Escherichia coli lipopolysaccharide - induced experimental infection in piglets: clinical and laboratory findings

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SUMMARY

In six female piglets aged 2-3 months weighing 28 ± 3 kg, an experimental infection has been provoked via the intravenous application of Escherichia coli 0111:B4 lipopolysaccharide (LPS) at a dose of 10 µg/kg b.w. The changes in clinical and laboratory parameters were followed out up to hour 72 following the treatment.

It was determined that the first clinical signs, manifested with hyperthermia, anorexia and decreased activity appeared as early as 40 min after the LPS application. The infection was characterized with an initial leukopenia, followed by leukocytosis.

The most prominent biochemical changes were expressed in hyperproteinemia, disproteinemia, increased serum sialic acid and urea levels and increased serum alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) activities.

KEY-WORDS: infection - lipopolysaccharide - clinical, haematological, biochemical findings - piglet.

RÉSUMÉ

Infection expérimentale induite par un lipopolysaccharide d’Escherichia coli chez des porcs: recherches en clinique et au laboratoire. Par I. BORISSOV et M. ANDONOVA

Chez 6 porcelets femelles âgés de 2-3 mois et pesant 28 ± 3 kg, une infection expérimentale a été induite par injection, en voie intraveineuse, de 10 µg/kg d’un lipopolysaccharide d’Escherichia coli (O111 : B4).

Les premiers signes cliniques (hyperthermie, anorexie et diminution de l’activité motrice) sont apparus 40 minutes après l’injection.

L’infection s’est manifestée par une leucopénie suivie d’une leucocytose.

Les principales modifications biochimiques furent une hyperprotéinémie, une dysprotéinémie, une augmentation des taux sériques d’acide sialique et de l’urée ainsi qu’une augmentation de l’activité enzymatique de l’alanine aminotransférase (ALAT) et de l’aspartate aminotransférase (ASAT).

MOTS-CLÉS: infection - lipopolysaccharide - indices cliniques, hématologiques, biochimiques - porc.

Introduction

Lipopolysaccharide (LPS) from the outer cell wall of Gram-negative bacteria, when given in purified form, reproduces many of the effects of live bacteria [28]. Endotoxin causes pathophysiological effects resulting in altered responses in cardiovascular [6, 19], neurologic, hepatic [5, 17, 22], gastrointestinal, immune and endocrine systems [11, 17, 26, 29].

The effects on an individual are attributable to direct LPS effects, adaptive or homeostatic changes in response to LPS, as well as to indirect effects, secondary to other factors generated by the interaction of endotoxin with body tissues [7].

Without delineation of principal general non-specific defence systems and the LPS-induced pathophysiological changes, the specific treatment could not be effective.

The aim of the present experiment was to study the principal pathogenetical mechanisms during the course of an experimental infection, provoked via the intravenous application of Escherichia coli lipopolysaccharide to pigs through the performance of clinical and laboratory studies.

Material and methods

ANIMALS

The experiments were performed with six 2-3 month old female piglets, Landrace x Large White crossbreeds, weighing 28 ± 3 kg.

The experimental group was formed 2 weeks prior to the study. The animals were housed in standard conditions-temperature of 20 °C and humidity of 60 %.
The pigs were canulated with heparinized polyurethane catheters (Vygonlon-T-Vicon, Germany) in the jugular vein. The study was performed only on female animals to exclude the potential influence of sexual hormones on studied parameters. The effect of circadian rhythm was eliminated by sampling blood at 8.00 a.m. before feeding for each experimental interval (hours 24, 48, 72).

The pigs were inoculated intravenously with 10 µg/kg b.w. LPS (Escherichia coli 0111:B4-phenol extract purified) (Sigma) in sterile 0.85 % saline solution.

**BLOOD SAMPLE COLLECTION**

The blood was collected before (0) and at hours 1, 2, 4, 24, 48 and 72 after the LPS challenge. Blood samples were either centrifuged (4 °C) within 15 minutes of collection (plasma) or allowed to clot at 20 to 22 °C prior to centrifugation (serum).

At the time at which each blood sample was collected, rectal temperature was recorded.

**HAEMATOLOGICAL PARAMETERS**

The leukocyte and erythrocyte counts were counted in the Bürker chamber. Haemoglobin (Hb) contents was determined by an acid-base analyser (ABL-3, Radiometer, Denmark) after correction to core body temperature. The haematocrit (Hc) level was determined by the microhaematocrit method.

The erythrocyte sedimentation rate (ESR) was determined according to the micromethod of Westergren (mm/1h).

**METABOLIC VARIABLES**

Serum total protein concentrations (TP) were determined by the Biuret reaction [4].

The serum albumin concentrations were determined by agar microelectrophoresis and were calculated from TP and the albumin percentage.

The serum levels of urea, creatinine, glucose, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were determined using commercial clinical chemistry kits (Bioagrogen-France).

Serum sialic acid was determined on the basis of a modification of the method of Hess [25] in relative units.

**STATISTICAL ANALYSIS**

The statistical significance of the results was determined using a one-way analysis of variance (ANOVA) at a level of 0.05.

**Results**

**CLINICAL CHANGES**

All pigs that received i.v. 10 µg/kg b.w. E.coli LPS developed signs of sickness characteristic of an acute phase response (APR). Forty to sixty minutes after the LPS injection the pigs became anorectic. This was further accompanied by lethargy, somnolence and decreased general activity.

Endotoxin administration resulted in characteristic changes in body temperature, expressed in statistically significant increase between posttreatment hours 1 and 4 with a peak at hour 2 (41.08 ± 0.19 °C ; p < 0.001) vs the initial average value of 39.52 ± 0.11 °C. After the 4th hour, the body temperature began to decrease and at the end of study (hour 72) it reached the baseline values (fig. 1).

**HAEMATOLOGICAL FINDINGS**

The results of haematological examinations are shown in table I. The leukocytes reacted the most rapidly, decreasing to 11.40 ± 1.14 G/l as early as hour 2 compared to the baseline value of 19.15 ± 0.47 G/l. A tendency towards increase was however observed afterwards. At the end of the study, a leukocytosis was registered (22.90 ± 2.09 G/l).

There were no significant changes in haemoglobin content and erythrocyte counts (table I). The analysis however showed a tendency towards decrease between hours 24 and 48 after the LPS application.

Similar changes occured in haematocrit as well. Up to posttreatment hour 4, it was close to initial levels while at hour 24 it decreased significantly (p < 0.05) reaching 0.29 ± 0.004 l/l at hour 48 (p < 0.01 vs baseline of 0.35 ± 0.013 l/l). At hour 72 after the treatment, the hemoglobin content, the haematocrit and the erythrocytes remained lower that the initial levels.

Another parameter with statistically significant changes was the erythrocyte sedimentation rate (table I). It was influenced not only by cellular factors (Hb, Hc) but by humoral factors as blood proteins, cholesterol, biliary salts, pigments etc., that have to be taken into account for the interpretation. The ESR reacted to the LPS administration rapidly with acceleration at hour 4 (p < 0.01 vs initial values).

**BIOCHEMICAL FINDINGS**

Changes that occurred in biochemical parameters prior to and after the E. coli LPS challenge are presented in table II. An increase in total protein levels was registered at hours 2 and hour 4 (average values of 73.50 ± 4.78 g/l vs initial values of 64.50 ± 2.99 g/l). Until the end of the study, the total protein content remained higher than baseline.

The results of serum protein electrophoresis showed that from all liver synthetized protein fractions, the albumin followed the tendency of change of total protein (table II). Changes were observed only in α1-globulins. It increased by hours 4-24 and reached its peak at hour 72 after the treatment: 4.57 ± 0.35 g/l.

Creatinine concentrations did not change statistically significantly following LPS application. There was a tendency towards decrease up to hour 4 but afterwards creatinine levels increased reaching 215.7 ± 66.04 µmol/l vs the initial average level of 182.0 ± 25.56 µmol/l. The changes in blood urea nitrogen were however more prominent. They were significantly elevated with a peak at posttreament hour 24 (9.19 ± 2.12 mmol/l ; p < 0.01 vs hour 72). At the end of study, blood urea nitrogen levels were similar to initial ones.
ESCHERICHIA COLI LIPOPOLYSACCHARIDE - INDUCED EXPERIMENTAL INFECTION IN PIGLETS

Fig. 1. — The effect of the intravenous application of *E. coli* LPS in piglets (10 µg/kg b.w.) on body temperature (°C) ; n = 6. Values significantly different (***) - p < 0.001) from baseline values.

### Table I.
Changes in haematological indices after intravenous *E. coli* LPS inoculation (10 µg/kg b.w.) in piglets (n = 6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0h</th>
<th>1h</th>
<th>2h</th>
<th>4h</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes (G/l)</td>
<td>19.15±0.47</td>
<td>14.17±2.09</td>
<td>11.40±1.14</td>
<td>12.08±1.23</td>
<td>15.42±1.50</td>
<td>18.10±2.20</td>
<td>22.90±2.09</td>
</tr>
<tr>
<td>Erythrocytes (T/l)</td>
<td>6.33±0.27</td>
<td>6.37±0.20</td>
<td>6.44±0.26</td>
<td>6.31±0.18</td>
<td>5.78±0.36</td>
<td>5.68±0.21</td>
<td>5.92±0.11</td>
</tr>
<tr>
<td>Hb (g/l)</td>
<td>124.3±6.5</td>
<td>121.7±3.3</td>
<td>126.3±7.9</td>
<td>119.3±3.2</td>
<td>108.3±6.2</td>
<td>106.7±5.1</td>
<td>108.0±2.9</td>
</tr>
<tr>
<td>Ht (L)</td>
<td>0.35±0.013</td>
<td>0.33±0.007</td>
<td>0.33±0.013</td>
<td>0.33±0.007</td>
<td>0.31±0.008</td>
<td>0.29±0.004</td>
<td>0.31±0.004</td>
</tr>
<tr>
<td>ESR (mm/1h)</td>
<td>2.33±0.33</td>
<td>3.33±0.56</td>
<td>3.00±0.45</td>
<td>4.50±0.92</td>
<td>2.67±0.33</td>
<td>2.67±0.33</td>
<td>2.33±0.33</td>
</tr>
</tbody>
</table>

*a* (p < 0.05) ; *a*1 (p < 0.01) ; *a*2 (p < 0.001) significantly different from baseline values.

### Table II.
Changes in biochemical indices after intravenous *E. coli* LPS inoculation (10 µg/kg b.w.) in piglets (n = 6).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>0h</th>
<th>1h</th>
<th>2h</th>
<th>4h</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
</tr>
</thead>
<tbody>
<tr>
<td>total protein (g/l)</td>
<td>64.50±2.99</td>
<td>65.33±3.50</td>
<td>73.50±4.16</td>
<td>73.50±4.78</td>
<td>71.00±2.46</td>
<td>67.17±1.40</td>
<td>70.17±2.14</td>
</tr>
<tr>
<td>albumin (g/l)</td>
<td>16.73±0.77</td>
<td>16.45±1.27</td>
<td>17.97±1.45</td>
<td>18.38±1.01</td>
<td>18.77±0.72</td>
<td>17.25±1.04</td>
<td>17.05±1.58</td>
</tr>
<tr>
<td>α1 globulins (g/l)</td>
<td>3.32±0.19</td>
<td>3.50±0.20</td>
<td>3.38±0.15</td>
<td>3.88±0.31</td>
<td>4.22±0.66</td>
<td>3.22±0.17</td>
<td>4.57±0.35</td>
</tr>
<tr>
<td>α2 globulins (g/l)</td>
<td>12.67±1.19</td>
<td>13.02±1.30</td>
<td>15.43±1.47</td>
<td>15.03±1.03</td>
<td>13.35±0.54</td>
<td>13.75±0.62</td>
<td>12.63±1.27</td>
</tr>
<tr>
<td>β globulins (g/l)</td>
<td>12.30±0.41</td>
<td>11.98±0.63</td>
<td>14.33±1.00</td>
<td>13.48±1.61</td>
<td>14.02±0.41</td>
<td>13.02±0.89</td>
<td>12.83±0.97</td>
</tr>
<tr>
<td>γ globulins (g/l)</td>
<td>19.48±1.05</td>
<td>20.38±1.94</td>
<td>22.38±2.20</td>
<td>22.72±2.52</td>
<td>20.65±2.03</td>
<td>19.93±1.24</td>
<td>19.68±1.74</td>
</tr>
<tr>
<td>creatinine (µmol/l)</td>
<td>182.0±25.56</td>
<td>148.2±26.19</td>
<td>126.2±21.32</td>
<td>183.3±39.45</td>
<td>215.7±66.04</td>
<td>199.7±18.88</td>
<td>150.5±26.98</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>5.90±0.91</td>
<td>8.74±1.47</td>
<td>8.13±1.43</td>
<td>8.30±1.37</td>
<td>9.19±2.12</td>
<td>4.53±0.46</td>
<td>3.48±0.29</td>
</tr>
<tr>
<td>Sialic acid (E)</td>
<td>235.7±19.96</td>
<td>232.0±10.30</td>
<td>239.0±17.37</td>
<td>278.5±33.34</td>
<td>206.5±16.24</td>
<td>212.7±32.43</td>
<td>201.8±11.36</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.46±0.54</td>
<td>7.02±0.58</td>
<td>6.13±0.44</td>
<td>5.82±0.45</td>
<td>4.31±0.90</td>
<td>4.45±1.12</td>
<td>3.53±0.32</td>
</tr>
</tbody>
</table>

*a* (p < 0.05) ; *a*1 (p < 0.01) ; *a*2 (p < 0.001) - significantly different from baseline values. - *b* (p < 0.05) ; *b*1 (p < 0.01) ; *b*2 (p < 0.001) significantly different from values at 72h.

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The sialic acid concentrations increased as early as hour 2 after the LPS treatment (table II). The highest value was recorded at hour 4 (279 ± 33 E vs 236 ± 20 E). At the end of the experiment, a tendency towards decrease was observed.

At the early phase of the infection (up to hour 4), an increase of blood glucose was determined (7.02 ± 0.58 mmol/l at hour 1). After hour 24, there was a tendency towards the development of hypoglycemia but the differences were insignificant.

Figures 2 and 3 present the changes in ASAT and ALAT activities. The infection was accompanied by significant elevation as early as hour 2 and 4 after the LPS administration. An ASAT peak was observed at hour 4 (14.31 ± 2.15 U/l), when the activity was almost thrice than baseline (5.94 ± 1.33 U/l - p < 0.001). Similar changes occurred in ALAT dynamics too (11.35 ± 3.13 U/l at hour 4 and average baseline activity 8.36 ± 1.94 U/l).

Discussion

The systemic reaction to the intravenous application of LPS was characterized with multiple changes in both clinical and laboratory parameters. They were non-specific, but gave a lot of information about the phase of disease, its evolution and the severity of the pathological changes that followed. Those changes were significant for the elucidation of some pathogenetical mechanisms that characterized the course of acute phase response to the experimental E. coli LPS infection.

After the LPS administration, the body temperature increased at hour 1 and reached its maximum at hour 2. Fever is one of the physiologic responses that seems to be adaptive by providing a more suitable internal environment enhancing adaptive and innate immunity [21]. Fever was most probably due to the fact that LPS, being included in the lipopolysaccharide binding protein complex, interacted with CD14 receptors on monocyte-macrophageal system cells, leading to cytokine mediator synthesis; in particular tumor necrosis factor α (TNFα), interleukin-1 (IL-1) and IL-6 [12]. Those mediators are reported to be the principal endogenic factors, participating in the mechanism for increase of body temperature.

Apart its pyrogenic effect, those mediators increase the vascular permeability, activate the synthesis of adhesion molecules and their expression upon endothelial cells thus contributing to the adhesion and transmigration of leukocytes.
[This mechanism is most probably responsible for the decrease in leukocyte counts observed at hour 2 after the LPS treatment in our studies (table I).

Certain aspects of leucocyte trafficking are relatively well dissected at the molecular level; the identification of selectins, integrins, chemokines and proteases [18]. In contrast, the expected significant increase of leukocytes at hour 24 after the LPS treatment might have resulted from the inflammatory response to endotoxin [13]. Further evidence in this connection is another systemic reaction manifested with ESR acceleration especially at hour 4 after the LPS administration. In our opinion, the observed ESR changes are a result of the increase in plasma factors (blood proteins - table II), because there were no significant changes in erythrocyte count, haemoglobin and haematocrit values.

The parallel interpretation of blood picture and the proteins is more informative for the evaluation of the infection process. The increase in α1-globulins could be regarded as an early indicator for the initial stage of acute infection. The data for sialic (N-acetyl neuraminic) acid concentrations are further supporting this thesis.

TURNER et al. [27] interpret the sialic acid as a marker of acute phase proteins. A high level of serum sialic acid in human patients with inflammatory diseases has also been found [22, 24].

It must be taken into account that the glycoprotein level, whose component is sialic acid, depends greatly on the liver status. This organ has also a primary role in the synthesis of urea-end product of protein metabolism. Blood urea levels are also elevated in our studies. They could be due to renal disorders, caused by the LPS in blood, as well as to extrarenal causes. The serum creatinine levels were however not statistically significantly changed although being elevated between hours 24 and 48. As creatinine concentration is a much more specific marker for impaired renal function, we suggest that urea changes were due to extrarenal causes. The elevations in serum transaminases activities (fig. 2, 3) are another evidence supporting the suggestion for parenchymatous changes.

It is known that LPS stimulates the release of contra-insulin hormones, like glucagon and glucocorticoids [1] responsible for hyperglycemia, for up to the 1-2 h of the endotoxin effect. On the other hand, LPS increases insulin secretion [2].

Fig. 3. — The effect of the intravenous application of E.coli LPS in piglets (10 µg/kg b.w.) on ALAT activities (U/l) ; n = 6.

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Hyperinsulinemia was probably the basis of the prolonged decrease of blood glucose after hour 4 following LPS challenge in our studies (table II).

Conclusions

The development of experimental infection in pigs provoked by the intravenous application of *E. coli* LPS was accompanied by clinical changes manifested by hyperthermia, anorexia and decreased activity.

The infection was characterized with decrease of leukocytes up to hour 24 and leukocytosis in the later hours. The other haematological parameters were not changed.

The most prominent biochemical changes were a hyperproteinemia, a disproteinemia, increase in the levels of sialic acid and urea as well as statistically significant increase in ASAT activity.

References


