Using of Brucellins and Their Fractionation Peaks in Immunization Against Brucellosis

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Abstract

A number of criteria have been considered during this study. These criteria include preparation and fractionation of brucellins using chromatography. Several peaks were obtained in each brucellin fractionation.

The third criteria was immunization of 5 groups of guinea pigs .The fourth criteria was the using of serum of immunized animals in ELISA test against peaks resulted from fractionation . Peak 1 of Rev1 brucellins show the highest positive results and considered as the responsible part of immunization against brucellosis .

استخدام القمم الناتجة عن التحليل بعمود الفصل في التمنيع ضد مرض البروسيلوس

الخلاصة

تضمنت هذه الدراسة العديد من المحاور وهي: تحضير وتجزئة البروسلين باستخدام عمود الفصل (الكروموتوغرافي) إذ تم استحصال عدة قمم لكل بروسلين.

تم تمنيع خمسة مجاميع من خنازير غينيا لدراسة القابلية التمنيعية لكل قمة. ثم تم استخدام مصل الحيوانات الممنعة لقياس القابلية المناعية بجهاز فحص المقايسة المناعية بالانزيمات (ELISA) مقابل القمم المفصولة بعمود الفصل وتبين ان القمة الأولى للبروسلين Rev 1 أعطت أعلى نتيجة موجبة واعتبر هو الجزء الأكثر كفاءةً في التمنيع ضد الـ Brucellosis .

Introduction

Brucellosis is one of the most important zoonotic diseases through over the world due to its economic and hygienic effects[1].

The studies of using brucellins were started in early eighteenth [2] who used peptidoglycan extracted from *Brucella abortus* to immunize mice against brucellosis.

In 1986 Merieux institute prepare peptidoglycan extracted by phenol from *Brucella suis* to vaccinate guinea pigs against the same bacterial infection. [3].

R- Lipopolysaccharide was used with addition of outer membrane protein 31 in vaccination against *Brucella ovis*(4).

Lipid "A" was used to support immunity against Brucellosis [5]. Non- covalent complex of *N. meningitidis* with cellular protein and Lipopolysaccharide of *Brucella melitensis* was used intranasally and a strong immunity was detected against Brucellosis [6].

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Our study was designed to detect the more specific part of brucellins, which play the specific role in immunization by fractionation of the brucellin and examining their parts in ELISA test.

Material and Methods

1- Vaccination strains, which used are *Brucella abortus* S19 and *Brucella melitensis* Rev 1. They brought from Brucellosis and Tuberculosis control center/ Ministry of Agriculture.

2- *Brucella* was cultured and harvested on Trypticase soya agar, which prepared as instructions of BBL Company.

3- Two brucellins were prepared from each strain. The First according to [7] i.e. extraction by Trichloroacetic acid (TCA) and the Second according to Merieux [8], i.e. extraction by phenol.

4- Fractionation was done by Column Chromatography using 80×2.5 ml. tubes, Sephadix G150, filtration volume was 2 ml in an average of 80 tubes, standard solution speed was 120 ml/ h and the separation system was 2070 Ultrorac of LKB Company.

5- Optical densities were read using ultraviolet spectrophotometer at 280 Nanometer wave length and protein concentration was done using Biuret method.

6- Five groups of 10 guinea pigs in each group were used. Each group immunized with a type of Brucellin in two doses with 14 days intervals. The fifth group was the control group, which gave PBS instead of brucellin in the same doses.

7- The peaks resulted from Chromatography examined in ELISA test against serum of different groups.

8- ELISA test was done using Synbiotic Kit and according to this company instructions and the reading 0.934 nanometer was the separating reading between negative and positive results.

Results

The results of Chromatography of S19 Bercovich brucellin show 2 peaks, the First at the tubes 10-33 and the second at the tubes 34-59. Protein concentration of peak one was 1.3 mg /ml. and peak 2 was 1.2 mg / ml.

Brucellin of S19 Merieux show 3 peaks at tubes 10-22, 30-40 and 40-52 respectively.

Peak1 have protein concentration 0.9 mg/ ml., peak 2 was 0.7 mg / ml. and peak 3 was 0.5mg / ml.

Rev. 1 brucellin extracted by Bercovich method show 3 peaks at 15-34, 35-45 and 46-55 respectively. With protein concentration of Peak1 was 1.6 mg/ ml., peak 2 was 0.9 mg / ml. and peak 3 was 0.9mg / ml.

Merieux brucellin of Rev. 1 strain show 4 peaks at 6-23, 24-38, 39-54 and 59-65 respectively. The protein concentration was 1.8 mg/ ml in Peak1., 0.6 mg / ml. in peak 2 , 1.6 mg/ ml in peak 3 and 0.6mg / ml. in peak 4.

Results of Chromatography were shown in the figures 1,2,3,4.

The results of ELISA Shows the highest optical density to peak 1 of Bercovich Rev.1 brucellin and of peak 1 of Merieux Rev.1 brucellin when examined against the serum of animal immunized with Rev. 1 Bercovich brucellin .

They were 2.311 and 1.939 nanometer respectively.

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These two peaks show O.D. of 1.993 and 1.617 nanometer respectively when examined against the serum of animal immunized with Rev. 1 Merieux brucellin.

Other peaks show lower O.D. and Some of them show negative results.

The results of ELISA test were shown in table 1, 2.

Discussion

The presence of high protein peaks in brucellin prepared according to Bercovich supported by the study of [3], they got no peaks when they prepare brucellin using Ethanol and Ammonium sulphate. Protein concentration of Peaks were nearly the same concentration that mentioned by [9] in their study on Merieux brucellin of *Brucella abortus*.

In this study, we show differences in peaks numbers between brucellins of Bercovich and Merieux. Peaks in Merieux was highest than Bercovich this may be due to the role of phenol in separation of pyramine bounded[10].The highest O.D. of peak 1 of Bercovich brucellin and peak1 of Meriux brucellin may refer to their specificity due to their contents of antigens that are responsible for immune responces.

References

[1]OIE Manual of diagnostic tests and vaccines for terrestrial animals, 5th edition, 2004 part 2, section 2.3, chapter 2.3.1.

[2]Bosseray, N. (1983). Vaccine and serum – mediated protection against *Brucella* infection of mouse placenta. Br. J. Exp. Pathol. 64: 617-625.

[3]Protocol Institute Merieux (1986). Vaccine against Brucellosis for human use Lyon.

[4]Cox, J. C. and Coulter, A. R. (1997). Adjuvants - a classification and review of their modes of action. Vaccine 15: 248-256.

[5]Tabatabai, L. B.; Pugh, Jr. G. W.; Stevens, M. G.; Phillips, M. and McDonald, T. J. (1992). Monophosphoryl lipid A-induced immune enhancement of *Brucella abortus* salt-extractable protein and lipopolysaccharide vaccines in BALB/c mice. Am. J. Vet. Res. 53: 1900-1907.

[6]World Health Organization (WHO) (1998). The development of new/improved Brucellosis vaccines: report of a WHO meeting 11-12 December 1997, Geneva, WHO, Geneva 19-21.

[7]Bercovich, Z.; Laak, E. A. ter, and Lipzigj, H. H. Van. (1992). Detection of brucellosis in dairy herds after an outbreak of the disease using a delayed-type hypersensitivity test. Prev. Vet. Med. 13: 277-285.

[8]Woodard, L. F. and Toone, N. (1980). Allergic activity and biochemical analysis of three soluble antigen preparations from *Brucella abortus* strain 45/20. Am. J. Vet. Res. 41 (1): 114-116.

[9]Duclose, P. J.; Bentejac, M. C.; Serre, A. and Bascoul, S.(1989). Skin test reaction to a phenol-soluble antigen of *B. abortus* among veterinary students, Lyon, France, International. J. Epidemol. 18 (2): 446-450.

[10]Kabat, A. (1976). Structural concepts in immunology and immunochemistry 2nd edition , New York. HOH Rinehart and Winston.

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Table (1) Show O.D. of peaks of Berchvich brucellins.

Rev1				S19		Type of brucellin
P4	P3	P2	P1	P2	P1	
0.071	0.621	1.091	2.119	1.002	1.371	S19 Berchvich
0.089	0.501	1.003	2.001	1.017	1.311	S19 Meriux
0.063	0.923	1.130	2.311	1.101	1.181	Rev1 Berchvich
0.055	0.612	1.033	1.993	0.812	1.212	Rev1 Meriux

Table (2) Show O.D. of peaks of Merieux brucellins

Rev1			S19			Type of brucellin
P3	P2	P1	P3	P2	P1	
0.810	1.003	1.911	0.903	1.001	1.130	S19 Berchvich
0.603	0.801	1.599	0.811	0.821	1.119	S19 Meriux
0.822	1.019	1.939	1.001	0.893	1.091	Rev1 Berchvich
0.731	0.812	1.617	0.601	0.803	1.103	Rev1 Meriux

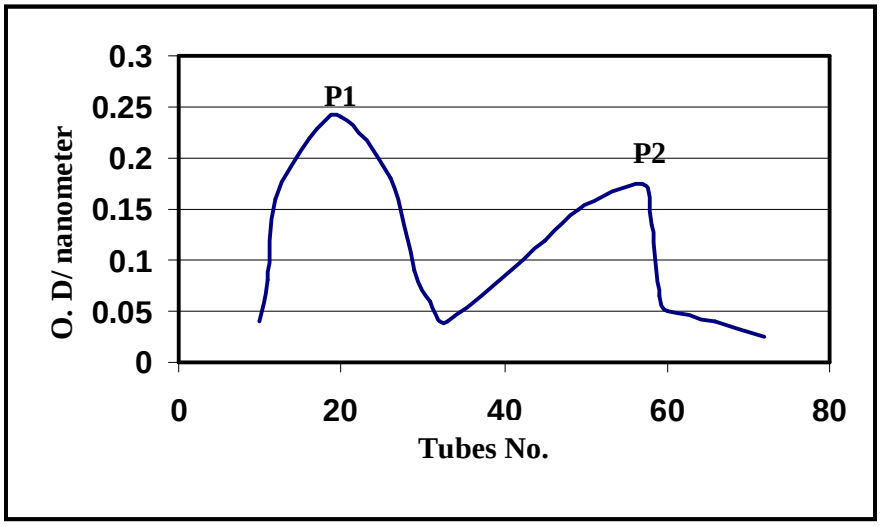

Figure (1) Show results of S19 Bercovich brucellin fractionation.

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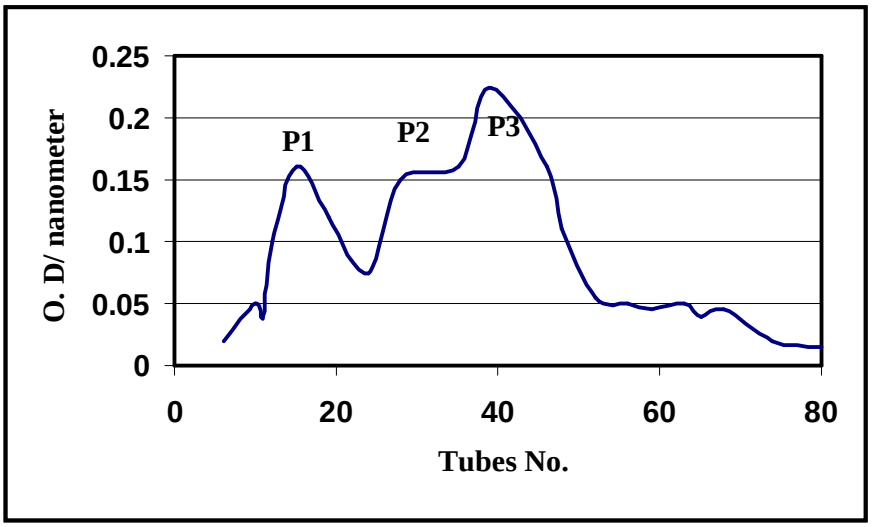

Figure (2) Show results of S19 Merieux brucellin phenol fractionation.

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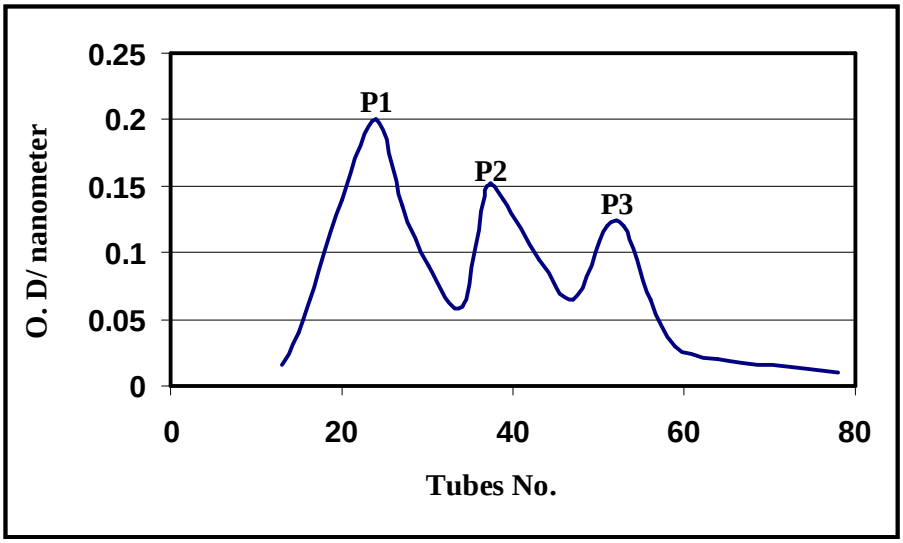

Figure (3) Show results of S19 Merieux brucellin fractionation.

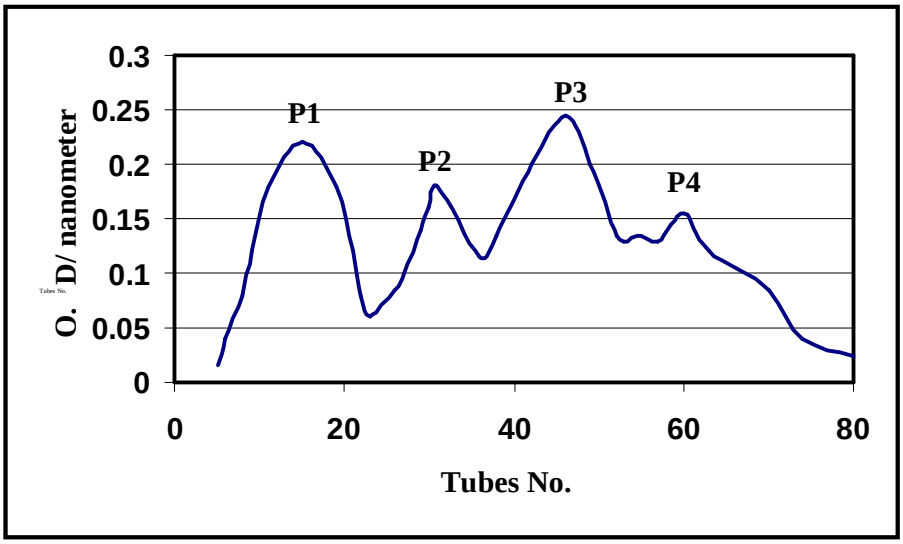

Figure (4) Show results of Rev1 Merieux brucellin fractionation.

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