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Studies on the experimental chemotherapy of *Angiostrongylus malaysiensis* infection in rats with mebendazole and levamisole

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Summary

The effect of levamisole hydrochloride and mebendazole on *Angiostrongylus malaysiensis* infection in albino rats was studied. Animals at different stages of infection were treated with various oral doses of levamisole and mebendazole with the aim of finding an effective treatment regime. Levamisole was most effective for treating rats seven days after infection but its efficacy dropped as infection progressed. Mebendazole given at a dose of 1 mg/kg for five days was more effective against early larval stages (97·39% efficacy). At 5 mg/kg for five days mebendazole was more effective than levamisole against all stages of the infection.

Introduction

In Malaysia, *Angiostrongylus malaysiensis* was first reported as *A. cantonensis* by SCHACHER & CHEONG (1960). LIM *et al.* (1965) found morphlogical differences between the Malaysian "*A. cantonensis*" and that described by CHEN (1935) and MACKERRAS & SANDARS (1955). Based on the difference in both morphology and pathogenicity (CROSS & FRESH, 1969), a new species *A. malaysiensis* was proposed (BHAIBULAYA & CROSS, 1971). The Malaysian species has been reported to be pathogenic to man. The first five human cases of eosinophilic meningoencephalitis in Malaysia were reported from Sarawak, East Malaysia (WATTS, 1969) and the sixth case was allegedly found in Kuala Lumpur (BISSERU *et al.*, 1972). Larval worms were recovered from three of these patients from Malaysia but the worms were not available for re-examination and the authors assumed them to be larvae of *A. cantonensis*.

Various drugs have been used against *A. cantonensis* infection in experimental models by different research workers. Diethylcarbamazine was found to be ineffective in rats infected with *A. cantonensis* (NISHIMURA, 1965). Although most angiostrongylosis cases in man were mild, fatalities were not infrequent (PUNYAGUPTA, 1979). As no specific treatment was known, some workers have relied on symptomatic treatment (DESCHIENS & COURDURIER, 1967). Analgesics such as salicylates (BAILEY, 1948) and steroids (HORIO & ALICATA, 1969) have been used to relieve the symptoms but, to date, there is no effective specific therapy for the infection or the disease (PUNYAGUPTA, 1979).

Materials and Methods

300 outbred albino rats (four to six weeks old) from the Division of Animal Resources, Institute for Medical Research, Kuala Lumpur were used in the four experiments.

Infective 3rd-stage larvae (L3) were collected from naturally infected snails and slugs, e.g., *Quantala striata*, *Macrochlamys resplendens*, *Microparmarion malayanum* and *Laevicaulus alte*. The snails 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 min and were collected with a pasteur pipette under the dissecting microscope. L3 were collected in saline, divided into standard infective doses of 150L3 per rat and intubated into the stomach.

Drugs were given orally by stomach tube as a suspension in distilled water.

The drug trials were divided into four different experiments and worms obtained at autopsies at the end of each were examined for evidence of drug effect. Histopathological studies were also carried out. Percentage efficacy of the drug regime was calculated as:

$$\frac{\text{No. of worms recovered from experimental group}}{\text{No of worms recovered from control group}} \times 100$$

In Expt. 1 (Table I), 50 rats each infected with 150L3 were divided into eight groups of five, except for Group 9 (controls) with 10 animals. Mebendazole was given at dosages of one, 5, 25 and 50 mg/kg body-weight respectively to Groups 1 to 4. Levamisole at similar dosages were given to Groups 5 to 8. All groups were treated for five days from day 7 after infection and autopsied 28 days later.

In Expt. 2 (Table II), 50 rats each infected with 150L3 were divided into groups of five, except for Group 9 (controls) with 10 animals. Mebendazole was given once to Groups 1 to 4 as a single dose of 25 mg/kg body-weight on days 7, 14, 21 and 28 after infection respectively. Similarly 50 mg/kg body-weight of levamisole was given to Groups 5 to 8. All animals were autopsied 38 days after infection.

In Expt. 3 (Table III), 50 rats each infected with 150L3 were divided into 10 groups, each of five animals. Levamisole was given daily, for five days, at a standard dose of 5 mg/kg body-weight to Groups 1 to 4 and 10 mg/kg body-weight to Groups 5 to 8 at 7, 14, 21 and 28 days respectively after infection. Another 125 rats, each infected with 150L3 were divided into five groups of 25 animals and treated for five days with 5 mg/kg body-weight of mebendazole at 7, 14, 21 and 28 days respectively after infection (Groups 9 to 12). The 13th group acted as control. All animals were killed 38 days after infection.

In Expt. 4 (Table IV) 25 rats, each infected seven

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days previously with 150L3, were divided into four groups of five animals. Groups 1 to 4 were given levamisole single doses of 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg body-weight respectively per group, the fifth group being the control. All animals were killed 32 days after infection.

Results

Mebendazole given at various doses (one, 5, 25, 50 mg/kg doses) seven days after infection was shown to be highly effective (Table I). An efficacy of 97·39% was obtained even at 1·0 mg/kg, worms (only eight) being recovered from one animal only. These worms were changed morphologically, numbers of eggs in females and of spermatids in males being reduced and internal organs showing signs of damage. All rats were symptom-free, even though worms were present. In Group 4, one animal died during the experiment and at autopsy the brain was found to be haemorrhagic.

Group 5 showed that a dosage of levamisole as low as 1 mg/kg body-weight was sufficient to produce a significant drop in the worm burden (5·6 ± 2·6 worms) when given seven days after infection. The 5, 25 and 50 mg dosages showed 100% efficacy. The lungs of all the treated rats appeared normal, although one rat in the 1 mg treatment group which died before the end of the experiment had 13 worms in the lungs.

None of the animals in the control group died during the experiment and there was a mean worm recovery of 59·35 ± 10·2.

A single dose treatment of 25 mg/kg given once at various stages of infection (Table II) showed that mebendazole had an efficacy of 78·12% in the seven-day post-infection period. Very high mean worm recoveries were seen in the 14 and 21-day post-infection groups (41 ± 14·5 and 61 ± 8·5 respectively) compared with the control group (52·15 ± 4·6). The lungs of all the infected rats were enlarged and granulomatous and the brain of one animal (Group 3) was found to contain fluid and collapsed as soon as it was removed from the cranium. Another rat in Group 3 died soon after treatment and 31 young adult worms were found in the lungs even at that early stage.

Levamisole given as a single dose of 50 mg/kg at various stages (Table II) showed that the 7-day and 14-day post-infection treatment groups had very significant cure rates (99·6% and 100% efficacy respectively). In the 28-day post-infection treatment

Table I—Efficacy of various doses of mebendazole (MEB) and levamisole (LEV) in rats infected with 150L3 of *Angiostrongylus malaysiensis* at 7 days after infection

Groups*	Drug	Dosage mg/kg×5 days	Mean worm recovery	Efficacy %
1	MEB	1	1·6	97·39
2		5	0	100
3		25	0	100
4		50	0	100
5	LEV	1	5·6±2·6	90·2
6		5	0	100
7		25	0	100
8		50	0	100
9	—	Control	59·35±10·2	—

*5 animals per group

Table II—Efficacy of single dose of levamisole (LEV) (50 mg/kg) and mebendazole (MEB) (25 mg/kg) in rats infected with 150L3 of *Angiostrongylus malaysiensis*

Groups	Drugs	Larval stage (Days-post infection)	Mean worm recovery	Efficacy %
1	MEB	7	9·8± 4·3	78·12
2		14	41 ±14·5	8·48
3		21	61 ± 8·5	0(−37·94)
4		28	19·4± 5·8	56·69
5	LEV	7	0·2	99·6
6		14	0	100
7		21	42·8± 9·24	28·06
8		28	28·4± 4·4	52·26
9	—	Control	52·15±4·6	—

Table III—Efficacy of levamisole (LEV) and mebendazole (MEB) in rats infected with 150L3 of *A. malaysiensis* at different stages of infection

Group	Drug	Dosage mg/kg×5 days	Days post-infection	Mean worm recovery	Efficacy
1		5	7	0·2	99·63
2	LEV	5	14	19·4 ± 8·9	64·57
3		5	21	42·4 ±14·2	22·58
4		5	28	63 ± 3·5	0(−15·02)
5		10	7	0	100
6	LEV	10	14	7·6 ± 7·1	86·12
7		10	21	37·6 ± 4·43	31·34
8		10	28	42·2 ± 9·84	22·95
9		5	7	0·1	99·82
10	MEB	5	14	5·48± 1·2	91·36
11		5	21	20·48± 2·8	64·07
12		5	28	8·17± 2·16	85·66
13	Controls	—	—	55·89±	—

Table IV—Efficacy of various single doses of levamisole given to rats infected with 150L3 of *A. malaysiensis* at 7 days after infection

Group	mg/kg body wt	Mean worm recovery	Efficacy %
1	10	2·2±1·42	95·7
2	20	0	100
3	30	0·2	99·60
4	40	0	100
5	Control	51±5·65	—

group the efficacy was 52·26% but in the 21-day post-infection treatment group it was only 28·06%. One rat in both Groups 5 and 7 and two in Group 6 died before the end of the experiment and at autopsy brain haemorrhage was seen. The lungs in the other infected rats (including control) were enlarged and granulomatous.

Mebendazole when given as a dose of 5 mg/kg for five days showed significant efficacy at seven (99·8%) and 14 days (91·36%) after infection. In the 28-day post-infection treatment group, the efficacy was 85·66% but in the 21-day post-infection treatment group it dropped 64·07% (Table III). One rat from Group 12 died before the end of the experiment and 44 worms were found in the lungs. Another rat in Group 12 was found to be dying and the dosage was increased to 10 mg/kg daily for four days. The rat recovered and at autopsy 15 worms were recovered from the lungs which were slightly enlarged.

Similarly levamisole (5 mg/kg and 10 mg/kg body-weight) given over a five-day period at various stages of infection showed high efficacy against the early stages of the infection (seven days after infection). It was also apparent that the effectiveness increased with the 10 mg/kg dose while the cure rate decreased as the infection progressed. Except for the seven-day post-infection groups in both treatment groups, all rats in the other treated groups had enlarged and granulomatous lungs.

Discussion

In the present study, mebendazole and levamisole have been shown to be very effective against larval *A. malaysiensis* when administered in various doses during early infection (Table I). Their larvicidal effectiveness were seen even with doses as low as 1 gm/kg body-weight but the drugs were less effective against later stages of the worms.

When treated with mebendazole, glycogen loss in encysted *T. spiralis* larvae was similar to that seen in adult *A. lumbricoides* (DE NOLLIN & VAN DEN BOSSCHE, 1973). The effect on *A. malaysiensis* larvae may be similar. The 78·12% efficacy with a single dose of 25 mg/kg mebendazole given once, 97·39% efficacy for 1 mg/kg × 5 days and 99·82% efficacy for 5 mg/kg × 5 days, showed that seven-day old *A. malaysiensis* larvae were definitely killed by mebendazole (Tables I, II & III).

Levamisole had been shown to be very effective against early larval *A. malaysiensis* (90 to 100% efficacy) regardless of dosages and duration of treatment (Table I and II). Similar results were obtained against *A. cantonensis* (95% efficacy) in multimammate rats using doses of 5 × 6·25 and 5 × 12·5 mg/kg (LAMMLER & WEIDNER, 1975), against *Ancylostoma caninum* larvae in multimammate rats (100% efficacy) with 25 mg × 5 days dose (LAMMLER & GENDI, 1978), and against cattle lungworm (100% efficacy) with single dose of 7·5 mg/kg (PRATZ, 1978).

Mebendazole has been observed not to penetrate the brain tissue of normal animals (Van den Bossche, personal communication). In the present study, however, *A. malaysiensis* larvae in the brain have been observed to be affected by the drug. This may be due to an alteration of the integrity of the blood-brain barrier as a result of the *A. malaysiensis* infection, allowing penetration of the drug into the brain tissue. During the one to 14-day post-infection period the worms migrate actively through the brain tissue and grow rapidly (LIM & RAMACHANDRAN, 1979). This rapid growth period may be correlated to a period of high metabolic activity which would possibly result in maximum absorption of the drug, thus explaining the high efficacy. At the 14-day post-infection period, there was steady growth and the worms were moving to the surface of the brain where they remain until their final migration to the lungs (LIM & RAMACHANDRAN, 1979). The reduced activity of the worms and their presence at this stage of the infection in the cerebro-spinal fluid would probably minimize their contact with the drug in the blood and could explain the observed drop in the efficacy of the drug given as a single dose between 14 and 24 days after infection (Tables II & III). The migration to the lungs starts as early as 21 days after infection, as seen from the recovery of worms from this site in the present study. The slight reduction in worm burden during this period (21 days; Tables II & III) may, therefore, be due to the effect of the drug on some of the early migrating worms in the blood stream. The growth of the worm is gradual until 24 days after infection (LIM & RAMACHANDRAN, 1979) and worms recovered 33 days after infection have been found to be 1·5 times longer than those found 24 days after infection. This gradual growth between 21 and 24 days, with a reduction is metabolic activity, may account for the fact that *A. malaysiensis* larvae treated during this period are able to tolerate the drug, resulting in a relatively high worm count (Tables II & III). Since the worms grew rapidly after day 24 and the migration of most of them to the pulmonary artery started at this time, the drug present in the blood 28 to 32 days after infection would be able to act on them. Hence the low recovery of worms from the group treated 28 days after infection (Tables II, III & IV).

Although levamisole has been shown to kill nemtodes by affecting their neuromuscular system (VAN DEN BOSSCHE, 1978), this effect was not evident during the migratory phase of the *A. malaysiensis* infection (14 days and more post-infection) in this study.

The damage in the lungs of untreated infected rats was caused by the first-stage larvae and the eggs which become calcified in the lung tissues. In rats treated with mebendazole and levamisole the worms produced malformed eggs which resulted in a drop in the number of first-stage larvae. This, is turn, was responsible for the difference in pathology of the lungs (slight enlargement) in treated rats as compared

to the untreated controls. Similar studies on the effect of levamisole on growth and egg production in adult nematodes have been described by a number of workers (LAMMLER *et al.*, 1971a, b; LECHAT *et al.*, 1974; MAK *et al.*, 1974; MILLER *et al.*, 1978; NAGATY *et al.*, 1978; PRATZ, 1978).

Our results showed that in the mebendazole-treated groups the internal organs of worms recovered at autopsy from the 5-day treated groups had been affected. The alimentary tract was very brittle and there were very few spermatids and eggs present in the males and females respectively. The lungs of infected treated animals in these groups were not granulomatous but were slightly enlarged. Mebendazole reduced the production of viable eggs which cause granuloma formation in the lungs of infected rats. The worms recovered were thin, unhealthy and smaller than normal, suggesting the mebendazole had a prolonged effect on the worms, probably starving them to death by preventing glucose uptake. Thus the over-all effect of the drug on the adult worm is to reduce the fecundity, resulting in reduced pathology in the lungs and slow death of the worm due to intestinal cell necrosis, as in *Ascaris suum* and *Syngamus trachea* (BORGERS *et al.*, 1975).

Results of this comparative study on levamisole and mebendazole show mebendazole to be the drug of choice, being effective against all stages of the worm, whereas levamisole was effective only against the early larval stages. Nevertheless, both drugs could possibly be used in human eosinophilic meningoencephalitis as it is the early larval stages (one to 20 days incubation period) that cause the disease (PUNYAGUPTA, 1979). Neither drug showed any apparent adverse effect on the treated animals.

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