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Evaluation of the effect of oxamniquine, praziquantel and a combination of both drugs on the intramolluscan phase of *Schistosoma mansoni*

A.C.A. Mattos [a], G.C. Pereira [a], L.K. Jannotti-Passos [b], J.R. Kusel [c], P.M.Z. Coelho [a,d,*]

[a] *Laboratório de Esquistossomose, Centro de Pesquisas René Rachou/Fiocruz, Av. Augusto de Lima 1715, Barro Preto, 30.090-002 Belo Horizonte, MG, Brazil*
[b] *Moluscário, Centro de Pesquisas René Rachou/Fiocruz, Belo Horizonte, MG, Brazil*
[c] *University of Glasgow, Glasgow G12 8TA, Scotland, UK*
[d] *Santa Casa de Misericórdia, Belo Horizonte, MG, Brazil*

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Abstract

The activity of oxamniquine (OXA), praziquantel (PZQ), and a combination of both drugs was evaluated at the intramolluscan phase of *Schistosoma mansoni*. *Biomphalaria glabrata* snails infected with *S. mansoni* were treated with 500 mg/kg OXA, 1000 mg/kg PZQ or with 250 mg/kg OXA and 500 mg/kg PZQ, in association, at the pre-patent and patent phases of infection. The results showed that either treatments with OXA or PZQ, alone, at the pre-patent period, delayed the parasite's development, increasing the pre-patent period by approximately 10 days. At the same pre-patent period, treatment with a combination of OXA/PZQ delayed the parasite's development even more, extending the pre-patent period up to 56 days. At the patent period, treatment with OXA and PZQ, alone, interrupted cercarial shedding. When the snails were treated with 1000 mg/kg PZQ, the cercarial production was re-established 15 days after treatment, but in lower numbers than those obtained before treatment, whereas the snails treated with 500 mg/kg OXA were able to shed cercariae in similar quantities to those observed before treatment. The association 250 mg/kg OXA + 500 mg/kg PZQ, at the patent period, not only discontinued cercarial shedding, but also led to the "cure" of the snails, showing a synergistic effect of this combination of drugs. These results suggest that this model will be useful for selection of resistant parasites, as well as for screening new antischistosomal drugs.

Keywords: *Schistosoma mansoni*; Praziquantel; Oxamniquine; Synergistic effect; Intramolluscan phase

1. Introduction

At present, praziquantel (PZQ) and oxamniquine (OXA) are the drugs available for treatment of schistosomiasis. According to Foster et al. (1971) and Cioli et al. (1995, 2004), all phases of *Schistosoma mansoni* life cycle can be treated with OXA and PZQ, but cercariae and adult worms are more susceptible to these drugs. Previous studies demonstrated that effective

* Corresponding author at: Laboratório de Esquistossomose, Centro de Pesquisas René Rachou/Fiocruz, Av. Augusto de Lima 1715, Barro Preto, 30.090-002 Belo Horizonte, MG, Brazil. Tel.: +55 31 3349 7740; fax: +55 31 3295 3115.
E-mail address: coelhopm@cpqrr.fiocruz.br (P.M.Z. Coelho).

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drugs against adult and immature worms of *S. mansoni*, in the vertebrate host, including OXA and PZQ, are also effective at the intramolluscan phase of the parasite, leading to interruption of cercarial shedding (Warren and Weisberger, 1966; Warren, 1967; Coles, 1979; Touassem and Combes, 1986; Yi and Combes, 1987; Coelho et al., 1988; Riley and Chappell, 1990). Mattos et al. (2006), using *in vitro* transformed *S. mansoni* sporocysts, reported that PZQ acts similarly on the parasite's intramolluscan stage and the adult worms, causing musculature contraction and damage to the tegument. The energy metabolism of the cercaria and miracidium involves the breakdown of glucose under aerobic conditions (Horemans et al., 1992; Tielens et al., 1991). After entry of cercaria and miracidium into the final or intermediate host, respectively, the schistosomulum (Horemans et al., 1992) and sporocyst (Tielens et al., 1992) use glucose by anaerobic glycolysis, but the aerobic pathways are still functional (Tielens et al., 1992; Horemans et al., 1992). The sporocyst also has mechanisms well adapted to the snail environment in its utilization of glutamine (Khayath et al., 2006). The adult has been shown to have both anaerobic and aerobic metabolism (van Oordt et al., 1985). There is therefore considerable similarity between the different life cycle stages in the pathways of energy metabolism, although some specific differences do exist. Moreover, according to Coles (1973), enzymes present in sporocysts migrate, under electrophoresis, in a similar manner, to those present in adult worms. The data suggest similarity between the metabolic routes of these two different phases of *S. mansoni* life cycle (Coelho et al., 1988). Recent work has shown that the sporocyst and the mammalian stages of the schistosome share a great many genes. This is shown in the work of Verjovski-Almeida et al. (2003) and Dillon et al. (2006). The adult worm even shares a tegumental antigen not found in the schistosomulum (Stein and David, 1986) and an ultrastuctural examination of the damage inflicted on the sporocyst by the haemocytes of the snail resembles the effects of macrophages on schistosomula (Bayne et al., 1980). Our conclusion is therefore that the sporocyst has a great many similarities to the mammalian stage both in gene expression and in metabolism.

Oxamniquine and PZQ have different mechanisms of action, thus the combination of these drugs may generate a synergistic effect (Delgado et al., 1992). Some studies showed a marked synergistic action of that combination, either in experimental model or in humans (Shaw and Brammer, 1983; Campos et al., 1985, 1989; Botros et al., 1989; Delgado et al., 1992), whereas other studies do not clearly show this effect (Pugh and Teesdale, 1983; Creasey et al., 1986; Gryscheck et al., 2004). The works by Yi and Combes (1987) and Coelho et al. (1988) suggest that infected snails exposed to drugs can be an alternative and inexpensive method for drug screening.

The aim of this study was to assess the activity of OXA and PZQ alone, as well as of a combination of both drugs on the parasite's intramolluscan phase.

2. Materials and methods

2.1. Molluscs, parasites and infection

Biomphalaria glabrata (Barreiro de Cima strain), and *Schistosoma mansoni* (LE strain), routinely maintained at the Research Center René Rachou/Fiocruz, Brazil, were used in this study. The snails were individually exposed to 10 miracidia, according to the following technique: in order to perform infection, plates for cell culture with 24 wells (2.5 ml capacity each) were utilized. A mollusc specimen of approximately 8 mm diameter was placed into each well. Ten miracidia were then added and the volume was made up to 2.5 ml with dechlorinated water. The plates were kept under artificial light for 4 h, and then the snails were transferred to aquaria with dechlorinated tap water at $27 \pm 1\,^{\circ}C$ and continuous aeration. Three experiments were carried out at the prepatent period of infection, and three at the patent period. For each experiment different batches of miracidia from the LE strain of *S. mansoni* were used and the number of snails used are shown in Table 1. In the experiments

Table 1
Experimental groups and treatments

Experiments	No. of snails/group	Treatment days[a]	Drugs/doses (mg/kg)
Pre-patent period, experiment 1	15	18–22	OXA (500) or PZQ (1000)
Pre-patent period, experiment 2	30	18–22	OXA + PZQ (250 + 500)
Pre-patent period, experiment 3	30	18–22 and 32–36	OXA + PZQ (250 + 500)
Patent period, experiment 4	15	32–36	OXA (500) or PZQ (1000)
Patent period, experiment 5	30	32–36	OXA + PZQ (250 + 500)
Patent period, experiment 6	50	32–36 and 42–46	OXA + PZQ (250 + 500)

[a] Days after exposure to miracidia.