Effect of two hydatid cyst antigens on the growth of melanoma cancer in C57/black mice

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Abstract Hydatid cyst is the larval stage of *Echinococcus granulosus*. In previous studies inhibitory effect of this parasite on cancer cell growth in culture medium has been shown. In this study effect of hydatid cyst antigens on tumor growth in experimental animals has been investigated. Two antigens of hydatid cyst including protoscolices excretory secretory antigen and hydatid fluid absorbed on alum as adjuvant were injected to two groups of C57/black mice as case groups. Control groups were injected with only saline and alum. All mice then were injected with melanoma cells. Both antigens reduced the tumor size in mice in case groups. The difference of tumor size in mice in case groups and control group was statistically significant. In conclusion, anti-tumor effect of hydatid cyst antigens may be related to antigenic similarities which exist between hydatid cyst and cancer cells.

Keywords Hydatid cyst · Antigen · Cancer · Melanoma

Introduction

Hydatid cyst is the larval stage of *Echinococcus granulosus*. Adult worms live in canines intestine and human is considered as accidental intermediate host and infected by ingestion of parasite ova in vegetables or water contaminated by definitive hosts' feces (McManus et al. 2003). The word, Hydatid, is derived from Greek which means the bottle of water (Milicevic 1994). Cystic structure is composed of outer laminated and inner germinal layers. These two adjacent layers filled with hydatid cyst fluid. Germinal layer generates brood capsules and protoscolices (McManus et al. 2003; Moro and Schantz 2009). Protoscolices are the larvae of the cyst, which are created by the asexual division of the germinal layer. The parasite protoscoleces are attached to the base of the germinal layer and when fully differentiated, based connection is interrupted and protoscoleces suspend in hydatid fluid (Eckert and Deplazes 2004; Moro and Schantz 2009). Hydatid cyst fluid, cyst membrane and protoscoleces have a complex mixture of antigens which stimulate the immune system (Kagan and Norman 1961). So one of the features of hydatid cyst is stimulation of cellular and humoral defense immune system (Rigano et al. 2004).

Cancers and parasites have some common features (Klinkert and Heussler 2006) and presence of common antigens between cancers and parasites has been reported (Baldus et al. 2004; Thomas and Harn 2004). According to previous researches, some parasites have capability of anti-tumor activities (Darani and Yousefi 2012; Kim et al. 2007; López et al. 2010; Oikonomopoulou et al. 2013; Pidherney et al. 1993; Plumelle et al. 1997) and so parasite antigens have been considered as possible targets for cancer immunotherapy (Darani and Yousefi 2012). In a retrospective study with a large population it has been shown that the

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prevalence of hydatid cysts in patients with cancer was significantly lower than the control group (Akgül et al. 2003). Yousofi Darani et al. (2012) showed that alive protoscoleces of hydatid cyst inhibited the growth of fibrosarcoma cells in culture medium (Yousofi Darani et al. 2012). In another study effect of hydatid cyst antigen on inhibition of cancer cell growth has also been shown in vitro (Aref et al. 2013). In an experimental study it has also been shown that alive hydatid cyst protoscoleces reduced tumor growth in mice challenged with fibrosarcoma cancer (Chookami et al. 2014). Eventually, cross reaction between hydatid cyst antigens and cancer cells was shown (Daneshpour et al. 2014). The aim of the present study was to investigate the effect of different hydatid cyst antigens on the growth of melanoma cells in animal model.

Materials and methods

Study population and in inoculation of antigens and tumor cells

In this investigation, the study population consisted of C57/Black mice and totally 18 mice in three groups were tested. The first and the second groups were injected with hydatid cyst fluid (case group) and excretory secretory antigen (ES antigen) of protoscolices (case group). Both antigens inject with alum as adjuvant. The third group was injected with isotonic saline absorbed on alum alone (control group). All mice received up to four booster injections every fortnight. Following the last injection all mice were injected with 10^6 melanoma cells in their chest site subcutaneously. Afterward the tumor growth was observed in mice and the tumor size was measured using a calipers and tumor area for each mouse was calculated using the following formula (24).

$$\text{Tumor area} = \times \frac{(\text{Diameter 1} + \text{Diameter 2})^2}{4}$$

Data analyzed with non-parametric statistical tests, such as Mann–Whitney test.

Antigen preparation

Different antigens of hydatid cyst were prepared as described before (Aref et al. 2013). Briefly *Echinococcus granulosus* hydatid cysts were collected from sheep or cattle from a slaughter house in Isfahan, Iran. Hydatid fluid of cysts were aspirated, and examined for the existence of protoscolices. The fluids were then centrifuged and the supernatant was concentrated. To prepare ES antigen of protoscolices, culture medium was added to alive protoscolices, and after 48 h incubation it was centrifuged and the supernatant was kept as ES antigen.

Culturing of tumor cells

Cell culture was performed as we published before (Yousofi Darani et al. 2012). Briefly Melanoma cell line was purchased from Pasture Institute (Tehran, Iran). Cells were cultured in RPMI 1640 medium (Bio-Rad, USA) and supplemented with 50 µg/mL gentamicin, and 10 % fetal calf serum (FCS).

Ethical concerns

All animals were handled and kept according to the guidelines for the welfare and use of animals in cancer research (Workman et al. 2010).

Results

The mean tumor size in mice injected with hydatid cyst fluid, excretory secretory antigen and saline were, 690, 325, 2,050 mm², respectively. The difference of tumor size between hydatid cyst fluid antigen and control group was statistically significant (Fig. 1). Also the difference of tumor size between ES antigen injected mice and control group was statistically significant (Fig. 2).

Discussion

Our results show that injection of mice with hydatid cyst fluid or excretory secretory antigen of protoscolices and subsequent challenge them with melanoma cells, resulted in inhibition of tumor growth compared to control group. In agreement with these results it has been shown that hydatid cyst fluid induced anticancer activity in mice with colon

Fig. 1 Mean tumor area (mm²) in mice injected with hydatid cyst fluid (case group), in comparison with those of mice injected with saline (control group)

Fig. 2 Mean tumor area (mm^2) in mice injected with excretory secretory antigen of protoscolices (case group), in comparison with those of mice injected with saline (control group)

cancer (Berriel et al. 2013). Also there are scientific evidences indicating the relationship between some bacteria and parasites infections with tumor growth and their anti-cancer activity (Darani et al. 2009; Lamm 1985; Yousofi Darani et al. 2012). For example, it has been shown that *Trypanosoma cruzi* infection can cause the development of resistance to tumor growth in mice, and also toxic effects of parasite extracts on cultured cancer cells have been shown in vitro (Atayde et al. 2008; Kallinikova et al. 2000). Also, use of parasite antigens for the immunotherapy of cancers has been proposed (Darani and Yousefi 2012). However it has been shown some forms of cancers occurs more frequently in patients with parasitic infections (Abdel-Rahim 2002). Also it is worthwhile to mention that all mice in the beginning of experiments were 8 weeks old, so it was unlikely that this mice have hydatid cyst infection or cancer.

How parasites interact with tumor antigens and mechanisms of anti-tumor effects of these antigens is still unknown. It is likely that the immune responses evoked by parasitic antigens, may have non-specific effects on tumor cells grow. Several studies have investigated the effects of parasite on the immune system motivation (Korbel et al. 2004; Papazahariadou et al. 2007). In a large retrospective study it has been found that hydatid cyst incidence is significantly lower in cancer patients than in healthy individuals (Akgül et al. 2003). The mechanism of anticancer activity of hydatid cyst antigens is also not completely clear. However this effect may be related to antigenic similarities which exist between hydatid cyst molecules and melanoma cells. As an example Tn antigens are jointly found in both hydatid cyst and the cancer cells (Alvarez Errico et al. 2001; Osinaga 2007). Based on this conception immune response raised against the hydatid cyst antigens may interact with antigens on tumor cells. Anti-cancer

activities of hydatid cyst antigens have been shown in other investigations. Yousofi Darani et al. (2012) showed that hydatid cyst alive protoscolices induced cell death in fibrosarcoma cells in vitro (Yousofi Darani et al. 2012). Also Aref et al. (2013) showed that hydatid cyst molecules increase tumor cells death or decrease alive tumor cells in cell culture medium (Aref et al. 2013). Other non-immunological mechanisms may be involved in induction of anticancer activity of hydatid cyst in culture medium. In agreement with our results it has also been shown that vaccination with the liquid of hydatid cyst can cause a partial protection against colon cancer in mice (Berriel et al. 2013).

To find more about anticancer activity of hydatid cyst antigens and the mechanisms involved in anti-tumor activity of this parasite further investigation is recommended.

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