Original Article

Effect of mefloquine on worm burden and tegumental changes in experimental Schistosoma mansoni infection

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A R T I C L E   I N F O

Article history:
Received 3 February 2014
Accepted 2 March 2014

Keywords:
Schistosoma mansoni
Mefloquine
Mice
Electron microscopy

A B S T R A C T

There is an important need to develop alternative anti-schistosomal drugs, as current treatment depends mainly on praziquantel (PZQ). This work aimed to study the in vivo effect of mefloquine on worm burden and tegumental changes on both the juvenile and adult worms in experimental Schistosoma mansoni infection.

We studied the effect of this compound in mice infected with cercaria of Schistosoma mansoni then treated with a single oral dose of 400 mg/kg mefloquine, 3 and 7 weeks after infection and worms were recovered two, three and seven days following treatment. Worm burden was calculated and alterations on the tegumental surface of schistosomula were examined by electron microscopy. The total worm burden reduction in juvenile was 94.5% and in adults was 74.8%. The electron microscopy examination showed tegumental changes in the form of retracted ventral sucker and oral sucker, fusion of tegumental ridges, pitting of the tegument and corrugations with swelling of the tegument in parts and shrinkage in the other parts with formation of deep furrows, disruption and peeling of the tegument with loss of spines and blebbing. Mefloquine has a promising effect in treatment of schistosomiasis.

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1. Introduction

Schistosomiasis is a chronic disease that exacerbates poverty [1]. About 800 million individuals are at risk and over 230 million people are thought to be infected [2,3].

In schistosomiasis, there is no vaccine available yet and the current mainstay of control is chemotherapy. Praziquantel (PZQ) is the drug of choice for the treatment of schistosomiasis because of its safety, broad-spectrum activity, and reasonable cost [4].

It might be possible the use of mefloquine which is anti malarial drug also as anti schistosomal drug, reduces the burden of schistosomiasis [5]. The antimalarials artemether and mefloquine have promising antischistosomal properties [6].

2. Materials and methods

2.1. Parasites

Cercariae of Schistosoma mansoni were obtained from infected Biomphalaria alexandrina snails, which were reared and maintained at Schistosome Biological Supply Program (SBSP), Theodor Bilharz Research Institute, Giza,
Egypt. This strain of *S. mansoni* was obtained originally from Lowell University, Lowell, Massachusetts, U.S.A., and has been passed through out bred mice and *B. alexandrina* snails.

2.2. Experimental animals

This study was conducted on laboratory albino mice strain CD1, clean from parasitic infection were used. They were all males (to spare any possible effect of pregnancy hormones in females on the immune system of the mice) weighting 20–25 g at the beginning of the experiment and had similar age (3–5 weeks). Animals were fed on a standard diet composed of about 24% proteins, 4% fat and about 4.5% fiber.

2.3. Experimental design

Mice were infected subcutaneously with 80 ± 10 *S. mansoni* cercariae then mefloquine was given at 21 and 49 days post infection. Mice were grouped into:

**Group I:** Treated with single oral dose of mefloquine (400 mg/kg) at 21 days post infection to study the effect of mefloquine on juvenile stage of *S. mansoni*.

**Group II:** Treated with single oral dose of mefloquine (400 mg/kg) at 49 days post infection to study the effect of mefloquine on adult stage of *S. mansoni*.

**Group III:** Control group of infected untreated mice at 21 days.

**Group IV:** Control group of infected untreated mice at 49 days.

Each group contains 20 mice which were sacrificed by decapitation [7] at different intervals two days, three days and one week post treatment to count the worms and detect the tegumental changes at these intervals.

**Group V:** Uninfected and untreated (healthy control group).

2.4. Drug preparation and adjustment of the dose

Mefloquine drug (Mepha Ltd., Aesch-Basel, Switzerland), each Lactab contains: mefloquine base 250 mg (in the form of 275 mg mefloquine hydrochloride).

The dose was 400 mg/kg [8].

The drug supplied as powder is suspended in 7% Tween, 80.3% absolute alcohol and distilled water shortly before use in dose 400 mg/kg orally once.

In case of mice harboring adult *S. mansoni* worms (49 days post infection) the mice were weighting 24–25 g each, while in case of mice harboring juvenile *S. mansoni* worms (21 days post infection) the mice were weighting 19–21 g each.

2.5. Recovery of parasites

Recovery of juvenile and adult schistosome worms was achieved by Porto-mesenteric perfusion of livers of *S. mansoni* infected mice.

The collected worms were then counted under a stereo-microscope [9].

2.6. Scanning electron microscopy (SEM) examination

Scanning electron microscopy examination was performed according to Hassan et al. [10], to determine the extent of damage on the surface of treated worms in comparison to the untreated worms.

2.6.1. Preparation of worms: [10]

Adult male worms of *S. mansoni* perfused from the hepatic and portomesentric vessels of infected mice were collected in glutaraldehyde buffer solution (25%) as a fixative over night at 4 °C, then washed out of any of the fixative by keeping them over night at 4 °C in phosphate buffer then passed into rising concentrations of alcohol (30%, 40%, 50%) each for 15 min and kept in 70% alcohol until the time of examination.

Before examination, they were washed twice for 30 min in 80% and 90% alcohol respectively. The last wash was for one hour in 100% alcohol.

Worms were then mounted on stainless steel holders and put in a drier for about 30 min and then subjected to sputter coat of gold, the different parts of worms were examined using Joel JEM-1200 scanning electron microscope, provided with a camera fitted to it. Areas in the worms that showed specific changes were examined and photographed mainly, suckers and the tubercles on the tegument.

2.7. Statistical analysis

The collected data will be tabulated and analyzed using IBM personal computer using SPSS 16 microstate soft ware package.

ANOVA (analysis of variance) “f” test was used as the test of significance. *P* value was considered significant if it was <0.05.

Bonferroni test was used as the multiple comparison tests after obtaining significant result by “f” test i.e. post hoc comparison to determine which pair was significantly different [11].

3. Results and discussion

The results are shown in Table 1 and Figs. 1–12.

Treatment of schistosomiasis relies almost exclusively on praziquantel. However, drug resistance is a real threat, particularly in the light of large-scale administration of praziquantel [12]. Praziquantel shows also a deficiency in its spectrum of activity, it has moderate activity against juvenile worms so there is a need for the development of new drugs for the treatment of schistosomiasis [13].

Reduction in worm burden is an important parameter for assessment of anti schistosomal activity of drugs in laboratory animals [14].

The rule served by the tegument in the immune evasion and parasite homeostasis was studied by Otubanjo et al. [15]. They revealed the importance of the tegument as a
target for antischistosomal drugs. Each drug induces characteristic changes in the tegument. The ultrastructure of the worm after drug administration clarify the procedure of killing of these worms [10].

In the present work, we studied the effect of mefloquine on the worm burden and tegument of juvenile and adult *S. mansoni* worms by scanning electron microscopy.

Concerning the effect of mefloquine on the total worm burden, we found that mefloquine has a great effect both on juvenile and adult worms reaches maximum (95.8% and 72.8% reduction respectively) 7 days after treatment. These results are in parallel with that reported by Keiser et al. [8] who reported that in juvenile infection (21-day post infection), total worm burden reduction of 97.2%, and total worm burden reduction in mature infection 77.3% was observed with a single oral dose of mefloquine (400 mg/kg). Keiser et al. [16] also reported that the total worm burden reduction in juvenile infection of 97.8% and in mature infection of 77.8% was observed with a single oral dose of mefloquine (200 mg/kg) was achieved with a single-dose oral regimen (200 mg/kg).

On the other hand Van Nassauw et al. [17] reported that, mefloquine at 150 mg/kg had no effect on worm burden, but significantly reduced the number of eggs in the first three developmental egg stages and concluded that, mefloquine significantly reduces egg production in *S. mansoni*-infected mice. This controversy regarding the effective dose needs more studies.

Basra et al. [18] used mefloquine as intermittent preventive treatment against malaria in pregnancy in a clinical trial in Gabon, it showed promising activity against concomitant *Schistosoma haematobium* infection leading to an important reduction of egg excretion in pregnant women.

Concerning the tegumental changes, we found that schistosomes and adult worms recovered from treated mice showed a variable degree of tegumental changes in the form of retracted ventral sucker and oral sucker, fusion of tegumental ridges, pitting, deep furrows, disruption and peeling of the tegument with loss of spines and blebbing. Also corrugations with swelling of the tegument in parts and shrinkage in the other parts with formation of vesicles and deep furrows. These changes were more prominent in female worms.

Blebbing is an indicator for stress and has been observed in previous studies evaluating anti-schistosomal drugs by scanning electron microscopy [19].

These results are in parallel with that reported by Pica-Mattoccia and Cioli [20]. They reported that following in vitro exposure to mefloquine, the tegument of female worms was slightly more affected and died more rapidly when compared to male worms.

These results are also similar to that, reported by Mannack et al. [21] They reported that a single oral dose of mefloquine (400 mg/kg) to *S. mansoni*-infected mice caused localized blebbing on the tegument appeared after one day and became more sever three days after treatment particularly in female worms. Moreover they found that the oral suckers were shrunken with deep furrows, their inner surface was covered by small spines while the outer margin were not affected. Moreover Mannack [22] revealed by SEM blebbing, shrinking and sloughing on the tegument of adult *S. mansoni* worms following mefloquine administration in
mice and concluded that mefloquine possesses excellent antischistosomal properties.

In related experiments but on Schistosoma japonicum Zhang and Xiao [23] studied the effect of 200 mg/kg mefloquine on 14 days old S. japonicum in mice and reported tegumental changes in the form of swelling and peeling and stated that it exhibits a potential and fast killing effect and induces sever histopathological lesions. In another study on juvenile S. japonicum, Xiao et al. [24] studied the ultrastructural alteration induced by 400 mg/kg mefloquine in mice and found tegumental alteration characterized by emergence of irregular and elongated cytoplasmic processes and concluded that mefloquine causes extensive ultrastructural damage to juvenile S. japonicum.

So it is clear that mefloquine has two advantages over praziquantel first it showed a significant effect on the worm burden and tegument of both juvenile stages and adults of S. mansoni but praziquantel had mild or moderate effect.

Figs. 2-12. Explanation of scanning electron micrograph (SEM) of S. mansoni figures: (2) Schistosomule two days post treatment showing retracted ventral sucker and fusion of tegumental ridges (2000×). (3) Schistosomule three days post treatment showing pitting of the tegument (5000×). (4) Schistosomule seven days after treatment showing furrows on the tegument (2400×). (5) Schistosomule seven days post treatment showing retracted oral sucker (6000×). (6) Male after 2 days with mefloquine treatment showing disruption and peeling of the tegument with loss of spines and flattening of the ventral sucker (vs) (600×). (7) Male after 2 days with mefloquine treatment showing deformity of the oral sucker (os) (1000×). (8) Female after 2 days post mefloquine treatment showing shedding of the tegument from the basement membrane (1200×). (9) Female two days post mefloquine treatment showing disruption and peeling of the tegument (1600×). (10) Male after 3 days post mefloquine treatment showing shrunken oral sucker with deep furrows (2031×). (11) Female after 3 days with mefloquine treatment showing shrunken tegument with formation of deep furrows (112×). (12) Male 7 days after mefloquine treatment showing flat tegument with flat ventral sucker (1000×).
on juvenile schistosomula as indicated by Doenhoff et al. [13]. Second advantage is that malaria and schistosomiasis coexist in large areas in tropical and subtropical Africa and mefloquine can treat both infections. We conclude that both calculating the worm burden and SEM of tegumental changes are effective in follow up treatment in mice infected with S. mansoni and that mefloquine had advantages over praziquantel and may take place in future for treatment of schistosomiasis.

Conflict of interest

The authors have no conflict of interest to declare.

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Please cite this article in press as: Fakahany AF, et al. Effect of mefloquine on worm burden and tegumental changes in experimental Schistosoma mansoni infection. J Microsc Ultrastruct (2014), http://dx.doi.org/10.1016/j.jmau.2014.03.001