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Simvastatin and artesunate impact the structural organization of adult *Schistosoma mansoni* in hypercholesterolemic mice

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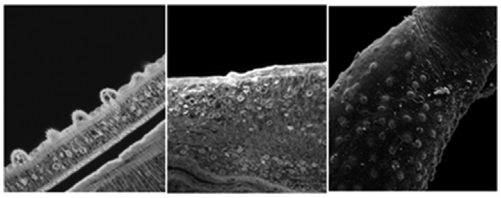
HIGHLIGHTS

- A cholesterol-rich environment provides favorable conditions for adult schistosomes.
- Sinvastatin and artesunato change the structural organization of *Schistosoma mansoni*.
- This drugs display antischistosomal activity in hypercholesterolemic mice.

GRAPHICAL ABSTRACT



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ABSTRACT

Experimental data have shown that simvastatin and artesunate possess activity against *Schistosoma mansoni* worms in mice fed standard chow. However, little is known regarding the roles of these drugs in mice fed high-fat chow. We have extended past studies by measuring the effects of these drugs on the structural organization of adult schistosomes in hypercholesterolemic mice. For this purpose, mice were gavaged with either simvastatin or artesunate at nine weeks post-infection and were euthanized by cervical dislocation at two weeks post-treatment. Adult worms were then collected and examined by conventional light microscopy, morphometry and confocal laser scanning microscopy. Plasma total cholesterol and worm reduction rates were significantly increased in mice fed high-fat chow compared with their respective control groups. Simvastatin and artesunate caused changes in the tegument, tubercles, and reproductive system (testicular lobes, vitelline glands and ovarian cells), particularly when administered to mice fed high-fat chow. In particular, the tegument and tubercles were significantly thinner in artesunate-treated worms in mice fed high-fat chow compared with mice fed standard chow. This study thus demonstrated that simvastatin and artesunate have several novel effects on the structural organization of adult worms. Together, these results show, for the first time, that simvastatin and artesunate display antischistosomal activity in hypercholesterolemic mice.

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1. Introduction

Schistosomiasis mansoni is caused by a blood-dwelling fluke (*Schistosoma mansoni*) that is transmitted via freshwater snails in Africa, Brazil, Caribbean, China, Egypt and Venezuela (Chitsulo et al., 2000). It is known that schistosomes do not synthesize

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lipids, instead taking up these fundamental compounds from their host's bloodstream (Meyer et al., 1970). Over the last few decades, human studies have demonstrated an association between schistosome infection and metabolic syndrome (Chen et al., 2013). Moreover, patients from endemic areas in China showed a lower prevalence of metabolic syndrome and low high-density lipoprotein cholesterol (Shen et al., 2015a,b). Our previous studies also showed that long-term feeding with a high-fat diet caused histological and parasitological effects on the outcome of murine schistosomiasis mansoni (Neves et al., 2007a; Alencar et al., 2009; Alencar et al., 2012).

Although praziquantel (PZQ) is a highly effective drug for the treatment and control of schistosomiasis in mass drug administration programs (Fenwick et al., 2006), its most important limitation is a decrease in or lack of activity against younger parasite stages (Doenhoff et al., 2009). In addition, reduced susceptibility to PZQ and PZQ resistance are not insignificant events (Wang et al., 2012). The search for schistosomicidal agents or combination therapy represents one potentially powerful strategy for overcoming drug resistance and enhancing drug efficacy (Greenberg, 2013, 2014; Neves et al., 2015).

Laboratory-based studies have revealed that artemisinin derivates, best known for their antimalarial properties, also have potential effects against the juvenile forms of *S. mansoni* (Li et al., 2012). Moreover, a hypolipidemic effect has been reported for artesunate (Wang et al., 2013). An increasing number of publications have additionally reported that statins show pleiotropic effects in reducing hyperlipidemia (Wang et al., 2008; Rutishauser, 2011) and in treating schistosomiasis (Rojo-Arreola et al., 2014). It is known that artesunate and statins cause morphological changes to the tegument and reproductive system of female schistosomes, resulting in low egg production (Shuhua et al., 1989, 2002, Xiao et al., 1996; Shuhua et al., 2002, Araújo et al., 1991; Xiao et al., 2000; De Clercq et al., 2000, Shaohong et al., 2006; Mitsui et al., 2009; Zhang et al., 2014). However, whether treatment with antischistosomal compounds is effective against schistosomiasis in mice fed a high-fat diet remains largely unknown. In the present study, the main research question was the influence of simvastatin and artesunate on the structural organization of adult schistosomes, as evaluated by light, confocal laser and scanning electron microscopy. Artesunate and simvastatin each caused changes in the tegument, tubercles, and reproductive system (testicular lobes, vitelline glands and ovarian cells), particularly when administered to mice fed a high-fat diet. In particular, the tegument and tubercles were significantly thinner in artesunate-treated worms from the high-fat group compared with worms from the mice fed a standard diet. Taken together, these results show that simvastatin and artesunate display antischistosomal activity in hypercholesterolemic mice.

2. Materials and methods

2.1. Ethics statement

All procedures were performed in accordance with valid international guidelines for animal experimentation (Ellery, 1985) and adhered to the rules of Brazilian Federal Law 11,794/2008 (Marques et al., 2009). The experimental protocol was approved by the local committee (The Ethics Commission on Animal Use - Oswaldo Cruz Foundation), reference number: CEUA L-0036/07.

2.2. Experimental model and diets

Conventionally raised three-week-old female Swiss Webster mice (Laboratory Animals Breeding Center, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil) were housed in polypropylene cages (40 × 33 cm) with stainless steel screened covers. Throughout the experimental period, the mice were maintained under controlled temperature (21 ± 1 °C), humidity (60± 10%) and photoperiod (0700–1900 h) conditions, with water and food provided *ad libitum*.

One group of mice received high-fat chow (HFC) containing pork lard, egg yolks, wheat flour, corn starch, and casein balanced with vitamins and minerals (47% carbohydrates, 24% protein, 29% lipids) (5.7 kcal/g body wt/day) for six months. This diet has previously been demonstrated to induce dyslipidemia in Swiss mice (Neves et al., 2006a). Another group received standard chow (SC) (Nuvilab CR-I-NUVITAL Nutrients Ltda., Colombo, Paraná, Brazil) composed of 12% fat, 28% protein and 60% carbohydrates (4.6 kcal/g body wt/day).

3. Experimental infection protocol

3.1. Schistosoma mansoni life cycle

The Brazilian strain (BH, Belo Horizonte) of *S. mansoni* was maintained at the Laboratory of Malacology (Oswaldo Cruz Institute, Rio de Janeiro, Brazil) in *Biomphalaria glabrata* snails and Swiss Webster mice (Paraense and Corrêa, 1981). After feeding on their respective diets, the mice were subcutaneously infected with ~85 cercariae, as previously described (Martinez et al., 2003).

3.2. Chemicals, treatment schedule and experimental groups

The mice received artesunate obtained from the Oswaldo Cruz Foundation or simvastatin (Medley, Campinas, SP, BRAZIL) dissolved in 1000 μl water and 250 μl Cremophor (Sigma Chemical Company, St. Louis, MO, USA). Nine weeks post-infection, the animals were gavaged with the appropriate pharmacological compound and assigned to six groups, as follows: groups SCA (n = 7) and HFCA (n = 5), a single oral dose of 300 mg/kg artesunate; groups SCS (n = 6) and HFCS (n = 5), a single oral dose of 200 mg/kg simvastatin ((Medley, Campinas, SP, BRAZIL); and untreated mice fed either HFC (UHFC group, n = 5) or SC (USC group, n = 5), which were used as controls.

3.3. Assessment of drug efficacy

The mice were euthanized at two weeks post-treatment by cervical dislocation. A midline incision was then made in the thorax and abdomen of each mouse. Adult worms recovered from the portal system and mesenteric veins were counted and sexed with a stereomicroscope to determine their infectivity, which was assessed as the percentage of cercariae maturing into adult worms (Freire et al., 2003). The percentage reduction in worm burden in each drug-treated group (Cioli et al., 2004) was calculated according to the following formula: Reduction (%) = (No. of worms in control group - No. of worms in treated group)/(No. of worms in control group) × 100.

Recovered schistosomes were prepared for conventional light microscopy processing, as follows: fixation in AFA (70% alcohol, 2% formalin and 2% glacial acetic acid) at room temperature, staining with 2.5% hydrochloric carmine, dehydration in an alcoholic series (70% GL, 90% GL and absolute), clarification in methyl salicylate with Canada balsam (1:2), and whole mounting on glass slides (Neves et al., 1998).

3.4. Scanning electron microscopic examination

The worms were washed in saline solution and fixed in AFA. For