Evaluation of in Vivo Activity of Tribendimidine against *Schistosoma mansoni*, *Fasciola hepatica*, *Clonorchis sinensis* and *Opisthorchis viverrini*

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Abstract

We examined the in vivo activity of tribendimidine against selected trematodes. A single 150 mg/kg dose of tribendimidine achieved a 99.1% reduction of *Clonorchis sinensis* in rats. 400 mg/kg tribendimidine reduced *Opisthorchis viverrini* in hamsters by 95.7%. High doses of tribendimidine showed no activity against *Schistosoma mansoni* and *Fasciola hepatica*.

Keywords: Tribendimidine, in vivo studies, *Fasciola hepatica*, *Clonorchis sinensis*, *Opisthorchis viverrini*, *Schistosoma mansoni*, food-borne trematodiasis, schistosomiasis
One-quarter of a billion people are infected with parasitic trematode worms worldwide. Schistosomiasis, caused by a blood fluke of the genus Schistosoma affects an estimated 207 million people (10). Disease-associated symptoms occur in 120 million people and 20 million people suffer from severe morbidity (12). The three most important human schistosomes are Schistosoma haematobium, S. japonicum and S. mansonii. An estimated 40 million people are infected with food-borne trematodes, which comprise the liver flukes (Clonorchis sinensis, Fasciola spp, Opisthorchis viverrini), the lung flukes (Paragonimus spp.), and the intestinal flukes (Echinostoma spp., heterophyids) (13). Food-borne trematodiasis is an emerging public health problem and is of considerable veterinary significance, but its global burden remains to be investigated (6).

Tribendimidine, an aminophenyldimidine derivative of amidantel, is a broad spectrum anthelminthic drug, developed in China since the mid-1980s (9). Tribendimidine has recently been approved by the Chinese authorities based on the good safety and therapeutic profile against soil-transmitted helminthiasis (9, 14, 15). In a recent in vivo investigation, we have demonstrated that tribendimidine is active against an intestinal trematode, Echinostoma caproni (4). Here we extent our investigations and screen for in vivo activity of tribendimidine against a range of clinically-significant trematodes, S. mansonii, Fasciola hepatica, C. sinensis and O. viverrini.

Animal studies were approved and carried out according to Swiss national regulations. Female NMRI mice (n = 10; age, ~5 weeks; weight, ~24 g) and female Wistar rats (n = 28; age, ~5 weeks; weight, ~100 g) were purchased from RCC (Ittingen, Switzerland). Male Syrian Gold hamsters (n = 14; age, ~4-16 weeks; weight, ~100-200 g) were purchased from Charles River (Sulzfeld, Germany). Animals were kept in groups of 4-5 in environmentally-controlled conditions, with free access to water and rodent diet.

Cercariae of S. mansonii (Liberian strain) were obtained from infected Biomphalaria glabrata following routine procedures at our laboratories. F. hepatica metacercariae (Cullompton
isolate) were purchased from G. Graham (Addlestone, UK). *C. sinensis* and *O. viverrini* metacercariae were isolated from freshwater fishes caught in Guanxi province (China) and Khon Kaen province (Thailand), respectively, as described previously (3).

Tribendimidine was synthesized and provided by the National Institute of Parasitic Diseases (Shanghai, China). The drug was prepared in a suspension in 7% Tween-80 and 3% ethanol. With the exception of our adult *S. mansoni* screen in mice, where we routinely use a 400 mg/kg single oral dose (2), we initially used a 150 mg/kg single oral dose of tribendimidine in rats or 200 mg/kg in hamsters. Depending on the results obtained, these doses, sequentially, were lowered or increased.

Fifteen rats were infected orally with 25 metacercariae of *F. hepatica*. Eight weeks postinfection five rats were treated with single 150–800 mg/kg oral doses of tribendimidine (only result of highest dose shown). The remaining rats were left untreated, hence they served as controls. One week posttreatment rats were euthanised by CO₂ and all flukes were removed from the liver and bile ducts and counted.

Thirteen rats were infected orally with 40 *C. sinensis* metacercariae. Four weeks postinfection two groups of four rats each were given a single 75 or 150 mg/kg oral dose of tribendimidine. The remaining rats served as controls. Killing and dissection of rats was done as described above.

Fourteen hamsters were infected orally with 45 *O. viverrini* metacercariae. Four weeks postinfection four hamsters each were treated orally with a single 200 or 400 mg/kg dose of tribendimidine. The remaining hamsters served as controls. Hamsters were killed and dissected one week posttreatment, and *O. viverrini* were removed from the liver, gall bladder and bile ducts and counted.

Ten mice were infected subcutaneously with 80 *S. mansoni* cercariae. Seven weeks postinfection, half of the mice were treated with a single 400 mg/kg oral dose of tribendimidine. The remaining mice served as controls. Twenty-eight days posttreatment animals were killed by
blood letting, *S. mansoni* were removed from the liver and mesenteric veins, sexed and counted.

Mean worm burden in the different treatment and control groups were calculated. Statistical analyses were done in version 2.4.5 Statsdirect (Statsdirect LtD; Cheshire, UK). Differences in the median of the responses between the treatment and the respective control groups were examined with the Kruskal-Wallis (KW) test. A difference in median was considered to be significant at a level of 5%.

We observed high in vivo activities of tribendimidine against *C. sinensis* and *O. viverrini*. A 99.1% worm burden reduction was achieved with 150 mg/kg tribendimidine administered to *C. sinensis*-infected rats. At half this dose the worm burden reduction was still significant (68.9%; KW = 5.46; \(P = 0.019\); Table 1). A single 400 mg/kg oral dose of tribendimidine given to hamsters infected with adult *O. viverrini* reduced the worm burden by 95.7%. At half this dose a worm burden reduction of 61.4% was obtained, which was highly significant (Table 2).

On the other hand, tribendimidine lacked in vivo efficacy against adult *S. mansoni* and adult *F. hepatica*; high doses showed no worm burden reduction at all (Tables 3 and 4).

We were somewhat surprised that the four biologically-related trematodes examined, which are in the adult stage all harbored in the liver, either in the veins (*S. mansoni*) or the tissue and bile ducts (*C. sinensis*, *F. hepatica* and *O. viverrini*), differ greatly in their susceptibility to tribendimidine in rodents. Elucidations on the mechanism of action of tribendimidine might help to clarify why *S. mansoni* and *F. hepatica* harbored in mice and rats, respectively, are not susceptible to tribendimidine. Pharmacokinetics and the choice of the host model might also play a role in the differential activities of tribendimidine observed.

For over two decades treatment of opisthorchiasis and clonorchiasis relies on praziquantel (5). Fortunately, parasite resistance to praziquantel is not a major public-health issue thus far. However, low cure rates have already been reported after administration of praziquantel to clonorchiasis patients in Vietnam (11). Hence, the development of new, orally-active, single dose
trematocidal drugs – in the period while praziquantel remains effective – would be an important step forward. Since tribendimidine is a safe and highly efficacious drug for treatment of soil-transmitted helminth infections (15) a phase II clinical trial could be initiated with an emphasis on opisthorchiasis or clonorchiasis. In addition, it is important to note that the latter two diseases often geographically overlap with soil-transmitted helminthiasis (1, 7, 8). Consequently, the effect of tribendimidine on concurrent infections with soil-transmitted helminths and either C. sinensis or O. viverrini should be assessed.

Acknowledgements

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References


Figure legend:

Figure 1: Chemical structure of tribendimidine
Figure 1:
TABLE 1: Effect of single oral doses of tribendimidine against adult *C. sinensis* harbored in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of rats cured*/investigated</th>
<th>Mean worm burden (SD)</th>
<th>Total worm burden reduction (%)</th>
<th>KW</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>0/5</td>
<td>29.0 (12.2)</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Tribendimidine</td>
<td>150</td>
<td>3/4</td>
<td>0.25 (0.5)</td>
<td>99.1</td>
<td>6.20</td>
<td>0.012</td>
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<td></td>
<td>75</td>
<td>1/4</td>
<td>9.0 (7.4)</td>
<td>68.9</td>
<td>5.46</td>
<td>0.019</td>
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</table>

SD, standard deviation; KW, Kruskal-Wallis

*signifies the number of rats without flukes
**TABLE 2: Effect of single oral doses of tribendimidine against *O. viverrini* harbored in hamsters**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of hamsters cured*/investigated</th>
<th>Mean total worm burden (SD)</th>
<th>Total worm burden reduction (%)</th>
<th>KW</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
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<td>0/6</td>
<td>5.83 (3.3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Tribendimidine</td>
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<td>0.25 (0.5)</td>
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<td>6.79</td>
<td>0.009</td>
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<td></td>
<td>200</td>
<td>1/4</td>
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<td>61.4</td>
<td>4.20</td>
<td>0.040</td>
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</tbody>
</table>

SD, standard deviation; KW, Kruskal-Wallis

* signifies the number of hamsters without flukes
TABLE 3: Effect of a single oral dose of tribendimidine against adult *F. hepatica* harbored in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of rats cured* / investigated</th>
<th>Mean worm burden (SD)</th>
<th>Total worm burden reduction (%)</th>
<th>KW</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>0/10</td>
<td>8.4 (4.2)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tribendimidine</td>
<td>800</td>
<td>0/5</td>
<td>9.4 (1.6)</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

SD, standard deviation; KW, Kruskal-Wallis; NA, not applicable

* signifies the number of rats without flukes
TABLE 4: Effect of single oral dose of tribendimidine against adult *S. mansoni* harbored in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>No. of mice cured*/investigated</th>
<th>Mean total worm burden (SD)</th>
<th>Total worm burden reduction (%)</th>
<th>KW</th>
<th>P value</th>
<th>Mean female worm burden (SD)</th>
<th>Total female burden reduction (%)</th>
<th>KW</th>
<th>P value</th>
</tr>
</thead>
<tbody>
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<td>Control</td>
<td>–</td>
<td>0/5</td>
<td>38.2 (13.8)</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>18.8 (6.2)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tribendimidine</td>
<td>400</td>
<td>0/5</td>
<td>39.2 (7.6)</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>19.2 (4.2)</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

SD, standard deviation; KW, Kruskal-Wallis; NA, not applicable.

* signifies the number of mice without flukes