ORIGINAL ARTICLE

doi: 10.5216/rpt.v42i3.26930

TEGUMENTARY CHANGES IN TWO DIFFERENT STRAINS OF *Schistosoma mansoni* TREATED WITH ARTEMISININ AND ARTESUNIC ACID

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ABSTRACT

Different strains of *Schistosoma mansoni* can respond differently to conventional and experimental treatments. Therefore, the responses to potential schistosomicidal drugs must be checked with different parasite strains. This work aimed to analyze changes caused by different concentrations of artemisinin or artesunic acid administered on different days in the teguments of adult *S. mansoni* belonging to two Brazilian strains (BH and SJ), using scanning electron microscopy (SEM). Infected mice were treated with 300 or 500 mg/kg of artemisinin and artesunic acid, 30 or 45 days post infection. Fifteen days after treatment, worms were examined by SEM. Altered teguments were observed in males and females on both days of treatment with both compounds, but the injury with artesunic acid was more intense. The treatment utilizing 500 mg/kg of artesunic acid against the BH strain 30 days after the infection proved to be particularly effective, resulting in erosion, peeling, sensory structure damage, and vesicle formation on the tegument of males and females. It is concluded that artemisinin and artesunic acid showed qualitatively similar tegumentary changes in both genders of the BH and SJ parasite strains. However, changes induced by artesunic acid were more severe for the BH strain, which demonstrates greater susceptibility of this strain to this experimental treatment.

KEY WORDS: *Schistosoma mansoni*; BH and SJ strains; artemisinin; artesunic acid; scanning electron microscopy.

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Received for publication in: 12/3/2013. Reviewed in: 10/9/2013. Accepted in: 25/9/2013.

RESUMO

Alterações tegumentares em duas diferentes linhagens de *Schistosoma mansoni* submetidas a tratamento com artemisinina e ácido artesúnico

Diferentes linhagens de *Schistosoma mansoni* podem responder de formas distintas a tratamentos experimentais. Portanto, ensaios com potenciais fármacos esquistossomicidas necessitam ser realizados com várias linhagens. Este trabalho visou analisar as alterações causadas por diferentes concentrações de artemisinina e ácido artesúnico, administradas em diferentes dias, sobre o tegumento de adultos de *S. mansoni* de duas linhagens brasileiras (BH e SJ), utilizando-se microscopia eletrônica de varredura (MEV). Os camundongos infectados foram tratados com 300 e 500 mg/kg de artemisinina ou ácido artesúnico, 30 ou 45 dias após a infecção. Após 15 dias do tratamento, os vermes foram analisados pela MEV. A destruição do tegumento foi observada em machos e fêmeas nos dois dias de tratamento com ambos os compostos, mas observou-se que o ácido artesúnico provocou uma destruição mais intensa. O tratamento realizado com 500 mg/g de ácido artesúnico contra parasitos da linhagem BH, 30 dias após a infecção, mostrou ser o mais efetivo, resultando em erosão, descamação, destruição das estruturas sensoriais e formação de vesículas nos machos e nas fêmeas. Ficou evidenciado que as linhagens BH e SJ apresentaram alterações tegumentares diferentes quanto à intensidade, com ambos os compostos. No entanto, a destruição do tegumento foi mais intensa na linhagem BH, especialmente com o ácido artesúnico, o que demonstrou maior suscetibilidade desta linhagem a esse tratamento experimental.

DESCRITORES: *Schistosoma mansoni;* linhagens BH e SJ; artemisinina; ácido artesúnico; microscopia eletrônica de varredura.

INTRODUCTION

The surface of *Schistosoma mansoni* consists of two opposite lipid bilayers that are very close to one another and have the form of a cell membrane. As the tegument has no lateral membranes, its cytoplasm extends as a continuous unity, or syncytium, around the body of the worm, forming a syncytial complex that is responsible for nutrient absorption (glucose, amino acids, among others), metabolite excretion (lactic acid and others) and protection against attacks by the immune system of the host. Accordingly, the tegument of *S. mansoni* is a crucial target of drugs with attested schistosomicidal activity and new drugs that are intended to combat this worm (Hoockley & McLaren, 1973; Shuhua et al., 2000).

The first drug that was widely used for *S. mansoni* schistosomiasis treatment was oxamniquine. This medicine, discovered at the end of the 1960s, was described by Richards and Foster (Cioli et al., 1993, 1995) for the treatment of *S. mansoni* schistosomiasis. *In vivo* tests showed the schistosomicidal potential of oxamniquine against *S. mansoni,* especially in male worms (Foster & Cheetham, 1973). This drug can cause structural alterations in the tegument of the adult parasite (Khon et al., 1982; Ferrari et al., 2003), including vacuolization, erosion of the tegument surface and tubercle damage (Magalhães-Filho et al., 1987). However, some studies indicated disadvantages in the use of oxamniquine, such as side effects on the central nervous system (Davis, 1993, Soyez et al., 1996) and the low activity

in the period between the 3rd and the 9th week of infection, which coincided with the stage of egg production (Frézard & Melo, 1997).

The use of praziquantel (PZQ) for treatment of schistosomiasis was described in 1977 by Gönnert & Andrews. *S. mansoni* schistosomiasis treatment is now based on the use of this drug, a low-cost, well-tolerated and broad-spectrum antihelminthic that is highly effective against all *Schistosoma* species that cause infection in humans (Cioli & Pica-Mattoccia, 2002). Preclinical assays have also proven the efficiency of praziquantel in the treatment for 5-6 week infections, but failures were reported in cases of 1-5 week infections (Gönnet & Andrews, 1977; Webbe & James, 1977; Xiao et al., 1985; Sabah et al., 1986). This drug causes extensive damage to the tegument of adult *S. mansoni* (Becker et al., 1980). The morphological changes produced by this drug are followed by an increase in the number of antigens on the surface of the worm. These antigens are identified and connected to the host's immune response, which is required to complement the activity of the drug (Doenhoff et al., 1987; Brindley et al., 1989), causing the surface of the parasite to disrupt, which results in its death (Brickes et al., 1983).

After the development of praziquantel, there was not a high rate of progress in the development of therapies for *S. mansoni* schistosomiasis. The extensive use of both praziquantel and oxamniquine has produced cases of tolerance and resistance in some strains of *S. mansoni* (Parise-Filho & Silveira, 2001; Katz, 2008). The development of resistance in *Schistosoma* sp. has already been proven in experiments showing that sub curative doses of PZQ on several generations resulted in parasites that were less sensitive to the drug (Fallon & Doenhoff, 1994). Therefore, developing new effective schistosomicidal drugs is extremely urgent.

Research on bioactive natural products extracted from plants has been performed in the search for a new medicine that can replace the current drugs or complement them (Matos, 1994; Doenhoff et al., 2009). In this regard, artemisinin and artesunic acid, which are extracted from *Artemisia annua* (Qinghaosu) and are currently used as antimalarial drugs, have been tested for the treatment of schistosomiasis since 1980 (Utzinger et al., 2001). These compounds have reportedly shown activity against *S. mekongi, S. japonicum* and different strains of adults and schistosomula of *S. mansoni*, such as LE, Liberian and Puerto Rico.

Tegumentary changes such as erosion, loss of tubercles in males, vesicle formation and collapse of the tegument in adult worms and schistosomula have been observed in strains of *S. mansoni* treated with artemisinin and artesunic acid (Araújo et al., 1999; Shuhua et al., 2000; Utzinger et al., 2002; Lu et al., 2004; Li et al., 2005; Lu et al., 2006). These data show the potential of both compounds as chemotherapeutic drugs in the treatment of schistosomiasis (Utzinger et al., 2000).

However, different strains of *S. mansoni* are known to respond differently to the same treatment. Such behavioral differences among the parasite strains in the vertebrate host are due to the worm's genotypic expression (Yoshioka et al., 2002).

Consequently, it is necessary to test the activity of potential schistosomicidal drugs on different strains of the worm.

The present work aimed to analyze the activity of both artemisinin and artesunic acid on the tegument of two Brazilian strains of *S. mansoni*, namely BH and SJ, by scanning electron microscopy (SEM).

MATERIAL AND METHODS

Parasite, intermediate and definitive hosts

Cercariae of BH (Belo Horizonte, Minas Gerais, -19°55'15"/-43°56'16", Brazil) and SJ (São José dos Campos, São Paulo, -23°10'46"/-45°53'13", Brazil) strains of *S. mansoni* were obtained from the infection of two species of mollusc, *Biomphalaria glabrata* and *B. tenagophila*, respectively, the former being sympatric with the first strain and the latter, with the second strain. As suggested by Souza et al. (1987), the molluscs were kept at the Department of Animal Biology – Institute of Biology – Unicamp. Swiss female mice (*Mus musculus*), provided by the Multidisciplinary Centre for Biological Investigation – Unicamp, were used as definitive hosts.

Planorbidae molluscs were exposed to light at 28ºC for two hours (Pellegrino & Macedo, 1955) and individually infected with newly hatched miracidia. *B. glabrata* specimens were infected with the BH strain, and *B. tenagophila* specimens were infected with the SJ strain. Forty days after the infection, the molluscs were individually exposed to light at the same temperature to release the cercariae. The infection of the definitive hosts, which were thirty days old, was performed individually by inserting their tails into the cercarial suspension (Olivier & Stirewalt, 1952). The protocol for these infection experiments (997-1) was approved by the Ethics Commission for Animal Experimentation (CEEA) of the Institute of Biology of Unicamp, in accordance with the ethical principles adopted by the Brazilian Association of Animal Experimentation (COBEA).

Drug, treatment and worm recovery

Artemisinin and artesunic acid were provided by the Chemical, Biological and Agricultural Pluridisciplinary Research Centre (CPQBA) – Unicamp.

PBS buffer solution was used to dissolve the samples. Mice were divided into two large groups, the first being treated 30 days after the infection (to observe the effect of the treatment on young adult worms) and the second being treated 45 days after the infection. Infected mice were treated intragastrically with 300 or 500 mg/kg of artemisinin or artesunic acid for five consecutive days (De Clercq, 2000a; 2000b). The group of control animals received only PBS buffer, also by the intragastric route. Fifteen days after the treatment, mice were sacrificed by cervical

dislocation, and the worms were retrieved by perfusion of the hepatic portal system (Yolles et al., 1947).

Scanning electron microscopy (SEM)

Worms were washed in NaCl solution (0.9%) and separated into three groups: males, females and mated pairs. While still alive, they were fixed in Karnovsky's solution (2.5% glutaraldehyde and 4% paraformaldehyde) buffered with sodium cacodylate at 0.1M, pH 7.4, for 10 hours. Then, they were washed in the same solution, dehydrated in growing concentrations of ethanol, post-fixed in osmium tetroxide at 1%, critical point-dried, mounted on aluminum stubs, coated with gold using Sputter Coater and examined with a "Jeol JSM 5800 LV Scanning Electron Microscope" (Silveira, 1998).

RESULTS

The characteristics of the tegument of the untreated control group (Figure 1-a; b; c; d; e; f) were compared with those of groups treated, with each schistosomicidal drug.

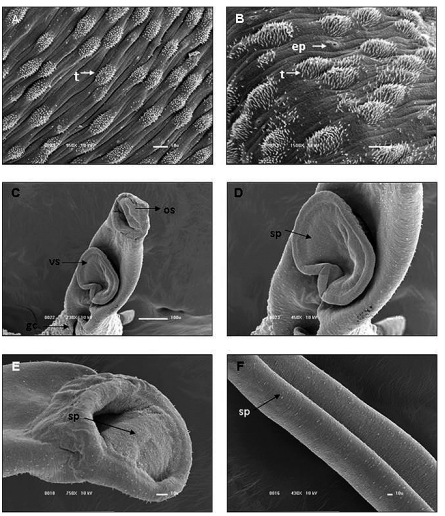

Figure 1. Normal tegument of adult male and female *Schistosoma mansoni* worms (control group). A and B – normal tegument of a male worm. C, D and E – suckers of a normal male worm. E and F – normal tegument of a female worm. t – tubercles with spines, male worm; ep – excretory pore; os – oral sucker; vs – ventral sucker; gc – gynaecophoric canal; sp – sensory papillae.

Males and females of the BH strain that were subjected to treatment with artesunic acid 30 days after the infection showed large tegumentary changes with both doses, 300 and 500 mg/kg (Figure 2- a, b). On the dorsal surface of females, the tegument was largely altered, and extensive peeling and erosion were observed in addition to the disappearance of some areas of the sensory structures (Figure 2-a). On the dorsal surface of males, particularly with the dose of 500 mg/kg, the tegument was largely shed and eroded, as on the dorsal surface of females. It was not possible to verify the presence of spines on the tubercles because these structures were largely damaged (Figure 2-b). The appearance of some vesicles occurred in both sexes (Figure 2- a, b), but changes were not detected on their suckers.

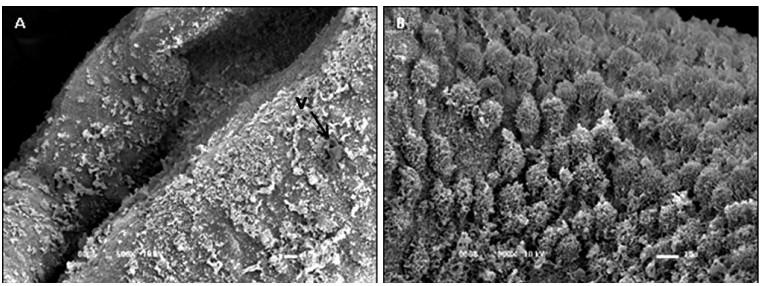

Figure 2. Activity of artesunic acid on the tegument of adult *Schistosoma mansoni* worms belonging to the BH strain. A. Tegumentary changes in a female worm subjected to treatment with 300 mg/kg of artesunic acid (the treatment was carried out 30 days after the infection). B. Activity of artesunic acid (500 mg/kg) on the tegument of a male worm, resulting in tubercle damage (treatment carried out 30 days after the infection). v – vesicle.

The treatment with artemisinin in the same doses, on the same day and for the same strain of worms showed less evident and less extensive tegumentary changes in both males and females (Figure 3- a, b). Females showed peeling of the tegument in the treatment with the smaller dose 30 days after the infection (Figure 3-a). With the dose of 500 mg/kg, it was possible to observe the presence of leukocytes of the host adhered to the tegument of males (Figure 3-b). In the treatment performed with artemisinin 45 days after the infection, male worms had their tubercles and spines destroyed, and the damage was more intense on that day than on the 30th day. Excretory pores of such males also became swollen (Figure 3-c).

Regarding the worms of the SJ strain that were subjected to the treatment with artesunic acid, changes in suckers and tegument were observed in males 45 days after the infection with the dose of 300 mg/kg (Figure 5-a; b). Adhesion of the leukocytes of the host could be observed in these structures, and the number of leukocytes was proportional to the administered dose of artesunic acid (Figure 5-a).

On the dorsal surface of males, spines and tubercles showed few changes, but some of the tubercles were destroyed. In addition, swelling of the tubercles appeared on the tegument (Figure 5-c).

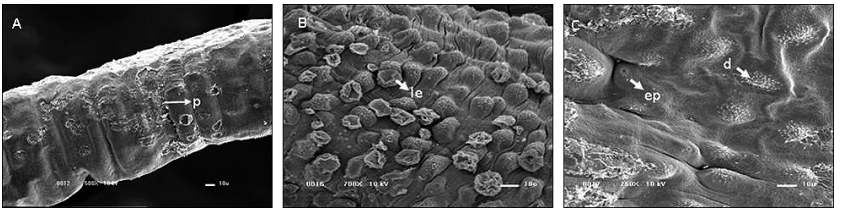

Figure 3. Activity of artemisinin on the tegument of adult *Schistosoma mansoni* worms belonging to the BH strain. A. Tegumentary changes in a female worm subjected to treatment with 300 mg/kg of artemisinin 30 days after the infection. p – peeling. B. Leukocytes of the host adhered to the tegument of the male worm (500 mg/kg of artemisinin, 30 days after the infection). le – adhered leukocytes. C. Change in tubercles of male worms (300 mg/kg of artemisinin 45 days after the infection). p – peeling; ep – excretory pore; d – tubercle damage.

The treatment with artemisinin for worms of the SJ strain showed changes in the tegument only 45 days after the infection, with the dose of 300 mg/kg (Figure 4-a). In males, the tegumentary changes on the dorsal surface were more intense with the dose of 500 mg/kg, administered in the same time period, and spine loss was observed on some tubercles (Figure 4-b). There were leukocytes of the host adhered to the tegument (Figure 4-a; b).

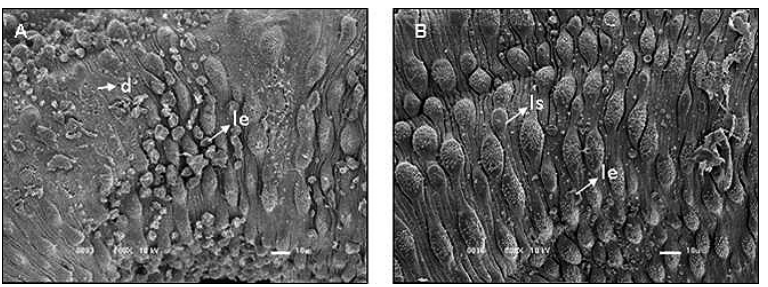

Figure 4. Activity of artemisinin on the tegument of adult *Schistosoma mansoni* worms belonging to the SJ strain. A. Tegumentary changes, including tubercle damage, in a male worm subjected to treatment with 300 mg/kg of artemisinin 45 days after the infection. d – tubercle damage. B. Tegumentary changes in a male worm, including loss of spines and some tubercles (500 mg/kg of artemisinin 45 days after the infection). le – leukocytes of the host; ls – tubercles showing loss of spines.

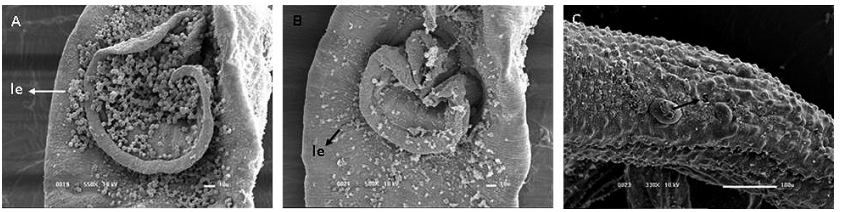

Figure 5. Activity of artesunic acid on the tegument of adult *Schistosoma mansoni* worms belonging to the SJ strain. A. Ventral sucker of a male worm subjected to treatment with 500 mg/kg of artesunic acid 45 days after the infection, with leukocytes adhered to the oral sucker. le – leukocytes of the host. B. Host leukocytes adhered to the oral sucker of a male worm (300 mg/kg of artesunic acid 45 days after the infection). C. Swollen tubercle of a male worm (300 mg/kg of artesunic acid 45 days after the infection). s – swollen tubercle.

DISCUSSION

The tegument of *Schistosoma* spp. is an important target of the activity of schistosomicidal drugs. Changes in the tegument of the parasite have been described after the use of drugs such as praziquantel, such as swelling of certain structures, vacuolization, blisters and surface shedding (Shuhua et al., 2000).

Tegumentary changes produced by these drugs are a necessary mechanism to cause the death of the worm. Injuries to the suckers, for instance, render the worms unable to adhere to blood vessel walls, while damage inflicted upon the tegument compromise its action and destroy the immune system of the worm, making it vulnerable to the immune system of the host (Xiao et al., 2000). Changes in worms caused by artemisinin observed in this work were in accordance with those described by Shuhua et al. (2000) after treatment with a single dose of artemether of 400 mg/kg against the Liberian strain 42 days after the infection. In both treatments with artesunic acid and artemisinin, tegumentary changes included loss of spines and damage to tubercles in males and shedding of the tegument in females. Afterwards, exposition of sensory structures, erosion of the tegument and vesicle formation can occur, as well as adhesion of leukocytes from the host, which harm the surface of the tegument and the suckers and are possibly the cause of worm death.

According to Shuhua et al. (2001, 2002) and Guo et al. (1997), the changes inflicted upon the tegument of *S. mansoni* by artesunate are similar to those caused by artemether. Shaohong et al. (2006) reported changes on tubercles and spines of male worms treated with artesunic acid after 60 days of infection, whereas sensory structures were not affected. The authors also reported the adhesion of leukocytes of the host to the tegument of male and female worms. In this work, a severe injury to the tegument of young male and female worms was observed when they were

subjected to treatment with artesunic acid 30 days after the infection. The treatment was particularly effective against the BH strain.

Studies performed with artemisinin and artesunic acid against *S. mansoni* and *S. japonicum* have indicated that the younger worms are the most susceptible to the drugs. In the present study, the damage caused by the treatments performed after 30 days of infection (when the worms are still young) was more intense with both compounds than after 45 days. The age-based susceptibility of the parasites can be related to the chemical structures and the pharmacodynamic properties of these drugs (Shuhua et al., 2000; Utzinger et al., 2000).

Concerning the effects of the two treatments on the BH and SJ parasite strains in the present investigation, it was noticed that the treatment with artemisinin was more harmful to the BH strain (higher degree of damage to spines and tubercles and increased swelling of excretory pores in males, stronger shedding in females and more adhesion of leukocytes in both sexes) than to the SJ strain. This difference was most conspicuous in the treatment performed 30 days after infection. The use of artesunic acid against the SJ strain resulted in some important but less severe lesions, mainly at the latest time point of the experimental period (45 days).

According to Xu (1998), artesunic acid is less toxic than artemether, but, as noted by Ultinger (2002), the former is less effective against *S. mansoni*. However, Shaohong et al. (2006), using the Puerto Rico strain, have proven the superior effect of artesunic acid. In the present work, it was noticed that the age of the worm and its strain make it more or less susceptible to the treatment. SEM observations made it possible to conclude that the damage of the tegument of male and female worms was more intense with the use of artesunic acid against the BH strain 30 days after the infection.

According to Magalhães et al. (1975), the strains of *Schistosoma* spp., in particular BH and SJ, have different intermediate hosts, biological behaviors, morphological characteristics and pathogenic activity, whereupon they can present different responses to the activity of the same drug. Therefore, a strong need exists for comparative studies of the strains so that their susceptibility to different drugs can be analyzed.

Derivatives of artemisinin (arteether, artemisone, artelinic and artesunic acids, among others) are used for malaria treatment, and their activity against *Schistosoma* spp. was described for the first time in the 1980s in China. The administration of this compound in animals that were experimentally infected with *S. japonicum* was shown to reduce their worm burden (Chen et al., 1980).

Clinical assays performed with Senegalese children under 6 years old in areas with overlapping distributions of malaria and urinary schistosomiasis (caused by *S. haematobium*) showed that amodiaquine or sulfadoxine/pyremethamine co-administered with artesunate contributed to the activity observed against *Schistosoma* spp. infections; one can reasonably assume that artesunate was the active drug (Boulanger et al., 2007). These data suggest that in some regions of

the globe where areas for malaria and schistosomiasis are co-endemic, the use of artemisinin derivatives, intending to treat malaria cases, can reduce the morbidity rate of endemic schistosomiasis (Lescano et al., 2004; Lu et al., 2010).

In Brazil, areas where malaria and schistosomiasis are co-endemic are a rarity. However, the present work is important because it aimed to compare the activity of artemisinin and artesunic acids in two strains of *S. mansoni* that have significant epidemiological relevance in this country. Recent preclinical assays demonstrated the efficacy of artesunic acid for juveniles (7 and 21 day old) of the *S. mansoni* Egyptian strain, while also protecting the host from the damage caused by the parasite eggs and opening a discussion about the use of this compound in the control of schistosomiasis in areas where malaria is not endemic (El-Beshbish et al., 2013).

The activity of artemisinin derivatives for juvenile stages of *Schistosoma* spp., a period in which treatment with PZQ fails, makes artemisinin derivatives potential candidates for schistosomiasis prophylactic drugs (Yue et al. 1984; Lu et al. 2010).

Regarding toxicity, clinical tests proved that artemisinin and its derivatives are well tolerated when administered orally (Nontprasert et al., 2002).

According to Shaohong et al. (2006), the use of artesunic acid in the treatment for *S. mansoni* schistosomiasis in humans is close to being implemented, as the half-life of the drug is too short to allow the parasite to develop any sort of tolerance or resistance. Nevertheless, it is first necessary to study the effect of artesunic acid on the worm, i.e., to determine what mechanism causes the death of the worm – which highlights the importance of SEM – and to study its activity on different strains of *S. mansoni* (El-Beshbish et al., 2013).

ACKNOWLEDGMENTS

The authors thank FAPESP for financial support.

REFERENCES

1. Araújo N, Kohn A, Katz N. Therapeutic evaluation of artesunate in experimental *Schistosoma mansoni* infection. *Rev Soc Bras Med Trop 32:* 7-12, 1999.
2. Becker B, Mehlhorn H, Andrews P, Thomas H, Eckert J. Light and electron microscopic studies on the effect of praziquantel on *Schsitosoma mansoni, Dicrocoelium dendriticum,* and *Fasciola hepatica* (Trematoda) in vivo. *Parasitol Res 63:* 113-128, 1980.
3. Brickes CS, Depenbusch JW, Benett JL, Thompson DF. The relationship between tegumental disruption and muscle contraction in *Schistosoma mansoni* exposed to various compounds. *Parasitol Res 69:* 61-67, 1983.
4. Brindley PJ, Strand M, Norden AP, Sher A. Role of host antibody in the chemeotherapeutic action of praziquantel against *Schistosoma mansoni:* identification of target antigens. *Mol Biochem Parasit 34:* 99-108, 1989.
5. Boulanger D, Dieng Y, Cisse B, Remoue F, Capuano F, Dieme JL, Ndiaye T, Sokhna C, Trape JF, Greenwood B, Simondon F. Antischistosomal efficacy of artesunate combination therapies

administered as curative treatments for malaria attacks. *Trans R Soc Trop Med Hyg 101:* 113-116, 2007.
6. Chen DJ, Fu LF, Shao PP, Wu FZ, Fan CZ, Shu H, Ren CS, Sheng XL. Studies on antischistosomal activity of qinghaosu in experimental therapy. *Zhong Hui Yi Xue Zha Zhi 80:* 422-428, 1980.
7. Cioli D, Pica-Mattoccia L, Archers S. Drug resistance in schistosomes. *Parasitol Today 9:* 162-166, 1993.
8. Cioli D, Pica-Mattoccia L, Archers S. Antischistosomal drugs: past, present and future? *Pharmacol Ther 68:* 35-85, 1995.
9. Cioli D, Pica-Mattoccia L. Praziquantel. *Parasitol Res 90:* S3-S9, 2002.
10. Davis A. Antischistosomal drugs and clinical pratice. *In*: Jordan P, Webbe G, Sturrock RF. *Human Schistosomiasis*. CAB International. United Kingdom, 1993.
11. De Clercq D, Vercruysse J, Veré P, Niasse F, Kongs A, Diop M Efficacy of artesunate against *Schistosoma mansoni* infections in Richard Toll, Senegal. *T Roy Soc Trop Med Hyg 94:* 90-91, 2000a.
12. De Clercq D, Vercruysse j, Veré P, Niasse F, Kongs A, Diop M. What is the effect of combining artesumate and praziquantel in the treatment of *Schistosoma mansoni* infections? *Trop Med Int Health 5:* 744-746, 2000b.
13. Doenhoff MJ, Sabah AA, Fletcher C, Webbe G, Bain J. Evidence for an immune-dependent action of praziquantel on *Schistosoma mansoni* in mice. *T Roy Soc Trop Med Hyg 81:* 947-951, 1987.
14. Doenhoff MJ, Hagan P, Cioli D, Southgate V, Pica-Mattoccia L, Botros s, Coles G, Tchuem-Tchuente LA, Mbaye A, Engels D. Praziquantel: its use in control of schistosomiasis in sub-Saharan Africa and current research needs. *Parasitology 136:* 1825-1835, 2009.
15. El-Beshbish SN, Taman A, El-Malky M, Azab MS, El-Hawary A, El-Tantawy DA. *In vivo* effect of single oral dose of artemether against early juvenile satges of *Schistosoma mansoni* Egyptian strain. *Exp Parasitol* doi: http://dx.doi.org/10.1016/j.exppara.2013.07.006, 2013.
16. Fallon PG., Doenhoff MJ. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug especific. *Am J Trop Med Hyg 53:* 61-62, 1994.
17. Ferrari MLA, Coelho PMZ, Antunes CAP, Tavares AS, Cunhas AS. Efficacy of oxaminiquine ando praziquantel in the treatment of *Schistosoma mansoni* infection: a controlled trial. *B World Health Organ 81:* 190-196, 2003.
18. Foster R, Cheetham BL. Studies with the schistosomicide oxamniquine (UK-38 4271). I: activity in rodents and *in vitro*. *Trans R Soc Trop Med Hyg 6:* 674-684, 1973.
19. Frézard F, Melo AL. Evaluation of the schistomicidal efficacy of lipossome – entrapped oxamniquine. *Rev Inst Med Trop Sao Paulo 39:* 97-100, 1997.
20. Guo Y, Xu PS, Xuan YX, Wu LJ, Li SW. Observation on the effect of artesunate on ultrastructure of schistosomula with electron microscope. *Chinese Journal of Schistosomiasis Control 9:* 34-36, 1997.
21. Gönnert R, Andrews P. Praziquantel, a new broad-spectrum antischistosomal agent. *Parasitol Res 52:* 129-150, 1977.
22. Hoockley DJ, Mclaren DJ. *Schistosoma mansoni:* Changes in the outer membrane of the tegument during development from cercaria to adult worm. *J Parasitol 3:* 13-25, 1973.
23. Katz N. Terapêutica experimental da esquistossomose mansoni. *In:* Carvalho OS, Coelho PMZ, Lenzi HL Schistosoma mansoni & *esquistossomose uma visão multidisciplinar*, Fiocruz, Rio de Janeiro, 2008.
24. Kohn A, López-Alvarez ML, Katz N. Transmission and scanning electron microscopical studies in the tegument of male *Schistosoma mansoni* after oxamniquine treatment. *Annales de Parasitologie Humaine et Comparée 57:* 285-291, 1982.
25. Lescano SZ, Chieffi PP, Canhassi RR, Boulos M, Amato-Neto V. Atividade antiparasitária do artemether na esquistossomose mansônica experimental. *Rev Saúde Públ 38*: 71-75, 2004.
26. Li YS, Chen HG, He HB, Hou XY, Ellis M, Mcmanus DP. A double-blind field trial on the effects of artemether on *Schistosoma japonicum* infection in a highly endemic focus in southern China. *Acta Trop 96:* 184-190, 2005.

27. Lu SH, Yan XL, Li SW, Shi JF, Liu X, Yan XH, Yan MJ, Lou JL, Kumagai T, Wen LY et al. Prophylatic effect of artesunate against experimental infection of *Schistosoma mansoni. Chinese Journal of Parasitology Parasitic Diseases 22:* 20-23, 2004.
28. Lu SH, Kumagai T, Qinghua A, Xiaolan Y, Ohmae H, Yabu Y, Siwen L, Liyong W, Maruyama H, Otha N. Evaluation of the antihelmintic effects of artesunate against experimental *Schistosoma mansoni* infection in mice using different treatment protocols. *Parasitol Int 55:* 63-68, 2006.
29. Lu G, Hu X, Huang C, Lu Y, Wu L, Lihua L, Xu J, Yu X. Effect of artmether, hemin and Fe^{3+} on recombinant lactate dehydrigenase from *Schistosoma japonicum*. *Asian Pacific Journal of Tropical Medicine 3:* 930-933, 2010.
30. Magalhães-Filho A, Melo MEB, Padovan PA, Padovan PP. *Schistosoma mansoni*: structural damage after treatment with oxamniquine. *Mem Inst Oswaldo Cruz 82:* 347-352, 1987.
31. Magalhães LA, Alcântara FG, Carvalho JF. Alguns dados referentes ao estudo parasitológico e anatomopatológico de duas linhagens de *Schistosoma mansoni* Sambon, 1907. *Rev Saúde Pública 9:* 1-5, 1975.
32. Matos FJA. *Farmácias vivas: sistema de utilização de plantas medicinais. Projeto para pequenas comunidades, 2th ed*. EUFC, Fortaleza, 1994.
33. Nontprasert A, Pukrittayakames S, Dondorp AM, Clemens R, Looareesuwan S, Whitw NJ. Neuropathologic toxicity of artemisinin derivatives in a mouse model. *Am J Trop Med Hyg 67:* 423-429, 2002.
34. Olivier L, Stirewalt MA. An efficient method for exposure of mice to cercarie of *Schistosoma mansoni. J Parasitol 38:* 19-23, 1952.
35. Parise-Filho R, Silveira MAB. Panorama atual da esquistossomíase no mundo. *Brazilian J Pharm Sci 37:* 123-135, 2001.
36. Pellegrino J, Macedo DG. A simplified method for the concentration of cercariae. *J. Parasitol 41:* 329-330, 1955.
37. Sabah AA, Fletcher C, Webbe G, Doenhoff MJ. *Schistosoma mansoni:* chemotherapy of infections of different ages. *Exp Parasitol 61*: 294-303, 1986.
38. Shaohong L, Kumagai T, Qinghua A, Xiaolan Y, Ohmae H, Yabu Y, Siwen L, Liyong W, Maruyama H, Otha N. Evaluation of the antihelmintic effects of artesunate against experimental *Schistosoma mansoni* infection in mice using different treatment protocols. *Parasitol Int 55:* 63-68, 2006.
39. Shuhua X, Binggui S, Chollet J, Utzinger J, Tanner M. Tegumental changes in adult *Schistosoma mansoni* harbored in mice treated with artemether. *J Parasitol 86:* 1125-1132, 2000.
40. Shuhua X, Chollet J, Utzingeti J, Matile H, Jinyan M, Tauuneti M. Artemether administered together with haemin damages schistosomes *in vitro. Tr Roy S Trop Med Hyg 95:* 67-71, 2001.
41. Shuhua X, Binggui S, Utzinger J, Chollet J, Tanner, M. Transmission electron microscopic observations on ultrastructural damage in juvenile *Schistosma mansoni* caused by artemether. *Acta Trop 81:* 53-61, 2002.
42. Silveira M. Preparo de amostras biológicas para microscopia eletrônica de varredura. *In:* Souza W. *Técnicas básicas de microscopia eletrônica aplicadas às Ciências Biológicas.* Sociedade Brasileira de Microscopia, Rio de Janeiro, 1998.
43. Souza CP, Araújo N, Carvalho OS, Freitas JR. Potencialidade de *Biomphalaria tenagophila* do Lago da Pampulha, Belo Horizonte, MG, como hospedeira do *Schistosoma mansoni. Mem Inst Oswaldo Cruz. 82:* 67-70, 1987.
44. Soyez H, Schacht E, Vanderkerden S. The crucial role of spacer groups in macromolecular prodrug design. *Adv Drug Delivery Rev 21*: 81-106, 1996.
45. Ultinger J, Chollet J, Tu Z, Xiao S, Tanner M. Comparative study of the effects of artemether and artesunate on juvenile and adult *Schistosoma mansoni* in experimentally infected mice. *Tr Roy S Trop Med Hyg 96:* 319-323, 2002.
46. Utzinger J, N'goran EK, N'dri A, Lengeler C, Xiao SH, Tanner M, Oral artemether for prevention of *Schistosoma mansoni* infection: randomised controlled trial. *Lancet 355:* 1320-1325, 2000.
47. Utzinger J, Xiao SH, N´Goran EK, Bergquist R, Tanner M. The potential of artemether for control of schistosomiasis. *Int J Parasitol 31:* 1549-1562, 2001.

48. Utzinger J, Chollet J, Tu ZW, Xiao SH, Tanner M. Comparative study of the effects of artemether and artesunate on juvenile and adult *Schistosoma mansoni* in experimentally infected mice. *Tr Roy Soc Trop MedHyg 96:* 318-323, 2002.
49. Webbe G, James C. A comparison of the susceptibility to praziquantel of *Schistosoma haematobium, S. japonicum, S. mansoni, S. intercalatum* and *S. mattheei* in hamsters. *Parasitol Res 52:* 169-177, 1977.
50. Xiao SH, Catto BA, Webster LT. Effects of praziquantel on different developmental stages of *Schistosoma munsoni in vitro* and *in vivo*. *J Infect Dis 151:* 1130-1137, 1985.
51. Xiao SH, Hotez PJ, Tanner, M. Artemether, an effective new agent for chemoprphylaxis against schistosomiasis in China: its in vivo effect on the biochemical metabolism of the asian schistosome. *Southeast Asian Journal of Tropical Medicine and Public Health 31:* 724-732, 2000.
52. Xu M. The progress on prophylatics studies of artemisinin, artemether and artesunate against schistosomiasis japonica. *Chinese Journal of Schistosomiasis Control 10:* 251-253, 1998.
53. Yolles TK, Moore PV, De Ginsti DL, Ripson CA, Meleney HE. A technique for the perfusion of laboratory animals for the recovery of schistosomes. *J Parasitol 33:* 419-426, 1947.
54. Yoshioka L, Magalhães EMZ, Magalhães LA, Linhares AX. *Schistosoma mansoni:* estudo da patogenia da linhagem santa rosa (Campinas, SP, Brazil) em camundongos. *Rev Soc Bras Med Trop 35:* 203-207, 2002.
55. Yue WJ, You JQ, Mei JY. Effects of artemether on *Schistosoma japonicum* adult worms and ova. *Acta Pharmacologica Sinica. 5*: 60-63, 1984.