Abstract. State of spatial memory and antioxidant system activity of rats in the dynamics of development of blast-induced traumatic brain injury. Kozlova Yu.V., Maslak H.S., Abramova O.E., Koldunov V.V., Khudyakov O.E. The main purpose of this study was to investigate the changes in spatial memory and catalase activity in dynamics in the blast-induced traumatic brain injury (bTBI). The experiment was carried out on 67 albino male Wistar rats, which were randomly divided into three groups: I group – experimental (n=34), animals were subjected to inhalation anesthesia with halothane, fixed and was simulated blast-induced traumatic brain injury was simulated by generating a shock wave with an overpressure of 26.4±3.6 kPa, II group – sham (n=34), animals which were subjected only to inhalation anesthesia and fixation and III group – intact (n=34). After the study of behavior, euthanasia was performed, blood and brain were taken. Histopathological examination of the brain showed disturbances both of hippocampal neurons and microcirculation. In the dynamics of the post-traumatic period of mild bTBI there was observed a significant (p<0.01) impairment of rats spatial memory in the experimental group, which was established as a prolongation of latent time of the search in the Barnes maze: by 39% – on day 1, by 76% – on day 3, by 65% – on day 7 and by 61% – on day 14 of the study. The analysis of catalase activity revealed a significant decrease of this enzyme activity in blood plasma of the rats in the experimental group in comparison with rats of sham and intact groups: on day 1 – by 35% (p<0.01), on day 3 – by 27% (p<0.01) and by 12% on day 7 of the post-traumatic period (p<0.05), which indicates the involvement of catalase in the reaction of hydrogen peroxide inactivation. Correlation analysis between spatial memory and catalase activity in experimental rats showed a negative relationship of medium degree on day 1 (r=-0.5, p<0.01) and a negative relationship on day 7 of post-traumatic period (r=-0.2, p<0.01) of weak degree on day 3 of post-traumatic period, indicating increased formation of free radicals during this period. One-way analysis of variance showed a significant (H1, p<0.01) effect of changes in the catalase activity of rat blood plasma on the state of spatial memory in the Barnes maze during 14 days of the post-traumatic period. It was established that oxidative stress is an important link in pathogenesis of the spatial memory disorders in animals with mild bTBI in the first week of the post-traumatic period.
Related to military conflicts and domestic explosions, even mild brain trauma attracts attention due to impaired behavioral and cognitive functions of the central nervous system (CNS). However, the pathology of these changes is largely unclear, what leads to the impossibility of effective treatments developing [13]. Behavioral and cognitive impairments (memory loss) are observed in the acute and remote post-traumatic period, which are directly related to both primary blast wave injury and secondary brain tissue damage [11]. In turn, the primary effect of the blast wave is the rapid displacement of the head and concussion, the impact of displaced fluids, the cavitation effect. All these effects lead to neurons and blood vessels damage [4]. Previous studies indicate diffuse axonal damage, blood-brain barrier dysfunction, which are triggering and creating conditions for the action of secondary pathological factors on the brain, among which are biochemical mechanisms, such as development of oxidative stress [2, 8].

It is known that oxidative stress occurs with excessive formation of free radicals (hydrogen peroxide and others) during the pathological processes development, in particular with brain damage. Modern researches show a negative effect of oxidative pressure on the CNS in various pathological processes, the clinical symptoms of which are changes in psycho-emotional state [15]. It is related to the fact that the brain is very sensitive to free radicals due to active consumption of oxygen and high content of lipids in the neurons structure. The key enzyme that inactivates hydrogen peroxide is the enzyme of the antioxidant system — catalase [14]. It is known that decreasing in its activity indicates an increasing in use and this can be used for the diagnosis and timely treatment of oxidative stress in the case of blast-induced traumatic brain injury (bTBI). Therefore, researching and understanding of changes in memory and activity of the antioxidant system enzymes, in particular catalase, in mild bTBI is relevant and a priority, which will contribute to the development of preventive and curative measures based on the pathogenesis.

MATERIALS AND METHODS OF RESEARCH

The experiment was carried out on 102 albino male Wistar rats (body mass 220-270 g, age 6-7 months). The animals were kept in standard conditions and on the standard diet of the Dnipro state medical university (DSMU) vivarium [5], all researches were conducted in accordance with modern international requirements and norms of humane attitude of animals (European Convention, 18.03.1986 (Strasbourg); Declaration of Helsinki, 1975, revised and supplemented in 2000, Law of Ukraine No. 3447-IV, 21.02.2006), attested by an extract from the minutes of the commission on biomedical ethics meeting of DSMU No. 3, 2.11.2021.

Selected rats were divided into two groups: group I — experimental, Exp (n=34), animals were subjected to inhalation anesthesia with halothane (Halothan Hoechst A.G., Germany), fixed in a horizontal position on the abdomen at a distance of 5 cm with a head to muzzle end and simulation of explosion-induced brain injury by generating a shock wave with an overpressure of 26.4±3.6 kPa on a self-made device [6], II group — control, sham, Sh (n=34), animals were subjected only to inhalation anesthesia with halothane and fixation in a horizontal position and III group — intact, Int (n=34). Sham and intact groups were created to limit the action of additional pathogenic factors (anesthesia, fixation). Excess pressure was measured using an electronic manometer APZ 3420G (“PIEZUS”, Russia). Immediately after the study of behavior, the animals were euthanized with halothane, followed by blood sampling for biochemical examination and brain extraction for histopathological analysis.

Studies of spatial memory were performed on 1, 3, 7 and 14 days of the post-traumatic period using the Barnes maze, represented by an arena with a diameter of 122 cm made of black plastic, which is fixed at 100 cm from the floor surface on a metal stand. Along the perimeter of the arena 20 holes with a diameter of 10.5 cm were located at equal distances from each other and from the center. One of the holes has been turned into escape chamber. The rest of the holes are fake chambers — not deep, in which it is impossible to hide. In the center of the arena was a start chamber that could be removed.
Before testing, all animals were trained while 5 days, after it only those animals were selected which found shelter during 5 minutes [8]. Rats were brought from the vivarium to the laboratory in 30 minutes before the experiment. Each animal was placed in a escape chamber for 2 minutes, then removed and moved to a start chamber in the center of the arena, which was immediately removed, and the rat was in the open area. The latent time of the animal entering the escape chamber was recorded and estimated.

Catalase activity (EC1.11.1.6; Systematic name: hydrogen-peroxide:hydrogen-peroxide oxidoreductase) was determined by the rate of hydrogen peroxide decrease (utilization) in the incubation medium. The concentration of hydrogen peroxide was determined by reaction with ammonium molybdate, which gives a stable colored complex, measured colorimetrically at a certain wavelength (405 nm).

The rats blood plasma was analyzed, and was stored at a temperature of -20°C. Rat blood plasma was transferred by 50 μl into centrifuge tubes with a volume of 1.6 ml at the rate of 1 control and 1 experimental sample for 1 test sample. To the control samples 50 μl of 0.2% sodium azide (NaN₃) was added to inhibit catalase activity, after which to all tubes 1 ml of 0.03% hydrogen peroxide (H₂O₂) was added, incubation of the reaction mixture was carried out in the darkness at a temperature of +25°C for 1 minute (thermostat TC-80) to neutralize the molecules of hydrogen peroxide by catalase for a set time. Immediately thereafter, catalase activity was inhibited by adding 50 μl of 0.2% sodium azide (NaN₃) to each of the test tubes. To the mixture in all (control and experimental) tubes 0.5 ml of 4% ammonium molybdate ((NH₄)₂ MoO₄) was added to form a stable colored complex with intact molecules of hydrogen peroxide, after which the mixture in the tubes was centrifuged for 2 min at 300 g (centrifuge MPW-55, Poland) for precipitation of insoluble complex. The supernatant was taken in 200 μl into the microplate well (the test sample was duplicated) and the absorption of the supernatant was measured using a microplate photometer Multiscan FC (Thermo Fisher Scientific, China, 2019) at 405 nm [13].

Catalase activity (μmol/s*l) was calculated by the formula:

\[ E = \frac{\Delta A \times V \times K}{\varepsilon \times t} \]

where E – catalase activity, μmol/s*l;
\( \Delta A \) – the difference in optical density between the control and test samples, (Ac – At);
V – sample volume, ml;
K – conversion factor per 1 liter of blood plasma, 10000;
t – incubation time, sec;
\( \varepsilon \) – the molar extinction coefficient of hydrogen peroxide, 22.2 M⁻¹•cm⁻¹.

For histopathological analysis, the extracted brain was fixed for 24 hours in 10% buffered formalin solution. After fixation, the brain was cut in the frontal plane into particles, followed by filling them with paraffin and making blocks. At the initial stage, paraffin sections were prepared with staining by hematoxylin and eosin in accordance with accepted standards [4]. Stained specimens were dehydrated in alcohols and clarified in xylene. Then the slices were placed on a glass slide with fixation in Canadian balm. The obtained specimens were studied using a Zeiss Imager A2 microscope (eyepiece x10; lens x10, 20, 50 μm), and photographed (Axioacam 512 color) of different parts of the hippocampus was performed with subsequent analysis.

Statistical processing of the results was performed using the software product STATISTICA 6.1 (StatSoftInc., the serial No. AGAR909E415822FA). Mathematical processing included calculations of arithmetic mean values (M) and standard deviations (M±SD). To determine the degree and nature of the links between the parameters of the study, we used a comparative analysis (Mann-Whitney U-test) at confidence thresholds p<0.01, p<0.05, to establish correlations between the study parameters Spearman's correlation coefficient was used, to determine the effect of indicators on each other one-way analysis of variance ANOVA was used [1].

RESULTS AND DISCUSSION

Histopathological examination of the rats brain with mild bTBI on the 1st day of the post-traumatic period showed heterochromatosis and uneven with a decrease in the density of neurons the pyramidal layer of the hippocampus, increased intercellular space, pericellular edema, and signs of hemorrhage, free location of single erythrocytes outside the vessels (Fig. 1).

Comparison of the results between sham and intact groups did not show significant differences, which indicates a minor toxic effect of halothane. Therefore, further analysis was carried out between experimental and intact groups of rats. The latent time of shelter search in animals of the experimental group was significantly (p<0.01) longer in all terms of the study (Fig. 2). So, on the 1st day of the post-traumatic period increasing in search time by 39% (p<0.01) was found in experimental rats, on the 3rd day – by 76% (p<0.01), on 7th day – by 65% and on 14th day – by 61% in comparison with intact rats.

Comparison of blood plasma catalase activity of three groups of animals showed a significant (p<0.01, p<0.05) reduction of this enzyme in the experimental group of rats on 1st day – by 35% (p<0.01), on 3rd day – by 27% (p<0.01), and on the 7th day of the post-traumatic period – by 12% (p<0.05) (Fig. 3).
Correlation analysis carried out between the indicators of spatial memory in the Barnes maze and the indicators of catalase activity in the rats blood plasma of both experimental groups indicated the presence of a medium negative link on the 1st day \((r=-0.3, p<0.01)\), weak negative link on the 3rd day \((r=-0.2, p<0.01)\), weak positive link on the 7th day \((r=+0.01, p<0.05)\) and strong positive link \((r=+0.6, p<0.01)\) on the 14th day of the post-traumatic period.

The one-way analysis of variance identified a significant \((H1, p<0.01)\) effect of changes in the catalase activity on the state of spatial memory in the Barnes maze in all terms of the investigation.

From clinical studies it is known that in victims of the explosion there is a memory impairment, in particular spatial, even with minor trauma, the pathogenesis of which is subject to precise research [7].

The sensitivity of the Barnes maze to detect spatial memory disorders in experimental traumatic brain injury (TBI) has been repeatedly proven [8, 11]. The method of spatial memory studying in an experiment using the Barnes maze is based on a quite simple and stress-free investigation of memory and subsequent spatial navigation in an open area. It is considered that reducing the time of finding an escape chamber during training reflects the preservation of rats memory [8]. The results of our studies indicate a significant disorder of spatial memory in rats with mild bTBI in the Barnes maze. This is evidenced by a significant increase in latent time of shelter search in rats of the experimental group.
Oxidative stress is considered the main cause of secondary damage to brain neurons in the early period of TBI [2]. The activity of free radical processes is evidenced by the reaction of antioxidant enzymes, in particular catalase, which is involved in the decomposition of toxic hydrogen peroxide into water and molecular oxygen [9]. The pathogenic effect of hydrogen peroxide is the oxidation of neuronal membrane lipids, cell proteins, mitochondrial disorders with the development of energy deficiency and dysfunction and death of brain neurons. The spread of free radicals throughout the body leads to homeostasis disorder and deterioration of the general condition. Therefore, it is believed that catalase activity is an important marker of the dynamics of the post-traumatic period. In a study [2] on the diagnostics of mild trauma in terms of elucidation of expert criteria, it was found that during the first week after brain injury, catalase activity decreases compared with the sham group.

In our experiment, during the first week after the reproduction of bTBI we observed a significant (p<0.01, p<0.05) reduction in plasma catalase activity in rats of the experimental group, indicating the involvement of the studied enzyme in the inactivation of hydrogen peroxide.

Thus, after the impact of traumatic factor – the blast wave, on the brain, in the hippocampus there are acute primary changes of neurons in the form of heterochromia, pericellular edema and vascular disorders of the microcirculatory tract, as confirmed by histopathological examination. These primary disorders are a trigger for the development of secondary damage factors, namely the development of oxidative stress, which, according to our research, continues for a week after injury.

Considering the identified primary traumatic changes in the microcirculatory tract with impaired blood supply to the neurons of the hippocampus, we believe that the development of oxidative stress is associated with hypoxia of the brain [12, 14]. All identified changes are links in the pathogenesis of spatial memory disorders in mild bTBI and require treatment in the acute post-traumatic period.

**CONCLUSIONS**

1. Histopathological examination of the brain revealed heterochromatosis and uneven with a decrease in the density localization of the neurons of the pyramidal layer of the hippocampus of CA3 increased intercellular space, pericellular edema and microcirculatory tract disorders.

2. In the dynamics of the post-traumatic period of mild bTBI there was observed a significant (p<0.01) impairment of rats spatial memory in the experimental group, which was established as a prolongation of latent time of shelter search in the Barnes maze by 39% on the 1st day, by 76% on the 3rd day, by 65% on the 7th day and by 61% on the 14th day of the investigation.

3. The analysis of catalase activity revealed a significant decrease of this enzyme in blood plasma of the rats in the experimental group in comparison with rats of sham and intact groups: on the 1st day by 35% (p<0.01), on the 3d day – by 27% (p<0.01) and on the 7th day of the post-traumatic period – by 12%
(p<0.05), which indicates the involvement of catalase in the reaction of hydrogen peroxide inactivation.

4. A significant decrease in catalase activity in blood plasma found in experimental rats showed a negative relationship of medium degree on day 1 (r=-0.3, p<0.01) and a negative relationship (r=-0.2, p<0.01) of weak degree on day 3 of post-traumatic period, indicating increased formation of free radicals during this period.

5. One-way analysis of variance showed a significant (H1, p<0.01) effect of changes in the catalase activity of rat blood plasma on the state of spatial memory in the Barnes maze during 14 days of the post-traumatic period.

6. It has been established that oxidative stress is an important link in the pathogenesis of spatial memory disorders in animals with mild bTBI in the first week of the post-traumatic period.

**Contributors:**

- Kozlova Yu.V. – conceptualization, research, data analysis, writing;
- Maslak H.S. – methodology, research, resources, data analysis;
- Abramova O.E. – research, software, data analysis;
- Koldunov V.V. – conceptualization, methodology;
- Khudyakov O.E. – methodology, resources.

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