Histopathological study in mice immunized against hydatidosis by hydatid cyst antigens

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Abstract

This study was designed to examined the histopathological lesions were occurred post-immunization against hydatid cyst infection in mice. hydatid cyst fluid HCFAs and excretion secretion antigens ESAgs were used for immunization against infection with protoscoleces of hydatid cysts, seventy-four of white male-mice were divided randomly into three groups each one subdivided into three subgroups which immunized by antigen concentrations (12.5, 25 and 50µg/ml); 1st and 2nd groups immunized by HCFAgs and ESAgs, respectively, 3rd group immunized by a mixture of HCFAgs and ESAgs, two doses (20µg/gm of body weight) S/C, 14 intervals. The 4th group; infected with protoscoleces. 5th group; injected with PBS as negative control group. Results revealed significant reduction of hydatid cysts growth recorded in immunized animal groups exclusively with a mixture antigens (HCF and ESAgs). The histopathologic examination of immunized mice revealed the infiltration of mononuclear cells mainly; lymphocytes and macrophages diffusely, focally and around blood vessels with proliferation of granulomatous lesions when compared with growth of hydatid cysts in positive control group and no growth in negative control group. In conclusions, the efficacy of HCF and ESAgs as prophylactic therapy in immunization where used alone or as a mixture against hydatid cyst infection.

Keywords: Hydatid cyst, Immunization, Histopathology, Mice.

Introduction

Echinococcosis is also referred to as hydatid disease, hydatidosis, or echinococcal disease, is a parasitic disease of tapeworms in the genus Echinococcus. (Bortoletti et al., 2004 and Piarroux et al., 2013). It affects both humans and other mammals, such as sheep, dogs, rodents and horses. In the human manifestation of the disease, Echinococcus granulosus, E. multilocularis, E. oligarthus and E. vogeliare localized in the liver (in 75% of cases), the lungs (in 5-15% of cases) and other organs in the body such as the spleen, brain, heart and kidneys (in 10-20% of cases). In the patients who are infected with E. granulosus and therefore, have cystic echinococcosis, the disease develops as a slow-growing mass in the body. The development of hydatid disease associated with cystic hydatidosis is still an important health problem (Sami et al., 2010). Many years ago the studies interests with the therapy treatment of hydatidosis like chemotherapy surgery but with some difficulties depends on the site, size and numbers of hydatid cysts in the infected host (Anonymous, 1979), so the present study was designed to investigates the histopathological study in mice responses by immunized against extracted antigens from the hydatid cyst itself to prevent or reduced the sizes of growing cysts.

Materials and Methods

Lab animals: Seventy-four of male white mice (Balb/c) of both gender, ranged (6-7) weeks old and weighted between 20-25gm which were obtained from the (National Center of Researches and Drugs Monitor in Baghdad).

Preparations of antigens:
1- HCFAs according to (McVie et al., 1997)
2- ESAgs according to (Aure and Aspock,1986).
3- Preparation of live Protoscoleces (PSC) and estimating the challenge dose (Morel, 1984).
4- Histopathological technique by (Godkar and Godkar, 2003) which includes:
   a) The tissue specimens preserved in 10% of neutral buffer formalin.
   b) Processing of tissue samples automatically by histokinette.
   c) Sectioning the paraffin blocking into slices about 4-5 μm in thickness by rotary microtome.
Experimental design: Seventy-four of male white mice were divided randomly into five groups and treated as following:

First group: (n=18) mice divided into three subgroups each one (n=6) immunized (day 0) with hydatid cyst fluid antigens (HCFAs) 20 ml S/C with concentrations 12.5, 25 and 50 µg /ml (Bradford, 1976). At day 21 the booster dose was (10µg/ml) S/C.

Second group: (n=18) as in the first group but immunized with Excretion-Secretion antigens (ESAs).

Third group: (n=18) as in the first group but with a mixture of HCFAs and ESAs.

Fourth group: (n=10) infected with live 3000 protosccoleces I/P as positive control group.

Fifth group: (n=10) injected with 20 ml of PBS S/C as negative control group.

1- Post-immunization (28 days), all immunized groups infected (challenge dose) with live 3000 protosccoleces I/P (Smyth & Davies, 1974).

2- Ninety days post-challenged infection all animal groups sacrificed to detect the reduction percentages growth of hydatid cysts in internal organs as (Heath,1976) and preserved the tissues in 10% formalin to examined the histopathologic changes post-immunization and compared with infected mice in positive control and negative control groups.

Reduction% = \[
\frac{\text{Mean No. of cysts in positive group} - \text{Mean No. of cysts in immunized}}{\text{Mean No. of cysts in positive group}} \times 100
\]

Results and Discussion

Many researches were interested about the preparation of specific vaccines aimed to stimulate the specific immune responses (humoral and cellular immunity) against the growth of hydatid cysts infection caused by Echinococcus granulosus so they used antigens that prepared from the cyst itself.

1- The reduction % of hydatid cysts growth: as in the (Table 1) the higher percentage of growth reduction was appeared in mice immunized with HCF and ESAs (97.4%), ESAgs (84.0%) then HCFAs (76.1%) as comparing to highly growth of hydatid cysts and their distribution in internal organs which were differed in their sizes, shapes in positive control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Reduction %</th>
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<tbody>
<tr>
<td>1st</td>
<td>76.1%</td>
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<tr>
<td>2nd</td>
<td>84.0%</td>
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<tr>
<td>3rd</td>
<td>97.4%</td>
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<tr>
<td>4th</td>
<td>0%</td>
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<tr>
<td>5th</td>
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Ibrahim (2012) who used a mixture of HCF and protosccoleces antigens (7.5 and 15µg/ml) conducted the immunostimulatory effects of these antigens in reduction growth of hydatid cysts post-challenge, Lightowlers et al. (2003) expressed the activity of different (mixture) antigens in induction the immune response as comparing with using single antigens as occurred in the present study were the animal groups immunized with HCF, ESAgs separately was not efficient in reduction the growth of hydatid cysts as a mixture antigens were appeared more effective in reduction the cystic growth and induction strong immune response against parasitic infection. Al-Azawi and Abdul Majeed, (2014); Al-malki (2012); Faleh (2002) recorded the efficacy of stimulating immune responses post immunization with antigens in preventing the growth of cysts.

Aure and Aspock (1986) improved the activity of SEAs as immunostimulatory (humoral and cellular) may due to containing different antigens as comparing with HCFAs alone that may be due the low content of parasitic antigens which reflect the high reduction percentages in mice immunized with a mixture (97.4%) by SE (94%) but in positive control group there were secondary growth of hydatid cysts single or in clusters adhered to the internal organs (Alkinani,1988).

2- Histopathologic examination: which included study the gross and histological changes in liver and spleen tissues of immunized mice and infected animals (positive control) as comparing with negative control group.

1- Mice immunized with (ESAgs/12.5 µg/ml): the histologic changes were varied from degenerative changes (acute cellular swelling) to the necrosis of hepatocytes, slight infiltration of inflammatory cells in dilated sinusoids (figure-1) and around dilated bile ducts in portal areas. In spleen there was focal deposition of amyloid-like substance appeared acidophile-like material replaced the lymphoid follicles (Figure 2) and hemorrhage in red pulp also noted.

2- Mice immunized with (ESAgs/25µg/ml) there was mild to moderate infiltration of inflammatory cells
mainly mononuclear cells (lymphocytes and macrophages) in liver parenchyma, moderate to marked hyperplasia of lymphoid follicles (reactive hyperplasia) in white pulp and proliferation of reticuloendothelial C.T in red pulp (Figure 3).

3- Liver tissues of mice immunized with (ESAgs/50µg/ml) there were degenerative changes of hepatocytes (vacuolar degeneration) which appeared enlarged, infiltration and focal aggregations of inflammatory cells (lymphocytes and macrophages) in portal areas also in liver parenchyma with deposition of eosinophilic material in sinusoids (Figure 4). In spleen slight to moderate changes as in NO3.

4- The liver tissue of mice immunized with (HCFAgs/12.5 and 25µg/ml), showed multifocal aggregations of inflammatory cells in parenchyma and in portal areas, necrosis of hepatocytes with eosinophilic substance between hepatocytes. Hyperplastic changes in spleen noticed.

5- Mice immunized with (HCFAgs/50µg/ml) there was infiltration and focal aggregations of inflammatory cells in liver parenchyma along the sinusoids (Figure 5) and in portal areas. The sections of spleen showed thickening of capsule and proliferation of lymphoid follicles, presence of large numbers of megakaryocytes in white pulp, sever congestion of blood sinuses in red pulp

6- Liver of mice immunized with a mixture of (HCEF ES and Ags 12.5 and 25µg/ml) the histologic lesions appeared similar to that changes occurred as above, beside apoptosis seen in centrilobular and extend outward (Figure 6). In spleen moderate hyperplasia of lymphoid follicles seen (Figure 7).

7- In mice immunized with a mixture of (HCF& ESAgs 50µg/ml) the histological sections of liver revealed extensive hepatic necrosis and infiltration of infl cells (Figure 8) apoptosis (Figure 9) also seen marked hyperplasia of lymphoid tissue seen.

8- Mice infected with protoscolices (positive control) there were sever damaged in liver parenchyma appeared hemorrhagic with sever necrotic hepatic tissue (Figures 10 and 11) heavy infiltration of inflammatory cells mainly (lymphocytes, macrophages and foreign body giant cells) (Figure 12).

9- No significant histopathological lesions recorded in negative animals group.
Figure (2): Histologic section in liver of mouse immunized with (ESAgs 12.5 µg/ml); acute cell swelling of hepatocytes (enlarged and acidophilic) (thick arrow) and focal infiltration of lymphocytes in dilated sinusoides (thin arrow) (H&E stain, 40X).

Figure (3): Histologic section in spleen of mouse immunized with (ESAgs 25 µg/ml); in red pulp proliferation of reticuloendothelial tissue and congestion of blood vessels sinuses (H&E stain, 40X).
Figure (4): Histologic section in liver of mouse immunized with (ESAgs 50µg/ml); perivascular cuffing of mononuclear cells around congested blood vessel (thick arrow), in portal area and deposition of amyloid-like substance around sinusoidal capillaries (thin arrow) (H&E stain, 40X).

Figure (5): Histologic section in liver of mouse immunized with (ESAgs 50µg/ml); infiltration of mononuclear cells in portal area (thick arrow) (H&E stain, 40X).
Figure (6): Histologic section in mice immunized with HCF Ags/50µg/ml; focal perivascular cuffing of mononuclear cells around dilated central vein (thick arrow) and proliferation of kupffer cells in dilated sinusoids (thin arrow) (H&E stain, 40X).

Figure (7): Histological section in liver of mice immunized with HCF ES&AgS/25µg/ml; showed granulomatous lesion (between arc) and perivascular cuffing at the right of figure (H&E stain, 20X).
Figure (8): The granulomatous lesion (in figure-6): consist from aggregation of lymphocytes, macrophages and epitheloid cells (acidopholic) (black arrow) and foreign-body giant cells (blue arrow) (H&E stain, 40X).

Figure (9): Hyperplasia of lymphoid follicle in white pulp in spleen of mouse immunized by a mixture of HCF ES&Ags 25µg/ml (H&E stain, 20X).
Figure (10): Necrosis of hepatocytes (thin arrow) in mouse immunized with a mixture of HCF&ES Ags 12.5 and 25µg/ml and focal aggregation of mononuclear cells in dilated sinusoids (blue arrow) (H&E stain, 40X).

Figure (11): Apoptosis in liver parenchyma of mouse immunized with a mixture of HCF &ES Ags 12.5 and 25µg/ml (H&E stain, 40X).
Figure (12): Histological section in liver of mouse infected with protoscoleces, showed intensive infiltration of inflammatory cells (to the right) in liver parenchyma with necrotic hepatocytes and hemorrhage (H&E stain, 20X).

Figure (13): Histological section in liver of mouse infected with protoscoleces, showed severe damage of liver parenchyma; hemorrhage with severe necrotic hepatocytes and intensive infiltration of inflammatory cells (H&E, 40X).
Figure (14): Histologic section of liver (as in Figure 12), showed mononuclear cells infiltration mainly lymphocytes, macrophages and foreign-body giant cells (H&E, 40X).

The histopathological changes were ranged according to the type of antigen and its concentration in immunized mice; from mild to moderated infiltration of inflammatory cells and focal aggregations of mononuclear cells mainly lymphocytes and macrophages in tissues of internal organs with HCF and ESAgs (12.5µg/ml) that explained the study of Rahimi et al. (2011) who demonstrated a high level production of IFN-γ and IL-12 (Th1 responses) which produced by immune cells (inflammatory cells) and the secretion of IL 10 and IL4 (Th2 responses) in response to the crude antigens (in vitro and in vivo) followed by the E/S and the immunodominant antigens, respectively, because these cytokines which are the driving and activating the lymphocytes to produce IL2 which important to stimulation of macrophages (Martínez, et al., 2009) so it could the suitable candidate for vaccination in experimental lab animals (Fraize et al., 2004 and Rahimi et al., 2011). HCFAg (25 and 50µg/ml) there were heavy infiltration of mononuclear cells in liver tissue that expressed the stimulation of specific cell-mediated immunity to the formation of granulomatous lesions in some immunized mice single and distributed mostly on liver and lungs which characterized microscopically by infiltration of mononuclear cells; Lymphocytes, macrophages, foreign body giant cells besides the eosinophils and plasma cells around protoscolosis (Ali-Khan.1974). The appearance of hydatide cysts in some immunized mice may due to the high dose of challenge and the activity of protosvolices in cyst or due to the suppression of immune response by these parasites. The deposition of amyloid-like substance with ESAgs 12.5 and 50µg/ml which appeared acidophilic and homogenized around the lymphoid follicles in white pulp and sinusoidal capillaries may be due to high concentrations that injected through immunization which influenced on immune cells functions that agreed with Ibrahim,2012 who revealed amyloid-like substance deposition post-immunization against tumor cells. it suggested that the transformation of soluble proteins and peptides into insoluble amyloid fibrils reflects a series of conformational alterations that involve formation of amyloidogenic intermediates; self-association and stabilization of these components through interactions between β-sheets that lead to protofilaments/protofibrils; and, finally, interaction of the components to form the mature fibril (Dobson, 2004).

Conclusions

The efficacy of a mixture antigen (HCF & ES) in stimulating cell mediated immunity against hydatid cyst infection and its relationship with excessive concentration in inducing perfect immunity.

References

Immunization mice with DNA from


