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Fasciola Hepatica Extract Induces Cell Death of Mammalian Cells

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Abstract

Background—Fascioliasis is a neglected tropical disease that affects poor people from poor and developing countries. In the world, it has been estimated that at least 2.6 million people are affected with this disease. The International agency for Research on Cancer, states that *O. viverrini* and *C. sinensis*, also liver flukes, are considered as definitive causes of cholangiocarcinoma. However, fascioliasis caused by *F. hepatica* has not been associated with cancer to date. There are not any known causative associations between this parasite and liver cancer (cholangiocarcinoma).

Methods—Chine Hamster Ovary (CHO) cells were treated with *F. hepatica* extracts and cell proliferation was assessed by using the indirect method for estimating cell number based on the mitochondrial activity with MTS cell proliferation reagent. We observed unexpected death of these cells when treated with *F. hepatica* extracts.

Results—We now hypothesize that this parasite could be used as a medically-important trematode pathogen in cancer therapy.

Keywords

Fasciola hepatica; mammalian cells; cholangiocarcinoma; *O. viverrini*; *C. sinensis*; Chine Hamster Ovary (CHO)

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This article does not contain any studies with human participants or animals performed by any of the authors.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

This article does not contain any individual person's data in any form. "Not applicable" in this section.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

1. INTRODUCTION

Fascioliasis is a neglected tropical disease that affects poor people from poor and developing countries [1, 2]. In the world, it has been estimated that at least 2.6 million people are affected with this disease [3]. This parasitosis is very common. Almost one-third of world population has been diagnosed with this infection. Diagnosis is currently performed through the use of high-sensitive serological and parasitological tools. Although most patients are asymptomatic, patients with acute infection normally present fever and abdominal pain, while patients with chronic infection present biliary colic, cholecystitis and cholangitis [4]. Conversely, there is a limited number of studies to evaluate the natural history of patients affected with fascioliasis to assert chronic inflammation, liver fibrosis stages and carcinogenesis. Also, in patients who were treated there is a lack of studies relating to post-infectious liver damage. Therefore, the long- term effects of fascioliasis are unknown. According to the International Agency for Research on Cancer (IARC), two other liver flukes (*O. viverrini* and *C. sinensis*) have been recognized as definitive causes of cancer [5]. However, fascioliasis caused by *F. hepatica* has not been associated with cancer to date.

While performing several experiments with parasite extracts using cell lines, we observed unexpected death of these cells when treated with *F. hepatica* extracts. Therefore, to assert the anti-cancer potential of *F. hepatica* we now hypothesize that this parasite could be used as a medically-important trematode pathogen.

2. MATERIAL AND METHODS

2.1. *F. hepatica* and *S. haematobium* Extract Production

Fasciola hepatica worms were collected from the livers of infected bovines in slaughter houses.

S. haematobium adult worms were collected by perfusion of the hepatic portal system of golden hamsters and balb/c mice respectively, at 7 weeks after infection with 100 cercarie.

The worms were suspended in PBS and then sonicated. The protein extract was then ultracentrifuged and the protein concentration was estimated using a micro BCA protein assay reagent kit [6, 7]

2.2. Animals and Experimental Infections

Ten golden hamsters were experimentally infected with 100 cercariae of *S. haematobium*. Hamsters were infected by member's extremities. The control animals consisted of 10 littermates. The cercariae were obtained by shedding of snails infected with miracidia.

2.3. Cell Lines

CHO cells were cultured and maintained at 37 °C in a 5% CO2 humidified atmosphere in CHO medium (Sigma) with 10% FBS and 1% penicillin/streptomycin (Sigma). Cells were passaged every 5 days. Before treatments cells were serum-starved for 16 h [6, 7]

2.4. Proliferation Assay

The CellTiter 96 AQ non-radioactive cell proliferation assay (Promega) was used to assess cell viability. The assay is composed of the tetrazolium compound MTS and an electron coupling reagent, PMS. MTS is reduced by viable cells to formazan, which can be measured with a spectrophotometer based on the amount of absorbance at 490 nm. Formazan production is time-dependent and proportional to the number of viable cells. CHO cells were cultured in 0.1 ml CHO media in 96-well flat-bottomed plates. Cultures were seeded at 1 000 cells/well and allowed to attach overnight. After the indicated time of incubation with the appropriate medium, 20 microl reagent was added per well, and cells were incubated for 1 h before measuring absorbance at 490 nm. Background absorbance from the control wells was subtracted. Studies were performed in triplicate for each experimental condition [6, 7].

2.5. Trypan Blue Exclusion Assay

The trypan blue exclusion method was used to assess cell viability. CHO cells were plated onto 24-well plates at approximately 1000 cells per well and incubated for 24 h. The cells were treated with *F. hepatica* and *S. haematobium* extracts at 50 microg/ml for 24 h. On days 0–4 after treatment, the cells were harvested by trypsinization and counted under a microscope after trypan blue staining. Three independent experiments were carried out based on the following formula: cell viability % = number of cells in drug treatment group/ number of cells in control group 100%.

2.6. Statistical Analysis

All experiments were done in triplicate and Standard Deviation was calculated for all experiments.

3. RESULTS

To begin investigating the effect of *F. hepatica* on cell viability and proliferation, CHO cells were seeded on 96-well plates, starved overnight, treated with increasing concentrations of *F. hepatica* extracts for 24 h, cultivated for 24, 48 and 72 h and then analyzed by MTS assay (Fig. 1). We compared the effects of *F. hepatica* extracts with *S. haematobium* extracts that increase proliferation of these cells as our group has demonstrated previously [6, 7]. The growth curve shows that treated cells show no growth while control cells and cells treated with *S. haematobium* extracts proliferate significantly. To evaluate the effect of *F. hepatica* on cell viability we used a trypan blue exclusion assay. We also used *S. haematobium* extracts to compare these results. Cell numbers were assessed by direct daily counting (Fig. 2).

4. DISCUSSION

Although brief we believe that our results could prompt other researchers to investigate if this parasite hinders cancer, focusing on phenotypic effects of this parasite extracts on informative cancer cell lines, e.g., cholangiocarcinoma cell lines. Therefore, this work addresses the possible anti-cancer role of *F. hepatica* extracts. Fascioliasis is a food borne

disease caused by infection with a fluke termed *Fasciola hepatica*. There are not any known causative associations between this parasite and liver cancer (cholangiocarcinoma).

While performing several experiments with parasite extracts using cell lines, we observed unexpected death of these cells when treated with *F. hepatica* extracts. Our results clearly show that extracts of *F. hepatica* induce cell death of CHO cells in culture. This effect seems to be specific since extracts of *S. haematobium* produce the opposite effect increasing proliferation of CHO cells.

Although there are not any reports in the literature addressing the effect of *F. hepatica* extracts on proliferation of mammalian cells in culture, the induced cell death in culture by *F. hepatica* is not surprising. Tsocheva *et al.* [8] isolated biologically active substances (BAS) from the tissues of *Fasciola hepatica*. These authors found a marked inhibiting effect of the BAS on hepatoma MC29 cell line proliferation and a slight inhibiting effect of the BAS on myeloma cell culture proliferation [8]. Several authors have attempted and succeeded in demonstrating the suppressive effects of *Fasciola* excretory/secretory extracts on lympho-proliferation. For example, Sharaf *et al*. [9] demonstrated that these extracts had significant suppressive effects on lympho-proliferation, up to 74%. Also, other team of researchers [10] demonstrated that rats infected with *F. hepatica* significantly decreased proliferation of spleen mononuclear cells. These authors hypothesize that a mechanism to avoid an immune response during the first stages of liver penetration could explain the suppression observed in spleen proliferative responses.

In the light of the results presented here, we demonstrate for the first time that *F. hepatica* extract induces cell death of mammalian cells. Further studies are necessary to identify and characterize this effect. We propose in the future to investigate if this parasite hinders cancer, focusing on phenotypic effects of this parasite extracts on informative cancer cell lines, e.g., cholangiocarcinoma cell lines. Therefore, to assert the anti-cancer potential of *F. hepatica* we now hypothesize that this parasite could be used as a medically-important tramatode pathogen.

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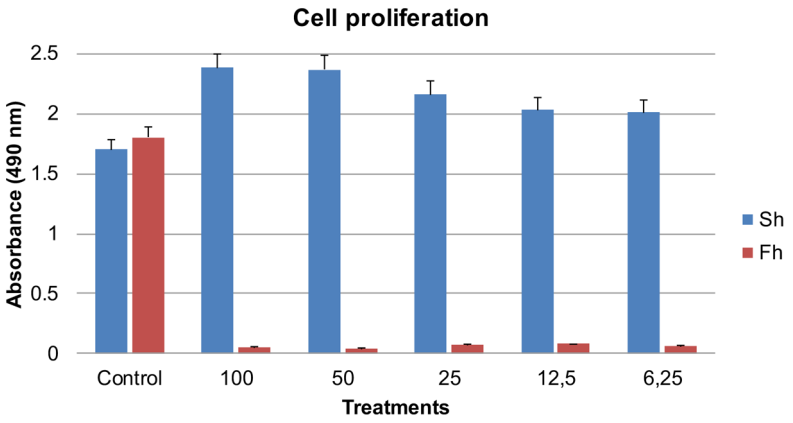

Fig. 1.

Cell proliferation assay of *Fasciola hepatica* and *Schistosoma haematobium* extracts-treated cells. The growth curve shows that treated cells with *F. hepatica* showed no growth while cells treated with *S. haematobium* proliferated significantly faster and more than control cells. Experiments were done in triplicate and the bars show standard deviation.

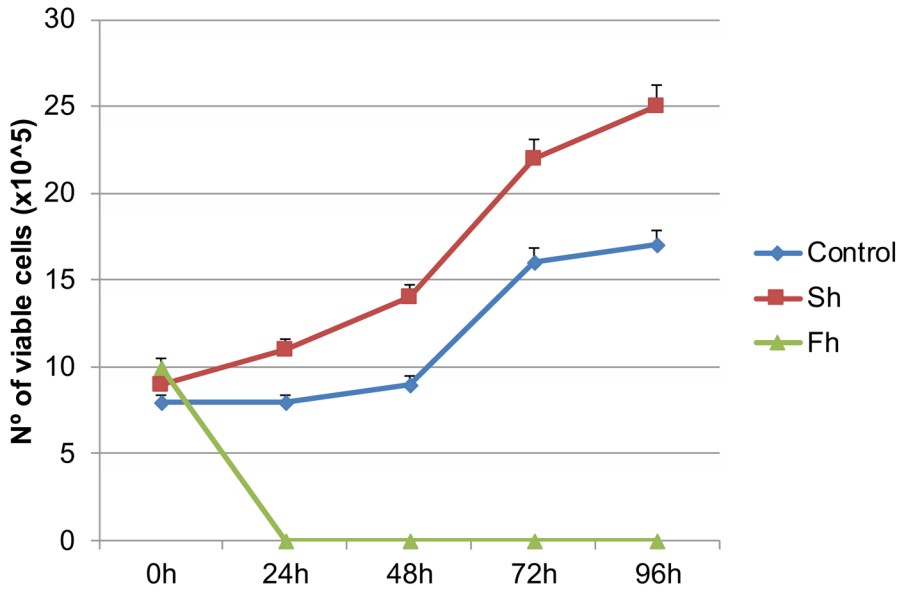

Fig. 2.

Trypan exclusion assay of control and *F. hepatica* and *S. haematobium*-treated cells. We used the concentration of 50 microg/ml of *F. hepatica* and *S. haematobium* extracts. We confirmed the induced cell death of CHO cells when treated with *F. hepatica* extracts and increase in cell viability when treated with *S. haematobium* extracts by trypan exclusion assay. Cell numbers were assessed by direct daily counting. The experiments were done in triplicate and the curve shows standard deviation.