

Studies on Alterations in Acid Phosphatase Activity,
Body Weight and Ultrastructure of Adult
Angiostrongylus cantonensis in Rats Treated with
Flubendazole at a Subcurative Dose

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Abstract: Physiological effects of flubendazole on adult *Angiostrongylus cantonensis* were studied. Administration of flubendazole for 3 consecutive days (48–50 days post-infection) at 10 mg/kg/day did not affect the number of worms and weight of adult female *A. cantonensis* recovered from rats 16 hr after termination of medication while it lowered the phosphatase activity by the intact worms. The possible modes of action of the drug were discussed together with electron-microscopic observation of the body wall of the worms recovered from the treated and non-treated rats.

Key words: *Angiostrongylus cantonensis*, flubendazole, modes of action

INTRODUCTION

Flubendazole is an anthelmintic of wide range covering many nematodes and cestodes (Thienpont et al., 1978). It also possesses chemotherapeutic activity against larval and adult *Angiostrongylus cantonensis* (Maki and Yanagisawa, 1983, 1985, 1986). Maki and Yanagisawa (1986) first reported that more than 80% of adult *A. cantonensis* in rats can be eliminated following oral administration of flubendazole at 10 mg/kg/day for 10 consecutive days. However, no information is available on modes of action of this drug regarding anthelmintic effects on this nematode.

Much attention has been paid to acid phosphatase activity in the body wall of adult *A. cantonensis* (Yanagisawa et al., 1970; Maki and Yanagisawa, 1980a, b) and transcuticular

absorption of glucose by this nematode (Yanagisawa et al., 1970). In an attempt to examine effects of this drug on the body wall of the nematode, the present studies were carried out. This communication describes the observation on changes in acid phosphatase activity, body weight of worms and ultrastructure of adult worms from rats administered flubendazole at a subcurative dose.

MATERIALS AND METHODS

The life cycle of *A. cantonensis* has been maintained according to the method by Maki and Yanagisawa (1980b). Three Wistar rats weighing about 250 g were inoculated with 50 third-stage larvae, orally given pure powdered flubendazole (Janssen Pharmaceutica, Belgium) suspended in 1% Tween 80 at 10 mg/kg/day for 3 consecutive days (48–50 days postinfection) and dissected 16 hr after the termination of the treatment. Three rats in control groups were given 1% Tween 80 alone. Recovered female and male adult worms were separately incubated in 5 ml Veronal buffered saline containing 5 mM *p*-nitrophenyl phosphate in a 50 ml flask. Each flask contained 5 females or 8 males. The method was based on the reports by Maki and Yanagisawa (1979, 1980a). The activity of the substrate hydrolysis was measured by the method described in the previous papers (Maki and Yanagisawa, 1979, 1980a) and expressed with $\Delta A_{405}/\text{hr}/\text{worm}$ or $\Delta A_{405}/\text{hr}/\text{mg}$ dry weight after drying worms at 90°C for 3 hr. Data were analyzed statistically using Student's *t*-test. P values less than 0.05 were considered to be significant.

Ultrastructure of female and male *A. cantonensis* adults from rats were studied as follows. Immediately being recovered from rats, 5 females and 5 males in control group, and 3 females and 4 males in experimental group were fixed for 2 hr in 2% paraformaldehyde, 1% glutaraldehyde solution (pH 7.2) at 4°C, rinsed three times in the buffer on the following day and cut so as to obtain approximately 2 mm long pieces at the middle parts of the body. Then the specimens were postfixed overnight in 2% osmium tetroxide in phosphate buffered solution. After being passed through *n*-butyl glycidyl ether, they were embedded in epoxy resin. The specimens were cross-sectioned with a Reichert ultramicrotome. Following the double-staining with uranyl acetate and lead citrate, one section from the respective specimen was examined with the Hitachi HS-8 electron microscope. The observation was made in a quarter area of a cross-section.

RESULTS

Flubendazole administered at 10 mg/kg/day for 3 consecutive days to rats harbouring adult *A. cantonensis* showed no decrease in number of worms in rats dissected 16 hr after the termination of medication (Table 1). However, phosphatase activity mainly due to cuticle and/or hypodermis (Yanagisawa et al., 1970; Maki and Yanagisawa, 1980a, b) and dry weight of male worms in experimental groups were significantly lower than those in control groups (Table 1). The statistical difference in the dry weight of female worms was not significant.

The electron microscopic observation revealed no significant difference between experimental and control groups in regard to hypodermis, muscle layer, intestine or reproductive organs under the present experimental conditions. There might be a possible difference on the surface of cuticular layer. A thick surface coat (glycocalyx-like materials) was seen as in Fig. 1 all over the cuticular surface of 5 females and one male in the control group. The other 4 males in the control group had a thin surface-coat as in Fig. 2. On the other hand, the present observation could not demonstrate any surface-coats in either of the worms (4 males and 3 females) from rats treated with flubendazole as shown in Fig. 3. However, the electron-microscopic observation was confined to so limited parts of the specimen that a sound conclusion on the comparison must await further studies.

Table 1. Effects of flubendazole administered 48–50 days post-infection for 3 consecutive days at 10 mg/kg/day on number of worms recovered, phosphatase activity and weight of *A. cantonensis* from rats 16 hr after termination of medication

Parameter measured	Treatment		% Change
	Flubendazole	Control	
Number of worms recovered (mean \pm S.E., n=no. rats)	21.3 \pm 3.0 (n=3)	23.3 \pm 4.9 (n=3)	- 8.6*
mg dry weight of recovered worms from vessels (mean \pm S.E., n=no. vessels)			
Females (5 worms/vessel)	3.5 \pm 0.2 (n=5)	4.3 \pm 0.3 (n=5)	-18.6**
Males (8 worms/vessel)	2.0 \pm 0.1 (n=4)	2.5 \pm 0.1 (n=4)	-20.0***
Phosphatase activity ΔA_{405} /hr/worm (Mean \pm S.E., n=no. vessels)			
Female	40.1 \pm 0.3 (n=5)	62.9 \pm 3.7 (n=5)	-36.2****
Male	16.4 \pm 1.2 (n=4)	23.7 \pm 1.2 (n=4)	-30.8****
ΔA_{405} /hr/mg dry wt. (Mean \pm S.E., n=no. vessels)			
Female	58.0 \pm 1.3 (n=5)	73.2 \pm 1.3 (n=5)	-20.8****
Male	65.5 \pm 2.9 (n=4)	75.0 \pm 1.2 (n=4)	-12.7***

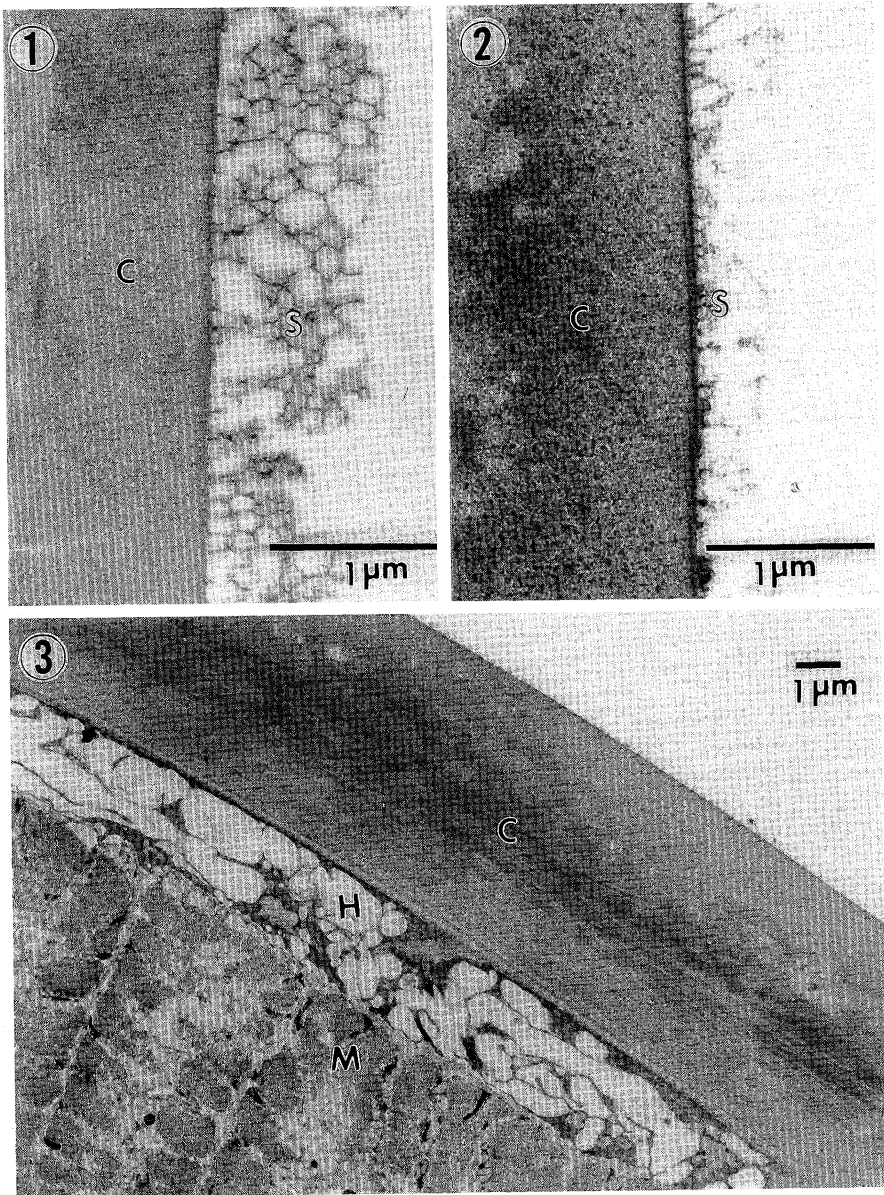
* $P > 0.5$

** $0.05 < P < 0.1$

*** $0.01 < P < 0.05$

**** $P < 0.01$

compared to each control, Student's *t*-test



- Fig. 1.** Cross section of the cuticle (C) and a thick surface-coat (S) of adult female *A. cantonensis* from the control group.
- Fig. 2.** Cross section of the cuticle (C) and rather a thin surface-coat (S) of adult male *A. cantonensis* from the control group.
- Fig. 3.** Cross section of cuticle (C), hypodermis (H) and muscle layer (M) of adult female *A. cantonensis* from the experimental group. No surface coat could be found on the cuticle under the present experimental condition.

DISCUSSION

Worm reduction was not observed at least 16 hr post-flubendazole therapy at the present dose, though more than 80% of adult worms in rats was eliminated following administration of flubendazole at 10 mg/kg/day for 10 consecutive days (Maki and Yanagisawa, 1986). In other words, the dose given under the present condition is subcurative.

However, acid phosphatase activity by female and male worms, and the dry weight of male worms was significantly affected even at the subcurative dose (total dose of 30 mg/kg).

The difference at the subcurative dose between the former (number of worms recovered) and the latter (acid phosphatase activity and male body weight) suggests that worm reduction, together with the possible other changes, is secondary to the decrease in the latter observation. It is probable that the decrease in the phosphatase activity by female and male worms might be related somehow with the subsequent effects of flubendazole at higher doses on adult *A. cantonensis*.

Flubendazole probably exerts its anthelmintic effect on adult *A. cantonensis* through a wide range of interrelated physiological systems including aspects in the present studies. Studies on mechanism of action of benzimidazoles in parasitic nematodes were reviewed by Lacey and Prichard (1986): inhibitory effect on fumarate reductase, glucose uptake, glycogen storage, protein secretion, disintegration of the microtubular framework in nematode cells and others. Modes of action of flubendazole against *A. cantonensis* merit further studies.

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