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Studies on Chemotherapy of Parasitic Helminths (XXIII) Effects of Ivermectin on *Angiostongylus cantonensis* in Rats

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The avermectins are novel antiparasitic agents produced by *Streptomyces avermitilis*, a new species of actinomycete (Burg *et al.*, 1979). They have been isolated (Miller *et al.*, 1979) and identified as a series of macrocyclic lactones (Albers-Schönberg *et al.*, 1981) and shown to be active against a wide variety of parasitic nematodes (Egerton *et al.*, 1979). A chemical derivative, ivermectin (22, 23-dihydroavermectin B_1 or MK-933)has been selected as a drug of special promise for the treatment of nematode and arthropod infections (Campbell, 1981/1982 ; Chabala *et al.*, 1980 ; Egerton *et al.*, 1980 , 1981 ; Klei and Torbert, 1980), and is the most potent broad-spectrum anthelmintic yet reported. Because of the medical importance of angiostrongylosis causing eosinophilic meningoencephalitis in the human, a nondefinitive host of *Angiostrongylus cantonensis*, studies have been carried out to determine the efficacy of certain drugs such as thiabendazole (Cuckler *et al.*, 1965 ; Nishimura, 1965/66), *l*-tetramisole (Jindrak and Alicata, 1969), mebendazole (Lämmler and Weidner, 1975 ; Hayashi *et al.*, 1982), avermectin B_{1a} (Ishii *et al.*, 1983) and flubendazole (Maki and Yanagisawa, 1983) against *A. cantonensis* in rats and other animals. However, satisfactory drugs are still inconclusive. In the present study, the effects of ivermectin against larval and adult stages of this parasite in rats, a definitive host, were examined prior to examining its efficacy on angiostrongylosis in nondefinitive hosts.

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Materials and Methods

Compound tested: Ivermectin (MK-933, L-640, 471-00W) was offered from Merck Sharp and Dohme Research Laboratories Co., LTD. and was dissolved in propylene glycol for administration to rats.

Animal treatment: Four-week-old male Wistar albino rats (80-90 g in body weight) were used in experiments as a definitive host. Infective third stage larvae of *A. cantonensis* were obtained from experimentally infected snails, *Biomphalaria glabrata*, by digestion using 0.2 % pepsin in 0.7 % HCl for 1 hr at 37 °C.

In the experiment A, effects of ivermectin on various developmental stages of this parasite in rats were preliminarily examined. All rats were inoculated orally with 20 larvae suspended in Tyrode's solution by a stomach tube, and divided into 9 groups of 3 rats each, i. e., 8 treated groups (A 1-A 8) and one non-treated, infected control group (A 9). Ivermectin was administered intraperitoneally with a single dose of 2.0 mg/kg of body weight 1/2 hr, 3 days, 1, 2, 3, 4, 5 or 6 weeks after infection. Infected control group received equal volumes of vehicle only. From 5 weeks after infection, the first stage larval counts in rat faeces were examined weekly, being represented as larvae per gram of faeaes per female worm recovered (LPG-PF). All rats of treated and control groups

were sacrificed 19 weeks after infection and the number of worms in the heart and lungs was examined to determine the recovery rate. Additionally, 2 rats used for the histological observations of their lung tissues, were administered with a single dose of 2.0 mg/kg 7 weeks after infection and sacrificed 3 weeks after treatment. Thin sections of lung tissues were stained by hematoxylin and eosin.

In the experiment B, effects of ivermectin on earlier stages of *A. cantonensis* in rats were examined. All rats were inoculated orally with 40 infective larvae, and were divided into 6 groups of 6 rats each, i. e., 5 treated groups (B 1–B 5) and one non-treated, infected control group (B 6). The drug in propylene glycol was administered intraperitoneally with a single dose of 2.0 mg/kg 1/2, 3, 12, 24 or 72 hr after infection. The first stage larval counts in rat faeces were done once at the time of sacrification. All rats of treated and control groups were sacrificed 12 weeks after infection, and the number of adult worms in the heart and lungs was examined for the recovery rate and the presence of the first stage larvae in lung tissues was ascertained. Microscopical observations of recovered female worms were also performed.

In both experiments, statistical significances were examined by means of Student's *t*-test.

Results

Experiment A: As summarized in Table 1, in each of groups treated 1/2 hr, 3 days and 4 weeks after infection (A 1, A 2 and A 6), there was a significant reduction in the recovery of the worm compared to that of non-treated, infected control group (A 9) ($p<0.05$). Especially in the A 1, no worm was recovered. The first stage larval count per female was shown as an average of 3 rats. As shown in Fig. 1, 3 patterns were roughly observed on LPGPF. In the groups treated 3 days, 1 or 2 weeks after infection (A 2, A 3 or A 4), the pattern was similar to that of infected group without treatment (A 9). In the groups treated 3 or 4 weeks after infection (A 5 or A 6), the larval output into faeces delayed and larvae appeared from 8 or 9 weeks after infection. In the groups treated after 5 or 6 weeks (A 7 or A 8), the larval count remarkably decreased 1 or 2 weeks after treatment. From 6 weeks after treatment, however, larvae reappeared in faeces. In the histological sections of lung tissues of rats 3 weeks after treatment, very few eggs and larvae were observed, and eggs in the uterus of female worm seemed to be degenerated (Fig. 2).

Table 1 Effects of ivermection on developmental and adult stages of *A. cantonesis* in rats when given 2.0 mg/kg intraperitoneally at various intervals after infection

Group	Time of drug administration after infection	No. of worms recovered (Mean ± S.D.)	Recovery rate (%)
A 1	1/2 hr	0 ± 0*	0
A 2	3 days	10.0 ± 2.6†	50.0
A 3	1 week	15.3 ± 1.5	76.7
A 4	2 〃	14.0 ± 1.7	70.0
A 5	3 〃	9.0 ± 5.3	45.0
A 6	4 〃	5.7 ± 4.5†	28.3
A 7	5 〃	11.7 ± 2.5	58.3
A 8	6 〃	13.0 ± 2.0	65.0
A 9	—	15.7 ± 1.5	78.3

Three rats were used in each group and 20 third stage larvae were given orally.
All rats were sacrificed 19 weeks after infection.
* Significantly lower than control group ($p<0.01$, Student' *t*-test).
† Significantly lower than control group ($p<0.05$).

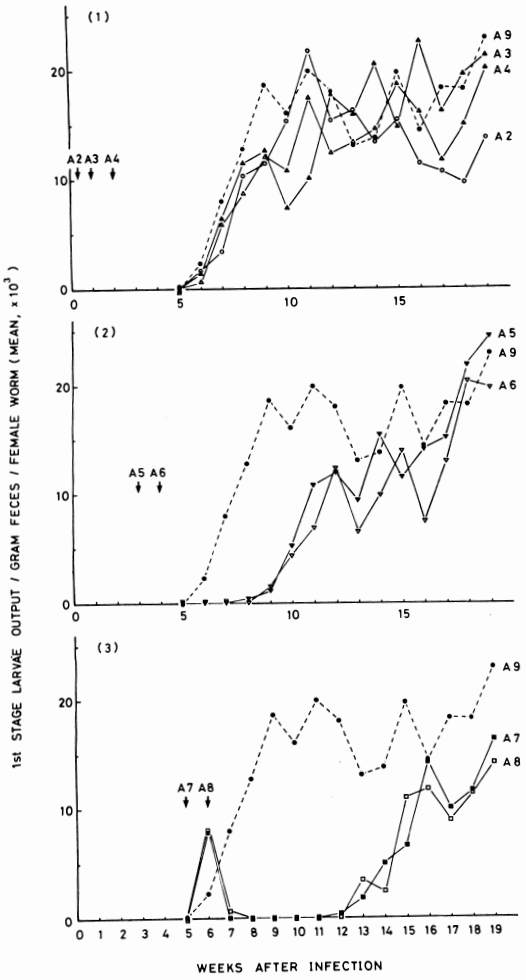

Fig. 1 Changes in the mean number of the first stage larvae per gram faeces per female worm of *A. cantonensis*. The mean number was calculated from LPG examined for three rats in each group. In the treated groups, ivermectin was administered intraperitoneally to rats with a single dose of 2.0 mg/kg at each interval postinfection (arrow). Three patterns (1, 2 and 3) were observed. In the group treated 1/2 hr after infection (A1), however, no worm was recovered. (1) ○—○ at 3 days postinfection (PI) (A 2), ▲—▲ at 1 week PI (A 3), △—△ at 2 weeks PI (A 4), ●···● non-treated, infected control (A 9); (2) ▼—▼ at 3 weeks PI (A 5), ▽—▽ at 4 weeks PI (A 6); (3) ■—■ at 5 weeks PI (A 7), □—□ at 6 weeks PI (A 8).

Experiment B: As summarized in Table 2, in each of treated groups, there was a significant reduction in the recovery of the worm compared to that of non-treated, infected control group ($p<0.01$). At 12 weeks postinfection when all rats were sacrificed, in the groups treated 1/2, 3 or 12 hr after infection (B 1, B 2 or B 3), no first stage larvae could be detected in faeces or even in the lung tissues of each rat. However, in the groups treated 24 or 72 hr after infection (B 4 or B 5), larvae could be detected in faeces on the same level of LPGPF as in the control group (B 6). In the sections of female worms recovered from B 2 (treated at 3 hr), the uteri contained eggs which seemed to be degenerated internally under a light microscope (Fig. 3).

Discussion

In the experiment A, significant reductions in recovery of the worm shown in groups of A 1, A 2 and A 6 indicate that this drug affected both the third larval stage and the immature adults which travel to the lungs, considering the developmental process of this worm in the definitive host (Alicata and Jindrak, 1970). Similar results on *A. cantonensis* in rats have been reported in mebendazole (Lämmler and Weidner, 1975; Hayashi *et al.*, 1982) and in avermectin B_{1a} (Ishii *et al.*, 1983). Furthermore, the results from the experiment B in which the drug was administered 1/2, 3, 12, 24 or 72 hr after infection, showed that the earlier was the stage of worms in rats, the lower the recovery rate was given. Namely, it is suggested that even at the third stage, the susceptibility to this drug gradually decreases as the day after infection increases in rats. Though the detailed mechanism of this action is not yet known, it may be the one that related to the morphological and/or physiological changes in *A. cantonensis* during development in the final host. As *A. cantonensis* develops to the young adults at most in humans, this vermicidal effect on its early stages may make ivermectin more meaningful. High larvicidal effect of flubendazole on this worm

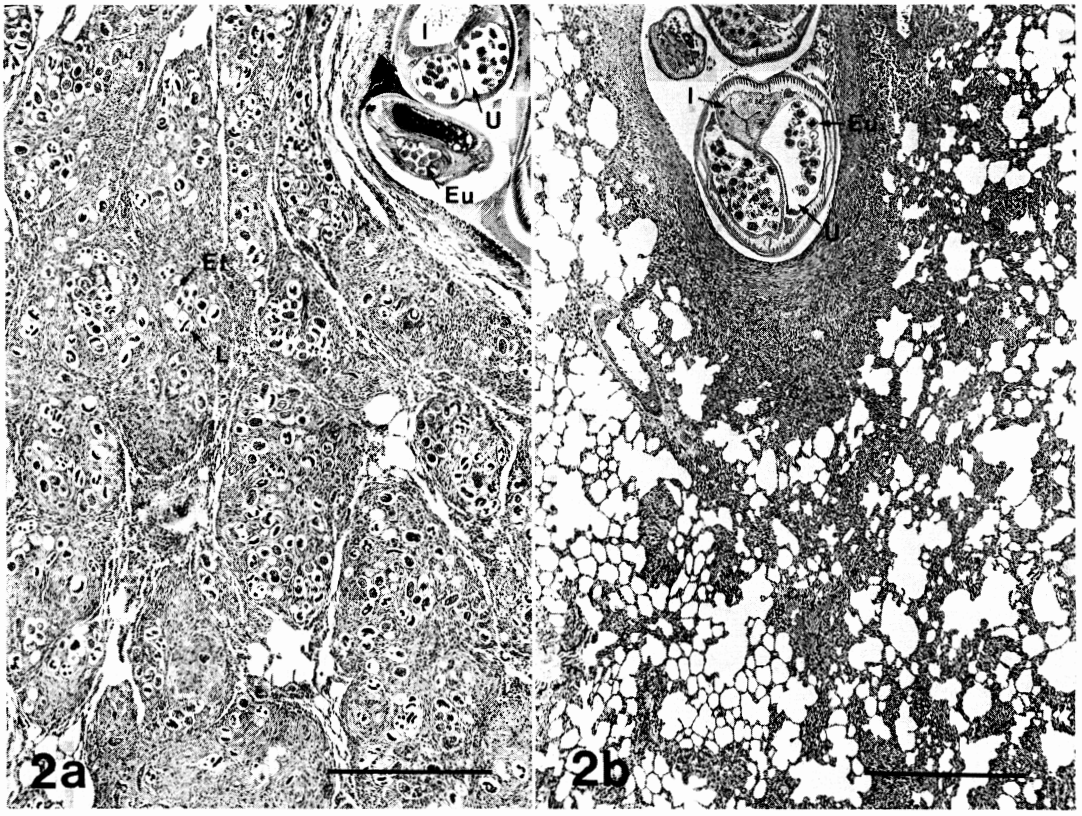

Fig. 2 Light micrographs of rat lung tissues in the non-treated, infected control (2a) and treated group at 7 weeks postinfection (2b). Note very few eggs and larvae in the lung tissue and degeneration of internal structures of eggs in the uteri 3 weeks after treatment in the treated group. El, egg in lung tissue; Eu, egg in uterus; I, intestine; L, first stage larva; U, uterus. Scale: 0.5 mm.

Table 2 Effects of ivermectin on earlier developmental stages of *A. cantonensis* in rats when given 2.0 mg/kg intraperitoneally at various intervals after infection

Group	Time of drug administration after infection (hr)	No. of worms recovered (Mean ± S.D.)	Recovery rate (%)
B 1	1/2	0.3 ± 0.8*	0.8
B 2	3	0.8 ± 1.2*	2.1
B 3	12	2.0 ± 1.8*	5.0
B 4	24	9.5 ± 1.8*	23.8
B 5	72	22.6 ± 1.7*	56.5
B 6	—	33.5 ± 2.4	83.8

Six rats were used in each group and 40 third stage larvae were givin orally.
All rats were sacrificed 12 weeks after infection.
* Significantly lower than control group ($p<0.01$, Student's t-test).

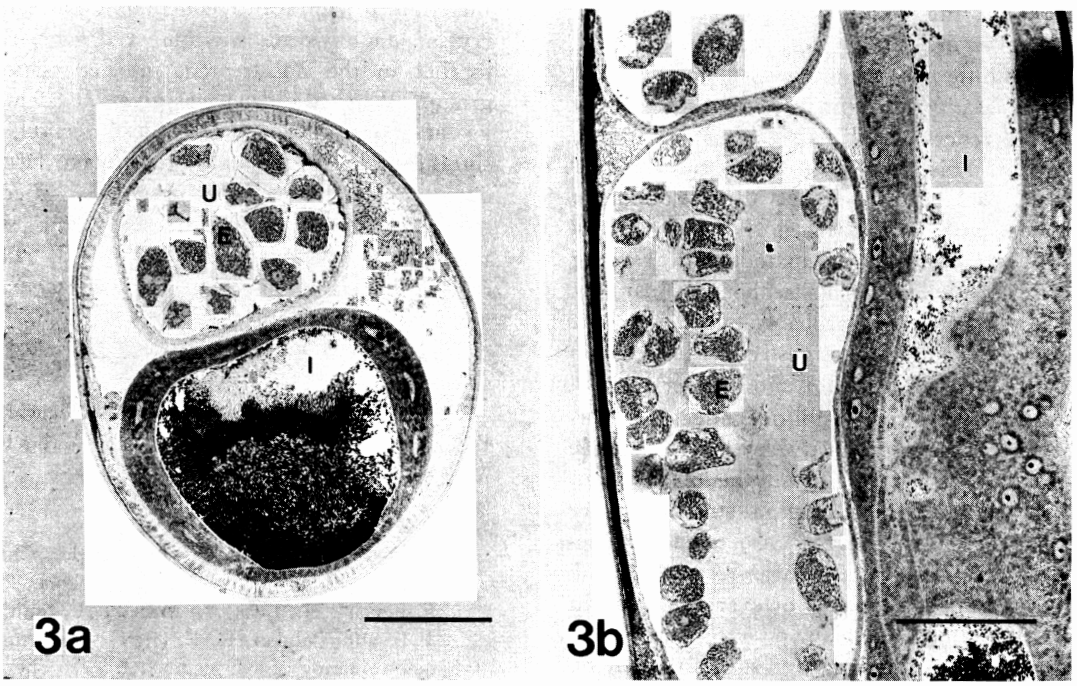

Fig. 3 Light micrographs of a female worm from control (3a) and treated group at 3 hr postinfection (3b). Rats were sacrificed 12 weeks after infection. Note the apparent degeneration of internal structures of eggs in the uterus of worm from the treated group. E, egg; I, intestine; U, uterus. Scale: 0.1 mm.

in the brain of mice has been reported (Maki and Yanagisawa, 1983). Therefore it is necessary to examine this effect using nondefinitive hosts such as mouse and monkey as the model of human infection.

In the treated groups of A 2, A 3 and A 4, the pattern of the first stage larval output was basically similar to that of control group (A 9), in which the normal larval output from adult worms in the lungs was observed from 5 to 6 weeks after infection. Thus, ivermectin has little inhibitory effects on the larval stages of worms in rat brain. However, about 4-week delay of output of the first stage larvae was observed in the treated groups of A 5 and A 6, and the decrease and temporary disappearance of the output were seen for about 4 weeks in the treated groups of A 7 and A 8. These findings show that ivermectin damages worms which are going to travel to the lungs and/or lay eggs in the lungs and causes the inhibition of the larval output. This inhibition lasted for 4 to 5 weeks after treatment. As to the mechanism of action of ivermectin on the larval output from adult female worm, following possibilities are speculated; 1) Eggs laid by worms cannot develop to the first stage larvae in the lung tissues, 2) The first stage larvae hatching from eggs cannot pass out from lung tissues, 3) As the worms are somewhat sustainedly paralyzed by this drug, they cannot lay the eggs for a few weeks, and 4) As the reproductive systems of worms are damaged by this drug, egg formation is inhibited for a few weeks. From observations on the rat lung tissues, neither laid eggs nor the first stage larvae could be observed in the lung tissues. Namely, because the first stage larvae are thought to pass out from lung tissues to faeces, it is difficult to support the first and second possibilities. Similar results have been reported in rats infected with *A. cantonensis* and treated with avermectin B_{1a} (Ishii *et al.*, 1983). On the other hand, from

the paralyzing action of ivermectin *in vitro* (Terada *et al.*, 1984), it is suggested that the anthelmintic may temporarily paralyze adult worms *in vivo* and this inhibition may result in the reduction of egg laying by female worms. This third possibility may be reasonable on thinking about the relationship between anthelmintic effects of ivermectin and other gastrointestinal nematodes. Regarding these nematodes, worms temporarily paralyzed by this drug are probably expelled by the peristalsis of the host digestive tract (Egerton *et al.*, 1980, 1981; Klei and Torbert, 1980).

Additionally an inhibitory effect of ivermectin on the reproductive system of female worms was suggested from the observations of eggs in uteri whose internal structures were damaged. This effect is also temporary because the larval output was restored 4 to 5 weeks posttreatment. However, as seen in the experiment B, this effect seems to be very intense against earlier stages so that no larvae could be detected even 12 weeks after infection. Similar effect on the reproductive system of *Dirofilaria immitis* was observed in dogs (Anantaphruti *et al.*, 1982). The suppression of microfilariae in circulatory system (Campbell, 1981/1982) may be also attributable from this effect.

After all, regarding the mechanisms of inhibitory action on the larval output from adult worms, the third and fourth possibilities described previously were suggested to be related. The paralyzing action of ivermectin on the motility of worms *in vitro* is less effective than that of avermectin $B_{1}a$ (Terada *et al.*, 1984). On the other hand, little effects of avermectin $B_{1}a$ against the reproductive system of female worms could be observed (Ishii *et al.*, 1983). Therefore, there might be differences between ivermectin and avermectin $B_{1}a$ regarding the mechanisms of major action on the adult worms of *A. cantonensis*.

Summary

Ivermectin was administered intraperitoneally to rats infected with *Angiostrongylus cantonensis*. When the drug was given at 2.0 mg/kg 1/2 to 72 hr and 4 weeks after infection, a significant reduction in the recovery of adult worms was observed compared to that in the non-treated, infected control group. When the drug was given at 2.0 mg/kg at more than 3 weeks postinfection, a significant inhibition of the first stage larval output in rat faeces was observed. Microscopical observations of recovered worms and rat's lung tissues were also performed. The *in vivo* effects of this drug on *A. cantonensis* were discussed.

Acknowledgements

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寄生蠕虫症の化学療法に関する研究 (XXIII)
Ivermectin の広東住血線虫に対する *in vivo* 作用

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広東住血線虫感染ラットに，ivermectinを腹腔内投与した．感染後30分から72時間目，および4週目に薬物を投与した群において，非投与対照群と比較し，成虫回収率に有意な減少が認められた．また，感染後3週目以後に薬物を投与した場合，第1期幼虫のラット糞便中への排出に，遅延および一時的停止等の変化が認められた．回収虫体およびラット肺組織の顕微鏡観察も行ない，本薬剤の作用機序について考察した．