[Jpn. J. Parasitol., Vol. 36, No. 2, 80–87, April, 1987]

Studies on Chemotherapy of Parasitic Helminths (XXX); Clinical and Pathological Changes in Mice Infected with *Angiostrongylus cantonensis* and Treatment with Mebendazole and Betamethasone

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(Received for publication; March 5, 1986)

Abstract

Experimental chemotherapeutic studies have been undertaken in mice infected with *Angiostrongylus cantonensis* as a model of human angiostrongylosis. Body weight, clinical signs, peripheral eosinophil levels, and pathological changes in the brain were monitored in mice infected with 50 larvae of *A. cantonensis*. All changes were detected about 10 days PI, the time when larvae develop into young adult worms. Significant correlations were seen between the inflammatory reactions in the brain and body weight. Administration of mebendazole at a daily dose of 5 mg/kg for 5 successive days was effective against larval and early young adult stages of the nematode. However, the drug was ineffective against late young adult worms. A combined treatment of mebendazole and betamethasone was effective against late stage young adult worms. The present study demonstrates that the mouse is a suitable animal model for human angiostrongylosis. The combined usage of mebendazole and betamethasone warrants further investigation for the treatment of human angiostrongylosis.

Key words: *Angiostrongylus cantonensis*, chemotherapy, brain pathology, mebendazole and betamethasone

Introduction

Angiostrongylus cantonensis is a cause of eosinophilic meningoencephalitis and is associated with various neurological symptoms when the parasite dies in aberrant hosts, including man (Alicata and Jindrak, 1970; Jindrak, 1975; Arseni and Chimion, 1978). In the Pacific Islands and Southeast Asia, many cases of human angiostrongylosis have been reported some of which are fatal (Alicata and Jindrak, 1970; Yii, 1976). Recently the infection has also been reported in Japan in rats (Tanaka *et al.*, 1982; Makiya and Onitake, 1983). Despite the importance of this disease and its serious manifestations, satisfactory clinical treatment has not been established.

Experimentally the efficacy of drugs against *A. cantonensis* has been tested in the multimammate rat *Mastomys natalensis* (Lämmler and Weidner, 1975) and the white rat (Hayashi *et al.*, 1982). The mouse has also been shown to be a useful model for human angiostrongylosis (John and Martinez, 1975; Maki and Yanagisawa, 1983), and the present study has been designed to determine clinico-pathological changes in this host and then to undertake chemotherapy.

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Materials and Methods

1. Experimental infections

Male ddY mice, 28 days old (15–21 g), were used in all experiments. Each mouse was inoculated orally with 50 *A. cantonensis* infective larvae following the method of Hayashi *et al.* (1982) unless otherwise stated.

2. Drugs

Mebendazole (methyl 5-benzoyl-2-benzimidazole-carbamate), was kindly donated by

Janssen Pharmaceutica and betamethasone was purchased from Sigma. Both drugs were suspended in olive oil and administered orally using a stomach tube. Control mice received vehicle alone.

3. Clinical and pathological examinations

Body weight was regarded as an index of the severity of the disease and determined at intervals of two or three days. Clinical signs were checked on most days. Blood smears stained with Wright solution were prepared for differential white cell counts to determine eosinophilia. Brains and other organs were fixed in 10% formalin after macroscopic examination. Haematoxylin and eosin stained sections were prepared from four areas of the brain; the frontal lobe, the central portion of cerebrum, the mesencephalon and medulla-cerebellum. In experiment II, the weight and the maximum width of each brain were determined prior to fixation. Quantification of histological changes in the brain was made according to the method of Hayashi *et al.* (1978) (Table 1). Statistical comparisons were made using the "Student's *t*-test".

4. Experimental design

Experiment I

In order to determine the optimum larval dose for therapeutic studies, four groups of 10 mice were infected with 0, 5, 50 and 200 infective larvae respectively. Body weight and clinical signs of each mouse were monitored for 28 days.

Experiment II

Seventy-seven infected and 30 non-infected control mice were used to study clinico-pathological changes. Fifteen infected and either 5 or 15 control mice were sacrificed on days 7, 10, 14 and 21 post infection (pi). Peripheral eosinophilia, neurological signs and histological changes in tissues were examined for each mouse. Body weight and clinical signs of the remaining 17 infected mice were monitored for 30 days.

Experiment III

This experiment was conducted to evaluate the timing of mebendazole administration and its effect. Six groups of 10 mice were used. Five groups were infected with *A. cantonensis* while another group acted as control. Four infected groups of mice were given a daily oral dose of mebendazole (5 mg/kg) for 5 successive days on days 1–5, 6–10, 11–15 and 16–20 pi. The remaining infected group served as a non-treated control. The control groups of mice received vehicle alone on days 6–10 pi. Body weight and clinical signs of all mice were monitored for 28 days following infection.

Experiment IV

This experiment was carried out to assess the effect of using betamethasone in addition to mebendazole in the treatment of *A. cantonensis*. Four infected groups of 10 mice each were used. Group 1 acted as a non-treated control and received vehicle alone on days 16–20 pi. Groups 2 and 3 were treated with mebenda-

Table 1 Score ranking of brain lesions for quantitative analysis

Severity of lesion	1	2	3
Meningitis	Slight and partial cellular infiltration observed in meninges	Slight infiltration covering a wide area or infiltrated cells forming a clear layer	Wide areas of the meninges area infiltrated, forming a thick layer
Perivascular cuffing	Small number of cells infiltrate around a few blood vessels	Many vessels are infiltrated with a small number of cells or a few vessels with a dense infiltration	Many vessels are infiltrated forming a thick layer of cells
Diffuse lesion	Slight and partial changes such as spongy state and reactive proliferation of glial cells observed	Changes are observed throughout the whole parenchyma, or major changes observed partially	Major changes found throughout the whole parenchyma
Nodular lesion	Score is expressed as to actual number per section		

zole at a daily dose of 5 mg/kg on days 16–20 pi. Groups 3 and 4 were treated with betamethasone (2.5 μg/mouse) on alternate days between 16 and 26 pi. Body weight and clinical signs were monitored in all mice for 28 days.

Results

Experiment I

Weight gain was similar in all groups of mice for the first week, but subsequently there was a marked decrease in these mice infected with either 50 or 200 larvae (Fig. 1A). Neurological signs such as convulsion and circling became more severe as the worm burden increased. High mortality was seen in mice receiving 200 infective larvae with all mice dying within 17 days. Same deaths were recorded in mice receiving 50 infective larvae but not with smaller numbers (Fig. 1B). Since mice infected with 50 larvae showed intermediate changes, this inoculum was chosen for subsequent experiments.

Experiment II

Changes in body weight of mice were similar to those receiving 50 larvae in Experiment I. Clinical signs such as depression and bristled hair were first observed 12 days pi. All mice exhibited these clinical signs 15 days pi, and some also showed wryneck and claudication. Clinical signs tended to become more intensive in the later stages of the infection. By 19 days other serious symptoms, hyperpnea, stiffness of the extremities, convulsions, circling, bloody tears, and coma were evident. All 17 non-sacrificed mice were alive on day 20 pi, but 9 out of them (52.9%) died before 30 days pi. The 8 remaining mice survived until 120 days pi when they were sacrificed.

Peripheral eosinophil count increased 10 days pi, reaching maximum levels (24.9±3.8%) 14 days pi. Thereafter eosinophil counts remained high (Fig. 2).

No significant pathological changes were detected in organs other than the brain. The mean weight of the brain of infected mice in-

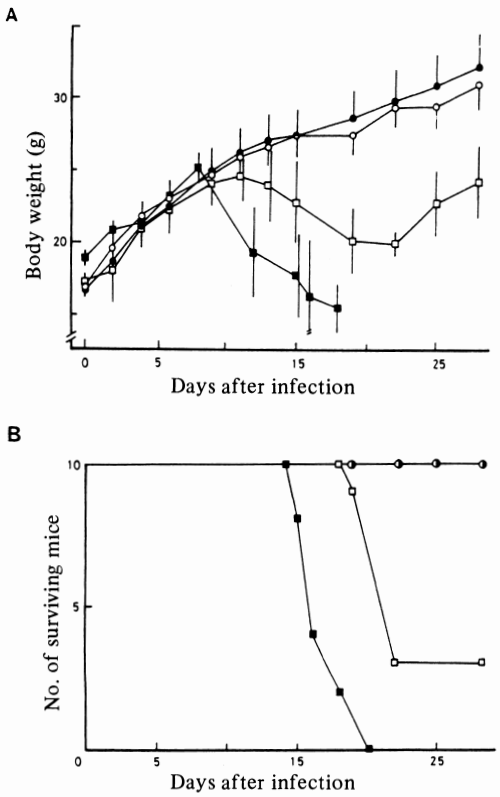

Fig. 1 Changes in body weight (A) and survival (B) of mice infected with *A. cantonensis*.
○: non-infected control; ●: 5 larvae;
□: 50 larvae; ■: 200 larvae
Vertical bars show mean ± SD.

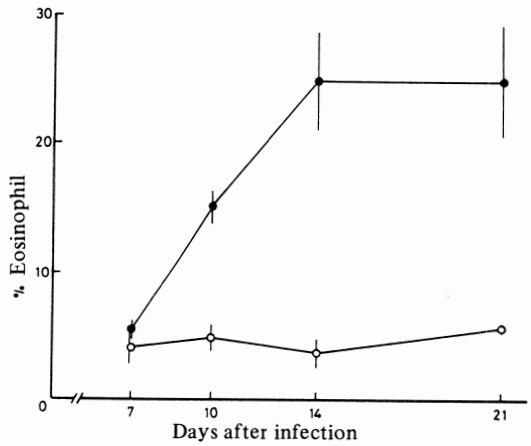

Fig. 2 Eosinophilia in mice infected with 50 larvae of *A. cantonensis*.
○: non-infected control; ●: infected
Each point is mean ± SE.

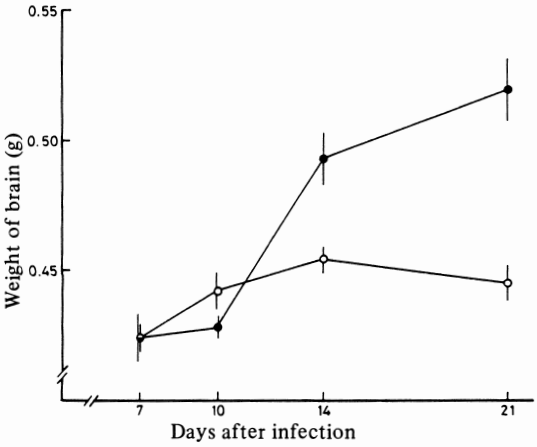

Fig. 3 Changes in the weight of the brain of mice after infection with 50 larvae of *A. cantonensis*.
○: non-infected control; ●: infected
Each point is mean ± SE.

creased and differed significantly from control mice 14 and 21 days pi ($P<0.01$) (Fig. 3). The brain width of infected mice was significantly greater at 21 days pi ($P<0.01$).

Dilatation of the skull and softened cranial bone were often observed macroscopically in the group sacrificed 14 days pi together with an increase in volume and cloudiness of cerebrospiral fluid. The pia mater became opaque and was hyperemic. The parenchyma of the brain was edematous. These changes became more evident 21 days pi. The parietal parenchyma was demonstrably thinner in some infected mice.

Histologically, few changes were observed 7 days pi. Many living larvae were found in the frontal lobe and central portion of the cerebrum. At 10 days pi many mice showed a slight meningitis with a cellular infiltration mainly consisting of eosinophils but with a few lymphatic and mononuclear cells. Slight changes in the brain parenchyma were seen in some mice consisting of perivascular cuffing, nodular lesions, reactive proliferation of glial cells, diffuse infiltration by inflammatory cells and a spongy appearance of the parenchyma consisting of tiny holes and degenerating patches. By 14 days pi, these changes became more distinct in all areas of the brain. Most worms were alive and found in the subarachnoid space or the cerebral ventricle. At 21 days pi, the changes in the brain became even more severe. Many dead worms were present frequently surrounded by inflammatory cells, mainly eosinophils. These lesions occasionally had a granulomatous appearance. The cerebral ventricle was frequently enlarged.

The meningitis (Fig. 4A), perivascular cuffing (Fig. 4B), nodular lesions (Fig. 4C) and the diffuse lesion of parenchyma including diffuse infiltration of inflammatory cells, reactive proliferation of glial cells, and the spongy state (Fig. 4D), were scored according to the severity of the lesion. The kinetics of the relative histological changes in the brain throughout the course of infection are shown in Fig. 5. The scores from the 4 brain portions were averaged for each mouse and then plotted against the ratio of the body weight to the average weight of control mice sacrificed simultaneously. Correlation coefficients were calculated for each change; i.e., -0.546 for the meningitis, -0.579 for the perivascular cuffing, -0.249 for the nodular lesion, and -0.638 for the diffuse lesion. All correlations were significant at 1% level except for the development of nodular lesions.

Experiment III

Changes in body weight and survival of mice following mebendazole treatment are shown in Figs. 6A and 6B for each group. In the non-treated controls, changes in body weight and clinical signs were similar to those reported for mice infected with 50 larvae in Experiments I and II. Five out of 10 mice died in this experiment. In the groups treated on days 1–5 or 6–10 pi, the gain in body weight was similar to that seen in the non-infected control group. There were clinical signs and no deaths occurred. In the group treated with mebendazole on days 11–15 pi, the gain in body weight decreased temporarily from 10 days ($P<0.01$) but quickly recovered. Clinical signs such as depression and bristled hair were noted from about 10 days pi but the severity was much less than in control mice. Results for the group treated on days 16–20 pi were similar to those of the con-

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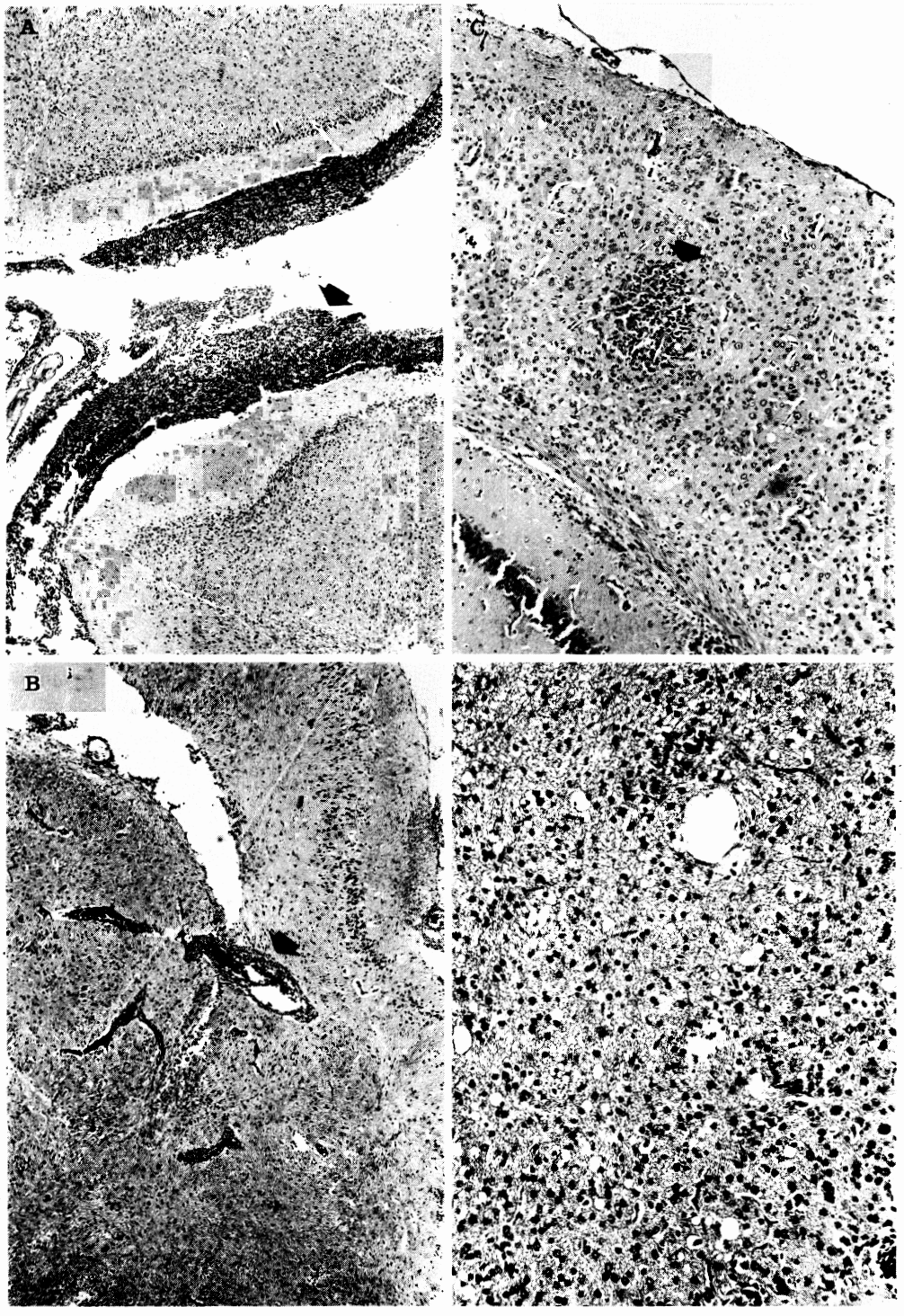

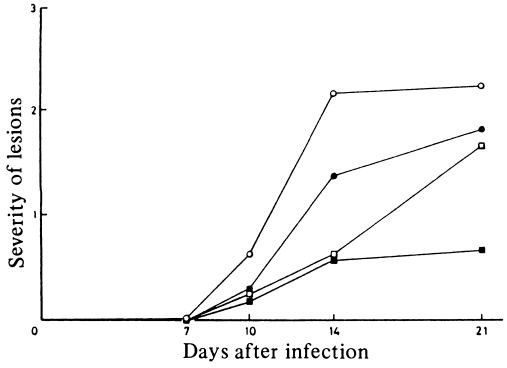

Fig. 5 Quantification of meningitis, perivascular cuffing, nodular lesion and diffuse lesion in the brain of mice following infection with *A. cantonensis* (for details see Table 1).
○: meningitis; ●: perivascular cuffing;
□: diffuse lesion; ■: nodular lesion

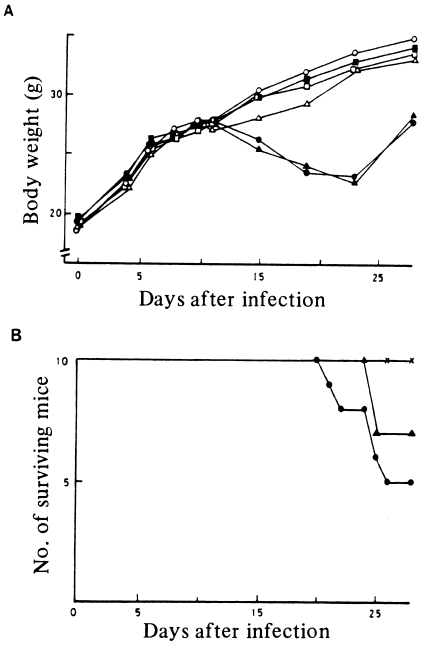

Fig. 6 Effects of mebendazole on body weight (A) and survival (B) of mice infected with *A. cantonensis*. Mebendazole was given orally at a daily dose of 5 mg/kg for 5 successive days. Days 1–5 (□), 6–10 (■), 11–15 (△) and 16–20 pi (▲). Non-infected (○) and non-treated (●) controls received vehicle only.
×: non-infected control and groups treated on days 1–5, 6–10 and 11–15 pi.

trol group, with 3 out of 10 mice dying.

Experiment IV

No mice died in the group treated with both mebendazole and betamethasone. In contrast, 4 mice died in the groups treated with either mebendazole or betamethasone alone and 7 mice died in the non-treated control group (Fig. 7B). Few clinical signs were observed in mice treated with both drugs, although the mice in this group did show slight depression and bristled hair. Changes in body weight were similar in all groups (Fig. 7A).

Discussion

Many similarities exist in the clinical signs and histopathological features in the brain of humans and mice infected with *A. cantonensis*. The disease may be fatal in some human infections and worms have been reported in the brain at autopsy (Rosen *et al.*, 1962; Jindrak and Alicata, 1965; Tangchai *et al.*, 1967; Yii, 1976). The results confirm that mice were dose-dependently affected by the parasite and that the most serious clinical signs, including death, were recorded in the mice with the heaviest infections. Clinical and pathological changes due to *A. cantonensis* were essentially similar to those described previously by John (1971) and John and Martinez (1975) for mice, and Rosen *et al.* (1962) and Yii (1976) for humans.

Fig. 4 Histopathological changes in the brain of mice infected with *A. cantonensis*. (A) Severe meningitis consisting of eosinophils and a few lymphatic cells and large mononuclear cells in the frontal lobe (arrow), 14 days pi (HE, ×30). (B) Perivascular cuffing showing eosinophils and lymphatic cells in the central portion of the cerebrum (arrow), 14 days pi (HE, ×30). (C) Nodular lesion possibly representing a traumatic tract in the cortex of the central portion of the cerebrum (arrow). The lesion contains degenerated brain tissues and leukocytes, 14 days pi (HE, ×75). (D) Lesion showing the diffuse infiltration of inflammatory cells, reactive proliferation of glial cells and spongy state in the frontal lobes, 21 days pi (HE, ×150).

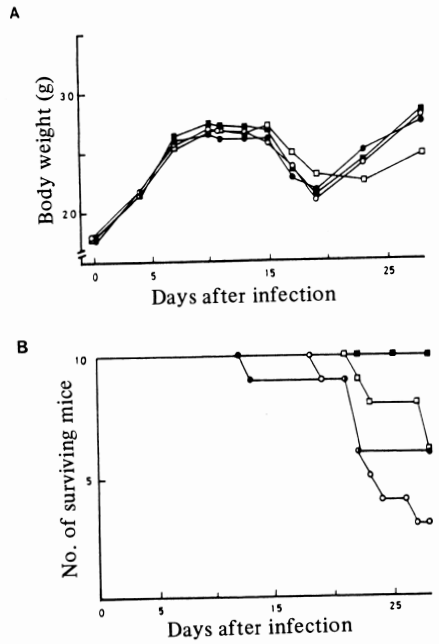

Fig. 7 Treatment of mice infected with *A. cantonensis* with mebendazole and betamethasone. (A) body weight (B) survival. Mebendazole was given at a daily dose of 5 mg/kg on days 16–20 pi, and betamethasone (2.5 μg/mouse) on alternate days between 16 and 26 days pi.
○: Non-treated control;
●: mebendazole;
□: betamethasone;
■: mebendazole and betamethasone

It is, therefore, possible that the pathogenesis of the disease in man and the mouse is similar, suggesting that the mouse is a suitable animal model for chemotherapeutic investigations of human angiostrongylosis.

From current histopathological results it is possible to suggest mechanisms for the pathogenesis of angiostrongylosis, since significant correlations exist between the degrees of inflammation in the brain and changes in body weight of mice. The primary lesion leading to neurological changes and death is the inflammation. Many pathogenic factors may be involved in the inflammatory reactions including the mechanical destruction of tissue by worms migrating through the brain, toxic substances released by living and/or dead worms and immune reactions elicited by the worms (Jindrak, 1975; John and Martinez, 1975; Arseni & Chimion, 1978). Although the critical pathogenic factor(s) have not been identified in the present study, it seems probable that young adults of *A. cantonensis* are involved in the abnormal hosts such as man and mouse. Clinical and pathological changes together with an eosinophilia were seen about 10 days pi. This coincides with the time when larvae developed to the young adult worm in mice (John, 1971).

Ideally *A. cantonensis* should be killed before the infective larvae reached the young adult stage in the brain, thus preventing the inflammatory disease. However if adult worms are present, the concomitant usage of an anthelmintic together with anti-inflammatory and/or anti-allergic drugs would appear to be useful to suppress the inflammatory reactions in addition to killing the worms.

Mebendazole is currently the drug of choice to treat *A. cantonensis* infections (Hayashi *et al.*, 1982). However this drug appears to be only effective against young adult worms and not mature worms (Lämmler and Weidner, 1975; Hayashi *et al.*, 1982). The present study demonstrated that mebendazole alone was effective up to 15 days pi in mouse angiostrongylosis but that between 16 and 20 days pi it was necessary to combine this with a steroid. Such a combination resulted in reduced mortality. It would seem likely that betamethasone is acting by suppressing the inflammatory reaction of the hosts.

Similar mechanisms have been proposed in other parasitic diseases. For example it has been shown that the adverse reactions which follow the treatment of *Dirofilaria immitis* with diethylcarbamazine in the dog can be largely prevented by prior administration of dexamethasone (Boreham *et al.*, 1985). Similar effects have been detected in human filarial infections treated with diethylcarbamazine Hawking (1979). Thus it is possible that the mouse infected with *A. cantonensis* could provide a useful model for a general phenomenon seen when parasites are killed by anthelmintics. A detailed

study of the autocoids released would be valuable. Body weight of mice treated with the combination of drugs was not improved. This suggests that other pathological mechanisms may be important.

Since the mouse is a suitable animal model for human angiostrongylosis, the present findings may be of value in the treatment of human cases. In particular the use of anti-allergic and/or anti-inflammatory drugs such as betamethasone may be a useful adjunct to therapy.

Acknowledgements

The author is grateful to Prof. M. Sano, Drs. M. Terada and H. Kino, and Mr. A. I. Ishii, Department of Parasitology, and to Dr. S. Nakamura, Department of Pathology, Hamamatsu Univesity School of Medicine, for their kind suggestions on experiments and for comments on this manuscript. Special thanks are due to Dr. P. F. L. Boreham, Queensland Institute of Medical Research, Australia, for his critical reviewing the manuscript.

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