ORIGINAL ARTICLE

EFFECT OF ARTESUNATE ON JUVENILE AND ADULT STAGES OF SCHISTOSOMA MANSONI IN MICE

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ABSTRACT

BACKGROUND: *Chemotherapy with praziquantel remains the mainstay of control of the schistosomiasis. The derivatives of artemisinin, artemether and artesunate are also known to exhibit antischistosomal activities. The aim of this study was to assess the effect of artesunate on different developmental stages of an Ethiopian strain of Schistosoma mansoni in mice.*

METHODS: *A total of 49 male Swiss albino mice were used and each was exposed to 100 cercariae of Schistosoma mansoni and randomly grouped into 9 groups. Mice in group 1 served as controls and were not treated. Mice in-group 2 were orally treated with 4 mg/kg of artesunate on day 7 and then with 2 mg/kg on days 14, 21, 28 and 35-post exposure to cercariae. Mice in groups 3, 4 and 5 were orally treated with 50, 100 and 200 mg/kg artesunate on days 7, 14, 21, 28 and 35 post-exposure to cercariae, respectively. Mice in-group 6 were orally treated with 4 mg/kg artesunate on day 50 and then with 2 mg/kg for four consecutive days. Mice in groups 7, 8 and 9 were treated with 50, 100 and 200-mg/kg of artesunate on days 50-56 post- exposure to cercariae, respectively. The mice were followed for worms development and reduction.*

RESULTS: *Doses of 100 and 200 mg/kg of artesunate administrated to mice on days 7, 14, 21, 28 and 35 post-infection resulted in total worm burden reductions of 61.4 -81.4%, which was significantly higher than the percentage of total worm burden reductions obtained with the same doses administrated on days 50-56 post-infection (20.7-21.4%, $P< 0.05$). Administration of 4 mg/kg and then 2 mg/kg or 50 mg/kg artesunate either between days 7-35 or on days 50-56 post-exposure to cercariae did not affect the juvenile stages or the adult worms of* **S. mansoni**.

CONCLUSION: *Artesunate appears to be more effective against juvenile stages of* **S. mansoni** *than adult worms in mice when it is administered at higher doses. Therefore, further studies are needed in humans to provide additional data on the efficacy of artesunate and other derivatives of artemisinin in the treatment of schistosomiasis. (Ethiop J Health Sci 2005; 15(1):31-38).*

KEY WORDS: *Artesunate, Schistosoma mansoni, Antischistosomal activity, Mice, Ethiopia*

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INTRODUCTION

Schistosomiasis remains one of the most important parasitic diseases of humans despite the various efforts made to control the disease. According to the estimation of World Health Organization (1) about 200 million people are actually infected with schistosome parasites while 650 million people are at risk of infection worldwide.

Schistosomiasis due to *S. mansoni* infection is a widely distributed disease in several localities of Ethiopia (2-5). Some 16 years ago, it was estimated that about 2.5 million people were infected with *S. mansoni* and 18 million people were at risk of infection (6) although these figures could be increased nowadays because of water resources development that play great role in the spreading of the disease (7,8).

Treatment with praziquantel is the current mainstay for morbidity control of the disease (9). However, fear of the emergence of praziquantel resistant schistosome strains (10-12) and absence/little efficacy of the drug against juvenile stages of the worms (13,14) has increased the need for search, and development of new antischistosomal drugs.

The antischistosomal activity of artemisinin derivatives, artesunate and artemether (the common antimalarial drugs), was first reported by Le and his co-workers in the early 1980s (15,16). Since then, subsequent studies in animal models have been shown the susceptibility of both juvenile and adult stages of different species of schistosomes to artesunate and artemether although the highest susceptibility has been confined to the juvenile stages of the parasites (17-21). Like Praziquantel, oral administration of the artemisinin derivatives to mice infected with schistosomes causes severe and extensive tegumental damage, alteration of glycogen content and energy metabolism, which finally lead to worm death (22, 23).

Moreover, a combined treatment with praziquantel and either artemether or artesunate appeared to be more efficacious than treatment with praziquantel alone against different species of schistosomes (24-27). However, validation studies need to be repeated in different schistosomiasis endemic countries since factors like species/strain of the parasite may affect the antischistosomal activity of these drugs. Therefore, the purpose of this study was to assess the anti-schistosomal property of various doses of artesunate in mice infected with an Ethiopian strain of *S. mansoni*.

MATERIALS AND METHODS

Infection of Mice

Cercariae of *S. mansoni* were obtained from *Biomphalaria pfeifferi* snails colleted from schistosomiasis endemic area of Ethiopia. A total of 49 male Swiss albino mice, weighing between 28-35 g (mean 30.85, SD=2.4) were selected and each mouse was exposed to 100 cercariae by tail dipping method (28). Then, mice were randomly allocated to 9 groups. Groups 1-5 each consisted of five mice while groups 6-9 each consisted of 6 mice. All groups were kept in animal house and provided with enough food and water until the end of the experiments.

Drug and Treatment

Artesunate tablets (Shin Poong Pharm, Seoul, Korea) were kindly provided by Mr. Fekede Balcha, Institute of Pathobiology, Addis Ababa University. The tablets were dissolved in distilled water by grinding repeatedly in grinding mortar. Then, the mice were orally treated using a mouse-feeding syringe. Mice in-group 1 served as control and were not treated. Mice in-group 2 were treated with 4 mg/kg of artesunate on day 7 and then with 2 mg/kg on days 14, 21, 28 and 35-post exposure to

cercariae. Mice in groups 3, 4 and 5 were treated with 50, 100 and 200 mg/kg artesunate on days 7, 14, 21, 28 and 35 post-exposure to cercariae, respectively.

Treatment of mice in groups 6-9 was started on day 50 post-infection. Mice in-group 6 were treated with 4 mg/kg artesunate on day 50 and then with 2mg/kg for four consecutive days. Mice in groups 7, 8 and 9 were treated with 50, 100 and 200 mg/kg artesunate for seven consecutive days, respectively.

Evaluation of effect of artesunate against juvenile stage of *Schistosoma mansoni*

Two weeks after the final dose, faecal samples were collected from all mice grouped under groups 1-5 into individual container. The faecal samples were processed by formal-ether concentration method and microscopically examined for the presence of eggs of *S. mansoni.* At the same time, fresh faecal sample was collected from each mouse into individual container and egg viability was checked by miracidial hatching (29). On the next day, all mice in the different treatment groups and the control were sacrificed, and adult worms were carefully collected from the portal and mesenteric veins of each mouse by perfusion as well as manually using needle and forceps. The female and male worms harvested from each mouse were counted, and the mean number of female and male worms was calculated for each group. Percentages of female, male and total worm reductions were determined by subtracting the mean of each group from the mean of the control group. Effect of the various doses of the drug was determined by comparing the mean number of female, male and total worms in the different treatment doses to that of the control group using Student's t-test, allowing for unequal variance.

Evaluation of effect of artesunate against adult worms and egg viability

In order to assess the effect of various doses of artesunate on egg viability, faecal samples were collected from mice grouped under groups 6-9 on days 1, 8, and 15 after finishing the respective treatment doses. The samples were processed by concentration method and microscopically examined for the presence of eggs. Egg viability was also checked by miracidial hatching method. On the same day, two mice from the different treatment groups were randomly sacrificed and perfused, to determine the presence, number and viability of adult worms. The effect of the drug was determined by comparing the mean counts of female, male and total worm in the different treatment doses to that of the control group using Student's t-test, allowing for unequal variance.

RESULTS

Effect of various doses of artesunate on development of Schistosoma mansoni

Mean of female, male and total worm counts, and percentage of worm reductions in mice treated with various doses of artesunate between days 7-35 post-exposure to *S. mansoni* cercariae are shown in Table 1. Results of faecal sample examination and worm counts revealed that administration of artesunate at doses of 4 mg/kg on day 7 and then 2 mg/kg on days 14, 21, 28 and 35 as well as 50 mg/kg on days 7, 14, 21, 28 and 35 post-exposure to cercariae did not affect either *S. mansoni* worm development or egg viability. Significant difference was not found between the mean of female, male and total worm counts of the control group and

groups treated either with 4 mg/kg on day 7 and then 2 mg/kg on days 14, 21, 28 and 35 or 50 mg/kg on days 7, 14, 21, 28 and 35 post-infection. However, statistically significant ($p < 0.05$) differences were observed between the mean of female, male and total worm counts of the control group and groups treated with 100 and 200 mg /kg artesunate on days 7, 14, 21, 28 and 35 post-infection. Moreover, adult worms or eggs were not obtained from the liver of one mouse among mice treated with 200 mg/kg of artesunate on days 7, 14, 21, 28 and 35 post-infection.

Effect of various doses of artesunate on adult worms of Schistosoma mansoni

For the second set of experiment, mean of female, male and total worm counts, and percentage of worm reductions in mice treated with various doses of artesunate 50 days after exposure to cercariae are shown in Table 2. One mouse died on day 52 among mice on treatment with 4 mg/kg artesunate on day 50 and then with 2 mg/kg for four consecutive days. Similarly, one mouse died on day 54 among mice on treatment with 50 mg/kg of artesunate.

Table 1. Mean Female, Male and Total Worm Count, and Percentage of Worm Reductions (15 Days Post-Treatment) in Mice Treated with Artesunate on Days 7- 35 Post-Exposure to *Schistosoma Mansoni* Cercariae, Addis Ababa, Ethiopia

Group	N	Dose (mg/kg)	Administration: Days after exposure to cercariae	Female worm count Mean (SD)	Female worm reduction (%)	Male worm count Mean (SD)	Male worm reduction (%)	Total worm count Mean (SD)	Total worm reduction (%)
1	5	Control	-	12 (4.0)	-	30 (7.5)	-	42 (9.4)	-
2	5	4 - 2	7,14, 21, 28, 35	11(4.0)	8.3	26.2 (5.5)	12.7	37.2 (10.4)	11.4
3	5	50	7,14, 21, 28, 35	13.2 (5.4)	None	31.8 (4.3)	None	45 (7.9)	None
4	5	100	7,14,21,28,35	8.2 (2.4)	31.7	8 (4.8)*	73.3	16.2 (5.7)*	61.4
5	5	200	7,14,21,28,35	3.2 (2.4)*	73.3	4.6 (3.6)*	84.7	7.8 (5.9)*	81.4

Group 2-5 tested versus group 1 (* $p < 0.05$), N = number of mice, SD = Standard deviation

Table 2. Mean Female, Male and Total Worm Counts and Percentage of Worm Reductions in Mice Treated with Artesunate on Days 50-56 Post-Exposure To *Schistosoma Mansoni* Cercariae (Combined Results For Days 1, 8 And 15 Post-Treatment), Addis Ababa, Ethiopia

Group	N	Dose (mg/kg)	Administration: days after exposure to cercariae	Female worm count Mean (SD)	Female worm reduction (%)	Male worm count Mean (SD)	Male worm reduction (%)	Total worm count Mean (SD)	Total worm reduction (%)
1	5	-	-	12 (4.0)	-	30 (7.5)	-	42 (9.4)	-
6	5	4 -2	Day 50-54	13.3 (2.8)	None	30.5 (7.2)	None	43.8 (6.1)	None
7	5	50	Day 50-56	14 (4.4)	None	30 (3.5)	0	44 (6.6)	None
8	6	100	Day 50-56	10.8 (2.6)	10	24.2 (4.8)	19.3	35 (6.1)	16.7
9	6	200	Day 50-56	9.2 (2.3)	23.3	23.8 (5.8)	20.7	33 (4.8)	21.4

Group 6-9 tested versus group 1, N = number of mice, SD = Standard deviation

From the remaining, two mice from the different treatment groups were randomly sacrificed at intervals of days 1, 8 and 15 after finishing the treatment, and the presence, number and viability of adult worms were determined. The results showed that the mean of female, male and total worm counts harvested from mice treated with various doses of artesunate 50 days post-infection were not significantly different from that of the control group. Eggs were detected in the faecal samples collected from mice dissected even 15 days post-treatment and they were viable (data not shown).

DISCUSSION

Administration of artesunate at high doses of 100 and 200 mg/kg on days 7,14, 21, 28 and 35 post-infection resulted in 61.4% and 81.4% total worm reductions, respectively which was significantly greater than percentage of total worm reductions (20.7-21.4%) obtained with the same doses administrated on days 50-56 post infection. Similar to this observation, a recent study by Utzinger et al. (21) has shown a high efficacy of artesunate (67-77% worm reductions) against juvenile stages of *S. mansoni,* but less active against adult worms at doses of 150 and 300 mg/kg administered on days 7, 21 and 35 post-infection. Similarly, another two independent studies in mice (17, 30) showed high efficacy of artesunate and artemether against immature (2-3 weeks old liver-stage) *S. mansoni* worms, but low efficacy of these drugs against adult worms although the cause of the stage-specific susceptibility is still not fully understood.

Studies in humans have shown that artesunate is effective for the treatment of both *S. mansoni* and *S. haematobium* infections when it is used in a combination with Praziquantel (24,25,31). It was also reported that treatment of *S. mansoni* infection with artesunate alone at the usual dose for the treatment of malaria in humans resulted in a profound egg reduction rate 5 to 10 weeks post-treatment (32) although study by Borrmann et al. (31) failed to confirm this finding in *S. haematobium*-infected Gabon children. Administration of 50 mg/kg of artemether to mice infected with *Schistosoma mansoni* was resulted in significant egg and worm reductions (33). In this study, an attempt was made to assess whether a low dose of artesunate has apparent effect on the juvenile stages or the adult worms of *S. mansoni* in mice. As compared to the control, the two low doses (4 mg/kg and then 2 mg/kg or 50 mg/kg) of artesunate administered either on days 7, 14, 21, 28 and 35 or on days 50-56 post-exposure to cercariae did not affect the juvenile stages or the adult worms of *S. mansoni.* Moreover, death was recorded in mice treated with 4 mg/kg and then 2 mg/kg or 50 mg/kg of artesunate on days 50-56 post-infection with *S. mansoni* cercariae. Hence, findings of previous studies (21, 30) and the present observations collectively show that high doses of artesunate are efficacious against schistosomiasis in mice, and these doses are appeared to be more effective against juvenile stages of the parasite than the adult worms.

In conclusion, the results of this study showed that artesunate appears to be more effective against juvenile stages of *S. mansoni* in mice when it is administered at higher doses. In sub-Saharan African countries where concomitant infections with malaria and schistosome parasites could be common public health problems and the socioeconomic status of the society is very low, drugs that exhibit both antimalarial and antischistosomal properties would be desirable. Therefore, further studies are needed to be conducted in humans to provide additional data on the efficacy of artesunate and other derivatives

of artemisinin in the treatment of schistosomiasis.

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