination at the skin could be mainly connected with the role of IgE antibodies, which are linked to receptors that are found on the surface of mastocytes at the site of penetration by their Fc portion, releasing a series of phenomena that would be of importance in the mechanism of protection at skin level, through the liberation of histamine and serotonine (GERKEN et al., 1980, 1984; SMITHERS & GAMMAGE, 1980). The parasites' death, caused by the action of oxamniquine, at skin level - a site of intense immunological response, as mentioned earlier - could condition an immunoprotective response against reinfection with normal cercariae, in a similar manner to that induced by the immunization technique with irradiated 400-500 cercariae (40-50 Krad). This method also causes the death of almost all the parasites in the skin.

A possible clinical application of the present results could be the treatment of a patient immediately after his contact with natural waters containing cercariae, although it is not usually possible to obtain a secure diagnosis of infection at this phase. The total dosage of 50 mg/kg divided into two doses of 12.5 mg/daily, for two days, should eliminate almost all the parasites on the first days after penetration of cercariae. It is important to note that the dose of 60 mg/kg, at a schedule of two doses of 15 mg/kg daily, for two days, has been used for treatment of schistosomiasis mansoni in Egypt, and this therapeutic schedule has been well tolerated by the population, in a general manner (OMS, 1980).

The treatment for schistosomiasis when carried out

at the early phase of infection would bring to the patient the advantage of preventing the pathology of the disease resultant of the egg-laying, since it is well established that the granulomas formed in the tissues involving the eggs constitute the fundamental elements of the pathogeny of the disease (WARREN, 1972). The conventional clinical disease conduct, related to chemotherapeutic treatment for schistosomiasis mansoni, preconizes the drug administration after detection of eggs in the feces and, as a result, granulomas can be seen at this phase of the disease in intestinal and hepatic tissues. It is worthwhile remembering that the efficacy of oxamniquine at the post-postural acute phase is not so marked than that observed at the chronic phase. This leads us to suppose that treatment, when carried out at the early phases of infection (first 5 days), could display a schistosomicidal efficacy even more intense than that observed at the post-postural acute phase.

Finally, parasites'death caused by chemotherapy, at the skin and lungs, could induce a partial protective immunity against reinfection by cercariae in man, as it has already been observed in experimental models (BICKLE & ANDREWS, 1985; MASTIN et al., 1985; COELHO et al. 1991).

RESUMO

Schistosoma mansoni: Avaliação da atividade da oxamniquina (Mansil*) em esquistossômulos com 24 horas após infecção.

Camundongos infectados transcutaneamente com cerca de 400 cercárias foram submetidos a tratamento com oxamniquina (400 mg/kg), 24 horas após a infecção. A recuperação dos esquistossômulos a nível da pele mostrou a atividade da droga nos parasitos e impediu praticamente sua migração para os pulmões. A recuperação a nível pulmonar (96 horas após tratamento) mostrou uma média de 0,6 vermes por camundongo no grupo tratado e 53,8 no grupo controle, não tratado. A perfusão do sistema porta, realizada aos 35 dias após infecção, mostrou claramente a eliminação de todos os parasitos no grupo tratado, enquanto foi recuperada uma média de 144,7 vermes no grupo controle, não tratado.

Estes achados vem comprovar a eficácia da oxamniquina na fase cutânea da infecção e mostra analogia com o sistema de imunização que usa cercárias irradiadas. Também se discute a aplicação destes resultados na clínica.

ACKNOWLEDGEMENTS

This work was supported by FAPEMIG, CNPq and FINEP, Brazil, and by WHO, Switzerland.

The authors are indebted to Mrs. Vera de Paula Ribeiro for translating the manuscript.

They also wish to thank Mr. Alberto G. dos Santos, Miss Zenir de Souza and Mrs. Alice Neni F. Balzuweit for technical assistance.

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Recebido para publicação em 26/04/1993
Aceito para publicação em 26/07/1993.