RESEARCH NOTE

LACK OF EFFICACY OF QUININE AND ARTEMETHER AGAINST ADVANCED THIRD-STAGE LARVAE OF *GNATHOSTOMA SPINIGERUM IN VITRO*

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Abstract. The efficacy of quinine and artemether- the effective blood schizontocide in malarial treatment- has been *in vitro* tested with the advanced third-stage larvae of *Gnathostoma spinigerum*. All larvae were collected from freshwater eel (*Fluta alba*) and exposed to the culture medium, each containing either quinine dihydrochloride or artemether at a final concentration of 20 μg/ml and 0.5 μg/ml, respectively for 21 consecutive days. Larval motility was assessed daily and the topographical changes were assessed using scanning electron microscope after 21-days of drug exposure. All worms moved actively for 21 days of study period and no change in surface ultrastructure was observed. Quinine and artemether at these concentrations have no effect on movement and topographical changes on the advanced third-stage larvae of this parasite.

The efficacy of several drugs has been conducted for the treatment of human gnathostomiasis caused by *Gnathostoma spinigerum* Owen, 1836 both *in vivo* and *in vitro*. Among them, quinine - an effective blood schizontocide in malarial treatment, was claimed to be an effective drug in human gnathostomiasis during clinical trial comparing with prednisolone. The clinical symptom of migratory swelling disappeared more rapidly in gnathostomiasis cases treated by quinine (Jaroonvesana and Harinasuta, 1973). However, its true effectiveness against the advanced third-stage larvae of *G. spinigerum* has not been proven. Hence, this *in vitro* study was undertaken using quinine equivalent to safe human plasma concentration for 21 days on third-stage larvae of *G. spinigerum*. Another potent effective blood schizontocide in malaria treatment, artemether, was also investigated. This drug has promising effect on the early infection of blood fluke, *Schistosoma japonicum*, in dogs, rabbits (Xiao *et al*, 1995a;b) and humans (Song *et al*, 1998) as well.

The advanced third-stage larvae of *G. spinigerum* (aL3) were collected from cysts in the liver of freshwater eel (*Fluta alba*). They were obtained by compressing liver between two thick transparent glasses. The larvae were then removed from the tissue and washed thoroughly in sterile physiologic saline solution (0.85% NaCl), containing 100 units/ml of penicillin G, 100 μg/ml of gentamicin and 100 μg/ml of amphotericin B before placing into a 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, NY, USA) and 10% fetal calf serum (SEROMED Biochrom KG, Berlin, Germany) containing 100 units/ml of penicillin G, 100 μg/ml of gentamicin and 100 μg/ml of amphotericin in each dish. All petri dishes were stored at 37ºC under 5% CO_2 in air.

Sixty active larvae were recruited for this study. Twenty larvae were incubated in a culture medium as the control group (10 larvae/dish). Another 40 larvae were divided into 2 groups. The larvae in the first group (10 larvae/dish) were exposed to quinine dihydrochloride (Government Pharmaceutical Organization of Thailand) at a final concentration of 20 μg/ml while those in the second group were exposed to artemether (Artenam®Arenco n.v.Belgium) at a final concentration of 0.5 μg/ml. The final concentrations of both drugs in the dish were performed according to the maximum concentration of drugs at a plasma level in patients (Karbwang and Na-Bangchang, 1993a,b). The culture medium as well as drugs were changed every 24 hours for 21 consecutive days and all petri dishes

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were maintained at 37ºC under 5% CO_2 in air. The larval motility of each group was assessed daily during this study period according to the criteria of Kiuchi *et al* (1987) before they were examined for topographical changes by SEM.

Following removal, all incubated larvae were briefly washed in 0.1 M phosphate buffer prior to fixation for electron microscopy. Cultured larvae were fixed in a fixative agent consisting of 2.5% glutaraldehyde at 4ºC for 24 hours. The fixed worms were subjected to postfix in 1% osmium tetroxide and dehydration in a graded alcohol series, followed by acetone and critical-pointed drying. The worms were then mounted on stubs and coated with gold. Ten larvae from each group were viewed with a JEOL-JSM840A scanning electron microscope at an accelerating voltage of 20 kV, and photographed with Kodak®Verichrome Panchromatic film VP 120.

Regarding the mobility of aL3 *G. spinigerum*, those incubated in quinine, artemether and in the control groups moved actively with the whole body for all 21 days. No dead and/or weaken worms were found. As for the examination of surface morphology under SEM, aL3 *G. spinigerum* in both drug-treated and control groups were of normal appearance as previously described (Anantaphruti *et al*, 1982; Ratanarapee, 1982; Maleewong *et al*, 1988). No change in surface ultrastructure was observed after the experimental period of 21 days (Fig 1).

The results from mobility and SEM showed that quinine and artemether were not effective on aL3 *G. spinigerum*. Disappearance of acute clinical symptoms after treatment with quinine proposed by Jaroonvesana and Harinasuta (1973) may not be the effect of drug in killing or immobilizing parasites. However, quinine itself has an anti-inflammatory effect (Santos and Rao, 1998) and inhibits the release of enzyme beta-glucoronidase from stimulated polymorphonuclear leukocytes (Fontagne *et al*, 1989). Disappearance of acute symptoms also occurred after both albendazole and placebo treatment (Kraivichian *et al*, 1992). Additionally, albendazole has proved to be effective on the motility of aL3 after 9 days of drug exposure (Sukontason *et al*, 1999). Disappearance of acute symptoms may be due to the course of disease itself and/or increment by drugs that have an anti-inflammatory property. Artemether, another potent blood schizontocide drug for human malaria parasites, has had a promising effect on the early infection of *Schistosoma japonicum* in dogs, rabbits (Xiao *et al*, 1995a,b) and humans (Song *et al*, 1998). It can reduce the phosphobycerate kinase and pyruvate kinase enzymes in the glycolytic pathway of this parasite (Xiao *et al*, 1998). However, all studies were only involved in the early infection by both immature and small sized parasites. Artemether

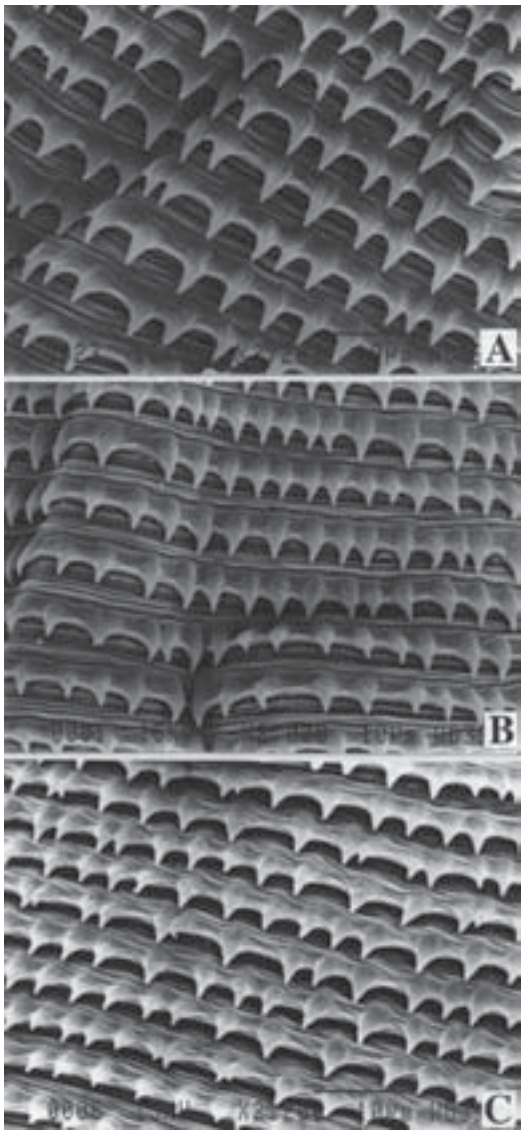

Fig 1–Scanning electron micrographs of advanced third-stage larvae *Gnathostoma spinigerum*. (A) Control. (B) Exposed *in vitro* to quinine dihydrochloride at a concentration of 20 µg/ml for 21 days. (C) Exposed *in vitro* to artemether at a concentration of 0.5 µg/ml for 21 days.

at a concentration of 0.5 μg/ml for 21 days has proved not to be effective on aL3 *G. spinigerum*. A higher concentration may or may not have any effect on this stage of parasite, however, the therapeutic index must be considered.

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