INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES

(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com

Research Article

A STUDY ON THE BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS ASSOCIATED WITH *Pasteurella multocida* LIPOPOLYSACCHARIDES IMMUNIZATION IN RABBITS

DR. KHALIL H. AL- JEBOORI* AND DR. SATTAR R. SUHAIL

Department of Pathology, College of Veterinary Medicine, Universioty of Baghdad, Iraq.
*Corresponding Author: khalilhassan1955@gmail.com

Abstract

This study was included extraction and purification of lipopolysaccharide (LPS) of *Pasteurella multocida* by using sepharose -6B as well as chemical analysis of LPS content . Half lethal dose (LD50 was 300 μ/ml) of purified Lps. The rabbits is considered more susceptible animal to *Pasteurella multocida* infection, so (15) rabbits were injected S/c by 30 μ of Lps /kg. The other (15) rabbits group were injected by phosphate buffer saline (PBS) as a control group, 2 doses at 2 weeks intervals. At 15th days and 27th days post immunization . The hematological and biochemical parameters were measured and it is found that there is no significant differences in the hematological and biochemical parameters in the group of LPS Immunization comparable to the control group, although slight increase the level of both hematological and biochemical parameters in the group of immunization with LPS , but still with normal level of control group .

Keywords: Lipopolysaccharide (LPS), *Pasteurella multocida,* Half lethal dose, hematological and biochemical parameters.

Introduction

Lipopolysaccharides (LPS) are the major part of the outer membrane of the cell wall of the Gram negative bacteria and play a critical role in the pathogenesis of the disease caused by any of Gram negative bacterial species (1) . LPS are the major part of the capsule of *pasteurella multocida* has a pivotal role in the determination of serogroup types of the bacteria and in some cases associated with lipoproteins . Also the LPS protect the bacteria against the dehydration (2) and responsible for the *pasteurella multocida* virulence and their transmission from host to other (3) . In addition, inhibit their phagocytosis by phagocytic cells and resistant to complement (4) .

LPS is thought to be responsible for toxicity and play important role in pathogenesis of the disease and a major cause of the endotoxic shook (5) . For the importance of the LPS in pathogenesis of the disease process in the different animal specieces this study aimed at:

1. Study the biochemical parameters total serum protein , globulin albumin , blood urea and Serum creatinine following immunization with LPS in rabbits .

2. Study the hematological parameters (total leukocytes count , erythrocytes count , hemoglobin and leukocyte differential count %) following immunization with LPS in rabbits .

Materials and Methods

Pasteurella multocida strain was supplied by Alkindi company for veterinary drugs and vaccines production, Baghdad, Iraq. This bacteria agent was reidentified to be sure *Pasteurella multocida* using cultural, biochemical , AP1-20 NE kit (Biomerieux , USA) (6) .

Lipopolysaccharides extraction and purification

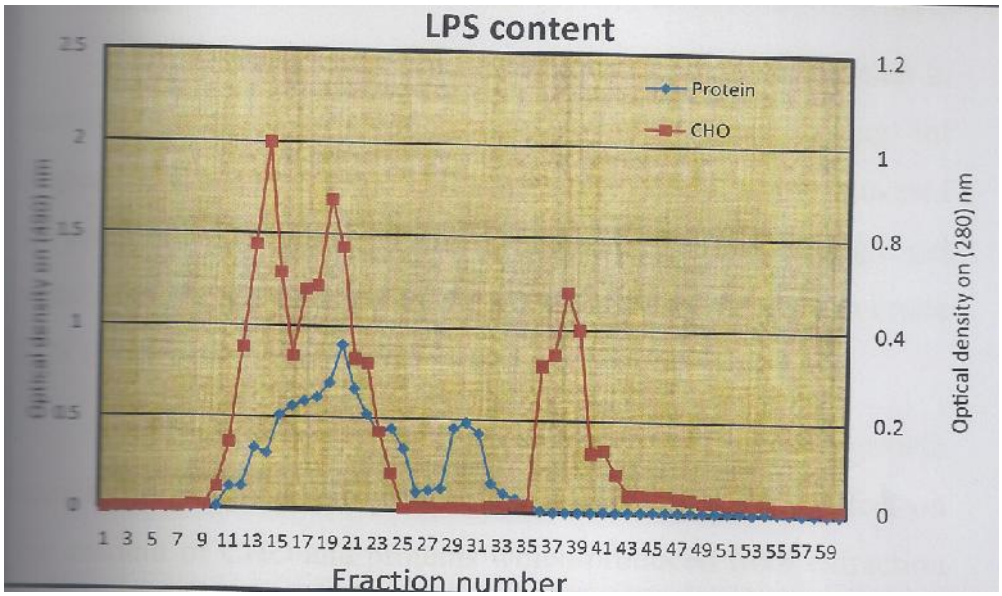
LPS were extracted with EDTA according to (7). shortly, the *Pasteurella multocida* growth suspension were admixed with EDTA, put in autoclave , following sterilization , the mixture treated with DNase , R Nase . finally with proteinase K solution at the 56^{o}C**.** After cooling the centrifugation and supernatant taken in dialysis tube against distal water then evaporated to obtain crude LPS , their purification was done by Gel filtration chromatography using sepharose – 6B and Gelatin in a column preparation , after adding and replacement of crude LPS according (8) . The absorbance of collected fractions was examined at wave lengh : 280 nm for protein concentration (9) measuring of carbohydrates concentration (phenol – sulphuric acid) according to (10) . Then the protein estimation of purified LPS were done by using Biuret kit (Bio system, Spain) (11). and the carbohydrate estimation was done according to (10) , method . The LD50 of LPS measured according to (12).method using serial concentrations (600, 450, 300 , 150 μ / ml) of LPS . The 15 rabbits we taken and injected S/C with 30 μ /kg body weight of rabbits at a dose of 1ml, after 15 days booster dose. The other group (15) of rabbits inoculated with PBS (control group).

At 15th and 27th days post inoculation blood samples taken for hematological parameters using (13) and for biochemical parameters by using (Agape , Switzerland , (11); linear chemical , Spain (11) and Bio system , Spain (14) .

Results and Discussion

Lipopolysaccharide extraction and purification

Three hundred (300 μ) of the LPS was extracted from 30 gm (weight) of *Pasteurella multocida* cells and the results of crude LPS replacement in column of sepharose- 6B were 60 collected fractions and three peaks . (Fig -1) of carbohydrates were Isolated , measured at 490nm and one peak of proteins when measured at 280nm . The carbohydrate concentration was 210 μ /ml in crude and 150 μ /ml for purified LPS and protein concentration was 6.5 μ /ml in crude and for purified 2.25 μ /ml . The LD50 of purified LPS was 300 μ /ml .



(Figure – 1) : Chromatogram showing gel filtration on C1-6B sepharose column (2.6x40cm) of LPS , fraction size (5 ml) , flow rate (20ml / 60 minutes)

Biochemical parameters

The results showed no significant differences (P < 0.05) between values of LPS immunized group and control group after 15th and 27th days post immunization but high level in LPS group compared to the control group (Table 1) of total protein , Albumin , globlulin , creatinine and blood urea, but still with normal range .

Table -1 : Biochemical levels in immunized (LPS) and control groups of rabbits .

Groups	Total protein (gm/dl) mean ± SE		Albumin (gm/dl) mean ± SE		Globulin (gm/dl) mean ± SE		S. creatinine (mg/dl) mean ± SE		B. Urea (mg/dl) mean ± SE	
LPS group	15^{th}. Day	27^{th} day	15^{th} day	27^{th}. Day	15^{th}. Day	27^{th}. Day	15^{th}. day	Day 27^{th}	15^{th}. Day	27^{th} day
	5.8 ± 0.415	6±0.681	3.3±0.204	3.3± 0.251	2.52±0.387	2.62±0.794	0.96±0.160	1±0.158	25.4±1.86	25.6± 1.63
Control group	5.46±0.132	5.48±0.146	3.0±0.143	3.0±0.100	2.42±0.220	2.48±0.226	0.67±0.05	0.63± 0.05	24.2±0.66	23.6± 1.43
There was no significant differences between groups and between periods (P< 0.05)										

Hematological parameters

The results of this study revealed that there is no significant differences in the level of hematological parameters between LPS group and control , but there is increase in the level of all hematological parameters in the LPS group compared to the control of total leukocytes count, differential leukocytes count, erythrocytes and hemoglobin count (Table -2), but with normal range .

Table -2 : Hematological parameters in immunized and control groups of rabbits .

Groups	Hb. Content (gm/dl) mean ± SE		Leukocytes count /μl mean ± SE		Neut. % mean ± SE		Lymph % mean ± SE		Mono % mean ± SE		Eosin.% mean ± SE		RBcs count /μl mean ± SE	
LPS group	15^{th}. Day	27^{th} day	15^{th} day	27^{th}. Day	15^{th}. Day	27^{th} Day	15^{th} day	Day 27^{th}	15^{th}. Day	27^{th} day	15^{th}. Day	27th day	15th Day	27th day
LPS group	11.28 ± 0.671	11.54 ± 0.974	7.08 ± 0.47	7.16 ± 0.34	52 ± 3.40	52.2 ± 4.63	48.6 ± 1.36	50 ± 1.70	2.6 ± 0.60	2.8 ± 0.58	3.4 ± 0.67	3.4 ± 0.40	6.04 ± 0.65	6.24 ± 0.53
Control group	11.22 ± 0.62	11.18 ± 0.81	6.6 ± 0.12	6.48 ± 0.41	50.6 ± 3.10	49 ± 2.93	45.8 ± 2.2	45 ± 2.82	1.8 ± 0.37	2 ± 0.31	3 ± 0.44	3.4 ± 0.67	5.98 ± 0.36	6.24 ± 0.22
There was no significant differences between groups and between periods (P< 0.05)														

The results of this study showed that the biochemical and hematological parameters of LPS group and control group showed no significant differences with slight elevation of all the biochemical and hematological parameters in the immunized (LPS) group compared to the control (PBS) group , but generally still with normal level range . A similar finding reported by (15) that the LPS gave the good immune response but no effect on hematological and biochemical parameters and the immunization by LPS enhance slight elevation of these parameters but still with normal range this elevation belong to that immunization process stimulate erythropoiesis in the bone marrow and lead to slight elevation of hematological parameters in peripheral circulation (16) . Also similar biochemical parameters were showed no significant differences between LPS group and control group , although slight elevation of the biochemical parameters in LPS group compared to the control group , but still with normal range . A similar finding were reported by (17) , (18) that the immunization do not effect on biochemical parameters which was evident in this study .

References

1. Wijewardana , T. G ; Wilson , C. F. ; Gilmour , N . J. and Poxton , I. R (1990) production of mouse monoclonal antibodies to *pasteurella multocida* type A and the immunological properties of a protective antilipopolysaccharide antibody . J. Med. Microbiol . 33: 217-222 .
2. Robert. L. S(1996) . The biochemistry and genetics of capsular polysaccharide production in bacteria . Annual Review of microbiology , 50 : 285 -313 .
3. Boyce. J. D; Chung , J .Y and Adler , B . (2000). *pasteurella multocida* capsule : composition , function and genetics . Journal of Biotechnology 83, 153-160 .
4. Jacques , M. ; Kobisch, M. ; Belanger , M. and Dugal, F (1993) . Virulence of capsulated and non capsulated isolates of *pasteurella multocida* and their adherence to porcine respiratory tract cells and mucus . Infection and Immunity , 61 : 4785-4792 .
5. Horadagoda , N. U; Hodgson , J. C. ; Moon , G. M ; Wijewardana , T. G and Eckersall , P. D. (2002) Development of a clinical syndrome resembling hemorrhagic septicemia in the buffalo following intravenous inoculation of *pasteurella multocida* serotype B : 2 endotoxin and the role of tumor necrosis foctor – alpha. Res. Vet. Sci. 72: 194-200 .
6. Wilson , M. A; Morgan , M. J. ; and Burger , G. E. (1993) . Comparison of DNA finger printing and serotyping for identification of avian *pasteurella multocida* isolates . J. Clin. Microbiol. 31 : 225-259 .
7. Chandan , V. ; Fraser , A. D. etal ., (1994) . Simple extraction of campylobacter lipopolysaccharides and protein antigen and production of their antibodies in egg yolk . Inter . J. Food Microbiol. 22: 189-200 .
8. Morrison , D. C and leive, L , (1975) Fractions of lipopolysaccharide from *Escherichia coli* prepared by two extraction procedures . J. Biol. Chem. 25(8) : 2911- 2919 .
9. Bruck , M. ; Butel . J. S. ; carroll, K. C. and Morse , S. A. (2007) Medical microbiology McGraw – Hill book company 24th. ed. P. 294.
10. Dubois , M. ; Gilles , K. L. ; Hamilton , J. K ; Robers , P. A. and Smith , F. (1956) colometric method for determination of sugar and related substances . Annals Chem , 25 : 350 -353 .
11. Tietz, N. M. (1999) Clinical guide to laboratory tests . 3rd. ed W. B. Saunders Co. Philadelphia , USA.
12. Dixon , W. J. (1980) Efficient analysis of experimental observations . Ann. Res. Pharmacol. Toxicol. 20; 441-462 .
13. Schalm , O. M; Jain , N. C. and Carroll , E. J. (1975) Veterinary hematology , 3rd.ed. Lea and Febigar , Philadelphia , USA.
14. Young , D. S. (1997) effects of drugs on clinical laboratory tests , 3rd. ed. Academic press , USA .
15. Hillyer , V. and Quesenberry , E. (1997) Ferrets , Rabbits and Rodents . Clinical Medicine and Surgery . W. B. Saunders company PP: 165-166.
16. Tizard, I. (1992) Veterinary Immunology : An introduction 4th.ed. W.B. Saunders C., Mexico PP:498.
17. Abbas , A. K and Lichtman , A. H. (2007) . Cellular and Molecular immunology , 2nd . ed. Amazon company , USA.
18. Roitt, I . M . (1998) Essential Immunology 6.ed. Black well scientific publication London .

