Effect of Passive Transfer of Anti-Hydatid Cyst Antigen Antisera on Melanoma Tumor Growth in Animal Model

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Abstract: *Introduction:* Hydatid cyst is the larval stage of *Echinococcusgranulosus,* a parasite responsible for hydatid disease in human and livestock. The Effect of different antigens of this parasite in preventing the growth of tumor cells has been demonstrated in various studies. Therefore, in this work the effect of passive transfer of antisera raised against different antigens of Hydatid cyst on melanoma cancer growth in animal model has been investigated.

Methods: In this experimental study, antisera against different antigens of hydatid cyst raised in rabbits. C57/black mice were injected with melanoma cells and then they received anti hydatid cyst antigen antisera. Control mice received normal rabbit serum or saline. Tumor size in the case and control groups was measured. Then, the data were analyzed using SPSS software and one-way Anova test.

Findings: The mean tumor area in mice that received antisera against hydatidcyst fluid, protoscolices crude antigen, excretory-secretory antigens of protoscolices and cuticular layer was not significantly different from tumor area of control mice.

Conclusion: The results of this study showed that injection of antisera against antigens of hydatid cyst had no significant effect on melanoma tumor growth. So it is recommended that effect of transfer of immune cells is investigated in future studies.

Keywords: Antiserum, Tumor, Antigens, Hydatid cyst, Passive immunization.

INTRODUCTION

Hydatid cyst is the larval stage of *Echinococcusgranulosus* tapeworm. Human is the intermediate host of this parasite and hydatid cyst is located in various viscera mainly in liver and lungs. Hydatid cyst consist of different components outwardly including: laminate and germinal layers, brood capsules containing protoscolices and hydatid fluid [1]. Antitumor effect of this parasite has been shown in cell culture [2, 3]. In other investigation it has been shown that hydatid cyst fluid induce antitumor activity against colon cancer in experimental animals [4]. Also immunization of mice with live protoscolices of this parasite resulted in significant reduction of melanoma tumor growth in C57/black mice [5].

Cancer cell antigens are usually less immunogenic than pathogen antigens. So it would be worthwile to use exogenic antigens for cancer immunotherapy. Due to existence of share antigens between parasites and cancers [6-8], parasite antigens can be considered as candidates for cancer immunotherapy [9].

Immunotherapy with antisera has received special attention since long times ago. Passive transfer of antisera was used for treatment of some infectious diseases in 1890 and then became more widespread [10, 11]. Now polyspecific antisera are used for treatment of different diseases especially for neutralization of snake venoms. Antibodies were used for treatment of white blood cells cancer in 1980 [12] and now they are used for treatment of different cancers [13]. So in this study effect of passive transfer of anti hydatid cyst antigen antisera in growth of melanoma tumor in mouse model has been investigated.

MATERIAL AND METHODS:

In this experimental study inbred C57/Black mice purchased from Pastor institute, Tehran, Iran were used as study population. Different antigens of hydatid cyst were prepared as described before [14]. Briefly *Echinococcusgranulosus* hydatid cysts were collected from sheep or cattle from a slaughter house in Isfahan, Iran. Hydatid fluid of cysts was aspirated, and examined for the existence of protoscolices. The fluids were then centrifuged and the supernatant was concentrated and collected as hydatid fluid. To prepare

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excretory secretory (ES) antigen of protoscolices, culture medium was added to a live protoscolices and after 48 hours incubation the medium was centrifuged and the supernatant was kept as ES antigen. Pellet of protoscolices were sonicated centrifuged and the supernatant kept as protoscolices crude antigen. Laminated layer of hydatid cyst minced and sonicated in isotonic saline and the supernatant kept as laminated layer antigen.

Different antigens of hydatid cyst; laminated layer antigen, protoscolices crude antigen, protoscolices excretory secretory antigen and Hydatid cyst fluid emulsified in frund's adjuvant and injected separately to four rabbits. Complete adjuvant used for the first injection and incomplete one for the boosters. Existence of antibody in rabbits sera were checked by ELISA test. The rabbits then were bled and their sera were collected at -20 until use. Prior to use the antisera were activated at 56 for one hour.

Mellanoma cells were purchased from Pastor Institute and cultured in RPMI medium. The cells were then harvested for injection to mice as we published before [2]. All mice were injected with 10^6 melanoma cells in their chest site subcutaneously. Then case groups injected with different anti hydatid cyst antigen antisera, 1 hour and 24 hours after cell injections. Control mice injected with either normal rabbit serum or isotonic saline. Every mouse was injected intravenously with 300 micro liter antiserum. Afterward the tumor growth was monitored in mice and the tumor size was measured using a calipers and tumor area for each mouse was calculated as we published before [15].

RESULTS

Mean tumor area in mice received anti hydatid cyst fluid or anti crude antigen of protoscolices antiseara, was not different from those of control mice. Mean tumor area of these mice has been presented in Table **1**. Also mean tumor area of mice received antiserum against excretory secretory antigen of protoscolices or antiserum against laminated layer antigen was not significantly different from mean tumor area of control mice. Mean tumor area of these mice has been presented in Table **2**.

DISCUSSION

Results of this investigation revealed that passive transfer of rabbit anti hydatid cyst antigen antisera to mice injected with melanoma cells induced no significant effect on solid tumor growth in comparison with control mice that received melanoma cells without

Table1: Mean Tumor Area in Mice which Received Melanoma Cells and Then Injected with Antiserum Against Hydatid Fluid or Antiserum Against Crude protoscolices Antigen or Saline (Control)

	Antiserum Against Hydatid Fluid Antigen	Antiserum Against Crude Protoscolices Antigen	Control
Mean of the First measurement	508.67	732.01	359.68
Mean of the Second measurement	1297.76	1379	912
Mean of the Third measurement	1050.32	1652	1266
Mean of the Fourth measurement	1089	All mice died	1590
Total Mean	986.70	1254	1032

Table2: Mean Tumor Area in Mice which Received Melanoma Cells and then Injected with Antiserum Against Excretory Secretory Antigen of Protoscolices, Antiserum Against Laminated Layer Antigen, Normal Rabbit Serum or Saline (Control)

	Antiserum Against Excretory-secretory Antigen	Antiserum Against Laminated Layer Antigen	Normal Rabbit Serum (control 1)	Isotonic Saline (control 2)
Mean of the First measurement	1855	2169	767	610
Mean of the Second measurement	1218	949	1336	908
Total Mean	1551	1559	1052	759

antisera passive transfer. However previous investigation showed that active immunization of mice with hydatid cyst fluid resulted in partial protection against subsequent injection of colon cancer cells [4] or melanoma cells [5]. Also it has been shown that injection of live protoscolices of hydatid cyst induced protection against subsequent injection of melanoma cells [5]. In agreement with our work Heimburg *et al*. showed that transfer of monoclonal antibody to Thomsen-Friedenreich antigen (TF-Ag), did not induce the killing of 4T1 tumor cells [16].

In this investigation passive transfer of immune serum did not result in protection against melanoma tumor in mouse model. The antibody in the immune sera may not be protective, although the protective role of passive transfer of antibody has been shown for some pathogens. As an example it has been shown that passively transferred immune serum provided significantly greater protection to BALB/c mice against attenuated Brucella abortus [17].

The other possible conception to explain why passive transfer of antisera was not protective against tumor growth is that, antibodies may need simultaneous involvement of cellular immunity to induce protection. In agreement with this conception Morrison *et al*. showed that passive immunization with immune serum conferred a marked level of protective immunity to murine *Chlamydia trachomatis* genital tract reinfection. This Ab-mediated protection was highly dependent on $CD4^+$ T cell-mediated adaptive changes that occur in the local genital tract tissues during primary infection [18]. In another work Zhigunova *et al*. showed that passive transfer of immune competent cell and serum resulted in suppression of tumor growth after transfer of spleen cells from animals immunized with Trypanosoma cruzi [19]. Also Kallinikova *et al*. showed that antibody against Trypanosomacruzi may have onco-protective effect [20]. According to results of our investigation it seems that anti tumor activity of hydatidcyst fluid in animal model may mainly related to cellular immunity. In this context Zeninan *et al*. showed that cell-mediated component of the immunity has a main role in the antitumor effect of T. cruzi [21].

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