MODELS OF GRAM NEGATIVE SEPSIS

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The aim of our research was that during the experimental model of sepsis, we induce apoptosis of pharenecymal cells and identify it by measuring relative mass of the examined organs. The experiment was carried out on 48 male rats of Wista strain. The model of sepsis was caused by caecal ligation and punctuation with clean culture of bacteria Escherichia coli. In order to follow the development of sepsis, time for observing the animals were hours 12, 24, 72 and 120 after surgical intervention. At these terms the animals were sacrificed and the following organs were taken out: heart, liver, spleen, lungs, kidneys, thymus and intestine. All the organs were cleaned and measured.

Relative mass of spleen in septic rats in the hours 72 and 120 was considerably above the control findings. A significant loss of relative mass of liver (hour 12), lungs (hour 12 and 24) and kidney (hour 24) was detected.

According to data in the literature about the recent research of sepsis model on animal, changes in relative mass of the observed organs in our experiment may be ascribed to apoptosis of pharenecymal cells, what is a consequence of uncontrolled secretion of pro-inflammatory cytokine.

Apoptosis presents an endogenous evolutionary conserved and controlled way of cell death where a cell participates actively, conducting a precise, genetically regulated program of cell self-destruction. Independent from the kind of cell population and circumstances that caused it, apoptosis is characterized by separating of deoxyriboonucleic acid, nucleus condensation, "boiling" of membrane plasma, change in distribution of membrane lipid and separation of a cell from extra-cell matrix (3). Traditionally, death of a cell has been considered exclusively as a passive phenomenon that is a consequence of pathological process, i.e. fatal damage of cell structure where a cell takes no important part, except in activating of reparatory mechanism. However, today it is clear that besides normal cell death, i.e. necrosis, there is another type of cell death, programmed cell death or apoptosis.

A broad spectrum of diseases where apoptosis plays and important role includes also sepsis. Today special intention is given to apoptosis of regulatory cells of inflammatory reaction, macrophages and neutrophile granulocytes, whose dysfunction causes inappropriate secretion of proinflammatory cytokine during the sepsis. It is known that reduced number of immune cells, death of parenchyma cells of different organs, as well ad dysfunction of neutrophile and monocytes/macrophages during the sepsis corresponds to uncontrolled induction of apoptosis (9, 15, 16, 17).
Materials and methods

Model of sepsis of caecal ligation and puncture (CLP) was reproduced by using technique of explanations and characteristic models according to Wichterman (20) with slight modifications (14). Surgical intervention was done on rats that received anesthesia through tiopentobarbitole. Abdominal incision was done in medial line two centimeter below caecum. The rats were divided in two groups: one consisting of 28 animals and control group consisting of 20 animal. The first group (SA) underwent surgical procedure of opening abdomen, emptying and binding up caecum (1/3 below jejoececal valve) and then the caecum was filled with inoculum that contained pure culture of gram positive bacteria Escherichia coli. In the control group (K) consisting of 20 animals, only abdominal incision was done (false operation). For the purpose of following the development of sepsis and measuring mass of the monitored parenchymatous organs, time for observing and sacrificing the animals were hours 12, 24, 72 and 120. At the mentioned time the animals were sacrificed, and the following organs were taken out: heart, liver, spleen, lungs, kidneys, thymus and intestine. All were cleaned and measured.

Results and discussion

Changes of relative mass is displayed in Table 1. Relative liver mass of rats with sepsis in the first half of experimental protocol was lower comparing to the control values, and was statistically significant after 12 hours. On the contrary, after 72 hours and 120 hours relative value of liver was above the level of control values. During the experiment relative kidney mass of animals with sepsis was considerably lower, except after 120 hours, i.e. comparing to control values, and was significantly lower on the hour 24 of the experiment. Relative mass of intestine, heart and thymus in septic animals was ranging, more or less, within the control values, and did not reach level of statistic importance, but relative mass of lungs in all the observed time was below the level of control values, and significantly lower after hours 12 and 24 of the experiment. From the Table it can be seen that relative mass of spleen in septic rats in the second half of experimental protocol was significantly higher comparing to control values.

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>EC-12</th>
<th>EC-24</th>
<th>EC-72</th>
<th>EC-120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>3.82±0.87</td>
<td>3.15±0.32*</td>
<td>3.65±0.30</td>
<td>4.20±0.38</td>
<td>4.07±0.12</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.77±0.06</td>
<td>0.67±0.18</td>
<td>0.70±0.02*</td>
<td>0.75±0.04</td>
<td>0.77±0.09</td>
</tr>
<tr>
<td>Intestine</td>
<td>14.08±2.31</td>
<td>13.84±2.28</td>
<td>13.14±2.25</td>
<td>14.21±1.36</td>
<td>14.42±1.38</td>
</tr>
<tr>
<td>Heart</td>
<td>0.34±0.04</td>
<td>0.31±0.05</td>
<td>0.34±0.03</td>
<td>0.37±0.05</td>
<td>0.33±0.02</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.72±0.12</td>
<td>0.60±0.05*</td>
<td>0.55±0.07*</td>
<td>0.66±0.10</td>
<td>0.71±0.08</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.33±0.05</td>
<td>0.32±0.12</td>
<td>0.34±0.07</td>
<td>0.53±0.08*</td>
<td>0.52±0.08*</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.21±0.06</td>
<td>0.25±0.04</td>
<td>0.20±0.02</td>
<td>0.21±0.06</td>
<td>0.21±0.03</td>
</tr>
</tbody>
</table>

+SD - standard deviation; EC-experimental group where sepsis was caused by pure culture of E.coli; *D<0.05.

It is known that during hyperdynamic phases of sepsis hypovalaemia occurs and provokes secretion of marrow hormone and adrenal gland, but also hypophysitis that results in mobilization of intracellular liquid. This causes decrease
of mass of intestinal organs. Also, there is an opinion that vasopressin in hypovolaemic condition stimulates glycogenolises and mobilizes water resources together with glycogen, especially in liver (2). Current research in model of sepsis on animals, speaks in favor of a theory that reduced mass of organs is a consequence of apoptosis of parenchymal cells (6, 8). Wang et al. (19) have discovered that apoptosis tymocyte, induced with interperitoneal inoculation of E. coli, started after 3 hours, i.e. 9 hours after inoculation S. pneumoniae, and culminated in hour 72. Hiromatsau et al. (5) also discovered that in model of caecal ligatation and puncture, induction of apoptosis, through corticosteroid, causes reduction in mass of thymus, spleen, lungs and intestine.

On the other hand, increase in relative mass of the monitored organs (first of all spleen and liver) may be ascribed to local inflammatory reaction and chemodynamic changes (7, 11, 12). Cytokines, that arise during inflammatory immune response, have strong effect on structure and function of endothel cells in microcirculation. Under influence of cytokine in endothelia cells, rearranging of aktin cytoketet filamenat occurs. Afterwards, out of them arise highly swollen cells that remain in contact with neighboring cells only through interdigital endings and, as such, they contribute fast extravasation of plasma. Protein of binding interstitium oedema, observed in system sepsis, is a result of increased microvascular permeability for plasma protein (4, 13). Reduction of transmembrae potentialy of parenchymal cells leads to intracellular increase of sodium concentration, and hereby also of intracellular liquid (swelling of cells) (13). The consequences is reduction of interstitial tissue and death of a cell.

Conclusion

In a model of sepsis the most marked changes in reduction of relative mass of parenchymatous organs, comparing to control values, was in the first half of experimental protocol. These changes can be ascribed to programmed death of cell-apoptosis, that is registered in broad spectrum of diseases, including also sepsis. Also, the first observation of apoptosis were noticed on experiments with the model of sepsis, where identified apoptosis of parenchymal cells of ileum, colon, lungs and in a smaller number in kidneys and skeletal muscles. Sepsis models on animals show that basic hematological changes present apoptosis of immune cells, as well as functional disturbance of regulatory cells in inflammatory response.

References