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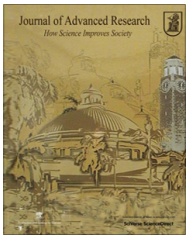

ORIGINAL ARTICLE

Effect of artemether on cytokine profile and egg induced pathology in murine *schistosomiasis mansoni*

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ABSTRACT

Artemether (ART), the methylated derivative of artemisinin, is an efficacious antimalarial drug that also displays antischistosomal properties. This study was designed to evaluate the immunomodulatory action of a single intramuscular dose (50 mg/kg body weight) of ART in comparison with PZQ treatment (42 days PI). ART administration was 7, 14, 21 and 45 days PI. ART effect was studied parasitologically, histopathologically and immunologically. It was found that maximum effect was reached when ART treatment interfered with 14 or 21 days old schistosomula. ART treatment 14 or 21 days PI was associated with shift from Th2 to Th1 predominancy (decrease in IL-4 and upgrading of serum IFN-γ levels). In conclusion, ART is a promising drug in control of *schistosomiasis mansoni* due to its reductive effect on worm burden and its role in improvement of hepatic granulomatous lesions.

Introduction

Schistosomiasis is a common intravascular trematode infection. It is one of the most prevalent parasitic diseases in the world, after Malaria [1]. Currently; praziquantel (PZQ) is the drug of choice for mass treatment of schistosomiasis. PZQ is active against all five human *Schistosoma* species [2]. To be left with only one drug for schistosomiasis treatment is a very dangerous situation; especially that PZQ does not prevent reinfection, mainly in high transmission areas. Furthermore, there is increasing concern about the possible development of parasite resistance and tolerance against PZQ. Recent attempts are directed toward natural products to design novel drugs that avoid the side effects of the synthetic medications. These may be from plant extracts and even camel milk [3]. One of these plant extracts is artemisinin, derived from the herb; *Artemisia annua*. Artemether (ART) is the methylated

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derivative of artemisinin. In addition to the amazing antimalarial effect of ART, it showed anti-parasitic properties toward many protozoan parasites such as *Leishmania*, *Toxoplasma gondii* and *Trypansoma* spp. [4].

Metazoan parasites as, *Schistosoma* spp., *Echinostoma caproni*, the liver fluke *Opisthorchi sviverrini*, *Clonorchis sinensis* [5] and *Fasciola hepatica* [6] are also greatly susceptible to ART.

One of the great advantages of ART therapy is its prophylactic action. The prophylactic effect of ART is defined by its ability to eradicate the developing stages of schistosomula, so that the egg-laying mature female worms are not allowed to develop in the vasculature [7].

The aim of the present study was to evaluate the immunomodulatory effect of a single intramuscular ART dose (50 mg/kg) on the cytokine profile in experimental *Schistosoma mansoni* infection.

Material and methods

Animals

Laboratory bred female, Swiss albino mice *Mus musculus* (CD-1 strain), each weighing 18–20 g, were used in this study. Experimental animals were obtained from Schistosome Biological Supply Center (SBSP) at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Mice were kept for 8 weeks (experiment duration) in air-conditioned animal house at 20–22 °C and maintained on food containing 24% protein. Mice were also given carrot, lettuce and milk as source of vitamins. *Animal experiments were carried out according to the internationally valid guidelines and in an institution responsible for animal ethics.*

Parasites and infection

The Egyptian strain of *S. mansoni* cercariae was obtained from SBSP at TBRI. Infection was performed by subcutaneous injection (s.c.) of *S. mansoni* cercariae (80 ± 10/mouse) [8].

Drugs

PZQ, was obtained in the form of Tablets (600 mg/Tablet) (Distocide, Epico, Corporation, Cairo). The drug was freshly prepared and administered orally as a suspension in 2% Cremophor (Sigma) in a dose of 500 mg/kg/b.wt. for two consecutive days, 42 days postinfection (PI). **ART**, suspended in ground-nut oil, in the form of intramuscular (i.m.) ampoules (80 mg/ampoule) with documented purity of 99.6% was purchased from Kunming Pharmaceutical Corporation (Kunming, China). This preparation is stable at room temperature for 4 years [18]. The drug was administrated i.m. as a single dose of 50 mg/kg/b.wt. according to the experimental design [9].

Experimental design

The experimental groups are illustrated in Table 1. Mice were euthanized 8 weeks postinfection (PI) by decapitation. Then, the blood was collected individually in plastic tubes without anticoagulant. Blood was allowed to stand for 1 h at 37 °C, then overnight at 4 °C and centrifuged at 2500 rpm for 15 min. The serum was obtained and kept in aliquots at −20 °C for cytokine assessment.

Worm burden

Individual worm burdens were examined after perfusing the hepatic and portomesenteric vessels of each animal. Infected mice were perfused.

Histopathology and granuloma measurement

Liver sections were microscopically studied to evaluate the pathological changes including portal tracts and schistosomal granulomatous reactions. Pieces of mice livers were fixed in 10% phosphate-buffered formalin, pH 7.2, processed into Paraffin blocks. Transverse sections (5 μm in thickness) were taken, 5 sections from each liver, using a microtome (Bright 5030, UK). Each section was at a distance of at least 300 μm from the proceeding one. Sections were mounted on glass slides. Deparaffinization was performed by dipping slides in 100% xylene and descending ethanol series (100%, 95%, 80% and 70%) for rehydration. Sections were stained with Hematoxylin and Eosin (H&E) and Masson Trichrome.

Mean hepatic granuloma number (MGN) and diameter (MGD)

Measurements were taken only for granuloma containing single egg in the center using an ocular micrometer. The number of granuloma in 5 successive low power fields (10 × 10) was counted and recorded for MGN [10]. The MGD of each granuloma was calculated by measuring two diameters of the lesion at right angles to each other [11].

Cytokine assay

Levels of the cytokines IL-4, IL-10 and IFN-γ were measured in serum using sandwich ELISA. Briefly, plates (Nunc, Roskilde, Denmark) were coated with capture antibodies with 100 μl of serum sample or recombinant cytokine. Following addition of the biotinylated detection antibody and streptavidin–alkaline phosphatase conjugate, the reaction was developed with p-nitrophenyl phosphate (PNPP) (Sigma). Absorbance at 405 nm was measured with a Benchmark reader (Bio-Rad Laboratories Inc., Hercules, Calif.). Assays were performed in duplicates. The cytokine concentration was obtained from a regression curve prepared with the help of Microplate Manager Software (Bio-Rad). The results were expressed as pg/ml.

Statistical analysis

The data were presented as mean ± standard error of mean (mean ± S.E). Statistical analysis of results was carried out using one-way analysis of variance (ANOVA). Comparison between two groups was done by the Student's *t*-test. All statistical analysis was performed with the aid of the SPSS computer program (version 13.0 Windows). The data were considered significant if ($P < 0.05$), highly significant if ($P < 0.01$) and very highly significant if ($P < 0.001$). Percent