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Evaluation of Praziquantel effects on *Echinostoma paraensei* ultrastructure

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ABSTRACT

Echinostomiasis is a food-borne, intestinal, zoonotic, snail-mediated helminthiasis caused by digenean trematodes of the family Echinostomatidae with seven species of the genus *Echinostoma* infecting humans or domestic and wildlife animals. *Echinostoma paraensei* is a peristomic 37-collar-spined echinostome belonging to the "revolutum group".

Praziquantel (PZQ) is the drug of choice for treatment and control of human schistosomiasis and food-borne trematodiasis. In the present study we used scanning and transmission electron microscopy to further elucidate the trematocidal effect of PZQ on adult *E. paraensei* and confirmed that this trematode is a suitable model to study anthelmintic drugs. Hamsters infected with *E. paraensei* were treated with a single dose of 30 mg kg^{-1} of PZQ. The worms were recovered 15, 30, 90 and 180 min after drug administration. There was a significant decrease in worm burden in the small intestine in the hamster-*E. paraensei* model at the intervals of 30, 90 and 180 min after the treatment. The worms displayed damage of the peristomic collar with internalization of the spines and erosion of the tegument of the circumoral head-collar of spines. Ultrastructural analysis demonstrated an intense vacuolization of the tegument, appearance of autophagic vacuoles and swelling of the basal infolds of the tegumental syncytium. There was no change in the morphology of cells from the excretory system of adult *E. paraensei*, however, there was an apparent decrease of stores of glycogen particles in parenchymal cells in PZQ-treated worms. Our results demonstrated that PZQ promotes surface and ultrastructural damage of the tegument of adult *E. paraensei* supporting the idea that this trematode may constitute a good model to investigate drug effects mechanisms *in vitro* and *in vivo*.

1. Introduction

Food-borne trematode infections are significant public health problems, most notably in Southeast Asia and the Western Pacific. Global estimates indicate that more than 40 million individuals are infected and 750 million are at risk (Saric et al., 2009). Echinostomiasis is a

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food-borne, intestinal, zoonotic, snail-mediated helminthiasis caused by digenean trematodes mainly of the genus *Echinostoma* (Graczyk and Fried, 1998). Echinostomes are hermaphroditic digenean parasites found worldwide in avian and mammalian definitive hosts (Davis, 2005), morphologically characterized by the presence of a head collar with spines around the oral sucker. The number and arrangement of collar spines is an important key for taxonomic purposes (Toledo et al., 2006).

Echinostoma paraensei Lie and Basch (1967) is a peristomic 37-collar-spined echinostome belonging to the "revolutum group". It was first isolated from *Biomphalaria glabrata* in Belo Horizonte, Minas Gerais State, Brazil (Lie and Basch, 1967). Its natural definitive host was identified by Maldonado et al. (2001) as the aquatic rodent *Nectomys squamipes* endemic to the Brazilian coast (Bonvicino et al., 2008).

Praziquantel (PZQ) has a very broad spectrum of activity against trematodes and cestodes, and it has advantages such as high efficacy, is well tolerated, has few and transient side effects, simple administration, and competitive cost (Cioli and Pica-Mattoccia, 2003; Keiser and Utzinger, 2007). It has become the only anti-schistosomal drug of choice for treatment against all the major species that infect humans (WHO, 2002). The exact action mechanism of PZQ on adult trematodes remains to be elucidated. Although, PZQ induces a rapid contraction of schistosomes and alters the tegumental surface (Mehlhorn et al., 1981), these changes have been attributed to the drug-dependent disruption of Ca^{2+} homeostasis (Greenberg, 2005).

For intestinal fluke infections, the World Health Organization (WHO) currently recommends a single 25 mg kg^{-1} oral dose of PZQ (Keiser and Utzinger, 2007). Keiser et al. (2006) and Ferraz et al. (2012) demonstrated the effect of PZQ on treatment of *Echinostoma caproni* and *E. paraensei* infected mice respectively and that a single dose of PZQ at 50 or 100 mg kg^{-1} resulted in killing all the trematodes. Recently Ferraz et al. (2012) showed that *E. paraensei* recovered from PQZ treated mice presented morphological alterations on adult *E. paraensei* tegument.

Food-borne trematodiasis is emerging public health problem (Keiser and Utzinger, 2005), and there is considerable concern regarding the development of drug-resistant parasite strains as unexpectedly low cure rates have been observed in clonorchiasis (Tinga et al., 1999) and schistosomiasis patients (Doenhoff et al., 2002) following praziquantel administration. Consequently, new anthelmintic drugs are needed, but the development process is long and costly.

Rodents infected with *Echinostoma* spp. are suitable models for anthelmintic studies because its life cycle in the laboratory is relatively simple and this model therefore produces rapid results and is more cost effective than host–parasite models with a longer development and a more complex life cycle (Saric et al., 2009). Moreover, the *Echinostoma*-mouse model is not only useful for anthelmintic studies on echinostomes *in vitro*, but can also serve as a prescreening to test for the *in vivo* trematocidal activity of different compounds.

Thus the aim of this work is to investigate the trematocidal effect of PZQ on adult *E. paraensei* worms recovered from the highly compatible hamster host after PZQ treatment using scanning and transmission electron microscopies.

2. Materials and methods

2.1. Animals and infections

The life cycle of *E. paraensei* was maintained in the Laboratório de Biologia e Parasitologia de Mamíferos Silvestres e Reservatórios – Instituto Oswaldo Cruz – Fiocruz – Rio de Janeiro, Brazil. Procedures performed using animals were in accordance with the ethical rules of the Commission of Ethics in the Use of Animals (CEUA) from Fundação Oswaldo Cruz (permit LW-0020/10).

Adult *E. paraensei* were collected during necropsies from the lumen of small and large intestines of 2-week-old hamsters (*Mesocricetus auratus*) experimentally infected by oral feeding with 100 metacercariae and using snails (*B. glabrata*) as first and second intermediate hosts (Souza et al., 2011).

2.2. Drugs

Praziquantel (PZQ) was purchased from Merck (Cestox® – Merck). For oral administration PZQ was dissolved in 2% Cremophor-EL (Sigma Chemical Co., St Louis, MO, EUA) and water to generate concentrations of 10 mg/mL (Fallon et al., 1995).

2.3. Treatment of E. paraensei infected hamsters and worm recovery

Two weeks after the infection, the animals were treated with a single oral dose of 30 mg kg^{-1} PZQ (Keiser and Utzinger, 2004) dissolved in 2% Cremophor-EL administered by gavage. Three to five hamsters were euthanized in a CO_2 chamber and necropsied at intervals of 15, 30, 90 and 180 min after the treatment and the worms were recovered. Infected untreated hamsters were used as a control group. The worms were collected from the lumen of small and large intestines and counted.

For statistical analyses we used the Primer software package (McGraw Hill version 1.0) applying the One-Way ANOVA and post-test Bonferroni and P values were considered statistically significant at $P < 0.05$. The results were expressed as raw values or a percentage reduction of worm burden at each time interval in comparison with the untreated control hamsters.

2.4. Scanning electron microscopy

For SEM, the adult worms were gently transferred to a Petri dish and washed with NaCl 0.9% and immediately fixed in AFA solution (Mafra and Lanfredi, 1998), then washed in 0.1 M cacodylate buffer, pH 7.2, postfixed in a solution containing 1% osmium tetroxide (OsO4) and 0.8% potassium ferricyanide ($K_3Fe(CN)_6$) in 0.1 M cacodylate buffer. After which the worms were dehydrated in a graded ethanol series (30–100%), critical point dried in CO_2, mounted on stubs, gold sputter-coated, and examined