Original Article

Effect of Hydatid Cyst Antigens Polyspecific Antisera on Breast Cancer Cells (4T1) Growth in Cell Culture Medium

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Abstract

Aim: Hydatid cyst is the *Echinococcus granulosus* larvae stage and is responsible for echinococcosis. Anticancer effects of hydatid cyst have been shown in human population, experimental animals, and *in vitro* works. However, the mechanisms of this anticancer activity are not clarified. Hence, in this work, the effect of antisera raised against hydatid cyst antigens on the growth of breast cancer cells has been investigated. **Materials and Methods:** In this experimental study, the various hydatid cyst antigens were prepared. To raise antisera, each of the hydatid cyst antigens (hydatid cyst fluid, germinal and laminated, protoscolex, and excretory-secretory [ES]) and toxoplasma trophozoite were injections to rabbits. The production of specific antibodies in rabbits determinate by enzyme-linked immunoassay, and then, the rabbit's blood was taken, and their sera isolated under the hood in sterile conditions. Before use, all antisera were inactivated at 56°C for half an hour and also placed under ultraviolet light for 20 min to disinfect. Breast cancer cells in the culture medium were purchased from the Pasteur Institute of Iran and growth in CO2 incubator in the Roswell Park Memorial Institute (RPMI) medium. After appropriate, the cells were counted and divided equally in eight-cell culture flasks and treated with different antisera of hydatid cyst. After 32-h incubation, the number of live cells was counted by trypan blue methods and compared with control groups. **Results:** Based on the results of this research, the difference between the number of live cells after treatment with antisera against hydatid cyst fluid, toxoplasma trophozoite, and ES antigen was significantly different from number of cells in flask treated with normal rabbit serum. **Conclusion:** In conclusion, due to the presence of common antigens between parasites and cancer, probably antibodies produced against hydatid cyst antigens may affect the growth of cells in the culture media.

Keywords: Breast cancer cell (4T), hydatid cyst, polyspecific antiserum

Introduction

The hydatid cyst is the larval stage of the *Echinococcus granulosus* that causes echinococcosis. It is also known as hydatidosis or hydatid disease in humans and domesticated livestock. The hydatid cyst has hemispheric shaped covered with multilayer wall and filled with hydatid cyst fluid. There are buds called protoscolex inside this cyst.[1] Cancer has been identified as one of the main causes of death in developed countries. Infection and parasitic diseases are the leading cause of death in developing countries. Various factors, such as air pollution and nutritional habits, may be responsible for raising number of death due to cancer in developed countries. There is a lot of scientific evidence that parasitic and bacterial infections have anticancer activity. In recent years, various researchers have reported a negative relationship between some parasitic infections and cancer. Statistically, due to the prevalence of parasitic infections and cancers, there is an inverse relation between cancer and the prevalence of parasitic infections.[2] Akgül *et al*., in a retrospective study, showed that the incidence of cancer was much lower in patients with hydatid disease than the normal people.[3] In animal models, it has been shown that certain parasites or parasitic compounds can inhibit cancer growth.[4-9] For example, the two parasite antigens of *Toxocara canis* and

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Toxoplasma gondii reduce the size of the fibrosarcoma tumor in the animal model.[7] *Strongyloides stercoalis* in patients with leukemia, increases life expectancy.[10] It has also been shown that *Trypanosoma cruzi* can reduce tumor size in mice.[4,6] A study by Yousofi Darani *et al*., the effect of hydatid cyst protoscolex on the proliferation and death of fibrocarcoma cells and kidney fibroblast cells of mouse kidney were studied. The results of this study indicated that hydatid cyst protoscolex inhibits WEHI-164 and BHK proliferation and induces cell death.[11] In another study, the effect of different hydatid cyst antigens on the growth of cancer cells in a cell culture medium has been shown.[12] Furthermore, the effect of cyst fluid antigen injection has been shown to inhibit cancer growth in mice.[13] The mechanism of the anticancer effect of hydatid cyst antigens is not known; probably, the anticancer property of the hydatid cyst antigen in an animal model is related to antibodies that have been developed against parasite molecules. The presence of common antigens between parasites and cancers has been reported in several studies.[14-22] Furthermore, it has been a cross-reaction between hydatid cysts and the serum of cancer patients.[23] Therefore, in this research, the effect of hydatid cyst antigens polyspecific antisera, on breast cancer cells (4T1), growth in cell culture medium has been studied.

Materials and Methods

Preparation of various hydatid cyst antigens

In this experimental study, the various hydatid cyst antigens are prepared as follows. At first, the hydatid cysts were isolated from liver and lung of infected sheep. Then, the hydatid cyst fluid was aspirated by syringe and then examined for the presence of protoscolexes. Hydatid cyst fluid was centrifuged for 2 min in 2000 rpm, and then, the supernatant was stored as hydatid cyst fluid antigen at -20°C. Then, the sediment at the bottom of tube containing protoscolex was washed with the normal saline solution and sonicated and stored at -20°C as protoscolex crude antigen. Germinal and laminated layer was isolated from hydatid cyst, homogenized and sonicated, and then stored at -20°C as cell wall of hydatid cyst antigen.

To prepare excretory-secretory (ES) antigen of protoscolices, culture medium was added to a live protoscolices, and after 48-h incubation, the medium was centrifuged, and the supernatant was kept as ES antigen.

Preparation of hydatid cyst antisera

To prepare antisera, each of the above antigens (hydatid cyst fluid, germinal and laminated, protoscolex, and ES antigen) was injected to rabbits with Freund's adjuvant. For the first injection, complete Freund's adjuvant, and for booster injection, the incomplete Freund's adjuvant was used. The production of specific antibodies in rabbits was checked by enzyme-linked immunoassay (ELISA) technique the production of appropriate amount of antibody, rabbit's blood was taken, and their serum isolated under the hood in sterile conditions. Before use, all antisera were inactivated at 56°C for half an hour and also placed under ultraviolet light for 20 min to disinfect.

Preparation of breast cancer cells (4T1)

Breast cancer cells in the culture medium were purchased from the Pasteur Institute of Iran and cultured in CO_2 incubator in the RPMI medium. After appropriate cell growth, the cells were harvested from the flask cell with 0.25% trypsin. After the cells were counted, they were divided equally in eight-cell culture flasks. Five hundred-µl antisera were prepared from the previous stage were added to each of the 1–5 flasks [Table 1]. Flasks 6 and 7 were added 500 µl of normal rabbit and 500 µl of RPMI medium, respectively. All of the flasks were placed in the CO_2 incubator. Flasks 6 and 7 were considered as negative control. The mean number of live cells (4T1) before treatment was 10×10^4 (No/ml) in each flask.

After 32-h incubation, the number of live cells was counted by trypan blue methods and compared with control groups. After counting the number of live cells, the mean of four repeat of test was calculated and analyzed with SPSS software (t-test) BM SPSS Statistics for Windows, Version 20 (IBM Corp., Armonk, N.Y., USA). The result of counting per flask is listed in Table 2.

Results

After preparation of various hydatid cyst antigens and injection into rabbit, the antibody titer in rabbits was measured by ELISA test which showed the antibody titer produced in rabbits increased 10 times than before injection. The 4T1 cell line grew well and was treated with deferent antisera, and after 32 h, the number of live cells was counted in each flask. For the treatment of 4T1 cells with each antiserum, the tests repeat four times. The result of counting per flask is listed in Table 2.

Based on the results of Table 2, the difference between number of live cells after treatment with hydatid cyst fluid, toxoplasma trophozoite, and ES antisera with negative control group (RPMI) was significant.

Discussion

In this experimental study, effect of hydatid cyst polyspecific antisera on breast cancer cells (4T1) growth in cell culture

Table 1: Treatment of breast cancer cell with deferent antisera

Flasks number	Treatment
Flask 1	500-µl hydatid cyst fluid antiserum
Flask 2	500-µl protoscolex antiserum
Flask 3	500-µl germinal and laminated layer antiserum
Flask 4	500-µl ES antiserum
Flask 5	500-µl toxoplasma trophozoite antiserum
Flask 6	500-µl normal rabbit serum
Flask 7	500-µl RPMI medium
Flask 8	500-µl 4T1 cell line antiserum

ES: Excretory secretory, 4T1: Breast cancer cell, RPMI: Roswell Park Memorial Institute

Table 2: Mean of number of live cells after treatment with deferent antisera

Number of tests	Number of live cells after treatment (no/ml)×10⁴							
	Hydatid cyst fluid antiserum	Protoscolex antiserum	Germinal and laminated layer antiserum	Toxoplasma trophozoite antiserum	ES antiserum	4T1 cell line antisera as positive control	Normal rabbit serum as negative control	RPMI medium as negative control
1	3	8	6	7	3	1	8	10
2	0	10	8	5	1	2	10	11
3	3	2	5	4	3	0	5	7
4	5	3	6	5	1	1	6	7
Mean	2.75	5.75	6.25	5.25	2	1	7.25	8.75
SD	2.06	3.86	1.25	1.25	1.15	0.81	2.21	2.06
Significant (two-tailed)	0.006≤0.05	0.23	0.09	0.034≤0.05	0.003≤0.05			

SD: Standard deviation, ES: Excretory secretory, 4T1: Breast cancer cell, RPMI: Roswell Park Memorial Institute

medium was investigated. The results showed that treatment with antisera against hydatid cyst fluid, toxoplasma trophozoite, and ES resulted in increasing dead cells. The number of dead cells in groups treated with those antisera was significantly higher than the number of cells in untreated groups.

Previous investigation showed that active immunization of mice with hydatid cyst fluid resulted in partial protection against subsequent injection of colon cancer cells[13] or melanoma cells. Furthermore, it has been shown that injection of live protoscolices of hydatid cyst induced protection against subsequent injection of melanoma cells.[18,24]

In agreement with our work, Chookami *et al*. showed effect of immunization with hydatid cyst antigens (protoscolices ES antigen and hydatid fluid) on tumor growth in experimental animals. Immunization with both antigens reduced the tumor size in mice.[25] Furthermore, in another study, different numbers of viable protoscoleces of hydatid cyst were injected to C57/black mice and then, melanoma cells were injected. In mice that received 100 or 500 protoscoleces, significantly smaller tumors were developed incomparison with the control group; but in the group received 1000 protoscoleces, there was no significant difference in tumor size compared to control group.[24] Probably, due to antigenic similarities between protoscoleces and cancer cells, the raised antibody against protoscoleces antigens may nonspecifically affect tumor growth in mice.

The results of this study showed that the number of live cells in the flask treated with antisera against hydatid cyst fluid was significant different with control flasks. Furthermore, Dorosti *et al*. investigated therapeutic effect of hydatid cyst liquid on melanoma tumor growth in mouse models. The results showed the mean tumor area, in groups which injected with hydatid cyst fluid (case groups), was less than the mean tumor area in the control groups, and this difference was statistically significant.

Anticancer effect of hydatid cyst antisera may be due to antigen similarity which exists between hydatid cyst and cancers.[26]

Conclusion

In this work, it was shown that treatment of with hydatid cyst antisera induced cell death in breast cancer cells. Hence, hydatid cyst antisera may have toxic effects on cancer cells. Due to the presence of common antigens between parasites and cancer, it is suggested that the effect of hydatid cyst antigens on breast tumor growth in mice also be investigated.

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Conflicts of interest

There are no conflicts of interest.

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