TRICLABENDAZOLE AND ITS TWO MAIN METABOLITES LACK ACTIVITY AGAINST SCHISTOSOMA MANSONI IN THE MOUSE MODEL

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Abstract. Some have claimed that triclabendazole, a safe and efficacious drug for the treatment of fascioliasis, also exhibits antischistosomal properties, but results are conflicting. We assessed the effect of triclabendazole and its two main metabolites against two different strains of Schistosoma mansoni harbored in mice. Low worm burden reductions (18.6–35.9%) were observed in mice infected with an Egyptian strain of S. mansoni and treated with a single dose of 120 mg/kg 3 days before infection or single/double doses of 120–200 mg/kg 7 weeks after infection. Triclabendazole failed to significantly reduce hepatic and intestinal tissue egg loads, and eggs of all developmental stages were observed. Administration of 400 mg/kg of either triclabendazole, triclabendazole sulphone, or triclabendazole sulphoxide to mice infected with a Liberian strain of S. mansoni resulted in worm burden reductions < 10%. In comparison, high worm burden reductions (82–100%) were observed in S. mansoni-infected mice treated with single oral doses of 400, 500, or 500 mg/kg twice a day praziquantel, regardless of the S. mansoni strain. We conclude that triclabendazole and its main metabolites display weak and inconsistent schistosomicidal activities.

INTRODUCTION

Schistosomiasis is a parasitic disease caused by the blood flukes Schistosoma mansoni, S. haematobium, S. japonicum, S. intercalatum, and S. mekongi. It is currently estimated that 779 million people are at risk of infection, with 207 million people being infected.1 The annual mortality rate exceeds 200,000 people,2 and the estimated burden of the disease is between 1.7 and 4.5 million disability-adjusted life-years (DALYs) lost each year.3 However, a recent meta-analysis suggests that the “true” burden of schistosomiasis might be significantly higher.4

Praziquantel-based morbidity control is the current mainstay of schistosomiasis control in high burden areas with the objective to regularly treat at least 75% of school-age children and other high-risk groups by 2010.5,6 Discovered in the mid-1970s,7 praziquantel has been widely and effectively used against schistosomiasis; >100 million doses have been administered in China and Egypt alone.8,9 Interestingly, unexpectedly low cure rates have been reported from S. mansoni-infected patients in Egypt and Senegal who were treated with recommended single oral doses of 40 mg/kg praziquantel.10,11 The pressing need to develop alternative anti-schistosomal drugs, while praziquantel remains effective, has been stressed.5,12

Triclabendazole is a safe and efficacious drug for the treatment of fascioliasis.13 Triclabendazole has also been tested against other major trematodes, including S. mansoni.13 Exposure of S. mansoni worms in vitro to a concentration of 15 μg/mL triclabendazole resulted in immediate contraction, destruction of the tegument, and death of the worms within 24 hours.14 However, results in the S. mansoni-mouse model are conflicting. On the one hand, a high worm burden reduction of 84% and cessation of oviposition have been reported after oral administration of 120 mg/kg triclabendazole to S. mansoni-infected mice.15 On the other hand, no effect on S. mansoni was observed when 500 ppm triclabendazole was added to standard rodent diet and given for 7 days.16 Interestingly, epidemiologic studies are underway in Egypt to assess the effect of triclabendazole administered to patients co-infected with Fasciola spp. and S. mansoni.17

The aim of this study was to assess the effect of triclabendazole and its two main metabolites, triclabendazole sulphone and triclabendazole sulphoxide, against S. mansoni harbored in mice. We used two different parasite strains. As a benchmark, praziquantel was also administered. Drug efficacy was evaluated on the basis of worm burden reduction, excretion and hatchability of eggs, tissue egg load, and oogram patterns.

MATERIALS AND METHODS

Drugs. For the experiments carried out in Basel, Switzerland, triclabendazole, triclabendazole sulphone, and triclabendazole sulphoxide were obtained from Novartis Animal Health (Basel, Switzerland). Praziquantel was provided by the Shanghai No. 6 Pharmaceutical Factory (Shanghai, China). Drugs were prepared in homogenous suspensions in 7% Tween-80 and 3% ethanol before oral administration. For studies with the Egyptian S. mansoni strain, done in Alexandria, Egypt, Fasinex suspension (Novartis Animal Health, Basel, Switzerland) and Distocide suspension (EIPICO, Cairo, Egypt) were obtained from the local pharmacy and the Egyptian Ministry of Health and Population.

Mice, parasites, and infection. All animal studies presented here had been approved by the local government based on national regulations. Female NMRI mice (N = 25, age: ~5 weeks, weight: ~24 g) and male CD-1 Swiss albino mice (N = 101, age: ~4 weeks, weight: ~20 g) were purchased from RCC (Itingen, Switzerland) and the Schistosome Biologic Supply Center, Theodore Bilharz Research Institute (Giza, Egypt), respectively. Mice were kept in groups of five under environmentally controlled conditions (temperature: ~25°C; humidity: ~70%; 12 hour light/dark cycle) and acclimatized for 1 week. They had free access to water and food.

Cercariae of S. mansoni (Liberian strain) were obtained from infected Biomphalaria glabrata following routine procedures at the laboratories of the Swiss Tropical Institute. Cercariae of S. mansoni (Egyptian/CD strain) were purchased from the Schistosome Biologic Supply Center. Female NMRI
mice were infected subcutaneously with 80 *S. mansoni* cercariae (Liberian strain), and male CD-1 mice were infected with 80 *S. mansoni* cercariae (Egyptian/CD strain) using the body immersion technique.

**Treatment.** Two sets of experiments were carried out at the High Institute of Public Health (Alexandria, Egypt) using an Egyptian *S. mansoni* strain. First, 15 mice were treated with a single oral dose of 120 mg/kg triclabendazole 3 days before *S. mansoni* infection. Second, mice with a 7-week-old adult *S. mansoni* infection were divided into four groups of 14 mice each and treated with triclabendazole at a single oral dose of either 120 or 200 mg/kg or a double dose on consecutive days at 120 or 200 mg/kg daily. As a benchmark, two groups of eight mice each received single or double doses of 500 mg/kg praziquantel, respectively. One group of 14 infected mice remained untreated and hence served as a control.

A third set of experiments was carried out at the Swiss Tropical Institute using a Liberian strain of *S. mansoni*. Seven weeks after infection, three groups of five mice each were treated with single oral doses of triclabendazole, triclabendazole sulphone, and triclabendazole sulphoxide at 400 mg/kg. Another group of five mice received a single oral dose of 400 mg/kg praziquantel, whereas the fifth group of mice remained untreated and served as a control.

**Fecal egg counts and hatching of *S. mansoni* eggs.** Five weeks after infection, fecal sampling from mice infected with an Egyptian strain of *S. mansoni* commenced and lasted until necropsy. Fecal samples were collected from each group of mice over a 2-hour period daily until *S. mansoni* eggs could be detected. Subsequently fecal sampling was done twice a week. Fecal samples were suspended in 2–4 mL of saline, and six aliquots of 50 μL were examined microscopically. The number of eggs per gram (epg) of feces was determined by multiplying the average number of eggs in each 50-μL sample by the total volume of the saline suspension and dividing this value by the weight of the sample in grams.

To assess the miracidial hatchability of eggs, stool samples were collected from each group of mice over a 2-hour period, suspended in 10 mL of saline, and centrifuged at 1,000 rpm for 1 minute. After washing with saline, the sediment was resuspended in 10 mL dechlorinated water and poured into a 1-L measuring flask. After adding 1 L of dechlorinated water, egg hatching was stimulated through exposure to artificial light.

**Evaluation of the worm burden.** Mice from the first two experimental series were necropsied 11 weeks after infection, and worms were recovered from the hepatic and portomesenteric vessels using the perfusion technique. The small and large intestines were placed in a Petri dish, and all *S. mansoni* in the mesenteric veins were removed, sexed, and counted under a stereoscopic microscope.

In the third set of experiments, mice were killed 11 weeks after infection and dissected by removing the liver and small and large intestines. The liver was placed into a 20 × 20-cm transparent plastic folder and gently compressed between two glass plates, and the parenchyma was examined at a 10-fold magnification under a stereoscopic microscope. *S. mansoni* were sexed and counted. The small and large intestines were put in a Petri dish, and all *S. mansoni* in the mesenteric veins were removed, sexed, and counted under a stereoscopic microscope.

**Oogram pattern and tissue egg load.** Three fragments of the small intestine (from the middle part of the small intestine) were cut longitudinally, washed with saline, compressed between two microscope slides, and examined under a low-power microscope. A total of 100 eggs per animal were observed, and the stage of each egg and the mean number of the different stages were recorded.

The tissue egg load was determined as follows. First, 0.3 g of liver and small intestine was taken from each mouse and digested overnight in 5 mL KOH (5%). Second, after complete digestion, the samples were vortexed, and three aliquots of 100 μL each were examined microscopically. All *S. mansoni* eggs were counted. The hepatic and intestinal tissue egg loads (expressed as epg) were determined by multiplying the number of eggs in each 100-μL sample by the total volume of KOH and dividing this value by the weight of the sample in grams.

**Statistical analysis.** Drug efficacy was assessed by comparing the mean number of total and female worms in any treatment group with that of the respective control groups. Differences were tested for significance using an unpaired two-tailed Student *t*-test, allowing for unequal variance. The data were considered significant if *P* < 0.05. Statistical analyses were done with version 9 of the STATA software (Stata-Corp., College Station, TX).

**RESULTS**

Viable *S. mansoni* eggs (i.e., eggs that developed into miracidia) were detected in fecal samples obtained from all experimental groups of mice infected with an Egyptian strain of *S. mansoni* and treated with different regimens of triclabendazole. Oogram pattern analysis of these mice revealed eggs of all developmental stages and no significant reduction in the number of eggs compared with the untreated control group (Table 1). On the other hand, in *S. mansoni*-infected mice treated with a single dose of 500 mg/kg praziquantel, immature eggs of the first stage were absent, the number of second to fourth stage immature eggs and the number of mature eggs strongly decreased, whereas a notable increase in dead eggs was recorded compared with the untreated control group. Neither immature nor mature eggs, but dead eggs, were found in mice treated with two doses of 500 mg/kg praziquantel (Table 1).

Treatment with different doses of triclabendazole failed to yield reductions in either hepatic or intestinal tissue egg loads, whereas a single or double dose of 500 mg/kg praziquantel resulted in hepatic tissue egg load reductions of 81.1 and 89.0%, and intestinal egg load reductions of 97.0 and 97.2%, respectively.

Table 2 summarizes the effect of various doses of triclabendazole in mice infected with an Egyptian strain of *S. mansoni*. There was no correlation between the total or female worm burden reductions and the dose of triclabendazole administered. In mice treated with a single dose of 120 mg/kg triclabendazole 3 days before infection with *S. mansoni*, low, but statistically significant, total (31.0%) and female (27.6%) worm burden reductions were achieved (*P* < 0.05). When mice harboring a 7-week-old adult *S. mansoni* infection were given a single or double dose of 120 mg/kg triclabendazole, moderate, but statistically significant, total worm burden reductions (35.5 and 29.8%, respectively) and insignificant female worm burden reductions (24.5 and 27.6%, respec-
Effect of triclabendazole administered at different doses to mice infected with an Egyptian strain of *S. mansoni* on different egg developmental stages and tissue egg load

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Hepatic</th>
<th>Intestinal</th>
<th>Immature</th>
<th>Mean percent egg developmental stages (SD)</th>
<th>Total worm burden reduction (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>14.2 (4.6)</td>
<td>27.4 (10.3)</td>
<td>8.4 (4.5)</td>
<td>12.7 (3.0)</td>
<td>12.5 (6.0)</td>
<td>15.5 (6.6)</td>
</tr>
<tr>
<td>Triclabendazole</td>
<td>120 (before infection)</td>
<td>18.9 (6.4)</td>
<td>35.2 (9.4)</td>
<td>9.1 (3.8)</td>
<td>11.9 (5.0)</td>
<td>15.4 (4.9)</td>
<td>14.9 (5.6)</td>
</tr>
<tr>
<td>200</td>
<td>15.5 (7.6)</td>
<td>29.5 (17.2)</td>
<td>7.9 (3.2)</td>
<td>14.0 (3.9)</td>
<td>15.5 (4.4)</td>
<td>12.1 (5.3)</td>
<td>49.5 (4.8)</td>
</tr>
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<td>2 x 200</td>
<td>14.2 (3.2)</td>
<td>29.5 (19.4)</td>
<td>8.2 (4.3)</td>
<td>11.2 (5.0)</td>
<td>15.2 (4.0)</td>
<td>14.6 (8.0)</td>
<td>49.2 (4.7)</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>500</td>
<td>2.7 (2.4)</td>
<td>0.8 (1.1)</td>
<td>0</td>
<td>2.8 (6.8)</td>
<td>0.6 (1.3)</td>
<td>0.6 (1.3)</td>
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<tr>
<td>2 x 500</td>
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<td>0.8 (0.7)</td>
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</table>

For comparison, results of praziquantel are also shown.

DISCUSSION

While the fascicidal efficacy of triclabendazole is well established, the currently available data on the drug’s schistosomical properties are conflicting. Consequently, we thoroughly studied whether triclabendazole is active against *S. mansoni* in the mouse model. We used various doses and treatment regimens of triclabendazole and also assessed whether the two main metabolites showed activity. In addition, we used two different *S. mansoni* strains, an Egyptian (CD) strain and a Liberian strain, because infectivity and drug susceptibility vary according to the *S. mansoni* strain used.

Our main finding is that triclabendazole, as well as its two main metabolites, lacks antischistosomal activity. Only low total and female worm burden reductions were observed after oral administration of triclabendazole, triclabendazole sulphone, or triclabendazole sulphoxide at doses as high as 400 mg/kg, regardless of the *S. mansoni* strain used. In addition, eggs of all developmental stages were present in mice treated with triclabendazole, and no significant reductions in either the hepatic and intestinal tissue egg loads were observed. Furthermore, eggs collected from fecal samples from treated mice were able to hatch and developed active miracidia.

Our results are in contrast to the previous study of Khalil and others, reporting a total worm burden reduction of 84% after the administration of a single oral dose of 120 mg/kg triclabendazole to mice harboring an Egyptian strain of *S. mansoni*. We replicated this experiment, but only found a total worm burden reduction of 35.5%. We have no explanation for this large difference in worm burden reductions because the same *S. mansoni* strain, route of administration, and distribution.
dose of triclabendazole, and parasitological methods were used to assess the worm burden. Interestingly, subsequent experiments with higher doses of triclabendazole failed to replicate a total worm burden reduction of 35.5%; instead worm burden reductions of only 6.6–31.3% were observed.

It is noteworthy that triclabendazole has a pronounced effect on schistosomes in vitro, but the drug and its two main metabolites failed to significantly reduce S. mansoni worms in our experiments in vivo. This finding is surprising because the liver is the target organ of both schistosomes and the biologically related Fasciola spp. Pharmacokinetics in the host might play a key role in the differing susceptibilities to triclabendazole between Fasciola and schistosomes in vitro, and might also explain the differing in vitro and in vivo activities of the drug on schistosomes. For example triclabendazole is highly protein bound in different animal species. While F. hepatica is known to be very susceptible to drugs that mainly bound to plasma proteins, schistosomes might be less affected by the protein bound triclabendazole.

Another aspect worth mentioning is the elevated death rate of S. mansoni–infected mice in the triclabendazole groups compared with the corresponding praziquantel or control groups (Tables 2 and 3). The LD<sub>50</sub> value of oral triclabendazole is > 8 g/kg in mice, and the LD<sub>50</sub> values of the sulphoxide and sulphone metabolites administered orally are > 5 g/kg in rats. S. mansoni is known to cause a downregulation of hepatic cytochrome P-450 activities in chronic infections in mice, and hence the safety margin of triclabendazole might decrease because of a S. mansoni infection in the mouse. We are not aware of studies that have investigated similarly high doses of triclabendazole against chronic infections of F. hepatica in rodent models, which might have revealed comparable changes of toxicity margins, because F. hepatica is also known to alter cytochrome P-450 activities.

Based on our findings, we speculate that triclabendazole, when administered as a dose of 10 mg/kg to fascioliasis patients, will have no or only a minimal effect against S. mansoni. This issue is currently under investigation in Egypt, where patients co-infected with F. hepatica and S. mansoni are given triclabendazole. We are curious to learn the results of this study. In case our findings of the lack of antischistosomal activity of triclabendazole and its metabolites in the mouse are confirmed in human studies, we are basically left with praziquantel as the only drug for treatment and control of schistosomiasis. The activity of oxamniquine is restricted to S. mansoni. The encouraging initial results obtained with the natural product myrrh (Mirazid), claiming a cure rate of 91.7–96.2% in S. mansoni–infected patients in Egypt, could not be confirmed in follow-up studies in humans or rodents. In light of these disappointing observations regarding triclabendazole and myrrh and no viable alternative to praziquantel, we conclude that establishment of an anti-schistosomal drug discovery and development portfolio is of pressing urgency.

References


