Effect of Simultaneous and/or Consecutive Administration of the Broad Spectrum Anthelmintic Flubendazole together with Praziquantel in Experimental *Schistosoma mansoni* Infection

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**Summary**

This study is a trial to demonstrate the effect of the broad spectrum anthelmintic drug flubendazole (methyl 5-(p-fluorobenzoyl)-2-benzimidazolecarbamate, CAS 31430-15-6), a mebendazole derivative, together with praziquantel (CAS 55268-74-1, EMBAY 8440, Biltricide®) in murine schistosomiasis mansoni. Moreover, the relationship between the posttreatment worm burden, oogram pattern, tissue egg load and hepatic granuloma volume was also investigated. Three main groups of Swiss albino mice infected with *Schistosoma mansoni* cercariae were used in the experiment. Group I included infected untreated control mice. Group II: Subgroup II (a): Animals received 1/3 the dose of praziquantel 25 days post infection. Subgroup II (b): Mice were given 1/3 dose of flubendazole 25 days post infection. Subgroup II (c): Animals received the combination (1/3 dose of flubendazole + 1/3 the dose of praziquantel 25 days post infection. Group III: Subgroup III (a): Mice were given 1/3 the dose of praziquantel 7 weeks post infection. Subgroup III (b): Mice received 1/3 dose of flubendazole 25 days post infection. 24 days later, 1/3 the dose of praziquantel was given. Mice given the consecutive drug regimen (flubendazole 1/3 single oral dose 25 days post infection, then praziquantel 1/3 oral dose for two successive days 24 days later, revealed a significant reduction in the recovery of adult schistosomes after portal perfusion (95.9 %), absence of immature stages of ova development, a higher level of dead ova in the oogram and the smallest granuloma mean diameter. These data were less conspicuous in mice given the simultaneous drug regimen.
Zusammenfassung

Die Wirkung von gleichzeitiger und/oder aufeinanderfolgender Behandlung mit dem Breitspektrum-Anthelminthikum Flubendazol zusammen mit Praziquantel bei experimenteller Schistosoma mansoni-Infektion


1. Introduction

Mebendazole (CAS 31431-39-7) is a benzimidazole broad-spectrum anthelmintic drug. The potent action of thiabendazole (a mebendazole derivative) on strongyloidiasis in patients with haematologic malignancies was recorded [17]. The clinical spectrum of albendazole (another mebendazole derivative), in children with neurocysticercosis was also studied [16]. Disappearance and reduction of the size of the lesions was noted in 91 % of these children. The high efficacy of albendazole on geohelminth infections including ascariasis, trichuriasis and ancylostomiasis in a dose of 100 mg orally twice daily for three successive days was recorded [12]. The authors studied the effect of albendazole on intraabdominal cystic echnococcosis and found that the drug was safe in treating hepatic simple cysts, peritoneal secondary cysts and splenic cysts [10]. Again, the drug combination albendazole and praziquantel was tried in hydatidosis. It showed a remarkable effect [19]. A report was given about the cure of two Schistosoma haematobium patients after treatment with mebendazole [1]. The effect of flubendazole (a mebendazole derivative) on Schistosoma mansoni infected mice was previously studied [13]. A 79.5 % worm reduction showed up when the drug was administered during the schistosome maturation phase (25 days post infection) [13]. In the present study, possible changes in adult worm recovery, oogram pattern, tissue egg load and hepatic granuloma volume were studied after using reduced doses (1/3 the curative ones) of flubendazole (methyl 5-(p-fluorobenzoyl)-2-benzimidazolcarbamate, CAS 31430-15-6), a mebendazole derivative, and praziquantel (CAS 55268-74-1, EMBAY 8440, Biltricide®) in Schistosoma mansoni infected mice.

2. Material and methods

A group of 100 Swiss albino mice of CD strain, weighing 18–20 g each, were infected with an Egyptian strain of Schistosoma mansoni cercariae. The strain used originated from the Nile delta, and was bred through mice and Biomphalaria alexandrina for six years. Animals were bred and supplied by the Theodor Bilharz Research Institute, Imbaba, Cairo (Egypt) through the Schistosome Biological Supply Program. The experimental animals were kept for seven weeks in air-conditioned rooms at 21 °C, with food containing 24 % protein content. The animal experiments were carried out according to the internationally valid guidelines in an institution responsible for animal ethics (Theodor Bilharz Research Institute) as described in [13]. Mice were divided in a randomized manner into three main groups: the first main group (I) received 100 Schistosoma mansoni cercariae by body immersion, and served as control infected untreated mice. The second main group (II) received 100 Schistosoma mansoni cercariae by body immersion and was divided into three subgroups: Subgroup IIa received 1/3 the curative dose of praziquantel (333.3 mg/kg b. w.) orally for two successive days 25 days post infection. Subgroup IIb was given 1/3 the curative dose of flubendazole 33.3 mg/kg b. w. single oral dose 25 days after infection. Subgroup IIc was

1) Manufacturer: Bayer, Leverkusen (Germany).
given the combination of 1/3 the curative doses of each drug (praziquantel + flubendazole) 25 days after infection. Sacrifice was done 7 weeks post infection. The third main group (III) received 100 Schistosoma mansoni cercariae by body immersion and was further subdivided: Subgroup III (a): Mice were given 1/3 the curative dose of flubendazole 33.3 mg/kg b. w. single oral dose 25 days post infection. 24 days later, 1/3 the curative dose of praziquantel was given (333.3 mg/kg body weight on 2 successive days). Animals were sacrificed 9 weeks post infection (2 weeks following the last dose of praziquantel). Flubendazole (Janssen Pharmaceuticals, Beerse, Belgium) was given orally in a readily prepared suspension single oral dose. The drug was supplied by Theodor Bilharz Research Institute. And so was praziquantel. Animals were sacrificed in randomized groups (depending on the number of survivors). Mice were perfused according to Duvall and Devitt [6] taking care to separate hepatic from portomesenteric worms. Livers were collected, fixed in 10 % formalin solution for subsequent preparation of hematoxlin and eosin stained sections for granuloma measurements according to Harris [7].

The proportion of eggs in various stages of maturity were studied using the oogram pattern according to Pellegrino et al [15]. One hundred eggs in the small intestine were examined microscopically and classified as immature, mature and dead in the infected untreated controls.

In drug treated mice, the number of eggs in the intestine is usually reduced, so the percentage was calculated from the total number of eggs seen. The number of eggs per gram tissue was calculated according to Kloetz [9].

Bilharzial hepatic granulomas were measured in stained sections by selecting lesions containing single eggs in their centers. The mean diameter of each lesion was obtained by measuring perpendicular diameters using an ocular micrometer. The volume of each lesion was calculated from its mean diameter using the formula:
\[ V = \frac{\pi r^3}{4} \times n \]

The mean volume per group represents the mean of 70 lesions and is expressed as mm³ x 10⁻⁴ according to Boros and Warren [3].

### 2.1. Statistics
Comparison was done between each treated group, and its respective untreated control. The percentage change between each two groups to be compared, was assessed using the formula:
\[ \text{Percent change} = \frac{\text{Mean value of the first group} - \text{mean value of the second group}}{\text{Mean value of the first group}} \times 100 \]

Mean value of the first group

Differences between the mean scores of any of the two groups to be compared, were tested for significance, using an unpaired 2-tailed Student's t-test. The data were considered significant if p values were less than 0.05.

### 3. Results

#### 3.1. Worm burden and distribution
The consecutive drug regimen (flubendazole 1/3 the curative dose 25 days post infection, then praziquantel 1/3 the curative dose 7 weeks post infection), was most effective in reducing total worm counts (95.9 % worm reduction). But when treatment was conducted simultaneously 25 days post infection, a 65 % worm reduction was recorded. The mean total worms and couples in the former group were 1.14 ± 0.5 and 0.0 ± 0.0, respectively, compared to 9.75 ± 1.0 and 2.3 ± 0.9, respectively, in the latter group. Comparing these data with the infected untreated control group (27.5 ± 1.4 mean total worms and 12.9 ± 0.9 mean total couples, the difference was statistically significant at p < 0.001 (Tables 1 and 2).

#### 3.2. Tissue egg load
More reduction in the mean total tissue egg load (hepatic and intestinal) was found in animals given the consecutive drug regimen (flubendazole 1/3 the curative dose 25 days post infection then praziquantel 1/3

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**Table 1: Worm load and distribution in S. mansoni infected mice simultaneously treated with 1/3 the curative doses of flubendazole and praziquantel versus infected untreated control animals.**

<table>
<thead>
<tr>
<th>Animal group</th>
<th>No. of animals</th>
<th>Time of treatment (days post infection)</th>
<th>Worm distribution</th>
<th>Mean Total worms</th>
<th>Mean total couples</th>
<th>Percent worm reduction</th>
<th>Percent uncoupling</th>
<th>Percent worms in the liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected control (I)</td>
<td>8</td>
<td>−</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>12.4±0.7</td>
</tr>
<tr>
<td>Infected (1/3) Praziquantel treated</td>
<td>8</td>
<td>25</td>
<td>4.0±0.9</td>
<td>0.6±0.3</td>
<td>6.0±0.4</td>
<td>3.4±0.6</td>
<td>14.0±0.8***</td>
<td>2.6±0.3***</td>
</tr>
<tr>
<td>Infected (1/3) Flubendazole treated</td>
<td>8</td>
<td>25</td>
<td>2.0±0.6</td>
<td>0.6±0.4</td>
<td>4.8±0.8</td>
<td>4.5±0.9</td>
<td>11.9±1.8***</td>
<td>3.3±0.6***</td>
</tr>
<tr>
<td>Infected (Flubendazole + PZQ) (1/3)</td>
<td>8</td>
<td>25</td>
<td>0.8±0.4</td>
<td>0.3±0.2</td>
<td>4.7±0.6</td>
<td>4.0±0.5</td>
<td>9.75±1.0***</td>
<td>2.3±0.9***</td>
</tr>
</tbody>
</table>

Statistically significant difference from infected untreated control at *** p < 0.001.
Praziquantel (PZQ) was given in 1/3 the curative dose (2 × 333.3 mg/kg) orally 25 days post infection. Flubendazole was given in 1/3 the curative dose (33.3 mg/kg) orally 25 days post infection. Animals were sacrificed 7 weeks post infection.
Animals were sacrificed 9 weeks post infection (2 weeks post PZQ treatment). Praziquantel (PZQ) was given in 1/3 curative dose (2 mg/kg) orally 7 weeks post infection. Flubendazole was given in 1/3 curative dose (33.3 mg/kg) orally 25 days post infection. Sacrifice was done 9 weeks post infection (2 weeks following the last dose of praziquantel).

Statistically significant difference from infected untreated control at ** p < 0.01, *** p < 0.001.

Flubendazole was given in 1/3 curative dose (33.3 mg/kg) orally 25 days post infection. Sacrifice was done 9 weeks post infection (2 weeks following the last dose of praziquantel).

Table 2: Worm load and distribution in S. mansoni infected mice simultaneously treated with 1/3 the curative doses of flubendazole and praziquantel in a consecutive manner versus infected untreated control animals.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>No. of animals</th>
<th>Time of treatment</th>
<th>Male hepatic</th>
<th>Female hepatic</th>
<th>Male intestinal</th>
<th>Female intestinal</th>
<th>Mean Total worms</th>
<th>Mean total couples</th>
<th>Percent worm reduction</th>
<th>Percent uncoupling</th>
<th>Percent worms in the liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected control (I)</td>
<td>8</td>
<td>–</td>
<td>1.4±0.4</td>
<td>0.8±0.3</td>
<td>12.0±0.7</td>
<td>13.0±0.5</td>
<td>27.5±1.4</td>
<td>12.9±0.9</td>
<td>–</td>
<td>–</td>
<td>8.0 %</td>
</tr>
<tr>
<td>Infected praziquantel treated (1/3)</td>
<td>8</td>
<td>7 weeks post infection</td>
<td>0.7±0.2</td>
<td>1.4±0.7</td>
<td>0.9±0.3</td>
<td>0.6±0.3</td>
<td>3.6±1.0***</td>
<td>0.0±0.0***</td>
<td>86.9 %</td>
<td>100 %</td>
<td>58.3 %</td>
</tr>
<tr>
<td>Infected (flubendazole + PZQ) 1/3</td>
<td>8</td>
<td>25 days + 7 weeks post infection</td>
<td>0.6±0.3</td>
<td>0.5±0.3</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>1.1±0.5***</td>
<td>0.0±0.0***</td>
<td>95.9 %</td>
<td>100 %</td>
<td>96.5 %</td>
</tr>
</tbody>
</table>

Statistically significant difference from infected untreated control at *** p < 0.001.

Praziquantel (PZQ) was given in 1/3 the curative dose (2 x 333.3 mg/kg) orally 7 weeks post infection. Flubendazole was given in 1/3 the curative dose (33.3 mg/kg) orally 25 days post infection. Sacrifice was done 9 weeks post infection (2 weeks following the last dose of praziquantel).

Table 3: Oogram pattern and tissue egg load (hepatic and intestinal) in S. mansoni infected mice simultaneously treated with 1/3 the curative doses of flubendazole and praziquantel in a simultaneous manner.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>No of ova/g tissue</th>
<th>Time of treatment</th>
<th>Total immature</th>
<th>Mature</th>
<th>Dead</th>
<th>No of ovum/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected control</td>
<td></td>
<td>–</td>
<td>71.9±3.1</td>
<td>25.6±2.7</td>
<td>2.5±0.9</td>
<td>14367±1071</td>
</tr>
<tr>
<td>Infected praziquantel treated (1/3)</td>
<td></td>
<td>25 days post infection</td>
<td>49.4±2.4***</td>
<td>36.3±2.8*</td>
<td>14.3±2.4***</td>
<td>8885±2046</td>
</tr>
<tr>
<td>Infected flubendazole treated (1/3)</td>
<td></td>
<td>25 days post infection</td>
<td>52.5±5.3***</td>
<td>32.5±3.8</td>
<td>15.0±2.7***</td>
<td>14546±2897</td>
</tr>
<tr>
<td>Infected (flubendazole+PZQ) 1/3</td>
<td></td>
<td>25 days post infection</td>
<td>48.6±4.5***</td>
<td>25.7±2.0</td>
<td>25.7±5.3***</td>
<td>12146±2362*</td>
</tr>
</tbody>
</table>

Statistically significant difference from infected untreated control at * p < 0.05, ** p < 0.01.

Table 4: Oogram pattern and tissue egg load (hepatic and intestinal) in mice treated consecutively with 1/3 the curative doses of flubendazole and praziquantel.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>No of ova/g tissue</th>
<th>Time of treatment</th>
<th>Total immature</th>
<th>Mature</th>
<th>Dead</th>
<th>No of ovum/g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected control</td>
<td></td>
<td>–</td>
<td>71.9±3.1</td>
<td>25.6±2.7</td>
<td>2.5±0.9</td>
<td>14367±1071</td>
</tr>
<tr>
<td>Infected praziquantel treated (1/3)</td>
<td></td>
<td>7 weeks post infection</td>
<td>0.0±0.0***</td>
<td>0.0±0.0***</td>
<td>0.0±0.0***</td>
<td>5044±863***</td>
</tr>
<tr>
<td>Infected (flubendazole+PZQ) 1/3</td>
<td></td>
<td>25 days + 7 weeks post infection</td>
<td>6.7±2.7***</td>
<td>93.3±2.7</td>
<td>5044±863***</td>
<td>3503±1173***</td>
</tr>
</tbody>
</table>

Statistically significant difference from infected untreated control at * p < 0.01, ** p < 0.001.

Animals were sacrificed 9 weeks post infection (2 weeks post PZQ treatment). Praziquantel (PZQ) was given in 1/3 curative dose (2 x 333.3 mg/kg) orally 7 weeks post infection. Flubendazole was given in 1/3 curative dose (33.3 mg/kg) orally 25 days post infection.

Statistically significant difference from infected untreated control at * p < 0.01, ** p < 0.001.

Animals were sacrificed 9 weeks post infection (2 weeks post PZQ treatment). Praziquantel (PZQ) was given in 1/3 curative dose (2 x 333.3 mg/kg) orally 7 weeks post infection. Flubendazole was given in 1/3 curative dose (33.3 mg/kg) orally 25 days post infection.

3.3. Percentage egg developmental stages (oogram pattern)

All stages of ova development were found in control infected untreated mice. However, mice given 1/3 the curative dose of praziquantel wether alone or in combination with 1/3 the curative dose of flubendazole (especially the consecutive drug regimen), revealed disap-
...pearance of the immature stages of ova development with rise in the dead ones in the oogram (67.1 ± 5.9 and 93.3 ± 2.7, respectively). The difference was statistically significant from infected untreated control mice at p < 0.001 (Tables 3 and 4).

3.4. Hepatic granuloma volume

The largest mean granuloma volume recorded was in untreated control infected mice (309 ± 9.7 μm). The mean granuloma volume was less in animals given the simultaneous drug combination regimen (1/3 the curative dose of flubendazole plus 1/3 the curative dose of praziquantel) 25 days post infection (283 ± 5.6 μm), and least in mice given the consecutive drug regimen (1/3 the curative dose of flubendazole 25 days post infection +1/3 the curative dose of praziquantel 7 weeks post infection) (219 ± 5.5 μm). The difference from infected untreated control mice was statistically significant at p < 0.05 for the former group, and p < 0.001 for the latter group (Table 5).

<table>
<thead>
<tr>
<th>Animal group</th>
<th>No. of lesions</th>
<th>Time of treatment</th>
<th>Hepatic granuloma mean diameter ± S.E. (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected control</td>
<td>10</td>
<td>–</td>
<td>309 ± 9.7</td>
</tr>
<tr>
<td>Infected flubendazole treated (1/3)</td>
<td>10</td>
<td>25 days post infection</td>
<td>286 ± 8.3</td>
</tr>
<tr>
<td>Infected praziquantel treated (1/3)</td>
<td>10</td>
<td>25 days post infection</td>
<td>298 ± 12</td>
</tr>
<tr>
<td>Infected 1/3 flubendazole +1/3 praziquantel</td>
<td>10</td>
<td>25 days post infection</td>
<td>283 ± 5.6*</td>
</tr>
<tr>
<td>Infected 1/3 praziquantel</td>
<td>10</td>
<td>7 weeks post infection</td>
<td>245 ± 7.4**</td>
</tr>
<tr>
<td>Infected 1/3 flubendazole +1/3 praziquantel</td>
<td>10</td>
<td>flubendazole: 25 days post infection praziquantel: 7 weeks post infection</td>
<td>219 ± 5.5***</td>
</tr>
</tbody>
</table>

Statistically significant difference from control group at * p < 0.05 at *** p < 0.001.
Highly significant difference from control group ** p < 0.001 at p < 0.001.
Flubendazole was given in a single oral dose of 33.3 mg/kg/b.w. single oral dose. Praziquantel was given in an oral dose of 2 × 333.3 mg/kg/b.w. Animals were sacrificed 7 weeks post infection.

4. Discussion

Broad spectrum anthelmintic drugs are usually prescribed by physicians to treat enteroparasitic infections. Thus, *S. mansoni* patients are quite often subjected to treatment with one of the broad spectrum anthelmintics available in our country. One of the aims of the present study, was to evaluate the possible deleterious effect of reduced doses (1/3 the curative ones) (Table 6) of flubendazole, a mebendazole derivative, and praziquantel in experimental schistosomiasis mansoni. This was evident by the hepatic worm shift, and the lower level of intestinal and hepatic worm and egg loads, especially in the group given the consecutive drug regimen (1/3 the curative dose of flubendazole 25 days post infection, then 1/3 the curative dose of praziquantel 7 weeks post infection). Again in this group, concurrently with the low level of hepatic worm recovery, a smaller hepatic granuloma mean diameter was recorded. This is consistent with previous findings of Askonas et al. [2], who stated that in infected mice treated with praziquantel there is an increase in the number of dead ova with consequent reduction of secreted antigen. The authors deduced that the stimulus for B-cell division ceases, when all the antigen has been removed. Reduction of granuloma size after praziquantel therapy was previously reported by Botros et al. [4], who noted diminution in the number of T-helper cells in the granulomata. Hassan et al. [8] and Botros et al. [5] suggested that this treatment induced reduction in granuloma size could be due to inhibition of the inflammatory mediators released at the site of granulomatous inflammation. Moreover, suppression of this reaction as a T-cell mediated response, cannot be ruled out. In favour for their conclusion, was the reduction in the number of Lyt1 T-lymphocytes in the granuloma after praziquantel treatment recorded by Botros et al. [5] and Hassan et al. [8]. In this work the observed reduction in granuloma size after flubendazole treatment is consistent with the previous assumption of Pancera et al. [14] and Nessim et al. [13]. On the other hand, Montenegro et al. [11] previously reported that with levamizole (one of the broad spectrum anthelmintic drugs belonging to the mebendazole group), when administered before *S. mansoni* infection, mice were usually more susceptible than resistant to the trematodes, which was probably due to the interference with the chemotaxis of blood polymorphonuclear leukocytes. Recently, Schmidt [18] reported that benzimidazole derivatives (including mebendazole and flubendazole) do not alter the replenishment of surface glycoconjugates in *S. mansoni* infected mice subjected to treatment. The diminution in the coating with glycoconjugates of the surface of the worms constitutes a secondary effect of benzimidazoles that might act synergistically with immune mechanisms [18].

In summary, the results obtained with flubendazole and praziquantel administration in reduced doses (1/3 the curative ones) to *S. mansoni* infected mice support...
the hypothesis of a partially deleterious effect upon trematodes. Flubendazole was administered 25 days post infection because it is known to act maximally during this schistosomal maturation period. The significance of the animal experiment has for human therapy is that mebendazole is frequently taken without medical prescription to treat supposed worm infections in humans. In underdeveloped countries, patients affected with Schistosomiasis may be harbouring concurrently other parasitic nematodes or cestodes such as *Anclylostoma, Ascaris, Enterobius, Taenia or Hymenolepis nana* infections. Therefore, mebendazole may act as a double weapon targeted against schistosomes, cestodes and/or nematodes. Accordingly, mebendazole can be recommended in humans as a safe and less expensive broad spectrum anthelmintic (Pancera et al. 1997, Nessim et al. 2000). Nevertheless, it is of utmost importance to point out the benefit of this simultaneous or consecutive drug administration. By reducing the doses of the drugs, we are trying to diminish the cost expenditure and the side effects of each drug when used solely. This may be of help especially in poor underdeveloped countries where schistosomiasis is a common public health problem.

5. References


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