PECULIARITIES OF LIVER FUNCTIONAL STATE IN THE EARLY PERIOD OF CRANIOSKELETAL INJURY COMBINED WITH BLEEDING, AND ITS CORRECTION BY CELL THERAPY

Zayets T.A., Gudyma A.A.

SHEI I.Ya. Horbachevsky Ternopil State Medical University of the Ministry of Healthcare of Ukraine

Summary

During the early manifestations of traumatic disease as a result of cranioskeletal injury (CSI) the content of total bile acids in bile is significantly reduced, biliary excretion is slowed, lithogenic properties of bile are increased. Intraperitoneal injection of fetal nerve cells in 12 hours after causing an injury accompanied by improvement of biligenic and biliary excretion liver functions, reduces the lithogenic properties of bile.

Keywords: crinoskeletal injury, hemorrhage, liver, biligenic, bile excretion, fetal cells.

Foreword. Recently in the structure of injuries the relentless upward trend in the frequency of concomitant injury is observed, which is characterized by severe complications and high mortality [11]. In Ukraine every year more than 70 thousand people get injured of varying severity, herewith polytrauma is the leading cause of death for people under the age of 40 [6].

The progress of multiple organ failure belongs to the severe complications of traumatic disease that develops in multiple trauma, and often becomes the immediate cause of death in period of early and late traumatic disease manifestations [8].

The efficacy of medicamentous prevention of multiple organ failure in polytrauma is still low. In this regard, in recent years the search of innovative approaches to the correction of the pathological process is conducted. Among them, an important place takes the usage of fetal nerve cells that due to the synthesis of biologically active compounds are able to neutralize a number of pathogenic mechanisms inherent in traumatic disease [4]. In the works of some authors the reduce of systemic manifestations of traumatic disease in conditions of experimental cranioskeletal injury (CSI) is shown. However, the efficacy of its influence on the functional condition of organs and systems in polytrauma is studied insufficiently.
The purpose of the work: to explicate the efficacy of fetal nerve cells applications in correction of biligenic and biliary excretion liver functions disorders in condition during CSI early manifestations.

Materials and methods. The experiments were performed on 54 non-linear white male rats which weight was 180-200 g and were kept on a standard diet of vivarium. The animals were divided into three groups: one control group and two experimental ones. The control group included 6 intact animals. In two experimental groups there were 24 animals in each – under sodium thiopental anesthesia (40 mg kg\(^{-1}\) body weight) a closed craniocerebral injury was modeled by the method of [8] our own modification. A specially designed device was applied to do one hit on each thigh that caused closed fracture of femurs. In 12 hours after the injury according to the recommendations [3] in one of the experimental groups suspension of cryopreserved fetal rat nerve cells, dose 5×10\(^6\) cells per 100 g of the animal, was intraperitoneally injected. The fetal nerve cells suspension was produced at the Institute of Problems of Cryobiology and Cryomedicine of the NAS of Ukraine (Kharkiv) by saving mechanical dissociation of rat embryos brain fragments of 11-day gestation and cryopreservation by means of program freezer WOP-6. The thawing of samples was performed in a water bath at 37 °C. In another experimental group the equivalent volume of saline was injected intraperitoneally.

In animals that survived, according to the methodological recommendations [7] the biligenic and biliary excretion liver functions were investigated by catheterization to anesthetized animals (thiopental sodium 60 mg per kilogram of animal body weight) common bile duct and bile sampling for 60 min. In the resulting bile the concentration of total bile acids, cholesterol were measured, holato-cholesterol ratio was calculated.

While working with laboratory animals we followed the international standards of humane handling of animals in accordance with the rules of the “European Convention for the protection of vertebrate animals used for experimental and other scientific purposes” (European Convention, 1984). Euthanasia of rats after bile sampling was carried out in conditions of anesthesia by total depletion from heart.

The obtained digital data were the subject for statistical analysis. Statistical significance of differences between experimental and control groups was evaluated by using the program STATISTICA 10.0 ("StatSoft, Inc.", USA).

The results of the research and their discussion. As shown in the Table 1 and Figure 1, in conditions of modeled CSI the content of total bile acids in bile in relation to the control group was significantly decreased, reaching a minimal level in 3 and 7 days (respectively 50.0 and
52.2 %, p <0.001), and during these terms the index was significantly lower in relation according to the first day of observation (p ≤ 0.05). Under the influence of the cell therapy the content of total bile acids in bile continued to remain statistically significantly lower than the control (p <0.001), but in 1 day the tendency of its value increase in comparison with group of untreated animals was recorded (15.0%, p <0.10). In 3 and 7 days the index became statistically significantly higher than in group of uncorrected animals (accordingly of 31.1 and 43.0%, p<0.05).

The content of cholesterol in bile (Table 1, Figure 2) after CSI in 1 day of the post-traumatic period is almost unchanged in comparison with the control group (p>0.05). Further the index decreased – in 3 days of 13.0 % (p <0.10), in 7 days – 17.4 % (p<0.05). At that time of observation the index was also statistically significantly less than in 1 day (p≤0.05). As a result of cell therapy the cholesterol content in bile was not statistically significantly different concerning to the control group and group of corrected animals in all terms of observation.

Holato-cholesterol ratio (Table 1, Figure 3) as a result of modeled injuries drastically decreased in 1 day and remained at the same level until the end of the observation period (an average of 37.5%, p <0.01). After correction the index increased, but remained below the control average of 25.9 % (p<0.05), moreover in 7 days it was statistically significantly higher than in group of uncorrected animals (33.2%, p <0.05).

In turn, the rate of bile (Table 1, Figure 4) under the influence of injuries was decreasing from the first to the seventh day: in 1 day – of 25.0 % (p<0.01), in 3 days – of 29.8 % (p<0.001), in 7 days – of 40.8 % (p<0.001). At the last term of observation the index was statistically significantly lower than in 1 and 3 days of post-traumatic period (p≤0.05). As a result of cell therapy application the biliary excretion rate became higher than in the group of uncorrected animals, but only in 7 days the index turned out to statistically significant (of 31.1 %, p <0.001).

The received results testify that the modeled CSI accompanied by a significant disorders of bile excretion liver function in the acute period and during the early manifestations of traumatic disease, that occurs by decrease of synthesis, excretion of total bile acids in all periods of observation and cholesterol excretion in 7 days of post-traumatic period. Total bile acid synthesis occurs in the endoplasmic reticulum of hepatocytes, that are the most sensitive to disorders of cellular homeostasis under the influence of shock, hypoxia, action of inflammatory mediators and endotoxins load that occurs in severe injury [10]. Herewith also swelling of organ, that slows the outflow of bile, is developed [5]. The reduction of holats synthesis leads to decreasing of holato-cholesterol ratio and increasing of lithogenic properties of bile.
The application of cell therapy reduces the disarrangement of studied indexes. In this situation, obviously, membrane-stabilizing effect of cell therapy is occurred, as shown in researches [1, 2], that provides functional usefulness of endoplasmic membranes. In addition, the received result may be caused by the anti-hypoxic effect of cell therapy. Stimulation of erythropoiesis is one of its mechanisms, that was recorded in conditions of the application of embryonic mesenchymal cells suspensions in the correction of other pathological processes [9].

Thus, single intraperitoneal injection of cryopreserved fetal nerve cells suspension is able to reduce the manifestations of liver dysfunction in conditions of experimental CSI, that attributes this method of traumatic disease correction to promising directions of multiple organ failure prevention during the early manifestations of traumatic disease.

Conclusions. 1. During the early manifestations of traumatic disease as a result of CSI the content of total bile acids in bile is significantly reduced, biliary excretion is slowed, lithogenic properties of bile are increased.

2. Intraperitoneal injection of fetal nerve cells in 12 hours after causing an injury is accompanied by improvement of biligenic and biliary excretion liver functions, reduces the lithogenic properties of bile.

Eventually the investigation of other parenchymal organs functional status and efficacy of cell therapy in the late manifestations of polytrauma is expected.

Literature


4. Holtsev A. N. The apoptotic processes in thymus and brain in the development of experimental allergic encephalomyelitis before and after treatment by fetal nerve cells /


**Table 1** – Dynamics of indices of biligenic and biliary excretion liver functions in response to cranioskeletal injury combined with bleeding corrected by cell therapy (М±m)

<table>
<thead>
<tr>
<th>Conditions of experiment</th>
<th>Control</th>
<th>Cranioskeletal injury</th>
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<tbody>
<tr>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt; day</td>
<td>3&lt;sup&gt;rd&lt;/sup&gt; day</td>
</tr>
<tr>
<td><strong>Total bile acids, g·l&lt;sup&gt;-1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without correction</td>
<td>1,80±0,11 (n=6)</td>
<td>1,13±0,06*** (n=6)</td>
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<tr>
<td>Cell therapy</td>
<td>1,30±0,05** (n=7)</td>
<td>1,18±0,08*** (n=7)</td>
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<tr>
<td><strong>p</strong></td>
<td>p&lt;0,10</td>
<td>p&lt;0,05</td>
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<tr>
<td><strong>Cholesterol, g·l&lt;sup&gt;-1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without correction</td>
<td>0,23±0,01 (n=6)</td>
<td>0,22±0,01 (n=6)</td>
</tr>
<tr>
<td>Cell therapy</td>
<td>0,24±0,02 (n=7)</td>
<td>0,21±0,01 (n=7)</td>
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<tr>
<td><strong>p</strong></td>
<td>p&lt;0,05</td>
<td>p&lt;0,05</td>
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<tr>
<td><strong>Holato-cholesterol ratio, standard units</strong></td>
<td></td>
<td></td>
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<tr>
<td>Without correction</td>
<td>7,77±0,57 (n=6)</td>
<td>5,21±0,49** (n=6)</td>
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<tr>
<td>Cell therapy</td>
<td>5,60±0,54* (n=7)</td>
<td>5,62±0,61* (n=7)</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>p&gt;0,05</td>
<td>p&gt;0,05</td>
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<tr>
<td><strong>Bile rate, ml·hour&lt;sup&gt;-1&lt;/sup&gt;·kg&lt;sup&gt;-1</strong></td>
<td></td>
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<tr>
<td>Without correction</td>
<td>2,293±0,108 (n=6)</td>
<td>1,719±0,086* (n=7)</td>
</tr>
<tr>
<td>Cell therapy</td>
<td>1,845±0,072*** (n=8)</td>
<td>1,760±0,056*** (n=7)</td>
</tr>
<tr>
<td><strong>p</strong></td>
<td>&gt;0,05</td>
<td>&lt;0,10</td>
</tr>
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**Notes:**
1. * – significance of differences in relation to the control group (p<0,05; ** – p<0,01; *** – p<0,001; # – p<0,10).
2. p – significance of differences between groups regarding corrected and uncorrected animals.
Figure 1. Deviation dynamics of total bile acids content in bile (percentage of control level) in animals with cranioskeletal injury combined with bleeding under the influence of cell therapy (1,3 – differences regarding the 1st and 3rd days statistically significant, p ≤ 0,05)

Figure 2. Deviation dynamics of cholesterol content in bile (percentage of control level) in animals with cranioskeletal injury combined with bleeding under the influence of cell therapy.
Figure 3. Deviation dynamics of holato-cholesterol bile ratio (percentage of control level) in animals with cranioskeletal injury combined with bleeding under the influence of cell therapy.

Figure 4. Deviation dynamics of bile rate (percentage of control level) in animals with cranioskeletal injury combined with bleeding under the influence of cell therapy.