Effects of Avermectin B1a on the motility of various parasitic helminths

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Summary. Using an isotonic transducer recently devised in Japan, it was observed that Avermectin B1a, a new anthelmintic, caused paralyzing effects on *Angiostrongylus cantonensis* and *Metastrongylus elongatus* at concentrations of more than 3.6×10^{-18} M.

Effects of drugs on the motility of parasitic helminths have been tested in vitro as one of the useful approaches to the investigation of anthelminthics[1-5]. With some exceptions for *Schistosoma mansoni*[6,7], however, these studies have been carried out exclusively on larger worms which were easily tested by kymographic procedures, such as *Ascaris suum* and *Fasciola hepatica.*

To record the motility of smaller worms, we have developed a method in which an isotonic transducer devised recently by the Nihon Koden Co., in Japan, is used[8]. We could definitely study the effects of drugs on the faint motility of smaller parasites in vitro by this method. In the present study, the in vitro effects of Avermectin B1a on various parasitic helminths were studied.

Materials and methods. Worms were obtained from the animals sacrificed at the Hamamatsu Slaughter-house or from those experimentally infected in our laboratory. Except for the plerocercoid of *Diphyllobothrium erinacei,* a helminthic preparation, a whole worm or an anterior portion of the worm (about 2 cm long), was suspended in Tyrode's solution in a thermostatically controlled organ bath (7 ml capacity) at 35 °C and gassed slightly with air. *D. erinacei* was suspended in Ringer's frog solution at 22 °C. The responses of the preparations to Avermectin B1a were

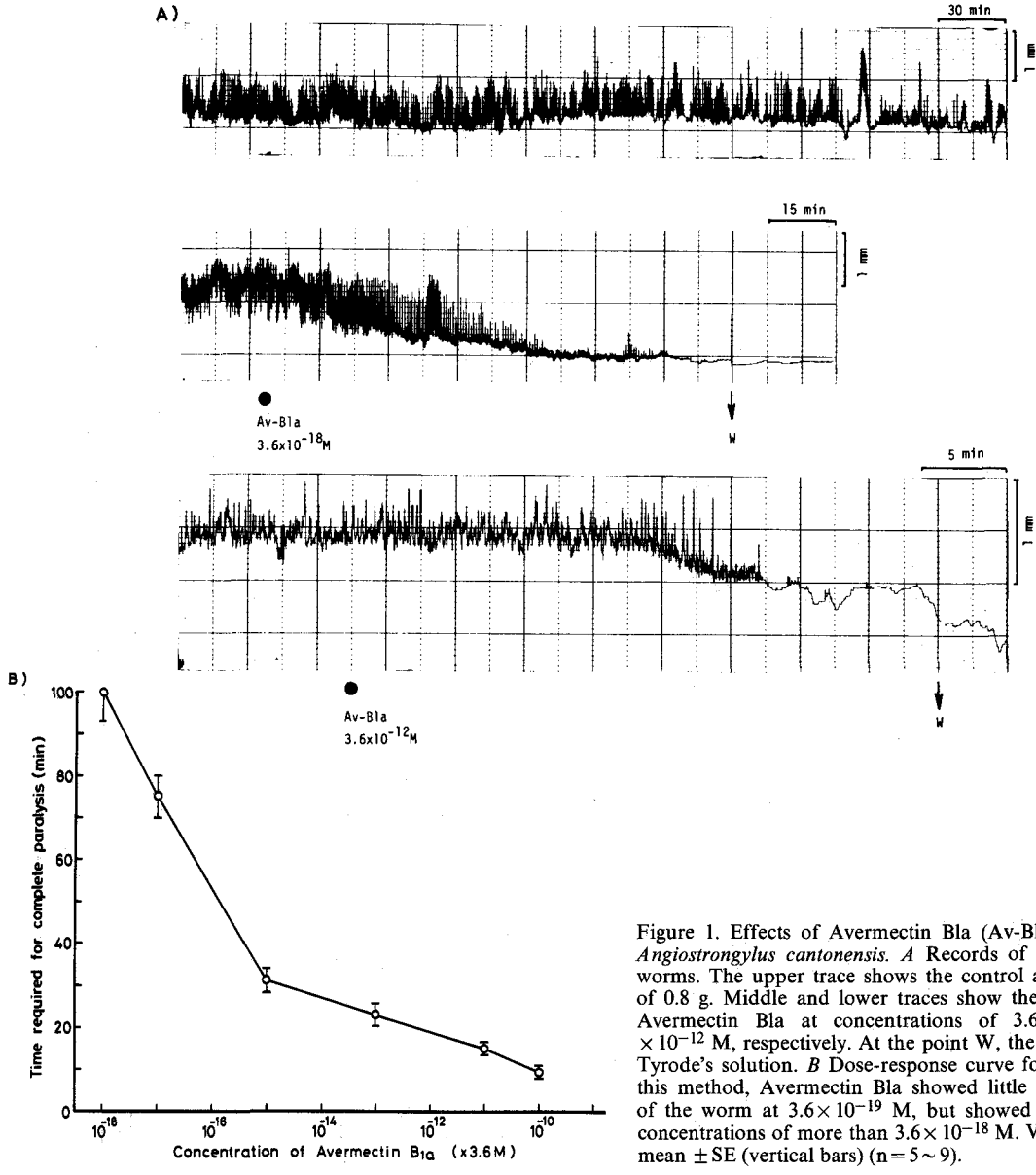

Figure 1. Effects of Avermectin Bla (Av-Bla) on the motility of *Angiostrongylus cantonensis. A* Records of the motility of whole worms. The upper trace shows the control activity with a tension of 0.8 g. Middle and lower traces show the paralyzing effects of Avermectin Bla at concentrations of 3.6×10^{-18} M and 3.6×10^{-12} M, respectively. At the point W, the worm was washed by Tyrode's solution. *B* Dose-response curve for Avermectin Bla. By this method, Avermectin Bla showed little effect on the motility of the worm at 3.6×10^{-19} M, but showed complete paralysis at concentrations of more than 3.6×10^{-18} M. Valves are expressed as mean ± SE (vertical bars) (n = 5 ~ 9).

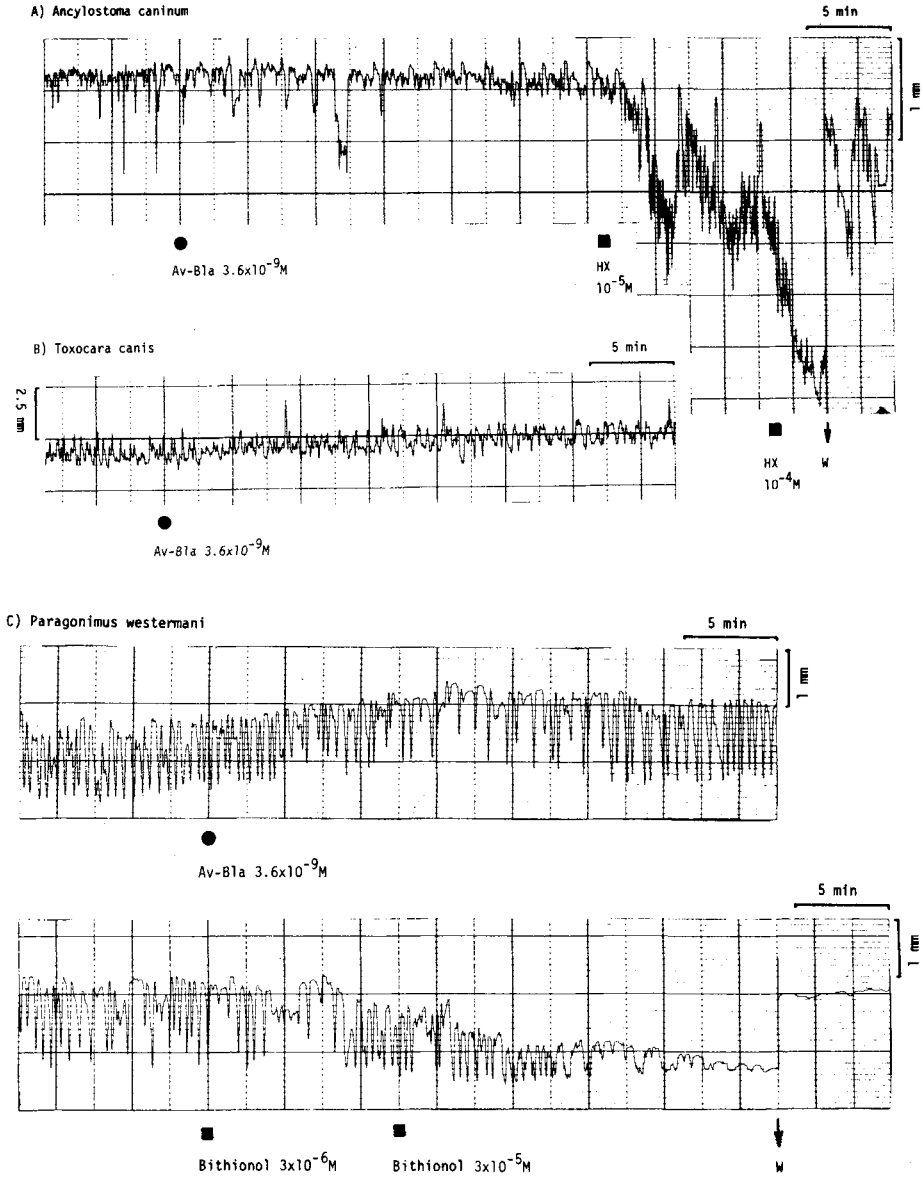

Figure 2. Effects of Avermectin B1a (Av-B1a), hexylresorcinol (HX) and bithionol on the motility of some parasitic helminths. *Ancylostoma caninum* was used as a whole worm with a tension of 0.6 g, *Toxocara canis* as an anterior portion of the worm with a tension of 3.8 g, and *Paragonimus westermani* as a whole worm with a tension of 0.3 g, respectively.

recorded isotonically on a recorder (Toa, EPR-100A) with an isotonic transducer (Nihon Koden, TD-112S). A suitable tension and a magnification for each worm were selected from the ranges of 0.2–3.8 g and 1.5–60-fold, respectively. Avermectin B1a was added to the organ bath by the single dose method. The time of contact with Avermectin B1a was usually 25–30 min for all preparations other than *Angiostrongylus cantonensis* and *Metastrongylus elongatus,* for which various doses of Avermectin B1a were given with various times of contact.

Results and discussion. Avermectin B1a is one of the antiparasitic macrocyclic lactones produced by the newly described actinomycete, *Streptomyces avermitilis.* There have been some reports referring to the in vivo effects of this compound against parasitic nematodes of domestic animals such as sheep, cattle, dogs and chickens[9,10]. When Avermectin B1a was given to animals as a single dose, this compound was effective against many nematodes such as *Ancylostoma caninum* (at 0.003–0.005 mg/kg), *Haemonchus contortus* (at 0.025 mg/kg), *Ascaridia galli* (immature worm, at 0.05 mg/kg) and *Dirofilaria immitis* (precardiac stage, at 0.1 mg/kg). There is, however, no report referring to the in vitro effects of Avermectin B1a on various parasitic helminths including cestodes and trematodes.

In our in vitro study, *A. cantonensis* and *M. elongatus,* among the many parasitic helminths, showed a significant susceptibility to Avermectin B1a. In the case of *A. cantonensis,* Avermectin B1a at a concentration of 3.6×10^{-18} M caused a sustained inhibition in motility with relaxation (fig. 1A). The time required to cause complete paralysis depended on the concentration of Avermectin B1a, varying from about 100 min at 3.6×10^{-18} M to about 10 min at 3.6×10^{-10} M (fig. 1B). As for *M. elongatus,* Avermectin B1a was similarly effective at concentrations of more than 3.6×10^{-18} M. These effects on both worms were, however, not vermicidal, because the worm could respond, to produce a transient movement, against mechanical stimuli after complete paralysis with this compound.

With Avermectin B1a at concentrations of less than 3.6×10^{-9} M, little effect was, however, observed on the motility of various parasitic helminths; i.e., nematodes: *D. immitis, Toxocara canis, A. suum, A. caninum, Trichuris*

vulpis, T. suis; trematodes: *F. hepatica, Paragonimus westermani, P. miyazakii;* cestodes: *Dipylidium caninum, D. erinacei* (plerocercoid). Figure 2 shows the effects of Avermectin B1a and other anthelminthics on the motility of some parasitic helminths. Avermectin B1a at 3.6×10^{-9} M showed little effect on the motility of *A. caninum, T. canis* and *P. westermani.* On the other hand, hexylresorcinol at $10^{-5} - 10^{-4}$ M, and bithionol at $3 \times 10^{-6} - 3 \times 10^{-5}$ M showed paralyzing effects on *A. caninum* and *P. westermani,* respectively.

Thus, under our experimental conditions, Avermectin B1a was especially effective against worms which belong to the Metastrongylidae. The ineffective result of this compound against *D. immitis* agreed with that reported in the in vivo experiment[9]. Though the action of Avermectin B1a against *A. caninum* was reported in the in vivo experiment[9], this compound showed little effect in our in vitro experiment in which the observation was made only for a shorter period.

We have obtained results suggesting that Avermectin B1a elicits paralyzing effects through a neuropharmacological mechanism including γ-aminobutyric acid (GABA) and acetylcholine (ACh) in *A. cantonensis*[11,12]. The nervous system found in *A. cantonensis* is similar to that described in *A. suum*[4,5,13]. Further experiments with higher concentrations of Avermectin B1a and/or longer periods of treatment may show the effectiveness of this compound against other nematodes such as *A. caninum* and *A. suum.*

It has been reported that the anthelminthic effects of many drugs are due to indirect paralytic or spastic action rather than to direct vermicidal action[14,15]. And their effects may also be due to host functions such as the peristalsis of the digestive tract, and trapping in the tissues which leads to killing through tissue reactions. Therefore, the anthelminthic effects of Avermectin B1a against *A. cantonensis* recently reported in rats[16] may be caused through the paralyzing action of this compound observed in the in vitro experiment.

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High magnesium content of *Escherichia coli* B[1]

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Summary. We have found that the intracellular concentration of magnesium in exponentially growing *Escherichia coli* B is much higher than has been previously assumed; it is about 100 mM. Results of equilibrium dialysis suggest that nearly all of this Mg is bound, probably most of it to nucleic acids. These findings could have important consequences for the study of protein-DNA interactions and the in vitro simulation of protein biosynthesis.

Knowledge of the intracellular concentrations of ions and other small molecular components is of prime importance for understanding protein-nucleic acid and protein-protein interactions which occur in nuclear material, and during the morphogenesis of viruses. An extensive study covering Mg, K, Na, ATP and polyamines will be reported in detail elsewhere. In the course of this work we discovered that the Mg content of *E. coli* B cells is 2.5–5 times higher than has generally been assumed[4].

It is well known that metabolic disturbances in cells which are packed in a centrifugation pellet (starvation of oxygen and nutrients) lead to massive leakages of ions; we therefore used a filtration method similar to that introduced by Epstein and Schultz[5]. During the deposition of cells from an exponentially growing culture (1 ml of a 2×10^8 bacteria/ml suspension) on a membrane filter (Millipore GSWP, 0.2 µm) under suction, the cells were maintained in the presence of O_2 and small amounts of nutrients. These conditions of continued metabolism were also maintained during washing. Control experiments showed that washing lead to plateaux of the internal ionic content, which were maintained through at least 2 cycles of washing, before the content of the most sensitive component, K, decreased to reach that of stationary phase cells. The washed deposit of cells on the membrane filter was then extracted with 1 M HNO_3, and the solution was analyzed by atomic absorption spectrophotometry.

A large size derivative of *E. coli* strain B (Hermann Epstein, B^E) was grown in 1% tryptone with 0.2% glucose and 0.1 M salt (NaCl or KCl) added, and harvested at 2×10^8 bacteria/ml. Tryptone was chosen as the basis of our growth medium because it has good buffering capacity, provides the possibility of achieving low ionic strengths, and has a low Mg-content (0.09 mM). We found the following internal contents and concentrations in cells grown in this way:

These contents are dependent on growth conditions; we confirmed Epstein and Schultz's[5] observations that K^+ is mainly used for compensating the outside osmolality of the growth medium. We found in addition that the Mg content is 55–50 and 170–140 mM, when the total molarity of the growth medium is 50 mM or 400 mM. Our values for the Mg content are about 4 times those reported by Lusk et al.[6]. The details of the procedures, in particular the new method