CHEMOTHERAPY AND CHEMOPROPHYLAXIS OF ANGIOSTRONGYLUS MALAYSIENSIS INFECTION IN RATS WITH LEVAMISOLE AND MEBENDAZOLE

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Abstract. The chemoprophylactic and chemotherapeutic effects of levamisole and mebendazole on Angiostrongylus malayensis infection in rats were studied. Both drugs were ineffective in preventing infection while the post-infection treatment showed 100% efficacy. Furthermore, levamisole and mebendazole when given in combination appeared to have an antagonistic effect.

Angiostrongylus cantonensis and A. malayensis (both lung-worms of rodents) were described in Peninsular Malaysia, the latter being more prevalent (Lim and Ramachandran 1979). When A. malayensis was first reported it was diagnosed as A. cantonensis (Schacher and Cheong 1960), until the Malaysian species was found to be morphologically (Bhaibulaya and Cross, 1971) and also pathogenically different (Cross and Fresh 1969) from that of the Thai and Hawaiian A. cantonensis. Six cases of eosinophilic meningoencephalitis have been reported in Malaysia and larval forms have been recovered from three of the cases. However, the worms recovered from these patients were not available for re-examination and the authors (Watts 1969, Bisenu et al. 1972) assumed that they were larvae of A. cantonensis.

Until now very few experimental drug trials have been carried out against angiostrongyliasis. In view of this and the serious medical importance of Angiostrongylus infection, we decided to carry out chemoprophylactic and chemotherapeutic studies against A. malayensis.

MATERIAL AND METHODS

Fifty outbred albino rats (4-6 weeks old) from the Division of Animal Resources, Institute for Medical Research, Kuala Lumpur were used in the experiments.

The infective 3rd-stage larvae (L3) were collected from naturally infected snails and slugs such as Quaonta striata, Macrochlamys resplendens, Micracornion malaysiense and Loovicolus alta. The slugs and slugs were macerated with a pair of scissors and allowed to settle in physiological saline (0.85% w/v). L3 migrated out of the snail tissue after about 15 minutes. The larvae were collected by the use of a Pasteur pipette under the dissecting microscope. L3 were collected in saline and divided into a standard infective dose of 100 L3 per animal for experiment 1 and 150 L3 per animal for experiment 2. The infective dose was fed to the animals by using a stomach tube.

Levamisole and mebendazole (source) tablets were crushed and dissolved in distilled water. The dosage of the drug was calculated according to the body weight of the animal. The drug was fed orally by using a stomach tube.

The trials were divided into two experiments. All animals were killed at the end of each experiment, and the worm burden was assessed. The efficacy was calculated as follows:

\[
\text{worm recovery in control group — worm recovery in treated group} \times 100
\]

\[
\text{worm recovery in control group}
\]

1) This study is a part of the thesis submitted to the University of Malaya in fulfillment for a Ph. D. degree by S. Ambu
Experiment 1. Twenty-five animals were divided into five groups of 5 animals per group. Groups 1 and 2 were treated with levamisole while groups 3 and 4 were treated with mebendazole at a dose of 20 mg/kg weekly for 5 weeks. All 25 rats were infected with 100 L3 each on the 6th week. The treatments in groups 2 and 4 were resumed from the 7th week and terminated on the 9th week. All animals were killed on the 10th week.

Experiment 2. Twenty-five animals infected with 100 L3 each were divided into 5 equal groups. Levamisole was given as a standard dose of 50 mg/kg body weight together with mebendazole at a dosage of 1 mg/kg, 5 mg/kg, 10 mg/kg, and 15 mg/kg body weight to groups 1—4, respectively. The treatment was given at 30 days after infection, once a week for 4 weeks. All animals were killed 1 week after the last treatment.

RESULTS

In experiment 1 (Table 1), levamisole used as a chemoprophylactic drug showed an efficacy of only 97.4%, but when the treatment was continued after infection there was a 100% cure rate, thus confirming the effectiveness of the drug in the early stages of infection.

Similarly, mebendazole had no chemoprophylactic properties at all (group 3) but when the treatment was continued after infection there was a 100% efficacy (group 4).

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug Dosage</th>
<th>Duration of treatment</th>
<th>Mean worm recovery</th>
<th>Efficacy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>levamisole</td>
<td>5 weeks pre-infection</td>
<td>42.6 ± 4.73</td>
<td>97.4</td>
</tr>
<tr>
<td>2</td>
<td>levamisole</td>
<td>5 weeks pre-infection</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>mebendazole</td>
<td>5 weeks post-infection</td>
<td>49.6 ± 6.24</td>
<td>94.5</td>
</tr>
<tr>
<td>4</td>
<td>mebendazole</td>
<td>5 weeks post-infection</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>Controls</td>
<td>47.2 ± 4.59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Combined therapy with levamisole (Leva) and mebendazole (MBZ) on rats infected with 100 L3 of *A. malayanensis*

<table>
<thead>
<tr>
<th>Group</th>
<th>Daily dosage in mg/kg × days</th>
<th>Mean worm recovery</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leva 50 + MBZ 1 mg × 5</td>
<td>3.8 ± 2.1</td>
<td>90.77</td>
</tr>
<tr>
<td>2</td>
<td>Leva 50 + MBZ 5 mg × 5</td>
<td>31.0 ± 5.7</td>
<td>24.75</td>
</tr>
<tr>
<td>3</td>
<td>Leva 50 + MBZ 10 mg × 5</td>
<td>15.6 ± 3.7</td>
<td>62.13</td>
</tr>
<tr>
<td>4</td>
<td>Leva 50 + MBZ 15 mg × 5</td>
<td>12.2 ± 3.7</td>
<td>76.38</td>
</tr>
<tr>
<td>5</td>
<td>Controls</td>
<td>41.2 ± 6.8</td>
<td></td>
</tr>
</tbody>
</table>

In the infected and treated groups the lungs were grossly enlarged and granulomatous, but in the pre-and post-infection treated groups they were normal in appearance.

In experiment 2 (Table 2), a combined therapy of levamisole and mebendazole on a 30-day-old infection showed that a combination of 50 mg levamisole and 1 mg mebendazole had an efficacy of 90.77%. Groups 3 and 4 had an efficacy of 62.13% and 70.38% respectively, while group 2 had an efficacy of 24.75%.

DISCUSSION

The present study showed that levamisole did not act as a prophylactic, the reasons being that the drug is rapidly excreted and not retained in the body for long periods (Macintyre 1979). The efficacy in pre-infection-treated group was 97.4% and in post-infection-treated group 100%. Thus it is apparent that the traces of levamisole present in the tissue are not sufficient to kill the worms.

Similarly, mebendazole has no prophylactic activity at all but it is extremely effective against the early stages of the parasite. Oral doses of the drug are rapidly excreted and the very small amount that is retained temporarily in the body (Van Wijnigen 1976) is apparently not sufficient to kill the worms.

The combined therapy with 50 mg of levamisole, 1 mg of mebendazole administered daily for 5 days is very effective against the worms 30 days after infection (Table 2). Levamisole affects the acetylcholine response and inactivates the fumarate reductase system of worms resulting in their passive elimination (Brymoens et al. 1979). Mebendazole affects the glucose transport system of the worms. This combined effect of the drugs on the neuromuscular system and the glucose uptake should result in quick death of the worms (Table 2). Bernberg et al. (1979) found a combined treatment of mebendazole (400 mg/kg) and levamisole (300 mg/kg) daily for 10 days to be effective in a perastin-like flukus infection. Levamisole is effective against *Brugia malayi* infection (Mac et al. 1974; Mak and Zaman 1980). Mebendazole is also larvicidal against *Brugia pahangi* in white rats (Mak 1981). Bennett et al. (1978) found that there was a synergistic effect between levamisole and mebendazole in the treatment of tetra-thyridia of *Mesocetoides corti*. Heath and Lawrence (1979) in the treatment of *Toxocara canis* and *Kabs* et al. (1978) in the treatment of school children with helmint infections found that the combination of the drugs was as effective as mebendazole given alone. It has been observed that when a low dosage of levamisole and a high dosage of mebendazole were given to mice infected with *Toxocara canis* forms, there was a significant reduction in cyst number and size (Bone 1980) but the results were not significant when the dosage of the two drugs was reversed. In the present study, in spite of the fact that the combined therapy with levamisole (50 mg/kg) and mebendazole (4 mg/kg) for 5 days was highly effective against adult *A. malayanensis*, the combination of these two drugs appeared to be more antagonistic than synergistic (Table 2).

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ХИМИОТЕРАПИЯ И ХИМИОПРОФИЛАКТИКА ПРИ ПОМОЩИ ЛЕВАМИСОЛА И МЕБЕНДАЗОЛА У КРЫС, ЗАРАЖЕННЫХ НЕМАТОДОЙ ANGIOSTRONGYLUS MALAYSIENSIS

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Резюме. Исследовали химиотерапевтические и химопрофилактические действия левамисола и мебендазола на крыс, зараженные нематодой Angiostrongylus malayensis. Лекарства не оказали химотерапевтического действия, но они оказались эффективными (97.4%) для лечения. При одновременном применении левамисола и мебендазола они оказывали антагонистическое действие.
REFERENCES


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