Evaluation of *Proteus vulgaris*fimbriae antigen by delayed type hypersensitivity (DTH)-skin test in rabbits

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Summary

The aim of this study is to evaluate the use of fimbriae antigens for immunization of rabbits against *Proteus vulgaris* bacteria results showed a higher significant difference (P<0.05) in erythema diameters in the immunized groups in compared with the control. There was no significant difference between both immunized groups 200 µg/ml and 100 µg/ml after 24 hours. Also, a higher significant differences (P<0.05) in the diameter of induration is recorded in both immunized groups compared to control site, a higher significant differences (P<0.05) in the immunized group (100 µg/ml) at the concentration 200 µg/ml compared with 100 and 50 µg/ml after 24 hours, as follow as after 48 hours except significant differences between 200 and 100 µg/ml concentration (P<0.01) and increase induration after 72 hours between both immunized groups; within groups and control site. Conclusion that the fimbrial antigen have the ability to elicit cellular immune response by delayed type hypersensitivity (DTH).

Key words: *Proteus vulgaris*, fimbriae, antigen.

تقييم مستضد الخمل لجرثومة المتقلبة الشائعة (*Proteus vulganis*) بفحص فرط الحساسية الجلدي المتاخر في الارانب

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الخلاصة

هدفت الدراسة الى معرفة امكانية استعمال مستضد الخمل (fimbriae antigen) في تمنيع الارانب ضد *Proteus vulganis* فاظهرت النتائج وجود ارتفاعا معنويا (P<0.05) في قطر الاحمرار (erythema) في المجموعتين الممنعتين بالمستضد مقارنة مع السيطرة، في حين لم تسجل هناك فرقا معنويا بين جرعتي التمنيع 200 مايكروغرام/مل و 100 مايكرغرام/مل بعد مرور 24 ساعة، كذلك كان هنالك ارتفاعا معنويا (P<0.05) في قطر التثخن (induration) في المجموعتين الممنعتين مقارنة مع السيطرة. وكان هذا الارتفاع معنويا (P<0.05) في المجموعة الممنعة بجرعة 100 مايكروغرام/مل عند تركيز 200 مايكروغرام/مل مقارنة مع تركيزي 100 و50 مايكروغرام/مل بعد مرور 24 ساعة، وكذلك بعد مرور 48 ساعة فيما عدا وجود فرق بين تركيزي 200 و100 مايكروغرام/مل. وكان ارتفاع التثخن معنويا (P<0.01) أيضا بعد مرور 72 ساعة بين المجموعتين الممنعتين وضمن كل مجموع ومع السيطرة. نستنتج من ذلك بان مستضد الخمل له القدرة على تحفيز الاستجابة المناعية الخلوية من خلال فرط الحساسية المتاخر (delayed type hypersensitivity-DTH).

Introduction

Bacterial disease of the intestinal tract can be highly communicable and may spread in epidemic fashion. Their agents enter the body mainly through the mouth in contaminated food or water as a result of direct contacts with infected persons (1). The genus *Proteus*, which contains bacteria considered now to belong to the opportunistic pathogens widely distributed in nature (2).

*Proteus vulgaris*is a rod-shaped bacillus, Gram negative bacterium that inhibits the intestinal tracts of animals, but it can also be found in soil, stagnant water, standing water, fecal matter, raw meats and dust; it is in the proteobacteia and considered to be pathogenic. In human; it can cause many different types of infection including urinary tract infections, wound infections and it is a common cause of sinus and respiratory tract infections (3). The invader, *Proteus vulgaris*have numerous virulence factors including fimbriae, flagella, outer membrane proteins, lipopolysaccharide, capsule antigen, urease, immunoglolin A protease, hemolysins, amino acid deaminases and swarming growth, enabling them to colonize and survive in higher organisms (2). It had many flagella completely around the organism (these are called peritrichous-flagella) (4).

Materials and Methods

1. *Proteus vulgaris* strain: The bacterial isolate was obtained from zoonoses unit/Veterinary Medicine College/ Baghdad University, which was documented by the central health laboratories of ministry of health. That maintance in urea base agar for preparation of fimbriae antigen according to the method described by (5) method and protein concentration was measured by Biuret method as described by (6).
2. Experimental Animals:Ten rabbits between 1-1.5 kg body weight were used which dividing randomly into two groups (each 5 animals). The first group was immunized subcutaneously (S/C) in a dose of 200 µg/ml by fimbriae antigen while the second group was immunized subcutaneously in a dose of 100 µg/ml of each animal by fimbriae antigen (7).

   All animals were given booster dose (as the same doses above of each group) after 14 days of the first immunization dose.
3. Delayed type hypersensitivity-skin test: This test was done after 28 days after immunization of each animals by using fimbriae antigen in a dose of 0.1 ml at concentration 200, 100, 50 µg/ml and phosphate buffer saline (pH 7.2) as control site, intradermal (I/D) in the flank region that was divided into four sites of injection (8). And the results were read after 24, 48 and 72 hours.

4.Stastical Analysis: Data were analyzed statistically by using student (t) test (9).

Results

The skin reaction (erythema and induration) was determined for all immunized animals. The results were showed that the first group which immunized with 200 µg/ml had a significant differences (P<0.05) at concentration 200 µg/ml compared with concentration 50 µg/ml in erythema diameter after 24 hours on the same hand of the second group that immunized with 100 µg/ml. while there is no significant differences (P>0.05) between the two immunized groups at different concentration, and significant differences (P<0.01) compared with control site, table (1).

Table 1: Erythema of delayed type hypersensitivity in the immunized rabbits with *Proteus vulganis* fimbriae antigen after 24 hours.

Mean±S.E. (mm) / Animals groups	Antigen concentration			PBS
	200 µg/ml	100 µg/ml	50 µg/ml	
First group (200 µg/ml)	6.75±0.25 aA*	6±0.4 aA	4.75±0.47 bA	1.5 ± 0.28
Second group (100 µg/ml)	6±0.4 aA*	5.5±0.28 abA	4.75±0.47 bA	

-*p<0.01

-Different small and capital letters mean significant difference (P<0.05) within and between groups respectively.

The induration (mm) was showed there was no significant differences in the first group while a higher significant differences (P<0.01) in the second group that immunized with 100 µg/ml also there was no significant differences (P>0.05) between both immunized groups on the other hand there was a higher significant differences (P<0.01) between both groups compared with the control site, table (2).

Table 2: Indurations of delayed type hypersensitivity in the immunized rabbits with *Proteus vulganis* fimbriae antigen after 24 hours.

Mean±S.E. (mm) / Animals groups	Antigen concentration			PBS
	200 µg/ml	**100 µg/ml**	**50 µg/ml**	
First group (200 µg/ml)	**6±0.40 aA***	**5.5±0.33 aA***	**5±0.40 bA***	**1.5 ± 0.33 B**
Second group (100 µg/ml)	**6.75±0.25 a*A***	**5.5±0.28 bA***	**5.5±0.50 bA***	

-*p<0.01

-Different small and capital letters mean significant difference (P<0.05) within and between groups respectively.

While there was a higher significant differences (P<0.01) within the first group; but the second group showed a significant differences between groups at concentrations 200 and 100 µg/ml with 50 µg/ml; the groups showed a higher significant differences (P<0.01) compared with control site after 48 hours, table (3).

Table 3: Indurations of delayed type hypersensitivity (DTH) in the immunized rabbits with *Proteus vulganis* fimbriae antigen after 48 hours.

Mean±S.E. (mm) Animals groups	Antigen concentration 200 µg/ml	100 µg/ml	50 µg/ml	PBS
First group (200 µg/ml)	6±0.40 aA*	5.5±0.33 aA*	5±0.40 aA*	1.5 ± 0.33 B
Second group (100 µg/ml)	6.75±0.25 bA*	5.5±0.28 bA*	5.5 ±0.50 a*A*	

-*p<0.01

-Different small and capital letters mean significant difference (P<0.05) within and between groups respectively.

At 72 hours the two immunized groups showed a significant differences (P<0.01) within each group and with control site, table (4).

Table 4: Indurations of delayed type hypersensitivity (DTH) in the immunized rabbits with *Proteus vulganis* fimbriae antigen after 72 hours.

Mean±S.E. (mm) Animals groups	Antigen concentration 200 µg/ml	100 µg/ml	50 µg/ml	PBS
First group (200 µg/ml)	6.12±0.31 a* A*	3.8±0.23 b* A*	2.3±0.23 C A*	0.75 ± 0.00 B
Second group (100 µg/ml)	5.6±0.23 a* A*	4.25±0.32 b* A*	2.75±0.14 C A*	

-*p<0.01

-Different small and capital letters mean significant differences (P<0.05) within and between groups respectively.

When compared the effect of concentration of immunized groups. Table 5 showed there was no significant differences (P>0.05) at concentration 200 µg/ml, but there was a higher significant differences (P<0.01) at concentration 100 and 50 µg/ml within groups after 24, 48 and 72 hours. Also there was a higher significant differences (P<0.01) between all concentrations at 48 and 72 hours compared with the control site.

Table 5: Indurations of delayed type hypersensitivity in the immunized rabbits with *Proteus vulganis* fimbriae antigen at concentration 200 µg/ml.

Hours Antigen concentration	Mean±S.E. (mm) 24	48	72
200 µg/ml	6 ±0.40 A	6.37 ±0.37 A	6.12 ±0.31 A*
100 µg/ml	5.5 ±0.28 aA	4.75 ±0.25 bB*	3.8 ±0.23 c*CE*
50 µg/ml	5 ±0.40 aA	3.5 ±0.20 bC*	2.3 ±0.23 C*DF*
Control P.B.S.	1.5 ±0.28 a* B*	1.3 ±0.23 aD*	0.75 ±0.0 bB*

-*p<0.01

-Different small and capital letters mean significant differences (P<0.05) within and between groups respectively.

At concentration 100 µg/ml, there was a higher significant differences (P<0.05) within the group 200 µg/ml after 24 and 48 hours with 72 hours. Also at concentration 100 µg/ml but at 50 µg/ml, there was a significant differences (P<0.01) at all period (24, 48 and 72 hours).

There was a higher significant differences (P<0.01 and P<0.05) between all concentration compared with control site in the same hand there was a significant differences (P<0.01) between all concentration at 72 hours, table (6).

Table 6: Induration of delayed type hypersensitivity in the immunized rabbits with *Proteus vulganis* fimbriae antigen at concentration 100 µg/ml.

Hours Antigen concentration	Mean±S.E. (mm)		
	24	48	72
200 µg/ml	6.75 ±0.25 aA	6.25 ±0.32 A	5.6 ±0.23 bA
100 µg/ml	5.5 ±0.28 aB*	5.62 ±0.23 C*aA	4.25 ±0.32 bB
50 µg/ml	5.5 ±0.50 a*B	3.37 ±0.23 bB	2.75 ±0.14 cC
Control P.B.S.	1.5 ±0.28 bD*	1.3 ±0.23 aC*	0.75 ±0.0 c

-*p<0.01

-different small and capital letters mean significant differences (P<0.05) within and between groups respectively.

Discussion

The delayed type hypersensitivity skin test is a simple test, which helps in the diagnosis of certain infectious diseases. The test detects cutaneous (skin) hypersensitivity to antigens which done to find whether the individual was already exposed (sensitized) to a particular antigens, the antigens were injected intradermally into the skin. Appearance of swelling (called induration) in 48 to 72 hours suggests that the individual was already exposed to the antigen, which were injected into the skin (10), that was agree with our results the highest indurations were recorded after 48 hours then they were declined at 72 hours. Generally CD_4^+ Th_1 subset of lymphocytes (TDTH lymphocytes) induce the DTH response. However, in few cases CD_8^+ T cells also induce DH responses; cytokines TNF and IL-1 induce expression of adhesion molecules on endothelial cells of blood vessels, which in turn lead to the infiltration of the site by moncytes and lymphocytes from blood, the infiltrating lymphocytes and monocytes secrete a number of cytokines and the area is inflammed, the peak of cellular infiltration is reached at 48 to 72 hours. The majority of lymphocytes are CD_4^+ T cells with small number of CD_8^+ T cell (9).

The inflammation involves the activation and directed migration of many different cells, especially neutrophils and macrophages from the blood

stream to sites of invasion. The binding of LPS to the protein CD_{14} which bind to the macrophages surface and activates them to triggers cytokines production. Pili and fimbriae are short projections that cover the surfaces of some Gram negative bacteria, Pili attach the bacteria to other bacteria and play a role in bacteria conjugation. Fimbriae may have an important protective function since they can present bacteria from sticking to body surfaces (11). The proteus species possess an extracytoplasmic outer membrane; contains a lipid bilayer, lipoproteins,polysaccharides, and lipopolysacchrides, various components of the membrane of proteus species initiates several events in the mucosal endothelial cells, including secretion of (interleukin-6) and (interleukins-8) (12). On the same hand, the antigens found on the outer membrane of *Proteus mirabilis* can potentially serve as target for vaccines, so far of the 37 identified immuno-reactive antigens, 23 are surface-bound proteins (13). Also who agree with (14) who refered to the cellular immune response of *Proteus vulganis* that showed a significant differences ($P<0.05$) in erythema of fimbriae antigen compared with LPS antigen after 24 hours, but there was no significant differences ($P>0.05$) were seen between both antigens in an induration after 24 hours of skin test; fimbriae antigen gave a higher antibodies titer than LPS antigen group and the bacterial isolates from spleen and liver were less in the fimbriae antigen followed LPS antigens, although, this agree with our results that the fimbriae antigens were gave a good cellular immune response and we can suggest to use it as a good vaccines for protection against *Proteus vulganis* in both doses (200 and 100 µg/ml) that were used with no significant differences between them.

References

1. Morello J A Mizer H E and Granato PA (2006). Laboratory manual and work book in microbiology applications to patient care. 8th ed. Th McGraw Hill Comp., 186-206.
2. Rozalski A Sidorczyk Z and Kotelka K(1997). Potential virulence factors of proteus bacilli. Microbial. Mol Bio Rev 61(1):65-89.
3. Baron S (1996). Medical microbiology. 4th ed.; University of Texas medical branch, Texas.
4. Vincent WH (2005). Infections cause by the genus proteus. Quest Diagnositic. 12:23-27.
5. Wary S K Hull S L Cook R G Barrish J and Hull R A(1986). Identification and characterization of auroepithelial cell adhesion from auropathogenic isolate of *Proteus mirabilis* Infect Immun 54(1):43-49.
6. Jozef H M and Harro P (2002). "Urea" in ulmann's encyclopedia of industrial chemistry. Whiley-VCH, Weinheim.
7. Li X Lockatell C V Lohanson D E Lane M C Warren J W and Mobley H L (2004). Development of intranasal vaccine to prevent urinary tract infection by *Proteus mirabilis*. Infect Immun Jan 72(1):66-75.
8. Hudsen L and Hay F C (1983). Practical Immunology 2nd ed. Black Well Scientific Publications, pp:328-340.
9. المحمد، نعيم ثاني احمد والراوي، خاشع محمود يونس، مؤيد احمد والمراني، وليد خضير (1986). مبادئ الاحصاء. دار الكتب للطباعة والنشر. جامعة الموصل.
10. Jayapal V (2007). Fundamentals of medical Immunology. 1st ed. Jaypee Brothers, medical Publishers LTD. New Delhi; pp:180-183.

11. Tizard I R (2009). Veterinary immunology an introduction. 8th ed. Saounders Elisevier. Pp:11-27, 81-88 and 369-379.
12. Struble K Bronze M S Jackson RL and Gonzalez G (2009). Proteus infections htp//emedicinemedscape.com.ct.
13. Nielubowicz G R Sara N S Harry L T M (2008). Outer membrane antigens of the uropathogen *Proteus mirabilis* recognized by the humoral response during experimental murine urinary tract infection Infect Immun 76(9):4223-4231.
14. Razook B R (2010). The isolation of *Proteus vulganis* from human and sheep urinary tracts to evaluate some antigens for immunization in rabbits. MSc thesis. Vet Med College-Baghdad University.