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Studies on Chemotherapy of Parasitic Helminths (XIV) Anthelmintic Effect of Mebendazole on *Angiostrongylus cantonensis* in rats

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Introduction

In spite of the importance of the disease caused by *Angiostrongylus cantonensis* (Nematoda; Metastrongylidae) and its widely extending distribution (Alicata and Jindrak, 1970; Jindrak, 1975), no satisfactory medical treatment for human cases has been known yet. Some drugs such as thiabendazole (Cuckler *et al.*, 1965; Nishimura, 1965/66) and *l*-tetramizole (Jindrak and Alicata, 1969), which are effective against *A. cantonensis* in rats, are effective only when used in a large dose which may cause side effects. Lämmler and Weidner (1975) studied effects of various drugs against *A. cantonensis* in rats and showed that mebendazole was effective against the larval stage in almost equal doses to its clinical ones (3–10 mg/kg) against intestinal nematodes such as *Ascaris, Enterobius,* and *Trichuris*. Recently Ambu and Mak (1981) reported a high efficacy of mebendazole against *A. malaysiensis* in rats.

Thus, it may be possible to introduce this drug for treatment of the human angiostrongylosis. Therefore, in the present studies, effects of mebendazole against *A. cantonensis* in rats were studied in detail, i.e., relationships between effects of mebendazole and factors such as timing of drug administration, form of the drug, dose and route of drug administration were studied.

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Materials and Methods

Mebendazole (methyl-5-benzimidazole carbamate) was offered from Jansen Pharmaceutica N.V.

Male Wistar albino rats, five weeks old, were used in all experiments as a definitive host. Infective larvae of *A. cantonensis* were taken from experimentally infected snails, *Biomphalaria glabrata*. After removal of shell by crushing, the snails were homogenized in Waring blendor and digested with 0.2% pepsin in 0.7% HCl for 1 hr. Each rat was given 40 larvae orally using a stomach tube.

In every experiment, infected rats were divided into treated and control groups consisting of five each. Exceptionally, some groups in experiment I consisted of ten or fifteen rats. After treatment with mebendazole at a given time, rats were sacrificed 49 days after infection unless otherwise described, and then the number of worms in the heart and lungs was examined. The effects of mebendazole were determined by the

reduction rate in the recovery of adult worms from the treated rats in comparison with the control.

In the experiment I, the relationship between the timing of administration of mebendazole and its effects was examined. Mebendazole was given orally at a single dose of 100 mg/kg as pure powder to each rat directly. Of all 18 treated groups, 16 groups were treated in a period of 1 hr to 49 days after infection and 2 groups were treated 5 hr or 30 min before infection. The rats treated 49 days after infection were exceptionally sacrificed 70 days after infection. In this experiment, the presence of eggs and the first stage larvae in the lungs was examined.

In the experiment II, the relationship between the dose of mebendazole and its effects was examined. All 9 treated groups were treated 4 days after infection at a single oral dose ranging from 0.01 to 150 mg/kg of mebendazole. Six groups treated with 20 mg/kg or less were given the drug with glucose, as a vehicle. Other three groups treated with 50 mg/kg or more were given the drug as pure powder. Besides these groups, two groups were provided as control groups with or without the vehicle.

In the experiment III, the relationship between the route of administration of mebendazole and its effects was examined. All 12 treated groups were treated 4 days after infection at a single dose ranging from 0.05 to 20 mg/kg of mebendazole suspended in propylene glycol. Of all 12 groups, 6 groups were given orally and other 6 groups intraperitoneally. Besides these groups, 2 control groups were orally or intraperitoneally given the vehicle only.

Results

I. *The relationship between the timing of administration of mebendazole and its effects (Experiment* I*)*

The effects of mebendazole-treatment before infection are shown in Table 1, and those after infection in Table 2. The reduction rates were 60% or less in the groups treated before or earlier than 10 hr after infection. Treatments from 24 hr to 7 days after infection caused the rates of more than 90%. The rate decreased in groups treated after 10 days after infection.

The worms recovered from rats treated in a period of 15 hr to 10 days after infection were macroscopically smaller in size than control worms (Fig. 1), though detail measurements were not performed. Some of the

Table 1 Effects of mebendazole administered before infection against *A. cantonensis* in rats

Treatment time before infection	No. of rats	No. of worms recovered (Mean±SE)	Reduction rate (%)
5 hr	5	16.0±2.10	52.2
30 min	5	14.6±3.70	56.4
non-treated	5	33.5±1.03	—

Each rat was given 40 larvae and administered the drug of 100 mg/kg.

Table 2 Effects of mebendazole administered after infection against *A. cantonensis* in rats

Treatment time after infection	No. of rats	No. of worms recovered (Mean±SE)	Reduction rate (%)
1 hr	4	22.0±3.81	25.4
3 hr	5	14.8±2.91	49.8
5 hr	5	12.0±2.65	59.3
7 hr	5	13.8±2.13	53.2
10 hr	5	11.8±3.12	60.0
15 hr	5	3.8±2.15	87.1
24 hr	5	0.6±0.40	98.0
2 days	5	1.2±0.97	95.9
3 days	10	0.3±0.15	99.0
6 days	5	0.0±0.00	100.0
7 days	5	0.4±0.40	98.6
10 days	15	3.3±1.25	88.8
11 days	5	3.8±2.33	87.1
14 days	15	9.6±1.66	67.4
24 days	5	23.6±1.63	20.0
49 days	4	24.3±2.50	17.6
non-treated	15	29.5±0.95	—

Each rat was given 40 larvae and administered the drug of 100 mg/kg.

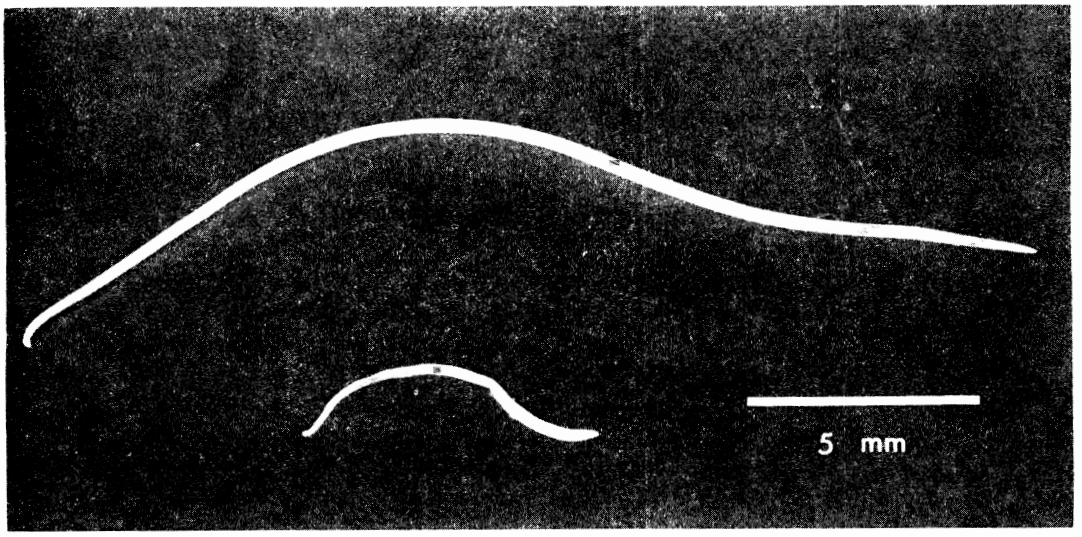

Fig. 1 Inhibitory effect of mebendazole on the development of *A. cantonensis*. Lower worm is from a rat treated 3 days after infection at a dose of 100 mg/kg. Upper worm is from a control rat. Both worms are female.

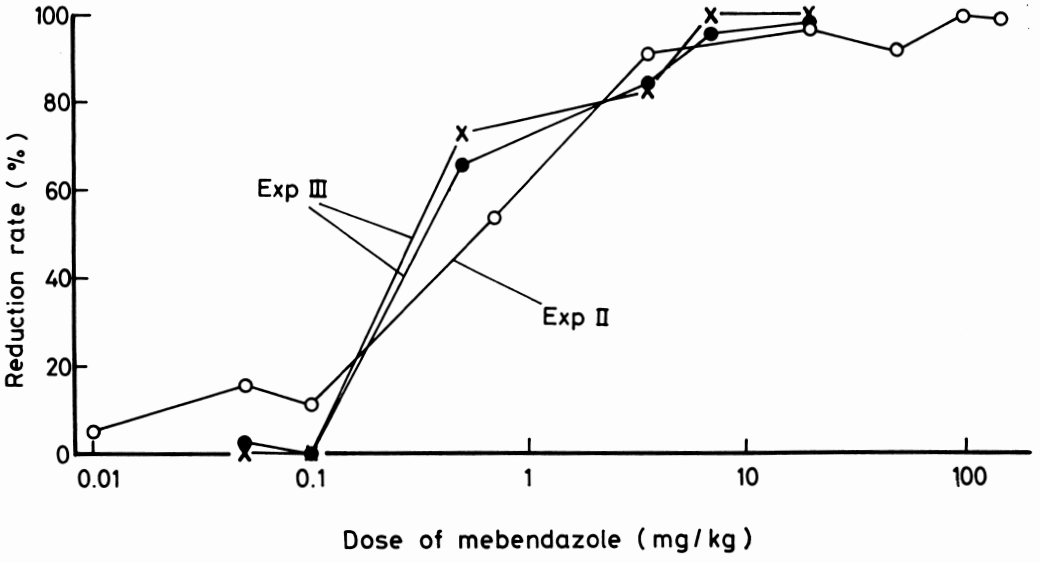

Fig. 2 Relationship between the dose of mebendazole by different routes and the reduction rate of *A. cantonensis*. All doses were given 4 days after infection. ○, oral treatment with powder mebendazole; ●, oral treatment with suspended mebendazole; ×, injection into abdominal cavity by suspended mebendazole.

worms were dead and their sex could not be distinguished. Larvae and eggs were not observed in the lungs of rats treated 24 hr, 3 days, or 7 days after infection, though a few living adult worms were recovered.

II. *The relationship between the dose of mebendazole and its effects (Experiment* II*)*

The results of experiments II and III are shown in Fig. 2. In the mebendazole-treatment on the 4th day after infection, the reduction rates more than 90% were observed in groups treated with 3 to 150 mg/kg, less than 20% in groups with 0.01 to 0.1 mg/kg. Though reduction rates in former groups were remarkable, the 100% reduction was not observed even in the group treated with 150 mg/kg. The dose which causes the 50% reduction was estimated to be about 0.6 mg/kg by the method of the probit analysis.

III. *The relationship between the route of administration of mebendazole and its effects (Experiment* III*)*

In the treatment with mebendazole suspended in propylene glycol on the 4th day after infection, the patterns of reduction rate in rats treated orally or intraperitoneally were similar. In both groups treated via different routes, the reduction rates more than 90% were observed in groups treated with 7 or 20 mg/kg. Particularly, the 100% reduction was observed in groups treated intraperitoneally. In the groups with 0.05 and 0.1 mg/kg, however, the reduction rates were less than 10%.

Discussion

According to Lämmler and Weidner (1975), mebendazole showed a larvicidal effect on *A. cantonensis* in rats by the successive five administrations of 6.25 mg/kg from 5 to 9 days after infection. In the present studies, this drug was similarly effective on the larval stage of *A. cantonensis* in rats by a single dose of 3 mg/kg or more. Remarkable effects such as the reduction more than 90% were shown when mebendazole was given in a period of 1 to 7 days after infection. The effect was, however, turned to decrease when the drug was given after the 10th day. The migration and development of larvae of *A. cantonensis* in rats have been described as follows (Mackerras and Sandars, 1955). After penetrating the digestive tract of rats, the 3rd stage larvae reach the central nervous system until about 4 days after infection. On about the 6–7th day, they molt to become the 4th stage larvae, and then on about the 11–13th day, they become young adults. The young adult worms migrate from the brain into the subarachnoid space and undergo further growth until 26 days after infection at the same site. Considering these migrating processes, it is suggested that mebendazole affects remarkably larvae of *A. cantonensis* in the central nervous system of rats, particularly in the brain. In other words, this drug is most effective against the 3rd and 4th stage larvae in the rat brain.

The larvicidal effects of oral mebendazole were remarkable at the dose of 3 mg/kg or more, while no wormicidal effect against the blood-dwelling adult worms was observed even when this drug was given at a higher dose of 150 mg/kg. Though it is said that mebendazole is poorly absorbed from the gastrointestinal tract of the host (Rollo, 1975), these results suggest that some of the orally administered drug are absorbed and act effectively against the larvae in tissues such as the brain. However, a poor absorption of mebendazole may not produce high blood levels as is effective against adult worms.

Though the reason of the difference in susceptibility between larvae and adult worms was not clear in the present studies, it may be associated with differences in the structure and the function of both stages. There may be some differences between both stages of this parasite regarding the absorption, distribution, or excretion of mebendazole. There may be also some differences regarding the specific activity or the affinity

of the drug between the stages. On the other hand, the nematocidal effect of mebendazole is due to its ability to inhibit the glucose uptake irreversively, which causes the gradual depletion of the energy in worms (Fierlafijn, 1971; Van den Bossche, 1972). In comparison with the adult worms, the larval stage must require much energy for their rapid growth and the basal metabolism. Therefore, it is most probable that such a energy requirement of larvae makes them more sensitive to mebendazole.

In comparison with the rat, a definitive host, the man is a nondefinitive host of *A. cantonensis,* in which the 3rd stage larvae can not develop to adult worms. Thus, serious symptoms of the human angiostrongylosis are almost caused by the larval stage of this parasite. Though rats were used as an experimental animal, the present studies showed that mebendazole was especially effective against the larval stage at the doses almost equivalent to its clinical ones (3–10 mg/kg) against human intestinal nematodes. From these results together with the finding on extremely low toxicity of this drug (Brugmans, 1971; Marsboom, 1973), the clinical doses of mebendazole may also have a clinical therapeutic value against the human angiostrongylosis by the oral administration of the commercial tablet.

Summary

The effects of mebendazole against *A. cantonensis* in rats were examined. The effect of the drug was determined by the reduction rate in the recovery of adult worms from the heart and lungs of rats. When the drug was given orally to each rat at a single dose of 100 mg/kg on various time before or after infection as pure powder directly, the drug was more effective in the larval stage (3rd and 4th stage) than in the adult stage. Namely the reduction rates more than 90% were observed in groups treated in a period of 1 to 7 days after infection. When the various doses of the drug were given 4 days after infection, the dose which causes the 50% reduction was estimated to be about 0.6 mg/kg by the method of the probit analysis. The reduction rates more than 90% were observed in groups treated with mebendazole of 3 mg/kg or more, which was almost equivalent to its clinical doses (3–10 mg/kg) against human intestinal nematodes. When mebendazole suspended in propylene glycol was given orally or intraperitoneally, no difference in reduction rate was seen between two routes.

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寄生蠕虫症の化学療法に関する研究（XIV）広東住血線虫感染ラットにおける mebendazole の抗寄生虫作用

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ラットに感染した広東住血線虫に対する mebendazole の効果を，成虫の回収率の変化を指標として検討した．Mebendazole は成虫に対してよりも，感染後1日から7日頃の第3期および第4期の幼虫に対して90%以上の回収率減少という著しい効果を示した．また，感染4日後に種々の用量を経口投与すると，50%の減少を示すのは約 0.6 mg/kg と推定された．また，3 mg/kg 以上で90%以上の回収率減少を示した．これは，人体腸管寄生線虫に対する臨床的用量にほぼ匹敵する．さらに，propylene glycol に懸濁し，経口および腹腔内投与を行なうと，両法によりほぼ同様の用量作用曲線が得られた．