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PRELIMINARY STUDIES ON THE ANTISCHISTOSOMAL ACTIVITY OF TOPICALLY APPLIED OXAMNIQUINE

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SUMMARY

A formulation of oxamniquine (6-hydroxymethyl-2-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline), uniformly mixed with plastifix (a neoprene of low molecular weight) dissolved in benzene was prepared to be used topically in mice. Groups of 10 animals, 7 weeks after exposure to 100 cercariae of *Schistosoma mansoni*, were shaved on the back and the oxamniquine formulation, containing 450, 225, and 112.5 mg of the drug per kg of treated mice, was rubbed on the skin. At the dose level of 450 mg/kg all animals presented oogram changes and practically 100% of the schistosomes were shifted towards the liver. With half of this dose, 20% of mice presented oogram changes and 55.6% of the worms were found in the liver. The chemoprophylactic activity of oxamniquine (1,000 mg/kg) was evaluated by smearing the mixture on the back of mice and then exposing the animals to 200 cercariae, by tail immersion, 1, 2, 4, 8, 16, and 30 days later. Complete protection was observed in all animals treated 4 days or less before exposure.

INTRODUCTION

The dermal application of drugs is long known and permits steady release of chemotherapeutic agents into the organism over a relatively long period. Such a method might, by maintaining a constant low blood level of an antischistosomal agent, enable cure or protection against infection. Elastomers are often favoured as matrices for slow release, solubility of the drug being desirable for steady release. The present preliminary investigation shows that oxamniquine is in fact active by the dermal route when applied in a soluble chlorinated rubber to the skin of experimentally infected mice.

MATERIALS AND METHODS

Formulation — Oxamniquine (6-hydroxymethyl-2-isopropylaminomethyl–7–nitro-1,2,3,4-tetrahydroquinoline) was supplied by Pfizer Química Ltda. Plastifix, a low molecular weight, soluble neoprene, supplied by Bayer do Brasil S.A., was used as a 10% solution in benzene, in which the drug was subsequently dissolved at a concentration of 7% calculated on the plastifix content.

Application — The dorsal region of each mouse was shaved and carefully washed and, after drying, the oxamniquine formulation was smeared onto the skin to give a uniform thin layer of about 4 cm^2.

Infection of animals — The L.E. strain of *S. mansoni* (Belo Horizonte, Brasil), shed by laboratory-reared and infected *Biomphalaria glabrata* was used in the present study. For infection mice weighing 18 to 20 g were exposed to 100 (antischistosomal effect)

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or 200 (chemoprophylaxis) *S. mansoni* cercariae by tail immersion, as described by PELLEGRINO & KATZ [3].

Evaluation of antischistosomal and chemoprophylactic activity — The parameters used in the assessment of antischistosomal activity were the percentage of oogram changes and the hepatic-shift of worms. Reduction in worm burden was not taken into consideration since the animals were sacrificed 8 days after treatment. Chemoprophylactic activity was evaluated by reduction in worm burden and by the percentage of animals with oogram changes. In this case mice were sacrificed and examined 40 days after exposure. The oograms of intestinal fragments were performed as described elsewhere (PELLEGRINO & FARIA [2]). Schistosomes from the liver, portal vein and mesenteric vessels were collected separately by perfusion (PELLEGRINO & SIQUEIRA [4]).

RESULTS AND DISCUSSION

The antischistosomal activity of the oxamniquine formulation is shown in Table I, where it is seen that at the dose level of 450 mg/kg the worms suffer an almost complete hepatic shift and egg-laying has ceased. At a half this dose a discrete effect is observed only and at 112.5 mg/kg no activity is detected.

The prophylactic activity of the same formulation is summarized in Table II, and here it is seen that at the 1,000 mg/kg level total protection is afforded for a period of 4 days, no schistosomes being found on perfusion 40 days later. When the interval between application and infection was increased to 8 days a 50% oogram change indicated some residual activity but this disappeared on further increase of the pre-infection period.

In retrospect it is probable that plastifix is not the ideal matrix for oxamniquine due to the slow migration rate of the drug, a large part of which is still retained in the polymer layer at the end of the experiment. However, the fact that protection was achieved 4 days after application when any initially absorbed oxamniquine would have been excreted (FOSTER [1]) illustrates the fact that migration of this drug does occur at an effective rate, from an external reserve into the blood stream. The method, which is apparently original in schistosomiasis treatment, deserves further examination using a variety of matrices and hosts and such a study will be the subject of a future publication.

RESUMO

Estudos preliminares sobre a atividade anti-esquistossomótica da oxamniquine aplicada por via dérmica

TABLE I

Antischistosomal activity of oxamniquine administered by the cutaneous route (plastifix dissolved in benzene). Mice were sacrificed and examined 8 days after treatment.

Dose (mg/kg)	Animals /dead	Mean worm burden	Distribution of schistosomes (%)			Percentage of animals with oogram changes
			Liver	Portal vein	Mesenteric vessels	
450	10/1	21.6	96.0	0.0	4.0	100.0
225	10/2	12.6	55.6	6.3	38.1	20.0
112.5	10/1	23.8	24.3	18.6	57.1	0.0
Control	10/1	28.2	20.6	13.5	65.9	0.0

TABLE II

Chemoprophylactic activity of oxamniquine (1,000 mg/kg) administered by the cutaneous route (plastifix dissolved in benzene). Groups of mice were smeared on the back with the oxamniquine formulation and, after different periods (1, 2, 4, 8, 16, and 30 days) exposed to 200 cercariae by tail immersion. For each experiment a group of 10 untreated mice was left as control. Animals were sacrificed and examined 40 days after exposure.

Period between treatment and exposure	Mice/ dead	Mean worm burden	Distribution of schistosomes (%)			Percent of animals with oogram changes
			Liver	Portal vein	Mesenteric vessels	
1	10/1	0.0	0.0	0.0	0.0	No eggs
Control	10/1	20.0	20.0	10.7	69.3	0.0
2	10/0	0.0	0.0	0.0	0.0	No eggs
Control	10/1	18.3	20.4	14.3	65.3	0.0
4	10/0	0.0	0.0	0.0	0.0	No eggs
Control	10/0	19.5	27.4	18.9	53.7	0.0
8	10/0	3.7	40.6	21.6	37.8	50.0
Control	10/0	21.3	17.4	27.2	55.4	0.0
16	10/0	16.3	17.4	27.2	55.4	0.0
Control	10/0	20.3	29.4	23.3	47.3	0.0
30	10/0	8.4	35.6	18.6	45.8	0.0
Control	10/0	17.4	26.9	21.6	51.5	0.0

Preparou-se uma formulação de oxamniquine (6-hidroximetil-2-isopropilaminometil-7-nitro-1,2,3,4-tetrahidroquinolina) uniformemente misturada com plastifix (um neopreno de baixo peso molecular), dissolvido em benzeno para aplicação dérmica em camundongos. Grupos de 10 animais, 7 semanas depois de expostos a 100 cercárias de *Schistosoma mansoni*, após terem sido cuidadosamente raspados no dorso, foram friccionados, nessa região, com a referida formulação contendo 450, 225 e 112,5 mg da droga por kg de peso de camundongo. Na dose de 450 mg/kg todos os animais mostraram alterações do oograma e praticamente 100% dos vermes foram deslocados para o fígado. Com a metade dessa dose, 20% dos animais apresentaram alterações do oograma e 55,6% dos vermes foram encontrados no fígado. A atividade quimioprofilática da oxamniquine (1.000 mg/kg) foi avaliada friccionando-se a mistura no dorso de camundongos previamente depilados, expondo-se em seguida os animais a 200 cercárias, por imersão da cauda, 1, 2, 4, 8, 16 e 30 dias depois. Proteção completa foi observada em todos os animais tratados 4 dias ou menos, antes da exposição a cercárias.

REFERENCES

1. FOSTER, R. — The preclinical development of oxamniquine. *Rev. Inst. Med. trop. São Paulo* **15** (Supl. 1): 1-9, 1973.

2. PELLEGRINO, J. & FARIA, J. — The oogram method for the screening of drugs in schistosomiasis mansoni. *Amer. J. Trop. Med. Hyg.* **14**:363-369, 1965.

3. PELLEGRINO, J. & KATZ, N. — Experimental chemotherapy of schistosomiasis mansoni. *In Advances in Parasitology*, **6**:233-290, 1968. Ed. Ben Dawes, Academic Press, New York.

4. PELLEGRINO, J. & SIQUEIRA, A. F. — Técnica de perfusão para colheita de *Schistosoma mansoni* em cobaias experimentalmente infestadas. *Rev. Brasil. Malar. Doenças Trop.* **8**:589-597, 1956.

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