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

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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. A 400-mg/kg dose of tribendimidine reduced Opisthorchis viverrini in hamsters by 95.7%. High doses of tribendimidine showed no activity against Schistosoma mansoni and Fasciola hepatica.

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 (11). Disease-associated symptoms occur in 120 million people, and 20 million people suffer from severe morbidity (2). The three most important human schistosomes are Schistosoma haematobium, Schistosoma japonicum, and Schistosoma mansoni. An estimated 40 million people are infected with food-borne trematodes, which comprise the liver flukes (Clonorchis sinensis, Fasciola spp., Opisthorchis spp.), 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 (7).

Tribendimidine, a symmetrical diamidine derivative of amidentane, is a broad-spectrum anthelminthic drug, developed in China since the mid-1980s (10). The chemical structure of Tribendimidine, a broad-spectrum anthelminthic drug, developed in China since the mid-1980s (10). The chemical structure of Tribendimidine is shown in Fig. 1. Tribendimidine has recently been approved by the Chinese authorities based on the good safety and therapeutic profile against soil-transmitted helminthiasis (10, 14, 15). In a recent in vivo investigation, we have demonstrated that tribendimidine is active against an intestinal trematode, Echinostoma caproni (5). Here we extend our investigations and screen for in vivo activity of tribendimidine against a range of clinically significant trematodes, S. mansoni, Fasciola hepatica, C. sinensis, and Opisthorchis 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 (Itingen, Switzerland). Male Syrian Gold hamsters (n = 14; age, ~4 to 16 weeks; weight, ~100 to 200 g) were purchased from Charles River (Sulzfeld, Germany). Animals were kept in groups of four to five under environmentally controlled conditions, with free access to water and rodent diet.

Cercariae of S. mansoni (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, United Kingdom). C. sinensis and O. viverrini metacercariae were isolated from freshwater fishes caught in Guangxi province (China) and Khon Kaen province (Thailand), respectively, as described previously (4).

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 (3), 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 Clonorchis sinensis, and 15 hamsters were infected rectally with 10,000 cercariae of Opisthorchis viverrini. The rats were infected on October 1, 2007, and the hamsters were infected on October 20, 2007.

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**F. hepatica.** Eight weeks postinfection, five rats were treated with single 150- to 800-mg/kg oral doses of tribendimidine (only result of highest dose shown). The remaining rats were left untreated, and hence, they served as controls. One week posttreatment, rats were euthanized 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. Kiling 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 1 week posttreatment, and *O. viverrini* flukes 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, and *S. mansoni* flukes were removed from the liver and mesenteric veins, sexed, and counted.

Mean worm burdens in the different treatment and control groups were calculated. Statistical analyses were done in version 2.4.5 Statsdirect (Statsdirect Ltd., Cheshire, United Kingdom). Differences in the median of the responses between the treatment and the respective control groups were examined with the Kruskal-Wallis (KW) test. 

**TABLE 2. Effect of single oral doses of tribendimidine against *O. viverrini* harbored in hamsters**

<table>
<thead>
<tr>
<th>Treatment</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>
<td>0/6</td>
<td>5.83 (3.3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tribendimidine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>3/4</td>
<td>0.25 (0.5)</td>
<td>95.7</td>
<td>6.79</td>
<td>0.009</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>1/4</td>
<td>2.25 (1.5)</td>
<td>61.4</td>
<td>4.20</td>
<td>0.040</td>
</tr>
</tbody>
</table>

*a Number of hamsters without flukes.

**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 significant (Table 2).

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

We were grateful to T. Smarn for his help in obtaining the metacercariae of *O. viverrini*.
REFERENCES