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PRELIMINARY LABORATORY TRIALS WITH OXAMNIQUINE AS A PROPHYLACTIC AGENT IN SCHISTOSOMIASIS

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SUMMARY

It was shown that oxamniquine (6-hydroxymethyl-2-isopropylamino-methyl-7-nitro-1,2,3,4-tetrahydroquinoline) is very active on early developing forms of *Schistosoma mansoni* in the peritoneal cavity of mice. Actually, a single intramuscular injection of oxamniquine, at the levels of 400, 200, and 100 mg/kg, killed all schistosomula within a few days. In order to evaluate the prophylactic activity of oxamniquine, prior to the exposure of mice to *S. mansoni* cercariae, the drug was administered as single i.m. doses of 400 and 200 mg/kg, 6, 4, 2, and 1 day before infection. A slight activity could be detected only in animals treated 24 hours before exposure (400 mg/kg). The antischistosomal activity of oxamniquine was more pronounced on early developing forms (up to 7 days) than on maturing schistosomes.

INTRODUCTION

It has been shown that oxamniquine (U.K. 4271) as well as its parent compound U.K. 3883 (both drugs belonging to the tetrahydroquinoline series) displays a prophylactic activity in rodents and primates against the infection with *Schistosoma mansoni* (CHEETHAM & MESMER [1]; FOSTER et al. [3]; PELLEGRINO & KATZ [6]). It was also demonstrated that U.K. 3883 and oxamniquine, as well as other members of the tetrahydro- and pyrazino-quinolines series, are able to kill early developing forms of *Schistosoma mansoni* in the peritoneal cavity of mice (PELLEGRINO et al. [7]; PEREIRA et al. [9]).

In the present paper the minimum effective dose of oxamniquine necessary to kill schistosomula of *S. mansoni* in the peritoneal cavity of mice was determined and the antischistosomal activity on maturing worms was evaluated.

MATERIALS AND METHODS

Infection of animals — Cercariae of the L.E. strain of *S. mansoni* (Belo Horizonte, Brazil), shed by laboratory-reared and infected *Biomphalaria glabrata*, were used in all experiments. Albino mice, weighing about 20 g, were infected with 100 ± 10 cercariae by the tail immersion method (PELLEGRINO & KATZ [5]) for the evaluation of antischistosomal activity.

Intraperitoneal inoculations were made from a concentrated pool of cercariae so as to inject about 400 larvae (in lml) per animal (PEREIRA et al. [10]).

Treatment of mice — Groups of 12 mice were treated with oxamniquine, i.m., at the dose levels of 400 and 200 mg/kg (single dose), 6, 4, 2, and 1 day and 3 hours before exposure to cercariae and 200 mg/kg, 2, 7, 14, 23, and 30 days after exposure. This was

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designed to evaluate the prophylactic as well as the curative activity of oxamniquine.

In order to determine the minimum effective dose of oxamniquine on the early developing forms of *S. mansoni* in the peritoneal cavity, groups of 10 mice, injected with 400 cercariae (i.p.), were treated, 3 hours later, with 400, 200, 100, and 50 mg/kg, i.m. (single dose).

Evaluation of antischistosomal activity — Seven weeks after exposure to cercariae the animals were killed by cervical fracture. Schistosomes were collected by perfusion of the hepatic-portal system (PELLEGRINO & SIQUEIRA [8]) and the oogram from intestinal fragments performed as described elsewhere (PELLEGRINO & FARIA [4]). The activity was evaluated by the degree of reduction in worm burden the hepato-shift of schistosomes and the percentage of animals with oogram changes.

Activity on the early developing forms of S. mansoni — Mice were killed by cervical fracture and the abdominal skin pulled out. Five ml of saline were injected into the peritoneal cavity and gentle massage was applied to the abdominal viscera. The peritoneum was opened, the liquid collected in a Petri dish and the larvae counted as described elsewhere (PEREIRA et al. [10]).

The activity on the early developing forms of *S. mansoni* was evaluated by the reduction in the number of larvae, 8 days after treatment.

RESULTS AND COMMENTS

Protective activity of oxamniquine prior to the exposure to cercariae — Alterations of the oogram were observed when mice were dosed 3 hours (200 mg/kg) and 1 and 2 days before exposure (400 mg/kg). It is interesting to note that no worms developed in animals treated 3 hours before exposure (200 mg/kg). No significant alterations in the mean worm burden as well as in the distribution of schistosomes were observed (Table I). It can be concluded from this experiment that the protective activity of oxamniquine is very limited as far as the interval between treatment and exposure to cercariae

TABLE I

Protective activity of oxamniquine (i.m.) prior to the exposure of mice to 100 ± 10 cercariae of *Schistosoma mansoni*. Worm burden, distribution of schistosomes and percentage of oogram changes

Treatment days before exposure	Dose (mg/kg/day) single dose	Number of animals	Animals dead	Distribution of schistosomes (%)			Mean worm burden	Percent oogram changes
				Liver	Portal vein	Mesenteric vessels		
6	400	12	1	23.1	17.9	59.0	10.6	0.0
6	200	12	2	17.9	25.1	57.0	17.9	0.0
4	400	12	1	23.1	19.5	57.4	14.9	0.0
4	200	12	2	21.2	17.9	60.9	20.7	0.0
2	400	12	2	28.1	7.4	64.5	12.1	20.0
2	200	12	1	27.3	16.4	56.3	21.0	0.0
1	400	12	1	27.8	11.1	61.1	8.2	54.5
1	200	12	2	28.3	3.3	68.4	12.0	0.0
0 (*)	200	12	2	0.0	0.0	0.0	0.0	100.0 (**)
Control	—	12	0	17.9	18.8	63.3	15.0	0.0

(*) Treatment 3 hours before exposure
(**) No eggs

is concerned. It will be shown that oxamniquine displays an outstanding effect on maturing schistosomes. In this respect, the development of a slow-release method aiming to increase the period of schistosomicidal activity would be helpful.

Antischistosomal activity of oxamniquine on maturing schistosomes — Table II shows that no worms developed in mice treated 2 and 7 days after exposure. The treatment consisted of a single dose (i.m.) of oxamniquine. A low mean worm burden was observed in groups treated 14, 23 and 30 days after exposure: 3.1, 2.5, and 0.6 worms, respectively. It is interesting to note that oxamniquine, in the experiment shown in Table II, was more effective on early developing schistosomes (up to 7 days).

Antischistosomal activity of oxamniquine on early developmental forms of S. mansoni *in the peritoneal cavity of mice* — Table III shows that no larvae could be recovered by peritoneal washing of mice 8 days after a single i.m. injection of oxamniquine at the levels of 400, 200 and 100 mg/kg. The animals dosed with 50 mg/kg presented a mean of 31.1 larvae, in contrast with the control group where about 30 percent of injected cercariae could be recovered as schistosome larvae (mean = 148.3).

In order to check a possible activity of oxamniquine on the process of transformation of cercariae into schistosomula, a group of 10 mice was injected, intraperitoneally, with 100 organisms per animal, 3 hours after the administration of the drug, i.m., at the level of 400 mg/kg (single dose). Peritoneal washings performed 3 hours after the intraperitoneal injection of cercariae revealed living schistosomula and a few cercariae. It can be concluded that in the experimental conditions used, oxamniquine does not interfere on the transformation of cercariae into schistosomula.

TABLE II

Antischistosomal activity of oxamniquine (i.m.) on maturing schistosomes. Mice were exposed to 100 ± 10 cercariae of *Schistosoma mansoni* and treated (200 mg/kg, single dose) 2, 7, 14, 23, and 30 days later. Worm burden, distribution of schistosomes and percentage of oogram changes

Treatment days after exposure	Number of animals	Animals dead	Distribution of schistosomes (%)			Mean worm burden	Percent oogram changes
			Liver	Portal vein	Mesenteric vessels		
2	12	0	0.0	0.0	0.0	0.0	100.0 (*)
7	12	0	0.0	0.0	0.0	0.0	100.0 (*)
14	12	0	40.5	0.0	59.5	3.1	50.0
23	12	0	60.0	0.0	40.0	2.5	91.7
30	12	2	83.3	0.0	16.7	0.6	90.0
Control	12	0	17.9	18.8	63.3	18.0	0.0

(*) No eggs

TABLE III

Four groups of 10 mice were injected, intraperitoneally, with about 400 cercariae of *Schistosoma mansoni* and treated, 3 hours later, with different doses of oxamniquine (i.m.). Eight days later schistosome larvae were recovered by peritoneal washing and counted under a dissecting microscope

Mice \ Doses of oxamniquine (mg/kg)	400	200	100	50	Control
1	0	0	0	53	317
2	0	0	0	10	275
3	0	0	0	0	159
4	0	0	0	17	43
5	0	0	0	0	77
6	0	0	0	0	9
7	0	0	0	85	203
8	0	0	0	73	69
9	0	0	d	42	174
10	0	d	d	d	157
Mean	0.0	0.0	0.0	31.1	148.3

d = dead animals

RESUMO

Ensaios preliminares de laboratório com a oxamniquine, como agente profilático na esquistossomose mansoni

Foi demonstrado que a oxamniquine (6-hidroximetil-2-isopropilamino-metil-7-nitro-1,2,3,4-tetrahidroquinolina) é muito ativa sobre as formas iniciais de desenvolvimento do *Schistosoma mansoni* na cavidade peritoneal do camundongo. Uma única injeção intramuscular de oxamniquine, em doses que variaram de 100 a 400 mg/kg foi capaz de matar todos os esquistossômulos em poucos dias.

Com a finalidade de avaliar a atividade profilática, antes da exposição de camundongos a cercárias de *S. mansoni*, a oxamniquine foi administrada em doses únicas de 400 e 200 mg/kg, 6, 4, 2, e 1 dia antes da infecção. Uma ligeira atividade pode ser detectada somente nos animais tratados 24 horas antes da exposição (400 mg/kg).

A atividade antiesquistossomótica da oxamniquine foi mais evidente sobre as formas iniciais de desenvolvimento (até 7 dias) do que sobre esquistossomos em maturação.

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