Original Article

Immunomodulation of Echinococcosis with Estrogen, Progesterone and HCGL

Ghassan H.J. Al-Kaisy M.Sc *
Khalil - I. Mohammad M.Sc., Ph.D **
Majid M.M. Al-Jewari M.Sc., Ph.D ***

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 protoscolices 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) protoscolices 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 lo have anti echinococcosis activity through killing of protoscolices by activated body immune system.

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

Fac Med Baghdad
2008; Vol.50, No.1
Received April 2007
Accepted Oct.2007

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

*College of Education, Diayla university
*College of Dentistry, Baghdad university
***College-of science, AL-Mustansyria university

forms of Echinocaccosis like cystic echinococcosis. Alveolar echinococcosis and polycystic echinococcosis. In addition to systemic immunological reactions may be observed like urticaria, asthma, anaphylaxis, or membraneous nephropathy (2). Presenting symptoms and signs of CE liver, tumor-hepatomegaly, cholestiasis, 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 metacestoes 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 *Rhizobhum meliloti* polysaccharide posses a good protection agaisnt 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 Bradiford (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 animalw 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 HCGL and infected.
6. Group of animals treated with 30 μg/gm B.wt of HCGL alone.
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 0.051nl of protoscolices solution in the right footpad while the left food pad was injected with 0.05ml of PBS and served as a control. The foodpad thickness was measured with a veriner calipser after (4) hrof 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.

- Periotoneal 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 pencillin 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):

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

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 (Pc0.05) 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 potoscolices

Groups	White blood cells count/cu.mm and differential				
	Total Count	Neutrophil	Lymphocyte	Monocyte	Acidophils
Positive Control	9638.0±294.3	69.40±	18.80±1.74	6.80±1.71	5.00±0.44
Negative Control	7800±61.20	57.40±1.28	37.20±1.46	5.00±0.31	4.00±0.24
Treated with estrogen	9030.0±148.79	87.00±1.94	8.20±1.93	4.40±0.40	4.00±0.34
Treated with progesterone	5994.0±005.75	64.40±1.53	25.60±0.92	9.20±1.82	1.8±0.29
Treated with HCGL alone	10030.0±32.75	89.50±1.98	5.60±1.43	4.20±0.58	1.8±0.38
Treated with estrogen and infected	9050.0±559.01	74.60±2.82	18.20±2.76	4.0±0.44	3.6±0.39
Treated with progesterone and infected	7120.0±281	68.40±4.26	20.40±3.82	7.80±1.11	3.40±0.40
Treated with HCGL and infected	10196.0±132.15	91.20±1.20	3.80±0.58	4.00±0.89	5.02±0.3
LSD	819.15	6.91	6.22	2.92	0.87

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

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

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

Groups	Arthus Index
Positive control	0.04
Negative control	0.05
Treated with estrogen alone	0.08
Treated with progesterone alone	0.09
Treated with HCGL antigen alone	0.13
Treated with estrogen and infected	0.09
Treated with progesterone and infected	0.07
Treated with HCGL and infected	0.06
LSD	0.08

Table 4: Effect of estrogen, progesterone and HCGL antigen on phagocytic index in peritoneal macrophage isolated from mice treated and infected with protoscolices

Groups of mice	Phagocytic Index %
Positive control	23.20
Negative control	9.20
Treated with estrogen alone	21.20
Treated with progesterone alone	11.00
Treated with HCGL antigen alone	14.40
Treated with estrogen and infected	46.00
Treated with progesterone and infected	30.40
Treated with HCGL and infected	14.40
LSD	8.40

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 Imrnunomodulators 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 crystalizables (FC) fragment and stimulate the phagocytosis by formation of phagosomes - lysomos 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:

1. *Thompson, R.C.A., Lymbery, A.J. & Constantine, C.C Variation in Echinococcus*

toward a taxonomic revision of the genus Adv. Parasitol. (1995) 35, 145-176.

2. *Wen, H.; Zou, P.F. & Xang, M.X. A rare case of mixed cystic and alveolar hydatidosis. Trans. Roy. Soc. Trop. Med. Hyg. (1992) 86: 290-291.*

3. *Dar, F.K. & Alkarmi, T. Iminunopathology of alveolar echinococcosis. Acta. Parasitol. (1997) 42: 1-6.*

4. *Suntsov, S.; Ozeretskorskaya, N.; Grigoryan, S. and Shcherbakov, A. Status of interferon during helminthiasis communication 1. Human Uniocular hydatidosis. Meditsinskaya Parazitologya Parazitarye Botezni (1 990) 4: 27-9.*

5. *Al-Taei, A.F. Activation of macrophages with Immunomodulators and the effect of this activation upon the infection with E. granulosus (1996) Ph.D Thesis, college of science, Al-Mustansyria University.*

6. *Fox, H.S.; Bond, B.L. and Parslow, T.G. Estrogen regulates the IFN-gamma promoter J. Immunol. (1991) 146: 4362-4367.*

7. *Smythe, J.D. and Barrett, N.J. Procedure - for testing the viability of human hydatid cyst following surgical removal especially after chemotherapy. Trans. Roy. Soc. Trop. Med. Hyg. (1980) 74: 649-652.*

8. *Wen, H. and Craig, P.S. Immunoglobulin G subclass response in human cystic and alveolar echinococcosis. Am. J. Trop. Med. Hyg. (1994) 97: 741-748.*

9. *Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantitation of protein utilizing, the principle of protein dye binding. Aral. Biochemistry (1997) 72L 248-254.*

10. *Garvey, J.S.; Cemer, N.E. and Sussodorf, D.H. Methods in immunology. A laboratorytext for instruction and research 3rd ed. W.A. Benjamin, Inc. Canada, (1997) PP. 425.*

11. *Triolo, A.J.; Osterhalm, J.L. and Kratky, M.T. Enhancement of the Arthus reaction and suppression of delayed type hypersensitivity (DTH) by pluronic F68, a detergent frequently used to prepare perfluoro carbon emulsions. Int. J. Imrnunophannicol. (1989) 11: 41- 48.*

12. *Ilcath. D.D. Resistance to E. granulosus infection in lambs J. Parasitol. (1981) 67: 797-799.*

13. *Al-Jorany, K.H.; Al-Ani, R.A. and Al-Shanawi, T.A. Increased candidacidal and leishmanidal activities of macrophagcs exposed to semi-perified rhizobium polysaccharides in vivo Iraq J. Biol. Sci. (1992): 14: 123.*

14. *Adam. D.O.: Eddson, P.J. & Koren, H. Methods fix studying mononuclear phagocytosis P. 63,261 Academic Press. New York (1981).*

15. *Sciki, K. and Sakabe, K. Sex hormones and the thymus in relation to thymocyte proliferation and maturation. Arch. Histol. Cytol. (1997) 6: 29-38.*

16. *Kaiscr. G.E. Phagocytosis - An Overview: http/www.Micro.msb. le.ac. uk/index.html. (2002).*

17. *Doring, G. and Dorner, F. A multicenter vaccine trial using the Pseudomonas aeroginosa flagella vaccine in patient with cystic iibrosis. Rehring Inst. Mitt. (1997) 98: 335-344.*

18. *Miller. L. and Hunt, J.S. Regulation of TNF-Alpha production in activated mouse rnacrophaes by progesterone. J. Immunol. (1998) 160: 5098-5104.*

19. *Baron, R.W. and Tanner, C.E. E. multiocularis in the mouse. The in vitro protoscolicidal activity of peritoneal macrophages. J. Parasit. (1977). 7: 489-495.*

20. *Sullivan, J. Immune system (http://wwmv.cellsalive.co. (2002).*

21. *Chao. T.C.; Vanalten, P.J. and Walter, R.J. Steroid sex-hormone and macrophages function-modulation of reactive oxygen intermediates and nitric release Am. J. Reprod. Immunol. (1994) 32: 43-52.*