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CHEMOPROPHYLACTIC PROPERTIES OF OXAMNIQUINE EMBONATE IN EXPERIMENTAL SCHISTOSOMIASIS (*)

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SUMMARY

Oxamniquine embonate was tested in mice aiming to prevent **Schistosoma mansoni** infection. The schistogram was used to evaluate the period of drug protection. The results showed that four days after drug administration the animals proved to be totally resistant to cercarial infection. It was found strong evidence that protection also occurs within a period of eight days after treatment.

INTRODUCTION

As stressed in the literature by CUSHING [2] and PELLEGRINO [8], the search for chemoprophylactic compounds against schistosomiasis must be increased, the finding of an effective, low toxic, cheap, **per os** administered substance should be the main goal of such research.

One of the many possible ways of achieving this goal is by using chemically modified schistosomicidal agents obtained by the method of molecular modification of drug design, especially the pro-drug approach, also called drug latentiation (HARPER [4], HIGUCHI & STELLA [5], ROCHE [15], SINKULA [17,18]). Outstanding for its wide utilization and easiness of attainment, among the several means to prepare pro-drugs, is the formation of salts, mainly of embonates, also known as pamoates (KOROLKOVAS [6], SAIAS, JONDET & PHILIPPE [16]).

Chemoprophylactic properties of known antischistosomal drugs especially oxamniquine and related compounds were focused in detail (PELLEGRINO, MELLO & PEREIRA [10], PELLEGRINO, PEREIRA & MELLO [12], PELLEGRINO et al. [13], PEREIRA, PELLEGRINO & MELLO [14]).

Oxamniquine itself proved efficacious in killing schistosomula early in the skin, thus preventing even the lung phase of the larvae (OLIVEIRA et al. [7]). However, this compound is efficient if given, at conventional doses of 200 mg/kg, single administration, only in the first week after infection, and 400 mg/kg is needed to prevent infection if the drug is given 24 hours before cercarial exposure (PELLEGRINO, PEREIRA & MELLO [11]). The same Authors were unable to prevent infection by giving the substance 48 hours or more before infection. A more prolonged protection was found with topically applied oxamniquine (mixed with plastifix — a low molecular weight neoprene). Protection was verified when the oxamniquine-plastifix was applied to tails of mice and later exposure of the tail to the cercariae up to four days later (PELLEGRINO, GILBERT & VALA-

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DARES [9]). Oxamniquine pamoate pellets have given 3 days complete protection against infections by **Schistosoma mansoni** cercariae, and 87% protection for 10 days (GILBERT et al. [3]).

In this paper, a study of oxamniquine embonate, designed to release slowy oxamniquine, the active moiety, is presented.

MATERIALS AND METHODS

Synthesis of oxamniquine embonate

Oxamniquine embonate was prepared by a reaction between two moles of oxamniquine with one mol of embonic (pamoic) acid (Fig. 1).

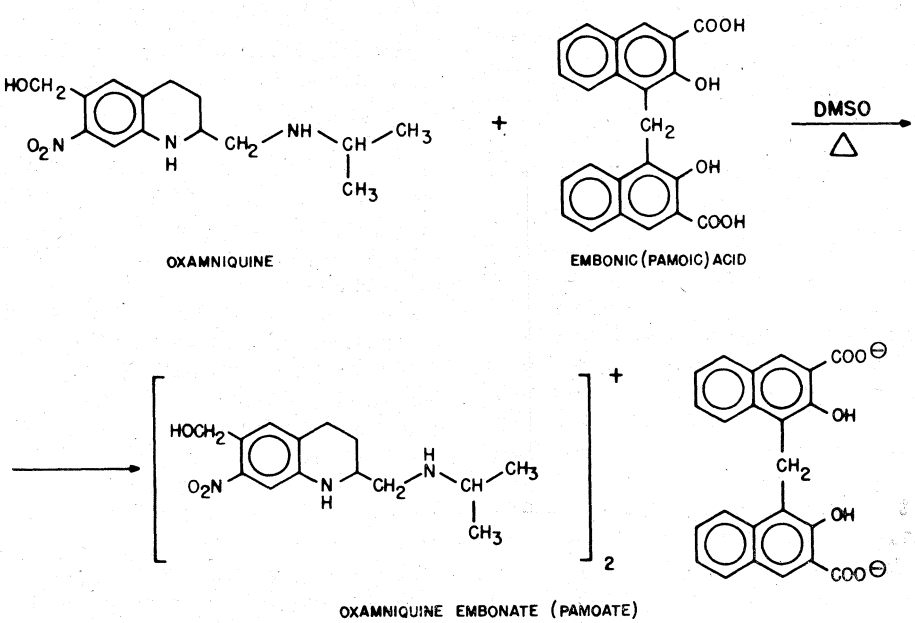

Fig. 1 — Synthesis of oxamniquine embonate

The procedure was the following: each reagent was dissolved separately in dimethylsulfoxide, with heating. Then both solutions were mixed and the mixture was stirred during 15 minutes. Afterwards, water was added until a precipitate was formed. Oxamniquine embonate thus prepared was collected by filtration, dried and analyzed. It is an orange powder, m.p. 235-237°C. Yield, 85%.

Infection of laboratory animals

Outbred albino mice were anesthetized (intraperitoneally) with 66.5 mg/kg of sodium pentobarbital. Their abdomens were shaved and each mouse was exposed to about 60 cercariae of **Schistosoma mansoni** shed by laboratory-reared-and-infected **Biomphalaria glabrata** (LE strain).

Drug administration

Oxamniquine embonate was given i.m., single doses, four days before the first infection. Six groups of mice were used. Groups A, B, C (five animals each) were infected 24, 20, and 14 days before the necropsy day, respectively. Groups D, E, F (10 animals each) received all the three infections. Groups A to D received no treatment. Groups E and F received 400 mg/kg of oxamniquine embonate and 200 mg/kg of oxamniquine itself, respectively, four days before the first infection.

Worm recovering and counting

Animals were sacrificed by cervical fracture. The mesenteric vessels and the liver were perfused with 0.85% saline using a pippeting machine. The mixture of blood and saline was

decanted, the supernatant discarded and the remaining material resuspended with saline. The procedure was repeated until few red blood cells remained. The worms were counted in a Petri dish taking note on the developmental stage of each larva (the schistogram) (BARBOSA et al.[1]).

RESULTS

Data are summarized in Table I. The schistogram of groups A to D indicated 2nd stage larvae were due mostly to the third infection, and not significantly due to the first or second infections.

In opposition, larvae of 5th stage were mostly due to the first infection.

By using oxamniquine embonate prophylactically, differences were seen when groups E (treated) and D (control) were compared. Besides differences in the percentual distribution, the absence of 5th stage larva was noted in group E. The oxamniquine embonate (400 mg/kg) killed all the larvae of the first infection, then protecting against infection at least for four days and there is evidence indicating protection up to eight days. Group E, which received the treatment with the oxamniquine embonate, and all the three infections showed practically the same schistogram percentual distribution of group C, suggesting infections A and B were suppressed by the compound.

DISCUSSION

It is widely known that the development of **Schistosoma mansoni** worms is very asynchronic in the definitive host. There is a definitive answer to that point, and it is possible to establish the time of infection if a percentual distribution of different stages (schistogram) is given (BARBOSA et al.[1]). Thus, if an animal receives three different inoculations within 10 days, it is possible to indicate, through the schistogram, some stages that belong to a specific infection, but 3rd and 4th stages may belong to all three infections. Since the schistogram is time consuming, when large numbers of compounds are tested, it is preferable to use single infections to evaluate how long a chemical may give protection against infection by perfusing the mature worms, 35 days later. However, if a small amount of the substance is available (as in the case of this experiment), the schistogram is helpful. In the same way, the numbers of laboratory mice are reduced to one third when multiple infections are used. For several different drugs single control groups (A, B, C) may be used. Worms can be preserved in 10% formalin for later examinations.

In all previous experiments with oxamniquine, in the base form, the compound did not afford protection after 48 hours of administration, even when high doses of 400 mg/kg were used, except if the compound, in a matrix of neoprene, was applied topically. It can be concluded that the oxamniquine embonate, as used in this experiment, and by GILBERT et al.[3], presents a slow-release activity, thus prolonging the protection time. This protection was found absolute within four days (no larvae from the first infection), and there is strong indication that the protection time is even larger. The similarity of schistogram between groups C and E reinforces this hypothesis.

RESUMO

Propriedades químio-profiláticas do embonato de oxamniquine na esquistossomose experimental

O embonato de oxamniquine foi testado em camundongos objetivando prevenir infecção por **Schistosoma mansoni.** Para avaliar o período de proteção foi usado o schistograma.

Os resultados mostraram que, quatro dias após a administração da droga, os animais mostraram-se totalmente resistentes à infecção cercariana. Houve também forte evidência de que, dentro do período de oito dias após o tratamento, ainda ocorre proteção.

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TABLE I

Distribution of different stages of **S. mansoni** worms in six groups of mice. Groups A, B, C received single infection. Groups D, E received all three infections. Drugs were administered i.m., single doses, four days before the first infection.

Group of mice	Time of infection (days before the necropsy day)	Treatment	Stages of S. mansoni larvae (1st to 6th) recovered from the portal system (mean number and standard deviation, percent distribution)					
			1st	2nd	3rd	4th	5th	6th
A	24	None	0.0±0.0	0.0±0.0	1.2±1.8 9.5%	7.2±3.7 57.1%	4.2±1.3 33.3%	0.0±0.0
B	20	None	0.0±0.0	0.4±0.5 1.6%	6.0±3.3 24.0%	17.6±4.6 70.4%	1.0±0.7 4.0%	0.0±0.0
C	14	None	0.4±0.9 2.4%	2.6±1.5 15.7%	11.4±6.9 68.7%	2.2±0.8 13.3%	0.0±0.0	0.0±0.0
D	24, 20, 14	None	0.0±0.0	1.4±2.0 3.4%	11.3±7.0 27.4%	20.8±9.9 50.4%	7.1±4.1 17.2%	0.7±1.1 1.7%
E	24, 20, 14	Oxamniquine embonate (400 mg/kg)	0.0±0.)	1.1±2.0 16.4%	4.3±3.4 64.2%	1.3±2.4 19.4%	0.0±0.0	0.0±0.0
F	24, 20, 14	Oxamniquine embonate (200 mg/kg)	0.0±0.0	2.7±2.3 6.1%	14.4±5.8 32.7%	20.3±4.8 46.1%	5.9±3.3 13.4%	0.7±1.1 1.6%

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