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Research Note

Studies on Chemotherapy of Parasitic Helminths (XIX) Further Examination on *in Vitro* Effects of Avermectin B1a

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There have been many reports regarding the *in vivo* efficacy of avermectin B1a (Av-B1a) as nematocides and pesticides (Campbell and Blair, 1978; Egerton *et al.*, 1979; Putter *et al.*, 1981). It has also become probable that these effects of Av-B1a are attributable to its action on the γ-aminobutyric acid (GABA) mechanism in animals including parasitic nematodes (Fritz *et al.*, 1979; Kass *et al.*, 1980, 1982; Putter *et al.*, 1981; Sano *et al.*, 1981a). Putter *et al.* (1981) described that Av-B1a is essentially nontoxic for organisms lacking the GABA system. Since there have been few reports regarding *in vitro* effects of this drug, we examined previously such effects of Av-B1a on the motility of various parasitic worms including cestodes, trematodes, and nematodes (Sano *et al.*, 1981b). In the experiments, a few worms such as *Angiostrongylus cantonensis* and *Metastrongylus elongatus* were extremely susceptible to this drug and the motility of these worms were paralyzed at concentrations as low as 3.6×10^{-18} M or more. Therefore, we examined the effects of this drug up to the concentration of 3.6×10^{-9} M against other worms, and showed that other nematodes as well as cestodes and trematodes were insensitive to Av-B1a at these lower concentrations. These results may disagree with the above-mentioned assumption that Av-B1a affects through the GABA mechanism in parasitic nematodes. Thus, further examination with higher concentrations of Av-B1a and longer period of treatment was carried out in this study.

Av-B1a was kindly offered from Merck Sharp & Dohme Research Laboratories. Worms were obtained from animals sacrificed at the Hamamatsu Slaughterhouse and the Shizuoka Prefectural Dog Center, or from animals experimentally infected in our laboratory. The isotonic transducer and visual observation methods previously described were used (Sano *et al.*, 1981c).

Against all nematode preparations examined, Av-B1a caused paralytic effects. Compared to metastrongylid nematodes such as *A. cantonensis*, however, the drug was remarkably less effective on other nematodes. The anterior preparation of *Toxocara canis* and the whole worm preparation of *Ancyclostoma caninum* were slightly affected with Av-B1a at the concentration of 3.6×10^{-7} M, though paralysis was caused after long exposure period of 6 hr or more (Fig. 1A). Av-B1a at the concentration of 3.6×10^{-6} M caused paralysis against anterior preparations of *T. canis* and *Dirofilaria immitis*, the muscle strip of *Ascaris suum*, and the whole worm preparation of *A. caninum*. The paralytic effect of Av-B1a in these preparations was char-

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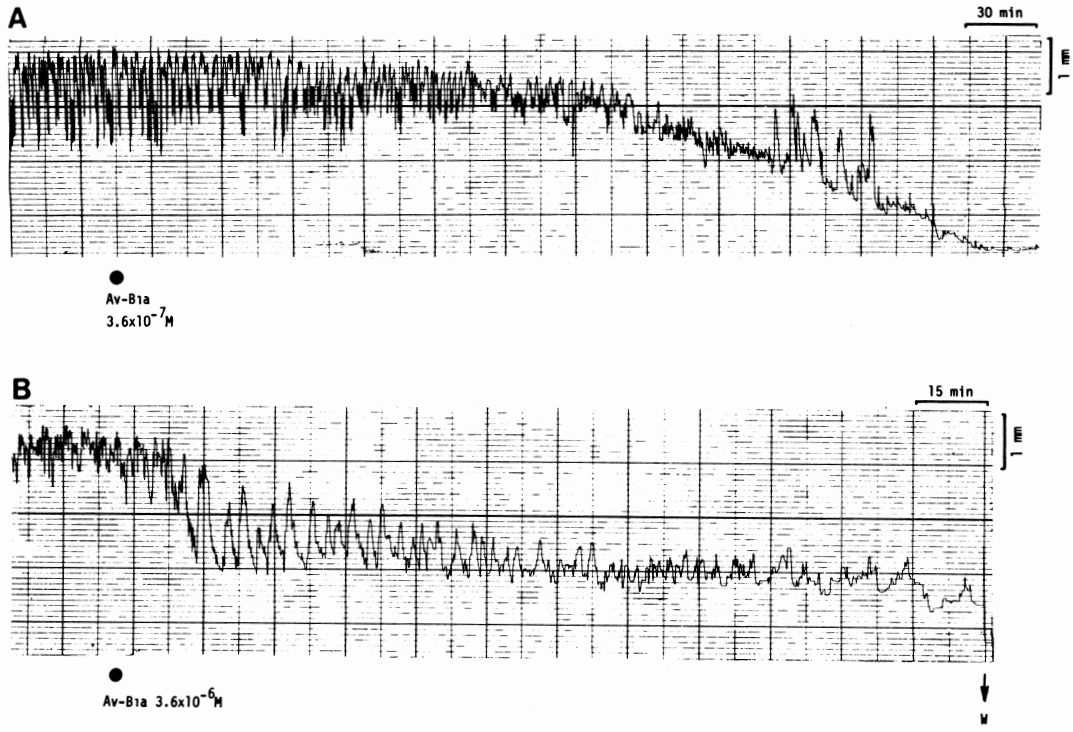

Fig. 1 Effects of avermectin B1a (Av-B1a) on the motility of the whole worm preparation of female *Ancylostoma caninum*. Worm preparation was suspended in Tyrode's solution with a tension of 0.6 g.

acterized by a decrease of rate, amplitude and tone (Fig. 1B, 2A). On the other hand, the whole worm preparation of *Trichuris vulpis* was less sensitive to this drug, and only slight inhibition in rate of the motility was caused by the concentration of 3.6×10^{-6} M. It was reported by Kass *et al.* (1980, 1982) that the whole worm preparation of *A. suum* was immobilized when 1.5 μg or more of Av-B1a was injected into the perienteric fluid and that the drug at the concentration of 5 μg/ml (6.1×10^{-6} M) blocks neural transmissions in the muscle strips of this worm. Since intact worm or anterior piece preparations of *A. suum* showed less susceptibility to almost neuropharmacological agents, this worm has been used as muscle strips with a longitudinal cut along the lateral line or eviscerated preparations (Baldwin and Moyle, 1949; Terada *et al.*, 1982). In our experiment, Av-B1a (3.6×10^{-6} M) had little effect against the anterior preparation of *Ascaris*, but paralyzed the muscle strip with a longitudinal cut along the lateral line (Fig. 2A, B). Differences in susceptibility to Av-B1a among nematodes may also be related to such factors as permeability across membranes and susceptibility of receptor sites.

On the other hand, Av-B1a at the concentrations of 3.6×10^{-6} M or less had little effect after long exposure up to 24 hr against cestodes such as *Diplogonoporus grandis, Taenia pisiformis, Dipylidium caninum* and plerocercoids of *Diphyllobothrium erinacei*, trematodes such as *Schistosoma japonicum, Fasciola hepatica, Paragonimus westermani* and *Metagonimus yokogawai*, and isolated host tissue

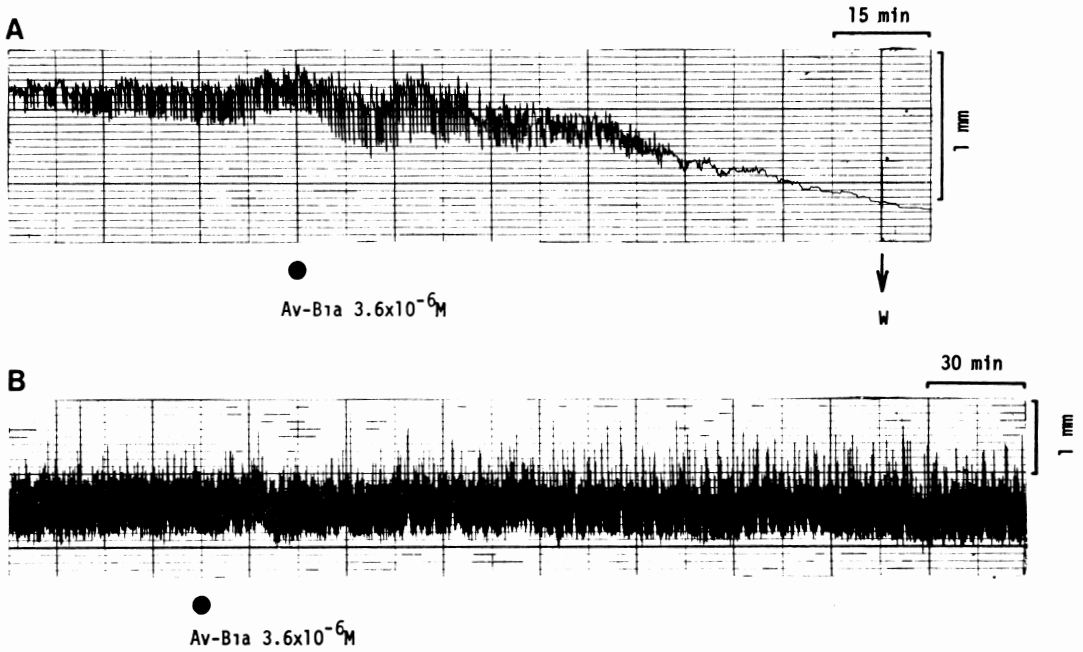

Fig. 2 Effects of avermectin Bia (Av-Bia) on the motility of the muscle strip (A) and the anterior preparation (B) of female *Ascaris suum*. The anterior 2 cm portion was suspended in Tyrode's solution with a tension of 1.0 g. After removing 1.5 cm portion of the anterior end of the worm, 2 cm portion with a longitudinal cut along the lateral line was used with a tension of 0.8 g.

preparations such as the frog rectus (guanidine-induced twitch response) and the mouse ileum. Higher concentrations of this drug should be examined against such preparations as cestodes, trematodes and isolated host tissues, if possible.

Besides the studies on the mode of action of Av-Bia (Fritz *et al.*, 1979; Kass *et al.*, 1980, 1982; Sano *et al.*, 1981a), these results from studies regarding *in vitro* spectrum of this drug may support the assumption that Av-Bia acts through the GABA mechanism in parasitic nematodes as well as other animals. However, there may be other mechanisms for the anthelmintic actions of Av-Bia, especially in higher doses, because we recently found that ivermectin, a closely related agent to Av-Bia, inhibits the production of microfilariae of *D. immitis* through inhibiting the embryonic development in the reproductive system of female worms (Anantaphruti *et al.*, 1982).

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短　報

寄生蠕虫症の化学療法に関する研究（XIX）．高濃度 avermectin B_{1a} の *in vitro* 作用について

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先に，著者ら（佐野ら，1981b）は avermectin B1a（以下 Av-B1a）の *in vitro* での作用スペクトルについて検討した．この実験では，擬円形線虫科の広東住血線虫などが Av-B1a に対し特異的感受性を示し，3.6×10^{-18}M 以上の濃度で弛緩性麻痺作用を受け，$3.6\times^{-10}$M では 10 min 以内に完全な麻痺を呈した．そこで他の虫種に対しては，Av-B1a の 3.6×10^{-9}M 以下の作用を 25～30 min にわたって観察した．その結果，他の線虫類は条虫類および吸虫類とともに 3.6×10^{-9}M 以下の濃度でも作用を受けなかった．これらの知見は，Av-B1a が線虫類の GABA 機構を介して作用するとの仮説（Kass *et al.*, 1980, 1982; 佐野ら，1981a）と矛盾する．そこで本研究では，更に高濃度の Av-B1a を用い，作用時間も更に長時間として，本薬物の作用スペクトルを検討した．その結果，検討したすべての線虫類標本は Av-B1a により弛緩的作用を受けた．しかし擬円形線虫科の広東住血線虫に比べ感受性は著しく劣り，3.6×10^{-6}M でも著しい作用の発現には 1～3 hr を要した．一方，条虫類および吸虫類に対しては，3.6×10^{-6}M でも全く影響が認められなかった．これら *in vitro* 作用スペクトルについての知見は，Av-B1a の作用機作に関する上記仮説を支持するものと考えられる．