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Morphofunctional Characteristics of the Hippocampus of White Rats in the Acute Period After Severe Traumatic Brain Injury During the Use of L-lysine Aescinat

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AIM OF STUDY
The study is devoted to the effect of L-lysine aescinat on the nervous tissue of the CA1 and CA3 fields of the hippocampus of the brain of white rats in the acute period after severe traumatic brain injury (TBI).

MATERIAL AND METHODS
TBI was simulated by applying a blow to the parieto-occipital region with a freely falling weight weighing 200-250 grams from a height of 50 cm using a special rail rack. The objectives of the study were: 1) comparative morphometric assessment of the degree of hydration, cyto- and glioarchitectonics of different layers of CA1 and CA3 fields after ischemia without treatment; 2) the effect of L-lysine aescinat on these indicators. We used histological (staining of sections with hematoxylin-eosin and Nissl), immunohistochemical (for NSE, MAP-2 and GFAP) and morphometric methods. On thin (4 μm) serial frontal sections of the hippocampus, neurons, astrocytes, microvessels and neuropiles were studied in control (intact animals, n=5) and 1 and 3 days after injury without treatment (n=10, comparison group) and with treatment (n = 10, main group). The number density of neurons was determined using the Nissl staining of cells and by the reaction to NSE. The cytoskeleton of neurons was studied by detecting MAP-2, and astroglia by GFAP. On color raster images (staining with hematoxylin and eosin, x100) using the Find Maxima plug-in filter, the zones of maximum brightness were determined, which were then analyzed using Analyze Particles from the ImageJ 1.52s program. Zones of maximum brightness corresponded to areas of the hippocampus with a high degree of hydration of the nervous tissue - edema-swelling. The nature of the distribution, statistical hypotheses, and plotting were checked using Statistica 8.0 software and R environment.

RESULTS In control animals, normochromic neurons without signs of changes in the cytoskeleton prevailed in all layers of fields CA1 and CA3, and a low degree of hydration of the nervous tissue was noted (the relative proportion of zones of maximum brightness was 5–8%). One and 3 days after TBI, there was a statistically significant increase in the focal content of dystrophic and necrobiotically altered neurons (95% confidence interval: 52–78%), manifestations of reactive gliosis were noted, and the proportion of zones of maximum brightness increased to 16%. Statistically significant layer-by-layer differences were revealed between the CA1 and CA3 fields of the hippocampus. The use of L-lysine aescinat had a statistically significant effect on the morphometric parameters of the nervous tissue of the hippocampus.

CONCLUSION In the early post-traumatic period after TBI, the degree of hydration of the nervous tissue of the hippocampus increased. Heteromorphicity of dystrophic and necrobiotic changes in different layers of CA1 and CA3 fields was noted. L-lysine aescinate had a statistically significant positive effect on these changes. To a greater extent, this is typical for the CA3 field. The revealed changes are considered not only as patho-, but also as sanogenetic structural mechanisms of protection and reorganization of the hippocampus in the acute post-traumatic period.

1. In the acute period (1−3 days) after severe traumatic brain injury, the degree of hydration of all components of the hippocampal nervous tissue increased. In the group without treatment, 3 days after injury, the relative volume of edema-swelling zones varied from 10 to 13% in CA1 (control 3-7%) and from 8 to 16% in CA3 (control 5–10%).
2. The heteromorphism of hydropic changes in the molecular layer, the layer of pyramidal neurons and the polymorphic layer was established. The maximum increase in the volume of free water (more than twofold) was characteristic of the molecular and polymorphic layer CA1, as well as the polymorphic layer CA3.
3. The use of L-lysine aescinat in the acute period significantly changed the manifestations of hydropic dystrophy. One day after injury, the volume of free water increased in comparison with animals without treatment, and then, after 3 days, decreased, but remained higher than in the comparison group. The maximum effect of the drug was noted in field CA3.

Keywords: traumatic brain injury, hippocampus, edema-swelling, immunohistochemistry, morphometry, in vivo study, Wistar rats, L-lysine aescinat
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*C*A1, *C*A3 — fields of hippocampus
DAB — diaminobenzidine
GFAP — glial fibrillary acidic protein
MAP-2 — microtubule-associated protein
MFL — multiform layer
MLL — molecular layer
NSE — neuron-specific enolase
ML — multiform layer
PNL — pyramidal neurons layer
STBI — severe traumatic brain injury
ZMB — zones of maximum brightness

INTRODUCTION

The most important reason for the development of unfavorable outcomes in the acute period after severe traumatic brain injury (STBI) is progressive intracranial hypertension due to the development of severe post-traumatic edema-swelling of the brain [1], which leads to secondary microcirculation disorders, ischemia and neuronal death [2-4]. That is, hydropic changes are traditionally considered as predominantly aggravating factors of the post-traumatic period [5–7].

According to modern concepts, the vascular component has the greatest pathogenetic significance in the development of cerebral edema [8, 9–11]. In the acute period of trauma, due to damage to the blood-brain barrier and an increase in its permeability, vasogenic edema develops, while in the gray matter of the brain, fluid accumulates intracellularly, mainly in the neuroglia. Then, due to the transfer of fluid from the outside to the intracellular space, cytotoxic edema develops [6, 12, 13].

It is known that STBI affects all parts of the brain structure according to the well-studied laws of primary and secondary damage in acute, subacute and long-term periods [11, 14-16]. In this regard, it is of particular importance to study the consequences of closed STBI on the hippocampus, which does not have direct contact with the traumatic agent, but has a significant effect on cognitive functions and memory [2, 4].

There are various ways to regulate water exchange, and, accordingly, the volume of free fluid and intracranial pressure [8, 17]. The drug L-lysine aescinat helps normalize the function of the blood-brain barrier, has an anti-exudative, anti-inflammatory effect [7, 8, 18]. However, the effect of L-lysine aescinat on the nervous tissue of the hippocampus of white rats under experimental traumatic brain injury has not been studied using histological and morphometric methods.

MATERIAL AND METHODS

The study was carried out on the basis of the Federal State Budgetary Educational Institution of Higher Education "Omsk State Medical University", approved by the ethical committee of the university (protocol No. 107 dated October 2, 2018). As experimental animals, we used 25 outbred sexually mature (110–120 days old) male Wistar runoff rats weighing 270–350 grams (Federal Research Center Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences). The animals were kept under standard laboratory conditions. The experiment was carried out in accordance with the "Rules for work using experimental animals" (Appendix to the Order of the Ministry of Health of the USSR dated Aug 12, 1977 No. 755) and the recommendations of the International Committee for the Science of Laboratory Animals, supported by WHO, Directive of the European Parliament No. 2010/63 / EU dated Sept 22, 2010 "On the protection of animals used for scientific purposes."

For the histological study of the hippocampus of the brain in the acute post-traumatic period (1 and 3 days), STBI was simulated, n = 20. For sedation, a solution of the drug Zoletil 100 (5-7 units) was used. Five minutes after the introduction of Zoletil 100, a free-falling weight of 200-250 g from a height of 50 cm was struck on the parieto-occipital region using a special rail stand.

After STBI, all surviving animals (n = 20) were randomly (number generator) divided into two groups. In the main group (n = 10), immediately after the injury and then once a day, 0.1 ml of the systemic angioprotector (L-lysine escinate) was diluted in 0.2 ml of 9% sodium chloride solution was injected with an insulin syringe into the lateral vein of the rat tail (in accordance with the average weight of the rat). The animals of the comparison group (n = 10) were not injected with the drug. Intact rats (n = 5) were the control group.

The takeaway of the animals of the main group and the comparison group from the experiment was carried out under anesthesia (Zoletil 100) 1 (n = 5) and 3 (n = 5) days after injury by bloodletting. The brain was fixed by perfusion: 100-125 ml of a solution of 0.9% sodium chloride and Fragmin (5000 units) and 30 ml of a 4% solution of paraformaldehyde in phosphate buffer (pH 7.2-7.4) were injected successively. After decapitation, the brain was removed and stored at a temperature of 3-5 ° C in a similar fixative. To make the samples, the material was embedded in homogenized paraffin (HISTOMIX®) using an STP 120 machine. On a sled microtome HM 450 (Thermo), serial frontal sections with a thickness of 4 μm were prepared, which were performed at the level of the hippocampus in the range from (-) 2.40 to (-) 3.36 mm from Bregma (anthropometric point located on the roof of the skull, crown of head) [19].

For histological examination, sections of the hippocampus were stained according to Nissl, hematoxylin, and eosin. Astrocytes were verified using immunohistochemical reaction for glial fibrillar acidic protein (GFAP), and neurons - using a microtubule-associated protein (MAP-2) and neuron-specific enolase (NSE). Sections were placed on polylysine slides. Monoclonal mouse antibodies (Bond Ready-to-Use Primary Antibody; Leica Biosystems Newcastle Ltd, UK) and polyclonal antibodies (NSE) were used as primary ones. For visualization of GFAP astrocytes and MAP-2 neurons, we used Novolink ™ (DAB) Polymer Detection System (Leica Biosystems Newcastle Ltd, UK). For NSE, polyclonal antibodies (rabbit source) to rat antigen (Cloud-Clone Corp.) were used. After reaction with primary antibodies, the sections were sequentially incubated with secondary antibodies, then with the DAB chromogen (3,3'-diaminobenzidine), counterstained with hematoxylin, and embedded in polystyrene.

The samples were photographed using a Leica DM 1000 microscope (x100 lens, GXCAM-DM800 Unique Wrap-Around 8MP AUTOFOCUS USB camera, pixel size 1.4x1.4 μm), the image was saved in files with the tiff extension (2592x1944 pixels). To achieve maximum contrast and image sharpness, the correction was performed using the Camera Raw filter (contrast, white balance, sharpness) in Photoshop CC. Further morphometric examination was carried out using the ImageJ 1.52s software. To identify areas of edema-swelling (maximum pixel brightness - Maxima) of the nervous tissue, we used the plug-in filter “Find Maxima” M. Schmid from the ImageJ 1.52s program (https://imagej.nih.gov/ij/docs/menus/process. html # find-maxima). For each period, 50 randomly selected fields of view (region of interest) of color images with an area of 19600 μm^2 were used. On the image masks obtained as a result of the operation of this filter, the relative area of the zones of maximum brightness (ZMB) was determined (“Analyze Particles”), which were then converted into the volume (V_maxima, μm^3). The non-uniformity of the ZMB distribution was assessed using the “Segmented Particles” watershed algorithm applied to the image brightness values, the “Maxima Within Tolerance” mode was used, and the “Prominence” value was selected to cut off empty large vessels (10–11).

To avoid systematic errors when testing statistical hypotheses using a random number generator in the Statistica 8.0 program, the visual fields were randomized from the obtained data sets (n = 50 was selected for each period). The distribution of the variation series was assessed using Shapiro – Wilk tests (Statistica 8.0). Statistical hypotheses were tested using the Mann – Whitney U-test and ANOVA (one-way analysis of variance) Kruskal – Wallis (StatSoft Statistica 8.0). Quantitative data in the work are presented as a median (50% quartile is a measure of the central tendency), 25–75% quartiles and ranges without outliers (Max – Min) are measures of the spread of the studied values. The median is a robust characteristic and is most suitable for this type of research [20]. During the statistical analysis, the null hypothesis was rejected at $p \leq 0.05$.

RESULTS AND DISCUSSION

Normally (control group), typical normochromic pyramidal neurons with a large nucleus predominated in CA1 and CA3 sections of the hippocampus stained with hematoxylin-eosin and thionine; no signs of hydropic or protein dystrophy were detected. The neuropil appears to be a homogeneous substrate without structural manifestations of edema-swelling of its components (dendrites, synapses, astrocyte processes) (Fig. 1 A, C). Neurons (NSE) and glial cells (GFAP) were clearly verified using immunohistochemical reactions (Fig. 2 A, B, C).

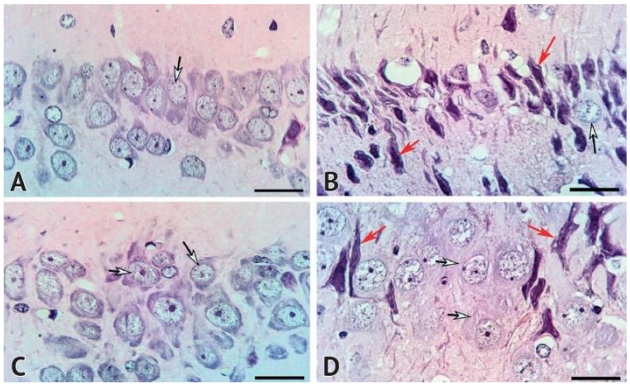

Fig. 1. CA1 (A, B) and CA3 (C, D) of the hippocampus of the brain in norm (A, C) and 3 days (B, D) after severe traumatic brain injury: normochromic pyramidal neurons (white arrows) prevail in norm); after injury, pycnomorphic neurons (red arrows) and manifestations of edema-swelling appeared. Hematoxylin-eosin staining; x100; scale — 25 microns

After STBI, typical structural signs of hydropic and protein dystrophy appeared: vacuolated, hypochromic and hyperchromic neurons without and with wrinkling (pyknomorphic), edema-swelling of neurons, dendrites, neuropil, edema of pericellular and perivascular processes of astrocytes, and oligoglytes (Fig. 1 B, D; 2 D – G). In addition to hydropic changes, signs of reactive neurogliosis were noted: the density of gliocytes and their processes increased (Fig. 2 B, C, E, G). The changes were focal: areas CA1 and CA3 with a complete predominance of normochromic neurons were combined with areas of damage, in which 52–78% (95% confidence interval) of neurons had signs of reversible or irreversible dystrophy. Hyperchromic changes predominated. In irreversibly altered pyknomorphic neurons, condensation and homogenization of the chromatophilic substance of the nucleus and cytoplasm took place, and the nucleolus "disappeared". The maximum number of such neurons was observed 3 days after injury (in some foci up to 80%) (Fig. 1C). Significantly, pericellular edema was not observed around all damaged neurons. This indicated the absence of a functional relationship between these qualitative variables (Fig. 1 B, D; 2 E – G).

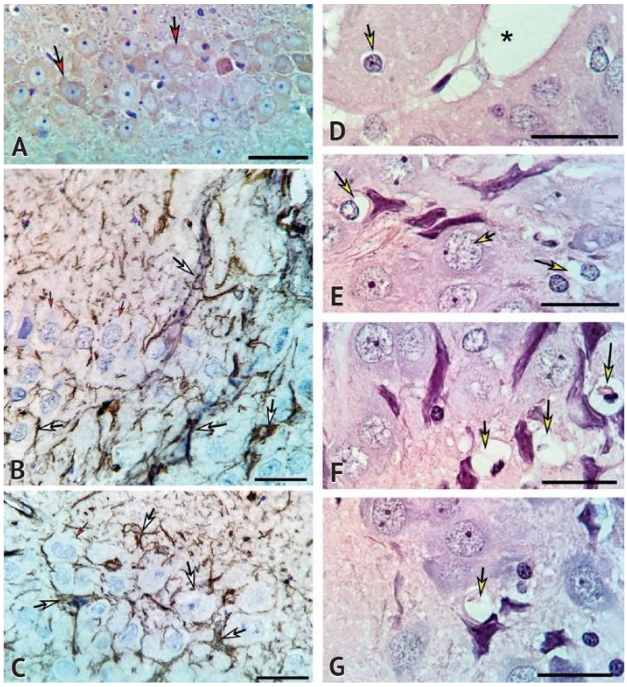

Fig. 2. Arbitrarily taken visual fields (A, B, D - CA1, C, E, F, G - CA3) of the hippocampus of the brain in norm (A) and 1 (d) and 3 days (B, C, E, F , G) after traumatic brain injury: A — distribution of NSE-positive perikaryons (red arrows), B, C — astrocyte processes (white arrows); D–G — various manifestations and compartments of free water accumulation (yellow arrows). Immunohistochemical reaction for NSE (A), GFAP (B, C); staining with hematoxylin and eosin (D–G). Magn x40 (A), x100 (B–G); scale — 25 microns

Thus, according to an overview histological study, after STBI in the hippocampus, the degree of hydration of the perikarion of neurons and neuropil increased, and free water was formed and redistributed between intracellular (nucleus and cytoplasm, cytoskeleton), cellular (neurons, glia, neuropil) and intercellular compartments of nervous tissue. That is, all its structural components took part in the process of water redistribution (Fig. 2 D – F; 3 B, D).

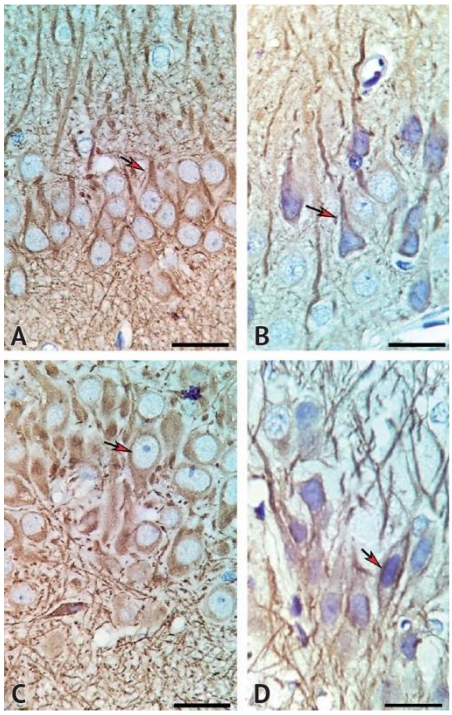

Fig. 3. Arbitrarily taken visual fields (A, B — CA1, C, D — CA3) of the hippocampus of the brain of a white rat in normal conditions (A, C), after 1 (B) and 3 days (D) after severe traumatic brain injury: MAP-2-positive perikarya and neuronal processes after injury underwent constriction, accumulated along the periphery of the perikarya, took a corkscrew shape, intercellular edema was noted, this is especially clearly seen in field CA3. Immunohistochemical reaction for MAP-2; x100; scale — 25 microns

Morphometric assessment of changes in free water content was carried out by the number and area of zones of bright pixels (ZMB - maximum hydration and free water) on color images identified using the Find Maxima plug-in filter in the ImageJ1.52 program (Fig. 4 A – D).

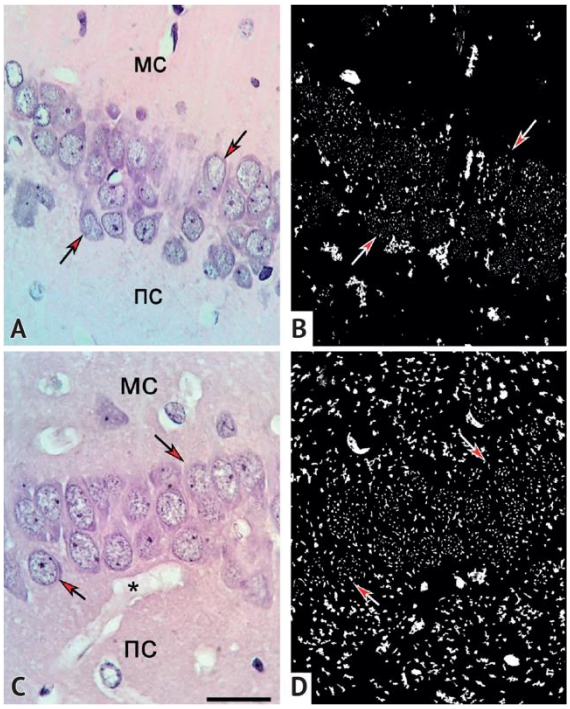

Fig. 4. CA1 hippocampus (A, B - control; 1 C, D — 1 day after injury): technology of morphometric measurement of areas of maximum image brightness: A, C - standard randomly selected area of interest; B, D — the result of applying the “Find Maxima” filter and the “Analyze Particles” plugin. The zones of maximum brightness have a complex shape; after an injury, the number of zones is visually greater than normal. Arrows — layer of pyramidal neurons, MLL — molecular layer, MFL — polymorphic. Hematoxylin-eosin staining; magn x100; scale — 25 microns

On raster images of nervous tissue (when stained with hematoxylin and eosin), the degree of hydration of its components was determined by the brightness of the pixels (from 0 to 255). The maximum value (230−245) was reached by areas containing free water, while in other areas the degree of hydration (chemical bond) of the neuropil had a range from 190 to 230 in terms of pixel brightness. After the injury, the amount of ZMP was even visually greater than normal. The ZMP had a complex shape and irregular outlines (Fig. 4 A, B).

Thus, the control group is characterized by a low degree of hydration (the ability of water to chemical association with hydrophilic substances - swelling) of the nervous tissue and almost complete absence of free water in it (a mechanical form of communication with the surrounding structures - edema).

In the control group, the volume of all ZMB (per 1 mm3) in the hippocampus did not exceed 5–8%. At the same time, in the CA1 field, the absolute value of this indicator - 0.054 (0.047−0.062) mm^3 - was statistically significantly less than in the CA3 field - 0.072 (0.065−0.079) mm3 ($p < 0.01$) (Fig. 5). This is due to the fact that the stratum lucidum of the CA3 epidermis contains a large number of apical dendrites of pyramidal neurons and giant synaptic terminals formed by the axons of mossy fibers on the bodies and dendrites of neurons. Therefore, this area in the image turned out to be lighter and contained more bright pixels (Fig. 3C).

We did not take into account large ZMB corresponding to the lumens of arterioles and venules.

In the acute post-traumatic period in both experimental groups, the volume of the ZMB significantly exceeded the control value in fields CA1 and CA3 2-fold, and also differed between the experimental groups in time after injury. The maximum increase in the total volume of ZMB in the hippocampus was revealed 3 days after injury. In the neuropil, the size of one ZMB corresponded to the size of sections of dendrites, astrocytic processes and large synapses - 0.4-3.5 μm. Larger ZMB (pericellular and perivascular edema), as a rule, were represented by a set of conglomerates of the corresponding astrocytic processes and their bodies (Fig. 3 D – G). After injury, the frequency spectrum shifted towards an increase in the content of larger ZMB, which was especially characteristic of CA1 (Fig. 5).

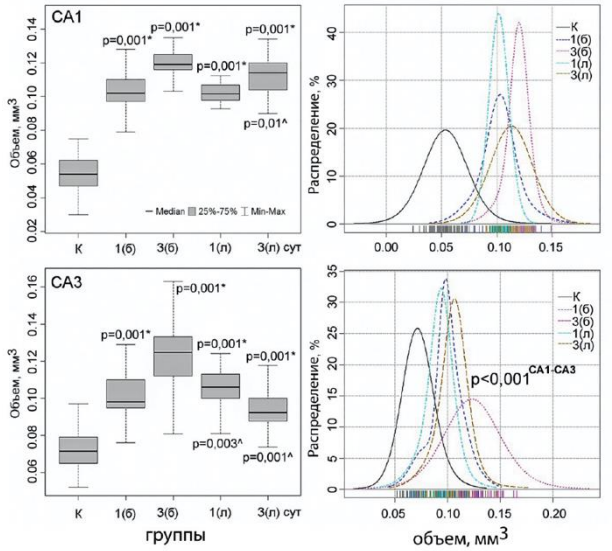

Fig. 5. Morphometric characteristics of changes in the degree of hydration of the nervous tissue of the CA1 and CA3 fields of the hippocampus in the acute post-traumatic period (1st and 3rd days) in groups without and with treatment: distribution of visual fields by the volume of zones of maximum brightness in 1 mm^3; * — differences in comparison with control and (^) between experimental groups are statistically significant ($p<0.001$, Mann–Whitney test). Material presented as median (50%), 25-75% quartile and no outlier range (Min-Max)

The use of the L-lysine aescinat led to a partial decrease in the total volume of edematous compartments (ZMB) only 3 days after injury. This was especially clearly seen in CA3 (Fig. 5). Probably, the latter indicated the effect of the drug on the degree of hydration of the nervous tissue of the hippocampus. During this period, the maximum accumulation of pyknomorphic neurons also occurred (Fig. 3 E – G).

We have established the heteromorphism of hydropic changes in the molecular layer, the layer of pyramidal neurons and the multiform layer of the hippocampus (Fig. 6 and 7). The maximum increase in the volume of free water was typical for the molecular and multiform layers of the CA1 field and the multiform layer CA3, where this indicator in the post-traumatic period increased more than 2-fold.

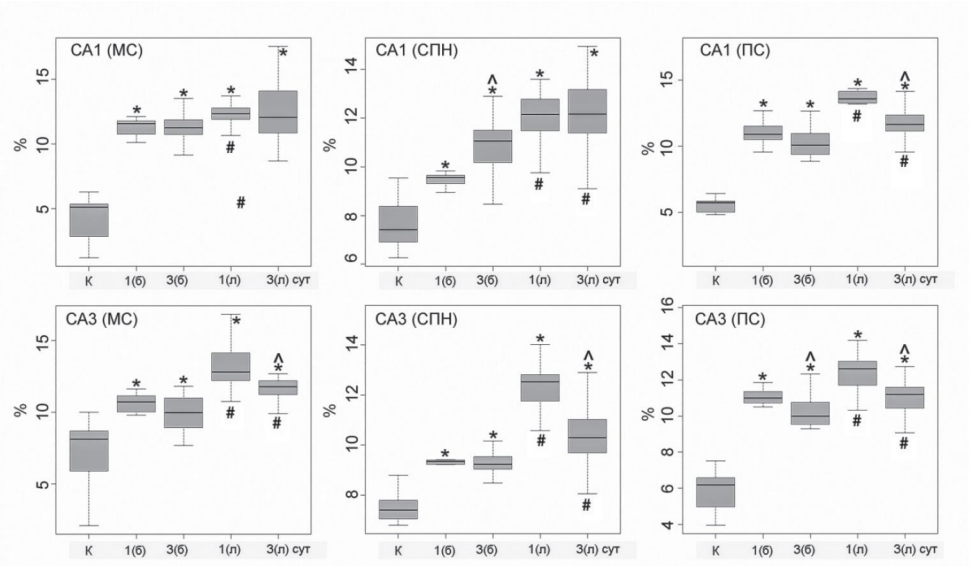

Fig. 6. Percentage of zones of maximum brightness in 1 mm^3 of nervous tissue of different layers of fields CA1 and CA3 of the hippocampus in the control and acute post-traumatic period (1st and 3rd days) in groups without and with treatment. Differences in comparison with the control (*), between the terms (^) and experimental groups (#) are statistically significant ($p<0.001$, Mann–Whitney test). Material is presented as median (50%), 25–75% quartile, and no outlier range (Min–Max). MFL – multiform layer; MLL – molecular layer; PL – pyramidal layer

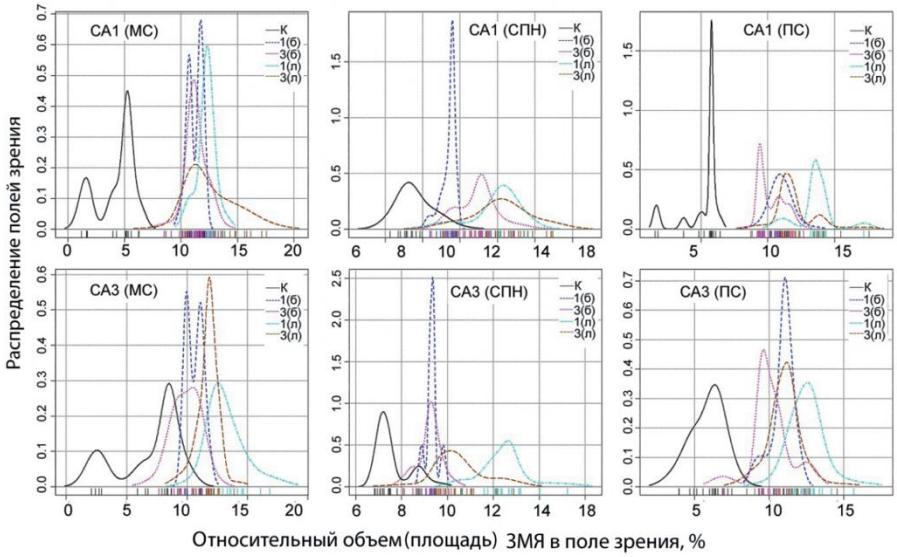

Fig. 7. Frequency distribution of visual fields by the relative volume (area) of the zones of maximum brightness in the molecular layer (ML), the layer of pyramidal neurons (PNL) and polymorphic layer (PL) of the CA1 and CA3 fields of the hippocampus in control and acute post-traumatic period (1st and 3rd day) in groups without and with treatment

After using L-lysine aescinat, the volume of ZMB was statistically significantly higher than in the comparison group without the drug. However, when paired comparison in terms of time within the main group, the volume of free fluid 3 days later decreased in all layers of the CA3 field and the multiform layer of the CA1 field. This did not happen in the comparison group (Fig. 6). This indicated the effect of the drug on the redistribution of fluid within the compartments of the nervous tissue. However, in the molecular layer and the layer of pyramidal neurons CA1, the volume of the ZMB remained at the level of the previous period (Fig. 6). Consequently, when using L-lysine aescinat, the degree of hydration of the nervous tissue of different layers of the CA1 and CA3 fields of the hippocampus changed ambiguously. It is likely that this depended on some peculiarities of the organization of their neurogliovascular microstructural complexes.

The significant difference in the change in the volume of free fluid over the layers of the CA1 and CA3 fields of the hippocampus was also evidenced by the frequency characteristics of the size distribution of the ZMB (Fig. 7). Thus, in the hippocampus, we revealed the heteromorphism of hydropic changes as a response to TBI.

DISCUSSION

The results of this experimental morphological study indicate that in the acute period after STBI in the CA1 and CA3 fields of the hippocampus, the degree of hydration of the nervous tissue (swelling) increases, when the part of the water chemically bound to macromolecules passes into a free form and is redistributed between nerve and glial cells (small focal edema). We believe that there are two main directions of free water flows from neurons to gliocytes: 1) at the level of processes (in the neuropil) and 2) from the bodies of neurons (cell layers) into the pericellular spaces. Moreover, in some cases, the exit of water from neurons is carried out only from the processes, while in others both directions are involved. This is supported by the identification in one field of view of the hippocampus of hyperchromic

neurons with an extreme degree of dehydration (pyknomorphic cells) without any signs of pericellular edema and with pronounced pericellular edema. The reason for these differences is not clear. We associate the found differences with the completeness of the realization of the potential of the drainage-detoxification system of an individual neuron or their group (column) in each specific case. It is likely that this may somehow influence the outcome of ischemic neuronal damage.

Post-traumatic changes in the degree of hydration of the nervous tissue were accompanied by changes in the tinctorial properties of perikaryons, neuronal processes, synaptic terminals, and astrocytes. This made it possible, using a special filter ("Find Maxima" from the ImageJ 1.52s program), to quantify them on color images, to identify the zones of maximum hydration of the nervous tissue and to determine the volume of these zones. It was found that in the control, the volume of free fluid in the hippocampus did not exceed 8-10% (Max), and in the acute period (1st and 3rd days) after TBI reached 16%.

The use of L-lysine aescinat had a statistically significant effect on the distribution of water in the nervous tissue of the hippocampus. It is significant that at first, 1 day after the injury, the use of this drug was accompanied by an increase in the volume of free water, while only after 3 days the value of this indicator decreased. This is not typical for the comparison group. The most effective effect of L-lysine escinate on the water balance in the area of the CA3 field. Probably, first of all, it promoted the removal of water and ischemic toxins dissolved in it from neurons, and then from astrocytes into blood vessels. This was superimposed on the natural mechanisms of nervous tissue sanitation, which are carried out using various drainage systems of neuroglial-vascular microstructural complexes [21, 22]. However, the pathobiochemical processes of hydropic reversible and irreversible dystrophy of neurons were only partially suppressed and developed in both studied groups in the same direction: swelling → edema → resorption of free water from the body and neuronal processes into the compartment of neuroglial cells (mainly plasma astrocytes and oligodendrogliocytes) →transfer to perivascular spaces → resorption from glial cells into vessels. All this happened in the acute period against the background of neuronal damage, dysfunction of the blood-brain barrier and, despite treatment, did not completely end. Such transformations are characteristic of reactively altered astrocytes and are described by other authors [23, 24]. The use of L-lysine aescinat, most likely, increased the outflow of water through the vessels and decreased pericellular edema. It is likely that the high activity of the drainage-detoxification function in the CA3 field in normal conditions and its adaptive and compensatory stimulation when using the drug could be the reason for the different sensitivity of CA1 and CA3 neurons to ischemia, described earlier in other works [25]. In this regard, the combination of activation of the drainage-detoxification function and the preservation of a high degree of hydration of neurons and astrocyte processes in the neuropil, found by us, can be considered as a new unexplored mechanism of sanogenesis after trauma. Probably, in the field CA3, a more complete removal of "ischemic toxins" occurs than in the field CA1 of the hippocampus. The data obtained can be used to study the phenomenon of selective damage to hippocampal neurons and the role of edema-swelling mechanisms in the rehabilitation of pathologically altered nervous tissue. To assess the activity of the drug L-lysine aescinat as a neuroprotector, it is necessary to conduct further experimental studies concerning the later periods of the post-traumatic period in the framework of the proposed direction.

CONCLUSION

1. In the acute period (1−3 days) after severe traumatic brain injury, the degree of hydration of all components of the nervous tissue of the hippocampus increased. In the group without treatment, 3 days after injury, the relative volume of edema-swelling zones varied from 10 to 13% in CA1 (control - 3-7%) and from 8 to 16% in CA3 (control - 5-10%).

2. The heteromorphism of hydropic changes in the molecular layer, the layer of pyramidal neurons and the multiform layer has been established. The maximum increase in the volume of free water (more than twofold) was characteristic of the molecular and multiform layer CA1, as well as the multiform layer CA3.

3. The use of L-lysine aescinat in the acute period contributed to a statistically significant change in the manifestations of hydropic dystrophy. One day after the injury, the volume of free water increased in comparison with animals without treatment and decreased 3 days later, but remained higher than in the comparison group. The maximum effect of the drug was noted in the CA3 field.

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