



Research Article

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Effect of Different Hydatid Cyst Molecules on Hela and Vero Cell Lines Growth *In vitro*

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Abstract

Background: Hydatid cyst is the larval stage of the tapeworm, *Echinococcus granulosus*. The prevalence of hydatid cyst in patients with cancer was lower than that of normal population. We previously showed that alive hydatid cyst protoscolices effected the growth of cancer cells in culture medium. In this work the effect of different hydatid cyst molecules on growth of cancer cells has been investigated *in vitro*.

Method: Crude protoscolices molecules, protoscolices excretory secretory (ES) molecules, laminated & germinal layers molecules and hydatid fluid molecules were prepared from cysts collected from slaughter house. These molecules were added to Hella or Vero cell lines. Following 48 hours incubation, the number of alive and dead cells were counted in comparison with appropriate controls. Jonckheere-Terpstra Test used for statistical analysis.

Results: In cell cultures treated with protoscolices ES molecules or hydatid fluid molecules the number of alive Hella cells decreased in comparison with control cell culture. Also in cell cultures treated with crude protoscolices molecules, protoscolices ES molecules or Laminated & germinal layers molecules the number of dead Hella cells increased. However with Vero cell lines, none of the molecules increased the number of dead cells or decreases the number of alive cells.

Conclusion: Hydatid cyst antigens effected Hella cells growth in culture medium. Further work is recommended to clarify the mechanisms of action.

Keywords

Hydatid cyst molecules; Cancer; Hella cells

Introduction

Hydatid cyst is the larval stage of the tapeworm, *Echinococcus granulosus*, a parasite responsible for hydatid disease or hydatidosis in human and livestock. This disease considered as one of the most important cosmopolitan zoonotic infection with different mammalian hosts being involved in the life cycle. It has been shown that in a large retrospective study of patients with cancer, the prevalence of hydatid cyst was significantly lower than in normal subjects [1]. Antitumor activity of some other parasites have also been demonstrated. For example anticancer activity of parasites such as *Trypanosoma cruzi*

[2-8] *Toxoplasma gondii* [9-15], *Toxocara canis* [16], *Acanthamoeba castellanii* [17,18] and *Plasmodium yoelii* [19] have been shown in experimental animals. *In vitro* investigations also revealed that some parasites such as *Trypanosoma cruzi*, hydatid cyst protoscolices, and *Toxoplasma gondii* show anticancer activities [7,16,20,21]. We previously showed that alive hydatid cyst protoscolices inhibited the proliferation of WEHI-164 and BHK cells and have the capacity to induce cell death in WEHI-164 cells *in vitro* [21]. In this work the effect of different hydatid cyst molecules including crude protoscolices molecules, excretory secretory molecules of protoscolices, Laminated & germinal layers molecules and hydatid fluid molecules on Hella and Vero cell lines growth *in vitro* have been investigated.

Materials and Methods

In this experimental study, *Echinococcus granulosus* hydatid cysts were collected from sheep or cattle from a slaughter house in Isfahan, Iran. Hydatid fluid were aspirated, collected and examined for presence of protoscolices. The fluids were then centrifuged at 2000×g, for 2 min, and the supernatant was concentrated and kept at -20 as hydatid fluid molecules. The sediment which was compact protoscolices washed with isotonic saline, sonicated and kept at -20 as crude protoscolice molecules. Also culture medium was added to compact alive protoscolices, and following 48 hours incubation centrifuged at 2000×g, for 2 min and the supernatant used as excretory secretory molecules of protoscolices (ES molecules). Laminated & germinal layers were removed from the cyst, homogenized and then sonicated and kept at -20 as Laminated & germinal layers molecules.

Culturing of tumor cells

Two cell lines including Hella and Vero cells were purchased from the Pasture Institute (Tehran, Iran). Cells were cultured as we performed before [21]. In each experiment, two culture flasks containing 10 mL culture medium with 100000 freshly prepared and viable Hella or Vero cells were used. The first flasks were treated with 500 µl of different molecules containing 5-10mg protein, while the second flasks were treated with 500 µl isotonic saline as control sample. All flasks were incubated in CO₂ incubator for 48h and then cell counting was determined for each flask after 48 h. Cell counting was performed using a Neubauer's chamber. Each experiment was performed in triplicate. In this study to evaluate the effect of different molecules on cancer cells, two criteria including increasing the number of dead cells or decreasing the numbers of alive cells were used. Trypan blue staining was used to discriminate between dead and alive cells. Jonckheere-Terpstra Test used for statistical analysis of the data.

Results

When crude extract of protoscolices was added to Hella cells a significant difference in number of dead cells between case and control groups was achieved (P=0.01). However the difference for alive cells was not significant (Figure 1). When excretory secretory molecules of protoscolices was used a significant difference was observed for both alive (P=0.014) and dead cells (P=0.02) (Figure 2). With the hydatid cyst fluid molecules the difference for alive cells was significant (P=0.031) and for dead cells was not significant (Figure 3). Finally

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when crude mixture of homogenized laminated & germinal layers was added a significant difference ($P=0.01$) was observed for dead cells. However the difference in alive cells was not significant (Figure 4). When different molecules incubated with Vera cells no significant effects on growth of those cells was detected.

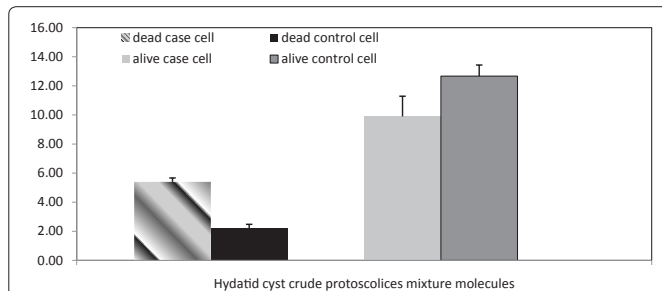


Figure 1: Mean of alive or dead cells in flasks of Hella cells treated with hydatid cyst crude protoscolices crude antigens (case groups) or isotonic saline (control group).

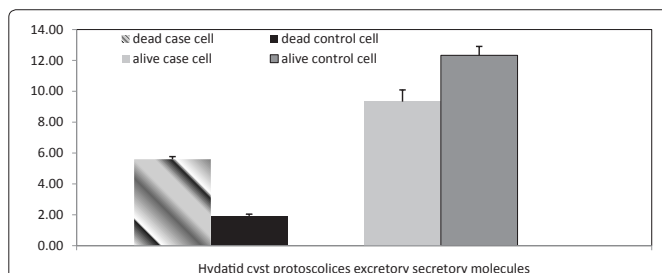


Figure 2: Mean of alive or dead cells in flasks of Hella cells treated with Excretory secretory antigen of hydatid cyst protoscolices (case groups) or isotonic saline (control group).

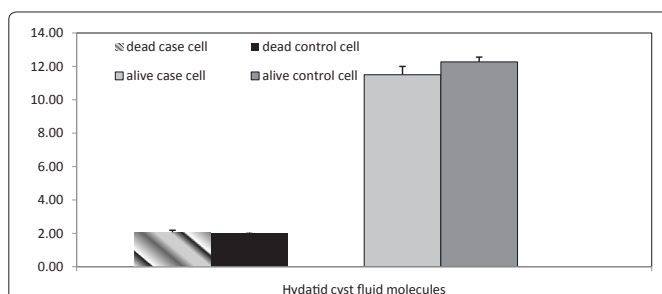


Figure 3: Mean of alive or dead cells in flasks of Hella cells treated with hydatid cyst fluid (case groups) or isotonic saline (control group).

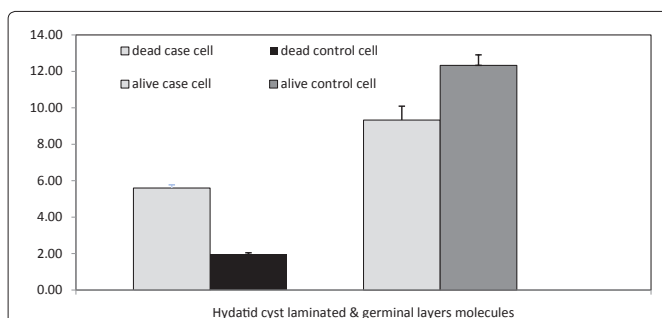


Figure 4: Mean of alive or dead cells in flasks of Hella cells treated with hydatid cyst laminated & germinal layers antigen (case groups) or isotonic saline (control group).

Discussion

Results of this investigation revealed that in cell cultures treated with crude protoscolices molecules, protoscolices ES molecules or Laminated & germinal layers molecules the number of dead Hela cells increased in comparison with control cell culture. Also in cell cultures treated with protoscolices ES molecules or hydatid fluid molecules the number of alive Hela cells decreased in comparison with control cell culture. However none of the molecules increased Vera dead cells or increased Vera alive cells

The results about Hela cells are consistent with our previous finding that Protoscolices of hydatid cyst induced cell death in WEHI-164 Fibrosarcoma cells and also inhibited the proliferation of baby hamster kidney fibroblasts in culture medium [21]. The effects of parasite antigens on inhibition of cancer cells have also been shown in other investigations. Atayde et al. showed that a *Trypanosoma cruzi* surface molecule gp82 recombinant protein induced cell death in melanoma cells [22]. In other investigation it has been shown that some parasitic antigens have stimulatory or inhibitory effects on certain cell lines. However some other antigen showed no effect on proliferation or death of cells in culture medium.

Rigano et al. investigated the effect of hydatid cyst fluid or antigen B on Human Dendritic Cell differentiation. They showed that Antigen B and hydatid cyst fluid upregulated CD86 expression and downmodulated CD1a expression [23]. Kanan and Chain explored the effect of hydatid cyst fluid on differentiation of human monocyte to dendritic cell. Their results showed that hydatid cyst fluid modulated differentiation of dendritic cells. The presence of hydatid cyst fluid also increased CD14 expression and decreased expression of CD1a [24]. Nono et al. showed that Excretory/Secretory-Products of *Echinococcus multilocularis* Larvae Induce apoptosis in dendritic cells *in vitro* [25]. Results of these investigations are in agreement with our finding indicating that hydatid cyst molecules are able to effect cells growth in culture medium.

Apart from *Echinococcus* antigens, effect of some other parasite antigens on different cell lines has also been investigated. Huby et al. showed that ES products of the intestinal nematode, *Nematodirus battus*, decreased the number of epithelial cells in culture medium. Inversely ES products of two other parasites stimulated cell growth [26]. In another study Huby et al. investigated effects of the excretory/secretory products of *Trichostrongylus colubriformis* on the growth of different cell lines. These products increased cell numbers of three epithelial intestinal cells. In contrast, the products inhibited the proliferation of epithelial ovarian cells and fibroblasts. Finally no effect was detected on the cell growth of hepatocytes [27]. Semnani et al. showed that *Brugia malayi* microfilariae induce cell death in dendritic cells [28]. These investigation indicate that in addition to hydatid cyst molecules, other parasite molecules also effect cells growth *in vitro*.

Results of our investigation showed that hydatid cyst molecules affected tumor cell growth either by increasing dead cells or by decreasing alive cells. From these results along with other findings about the effect of parasite antigens on cell growth it can be inferred that parasite antigens interfere with different cell lines growth *in vitro*. However the mechanisms that may be involved remain concealed and further work is recommended to discover these mechanisms.

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