RESEARCH NOTE

MOVABILITY OF ADVANCED THIRD-STAGE LARVA OF *GNATHOSTOMA SPINIGERUM* EXPOSED TO ALBENDAZOLE SULPHOXIDE *IN VITRO*

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Abstract. Movability of advanced third-stage larvae of *Gnathostoma spinigerum* exposed to albendazole sulphoxide (AlbSO), the active metabolite of albendazole, was determined *in vitro*. Larvae in control groups moved actively with the whole body for all 21 days of the study period. In larvae treated with AlbSO 1 µg/ml, the movement was significantly reduced after 11 days exposed to the drug and to be only a part of body on the 15th - 21st days. In larvae treated with AlbSO 2 µg/ml, the movement was initiated in decreasing after 9th days and to be only a part of body on the 12th - 17th days. Finally, worms were immobile but not dead on the 20th - 21st days. Although there was no larvae died at 21st days exposed to AlbSO in both concentrations; but all worms were sluggish and may die later. These lethargic worms may not be able to migrate in patients and leading to cure. Albendazole may not be benefit for acute symptom clearance; however, it can prevent the recurrent migratory swelling after the treatment of 21 day-course.

Gnathostoma spinigerum Owen, 1836 is a nematode causing human gnathostomiasis in Southeast Asia including Thailand (Daengsvang, 1981). The clinical manifestations in man are most commonly characterized by localized, intermittent, migratory swellings of the skin and subcutaneous tissues, often in association with localized pain, pruritus, and erythema (Rusnak and Lucey, 1993). The damage in many visceral organs may result from the migration of worms to deeper tissues. Damage to the central nervous system by *G. spinigerum* can cause eosinophilic myeloencephalitis and death (Daengsvang, 1981).

Albendazole, an anthelminthic drug, has been shown to be effective for the treatment of human gnathostomiasis (Kraivichian *et al*, 1992; Suntharasamai *et al*, 1992). The administration of albendazole significantly reduced the number of *G. spinigerum* larvae in mice at the dosage of 90 mg/kg twice daily (Maleewong *et al*, 1992). However, the mechanism by which albendazole reacts with gnathostome larvae is still unknown. Pharmacokinetic studies have shown that albendazole is metabolized mainly to the sulphoxide (AlbSO), an active metabolite (Marriner *et al*, 1986). To date there is no information regarding the movability of *G. spinigerum* exposed to AlbSO *in vitro*, and this study was therefore undertaken.

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The advanced third-stage larvae of *G. spinigerum* (aL3) were collected from cysts in the liver of freshwater eel (*Fluta alba*). The larvae were obtained by compression of liver between two thick transparent glasses. Larvae were removed from the tissue and washed thoroughly in sterile physiologic saline solution (0.85% NaCl), containing 100 U/ml of penicillin G, 100 µg/ml of gentamicin and 100 µg/ml of amphotericin before placing into culture medium. All larvae were cultured in two 35 mm diameter sterile petri dishes (10 larvae/dish) with 2 ml of culture medium of RPMI-1640 (GIBCO laboratories, Life Technologies, Inc, Grand Island, New York, USA) and 10% fetal calf serum (SEROMED Biochrom KG, Berlin, Germany) containing 100 U/ml of penicillin G, 100 µg/ml of gentamicin and 100 µg/ml of amphotericin in each dish. All petri dishes were stored at 37°C under 5% CO_2 in air.

Twenty larvae were incubated in culture medium as the control group (10 larvae/dish). Another 40 larvae were divided into 2 groups and exposed to AlbSO 1.0 and 2.0 µg/ml. Larval movability of each group was assessed according to the criteria of Kiuchi *et al* (1987).

The movability index of *G. spinigerum* larvae incubated with AlbSO at both concentrations as well as in the control groups is shown in Fig 1. Larvae

in control groups moved actively with the whole body for all 21 days of the incubation period. No dead and/or weaken worms were found. In larvae treated with AlbSO 1.0 μg/ml, movement was significantly reduced after 11 days exposed to the drug. Movement was with only a part of the body on the 15th - 21st days. In larvae treated with AlbSO 2.0 μg/ml, the movement decreased after 9th days. Movement was with only part of body on the 12th - 17th days of the incubation period. The worms were immobile but not dead on the 20th - 21st days.

Albendazole may be an effective anthelminthic drug for the treatment of gnathostomiasis. In symptomatic gnathostomiasis patients, albendazole at the dosage of 800 mg/day for 14 days (approximately 15 mg/kg/day) produced the outward migration of gnathostome larvae (Suntharasamai *et al*, 1992). However, 94.1 % cure was seen in the longer period of treatment for 21 days (Kraivichian *et al*, 1992). Nevertheless, the symptom clearance time of albendazole or placebo treated patients were not different (6.4 and 6.8 days respectively) (Kraivichian *et al*, 1992). The decrease in migratory swelling of human gnathostomiasis within 6-7 days after exposure to albendazole may not be a direct effect of the drug, since the onset in declining movement only occurred by 9-11 days in these experiments. The initial decrease in migratory swelling is probably due to the natural history of the disease itself, while recurrence of swelling is probably due to drug effects on the parasite itself.

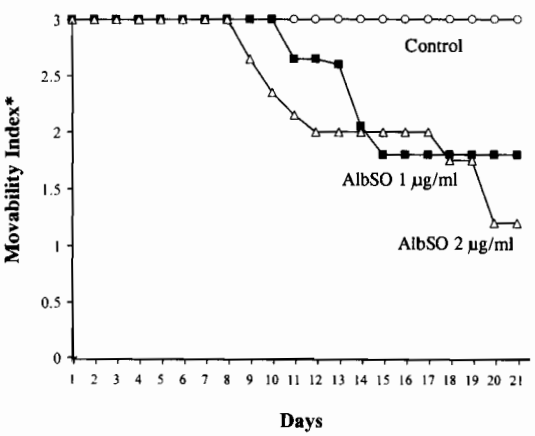

Fig 1–Average of movability index of the advanced third-stage larva *Gnathostoma spinigerum* incubated in albendazole sulphoxide 1.0 and 2.0 μg/ml (n=20 larvae/group)
*3 = Moving with whole body
2 = Moving with only a part of body in the observation period
1 = Immobile but not dead

The decreasing in movement of *G. spinigerum* larvae in this experiment was supported by scanning electron microscopic observations that the ultrastructural changes were swelling and sloughing of tegumental surface and detachment of spines (unpublished data). This damages may be the explanation for reduction in *G. spinigerum* larval movement since albendazole and AlbSO produce tubulin alterations which involved in muscle contraction of *Echinococcus granulosus* protoscolices (Serrano *et al*, 1995).

The result of decreasing larval movement of *G. spinigerum* after AlbSO administration confirmed the lack of benefit of albendazole in symptom clearance from the previous clinical study of Kraivichian *et al* (1992). Although there was no larvae died after 21 days exposed to AlbSO in both concentrations; but all worms were sluggish and might die later. These lethargic worms may not be able to produce further migratory swelling in human gnathostomiasis patients and lead eventually to cure in 21 day-course of albendazole treatment.

ACKNOWLEDGEMENTS

This work was supported by Faculty of Medicine, Chiang Mai University. We are greatful to Dr John Horton and Smith Kline and Beecham Research Ltd (UK) for supplying sample of albendazole sulphoxide.

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