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

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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 µg 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 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 shock (5). For the importance of the LPS in pathogenesis of the disease process in the different animal species 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, RNase. finally with proteinase K solution at the 56°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 length : 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, globulin, creatinine and blood urea, but still with normal range.
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 within normal range.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hb. Content (gm/dl) mean ± SE</th>
<th>Leukocytes count /µl mean ± SE</th>
<th>Neut. % mean ± SE</th>
<th>Lymph % mean ± SE</th>
<th>Mono % mean ± SE</th>
<th>Eosin.% mean ± SE</th>
<th>RBcs count /µl mean ± SE</th>
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</thead>
<tbody>
<tr>
<td>LPS group</td>
<td>11.28 ± 0.671</td>
<td>7.08 ± 0.47</td>
<td>52 ± 3.40</td>
<td>48.6 ± 1.70</td>
<td>2.6 ± 0.60</td>
<td>3.4 ± 0.67</td>
<td>6.04 ± 0.65</td>
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<td>11.54 ± 0.947</td>
<td>7.16 ± 0.34</td>
<td>52 ± 4.63</td>
<td>50 ± 1.37</td>
<td>2.8 ± 0.58</td>
<td>3.4 ± 0.40</td>
<td>6.2 ± 0.53</td>
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<td>6.48 ± 0.41</td>
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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


