Immunomodulation of Echinococcosis with Estrogen, Progesterone and HCGL

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Summary:

Background:
Echinococcosis is a zoonotic disease with high incidence. Some recent reports indicated an Immunopotentiation role of estrogen and progesterone against some pathogens.

Aim:
To investigate the Immunopotentiation effect or estrogen, progesterone and HCGL before infection with protostrongylus, in BALB/c mice through various parameters.

Methods:
BALB/C mice were divided into experimental groups with each group consisting of eight animals. The experimental groups were injected intradermally with estrogen, progesterone at doses of (12.5, 62.5) g/20 gm b.wt. respectively and with HCGL at doses of 30 g/20 gm b.wt. These animals were injected with (1000) protostrongylus of hydatid cyst, than sacrificed after four months. Total number and differential count of white blood cells, number and diameter of hydatid cyst in internal organ, immediate type hypersensitivity (Arthus reaction), and phagocytic index of peritoneal macrophages to yeasts at ratio (1:10) were estimated.

Result:
All mice treated with estrogen, progesterone and HCGL showed decrease in the number and diameter of hydatid cyst in, P<0.05 also increase in the phagocyte index in peritoneal macrophages and increase in arthus reaction comparing with control.

Conclusion:
Estrogen progesterone and HCGL antigen were found to have anti echinococcosis activity through killing of protostrongylus by activated body immune system.

Keywords:
Echinococcus, Immunodulation, progesterone, estrogens, HCGL antigen, activated macrophage.

Introduction:
Echinococcosis is azoonotic infection caused by adult or larval metacestode stage of cestodes belonging to the genus echinococcus (1). The parasites are perpetuated in life cycle with carnivores as definitive host, which harbor the adult eggs producing stage in the intestine, and intermediate host animals, in which the infective metacestodes develop after per-oral infection with eggs. Metacestodes also, develop in human's causing various

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forms of Echinococcosis like cystic echinococcosis. Alveolar echinococcosis and polycystic echinococcosis. In addition to systemic immunological reactions may be observed like urticaria, asthma, anaphylaxis, or membranous nephropathy (2). Presenting symptoms and signs of CE liver, tumor-hepatomegaly, cholestasis, jaundice secondary biliary cirrhosis, abscess and in the lungs. Lung tumor, chest pain, chronic cough, lung abscess, eosinophilic pneumonia, parasitic lung embolism (3). The ability of the metacestodes to avoid host immune system mechanisms in order to survive, and at the same time to simulate an effective host immune rejection of the later invasive organism by inhibition the interferon levels in serum, and also, production of interferon by lymphocyte from patients (4). Activation of body immune system which were obtained from mice treated with Esouletin and Rhizobium meliloti polysaccharide posses a good protection against Echinococcosis due to its immunological properties (5). Some studies demonstrated the ability of estrogen and progesterone to induce the stimulation of interferon-γ secretion (6). In this study progesterone, estrogen was attempted as Immunomodulatory structure against Echinococcus granulosus in experimental model.

Materials and Methods:
Males of BALB/c mice, 6-8 weeks of ages and 18-20 gm body weight, were obtained from institute for Embryo Research and Infertility, Al-Nahrain University.
Parasites and their viability:
Samples of E. granulosus were obtained from infected animals from butcher’s, the separation of echinococcosis cyst, then the examination of protoscolices viability and counting done according to Symth and Barrett, 1980 (7).
The antigen of hydatid cyst germinal layer was prepared according to Wen and Craig (1994) (8) than the total protein concentration was estimated according to methods of Bradford (1976) (9).
The animals were divided into eight groups and each contain eight animals as the following:

1. Groups of animals treated with 12.5 µg/gm Bwt of estrogen intradermally and infected.
2. Group of animal treated with 62.5 µg/gm B.wt of progesterone intradermally and infected.
3. Group of animals treated with 12.5 µg/gm B.wt of estrogen intradermally alone.
4. Group of animals treated with 62.5 µg/gm B.wt of progesterone intradermally alone.
5. Group of animals treated with 30 µg/gm B.wt of HCG and infected.
7. Group of animals treated with 0.1 ml/gm sterilized PBS and infected and served as a positive control.
8. Group of animals treated with 0.1 ml/gm of sterilized PBS and served as a negative control.
All these groups except treated alone and negative control were inoculated intraperitoneally with (1000) protoscolices/mice, after four months the animals were sacrificed and concern on the following parameter.
1 - Total of leukocyte and differential count according to methods of Garvey et al, 1977 (10).
2 - Estimation of Arthus reaction.
All treated groups of mice were injected intradermally with 0.05 ml of protoscolices solution in the right footpad while the left foot pad was injected with 0.05 ml of PBS and served as a control. The footpad thickness was measured with a vernier caliper after (4) hour antigen injection, then the difference considered the immediate type hypersensitivity (Arthus reaction) Triolo et al., (1989) (11).
3 - Estimation of hydatid cyst in internal organ and their diameter with the prophylactic index according to method of Heath, 1981 (12).
4 - Evaluation of phagocytic cells in treated and infected groups.
- Peritoneal macrophage cultures:
The medium was prepared by using tissue culture.
Media RPMI-1640 (flow laboratories); 4% heat inactivated fetal calf serum, 1% Lglutamine, supplemented with 100 unit/ml penicillin and 0.1 mg/ml streptomycin according to (13). Four milliliters of this
medium were injected into peritoneal cavity of killed mice. Recovered peritoneal lavage was subdivided into three 35mm petridish (Lux) one ml of lavage contain 1x10^7 Leukocyte.

To study macrophage, yeast interaction in vitro the washed freshly isolated macrophages from normal and treated mice were infected with yeast, least and phagocyte cells were suspended separately. Yeast were washed twice in sterile PBS, than added to macrophage lavage to prepare macrophage to yeast mixture at an approximate ratio of (1:10) according to (13).

**Assessment of Phagocytosis:**
After infection the experiment sample triplicate were incubated for (120 min) at 5% CO2, at 37°C. The phagocytosis was observed using microscope, then the phagocytic index was measured according to the formula (13):

\[
\text{Phagocytic index (P.I) \% = } \frac{\text{No. of yeast phagocytic cells}}{\text{Total phagocytic cells}} \times 100
\]

**Statistical Analysis:**
ANOVA test was used to compare the results.

**Results:**
I - Total white blood cells and differential count.
Table (1) shows the changes in W.B.C. count in mice treated with estrogen, progesterone, HCGL and infected with parasites, the number's of all treated groups were significantly increased (P<0.05) it reached (10196, 10030) cells/Cu.mm in groups of mice treated with HCGL and infected with parasites and treated alone respectively, comparing with positive and negative control which reached (9638, 7800) cell/cu.mm respectively.

Also, the number of white blood cells increasing in groups treated with estrogen, progesterone and infected with parasites reach (9050, 7120) cell/cu.mm respectively. Comparing with treated uninfected groups which reached (9030.0, 5994.00) cell/cu.mm respectively.

II - Total number's and diameter of hydatid cyst with the prophylactic index in mice treated with estrogen progesterone HCGL estrogen and infected with the protoscolices.

Table (2) shows the changes in the number and diameter of hydatid cyst, the numbers were decreased significantly (Pe0.05) in all groups comparing with positive control, which reached (13) while the diameter of hydatid cyst/mice reached (1.08 ± 0.07) mm comparing with groups of mice treated with progesterone and estrogen respectively, while the prophylactic index reach to 100% in groups of mice treated with HCGL.

III - Estimation of immediate hypersensitivity (Arthus reaction).
Table (3) shows the changes of Arthus reaction in group of mice treated with estrogen, progesterone, HCGL and infected with protoscolices, it reach (0.09, 0.07, 0.06) mm comparing with the control treated and non infected it reach (0.08, 0.09, 0.13) mm respectively, while the positive and negative control reach (0.04,0.05) mm respectively.

IV - Evaluation the phagocytic index:
Table (4) shows the changes of phagocytic index in peritoneal macrophage of mice treated with estrogen, progesterone HCGL and infected with protoscolices.

The phagocytic index increased significantly (P < 0.05) it reach (23.20%) in positive control comparing with (9.20%) in negative control, while the value reach (46%, 30.40%, 14.4%) in groups of mice treated with estrogen, progesterone HCGL and infected respectively. Comparing with other groups treated alone which reach (21.2, 11.0, 14.4%) respectively.
**Table 1:** Effect of estrogen, progesterone and HCGL antigen on total and differential count of white blood cells of mice treated and infected with protoscolices

<table>
<thead>
<tr>
<th>Groups</th>
<th>White blood cells count/cu.mm and differential</th>
<th>Neutrophil</th>
<th>Lymphocyte</th>
<th>Monocyte</th>
<th>Acidophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>9638.0±294.3</td>
<td>69.40±1.84</td>
<td>18.80±1.74</td>
<td>6.80±1.71</td>
<td>5.00±0.44</td>
</tr>
<tr>
<td>Negative Control</td>
<td>7800.0±61.20</td>
<td>57.40±1.28</td>
<td>37.20±1.46</td>
<td>5.00±0.31</td>
<td>4.00±0.24</td>
</tr>
<tr>
<td>Treated with estrogen</td>
<td>9030.0±148.79</td>
<td>87.00±1.94</td>
<td>8.20±1.93</td>
<td>4.40±0.40</td>
<td>4.00±0.34</td>
</tr>
<tr>
<td>Treated with progesterone</td>
<td>5994.0±6005.75</td>
<td>64.40±1.53</td>
<td>25.60±0.92</td>
<td>9.20±1.82</td>
<td>1.8±0.29</td>
</tr>
<tr>
<td>Treated with HCGL alone</td>
<td>10030.0±32.75</td>
<td>89.50±1.98</td>
<td>5.60±1.43</td>
<td>4.20±0.58</td>
<td>1.8±0.38</td>
</tr>
<tr>
<td>Treated with estrogen and infected</td>
<td>9050.0±559.01</td>
<td>74.60±2.82</td>
<td>18.20±2.76</td>
<td>4.0±0.44</td>
<td>3.6±0.39</td>
</tr>
<tr>
<td>Treated with progesterone and infected</td>
<td>7120.0±281</td>
<td>68.40±4.26</td>
<td>20.40±3.82</td>
<td>7.80±1.11</td>
<td>3.40±0.40</td>
</tr>
<tr>
<td>Treated with HCGL and infected</td>
<td>10196.0±132.15</td>
<td>91.20±1.20</td>
<td>3.80±0.58</td>
<td>4.00±0.89</td>
<td>5.0±0.3</td>
</tr>
<tr>
<td>LSD</td>
<td>819.15</td>
<td>6.91</td>
<td>6.22</td>
<td>2.92</td>
<td>0.87</td>
</tr>
</tbody>
</table>

**Table 2:** Effect of estrogen, progesterone and HCGL antigen on number and diameter of hydatid cyst/mm and prophylactic index in groups of mice treated and infected with protoscolices

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of hydatid cyst/mice ± SD</th>
<th>Diameter of hydatid cyst (mm) ± SD</th>
<th>Prophylactic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>2.60±0.87</td>
<td>1.08±0.07</td>
<td></td>
</tr>
<tr>
<td>Treated with estrogen and infected</td>
<td>0.80±0.20</td>
<td>0.48±0.13</td>
<td>69.23</td>
</tr>
<tr>
<td>Treated with progesterone and infected</td>
<td>0.40±0.24</td>
<td>0.40±0.30</td>
<td>84.61</td>
</tr>
<tr>
<td>Treated with HCGL and infected</td>
<td>0.00</td>
<td>0.00</td>
<td>Completely protect</td>
</tr>
<tr>
<td>LSD</td>
<td>1.05</td>
<td>0.36</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Effect of estrogen, progesterone, and HCGL antigen on immediate type hypersensitivity (Arthus reaction)/mm in groups of mice treated and infected with protoscolices

<table>
<thead>
<tr>
<th>Groups</th>
<th>Arthus Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>0.04</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.05</td>
</tr>
<tr>
<td>Treated with estrogen alone</td>
<td>0.08</td>
</tr>
<tr>
<td>Treated with progesterone alone</td>
<td>0.09</td>
</tr>
<tr>
<td>Treated with HCGL antigen alone</td>
<td>0.13</td>
</tr>
<tr>
<td>Treated with estrogen and infected</td>
<td>0.09</td>
</tr>
<tr>
<td>Treated with progesterone and infected</td>
<td>0.07</td>
</tr>
<tr>
<td>Treated with HCGL and infected</td>
<td>0.06</td>
</tr>
<tr>
<td>LSD</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Table 4: Effect of estrogen, progesterone and HCGL antigen on phagocytic index in peritoneal macrophage isolated from mice treated and infected with protoscolices

<table>
<thead>
<tr>
<th>Groups of mice</th>
<th>Phagocytic Index %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>23.20</td>
</tr>
<tr>
<td>Negative control</td>
<td>9.20</td>
</tr>
<tr>
<td>Treated with estrogen alone</td>
<td>21.20</td>
</tr>
<tr>
<td>Treated with progesterone alone</td>
<td>11.00</td>
</tr>
<tr>
<td>Treated with HCGL antigen alone</td>
<td>14.40</td>
</tr>
<tr>
<td>Treated with estrogen and infected</td>
<td>46.00</td>
</tr>
<tr>
<td>Treated with progesterone and infected</td>
<td>30.40</td>
</tr>
<tr>
<td>Treated with HCGL and infected</td>
<td>14.40</td>
</tr>
<tr>
<td>LSD</td>
<td>8.40</td>
</tr>
</tbody>
</table>

Discussion:
The present results of experimental studies with estrogen, progesterone and HCGL antigens have immunoenhancing activities for murine immune system in vivo in the intact immunized mice. Potentiation of host defense mechanism by estrogen, progesterone and HCGL antigens consists of multiple kinds of pathways among these, its ability to stimulation of lymphocyte and other white blood cells (Table-1). This activity may be due to stimulation of macrophages which play an important regulation role in antigen processing and monokine production (14). The increasing of eosinophils cells especially in group of mice treated with HCGL and infected with protoscolices due to stimulation of eosinophils chemotactic factor (ECF) (15).

The increasing of immediate hypersensitivity (Arthus reaction) in groups of mice treated with Immunomodulators and infected with protoscolices (Table-2), may be due to enhancing the synthesis of antibodies and formation of edema in addition to aggregation of polymorphonuclear leukocyte (16).

Progesterone and estrogen have a marked stimulating action on macrophages and lymphocytes activation (17). These effect are especially marked in failure the infection by prevent the parasites to penetration the tissue then leading to increasing the prophylactic index till reach (69.93, 84.6%) for estrogen and progesterone respectively (Table-3), in addition to that, hydatid cyst disappear in all organs of the infected mice and treated with HCGL. The mechanisms seems to be involved in the action of estrogen stimulation of interferon-γ and progesterone stimulate the tumor necrosis factor α (TNF-α) then activate the macrophages and stimulation of body immune system (18). Also, biological activities of HCGL appears to stimulating the functions of lymphocytes and macrophages which are controlled by the soluble mediators like INF-γ than determine the infectivity of the parasites by stimulation of antibody dependent cellular cytotoxicity (18). In addition to increasing of eosinophils which increase the secretion of perforin, Basic major protein and cationic protein (19, 20). Then these products kill the protoscolices. The increasing of phagocytic index (P<0.05) in groups of mice treated with immunomodulators comparing with the control (Table-4) may be due to enhance the explanation of factor crystallizables (FC) fragment and stimulate the phagocytosis by formation of phagosomes - lysosomes vacuoles (16) and enhance the phagocytosis (21).

Conclusions:
These results illustrated that estrogen, progesterone and HCGL antigen stimulate the body immune system by enhancing the activation of macrophages and increasing the prophylactic index to destroying the protoscolices of hydatid cyst, increasing Arthus reaction, stimulation of phagocytic cells and lymphocytes.

References:
I. Thompson, R.C.A., Lymbery, A.J & Constantine, C.C Variation in Echinococcus