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Some aspects of immune response to lipopolysaccharides of *Providencia rettgeri*

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Abstract:

LPS of *P.rettgeri* was extracted and purified in our previous study, the present study found that the injection of 150µg/ Kg of LPS (intramuscularly and subcutaneously) was able to stimulate humoral immune response at both systemic and mucosal levels which was detected by passive haemmaglutination test and the mean values of systemic antibody titer (6826.6) was higher than the mucosal (at appendix) antibody titer (512). Some cytokines concentrations such as IL-2 ,IL-4,IL-6 and IL-1ß were detected using ELISA kits. The study was observed that, the concentrations of IL-2 (3.21 pg/ml) , IL-4(5.84 pg/ml) and IL-6 (4831 pg/ml) were increased significantly in LPS immunized animals group compared with control group which were 2.05 , 2.34 and 3121.06 pg/ml for IL-2,IL-4 and IL-6 respectively, while the concentration of IL-1ß (115.7 pg/ml) was decreased significantly in LPS immunized animal group than control group which was 177.24 pg/ml.

introduction:

The genus *Providencia* is a member of the *Enterobacteriaceae* family which consists of eight species (Galac and Lazzaro,2011).*Providencia rettgeri* is one of five *Providencia* species that is known to cause various infections, especially the urinary tract infection(UTI) (Cho *et al*.,2010). The rise in importance of this species is associated with its tendency to cause nosocomial infections and with its marked resistance to numerous antibiotics (Shiroto *et al*.,2005).Endotoxins, also called lipopolysaccharides (LPS) is the major component of the outer membrane of the cell wall of Gram-negative bacteria, which is considered as a virulence factor of pathogenic bacteria, including *Providencia* (Penner *et al*.,1992).

The lipopolysaccharide (LPS), as in other members of the family *Enterobacteriaceae* , consists of three domains, an endotoxic glycolipid (lipid A), an O-polysaccharide (O-PS) chain or O-antigen, and an intervening core oligosaccharide (OS) region ,the O-antigen is the major surface antigen, and its serological O specificity, in contrast to that of other Gram-negative bacteria (Aquilini *et al*.,2010). The lipid A is the most conserved part of endotoxin and is responsible for most of the biological activities of endotoxin, i.e. its toxicity and the lipid A form of *P .rettgeri* warrants maximal immunostimulatory activities (Lukasiewicz *et al*.,2010).

Bacterial lipopolysaccharide play a critical role in the initiation of the proinflammatory events that contribute to the pathogenesis of the sepsis and the pathophysiologic mechanism(s) responsible for this illness are thought to result from the noncytotoxic interaction of LPS with mammalian host inflammatory mediator cells monocytes/macrophages constitutes a major mechanism responsible for innate immune response to Gram-negative infection (Luchi and Morrison,2000). LPS was a potent stimulator of macrophages ,B-cell and T-cell activation in vivo were recognized at same time in both early and late responses to LPS and macrophages released various multifunctional cytokines, such as IL-1 and TNF-α (Bachmann and Oxenius,2007). Endotoxin elicits a wide variety of pathophysiological effects, such as endotoxin shock, tissue injury, fever, sickness behavior and death ,animal studies

indicate that these effects are mediated by cytokines, particularly IL-1ß,IL-6,IL-2, and TNF-α (Ogikubo *et al*.,2004; Bagchi and Sinha,2005).Locally little studies about the *Providencia* especially, the virulence determinant like LPS. Therefore, this study was aimed to investigate some aspects of immune response occurring at mucosal and systemic levels and detection of some systemic cytokines concentration after immunization of rabbits with *Providencia rettgeri* LPS.

Materials and methods:

1-Bacterial strain:

P. rettgeri strains were isolated from urinary tract infections (UTIs). Traditional biochemical tests were used for final identification of bacterial isolates (Macfaddin ,2000) .And the confirmed identifications to species level were also carried out by using Hi25 E system (Enterobacteriacea identification system, Himedia,India).

2-Extraction and purification of LPS :

An isolate of *P. rettgeri* from a patient with UTI were cultivated for extraction.Endotoxin extraction was carried out according to method of Mirzaei *et al*., (2011) and purification of LPS was carried out according to methods of Mannel and Mayer (1978) ,Luchi and Mrrison (2000) and Perdomo and Montero (2006).

3-Bioassay of endotoxin :

This was carried out according to method of Rezania *et al*.(2011)

4-experimental animals:

Six (6) of local rabbits (*Oryctylagus conniculus*) ,each rabbit was about (1.5-2kg) of weight were selected as test experimental animals and left for two weeks for adapted conditions and kept at libitum and then divided into two groups .

5-Immunization Protocol:

First group of animals was injected with 1 ml of LPS dose (300µg/ml) intramuscularly and subcutanously , while the other group was injected with 1 ml of normal saline and considered as control group .LPS and normal saline doses were administrated weekly for four weeks, on the fifth week all groups were left for one week then they were ready for immunological investigations (Shnawa and Thewaini,2001). The sera were separated from the whole blood , then divided into 0.5 ml small tubes, and stored at - 20˚C till testing time.Entero mucosal sampling (Appendix) were collected from all immunized rabbits according to (Mancini,1965).

6-Passive haemgglutination test (PHA):

This was carried out by method of Garvey *et al*., (1977) to determine antibody titer in serum and mucosal rabbits that was immunized with LPS compared with control animals group.

7- Detection of secreted cytokines:

To determine the concentration of systemic cytokines :IL-1β, IL-6, IL-2 and IL-4.ELISAs were conducted using commercial assay kits (IL-1β Stress gen.com, USA; IL-6,Bender Med.system, USA; IL-2 and IL-4 ,eBioscience, USA) according to the manufacturers' protocols. All experiments were performed in triplicate. Results are shown as mean ± standard deviation(SD).

Results:

1-specific antibody response in rabbits:

LPS immunoprimed rabbits showed specific LPS mucosal and systemic antibodies .The systemic titer was higher (6826.6) than mucosal titer (512) (Table 1) and there were significant differences between systemic and mucosal antibody titer ($P< 0.05$) .

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Table (1) : Specific antibody in rabbits immunized with *P.rettgeri* LPS antigen.

Animal group	Systemic titer Mean ±S.D	Mucosal titer Mean± S.D	P-value†
Control group	20	2	0.003*
Immunized group with LPS(150µg/kg)	6826.6±295	512± 0	

*P< 0.05 is significance . † Value of P is for control group compared with LPS group , S.D=Standard deviation.

2-Detection of cytokines:

Systemic IL-2 and IL-4 concentrations showed significant increase (P<0.05) in rabbits which were immunized with LPS antigen than control rabbits group, and the mean values of IL-2 and IL-4 concentrations for LPS group were (3.21±1.02 pg/ml) and (5.84 ±0.9190pg/ml) respectively , while the mean values for control groups were (2.05± 0.101 pg/ml) and (2.34± 0.103 pg/ml), respectively (Table 2) . IL-6 concentration showed significant increase (P<0.05) in LPS rabbits group (4831.57±181.03 pg/ml) than control rabbits group (3121.06±438.56 pg/ml) as shown in table 2.

On the other hand, IL-1ß was showed significant decrease (P<0.05) in rabbits group which were immunized with LPS antigen compared with control rabbits group and the mean values of concentrations were (177.24±54.65pg/ml) for control group and (115.7±6.612pg/ml) for LPS group (Table 2).

Discussion:

The result in table (1) showed that systemic antibody titer in rabbits immunized with LPS antigen was higher than mucosal (appendix) titer and a significant differences between systemic and mucosal antibody titer (P< 0.05).

This result agree with local study of AL-Khafagee (2010) who found that LPS of *Citrobacter freundii* cause increase in systemic antibody titer which was higher than mucosal titer .

The antibody-producing cell of mice responses to a protein isolated from the outer membrane of *Proteus mirabilis* and this protein in turn significantly increased the immune response to lipopolysaccharide and also converted this response from predominantly immunoglobulin M to predominantly immunoglobulin G (Nielubowicz *et al*.2008).

The gut-associated lymphoid tissues (GALT) such as Peyer's patches or rabbit appendix represent large lymphoid aggregates in the intestinal wall and contain many follicles packed with proliferating B cells, progenitors of the plasma cells that manufacture antibody during immune response(Stevens ,2010).

The result in table 2 showed that there was significance increase in the concentrations of IL-2 and IL-4 in rabbits which were immunized with LPS antigen than control rabbits group .This result was converged with other studies such as Bagchi and Sinha (2005) who demonstrated that mice immunized with the 57 kDa outer membrane protein of *Shigella dysenteriae* was found to increase the level of IL-2 significantly and Bhatia and Basu (2007) who observed that, LPS-activated macrophage secretes inflammatory mediators like IL-2.

The lower level of IL-2 in serum could be due to the fact that production of cytokines is transient at early time points after the microbial encounter (Fidan *et al*.,2010).

IL-2-receptor stimulation via IL-2 did not significantly induce the release of IL-2 with consistent intracellular Ca+2 production, this indicate that phosphotidylinositol-3 kinase-mediated signals are up-regulated through intracellular free [(Ca+2)i], which is essential for Th1-type responses,the TCR up-regulates the protein kinase C (PKC)-mediated phosphorylation in CD4T cells, which in turn leads to IL-2 production (Bagchi and Sinha,2005) .

The Th2 cells which produce IL-4, are primarily responsible for antibody-mediated immunity ,IL-4 is one of the key cytokines regulating Th2 immune activities and helps drive antibody responses in a variety of diseases (Stevens,2010).

Our result also showed that IL-6 concentration was significantly increased in rabbits immunized with LPS compared with control rabbits group .On the other hand the result in table 2 was showed that there was significant decrease in the concentration of IL-1ß in LPS group than control group.This result agree with other study such as Ranallo *et al*.(2010) who observed that the level of IL-6 was elevated in mice inoculated with *Shigella flexneri* strains while variable concentrations of IL-1ß were found in mice inoculated with different strains and Jansky *et al*.(2003) who found that, the production of IL-1ß and IL-6 was lower than its level produced from peripheral blood mononuclear cells (PBMC) stimulated by *Borrelia*.

The low level of IL-1ß concentration in animal group immunized with LPS may be due to the effect of IL-6 which was at higher concentration in this group and this result was in agreement with Tilg *et al*.(1994) study which showed that IL-6 inhibits LPS-induced TNF-α and IL-lß production in cultured human monocytes and in mice in vivo and this may be due, in part, to the induction of IL-1 receptor antagonist (IL-l Ra) synthesis and the release of soluble TNF receptors result in low plasma levels of IL-1ß and TNF-α plasma in all samples tested. Nandi *et al* .(2012) demonstrated that IL-6 activate IL-10 production in serum of endotoxic group and then IL-10 inhibit the synthesis of proinflammatory cytokines and also has a suppressive effect on proinflammatory cytokines like IL-1ß,TNF-α,INF-γ and IL-12. In humans, circulating concentrations of IL-6 are highly correlated with increased mortality, a finding that has been attributed to its ability to persist in the circulation for a longer period of time than other proinflammatory cytokines (Song and Kellum, 2005).Several lines of evidence indicate that the primary target of LPS on monocytes/macrophages is the mCD14 molecule, which triggers, together with TLRs intracellular signaling pathways leading to cytokine production (Bachmann and Oxenius,2007).

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بعض أوجه الاستجابة المناعية لعديد السكريد ألشحمي لبكتريا *Providencia rettgeri*

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الخلاصة:

تم في دراستنا السابقة استخلاص وتنقية عديد السكريد ألشحمي من عزلة لبكتريا *Providencia rettgeri* ،وجد من خلال هذه الدراسة أن عديد السكريد ألشحمي عند حقنه في الأرانب بالجرعة 150 مايكروغرام/كغم في العضلة وتحت الجلد قد حفز الاستجابة المناعية الخلطية على المستويين ألجهازي والموضعي والتي تم حسابها باستخدام اختبار التراص الدموي المنفعل ،وكان معدل عيار الأضداد جهازيا (6826،) أعلى معنويا من عيار الضد الموضعي (في الزائدة الدودية) (512) . وتم قياس تركيز بعض السايتوكينات مثل IL-2 و IL-4 و IL-6 وIL-1ß باستخدام تقنية ELISA. وجد من خلال هذه الدراسة إن تراكيز كل من السايتوكينات IL-2 و IL-4 و IL-6 قد ارتفعت معنويا في الحيوانات الممنعة بعديد السكريد ألشحمي وكانت (3.21 و 5.84 و4831) بيكوغرام /مل على التوالي، مقارنة بتراكيزها في حيوانات السيطرة والتي كانت 2.05 و 2.34 و 3121.06 بيكوغرام/مل لكل من السايتوكاين-2 والسليتوكاين-4 والسايتوكاين-6على التوالي.في حين أن تركيز السايتوكاين IL-1ß قد انخفض معنويا في الحيوانات الممنعة بعديد السكريد ألشحمي ليصل إلى 115.7 بيكوغرام /مل مقارنة بتركيزه في مجموعة السيطرة والذي كان 177.24 بيكوغرام /مل.