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Study Some Immunological Parameters in Rabbits Immunized with *Cryptococcus neoformans*

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Abstract

The aims of this study were to prepare *Cryptococcus neoformans* killed whole cell antigen and evaluate their effects by using DTH- skin test and the differential white blood cells in local rabbits.

Fourteen animals of both sexes were divided randomly into two groups. The first group (7 animals) was immunized with 1 ml of *Cryptococcus neoformans* killed whole cell antigen (1x 10 cells/ml), subcutaneously. A booster dose was given after 14 days of the first dose. The second group (7 animals) was considered as control group. Based on results of DTH-skin test, no significant differences ($P< 0.05$) were recorded between the concentrations, 15 mg/ml and 7.5 mg/ml after 24, 48 and 72 hrs., but there was a significant differences ($P<0.05$) between these concentrations and 7.5 mg/ml and 3.75 mg/ml and control site. There was no significant difference between all types of cells (neutrophils, lymphocytes, monocytes, and eosinophils) of immunized and control groups.

Keywords: *Cryptococcus neoformans*, Local rabbit, Immune response, Experimental infection.

دراسة بعض المعايير المناعيه في الارانب الممنعة بخميره المكورات الخبيئة

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الخلاصة

هدفت الدراسة الحالية تحضير مستضد خميرة المكورات الخبيئه مقتولة الخليه الكاملة المعامله بلفورمالين و تقييم تاثيرها باستعمال فحص فرط الحساسيه المتاخر في الجلد وفحص عدد الخلايا البيض في الدم في الارانب المحليه . قسم اربعه عشر حيوانا ولكلا الجنسين عشوائيا الى مجموعتين المجموعه الاولى (سبعه حيوانات) حقنت بمقدار 1 مل في مستضد خميره المكورات الخبيئه مقتولة الخليه الكامله بتركيز 1×10 خليه /مل تحت الجلد . واعطيت جرعه تقويه بنفس المقدار بعد 14 يوما من الجرعه الاولى. حقنت حيوانات المجموعة الثانية (السيطرة) تحت الجلد 1مل من محلول دارئ الفوسفات الملحي (pH 7.2) اظهرت نتائج فحص فرط الحساسيه المتاخر في الجلد وجود فروقات معنويه ($p<0.05$) بين التراكيز 15 ملغم /مل و 7,5 ملغم/مل بعد مرور 48.72.24ساعه بينما لم تظهرفروقات معنويه ($p<0.05$) بين التراكيز المذكوره مع التركيز 7.5ملغم /مل ,3,75ملغم /مل ومنطقه السيطرة في هذا الفحص بينت النتائج عدم وجود فروقات

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معنويه ($p<0.05$) بين جميع انواع خلايا الدم البيض (العدله واللمفية ووحيده النواة والحمضه) في مجاميع الحيوانات الممتعة غير والغير ممنعة (مجموعة السيطره).

Introduction

Cryptococcus neformans var *neoformans* causes most cryptococcal infections. Tthe variety of this basidiomycetous fungus is found worldwide, isolated mainly from the dropping of pigeon(Clumba Livia), since it can contain high concentrations of creatinine and from debris pigeon or chicken dropping [1,2] it has been reported in wild and domestic mammals with sporadic cases in cats, dogs and sheep [3]. Also it was isolated from a severe outbreak of bovine mastitis [4,5]and isolated from caprine mastitis [6].

The incidence of infections by the encapsulated yeast *C. neoformans* has risen markedly over the past 20 years as a result of the HIV epidemic and increasing use of immunosuppressive therapies [7,8]. The major virulence factors of *C. neoformans* are its elaborate polysaccharide capsule, melanin, mannitol and mating type α. The principle constituent of capsular material is glucuronoxylomannann (GXM) that principly been implicated in multiple fungal mechanisms that evade or weaken host defense [9]. Previous vaccination attempts using one or more the cell wall components of such as mannoprotein(MP) which is in contrast to (GXM), the mannoprotein MP) a minor component of capsular material may be considered as an immunopotentaitin antigen involved in the induction of the cell mediated immune response[10], selected fungal subunits were also used for vaccines against *C. neoformans* [11].

The vast majority of global cases of cryptococcosis are caused by *Cryptococcus neoformans* , serotype A, D or AD [2]. Due to the importance of *C. neoformans* infections in human and domestic animals and the lack of attempts to prepare potential antigen against it infections, this research was conducted to detect the effect of killed whole cell antigen in local rabbits.

Materials and methods

1. *Cryptococcus neoformans* isolate was obtained from the zoonotic diseases unit\veterinary Medicine College_ Baghdad University. Killed whole cell antigen was prepared by using formaldehyde buffer saline 1% (KWCA)[12] and used in the immunization of rabbits and killed whole cell sonicated antigen (KWCSA) that used in delayed Type hyper - sensitivity (DTH)-skin test.
2. Immunization of rabbits Fourteen [14] local breed rabbits of both sexes (1.5-2 Kg. B.Wt.) were used, and divided randomly in to two groups: first group (7 animals) were immunized with 1ml of *C. neoformans* (Kwc Ag)containing 1 x 10 cells/ ml injected subcutaneously, and a booster dose 1ml(1x10 cells/ml) was injected subcutaneously 14 days later. The second group (7 animals) control was injected subcutaneously 1 ml of PBS (pH 7.2) and considered as a control.
3. Immunological tests.

a. Delayed Type Hype sensitivity (DTH)-skin test was done for all immunized rabbits in day 20 of immunization .as suggested by [13] with some modification, Using different protein concentrations of sonicated (KWCS) antigen of *Cryptococcus neoformans*, concentrated antigen used in a dose 15 mg /ml , 7.5 mg/ml and 3.75 mg /ml and PBS(PH 7.2) as a control region by intradermal injection of immunized animals.

b. Blood samples in day 20 of immunization 2ml of blood were collected from animals into EDTA containing containers and blood smear were prepared by staining Giemsa stain to estimate the differential white blood cell [14].

Statistical Analysis Data were expressed in absolute numbers and percentages .Proportions were compared at $P<0.05$ probability level, using the Statistical Package for Social Sciences (SPSS) version 16 software [15].

Results

Delayed Type Hyper sensitivity (DTH)-skin test

The result showed that the diameter of erythema (mm) after 24hrs.where (7.42±04) against concentrated antigen (15 mg/ml) and these values were significantly higher ($P<0.05$) than those at 72hr (4.57±0.42) and between 48 hrs. and 72 hr. but there was no significant differences($P<0.05$) between 24 hrs. and 48hrs.

The result showed that there was no significant difference in using 7.5 mg/ml after 24 hrs. (6.85±0.4) and 48 hrs.(6±0.3) than 72 hrs. (4.14±0.34) while in the concentration 3.75 also there was significant differences (P<0.05) at 24 hrs (5.57±0.42) and (4.54±0.29) at48hrs and (3±0.3) at 72 hrs . There was no significant difference (P< 0.05) recorded between the concentrations 15mg/ml and 7.5 mg/ml in all times but there was a significant difference between these concentrations and control site and also there was significant a difference was recorded between the concentration 3.75 mg/ml and control site. After 48 hrs. and 72hrs. also there was significant differences between all concentration and control site (PBS PH 7 .2) but there was a significant differences (P<0.05) between 15 mg/ml and 7.5 mg/ml than 3.75 mg/ml (Table-1).

Table 1- Means of skin erythema (mm) of immunized rabbits by *Cryptococcus neoformans*killed whole cell antigen.

TIME (HOURS) / CONCENTRATION	MEAN± S.E (mm)		
CONCENTRATed Ag (15 mg/ml)	7.42±0.04 Ca	6.00±0.53 Ca	4.57±0.42 Cb
1:2 Ag (7.5 mg/ml)	6.85±0.40 Ca	6.00±0.30 Ca	4.14±0.34 Cb
1:4Ag (3.75mg/Ml)	5.57±0.40 Ba	4.57±0.29 Bb	3.00±0.30 Bc
Control site PBS (PH 7.2)	1.28±0.18 Aa	1.28±0.18 Aa	1.00±0.00 Aa

- Capital letters, significant differences between groups, small letters significant differences with in groups.
- *P<0.05
- ~ N= 7 animals each treatment.

Differential white blood cells count:

There were no significant differences (P<0.05) between all types of cells (neutrophils, lymphocytes, monocytes, and eosinophils) between the immunized group and control group (Table-2).

Table 2- Means of differential white blood cells count in immunized rabbits by *Cryptococcus neoformans* killed whole cell antigens during DTH test.

Types of cells / Groups	Neutrophils %	Lymphocytes %	Monocytes %	Eosinophils %	Basophils %
Immunized group	36.71±5.37 A	53.00±5.59 A	5.00±0.57 A	4.85±1.93 A	0.42±0.29 A
Control group	44.85±3.77 A	47.57±3.49 A	5.57±0.61 A	0.59± 1.85 A	0.14±0.14 A

- N= 7 animals each group p<0.05

Discussion

Delayed Type Hyper sensitivity response is the principle pattern of cell mediated immunization protocols which elicited cell immune response in mediated immune response, that induced by CD4 and CD8 T cell [16] .The induration of the skin at the site of injection is may be due to accumulation of activated macrophages and other non-specific inflammatory cells in the dermis and between muscle fibers. The infiltration of these cells in the site of injection and a result of increase in blood vessels permeability will lead to formation of red appearance of skin. The antigen stayed in this site and help in the continuous stimulation of phagocytes that will accumulate and then digested the

antigen[17].These reflexes , the increase in the diameter of erythema that was recorded in this study by the synchronization between the antigen concentration and the diameter of erythema of *C. neoformans* sonicated antigen at the site of antigen injection . This finding is ingreement of [18] who revealed that the development of highly activated macrophages and their capacity to resist the infection by intracellular pathogens is correlated with onset of DTH and the expression depends in large part on the secretion of the cytokine by Th-1 cells [19]Generally CD4 Th1 subset lymphocytes (DTH lymphocytes) and in a few cases CD8 T cells also induce this response [20] that in agreement with our results in the increase the number of lymphocytes without significant differences in the immunized group compared with control group may be due to regeneration of lymphocytes from the bone marrow and migrated to the site of infection that is referred before [21], the phagocytic system is the earliest non-specific defense mechanism against microbe with the neutrophils, monocytes and eosinophils functioning as effectors and also responsible for ingestion And killing of bacteria, also complement system, resident macrophages and elicited inflammatory cells which were activated at the site of microbial Invasion[22] and the receptors found on lymphocytes which were require to recognize antigen. IL-l acts on bone marrow to stimulate the release Of neutrophils into the circulation causing neutrophilia ,and it chemotactic for neutrophils to attactes them to sites of yeasts activity by stimulating their oxidative metabolism also it is enhance the inflammation by degranulating basophils and mast cells and activation neutrophils and eosinophils to release their lysozomal enzyme [23].

TH1 cells, which induced via immunization with WSC AGs Activated the phagocytic cells which destroyed most disseminated C.neoformans in the internal organs [24]. The WSC Ags which used in the our study were containing all types of *C. neoformans* Ags particularly, MPs and these Ags are able to induce protective response against *C. neoformans* ,this observation was agreed with pietrella *et al*.[25] who provide evidence that MPs faster maturation and activation of human DCs and facilitate expression of co-stimulatory molecules such as CD40, CD86, CD80 and MHC class II and I.

According to these evidences we postulated that WSC AGs will stimulate production of INF- γ and TNF- α which activated the macrophages and increased fungicidal ability to kill and destroy the disseminated *C. neoformans.*

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