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A single dose of the antineoplastics hydroxyurea or cisplatin has praziquantel-like effects on *Schistosoma mansoni* worms and host mouse liver

Eman Eldeeb, Shereen Fahmy, Kadry Elbakry, Ayman Hyder*

Zoology Department, Faculty of Science, Damietta University, New Damietta 34517, Egypt

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ABSTRACT

Increasing resistance to praziquantel, the only available antischistosomal drug, is always developed by schistosomes. The recent description of stem cell-like neoblasts in schistosomes led to the idea of applying antineoplastic drugs as antischistosomal agents that may inhibit stem cell divisions and retard worm regeneration. Here, we explored the *in vitro* and *in vivo* effect of some antineoplastic drugs on *S. mansoni* worm and the host mouse liver. *S. mansoni* worms' viability was tested after exposure to either praziquantel or one of the antitumor drugs (hydroxyurea, cisplatin, methotrexate, and colchicine) *in vitro* for 24 and 48 h. The effect of two of them (hydroxyurea and cisplatin) on worm burden, tegument ultrastructure, and host liver structure and function was tested *in vivo* in *S. mansoni*-infected mouse model. All drugs affected variably the worm burden *in vitro*. Hydroxyurea and cisplatin, like praziquantel, damaged the worm tegument, reduced worm burden, and viable schistosome eggs, decreased anti-schistosome IgG, reduced egg-induced hepatic granuloma size and cellularity, restored liver organization and improved liver function as represented by serum alanine aminotransferase and albumin. In conclusions, a single dose of hydroxyurea and cisplatin had anti-schistosome effects and may offer a safe promising alternative to control of schistosomiasis. A direct link between antitumor drugs and inhibition of schistosome neoblasts remains to be proven.

1. Introduction

After vigorous control activities of the Egyptian government and the lower rate of prevalence of Schistosoma, the follow-up outcome was not as positive as imagined before. The prevalence of schistosomiasis in one recent study [1] was found to be 29% in some villages, with infection peaked to 50.8% in the young age group. *Schistosoma mansoni* was detected in 31 districts out of 35 in five governorates in the Nile Delta [2]. Internationally, the situation is not better. Approximately 779 million people live at risk of infection, and 230 million are infected [3]. Schistosomiasis is killing about 280,000 people every year in Africa and represents the second most common, after malaria, parasitic disease [4]. Thus, after 40 years of continuous treatment with Praziquantel, the only available anti-schistosomal medication, transmission of Schistosoma remains continuous and uninterrupted. In fact, the presence of only one drug against schistosomiasis is completely unsatisfactory, especially if it is known that immature worms survive praziquantel [5] and that emerging resistance to praziquantel is always developed by schistosomes [6], so that the treatment fails as reported in many instances [7]. This failure has been shown in Senegalese, Kenyan and Egyptian schistosoma-infected patients [8–10]. This inefficacy of praziquantel calls continuous efforts to develop alternative anti-schistosome drugs.

The blood-dwelling fluke of the genus Schistosoma can survive and lay eggs for decades in human. Adult schistosomes could modulate their growth, as a response to host immune signals [11], and could regenerate damaged tissues after exposure to sub-lethal doses of praziquantel [12]. These experiments revealed the flexibility and plasticity of schistosomes. In fact, the longevity of schistosomes, despite time and host immune attacks, reflects a powerful regenerative system. This regeneration may occur as a result of proliferation and differentiation of stem cells.

Recently, Collins et al. [13] described proliferating cells scattered throughout the parasite body that are akin to the previously described stem-cell-like neoblasts in planarians. It seems that these stem cells are essential to the longevity of Schistosoma in the host [14]. Antineoplastic drugs are drugs that prevent or inhibit cell maturation and proliferation. Because stem cells are always proliferating for self-renewal or differentiation, then antineoplastic drugs may represent a powerful defense against them. An antineoplastic agent may be an alternative drug against Schistosomiasis, targeting stem cell proliferation and differentiation in schistosome worms, and preventing the

* Corresponding author.
E-mail address: hyder@du.edu.eg (A. Hyder).

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regeneration of the parasite. Prevention of regeneration will expose the worm to the host attacks. This may lead to the eradication of the worm.

It was the aim of the present study to investigate whether antineoplastic drugs have a damaging effect on schistosome worms *in vitro* and *in vivo* in infected mice and to compare this effect to that of praziquantel.

2. Material and methods

2.1. In vitro culture of Schistosoma mansoni worms

All *in vitro* studies were conducted in sterile conditions under the laminar flow hood in tissue culture lab. Different antineoplastic drugs, in addition to Praziquantel, were added to DMEM culture medium (HyClone) supplemented with 10% fetal bovine serum (FBS, HyClone), 100 U/ml penicillin, 40 µg/ml Gentamicin sulfate, and 40 µg/ml Fluconazole. *In vitro* experiments were performed in 24-well flat-bottom culture plates. All drugs were prepared in different concentrations ranging from 1 to 100 µg/ml for Praziquantel, 10 to 200 µg/ml for Cisplatin, 1 to 100 µg/ml for Hydroxyurea, 0.01 to 100 µmol/L for Methotrexate, and 0.1 to 0.5 µmol/L for Colchicine. Collected worms were incubated at 37 °C, and 5% CO2, and scored for viability 24 and 48 h post incubation.

2.2. Animals

All animals received humane care. Study protocols comply with the institution's guidelines and animal research laws, and animal experiments have been conducted in accordance with the internationally valid ethical conditions and guidelines. The study protocols comply with Damietta University institution's guidelines. Female CD1 Swiss albino mice (Weight 20 g ± 2 g) bred and maintained at the Schistosome Biological Supply Center (SBSC), Theodor Bilharz Research Institute (TBRI), Egypt, were used in this study. The procedure of mice infection by subcutaneous injection of schistosomal cercariae were performed also in SBSC at TBRI. Every mouse was injected subcutaneously with 100 ± 10 *Schistosoma mansoni* cercariae as described by Oliver & Stirewalt [15].

2.3. Animal groups

Mice were maintained on normal chow and drinking water, *ad libitum*, throughout experiments, and divided into 5 groups: 1) normal mice, non-infected negative control group (C−); 2) *S. mansoni* cercaria -infected mice, non-treated, positive control group (C+); 3) Praziquantel (PZQ)-treated *S. mansoni*-infected mice, in which animals were orally administered 0.1 ml of PZQ (600 mg/kg body weight) as one dose on day 42 post infection [16]; 4) Cisplatin (CP)-treated *S. mansoni*-infected mice, in which animals were i.p injected with 8 mg/kg/day Cisplatin for 3 days started on day 42 post infection. [17]; and 5) Hydroxyurea (HU)-treated *S. mansoni*-infected mice, in which infected mice were i.p injected with 100 mg/kg/day Hydroxyurea for 3 days. Injections started on day 42 post infection with *S. mansoni* [18]. Two weeks after the last dose, perfusion of the hepatic portal vein was performed in different groups to collect worms.

2.4. Parasite acquisition by hepatic portal vein perfusion

Hepatic portal vein perfusion infected mice was carried out to obtain adult *Schistosoma mansoni* worms. This process was performed 6–7 weeks post-infection for *in vitro* experiments and Two weeks after the last dose in *in vivo* experiments. The method of perfusion was carried out according to Smithers & Terry [19]. The infected mouse was anesthetized and dipped after two minutes in water and fixed on the perfusion plate from its limbs. Abdomen and diaphragm were cut by scissors and forceps to expose the internal organs and blood vessels. The 21-gauge perfusion needle has been inserted for a distance of about 1 cm through the chest ribs into the ascending aorta. The blood vessels and organs were washed with the citrate buffer saline (0.85% NaCl and 1.5% Na-Citrate). The citrate buffer was used also for the perfusion process. A small cut was made in the hepatic portal vein to collect the perfusate. The citrate buffer saline perfusate, together with the blood containing worms, has been collected. Worms were collected, washed, counted, examined and processed for histology and ultrastructural examinations.

2.5. Mice intestinal schistosome egg picture

Mice intestines were analyzed for the presence and morphology of Schistosome eggs. Number and percentages of live mature, dead and immature eggs were calculated in the small intestine of mice of all infected groups. In each intestinal segment, the calculations were performed in a total of 25 eggs/segment. Eggs were classified according to Pellegrino et al. [20] as follows: (1) Viable immature (1st–4th stages) and mature eggs; (2) Dead immature (eggs with retracted embryo, semitransparent, darkened, or granular eggs) and dead mature eggs (calcified eggs, those with disintegrating miracidia, or eggs with retracted miracidia); (3) Shells and (4) Granulomas

2.6. Measurement of egg-induced hepatic granuloma size

Livers from host mice of different groups have been processed for histological examination. Granuloma size was studied according to Ohmae et al. [21] in livers stained with hematoxylin and eosin stain. The diameter of a single liver egg granuloma in liver sections of each mouse was light microscopically measured. Aggregated granuloma were not considered for this measurement. Percentage of change between control and treated groups was calculated according to the following equation:

$$percentage\ of\ change = \frac{Test\ \text{measurement} - Control\ mean}{Control\ mean} \times 100$$

2.7. Biochemical analysis

Albumin, alanine aminotransferase (ALT) and antischistosomal IgG were tested in mice serum of each group using commercial kits from local suppliers.

2.8. Statistical analysis

Values were expressed as arithmetic mean ± SEM. The difference between different groups was estimated by ANOVA. Whenever ANOVA gave significance, Student's *t*-test was applied as a *post hoc* to differentiate between every two groups. A p-value of $< .05$ between two groups was considered as significant.

3. Results

3.1. Antineoplastic drugs reduce the viability of S. mansoni worms in vitro

First, we thought to conduct some *in vitro* experimental trials to determine the effect of some anti-cancer drugs (Cisplatin, Methotrexate, Hydroxyurea, and Colchicine) on *S. mansoni* worm burden. For this purpose, freshly excised normal worms were cultured at 37 °C and 5% CO_2 in DMEM supplemented with 10% FCS and the necessary antibiotics and antifungal agents. Also, we applied a wide range of concentrations of different drugs. A group of worms was treated similarly with a wide range of concentrations of praziquantel to observe the similarity/dissimilarity trend between praziquantel and other antineoplastic drugs. The numbers of viable worms were recorded after 24 and 48 h of culture, and the survival rate of the *schistosoma mansoni*