IN VIVO EFFICACY OF LEVAMISOLE AGAINST LARVAL STAGES OF *ANGIOSTRONGYLUS CANTONENSIS* AND *A. COSTARICENSIS**

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INTRODUCTION

Human angiostrongylosis has been widely reported from various parts of the world. Among these zoonotic diseases, *Angiostrongylus cantonensis*, a nematode parasitic in the pulmonary artery and heart of wild rats, has been recognized as a causative agent of human eosinophilic meningoencephalitis (Alicata, 1962). This parasite has also a possibility of spreading from endemic areas to other regions in the world (Alicata, 1965; Ishii, 1984). Abdominal parasitosis described in Costa Rica is caused by *A. costaricensis* which usually parasitize in the mesenteric arteries (Morera, 1973). Since the first report in Costa Rica, many human cases have been reported throughout the Western Hemisphere, from Mexico to Brazil (Zambrano, 1973; Ziliotto *et al.*, 1975; Iabuki and Montenegro, 1979; Loría-Cortés and Lobo-Sanahuja, 1980). Because of the medical importance of these parasites, studies have been carried out to search for suitable drugs. In these studies, certain anthelmintics have been shown to be effective in animal infections with *A. cantonensis*. However, safe and satisfactory drugs are still inconclusive. On the other hand, there have been few studies on the *in vivo* efficacy of drugs against *A. costaricensis* (Terada *et al.*, 1987). For the treatment of human abdominal angiostrongylosis, some anthelmintics like diethylcarbamazine and thiabendazole have been used. However, whether the treatments were actually effective or not has never been confirmed yet (Loría-Cortés and Lobo-Sanahuja, 1980). In the present study the *in vivo* effects of levamisole against the larval stages of *A. cantonensis* and *A. costaricensis* were examined.

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MATERIALS AND METHODS

Compound tested Levamisole hydrochloride was purchased from Aldrich Chemical Co. Inc., and was dissolved in water for administration to experimental animals.

Animal treatment Five-week-old male ddY mice (25–27 g in body weight) and 4-week-old male Wistar rats (80–90 g in body weight) were used as host animals.

The isolation of infective third-stage larvae of *A. cantonensis* and *A. costaricensis* from

experimental intermediate host snails, *Biomphalaria glabrata* and infection of host animals with each of these infective larvae were carried out as described by Ishii *et al.*, (1983).

In experiment I, the relationship between the dose of drug and its effects against larval stages of *A. cantonensis* was examined in rats. Levamisole has been shown to be effective against the early stages of infection in rats at higher doses (Jindrak and Alicata, 1969). The present experiment dealt with the study of dose range which was effective on larval *A. cantonensis* in rats. Seven groups of 5 rats infected with 40 infective larvae of *A. cantonensis* each were given orally a single dose ranging from 0.1 to 100 mg/kg of drug on 6 days post-infection (pi). Additional non-treated control group of 5 rats received vehicle alone on 6 days pi. All rats (treated and control groups) were sacrificed 42 days pi and the number of worms in the heart and lungs was examined. The lung-body weight ratio on each rat was also determined by the calculation as 100 × lung weight / body weight, as this ratio was suggested to be a good indicator of drug effects in the infection (Ishii, 1987).

In experiment II, the relationship between the timing of administration of the drug and its effects on larval stages of *A. costaricensis* was examined in mice. Six groups of 10 mice infected with 20 infective larvae of *A. costaricensis* each were treated orally with a single dose of 30 mg/kg in a period of 1 to 6 days pi. Another group of 10 mice served as non-medicated controls. Body weight and number of surviving host animals in all groups were monitored until 42 days pi, and worms were recovered from the mesenteric arteries.

In experiment III, the relationship between the dose of drug and its effects was examined against larval *A. costaricensis* in mice. Five groups of 10 mice infected with 20 infective larvae of *A. costaricensis* each were given orally with 3 to 7 consecutive daily doses of 3 to 30 mg/kg from 3 days pi. A group of 10 mice served as the non-medicated control. Body weight and number of surviving mice in all groups were monitored until 42 days pi, and the worm recovery was done.

Statistical analysis In each experiment, statistical differences were examined by Student's *t*-test.

RESULTS

Experiment I. Effects of the dose of drug against larval stages of *A.cantonensis* in rats: Compared with the non-medicated control group, a significant reduction in worm recovery was seen in the group receiving a drug dose of 1.0 mg/kg or more (Table 1). Rats treated with a dose of 100 mg/kg of drug showed no worm burden. A significant decrease in host lung-body weight ratio was observed in the group receiving drug of 3.0 mg/kg or more (Table 1).

Experiment II. Effects of the timing of drug administration against larval stages of *A. costaricensis* in mice: As compared to the non-treated control mice, remarkable effects were observed in animals receiving a single dose of 30 mg/kg on 3, 4 or 5 days pi; no decrease in body weight and little death of host animals were seen (Fig. 1). One out of 10 mice died in the group treated on 3 or 4 days pi. In animals treated on 1, 2 or 6 days pi, effects were rather slight. Regarding worm burden, a significant difference was also seen in the group treated on 3, 4 or 5 days pi compared with the non-treated control (Table 2).

Table 1

Effects of levamisole on *A. cantonensis* in rats when given orally at various doses on 6 days post-infection.

Dose (mg/kg)	No. of rats positive / No. examined / No. used	No. of worms recovered (mean ± SD)	Rat lung-body weight ratio (mean ± SD)
0.1	5/5/5	30.8 ± 3.1	1.92 ± 0.37
0.3	5/5/5	28.4 ± 4.9	2.08 ± 0.58
1.0	5/5/5	19.0 ± 3.3*	1.50 ± 0.23
3.0	5/5/5	6.0 ± 3.5*	0.94 ± 0.14*
10	5/5/5	2.2 ± 1.6*	0.83 ± 0.05*
30	2/5/5	0.8 ± 1.3*	0.81 ± 0.03*
100	0/5/5	0.0 ± 0.0*	0.78 ± 0.04*
None	5/5/5	27.6 ± 3.9	1.83 ± 0.28

Each rat was given 40 third-stage larvae of *A. cantonensis* and sacrificed 42 days post-infection.
* : Significantly lower than the non-medicated control group ($P<0.05$, Student's *t*-test).

Table 2

Effects of levamisole on *A. costaricensis* in mice when given orally at 30 mg/kg once at various timinig post-infection.

Timing of drug administration after infection (day)	No. of mice positive / No. examined / No. used	No. of worms recovered (mean ± SD)
1	7/7/10	4.4 ± 2.6
2	5/7/10	2.3 ± 2.5
3	5/9/10	1.7 ± 1.6*
4	4/9/10	1.0 ± 1.2*
5	4/8/10	1.0 ± 1.3*
6	4/4/10	4.8 ± 2.5
None	3/3/10	5.6 ± 1.5

Each mouse was given 20 third-stage larvae of *A. costaricensis* and sacrificed 42 days post-infection.
* : Significantly lower than the non-medicated control group ($P<0.05$, Student's *t*-test).

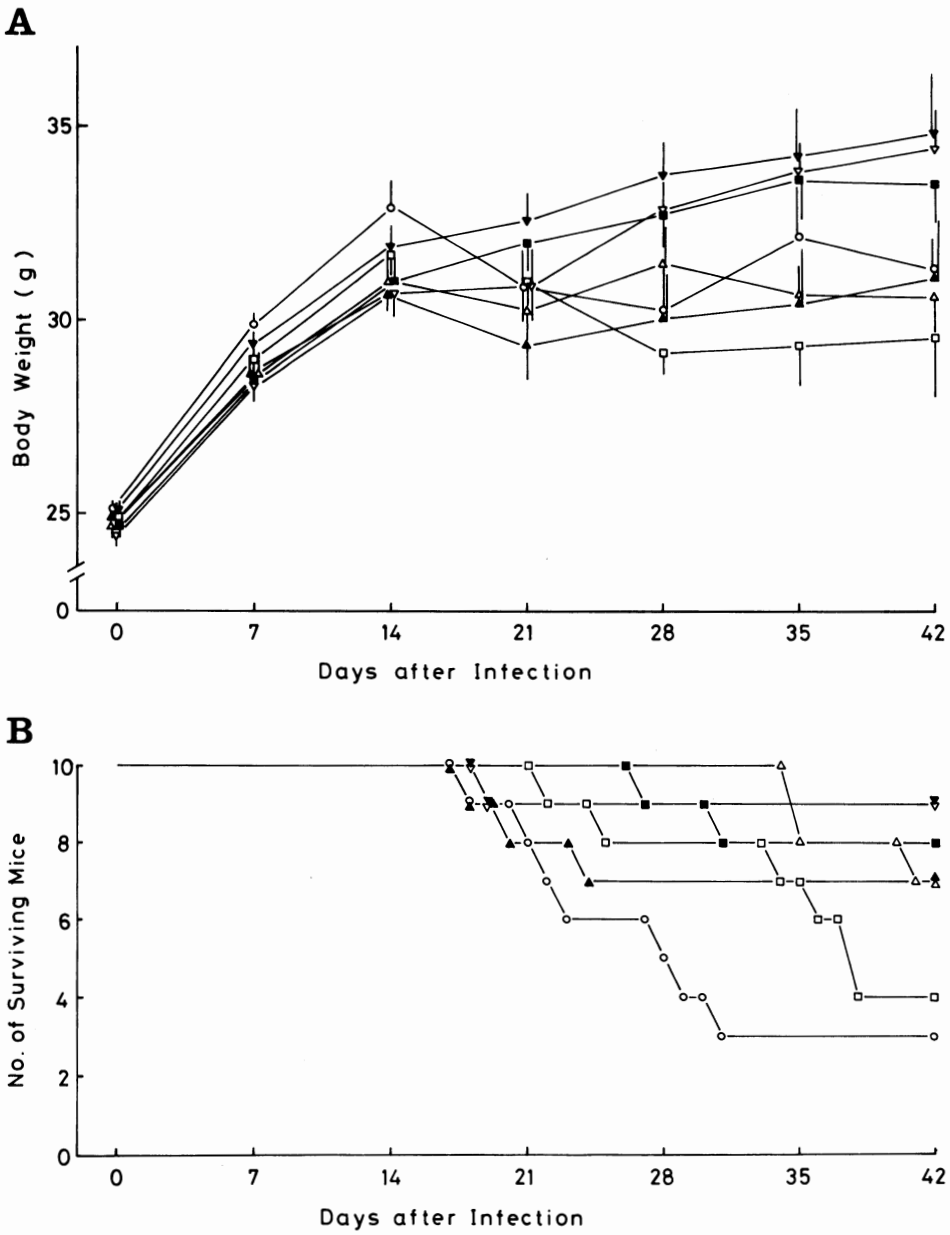

Fig. 1–Effects of levamisole on body weight (A) and surviving number (B) of mice infected with *A. costaricensis* (Experiment II). All mice were inoculated with 20 infective larvae and each group had 10 animals. A single dose of 30 mg/kg was administered on 1 day (▲—▲), 2 days (△—△), 3 days (▼—▼), 4 days (▽—▽), 5 days (■—■) or 6 days (□—□) postinfection. Control mice (○—○) received vehicle only. Each value represents the mean with SE shown as a vertical bar in (A).

Experiment III. Effects of the dose of drug against larval *A. costaricensis* in mice: As shown in Fig. 2, a noticeable difference was seen in the number of host death. No mice died and no decrease in body weight was seen in the group treated with 7 doses of 10 mg/kg, or 3 or 7 doses of 30 mg/kg. There was a significant difference in worm recovery between the non-medicated control and medicated group except the group receiving a dose of 3 mg/kg for 7 days (Table 3). No worm was recovered from all of 10 animals receiving 3 or 7 consecutive daily doses of 30 mg/kg.

DISCUSSION

In the present study levamisole had been shown to be very effective against larval stages of *A. cantonensis* in rats as well as of *A. costaricensis* in mice when administered orally during early infection. The efficacy against *A. cantonensis* in rats was seen from such parameters as number of worms recovered and host lung-body weight ratio. Anti-larval effects against *A. costaricensis* in mice were observed from worm recovery, changes in body weight and death of host animals.

Considering the migrating processes of *A. cantonensis* in man, it is reasonable to examine the efficacy of drugs on the early stages of infection. In previous studies, levamisole showed the *in vivo* effects on the larval stages of this parasite when given in doses of 40 to 160 mg/kg body weight (Jindrak and Alicata, 1969) or of 6.25 to 125 mg/kg (Lämmler and Weidner, 1975). In the present studies, larvicidal effects on *A. cantonensis* were seen even with a dose as low as 1.0 mg/kg. This effective dose is rather lower than those in previous reports. From the viewpoint of preventing adverse side-effect the present findings may be of value in further investigations for the treatment of human cases.

The anti-larval effects of levamisole were also remarkable in *A. costaricensis* infection in mice. Significant reduction in worm recovery was shown when given with a single dose of 30 mg/kg in a period of 3 to 5 days pi. In these treatment groups, however, 1 to 2 out of 10 mice died and half of the host animals were still positive for worm burden. The conspicuous *in vivo* efficacy was observed when given the drug in consecutive daily doses to animals. In the medicated groups, with 3 or 7 successive doses of 30 mg/kg, no decrease in body weight and no death of host animals were seen. The effective duration of treatment in *A. costaricensis* infection seems to be limited compared to that in *A. cantonensis* infection. There is also a difference in the effective dose level between both parasites. The process of migration of *A. cantonensis* in final hosts was examined in detail, and thereby the effect of the drug in relation to the localization of this parasite in the various organs of the host has been discussed (Jindrak and Alicata, 1969). The further studies on the migrating processes of *A. costaricensis* in final hosts should explain the differences between them.

From these results, it was suggested that levamisole had an effect on the development and growth of larval stages of both species of nematodes. Although levamisole has been shown to kill nematodes by affecting their neuromuscular system (Van den Bossche, 1978), this effect was not evident on the larval stages of these two parasites in this study.

As described in the introduction, anti-larval effects seem to be essential against

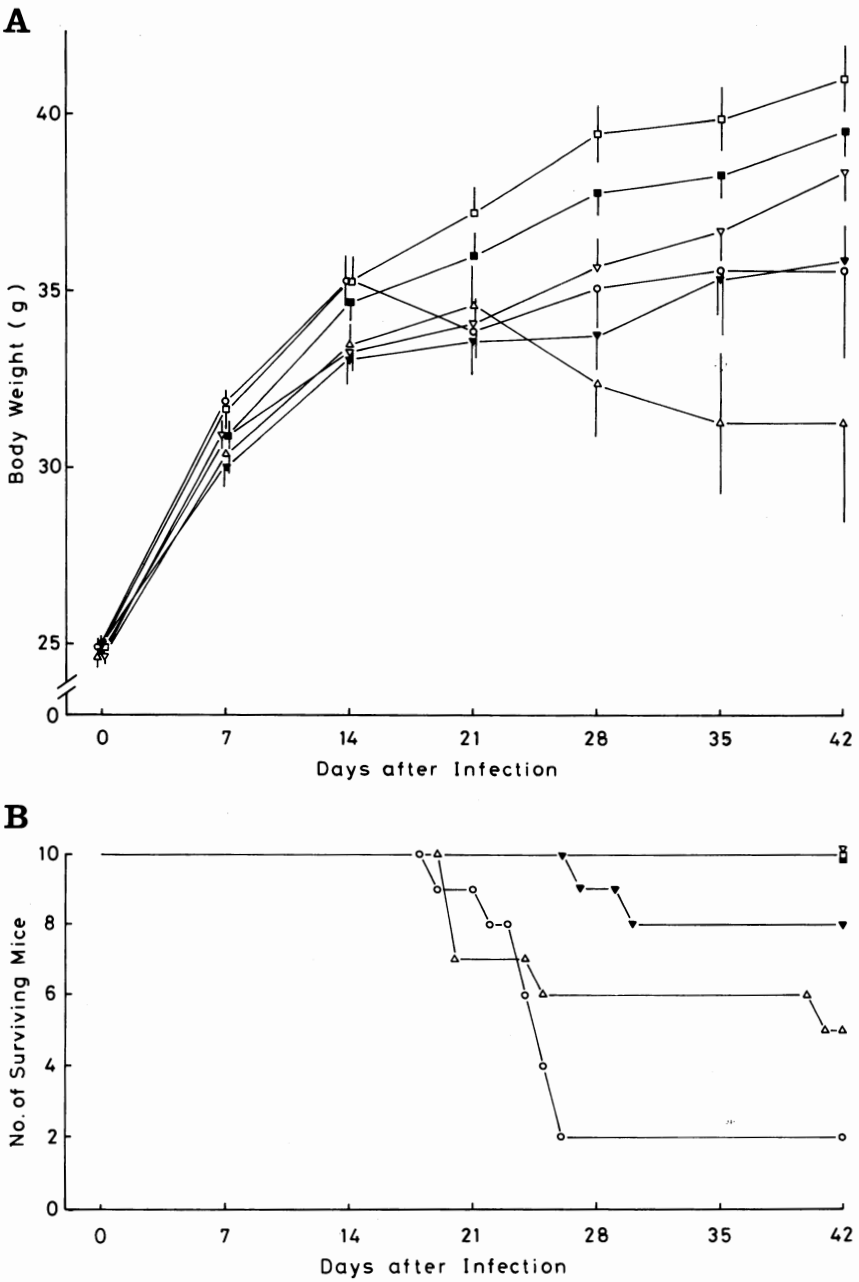

Fig. 2–Effects of levamisole on body weight (A) and surviving number (B) of mice infected with *A. costaricensis* (Experiment III). All mice were inoculated with 20 infective larvae and each group had 10 animals. Seven successive daily doses of 3 mg/kg (△—△), 10 mg/kg (▽—▽) or 30 mg/kg (□—□) and 3 consecutive doses of 10 mg/kg (▼—▼) or 30 mg/kg (■—■) were administered from 3 days postinfection. Control mice (○—○) received vehicle only. Each value represents the mean with SE shown as a vertical bar in (A).

Table 3

Effects of levamisole on *A. costaricensis* in mice when given orally at various doses from 3 days post-infection.

Dose (mg/kg × days)	No. of mice positive / No. examined / No. used	No. of worms recovered (mean ± SD)
3 × 7	5/ 5/10	5.0 ± 2.6
10 × 3	5/ 8/10	1.6 ± 1.6*
10 × 7	2/10/10	0.2 ± 0.4*
30 × 3	0/10/10	0.0 ± 0.0*
30 × 7	0/10/10	0.0 ± 0.0*
None	2/ 2/10	4.5 ± 0.7

Each mouse was given 20 third-stage larvae of *A. costaricensis* and sacrificed 42 days post-infection.
* : Significantly lower than the non-medicated control group ($P < 0.05$, Student's *t*-test).

human angiostrongylosis cantonensis, and such effects have been reported in animal experiments with drugs like thiabendazole (Cuckler *et al.*, 1965; Nishimura, 1965/66), *l*-tetramisole (Jindrak and Alicata, 1969; Lämmler and Weidner, 1975), mebendazole (Lämmler and Weidner, 1975; Hayashi *et al.*, 1982), flubendazole (Maki and Yanagisawa, 1983), avermectin Bla (Ishii *et al.*, 1983), ivermectin (Ishii *et al.*, 1985) and milbemycin D (Terada *et al.*, 1987). For human treatment satisfactory drugs are still unavailable inconclusive, and hence further investigations are needed.

On the other hand, *A. costaricensis* develops to adult stage in humans, studies on the anti-adult effects of anthelmintics on this nematode are desirable. It was reported that some anthelmintics including levamisole and diethylcarbamazine were effective on the *in vitro* adult worm motility of *A. costaricensis* (Terada *et al.*, 1986). This suggests that the effects of these drugs are promising on adult *A. costaricensis in vivo*.

SUMMARY

Anti-larval effects of levamisole were examined on *A. cantonensis* in rats and *A. costaricensis* in mice. 1) In rats inoculated with 40 infective larvae of *A. cantonensis* : Compared with a non-treated control group, a significant reduction in number of worms recovered was seen in the group receiving a single dose of 1.0 mg/kg or more. A significant decrease in host lung-body weight ratio was seen in the group receiving drug of 3.0 mg/kg or more. 2) In mice inoculated with 20 infective larvae of *A. costaricensis* : In the non-treated control group, a severe loss in body weight and death of host animals were observed. A single dose of 30 mg/kg on 3, 4 or 5 days post-infection remarkably inhibited these changes. At 30 mg/kg for 3 or 7 days levamisole was more effective than a single dose of the drug. These results suggest that levamisole has conspicuous *in vivo* effects against larval stages of *A. costaricensis* as well as *A. cantonensis*.

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