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Original Article

Is arachidonic acid an endoschistosomicide?

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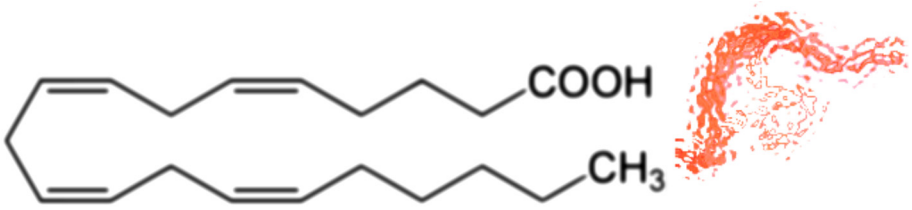
GRAPHICAL ABSTRACT



Arachidonic acid interacts with the surface double lipid bilayer shield of larval, developing and adult schistosomes, leading to its disintegration and eventual parasite attrition.

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ABSTRACT

Schistosoma mansoni and *Schistosoma haematobium* are intravascular, parasitic flatworms that infect >250 million people in 70 developing countries, yet not all people of the same community and household are afflicted. Regarding laboratory rodents, mice but not rats are susceptible to infection with *S. mansoni* and hamsters but not mice are entirely permissive to infection with *S. haematobium*. A recent Brazilian publication has demonstrated that resistance of the water-rat, *Nectomys squamipes* to *S. mansoni* infection might be ascribed to stores of arachidonic acid (ARA)-rich lipids in liver. Several reports have previously shown that ARA is a safe and effective schistosomicide *in vitro*, and *in vivo* in mice, hamsters and in children. *Schistosoma haematobium* appeared more sensitive than *S. mansoni* to ARA in *in vitro* and *in vivo* experiments. Accordingly, it was proposed that ARA increased levels might be predominantly responsible for natural attrition of *S. mansoni* and *S. haematobium* in resistant experimental rodents. Therefore, the levels of ARA in serum, lung, and liver of rats (resistant) and mice (susceptible) at 1, 2, 3, 4 and 6 weeks after infection with *S. mansoni* cercariae and between mice (semi-permissive) and hamster (susceptible) at 1, 2, 3, 4, and 12 weeks after infection with *S. haematobium* cercariae were compared and contrasted. Neutral triglycerides and ARA levels were assessed in serum using commercially available assays and in liver and lung sections by transmission electron microscopy, Oil Red O staining, and specific anti-ARA antibody-based immunohistochemistry assays. Significant ($P < .05$), consistent, and reproducible correlation was recorded between ARA content in serum, lung, and liver and rodent resistance to schistosome infection, thereby implicating ARA as an endoschistosomicide.

Introduction

Schistosoma mansoni and *S. haematobium* infect >250 million people in 70 developing countries with more than 800 million,

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namely children, at risk of the infection [1]. Yet, there is no instance where hundred percent or so of individuals are afflicted despite residing in endemic foci, and sharing community, household, and exposure to schistosome-infected water bodies. Indeed, "endemic normals" are repeatedly exposed to viable cercariae of *S. mansoni*, are seropositive by enzyme-linked immunosorbent assay (ELISA) against crude adult worm antigen and, yet, have no record of previous or current infection when judged by repeated stool examination [2–9]. The apparent lack of schistosome maturation and egg deposition in "endemic normals" was ascribed to antibody, lymphoproliferative, interferon-gamma (IFN-γ) responses to specific antigens, and/or specific antibody isotypes levels or ratios to soluble egg and worm antigens [2–9]. However, no consensus or solid explanations were reached.

Rodents too display differential susceptibility to schistosomes. Mice are susceptible to *S. mansoni* but are hardly semi-permissive to infection with *S. haematobium* [10–12]. To our knowledge, no explanation was provided for this phenomenon [13]. Conversely, nearly sterile resistance of laboratory rats (*Rattus norvegicus*) to *S. mansoni* infection [14–16] was attributed to the production of Th2 cytokines, interleukin (IL)-4, IL-5, and IL-13, in response to the invading larvae [17–21]. A recent study indicated that resistance of the water-rat, *Nectomys squamipes* to continuous infection with *S. mansoni* is associated with accumulation of lipids, principally arachidonic acid (ARA), in liver of naïve and naturally-infected animals [22].

Arachidonic acid, an omega-6 polyunsaturated fatty acid, is an essential constituent of biological cell membranes. Free unesterified ARA modulates the function of numerous ion channels, and several receptors and enzymes, via activation as well as inhibition, and readily induces apoptosis of normal and cancer cell lines [23–27]. It was previously shown that exposure to ARA (10 μM, 30 min) was effective in allowing specific antibody binding to otherwise hidden surface membrane antigens of *S. mansoni* and *S. haematobium* lung-stage schistosomula and adult worms [28,29]. Exposure to 20 μM ARA for 30 min elicited surface membrane disintegration and attrition of the schistosomula, likely as result of excessive ARA activation of the parasite tegument-associated neutral sphingomyelinase (nSMase) [29–31]. Further studies documented the ARA *in vitro* and *in vivo* schistosomicidal action on lung-stage and adult male and female *S. mansoni* and *S. haematobium* whereby *S. haematobium* appeared more sensitive than *S. mansoni* to ARA in *in vitro* and *in vivo* experiments [29–34]. These findings together prompted examination whether there is a correlation between laboratory rodents' resistance and susceptibility to infection with *S. mansoni* (rats vs. mice) or *S. haematobium* (mice vs. hamsters) and ARA levels in serum, lung, and liver in naïve hosts and weekly for 4 weeks after, as well as at the end of the experimental infection.

Experimental

Ethics statement

All animal experiments were performed following the recommendations of the current edition of the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, USA, and were approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Science, Cairo University (permit number CUIS 36 16).

Animals and parasites

Outbred, female CD-1 mice, albino rats (*Rattus norvegicus*), and Syrian hamsters (*Mesocricetus auratus*) were raised at the Schistosome Biological Materials Supply Program, Theodore Bilharz Research Institute (SBSP/TBRI), Giza, Egypt, and at the age of 6 weeks were maintained throughout experimentation at the animal facility of the Zoology Department, Faculty of Science, Cairo University. Cercariae of an Egyptian strain of *S. mansoni* and *S. haematobium* were obtained from SBSP/TBRI, and used immediately after shedding from *Biomphalaria alexandrina* and *Bulinus truncatus* snails, respectively. Infection of CD-1 mice and rats was with 100 ± 2 cercaria *via* whole body exposure [21], while hamsters were anesthetized, the abdomen shaved and wetted with sterile deionized water, and then exposed to 100 cercariae in 100 μL deionized water, protected from spilling by a sterile steel ring as described [12].

Experimental design

Experiment 1. A total of 30 rats and 30 mice were randomly assigned to groups of 12 uninfected, naïve hosts and groups of 18 that were exposed to 100 cercariae of *S. mansoni*. Three naïve and three *S. mansoni*-infected rats and mice were euthanized on day 7, 14, 21, and 28, and 6 of each infected group on day 40. Individual host serum was used for evaluation of circulating lipid levels. Lung and liver pieces of 2–3 mm^3 were immediately fixed in 4% paraformaldehyde (Sigma Chemical Co., St Louis, MO, USA) and destined to transmission electron microscopy, histological examination following haematoxylin and eosin staining of paraffin sections, and Oil Red O staining and immunohistochemistry assays of cryostat sections. Worm burdens as well as liver worm egg load in individual rats and mice (six per group) were evaluated at the last interval (40 days after the challenge infection with *S. mansoni* cercariae) as described elsewhere [12,33,34].

Experiment 2. A total of 30 mice and 30 hamsters were randomly divided into groups of 12 uninfected, naïve hosts and the rest exposed to 100 cercariae of S. *haematobium*. Three naïve and three *S. haematobium*-infected mice and hamsters were euthanized on day 7, 14, 21, 28, and 6 of each infected group on day 84, and provided serum and 2–3 mm^3 lung and liver pieces, which were processed and examined as mentioned above. Worm burdens as well as liver worm egg load in individual mice and hamsters (six per group) were evaluated at the last interval (84 days after the challenge infection with *S. haematobium* cercariae) as described elsewhere [12,33,34].

Serum lipids levels

Serum samples were assessed on an individual host basis, in duplicates, for enzymatic colorimetric (Multiskan EX, Labsystems, Helsinki, Finland) determination of total cholesterol (Cholesterol-LQ, CHRONOLAB SYSTEMS, S.L., Barcelona, Spain) and triglycerides (Triglycerides, CHRONOLAB) following the manufacturer's instructions. Levels of circulating unbound, free ARA were evaluated on an individual animal basis, and depending on serum availability in duplicate or quadruplicate wells, by competitive enzyme-linked immunosorbent assays (ELISA) using AA (Arachidonic Acid) ELISA Kit (Elabscience Biotechnology Co., Ltd, WuHan, People Republic of China; catalog No.: E-EL-0051) following the manufacturer's instructions. Absorbance readings (650 nm) of the ARA standard dilutions were plotted vs. concentration values in ng/mL using scatter graph [35]. For evaluating the concentration of the test samples, absorbance readings (650 nm) were fitted into the following obtained equation $Y = -0.0128\ X + 1.2044$, where Y represents the absorbance values and X the concentration values in ng/ml.

Transmission electron microscopy

Samples were fixed at 4 °C overnight in 4% paraformaldehyde, maintained at 4 °C in Dulbecco's phosphate-buffered saline,