EFFECT OF DIETHYLCARBAMAZINE CITRATE ON ANGIOSTRONGYLUS MALAYSIENSIS INFECTION IN RATS

STEPHEN AMBU, KWA BOO HOE,1) MAK JOON WAH,2) and INDER SINGH

Division of Medical Ecology and 2)Division of Filariasis, Institute for Medical Research and 2)Department of Zoology, University of Malaya, Kuala Lumpur, Malaysia

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SUMMARY: The aim of the study was to observe the effect of diethylcarbamazine citrate (DEC) on Angiostrongylus malaysiensis infection in albino rats. An attempt was made to vary the dose of DEC and treat infected animals at the larval and adult stages of infection. The doses were varied with an aim at finding an effective dosage. Animals were treated also during the pre-infection period to observe if the drug had any prophylactic properties.

The results obtained show that DEC given after infection was effective only when its administration was initiated on day 42 of the infection corresponding to the mature adult stage of the parasite. In addition, it had some prophylactic activity against the infection.

INTRODUCTION

Diethylcarbamazine (DEC) is effective against various nematode infections and is the drug of choice in the treatment of filariasis (Hawking, 1978). It has also been successfully used in the treatment of eosinophilic meningoencephalitis due to Angiostrongylus cantonensis (Watts, 1969; Williams, 1967; Alicata and Jindrak, 1970). However, experimental studies by Nishimura (1965) have shown DEC to be ineffective against A. cantonensis.

We studied the effects of various doses and duration of treatment with DEC on A. malaysiensis infection in experimentally infected rats. A. cantonensis and A. malaysiensis vary in the number of days taken to complete their life cycles, being 40–42 and 35 days, respectively (Lim and Ramachandran, 1979). A. malaysiensis is also less pathogenic than A. cantonensis in rats and in monkeys (Cross and Fresh, 1969; Cross, 1979).

MATERIALS AND METHODS

Outbred albino rats (4–6 weeks old) from the Division of Animal Resources, Institute for Medical Research, Kuala Lumpur, were used in all the experiments. A total of 110 rats were used for the three experiments.

* This study is a part of the thesis by Stephen Ambu submitted to the University of Malaya in fulfilment for a Ph. D degree.
The infective 3rd-stage larvae (L₃) were collected from naturally infected snails and slugs. L₃ were collected from snails and slugs macerated in saline and divided into standard doses of 150L₃ per animal, except in experiment 3, in which each animal was given only 100L₃. The infective dose was fed to the animal with a stomach tube.

Dosage: DEC tablets were crushed, suspended in distilled water and fed to the animals orally with a stomach tube.

All animals were killed at the end of each experiment and the worm burden was assessed.

Experiment 1: Seventy rats were divided into eight groups. Groups 1–4 had 10 animals per group, while Groups 5–7 five animals each. Group 8 with 15 animals served as control. Dosages of 1, 5, 25, 50, 100, 150 and 200 mg of DEC/kg body weight were given to groups 1–7, respectively. Treatment was started 7 days after infection and the drug was given daily for 5 consecutive days (Table I).

Experiment 2: Twenty-five rats were divided into five groups of five animals each (Table II). DEC was given to Groups 1, 2, 3 and 4 at a dosage of 50 mg/kg body weight with the fifth group serving as control. Group 1 was treated daily for 6 days, Group 2 for 8 days, Group 3 for 10 days and Group 4 for 12 days. Treatment was started 42 days after infection.

Experiment 3: Fifteen rats were divided into three groups of five animals per group (Table III). Groups 1 and 2 were treated weekly with a single standard dose of 20 mg of DEC/kg body weight for 5 weeks. All 15 rats were infected with 100L₃ in the sixth week. Treatment of Group 2 was resumed in the 7th week and terminated in the 9th week. Group 3 served as control.

RESULTS

Treatment of animals with DEC was started 7 and 42 days post-infection, respectively. By day 7, the infective larvae in the brain would have moulted to the fourth stage. The fourth moult occurs approximately 12 days post-infection and migration to the lungs in about 28 days. By day 42 all the worms would be in the lungs as adults.

In experiment 1, in which administration of DEC was started 7 days post-infection, all the infected animals survived until killed at the end of the experiment (31 days after infection). Worms were recovered from both the lungs and heart. The lungs of all animals were grossly enlarged and granulomatous. There was also a decrease in mean worm burden (range 34.70 ± 5.60 to 63.75 ± 9.82) in the treated groups when compared with the control group (66.70 ± 3.20). However, the worm reduction was statistically significant only in the group given DEC at a daily dose of 50 mg/kg. In Groups 1 to 4, a dose-response tendency was observed. Efficacy increased as the dose increased. However, at dosages above 50 mg/kg, the results obtained were inconsistent and even showed less efficacy than the lower dosages (Table I).
### TABLE I

**Efficacy of various doses of DEC given to rats infected with 150L₃ of Angiostrongylus malaysiensis 7 days post infection**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Dosage (mg/kg body wt.) × duration in days of treatment</th>
<th>Mean worm recovery</th>
<th>Efficacy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1×5</td>
<td>61.90± 5.23</td>
<td>7.21</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>5×5</td>
<td>55.50±10.27</td>
<td>16.80</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>25×5</td>
<td>46.60±6.21</td>
<td>30.14</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>50×5</td>
<td><strong>34.70±5.69</strong></td>
<td>47.98</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>100×5</td>
<td>66.75±9.82</td>
<td>4.43</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>150×5</td>
<td>50.25±5.84</td>
<td>24.67</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>200×5</td>
<td>60.40±6.78</td>
<td>9.45</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>—</td>
<td>66.71±3.22</td>
<td>—</td>
</tr>
</tbody>
</table>

* Efficacy = \[
\frac{\text{Worm recovery in control} - \text{Worm recovery in treated group}}{\text{Worm recovery in control}} \times 100
\]

** Significantly different from control at P<0.05, students t-test.

### TABLE II

**Efficacy of DEC at a dose given to rats infected with 150L₃ of Angiostrongylus malaysiensis 42 days post infection**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Duration in days of treatment</th>
<th>Mean worm recovery</th>
<th>Efficacy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>6</td>
<td>44.00±10.00</td>
<td>41.52</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>8</td>
<td>44.40±7.34</td>
<td>40.99</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>10</td>
<td>42.60±9.89</td>
<td>43.38</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>12</td>
<td>36.80±5.22</td>
<td>51.09</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Control</td>
<td>75.25±1.65</td>
<td>—</td>
</tr>
</tbody>
</table>

* Efficacy = \[
\frac{\text{Worm recovery in control} - \text{Worm recovery in treated group}}{\text{Worm recovery in control}} \times 100
\]

All four groups significantly different from the control at P<0.05, students t-test.

### TABLE III

**Comparative results of chemotherapeutic and chemoprophylactic trials with DEC at a standard single weekly dose of 20 mg/kg on rats infected with 100L₃ of Angiostrongylus malaysiensis**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of animals</th>
<th>Duration of treatment</th>
<th>Mean worm recovery</th>
<th>Efficacy %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>5 weeks pre-infection</td>
<td><strong>24.80±7.23</strong></td>
<td>47.45</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>5 weeks pre-infection</td>
<td>** 4.00±1.76**</td>
<td>91.52</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>3 weeks post-infection control</td>
<td>47.20±4.59</td>
<td>—</td>
</tr>
</tbody>
</table>

* Efficacy = \[
\frac{\text{Worm recovery in control} - \text{Worm recovery in treated group}}{\text{Worm recovery in control}} \times 100
\]

** Significantly different from control at P<0.05, student t-test.
In experiment 2, in which treatment was given 42 days post infection, a decreased worm burden was observed as the duration of treatment increased (Table II). In all treated groups, there was a significant reduction in the worm load of above 40% compared with the control group (75.25 ± 1.65). The lowest worm recovery was from Group 4 treated for 12 days (36.80 ± 5.22 worms). The lungs were all enlarged. Unlike in experiment 1, in which worms were recovered from both the lungs and the heart, no worms were found in the heart in this experiment.

Experiment 3 shows that as a chemoprophylactic drug, DEC had an efficacy of 47.45% and when the treatment was continued after the infection, the efficacy increased to 91.52%, being statistically significant (Table III). In the pretreated group, the lungs were enlarged and granulomatous but in the post treated groups, they were normal in appearance.

**DISCUSSION**

Nishimura (1965) treated *A. cantonensis*-infected rats with DEC for 5 consecutive days starting 14, 17 and 21 days after infection. In the present study, DEC was given 7 and 42 days after infection to test the efficacy against the early brain stages and mature adult worms, respectively. DEC has been used for treating eosinophilic meningoencephalitis and favourable results reported (Watts, 1969). The success of the treatment was mainly based on the disappearance of symptoms of the disease and general recovery of the patient but it remains to be studied as to whether DEC had any effect on the course of the illness.

Williams (1967) reported that DEC in combination with chloroquine halted the progress of the disease followed by disappearance of symptoms. Alicata and Jindrat (1970) reported the combined therapy with sulphadimidine or sulphafurazole with DEC. Various antibiotics and antihistaminics have no effect on the course of the disease. Analgesic (Bailey, 1948) and steroids (Horio and Alicata, 1961) have been used to successfully relieve the symptoms. This indicates the empirical use of drugs without actually knowing what action it has on the course of the disease.

In the present study, higher doses of the drug were not effective against larval stages of the parasite (Table I, Exp. 1). These results can be attributed to the fact that when infected animals are given high dosage of the drug, large amounts of the drug are excreted intact in the urine within 24 hr after treatment (Bangham, 1955; Ramachandran and Sharma, 1974). Furthermore, it is known that similar drugs at high dosages form insoluble precipitates, which are not absorbed (Van den Bossch, unpublished data).

The amount of DEC excreted was successfully quantitated by the quantitative calorimetric method (Ramachandran, 1973). Workers have found that in 24 hr a significant amount of DEC was found only in the kidney and 30.26% of the drug excreted in the urine (Ramachandran and Sharma, 1974), showing that the drug was quickly metabolized and excreted (Bangham, 1955b).

The drug has some prophylactic activity on the worm (Table III). In the
treatment of filarial infections, DEC has successfully been used over a long period of time (Chen, 1964). Duke and Moore (1961) treated patients with *Loa loa* infection with 200 mg of DEC three times a day for 20 consecutive days and found it to be effective and the dose well tolerated. The present study showed that weekly treatment of angiostrongyliasis with a 20 mg/kg dose had a significant effect on the worm burden. Hence, the dosage for the treatment of angiostrongyliasis may be increased and the duration prolonged for complete cure in animal infections. From the fact that the first symptoms of the disease in man develop within 12 to 28 days after infection, the ideal treatment would be such that relieves symptoms immediately. Nishimura (1965) showed that the drug had very little effect when given 14, 17 or 21 days post infection. Thus, the drug cannot be regarded as specific against angiostrongyliasis.

The chemoprophylactic effect in the present study may have been due to the effect of the drug on L₃ before the third moult in the brain. It has been reported that single doses of DEC have an effect on microfilaria even 4–8 days after treatment (Hawking et al., 1950). In *A. malaysiensis* infection, the worms undergo their 3rd moult in the brain between 4–6 days after infection. Hence, at this stage, the traces of DEC present in the circulation due to pretreatment might alter the structure of the larvae before the 3rd moult making them susceptible to treatment. However, the treatment may not kill the larvae immediately. Piessens and Beldekas (1979) showed that DEC increased the antibody-mediated adherence of leukocytes to *B. malayi* microfilariae. However, Kamiya and Tanaka (1969) reported that detectable antibodies due to *A. cantonensis* coincided with the appearance of larvae in the faeces, which suggests that the antigenic stimulation is furnished by adult worms or their metabolic products or both. In the present study, treatment was instituted before and during the early stages of the infection. Infective larvae and probably L₄ were killed by the drug. Whether the deaths of these early stages and the presence of surviviors could bring about immunological stimulation of the host needs to be studied. Similarly whether such immunological responses, combined with a possible immunomodulating effect of DEC, could have resulted in the death of the parasite remains to be studied.

Although DEC has been tested and found to be effective on domestic animal lung worms such as *Dictyocaulus viviparus* in cattle, *D. filaria* in sheep, *Aleurostrongylus abstrusus* in cats and *Metastrongylus apris* in pigs (Hawking, 1978), the same cannot be said for *Angiostrongylus cantonensis* or *A. malaysiensis* infection.

From the above observations, DEC does not seem to be a very effective drug for treating angiostrongyliasis since its effects on the worm is not significant when given during the post infection period. These results suggest that other drugs should be alternatively tested for their effects on angiostrongyliasis.

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