

# IN VIVO ACTIVITY OF DIHYDROARTEMISININ AGAINST SCHISTOSOMA MANSONI SCHISTOSOMULA IN MICE

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**Abstract.** Dihydroartemisinin, an anti-malarial agent, has been shown to exhibit activity against *Schistosoma japonicum* and *S. mansoni*. The purpose of the present study was to investigate the *in vivo* activity of dihydroartemisinin against juvenile *S. mansoni* and the changes to the genital system among worms surviving drug treatment. Mice were infected with 200 *S. mansoni* cercariae each and randomly assigned to groups. Dihydroartemisinin at a single oral dose of 300 mg/kg was given to mice on Days 14 or 16, 18, 20, 21, 22, 24, 26 or 28 post-infection, to assess the efficacy of dihydroartemisinin against juvenile *S. mansoni*. Mice were treated with dihydroartemisinin using various protocols with the total drug dose of 900 mg/kg, to investigate the efficacy of dihydroartemisinin against the schistosomula of *S. mansoni*. In addition, changes to the genital system among worms surviving dihydroartemisinin treatment, were recorded. An oral dose of dihydroartemisinin of 300 mg/kg was given to mice on Days 14, 16, 18, 20, 21, 22, 24, 26 or 28 days post-infection; this resulted in a 65.0-82.4% reduction in total worm burden and a 70.9-83.0% female worm burden. Better results were seen when treatment was given 20-24 days post-infection. Administration of multiple-dose and low-oral-dose dihydroartemisinin (at doses of 90, 180, 300 and 450 mg/kg) at different times, reduced total worm burdens by 88.7-99.1% and female worm burdens by 93.2-99.5%. The egg tubercles in mice livers were significantly reduced following treatment; in some mice no egg tubercles were found. These findings indicate dihydroartemisinin exhibits high *in vivo* activity against the schistosomula of *S. mansoni*. It causes damage to the genital system of worms, influences the development of *S. mansoni* worms, reduces the oviposition of surviving worms and enhances the formation of granulomas around tissue-trapped eggs, thereby reducing damage to the infected mammalian host.

**Keywords:** *Schistosoma mansoni*, dihydroartemisinin, schistosomula, *in vivo* effect, genital system, mice

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## INTRODUCTION

Schistosomiasis is a major tropical parasitic disease caused by infection due to *Schistosoma* species. The main pathology in schistosomiasis is due to formation of granulomas around tissue-trapped eggs and the resultant organ damage. The parasite expresses numerous glycoconjugates that provoke humoral and cellular immune responses among infected mammalian hosts (Amsden *et al*, 1980; Boros 1989; Wynn *et al*, 2004; Van de Vijver *et al*, 2006), resulting in a granulomatous reaction around trapped parasite eggs in the host liver, bladder and intestine (Sadun *et al*, 1969; Wynn *et al*, 2004). Granulomatous inflammation in the liver may result in fibrosis, scarring, portal hypertension, hemorrhaging and death in the worst cases (Boros, 1989). The egg is the major pathogenic agent in schistosomiasis (Sadun *et al*, 1969); decreasing or inhibiting oviposition of worms can reduce damage to the host. It is better to kill the schistosome larvae to prevent them from developing to maturity.

Dihydroartemisinin is the main metabolite of the mother compound artemisinins, as well as other artemisinin derivatives, such as artemether and artesunate (Li *et al*, 2011a). Artemether and artesunate are effective against *S. japonicum* and *S. mansoni* in experimentally infected mice (Utzinger *et al*, 2000, 2001, 2002; Xiao *et al*, 1995, 2000a,b, 2002a,b, 2006). Dihydroartemisinin exhibits *in-vivo* efficacy against juvenile and adult worms of *S. japonicum* and *S. mansoni* (Li *et al*, 2011b,c,d, 2012), and is more effective against 7-day-old schistosomula and 35-day-old adult worms of *S. japonicum* (Li *et al*, 2011b,c,d). Dihydroartemisinin has better efficacy against the schistosomula of *S. mansoni* aged 14-28 days (Li *et al*, 2012). However,

the activity of dihydroartemisinin against juvenile *S. mansoni* using different treatment protocols remains unclear. The present study used a mouse model to assess the effect of dihydroartemisinin against the schistosomula of *S. mansoni* using various treatment protocols, and investigated changes in the genital system of surviving worms following treatment.

## MATERIALS AND METHODS

### Drugs

The dihydroartemisinin used in the present study was kindly provided by the Chongqing Holley Wuling Mountain Pharmaceutical Company (Zhongduo Town, Youyang County, Chongqing, China; batch no. 20090201-2; 99.8% purity). The drug was ground in a ball miller with dimethyl sulfoxide (DMSO), Tween-80 and 1% carboxymethylcellulose sodium to give suspension solutions containing 3.6, 7.2, 12 or 18 grams of dihydroartemisinin/liter, 4-8% (v/v) DMSO and 0.5% (v/v) Tween-80. The volume of each dose given to the mice was 25 ml/kg using four different regimens (90, 180, 300 and 450 mg/kg). Drugs were administered by oral gavage feed to each mouse in doses related to body weight (a 20 g mouse bodyweight corresponding to a dose of 0.5 ml).

### Parasite, snail and mouse

*S. mansoni* cercariae (obtained from the Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan) were collected when released from infected *Biomphalaria glabrata* snails (obtained from the School of Clinical Veterinary Science, University of Bristol, UK) and maintained at the Key Laboratory of Technology for Parasitic Disease Prevention and Control, Ministry of Health, the People's Republic of China (Wuxi, China). Six to eight week-

old female mice of the ICR strain, each weighing 20-24 g, were purchased from Yangzhou University (Yangzhou, China), and given free access to food and water.

#### **Infection of mice and treatment protocol**

Each mouse was infected with 200 *S. mansoni* cercariae using the wading methods described previously (Smithers and Terry, 1965), and then randomly assigned into groups.

In the first experiment designed to assess the efficacy of dihydroartemisinin against juvenile *S. mansoni* aged 14-28 days, the infected mice were randomly divided into 10 groups of 10 mice each group, and treated with a single oral dose of the drug (300 mg/kg bodyweight) on Days 14, 16, 18, 20, 21, 22, 24, 26 or 28 post-infection (one group of mice was used for each treatment time). The remaining untreated mice served as controls.

The second experiment was designed to investigate the efficacy of dihydroartemisinin using various treatment protocols against the schistosomula of *S. mansoni*, at a total drug dose of 900 mg/kg. Mice in Group 1 were administered with dihydroartemisinin 90 mg/kg daily for 10 successive days on Days 15-24 post-infection. Mice in Group 2 were given dihydroartemisinin 180 mg/kg daily on Days 14, 16, 18, 19 and 21 post-infection. Mice in Group 3 were given dihydroartemisinin at 180 mg/kg daily on Days 15, 17, 19, 21 and 23 post-infection. Mice in Group 4 were given dihydroartemisinin at 180 mg/kg daily on Days 14, 17, 21, 24 and 28 post-infection. Mice in Group 5 were given dihydroartemisinin at 180 mg/kg daily on Days 17, 19, 21, 23 and 25 post-infection. Mice in Group 6 were given dihydroartemisinin at 180 mg/kg daily on Days 19, 20, 21, 22 and 23 post-infection. Mice in Group 7 were given

dihydroartemisinin at 180 mg/kg daily on Days 21, 23, 24, 26 and 28 post-infection. Mice in Group 8 were given dihydroartemisinin at 300 mg/kg daily on Days 17, 19 and 21 post-infection. Mice in Group 9 were given dihydroartemisinin at 300 mg/kg daily on Days 20, 21 and 22 post-infection. Mice in Group 10 were given dihydroartemisinin at 450 mg/kg daily on Days 20 and 21 post-infection. Untreated mice served as controls.

In the third experiment designed to investigate changes in the genital system of 60-day-old worms who survived dihydroartemisinin treatment, preparation and treatment of infected mice was conducted the same as in the second experiment for Groups 1, 4, 5 and 7. An additional group of infected mice not treated was used as a control. The mice were then sacrificed 60 days post-infection and any adult *S. mansoni* worms in the hepatic or portomesenteric veins were collected and fixed in 10% formaldehyde solution. H&E staining (Li, 2000) of the gonads was performed to observe changes in the vitelline glands of female worms.

#### **Dissection of mice and statistical assessment**

In all the experiments, the mice were sacrificed 60 days post-infection and any adult *S. mansoni* worms in the hepatic and portomesenteric veins were recovered (Smithers and Terry, 1965), sexed and counted by means of anatomical microscopy. The reductions in the total number of worms recovered and in the number of female worms recovered were then calculated, along with percentages and compared with worms recovered from untreated control mice. Statistical analysis was performed using SPSS 17.0 (SPSS, Chicago, IL). Statistical significance for each reduction was estimated using

Table 1  
Effect of dihydroartemisinin treatment (a single dose of 300 mg/kg) on recovery of adult *S. mansoni* from mice experimentally infected 60 days earlier.

Administration days post-infection	No. of mice investigated	Mean total worm burden (SD)	Reductions (%)	Mean female worm burden (SD)	Reductions (%)
14	8	14.13 (3.36) <sup>a</sup>	75.8	5.63 (2.13) <sup>a</sup>	78.3
16	8	13.75 (4.62) <sup>a</sup>	76.4	5.75 (2.55) <sup>a</sup>	77.9
18	8	13.63 (4.75) <sup>a</sup>	76.6	5.50 (2.56) <sup>a</sup>	78.8
20	8	12.13 (3.72) <sup>a</sup>	79.2	4.88 (2.23) <sup>a</sup>	81.2
21	8	10.25 (3.06) <sup>a</sup>	82.4	4.50 (1.69) <sup>a</sup>	82.7
22	8	10.50 (2.67) <sup>a</sup>	82.0	4.50 (2.45) <sup>a</sup>	82.7
24	7	10.43 (4.31) <sup>a</sup>	82.1	4.43 (2.37) <sup>a</sup>	83.0
26	8	18.63 (5.29) <sup>a</sup>	68.0	7.50 (2.45) <sup>a</sup>	71.2
28	7	20.43 (5.19) <sup>a</sup>	65.0	7.57 (1.62) <sup>a</sup>	70.9
None (control)	10	58.30 (15.03)	-	26.0 (7.04)	-

<sup>a</sup>  $p < 0.01$  vs control.

Fisher's least significant-difference (LSD) tests; a  $p$ -value  $< 0.05$  was considered statistically significant.

#### Ethical approval

The animal experimentation in this study was performed in accordance with the recommendations of the Guidelines for the Care and Use of Laboratory Animals, Ministry of Science and Technology, People's Republic of China [(2006)398]. Mice were sacrificed using carbon dioxide asphyxiation in sealed containers. This study was approved by the Ethics Review Committee of Jiangsu Institute of Parasitic Diseases and the Key Laboratory on Technology for Parasitic Disease Prevention and Control, Ministry of Health, People's Republic of China.

## RESULTS

#### Effect of dihydroartemisinin on schistosomula of *S. mansoni*

In the first experiment, treatment with a single oral dose of dihydroartemisinin

(300 mg/kg) on Days 14, 16, 18, 20, 21, 22, 24, 26 or 28 post-infection, resulted in reductions of total worm burdens of 75.8, 76.4, 76.6, 79.2, 82.4, 82.0, 82.1, 68.0 and 65.0%, and in reductions of female worm burdens of 78.3, 77.9, 78.8, 81.2, 82.7, 82.7, 83.0, 71.2 and 70.9%, respectively (Table 1). Greater efficacy was seen when treatment was given on Days 20-24 post-infection (Table 1).

#### Effect of dihydroartemisinin with different treatment protocols on juvenile *S. mansoni*

In the second experiment, the total worm burdens in groups 1-10 were reduced by 99.1, 96.2, 95.9, 95.0, 95.5, 96.8, 93.2, 89.9, 90.5 and 88.7%, while female worm burdens were reduced by 99.5, 97.1, 96.0, 95.9, 96.0, 96.6, 93.2, 90.3, 90.9 and 89.1%, respectively (Table 2). The greatest reductions were seen in Group 1, while better results were achieved in Groups 2, 3, 4, 5, and 6, respectively (Table 2). The egg tubercles in mouse livers were significantly reduced and in some mice no egg tubercles were found (Fig 1). The bodies



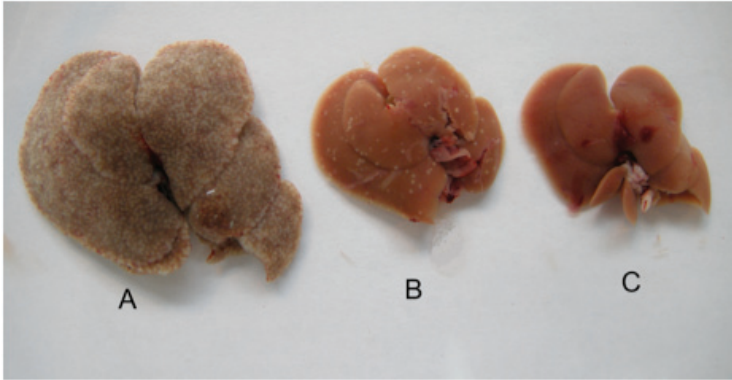


Fig 1—The liver obtained from mice 60 days post-infection with *S. mansoni*. A) The liver of a mouse in the control group with a large number of egg tubercles; B) the liver of a mouse in the treated group with only a few egg tubercles; C) the liver of a mouse in the treated group without egg tubercles observed.



Fig 2—60-day-old *S. mansoni* worms. A) Worms recovered from the control group; B) worms recovered from the treated group.

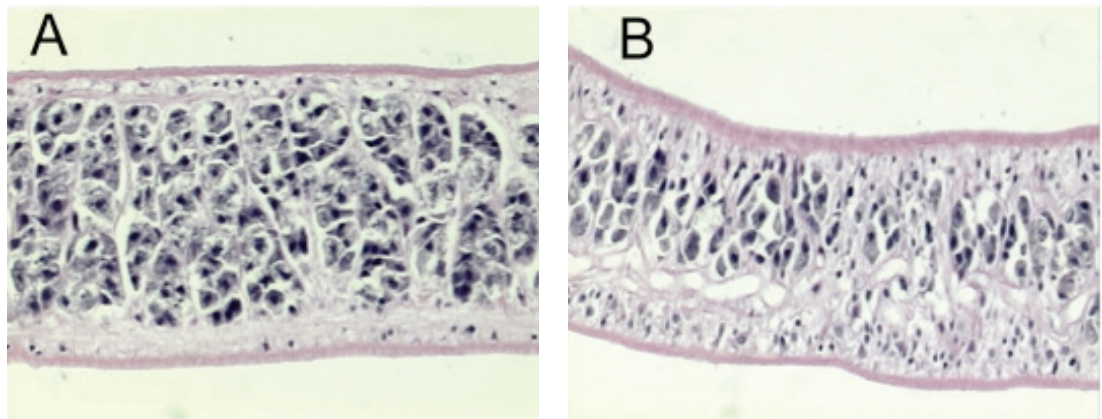


Fig 3—The vitelline glands of 60-day-old female worms of *S. mansoni* (HE staining, x200). A) The vitelline glands of female worms recovered in the control group; B) the vitelline glands of surviving female worms recovered in the treated groups.

of 60-day-old worms from mice without treatment were larger than those that survived treatment at 60 days (Fig 2).

#### Changes in vitelline glands of adult *S. mansoni* worms with and without dihydroartemisinin treatment

The vitelline glands of *Schistosoma* are more abundantly developed and distributed between the ovary and the worm tail, and around the cecum tube (Mao, 1990). The vitelline glands of 60-day-old female worms not treated with dihydroartemisinin were plump, while the vitelline glands of surviving treated female worms were atrophied (Fig 3).

#### DISCUSSION

Praziquantel is the current drug of choice to treat human schistosomiasis.

Table 2  
Effects of dihydroartemisinin with different treatment protocols (total dose of 900 mg/kg) on the recovery of adult *S. mansoni* from mice experimentally infected 60 days earlier.

Group	Administration days post-infection	Dosage (mg/kg)	No. of mice investigated	Mice without egg tubercles in liver	Mean total worm burden (SD)	Reductions (%)	Mean female worm burden (SD)	Reductions (%)
1	15, 16, 17, 18, 19, 20, 21, 22, 23, 24	90	10	10/10	0.5 (0.71) <sup>a</sup>	99.1	0.10 (0.32) <sup>a,b</sup>	99.5
2	14, 16, 18, 19, 21	180	8	5/8	2.13 (1.96) <sup>a</sup>	96.2	0.63 (0.74) <sup>a</sup>	97.1
3	15, 17, 19, 21, 23	180	8	6/8	2.25 (2.12) <sup>a</sup>	95.9	0.88 (0.99) <sup>a</sup>	96.0
4	14, 17, 21, 24, 28	180	9	5/9	2.78 (2.73) <sup>a</sup>	95.0	0.89 (0.93) <sup>a</sup>	95.9
5	17, 19, 21, 23, 25	180	8	5/8	2.50 (2.20) <sup>a</sup>	95.5	0.88 (0.99) <sup>a</sup>	96.0
6	19, 20, 21, 22, 23	180	8	6/8	1.75 (1.16) <sup>a</sup>	96.8	0.75 (0.71) <sup>a</sup>	96.6
7	21, 23, 24, 26, 28	180	8	3/8	3.75 (2.96) <sup>a</sup>	93.2	1.50 (1.20) <sup>a</sup>	93.2
8	17, 19, 21	300	8	2/8	5.63 (2.77) <sup>a</sup>	89.9	2.13 (1.55) <sup>a</sup>	90.3
9	20, 21, 22	300	8	3/8	5.25 (3.01) <sup>a</sup>	90.5	2.0 (1.60) <sup>a</sup>	90.9
10	20, 21	450	8	2/8	6.25 (3.69) <sup>a</sup>	88.7	2.38 (1.30) <sup>a</sup>	89.1
11	None (control)	-	10	-	55.5 (17.47)	-	21.90 (5.90)	-

<sup>a</sup>  $p < 0.01$  vs control, <sup>b</sup>  $p < 0.05$  vs Group 10.

However, it has been shown sustained drug pressure on *S. mansoni* under laboratory conditions promotes praziquantel resistance (Fallon and Doenhoff, 1994), as has been found in some schistosome-endemic areas of Africa and North America (Fallon *et al*, 1995; Stelma *et al*, 1995; Ismail *et al*, 1996; Melman *et al*, 2009). The likelihood of the development and spread of praziquantel resistance among schistosomes remains uncertain, but if it occurs, it could severely affect the treatment, control and prevention of schistosomiasis since praziquantel-based chemotherapy is part of national schistosomiasis control programs in many countries endemic for the parasite. However, there is a problem with praziquantel despite its success in controlling schistosomiasis: the drug targets mature adult worms but has only minor or no activity against the juvenile worm stages (such as schistosomula) (Doenhoff *et al*, 2008; Huang and Xiao, 2008).

It has been reported artemether causes severe degenerative changes in the ovaries and vitelline glands of female *S. mansoni* worms and in the testis of male worms (Xiao *et al*, 2004). In the present study dihydroartemisinin exhibited strong activity against the schistosomula of *S. mansoni* at a low dose following successive treatment. Dihydroartemisinin kills juvenile *S. mansoni* worms before they develop into adults and suppresses the parasite from laying eggs, thereby reducing the formation of egg granulomas and hepatic fibrosis. Dihydroartemisinin also affects the development of *S. mansoni*, causing damage to the vitelline glands of female worms. Therefore, dihydroartemisinin can inhibit and reduce oviposition among surviving worms reducing the likelihood of damage to the mammalian host.

The development of *S. mansoni* is lon-

ger than *S. japonicum* (Mao, 1990), giving a long time to control transmission of the parasite due to a longer schistosomulum stage. Dihydroartemisinin may be a better drug for the prevention and treatment of *S. mansoni* infections. Dihydroartemisinin, should be considered as a potential drug for the treatment of *S. mansoni* infections. Studies to assess the efficacy of dihydroartemisinin against *S. mansoni* human infections should be carried out in the schistosome-endemic foci to validate this hypothesis.

Our findings demonstrate dihydroartemisinin exhibited good activity against the schistosomula of *S. mansoni*. It causes damage to the genital system of worms, influencing the survival of *S. mansoni*, inhibiting oviposition and formation of egg granulomas, reducing damage to the infected mammalian host.

#### ACKNOWLEDGEMENTS

Thanks to Mr Yong-Liang Xu and Ms Jing Yang for their kind help during the animal experiments. This study was supported by the National Science & Technology Pillar Program of China (2009BAI78B06), Jiangsu Department of Health (X200911, X200912 and X201111), and Jiangsu Preventive Medicine Association (Y201031).

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