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Effects of Mebendazole Given Intermittently or Successively with Various Doses and Intervals on Murine Angiostrongyliasis Costaricensis After Worm Maturation

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Abstract

Effects of mebendazole against murine abdominal angiostrongyliasis after worm maturation were examined. The most reduction in worm recovery was observed in the groups treated with 4 daily successive doses of 10 mg/kg and with 4 intermittent doses of 5 mg/kg given weekly, suggesting involvement of different mechanisms of action for killing worms. However, from other findings including the hematologic values in host mice and worm body length, the best effects were seen in the groups treated with 4 intermittent doses of 5 mg/kg given weekly and with 8 daily successive doses of 1.25 mg/kg. In parameters with exception of the result on worm recovery, there was a similar tendency in degree of efficacy among the medicated groups. Thus, it is suggested that many secondary effects on the worms and host mice are probably caused by the consequence of mebendazole-inhibited glucose uptake in worms. Because the pathogenic factors in the abdominal angiostrongyliasis are suggested to be eggs and/or hatched larvae, it seems natural that the regimens inhibiting the reproductive activity consequently inhibit various symptomatic changes in host such as reduction in body weight and anemia. Conclusively, for treating murine angiostrongyliasis costaricensis by mebendazole effectively and safely after worm maturation, it seems the most important to inhibit egg formation and/or oviposition of worms for longer period.

Key words: *Angiostrongylus costaricensis*, adult stage, mice, mebendazole

Introduction

Efficacy of the chemotherapeutic treatment is influenced by various factors including route, dosage and regimen of the treatment. In addition, in the treatment of the diseases by tissue dwelling parasites, allergenic components of killed worms may cause adverse effects on the host (Hawking, 1979; Joubert *et al.*, 1985; Hayashi, 1987). Therefore, effective treatment will be established after these factors are taken into account through basic studies using experimental systems. We have established a model system of tissue dwelling nematode infections using *Angiostrongylus costaricensis*, a causative agent of human abdominal angiostrongyliasis (Morera, 1985), and the outbred ddY mouse (Japan SLC) (Terada *et al.*, 1991a, b).

Before worm maturation, we could treat the murine abdominal angiostrongyliasis almost completely by 5 successive doses of mebendazole (5 mg/kg) or PF1022A (10 mg/kg) (Terada *et al.*, 1993a, b). In the effective treatment there were no pathologic changes in host mice and no worms recovered from the mesenteric arteries. A complete wormicidal effect on adult *A. costaricensis* in mice was also observed by 10 successive doses of mebendazole at 10 mg/kg, but the regimen tended to stimulate host death (Terada *et al.*, 1992). There may be a possibility that such a host death is caused by direct side effects of mebendazole against host animal. However, it is known that LD_{50} value of oral mebendazole to mice is as large as 1280 mg/kg (Van den Bossche, 1985). Additionally, it was reported from [^{3}H]mebendazole charcoal-stable binding that mebendazole exhibits an apparently higher affinity

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for nematode tubulin, since nematodes had higher value of 60 to 70 pmoles/mg tubulin compared with sheep brain which showed 1 or less pmoles/mg tubulin. Consequently, it is suggested that less affinity of mebendazole for host tubulin is probably attributable to less toxicity against host animals (Lacey, 1990). Thus, host death in the above-described successive treatment with mebendazole is considered to be caused by allergenic reactions related to the killed worms.

It seems necessary for treating host after worm maturation to introduce some trials different from treating host before worm maturation. In the treatment of angiostrongyliasis cantonensis in rats, more worms were killed by 5 successive doses of milbemycin D at 5 mg/kg, but less pathologic changes in the host tissues occurred by 9 intermittent doses of 5 mg/kg given weekly (Terada *et al.*, 1987). As one of such trials, therefore, we studied in the present study, effects of mebendazole given successively or intermittently with various doses and intervals on murine angiostrongyliasis costaricensis after worm maturation.

Materials and Methods

Male ddY mice of 5-weeks-old were used as a final host in this experiment. Six groups of 10 mice each were inoculated orally with 10 *Angiostrongylus costaricensis* infective larvae following the method previously described by Ishii and Sano (1989).

Five groups were treated orally with 2 intermittent doses of 10 mg/kg 20 and 27 days post-infection (PI) (10 mg/kg intermittent), 4 intermittent doses of 5 mg/kg 20, 23, 27 and 30 days PI (5 mg/kg short intermittent), 4 intermittent doses of 5 mg/kg 20, 27, 34 and 41 days PI (5 mg/kg intermittent), 4 successive doses of 10 mg/kg 20 to 23 days PI (10 mg/kg successive) and 8 successive doses of 1.25 mg/kg 20 to 23 and 25 to 28 days PI (1.25 mg/kg successive), respectively. Mebendazole was suspended in 2% cremophor EL and administered using a metal catheter. Another infected group received vehicle only as non-treated control. In addition, one group of 10 mice without infection received vehicle as non-infected control.

All surviving mice were sacrificed at 44 or 45 days PI under over anesthesia with diethyl ether. Parameters for detecting chemotherapeutic effects of mebendazole were examined by the methods described by Terada *et al.* (1991a, b). Exceptionally, the worm body length was measured from photographs using a computerized image analyzer (Videoplan, Kontron Co., Munich, Federal Republic of Germany).

The results were statistically analyzed by the Student's *t*-test.

Results

Parameters in host mice

1. Body weight: Table 1 and Figs. 1–3

Non-infected control group kept its mean body weight increasing during the experiment. Non-treated control, however, started to decrease its weight from around 20 days PI, and the weight of surviving mice at dissection was 35.2 g.

Though surviving mice in all medicated groups also started to decrease their body weight from around 20 days PI, after starting treatment they started to gain body weight again. But the restored weight started to decrease again about 10 days after finishing the medication.

We regarded the period from the day of starting medication to the day when body weight started to decrease again as the length of duration of drug efficacy, which was included in Tables 1 and 2.

2. Host death: Table 1

Animals in the non-treated control started to die from 26 days PI and 4 out of 10 mice died till dissection. Only 1 mouse died in the groups of 5 mg/kg intermittent and 1.25 mg/kg successive, while 4 to 5 mice died in other medicated groups.

3. Relative spleen weight: Table 1

Compared with the relative spleen weight (g/100 g body weight) of 0.33 in the non-infected control animals, that of the non-treated control was 4.5 times high. The spleen weights in all medicated groups were less than that of the non-treated control, but a significant change was seen only in the 1.25 mg/kg successive group.

4. Pathologic changes on the serosal surface of cecum and ileum: Table 1

By means of macroscopic observation there were pinpoint yellowish-white foci on the serosal surface of cecum and ileum of all surviving mice in the non-

Table 1 Effects of mebendazole orally given with various doses and intervals on angiostrongyliasis costaricensis in mice: parameters in host mice at dissection

Parameters	Non-treated control	10 mg/kg intermittent 20 and 27 dpi	5 mg/kg short intermittent 20, 23, 27 and 30 dpi	5 mg/kg intermittent 20, 27, 34 and 41 dpi	10 mg/kg successive 20~23 dpi	1.25 mg/kg successive 20~23 and 25~28 dpi	Non-infected control
Duration (days) of drug efficacy[1)]	/	17	21	24 >	14	21	/
No. of mice surviving	6/10	6/10	6/10	9/10	5/10	9/10	10/10
Body weight (g)	35.2±1.0§	39.4±0.9	39.0±1.4	40.9±0.8	39.1±0.9	39.9±1.3	41.2±0.8†
Pathological changes in serosal surface							
Granulomatous changes around eggs	6/6	0/6	0/6	0/10	5/5	0/9	0/10
Petechiae	0/6	6/6	6/6	3/10[2)]	0/5	5/9	0/10
Not observed	0/6	0/6	0/6	7/10[2)]	0/5	4/9	0/10
Relative spleen weight (g/100 g body weight)	1.48±0.23§	1.32±0.14§	1.06±0.07§	1.02±0.16§[2)]	1.15±0.20‡	0.95±0.15*§	0.33±0.03†
Hematologic examination							
RBC ($\times 10^4$/mm^3)	509±52§	620±24*§	755±35†§	790±34†‡	676±43*§	732±50*‡	896±18†
Hb (g/dl)	7.6±0.8§	10.4±0.3†§	12.3±0.5†§	12.4±0.5†§	10.3±0.8*§	11.7±0.8†§	14.9±0.3†
Ht (%)	28.8±2.1§	32.8±0.8†§	38.0±1.3†§	39.0±1.7†‡	35.1±1.3*§	36.8±2.1*‡	43.7±0.7†

Significant difference from non-treated control: *$P<0.05$, $^{\dagger}P<0.01$.
Significant difference from non-infected control: $^{\ddagger}P<0.05$, $^{\S}P<0.01$.

1): The period from the day of starting of medication to the day when body weight started to decrease again was regarded as the duration (days) of drug efficacy in each regimen.

2): With exception for the group in which the data on the mouse died 44 days PI were included, various observation was done on surviving mice at dissection.

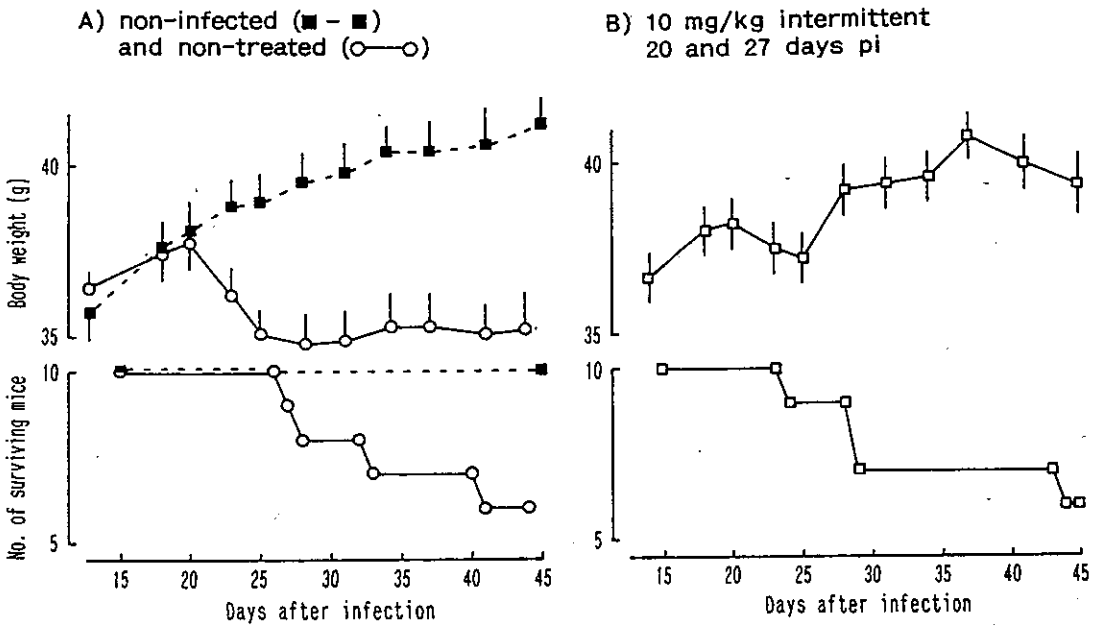

Fig. 1 Effects of mebendazole given intermittently at a dose of 10 mg/kg (B) on body weight (upper) and surviving number (lower) of mice infected with *A. costaricensis*. Results of non-infected and non-treated controls (A) are also shown. In Figs. 1–3, all mice were inoculated with 10 infective larvae and each group had 10 animals.

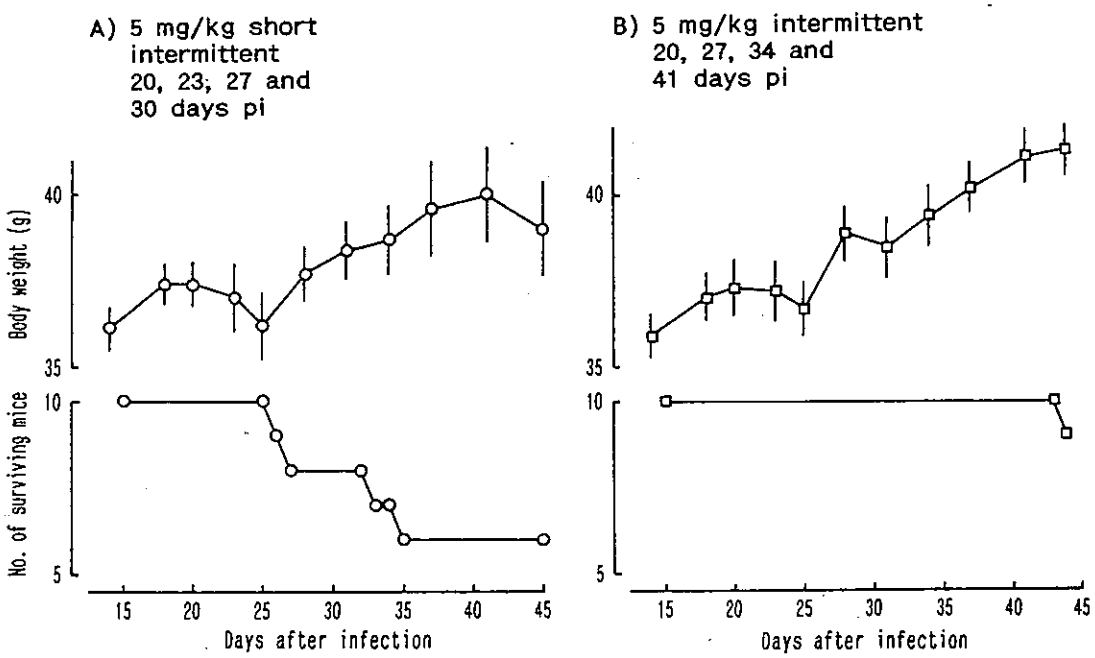

Fig. 2 Effects of mebendazole given short intermittently (A) and intermittently (B) at a dose of 5 mg/kg on body weight (upper) and surviving number (lower) of mice infected with *A. costaricensis*.

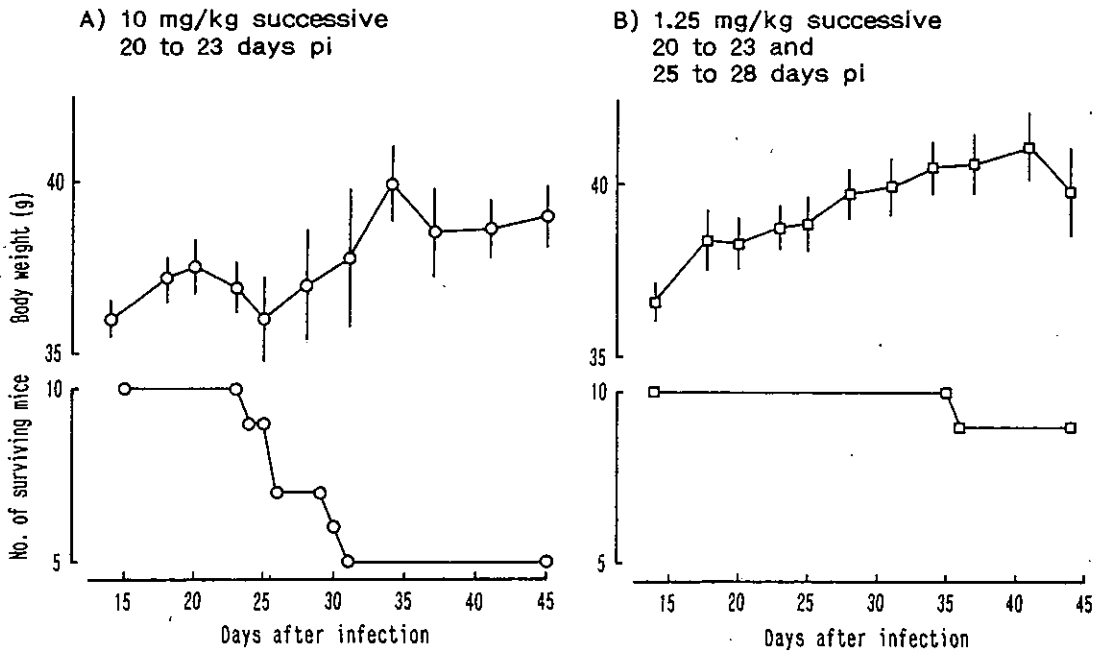

Fig. 3 Effects of mebendazole given successively at doses of 10 mg/kg (A) and 1.25 mg/kg (B) on body weight (upper) and surviving number (lower) of mice infected with *A. costaricensis*.

treated control and 10 mg/kg successive groups, showing accumulation of large number of eggs in the capillaries of the intestinal wall. There were many petechiae in the serosa of the terminal ileum in all or parts of other medicated groups.

5. Hematologic examination: Table 1

Red blood cell counts (RBC), hemoglobin content (Hb) and hematocrit (Ht) in the non-infected control animals were 896, 14.9 g/dl and 43.7%, respectively. These values decreased by 56.8, 51.0 and 65.9% in the non-treated control. All the values in the medicated groups showed significantly increase from those in the non-treated control, and the most remarkable effects were seen in the 5 mg/kg intermittent group.

Parameters in worms

1. Number of worms recovered from the mesenteric arteries: Table 2

The number of worms recovered was 6.8 from the non-treated mice, while all medicated groups showed recovery reduction and the smallest number of 4.0 with significant difference was seen in the 5 mg/kg intermittent and 10 mg/kg successive groups, respectively.

2. Body length of recovered worms: Table 2

The mean body length of the worms recovered from the non-treated control group was 30.0 mm in female and 17.2 mm in male, respectively. The length of both sexes of worms recovered from all medicated groups was significantly shorter than those of the non-treated control, and the worms from the 5 mg/kg intermittent group were the shortest.

3. Morphologic changes in recovered worms: Fig. 4

Compared with the worm recovered from the non-treated control, there were degenerative changes in the intestinal wall and a few eggs with degeneration in the uteri of the worms recovered from the 5 mg/kg intermittent group.

4. Output of the 1st-stage larvae in feces: Table 2

Except one mouse in which only female worms were detected, the 1st-stage larvae were observed in feces of all surviving mice in the non-treated control group. The larvae were observed in feces of 4 out of 5 surviving mice of the 10 mg/kg successive group, while no larvae were seen in all other medicated

Table 2 Effects of mebendazole orally given with various doses and intervals on angiostrongyliasis costaricensis in mice: parameters in worms at dissection

Parameters	Non-treated control	10 mg/kg intermittent 20 and 27 dpi	5 mg/kg short intermittent 20, 23, 27 and 30 dpi	5 mg/kg intermittent 20, 27, 34 and 41 dpi[1)]	10 mg/kg successive 20~23 dpi	1.25 mg/kg successive 20~23 and 25~28 dpi
Duration (days) of drug efficacy[2)]	/	17	21	24>	14	21
No. of worms recovered	6.8±0.9 (6)[a)]	6.3±0.6 (6)	5.0±0.7 (6)	4.0±0.5* (10)	4.0±0.6* (5)	4.6±0.6 (9)
Worm length (mm)						
Female	29.98±0.45 (17)[b)]	26.09±0.45† (17)	24.58±0.25† (20)	23.87±0.49† (20)	25.83±0.28† (11)	24.83±0.30† (20)
Male	17.23±0.24 (17)[b)]	16.10±0.27† (13)	15.20±0.26† (5)	14.97±0.21† (15)	14.73±0.22† (8)	15.62±0.27† (12)
1st-larvae in feces						
Detected	5/6	0/6	0/6	0/10	4/5	0/9
Not detected	1/6[3)]	6/6	6/6	10/10	1/5	9/9
Eggs in host intestine						
Many eggs with various stages including larvae	5/6	3/6	0/6	0/10	5/5	0/9
Many developing eggs without larvae	0/6	2/6	2/6	0/10	0/5	3/9
Few eggs without development	1/6[4)]	1/6	3/6	4/10	0/5	6/9
Not detected	0/6	0/6	1/6	6/10	0/5	0/9

Significant difference from non-treated control: *P<0.05, †P<0.01.

Numbers in parentheses show number of dissected mice (a) or observed worms (b).

1): The data on the mouse died 44 days PI were included.

2): The period from the day of starting of medication to the day when body weight started to decrease again was regarded as the duration (days) of drug efficacy in each regimen.

3): Only females were removed. 4): unfertilized eggs.

groups.

5. Eggs in the host cecum and terminal ileum: Table 2 and Fig. 5

Various stages of development of eggs and the 1st-stage larvae were observed in the tissues of all surviving mice in the non-treated control group with exception of a mouse parasitized by female worms alone. Larvae were also observed in all surviving mice of the 10 mg/kg successive group and in 3 out of 6 surviving mice of the 10 mg/kg intermittent group. Many eggs in various stages of cleavage were seen in 2 or 3 out of surviving mice of the 10 mg/kg intermittent, 5 mg/kg short intermittent and 1.25 mg/kg successive groups, respectively. No and only few one-cell eggs were observed in 6 and 4 mice, respectively, of the 5 mg/kg intermittent group. Fig. 5 shows only few one-cell eggs in the terminal ileum from the group.

Discussion

Regarding mode of action of mebendazole most of recent studies suggest that interruption of microtubular function represents the primary underlying mechanism and it causes secondarily various inhibition such as glucose uptake, fumarate reductase, egg hatching and larval development (Rew and Fetterer, 1986). Examining efficacy of mebendazole given intermittently or successively with various doses and intervals, we found that there were two different types in efficacy; i.e. one favorable to killing worms and another favorable to treating host animals safely as well as effectively.

Significant reduction in worm recovery was observed in the 10 mg/kg successive and 5 mg/kg intermittent groups, respectively. That is, the smallest worm recovery was seen in two different ways of treatment in successive and intermittent regimens. From these results, it is suggested that different mechanisms are probably related to killing action of worms by mebendazole. When host mice were treated successively with higher doses, worms may be killed abruptly by the mechanism mainly attributable to anthelmintic action of mebendazole, causing rather severe side effects probably related to killed worms. When host mice were treated intermittently with

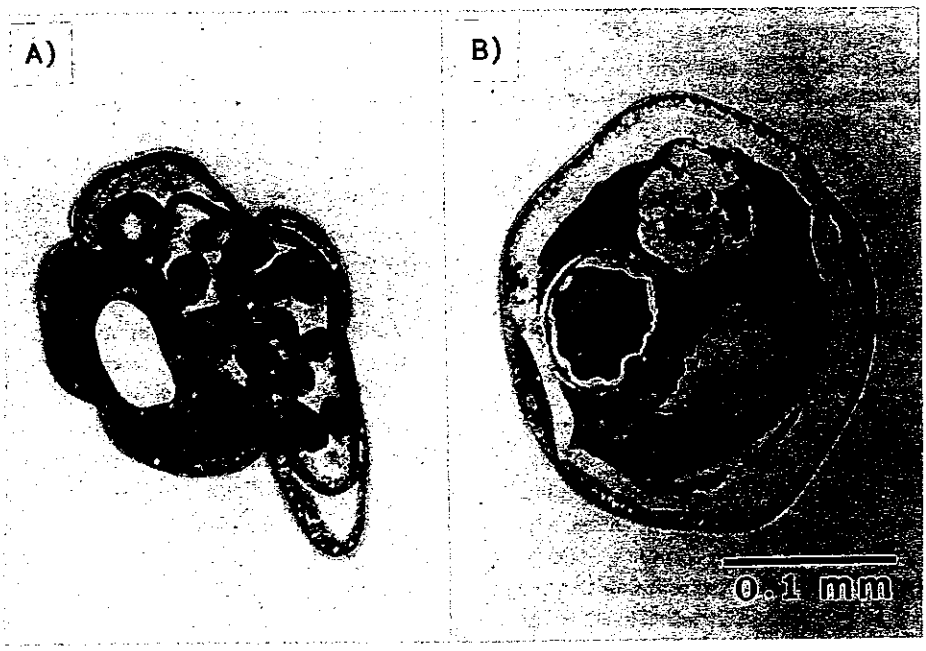

Fig. 4 Morphologic changes in the recovered worms from the non-treated control (A) and 5 mg/kg intermittent (B) groups. Degenerative changes in the intestinal wall and a few eggs with degeneration were observed in the uteri of the worms recovered from the treated group.

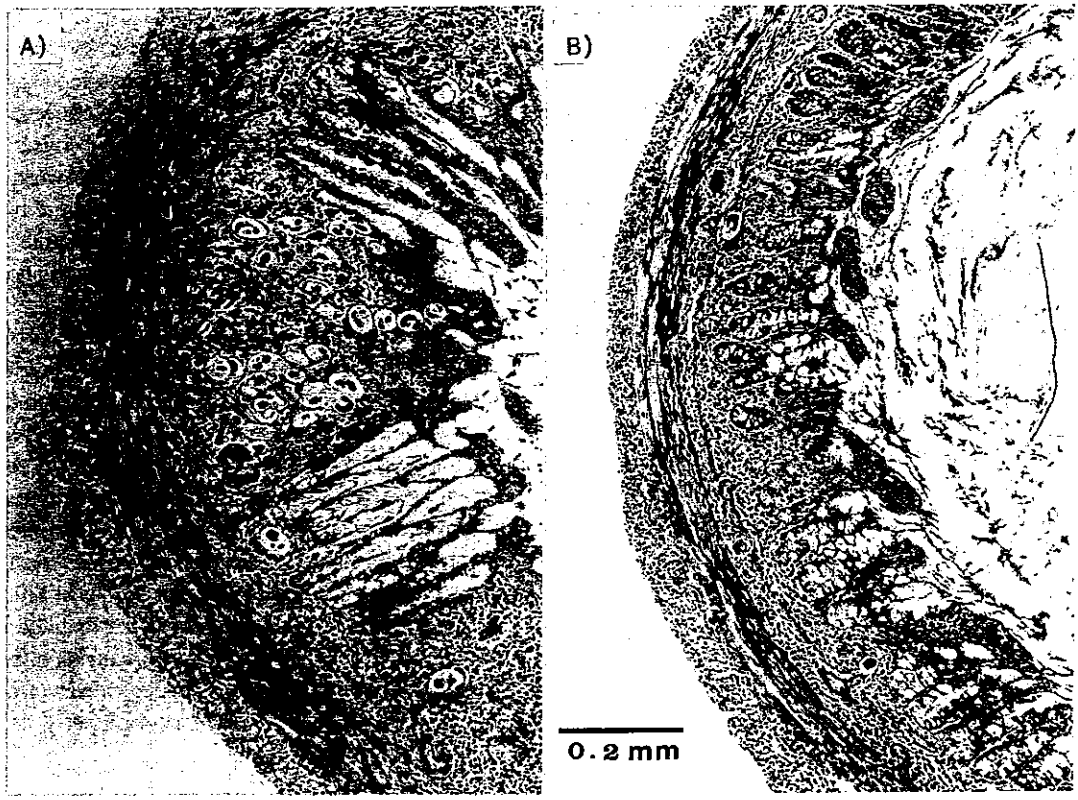

Fig. 5 Histopathologic figures of the host terminal ileum from the non-treated control (A) and 5 mg/kg intermittent (B) groups. Numerous eggs with various stages including larvae were observed in the tissues from the non-treated control, while only few eggs without development in the tissue from the treated group.

lower doses, the worms weakened by the anthelmintic may be killed more gradually through another mechanisms probably related to host defense mechanisms, being accompanied by fewer side effects.

On the other hand, from all parameters except the worm recovery, the best effects were seen in the 5 mg/kg intermittent and 1.25 mg/kg successive groups, respectively. In these parameters there was a similar tendency in degree of efficacy among the medicated groups. In the murine abdominal angiostrongyliasis, interruption of microtubular function probably occurs as the primary underlying mechanism and also others such as inhibition in egg hatching may be caused secondarily. However, it may be more easily understandable to suggest that many secondary effects on the worms and host mice are caused by the consequence of mebendazole-inhibited glucose uptake (Van den Bossche 1972; 1985) (Fig. 6). For example, energy depletion in the worms occurs, which in turn causes inhibition in their growth, and with participation of host defense mechanisms some weakened worms may be killed. Depletion of energy also inhibits the reproductive activity of the worms causing inhibition of oviposition, and the inhibition in the reproductive activity causes various changes in the worms and host animals. That is, there occur many changes including morphologic changes in worm uteri, disappearance of eggs and larvae in host tissues and blockage of output of the 1st-stage larvae in host feces. Because the pathogenic factors in the murine angiostrongyliasis costaricensis are suggested to be eggs and/or hatched larvae (Terada *et al.*, 1991a, b), it seems natural that the regimens inhibiting the reproductive activity consequently inhibit various symptomatic changes in host such as reduction in

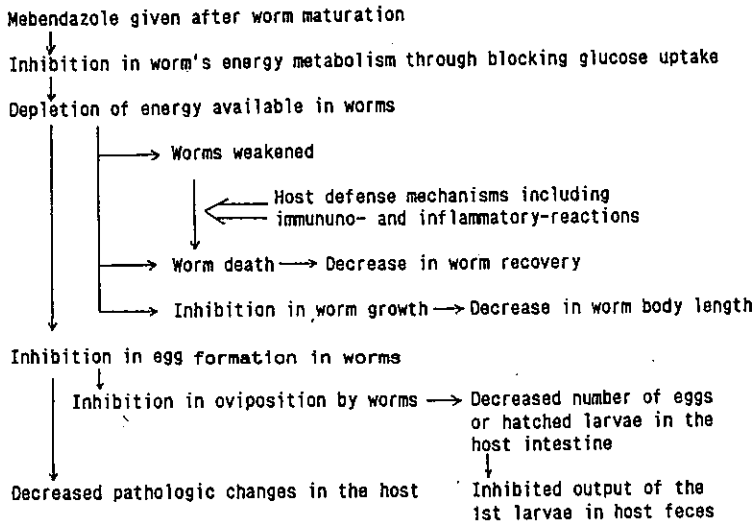

Fig. 6 A possible mechanism of action of mebendazole in murine angiostrongyliasis costaricensis after worm maturation.

body weight and anemia. Similar relationship between the primary paralysis caused by piperazine (Bueding *et al.*, 1959) or milbemycin D (Terada *et al.*, 1987) through gabergic stimulation and various complicated effects secondarily elicited is reported in *Ascaris lumbricoides* and *A. cantonensis*, respectively.

If we regarded the period from the day of starting medication to the day when body weight started to decrease again as the length of duration of drug efficacy, a good coincidence was observed between the duration of drug action and degree of efficacy of mebendazole from all parameters except the worm recovery. That is, the best effects and the longest duration of drug action were in the 5 mg/kg intermittent treatment. Thus, for treating murine abdominal angiostrongyliasis by mebendazole effectively and safely after worm maturation, it seems the most important to inhibit egg formation and/or oviposition for longer period. From the comprehensive standpoint of treating host animals safely and effectively, killing worms, and ease of treatment, the regimen with 5 mg/kg intermittently is also considered to be the best. In treatment of filariasis, such an intermittent treatment with lower doses of diethylcarbamazine has been recommended (Hawking, 1979).

If the best regimen in the present study could be given for a longer period, more worms should be killed gradually by the participation of host defense mechanisms. However, one mouse died on the day of dissection in the present intermittent treatment with 5 mg/kg, probably due to the allergenic reactions related to the killed worms. Thus, a combination with some other therapy such as anti-allergic and/or anti-inflammatory agents may be necessary for establishing treatment of the disease after worm maturation. In some parasitic diseases like filariasis (Hawking, 1979; Boreham *et al.*, 1985), human neurocysticercosis (Joubert *et al.*, 1985) and murine angiostrongyliasis cantonensis (Hayashi, 1987), good results were indeed reported by applying the combined therapy. Thus, it is necessary to introduce such a combination as another trials for establishing treatment of angiostrongyliasis costaricensis after worm maturation.

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References

1) Boreham, P. F. L., Atwell, R. B. and Euclid, J. M.

(1985): Studies on the mechanism of the DEC reaction in dogs infected with *Dirofilaria immitis*. Int. J. Parasitol., 15, 543–549.

2) Bueding, E., Saz, H. J. and Farrow, G. W. (1959): The effects of piperazine on succinate production by *Ascaris lumbricoides*. Brit. J. Pharmacol., 14, 497–500.
3) Hawking, F. (1979): Diethylcarbamazine and new compounds for the treatment of filariasis. Ad. Pharmacol., 16, 129–194.
4) Hayashi, M. (1987): Studies on chemotherapy of parasitic helminths (XXX). Clinical and pathological changes in mice infected with *Angiostrongylus cantonensis* and treatment with mebendazole and betamethasone. Jpn. J. Parasitol., 36, 80–87.
5) Ishii, A. I. and Sano, M. (1989): Strain-dependent differences in susceptibility of mice to experimental *Angiostrongylus costaricensis* infection. J. Helminthol., 63, 302–306.
6) Joubert, J., Joubert, M. J. and Lombaard, C. M. (1985): Neurocysticercosis – a comprehensive approach to medical treatment. South. Afr. Med. J., 68, 11–14.
7) Lacey, E. (1990): Mode of action of benzimidazoles. Parasitol. Today, 6, 112–115.
8) Morera, P. (1985): Abdominal angiostrongyliasis: A problem of public health. Parasitol. Today, 1, 173–175.
9) Rew, R. S. and Fetterer, R. H. (1986): Mode of action of antinematodal drugs. In Chemotherapy of Parasitic Diseases, ed. by Campbell, W. C. and Rew, R. S., Plenum Press, New York and London, pp. 321–337.
10) Terada, M., Dharejo, A. M., Ishii, A. I., Nacapunchai, D. and Sano, M. (1987): Effects of milbemycin D on adult *Angiostrongylus cantonensis* in rats. Parasitol. Res., 73, 375–380.
11) Terada, M., Ishih, A. and Sano, M. (1991a): Studies on chemotherapy of parasitic helminths (XXXVI). Indicative changes for experimental chemotherapy in mice infected with *Angiostrongylus costaricensis*. Jpn. J. Parasitol., 40, 415–423. (in Japanese)
12) Terada, M., Kino, H. and Sano, M. (1991b): Studies on chemotherapy of parasitic helminths (XXXVII). Growth of *Angiostrongylus costaricensis* in ddY mice. Jpn. J. Parasitol., 40, 521–527. (in Japanese)
13) Terada, M., Tungtrongchitr, A., Ishih, A. and Sano, M. (1992): Effects of mebendazole on abdominal angiostrongyliasis in mice after worm maturation: Preliminary trials. Jpn. J. Parasitol., 41, 481–486.
14) Terada, M., Ishih, A., Tungtrongchitr, A., Sano, M. and Shomura, T. (1993a): Effects of PF1022A on developing larvae of *Angiostrongylus costaricensis* in mice, with special reference to route, dose and formulation. Jpn. J. Parasitol., 42, 199–210.
15) Terada, M., Kino, H., Akyol, C. V. and Sano, M. (1993b): Effects of mebendazole on *Angiostrongylus costaricensis* in mice, with special reference to the timing of treatment. Parasitol. Res., 79, in press.
16) Van den Bossche, H. (1972): Biochemical effects of the anthelmintic drug mebendazole. In Comparative Biochemistry of Parasites (ed. by Van den Bossche, H.), pp.139–157, Academic Press, New York.
17) Van den Bossche, H. (1985): Pharmacology of anthelmintics. In Chemotherapy of Gastrointestinal Helminths (ed. by Van den Bossche, H., Thienpont, D. and Janssens, P. G.), pp.125–181, Springer-Verlag, Berlin, Heidelberg, New York and Tokyo.