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Antischistosomal activity of artemether in experimental Schistosomiasis mansoni

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ABSTRACT

Objective

To evaluate the effect of *intramuscular injection of artemether in mice experimentally infected with Schistosoma mansoni, at the time of infection, during schistosomula maturation and after the beginning of egg-laying.*

Methods

Eighty adult females Balb/c mice were divided into 8 groups with 10 animals each. Seven groups were infected with S. mansoni using 60 cercariae for each animal, inoculated subcutaneously, and the remaining group was maintained without infection. Among the seven infected groups, six were treated

with artemether, according to the following schedule: three groups received doses of 100 mg/kg on days 0, 20 or 60 after inoculation of the cercariae; the other three received 50 mg/kg of artemether, also on days 0, 20 or 60. At the end of the 9th, 10th and 11th weeks after infection all the mice infected with *S. mansoni* were submitted to fecal examination using the Kato-Katz technique. On the 80th day of the experiment, the surviving animals were sacrificed and submitted to perfusion of the portal system in order to recover the worms. Body, liver and spleen weights of each animal were determined at that time.

Results

A reduction in egg-laying and the number of worms recovered was observed in mice treated with artemether (50 or 100 mg/kg) on the 20th day after infection. The decrease in the number of worms was more notable among *S. mansoni* females. A significant decrease in liver and spleen weights was also seen on the 20th day among animals treated with 50 or 100 mg/kg of artemether and also among those that received the drug at a dose of 50 mg/kg 60 days after infection.

Conclusions

Evidence of the antischistosomal activity of artemether was shown, even at a dose of 50 mg/kg, when the drug was administered during the schistosomula maturation period in the portal system of the vertebrate host.

Keywords

Schistosoma mansoni. Artemisinins, therapeutic use. Schistosomiasis mansoni, drug therapy. Animal experimentation. Artemether.

INTRODUCTION

It is believed that at least 2.5 million people in Brazil carry *Schistosoma mansoni* and around 25 million individuals are exposed to the risk of contracting it.⁹

Two drugs have been widely used in the treatment of the disease, with good efficacy and low toxicity: oxamniquine and praziquantel. Over recent years, the latter has been used preferentially. In view of the possible development of tolerance or resistance to praziquantel, research into and production of new drugs for the prevention and cure of *Schistosoma mansoni* has become justified.⁴

Artemisinin derivatives, which are used with efficacy in the treatment of malaria, have also been shown to have anti-*Schistosoma* activity, especially artemether. The schistosomicide action of artemisinin was discovered in 1980 by Chinese scientists. In their study, when it was administered to animals infected experimentally with *Schistosoma japonicum*, it caused a marked reduction in worm load in comparison with control animals that were not treated (Chen et al *apud* Utzinger et al,¹¹ 2001).

In 1982, Le et al⁷ confirmed the schistosomicide property of artemether. Mice or dogs infected with *S. japonicum* and treated with this drug at various doses and utilizing different administration routes showed a significant reduction in worm load. The larval phases (schistosomulae) of *S. japonicum* were also shown to be susceptible to artemether. However, no effect was observed on trematode eggs.¹⁵ Subsequent studies have confirmed that other artemisinin derivatives have schistosomicide properties: artesunate,^{3,8} arteether¹⁴ and, recently, dihydroartemisinin.¹ In 1991 in Brazil, Araújo et al² studied artemether activity in hamsters and mice experimentally infected with *S. mansoni*, 45 days after penetration by the cercariae. Better results were also observed when the drug was administered via intramuscular route.

The present work has sought to evaluate the effect of the intramuscular administration of artemether in mice experimentally infected with *S. mansoni*, at the time of infection, during schistosomula maturation and after the beginning of egg-laying.

METHODS

Eighty adult female mice of the Balb/c lineage were utilized, divided into eight subgroups: six infected with *S. mansoni* and treated using artemether, one equally infected but not treated with artemether and, finally, one other subgroup that was not infected and not treated, as shown in Table 1.

Table 1 – Artemether treatment schedule, via intramuscular route, for the mice infected with *S. mansoni*.

Subgroup	No. of mice	Dose	Day of treatment
1	10	100 mg/kg of weight	zero
2	10	100 mg/kg of weight	20 a.i.
3	10	100 mg/kg of weight	60 a.i.
4	10	50 mg/kg of weight	zero
5	10	50 mg/kg of weight	20 a.i.
6	10	50 mg/kg of weight	60 a.i.
7	10	Infected control	Not treated
8	10	Free of infection	Not treated

a.i. = after infection

The mice in subgroups 1 to 7 were each infected with 60 cercariae of the BH strain of *S. mansoni*, via subcutaneous route. The BH strain of this trematode has been kept for more than 15 years in the schistosomiasis immunopathology laboratory of Instituto de Medicina Tropical de São Paulo (São Paulo Institute of Tropical Medicine), by utilizing specimens of *Biomphalaria glabrata* and hamsters. The inoculation of 60 cercariae was achieved by 1:6 dilution of an aliquot that contained 1800 cercariae per ml, with 0.2 ml injected into each mouse. The artemether, at doses of 50 and 100 mg/kg, was administered via intramuscular route to the mice in subgroups 1 to 6.

The effect of the artemether was evaluated by means of quantitative examination of feces, done according to the Kato-Katz method⁶ in the 9th, 10th and 11th weeks after the infection. Eighty days after the start of the experiment, the surviving animals were sacrificed and submitted to perfusion of the portal system for the recovery and counting of the worms present, according to the technique recommended by Pellegrino & Siqueira.¹⁰ At that time, the body, liver, and spleen weights of each mouse were determined.

Throughout the experiment the mice received feed and water *ad libitum* and their management was in accordance with the recommendations of the Colégio Brasileiro de Experimentação Animal (Brazilian College for Animal Experimentation).

The results were analyzed via non-parametric statistical tests, utilizing a significance level of 95% ($p=0.05$).

RESULTS

Table 2 shows the average number of *S. mansoni* eggs per gram of feces obtained from the different mouse subgroups in the 9th, 10th and 11th weeks after infection. In subgroups 2 and 5, treated with artemether on the 20th day after infection with doses of 100 mg/kg and 50 mg/kg, respectively, there was a marked reduction in the number of eggs found.

Table 2 - Results from the feces examinations (Kato Katz) between the 9th and 11th weeks after infection with *S. mansoni*, in the mice treated with artemether and untreated mice.

Subgroup (artemether dose)	No. of mice examined	Eggs per gram of feces		
		9 th week a.i.	10 th week a.i.	11 th week a.i.
1 (100 mg/kg, day 0)	10	239.0	168	158.4
2 (100 mg/kg, 20 days a.i.)	10	19.2	0	0
3 (100 mg/kg, 60 days a.i.)	6	75.4	85.7	0
4 (50 mg/kg, day 0)	9	163.0	125.3	112.0
5 (50 mg/kg, 20 days a.i.)	10	0	12.0	4.8
6 (50 mg/kg, 60 days a.i.)	10	103.2	138.0	7.2
7 (Untreated)	8	269.3	243.0	147.0

a.i. = after infection

Table 3 presents the data relating to the number of worms recovered after perfusion of the portal system of the surviving animals.

Table 3 – Average number of worms recovered from the mice infected with *S. mansoni* and treated with different doses of artemether.

Subgroup	No. of mice perfused	Females	Males	Worms
		N	N	N
1	10	3.0±3.85	9.0±4.07	12.0±6.97
2	10	0.1±0.31*	1.3±1.83*	1.4±1.9*
3	6	0.0	8.5±6.53	8.5±6.53
4	9	0.75±0.88	6.87±9.34	7.62±9.78
5	10	0.2±0.42*	2.8±6.83	3.0±6.81
6	10	0.125±0.35*	1.75±2.71*	1.85±2.95*
7	8	1.67±2.34	6.0±8.03	7.67±10.16

* Significant difference.

The ratios between the liver and spleen weights and body weights of the mice in the different groups studied are presented in Table 4. A significant difference was observed between the values found for animals in subgroups 2, 5 and 6 and those of the mouse subgroup that was infected but not treated with artemether (subgroup 7).

Table 4 – Ratios of spleen weight X 100/ body weight and liver weight X 100/ body weight in mice infected with *S. mansoni* and treated with artemether.

Subgroup	No. of mice examined	Spleen weight x 100/ body weight	Liver weight x 100/ body weight
1	10	1.137±0.44	6.817±1.68
2	10	0.675±0.09*	5.536±0.70*
3	6	1.078±0.24	6.732±0.56
4	9	1.262±0.80	7.426±2.31
5	10	0.704±0.22*	4.617±1.02*
6	10	0.705±0.18*	5.631±1.12*
7	8	1.46±0.60	8.021±1.85
8	10	0.402±0.09	4.443±0.80

* Significant difference

DISCUSSION

Schistosomiasis continues to occupy second position in the world among parasites, after malaria, in terms of the extent of endemic areas and number of people infected. Treatment using praziquantel is efficacious in the reduction of morbidity, although it fails to prevent reinfection. Thus, foci with high transmission rates still exist in endemic areas, despite the regular administration of this drug.¹³ In view of the possible development of tolerance and/or resistance to this drug, research into new alternatives for the prevention and cure of schistosomiasis have become justified.⁴

Artemisinin (qinghaosu) is the main active agent extracted from the leaves of *Artemisia annua*, a plant that is widely disseminated in China and which also grows naturally in Central Europe, the United States and Argentina. Its antimalarial activity was confirmed in 1971 and more than two million patients with malaria have been treated with this drug and its derivatives (artemether, artesunate and arteether) over that last 20 years.¹¹ The antischistosomal activity of artemisinin was discovered in 1980, when it was observed that its administration to animals experimentally infected with *S. japonicum* caused a marked reduction in the worm load, in comparison with control animals that were not treated.¹¹ These results were confirmed by the findings of Le et al⁷ (1982). In their turn, Yue et al¹⁵ (1984) verified the susceptibility of the schistosomulae of *S. japonicum* to artemether. They did not, however, observe any effect of this drug on the eggs of this trematode.

An experimental study made by Chinese researchers showed that artemether had greater activity against *S. mansoni* when the drug, used at a high dose (400 mg/kg), was utilized during the phase of schistosomula evolution (14 to 21 days after penetration by the cercariae), with lesser activity shown in relation to adult worms. A study done in Brazil (Araújo et al,² 1991) confirmed the moderate reduction in parasite load when artemether was used in mice carrying adult specimens of *S. mansoni*.

The present work sought to utilize lower doses of artemether, closer to those utilized in human beings in the treatment of malaria, with the objective of simulating situations that could arise in regions where malaria and schistosomiasis are endemic. The results obtained similarly suggested that there is greater activity of artemether on schistosomulae (Table 3). In the animals in subgroups 2 and 5 (infected and treated with 100 mg and 50 mg of artemether per kg of weight, on the 20th day after infection, at which time the maturation of the schistosomulae was taking place), fewer worms were

recovered after the perfusion of the portal system. These results were confirmed by the absence or low counts of eggs in the feces of mice in these subgroups in the 10th and 11th weeks after infection (Table 2). These findings partially coincide with those of Xiao & Catto¹² (1989) and Xiao et al¹³ (2000), who worked with mice infected with *S. mansoni* and observed that the parasites aged 14 and 21 days were the ones most susceptible to artemether. It should be stressed that lower mortality was observed in the subgroups treated with artemether (with the exception of subgroup 3, which received the drug when the *S. mansoni* specimens were already fully developed), than in subgroup 7 (mice infected with *S. mansoni* and not treated with artemether).

The animals in subgroups 3 and 6 – treated 60 days after infection, with 100 mg and 50 mg of artemether per kg of weight – presented lower numbers of female worms after the perfusion (Table 3) and the egg count was negative in the last week of the experiment (Table 2). Evidence was thus shown of the antiparasitic activity of artemether, especially in relation to schistosomulae and females of *S. mansoni*, even when lower doses of this drug were utilized.

In Brazil, there is practically no overlap between the endemic areas for malaria and schistosomiasis. However, in certain regions of the African continent, the transmission of these parasitoses takes place concomitantly, and the results from the use of praziquantel have been less promising than what was expected⁵. In these circumstances, the use of artemisinin derivatives with the aim of treating malaria cases may exert a significant influence on the reduction of the morbidity rate due to endemic schistosomiasis.

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