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Original Article

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

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ABSTRACT

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 manson* 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.

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,

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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 stereomicroscope [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