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In vitro effect of artemether and triclabendazole on adult *Fasciola gigantica*

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ABSTRACT

Triclabendazole "Fasinex®" is the drug of choice against fasciolosis because of its high efficacy against both mature and immature flukes, however parasite resistance against this drug is increasing. Hence, there is pressing need for new fasciolicidal drugs. In the present study, the in vitro effect of artemether on adult flukes was evaluated using scanning electron microscopy. After 24 h incubation with 10 μg/ml artemether, the tegument of the apical cone appeared to be slightly more swollen than normal. This swelling became so severe and the spines appeared sunken, with their tips protruding from a swollen and blebbed base, on increasing the concentration to 20 μg/ml. With the higher concentration of 30 μg/ml, extensive and severe tegumental swelling occurred in the apical cone region of the flukes. There were many blebs around ventral sucker, a number of which appeared to have burst causing lesion. The tegumental changes occurred following incubation in artemether were comparable with those observed with triclabendazole in its active sulphoxide metabolite form (TCBZ-SX).

1. Introduction

Fasciola gigantica is a trematode parasite closely related to *Fasciola hepatica*. It occurs in cattle and sheep in almost all tropical and subtropical regions of the world and is the causative agent of tropical fasciolosis. In Egypt, it is an important helminth parasite of livestock and is emerging as an important production and zoonotic disease. Direct and indirect losses ascribed to fasciolosis of all fluke species were estimated at 484.5 millions LE per year, according to the report of Central Organization of Mobilization and Computation, Cairo (2000). According to some estimates every year about 600 million domestic animals become infected worldwide. In the USA alone the economical losses due to *F. hepatica* were determined at over $ 2 billion (Schweizer et al., 2005).

At present there is no commercial vaccine available for the prevention of fasciolosis (McManus and Dalton, 2006) and hence its control based largely on chemotherapy. A benzimidazole derivative, triclabendazole (TCBZ) is the drug of choice as it is safe and efficacious against both juvenile and adult flukes (Fairweather and Boray, 1999a) and has been marketed since 1983 as a veterinary drug (Fasinex®) (Keiser and Utzinger, 2005). The sulphoxide metabolite of triclabendazole (TCBZ-SX) is the active form of this drug (Hennessy et al., 1987). Recent studies confirmed the occurrence of triclabendazole resistance in sheep and cattle from different parts of the world as e.g., Australia, Ireland, The Netherlands, Spain and the UK (Alvarez-Sanchez et al., 2006; Keiser et al., 2005). There are no drugs of comparable activity currently available for the treatment and control of fasciolosis, hence there is a pressing need for new trematocidal drugs.

Artemether is a semi-synthetic derivative of artemisinin and the most widely used antimalarial (Haynes, 2006).

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Artemisinin is extracted from the leaves of *Artemisia annua*, an herbal plant used for centuries in Chinese traditional medicine (Klayman, 1985). In 1982, Chinese parasitologists discovered that administration of artemether (methyl ether derivative of dihydroartemisinin) to mice infected with *Schistosoma japonicum* resulted in significant reductions of the schistosome worm-burden (Le et al., 1982). High worm-burden reductions were obtained with this drug in rodents with acute or chronic infections of *S. japonicum*, *S. mansoni*, *Clonorchis sinensis*, *Opisthorchis viverrini*, and *F. hepatica* (Utzinger and Keiser, 2007). Administration of artemether at single 200 mg/kg oral dose resulted in worm-burden reductions of 100% against both juvenile and adult *F. hepatica* (Keiser et al., 2006).

The tegument is one of the main absorptive surfaces for the uptake of drugs by the fluke. The adult fluke resident in the bile duct is bathed in bile and will be exposed to drugs in the flow of bile as the drug is excreted by the host. Consequently, the tegument is one of the tissues most immediately exposed to anthelmintics and is likely to represent a primary drug target (McKinstry et al., 2003). Scanning electron microscopy (SEM) has proved to be a useful tool for evaluating surface changes on the tegument and suckers of trematodes resulting from anthelmintic action (Fairweather and Boray, 1999b). Thus, a scanning electron microscopic study was undertaken to evaluate changes in attachment organs and tegument of adult *F. gigantica* following exposure to artemether and TCBZ-SX (Fasinex®, Ciba-Geigy) in vitro.

2. Materials and methods

2.1. Drugs

Artemether was obtained from Kunming Pharmaceutical cooperation (Kunming, PR China). Triclabendazole "Fasinex®" was purchased from Ciba-Geigy Company.

2.2. Effect of artemether and triclabendazole on adult flukes

Adult worms of *F. gigantica* were recovered from the bile ducts of buffalo slaughtered in a Cairo abattoir. Under sterile conditions in a laminar flow cabinet, flukes were washed in several changes of warm (37 °C), sterile complete RPMI 1640 culture medium containing antibiotics (penicillin, 50 IU/ml; streptomycin, 50 μg/ml). The flukes were subsequently transferred to fresh culture medium containing 50% (v/v) heat denatured rabbit serum, 2% (v/v) rabbit red blood cells; as recommended by Ibarra and Jenkins (1984), and artemether at three different concentrations 10, 20 and 30 μg/ml. Dilutions were made from a stock solution of artemether at 10 mg/ml, prepared with 60% (v/v) dimethyl sulphoxide (DMSO). The whole flukes were incubated for 24 h at 37 °C in an atmosphere of 5% CO_2. A positive control group was prepared by incubating whole flukes for 24 h in RPMI culture medium containing either 10 or 20 μg/ml TCBZ-SX. This level corresponded to maximum blood levels in vivo (Sanyal, 1995). The TCBZ was initially prepared as a stock solution in DMSO and added to the culture medium to give a maximum solvent concentration of 0.1% (v/v). Solvent control flukes were incubated for 24 h in RPMI 1640 culture medium containing 0.1% (v/v) DMSO. Normal control flukes were fixed immediately following the initial washing. Six flukes were examined for each concentration.

2.3. Scanning electron microscopy

Following incubation, the oral cone (including ventral sucker) of adult flukes was fixed intact for 12 h in a 3:1 mixture of 4% (w/v) glutaraldehyde in 0.12 M-Millonig's buffer, pH 7.4 and 1% aqueous osmium tetroxide. The specimens were washed repeatedly in double-distilled water, dehydrated through acetone, critical point dried in carbon dioxide, fixed to aluminum stubs and coated with gold–palladium. The specimens were viewed in a Jeol scanning electron microscope (Jeol Corp., Mitaka, Japan) operated at 15 kV.

2.4. Measurements technique

Measurements of the apical cone of control flukes and the two groups of treated flukes were made according to method proposed by Valero et al. (1996), using a computer image analysis system (ELICA QWin 500, Cambridge, England). The measurements included lineal biometric characters; cone length and width, maximum diameter of oral and ventral suckers, spine length and width as well as area of tegumental swelling around the ventral sucker.

3. Results

3.1. SEM of normal fresh and control flukes

SEM of the apical cone surface of the normal fresh fluke revealed smooth oral and ventral suckers with thick rims covered with transverse folds. The gonopore was situated two-thirds of the way back between the oral and ventral sucker (Fig. 1). The entire surface of the tegument, with the exception of the rims of both suckers, was seen to be covered in spines (Fig. 2). The size and shape of these spines were dependent on the area of the fluke on which they were present. Spines were larger on the anterior and anterior mid-body regions than on the posterior mid-body and tail regions. The spines from the anterior regions also differed in shape, having finger-like protrusion at their tips (Fig. 3). The tegumental surface of the fluke between the spines was seen to be made up of a meshwork of tiny ridges and pits (Fig. 2); this meshwork was present on all areas of the fluke, except the rims of both suckers. No significant differences were observed between fresh fluke and control fluke incubated for 24 h in solvent; 0.1% (v/v) DMSO (Figs. 4–6).

3.2. Effects of artemether

After 24 h incubation with 10 μg/ml artemether, the apical cone region appeared to be slightly more swollen than normal (Fig. 7). The swelling was more prominent on the ventral surface; around the ventral sucker and directly behind it (Fig. 8). Swelling was also present towards the lateral margins of the fluke. At higher magnification, the