MORPHOLOGICAL DISTURBANCES OF BRAIN STRUCTURES IN TRAUMATIC BRAIN INJURY AND THEIR CORRECTION WITH USE OF CYTOPROTECTORS

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Abstract. An experimental trauma was performed for the studying of morphological changes characteristics of the brain under the action of neuroprotectors soon after the brain injury. The closed craniocerebral injury was modeled by the free fall of a load on the parietooccipital area of the brain. We made repeat studies of the influence of succinate medicaments (cytoflavin and mexicor) on histological examination and morphometric analysis of the microcirculatory bed of the cerebral cortex. The experiments were made on the 1st, 3rd, 7th, and 12th day after the traumatic brain injury. An experimental morphological study has established that the use of neuroprotetors after the trauma recovers of brain tissue and positively affects the angio- and cytoarchitectonics of the cerebral cortex in the posttraumatic period.

Keywords: traumatic brain injury (TBI), microcirculation, neuroprotectors.

List of abbreviations
ETC – electron transport chain
NO - nitrogen oxide
RBC- red blood cells
ROS - reactive oxygen species
TBI - traumatic brain injury

Introduction

TBI (traumatic brain injury) is accompanied with pronounced changes in brain tissues. It's generally accepted that morphological changes in the brain caused by a mechanical impact on its tissue define the character and the expressiveness of the consequences of TBI (Gaetz, 2004). The primary damages appear at the moment of the trauma and initiate a cascade of pathochemical and pathophysiological changes which predetermines the development of secondary mechanisms of brain damage (Monson et al., 2019). The secondary disturbances of microcirculation, edema swelling, the disbalance between inhibitory and stimulating systems, the start of far mechanisms of apoptosis, and the inflammatory processes enlarge considerably the value of damage and reorganization of neuronal network of the brain. All of these things provoke pronounced additional pathological structural functional changes in the brain in the posttraumatic period (Kaur & Sharma, 2018; Regner et al., 2017). The restoration of the blood circulation after the metabolic damages under the conditions of hypoxia and ischemia, which take place in the posttraumatic period may contribute to the quickest most quickly normalization of the brain functioning. Therefore the aim of this paper was to study the effect of neuroprotectors having antihypoxic and antioxidant activity on morphological changes in brain structures in the posttraumatic period.

Methods

Animals. The study was performed on (n = 65) white outbreed female rats weighing 180 ± 20 g and 14 weeks old that were obtained from the laboratory animal nursery of the Kryukovo branch of the

Federal State Budgetary Institution Scientific center of biomedical technologies of the Federal Medical and Biological Agency. Animals had access to food and water ad libitum. The work was performed in accordance with the rules presented in the Guide to the Care and Use of Laboratory Animals and requirements of Order of the Ministry of Health of the Russian Federation no. 267 of June 19, 2003, «On the Approval of the Rules of Laboratory Practice in the Russian Federation». The research protocol was approved by the Local Ethics Committee for conducting scientific research involving animals as research objects of the Lobachevsky State University on July 4, 2014.

Rat model of Traumatic Brain Injury. Rats, not anesthetized, were fixed on a tablet, but the head was not fixed. TBI was modeled by a free-falling weight drop of 100 g from a height of 80 cm on the parietal-occipital region of the head (Kalish, Whalen, 2016). Immediately after the injury, rats were transferred to a special plastic cage, and they were monitored until normal behavioral patterns were restored. The mortality rate of falling weight was 0-10%, and a righting reflex time was 2–4 min. This indicates the development of minor trauma (Alder et al., 2011). After the injury, the animals experienced asphyxia, convulsions, bleeding, etc. 30 to 40 minutes after the injury, the animals returned to normal life and nutrition. The animals that died ($n = 2$ in total) during the experiments were discarded from this study.

Treatment. After TBI, in Group 1, 20 rats received an intraperitoneal injection of 2-ethyl-6-methyl-3-hydroxypyridine succinate (mexicor) for 10 days in a daily dose of 8.0mg/kg (a solution for intravenous and intramuscular administration, JSC EkoFarmInvest, Russia). In Group 2, 20 rats received an intraperitoneal injection of cytoflavin for 10 days in a daily dose of 0.2 ml/kg (a solution for intravenous administration, OOO NTFF «Polisan», Russia). In Group 3 (control group-CG), 20 rats received an intraperitoneal injection of physiological saline solution in the same volume. The administration of the drugs was started 1 hour after TBI. The values of the physiological norm of the studied parameters were determined in intact rats ($n = 5$).

Histopathology. Histological examinations were carried out on the 1st, 3rd, 7th and 12th days with rats suffered TBI after blood sampling. Animals intraabdominal injection of Na thiopental (100 mg/kg animal mass), then the decapitation took place, then brain (cerebrum) was extracted (n = 5/group/time point). After decapitation, the brain was quickly removed from each rat, dissected, and put into 10% buffered neutral formalin solution. Material fixation had been lasting for 72–96 hrs, then bits of parietal occipital regions of the brain was cut from the fixed material for the following histological investigation. For this purpose, they were embedded into paraffin (the media Histomix-extra, «Biovitrum», Russia were used). The slices 5–7 mkm thick on the rotation microtome Leica 450 RM (Leica Microsystems, Germany) from the received blocks were produced. The slices were colored with hematoxylin and eosin.

A senior pathologist performed an initial histopathological analysis. His analysis of the area (S, mm^2) of capillaries (on cross sections), the area of perivascular edema around the examined capillaries (S, mm^2) and the diameter of capillaries (d, mm^2) were visualized under a light microscope. In total, about 30 brain sections per rat were analyzed. Using 400× magnification, 20 randomized fields were selected randomly, and 10 vessels and/or 30 cells were counted in each field. Histological preparations were studied with the light microscope Leica DM1000 (Leica Microsystems, Germany), micro photos were got with the help of the digital camera Leica DFC290 (Leica Microsystems, Germany).

Statistical analysis. The received data were processed with the use of the application package BIOSTAT (Analystsoft < USA) and Microsoft Excel (Microsoft, USA) applying the methods of one-dimensional statistics. The results are given as M + SD, where M – arithmetic average, *m* – standard error of the mean. The authenticity of average differences was defined by Student's *t*-test. The differences were supposed to be reliable when the level of importance was $p < 0,05$.

Results

In the control group rats after the TBI the signs of regional blood flow disturbance in the brain. It manifested in vascular plethora, an abruption of vascular wall integrity, and the exit of RBCs beside the blood-flow predominantly in the injury zone. It was recognized in the capillaries the sludges, the RBC aggregates, the swelling of endothelium nuclei, the increase in precapillary edema surface by a factor of 2.7, a significant decrease in capillary diameter by 40% and, as a consequence, the decrease in ca-

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pillary flow surface by a factor of 2 relative to the intact group value (fig. 1 a, d; table 1). The neuron swelling, moderate vacuolization and homogenization of cytoplasm, deformation and shrink of nuclei, numerous large pericellular edema were observed (fig. 2 a, b). The karyoplasm cytolysis was discovered in some neurons. It manifests in swelling, displacement of the nucleolus, replacement of nucleus on the cell periphery or its total loss. It should be noted that the basic mass of altered neurons was situated in the regions which were far from the capillaries. The most considerable changes in the morphological state of vascular flow of the brain and neurons were observed 1–3 days after the injury.

Morphometric analysis showed that the increase in vessel diameter of microcirculatory flow was observed from the 3rd day of the experiment. It was a result of capillary plethora, the formation of RBC aggregates in the form of rouleaux, and an increase in pericapillary edema surface (fig. 1 d). It provoked the decrease in density of capillary flow, vacuolization and enlargement of perivascular and perineuronal space which reflected the phenomena of diffusive cerebral edema. The restoration of the blood circulation in brain cortex, the decrease in the surface of pericapillary and pericellular edema (fig. 1 k), and the decrease in a number of hyperchromic shrunken neurons (fig. 2 b, e) relative to the 3rd day value were observed from the 12th day of the experiment. The average diameter of the capillaries increased by 8% relative to the intact group value, the density of capillary flow decreased by 28% relative to the 7th-day value, but it was by 10% lower the norm. It makes evident that the disturbances in blood circulation in brain cortex preserves (fig. 1 g).

The tendency to the reducing of the pericapillary edema and the increase in the surface of the capillary flow due to involving of «plasmatic capillaries» in response to a higher oxygenic need of brain was registered on the background of cytoprotective therapy. When the mexicor treatment took place the decrease in edema surface and vacuolization of intercellular space, as well as a true increase in capillary diameter and the surface of capillary flow, were observed from the 3rd day of the posttraumatic period (fig. 1 e). A positive influence of mexicor on microcirculatory flow is proved by the restoration of endotheliocyte state, capillary diameter, and capillary flow surface which took place on the 7th day of the study (fig. 1 h). The above mentioned structural reforms made evident the reparative changes in the vessel wall. It should be noted that on the background of the blood supply normalization the deformation and the loss of neurons were pronounced less strongly compared to the control group. From the 3rd day of the posttraumatic period only, the neurons without shrink and destruction of organelles and with long undeformed scions and moderate pericellular edema predominated (fig. 2 f, g, h).

The study of the cytoflavin effect in the posttraumatic period showed that on the 3rd day after the trauma the capillary diameter was constricted, the endothelium had some signs of moderate edema because of strong perivascular edema and vacuolization of parenchyma (fig. 1f). According to the morphological analysis data, the surface of capillary flow did not differ statistically from the value of the control group at this period of study. The «switch» of the compensatory mechanisms contributing to the reduction of edema and of endothelium deformation under the treatment with cytoflavin was registered from the 7th till the 12th day (fig. 1 i, m). On the 7th day, the capillary diameter became wider by 23% compared to the 1st-day value. As a result, the pericapillary edema was lower by 26%, and the surface of the capillary flow was higher by 27% relative to the control group value. On the 12th day the capillary diameter kept constricted relative to the normal value, a little pericapillary edema preserved but the capillary flow surface was higher by 24% than in the mexicor group. In 30% of cases, there were neurons with the deformed cell membrane, small deformation of the nucleus, vacuolization of cytoplasm and pronounced pericellular edema (fig. 2 k, l).

Table 1

Characteristics of morphometric indicators of the capillary of the brain in the investigated groups of rats (M + m)

Value	Intact rats	Group	Period after TBI (day)			
			1	2	3	4
Diameter of the capillary, μm^2	5,55 ± 0,06	CG	3,25±0,05*	3,95±0,06*	5,21±0,05*	5,94±0,03*
		Group 1	3,53±0,04*	3,49±0,05*	4,75±0,03*▲	5,19±0,04*▲
		Group 2	3,45±0,07*	3,36±0,04 *	4,25±0,06*▲	4,86±0,05*▲
Area of the capillary, μm^2	41,9 ± 5,31	CG	114,86±12,32*	154,64±10,41*	129,69±9,87*	109,06±34,51*
		Group 1	120,62±9,85*	124,28±8,63*▲	98,16±9,45*▲	45,02±6,21*▲
		Group 2	366,39±25,17*	394,96±21,75*▲	242,19±16,1**	156,2±18,34*▲
Area of pericapillary edema, μm^2	124,2 ± 12,83	CG	375,79±27,51*	533,86±34,71*	277,04±28,08*	194,64±24,18*
		Group 1	345±26,82*	262,82±14,54*▲	144,29±11,05▲	117,75±12,47▲
		Group 2	750±34,47*	998±38,15*	956±31,84*▲	784±28,93*▲

Data are means ± SD. * – statistically important differences regarding the intact group data , $p < 0,05$, ▲ – statistically important differences of experiment from control, $p < 0,05$

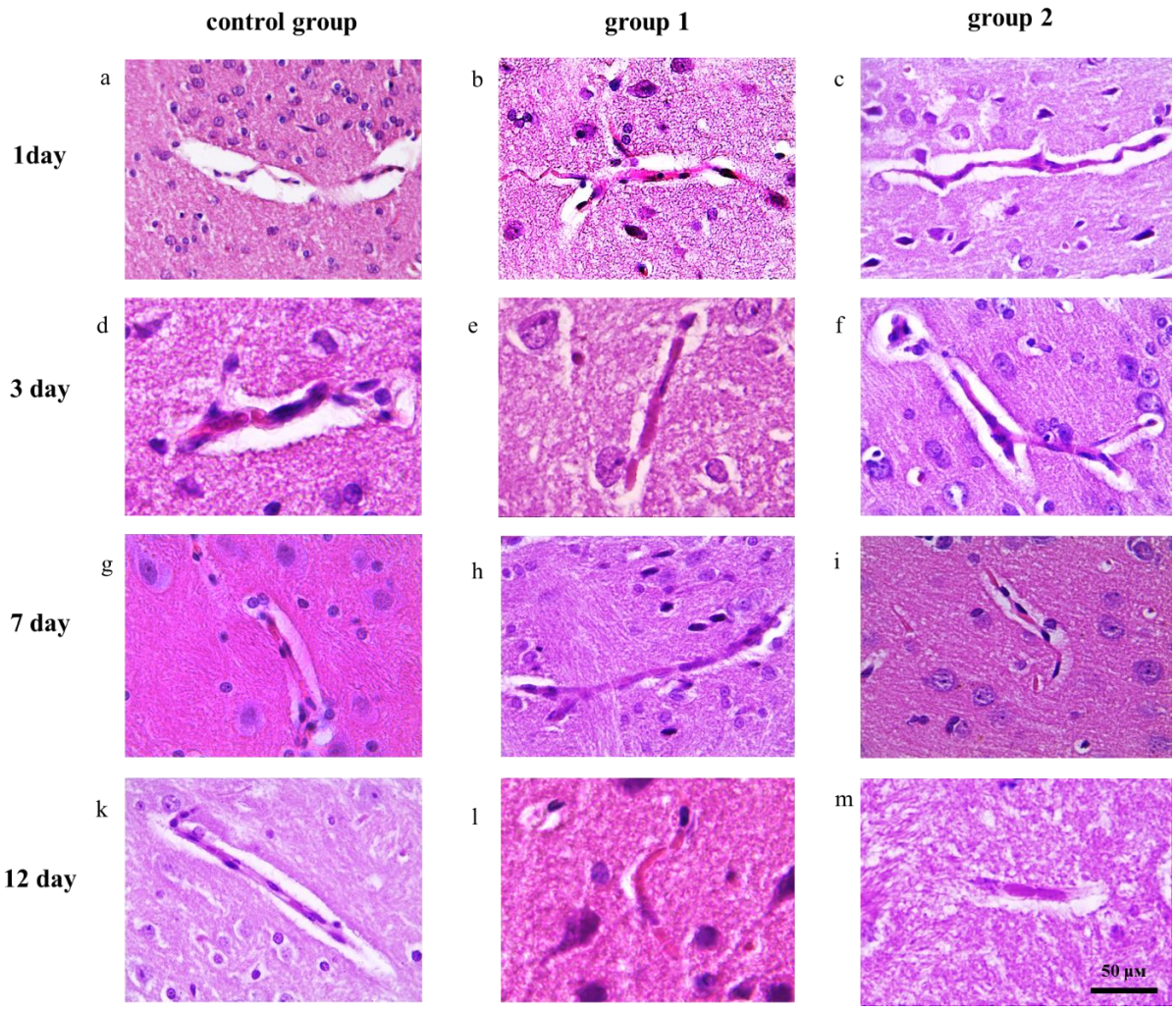

Fig. 1. The structure of brain microcirculatory mainstream of the rats after traumatic brain injury.
a – the brain capillaries were like sludges, highly expressed perivascular edema (magnification ×100); b – in capillary lumen there were sludges from erythrocytes, moderate endothelium edema, highly expressed perivascular edema (magnification ×200); c – erythrocytes located in the form of chains and strongly expressed perivascular edema were detected in the capillaries (magnification ×400); d – red blood cells aggregation, endothelium edema, highly expressed perivascular edema in the clearance were noticed in capillaries (magnification ×400); e – erythrocytes sludges, moderate perivascular edema were defined in capillaries (magnification ×400); f – erythrocytes, moderate endothelial edema, and severe perivascular edema were detected in the lumen (magnification ×400); g – in capillaries plethora, highly expressed endothelium and perivascular edema were noted (magnification ×200); h – capillaries were free laying erythrocytes in the capillary lumen, perivascular edema was not expressed (magnification ×400); i – individual erythrocytes were detected in the lumen, endothelial edema was pronounced moderately, and moderate perivascular edema was detected (magnification ×200); k – there were free laying erythrocytes in the capillary lumen, as well as moderate endothelium edema and moderate perivascular edema (magnification ×400); l – capillaries were free laying erythrocytes in capillary lumens. Around most of them, perivascular edema was absent (magnification ×400); m – there were two free-lying red blood cells in the lumen. There was no perivascular edema (magnification ×400)

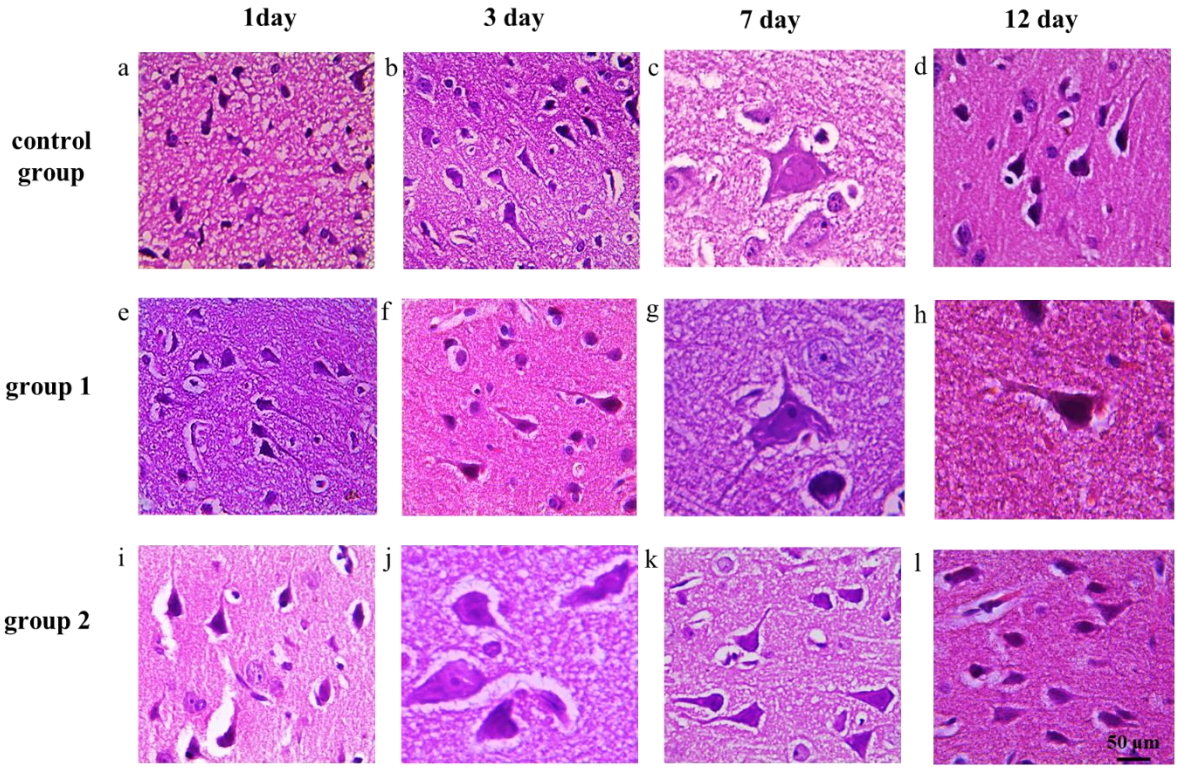

Fig. 2. Neurons of rat brain tissue after the traumatic brain injury.
a – there was a strongly pronounced pericellular edema around neurons and glia cells. The intercellular substance had an almost frothy appearance (magnification ×200); b – there was a strongly expressed pericellular edema around neurons and glia cells. Moderate amount of vacuoles was in the intercellular substance (magnification ×200); c – edema around the neurons and glia cells was moderate. Small number of vacuoles was in the intercellular substance. (magnification ×400); d – there was was a moderate pericellular edema around neurons and glia cells. Single vacuoles were in the intercellular substance (magnification ×200); e – there was a severe pericellular edema around neurons and glia cells. Moderate amount of vacuoles was in the intercellular substance (magnification ×200); f – there was a moderate pericellular edema around neurons and glia cells. Moderate amount of vacuoles was in the intercellular substance (magnification ×400); g – there was a weakly expressed pericellular edema around neurons and glia cells. Single vacuoles were in the intercellular substance (magnification ×400); h – the pericellular edema around the neurons and glia cells was absent. The intercellular substance was not vacuolated (magnification ×200); i – there was neurons and glial cells with severe pericellular edema. Intercellular substance with a moderate amount of vacuolated (magnification ×400); j – there was severe pericellular edema of neurons and glial cells. Moderate number of vacuoles in the intercellular substance (magnification ×400); k – there were moderate pericellular edema of neurons and glial cells. Moderate number of vacuoles in the intercellular substance (magnification ×400); l – there were moderate pericellular edema of neurons and mild pericellular edema of glial cells. Single vacuoles in the intercellular substance (magnification ×200)

Discussion

According to the results of morphological and morphometric analysis received in this work, the disturbances of microvascular flow and diffusive desolation of cortex cerebri areas are developed in rat's body in the posttraumatic period of TBI. One of the supposed mechanisms of the adverse effect of the trauma and of hypoxia which develops on its background is the disbalance of vasoregulative agents. Nitrogen oxide (NO) is one of these agents. It's a powerful vasodilator which plays a crucial role in neurovascular coupling regulating blood flow depending on neuronal activity (Wan et al., 2008). The surplus of NO negatively affects the functions of BBB, increasing its permeability by disturbing the structure of tight junctions between endothelia. The functional antagonist of No is endothelin 1, a powerful endogenous vasoconstrictor which is expressed in various types of cells in CNS. The increase in endothelin 1 expression in astrocytes enforces cerebral edema; it induces neurodegenerative processes and oxidative stress and provokes cognitive deficit (Michinaga & Koyama, 2019). When dystrophic changes in endotheliocytes become more frequent, the exit of triglycerides, glycoproteids, glycosaminoglycans from capillary bores and their lodgment in basal membrane takes place. It contributes to its thickening and dissection and, as a consequence, it reduces the elasticity and the filtration properties of the basal membrane (Boyarinov et al., 2020). Another target of TBI damaging action in the conditions of higher ROS forming is the activation of coagulating cascades which provokes the disturbance of interaction of blood cells with vessel wall thereby reducing the antithrombogenic potential of endothelium and disturbing the brain blood supply (Boyarinov et al., 2016; Shumilova et al., 2018).

The comparative analysis of cytoarchitectonics of animals in Groups 1 and 2 showed that after the treatment with neuroprotective therapy the "switch" of compensatory mechanisms contributing to the conservation of structural functional integrity, form and permeability of vascular capillary net as well as of neuron ultrastructure was observed from the 3rd till the 7th day of the study when mexicor was used, and from the 7th till the 12th day of the study when cytoflavin was used. The containment of claudication under the action of mexicor may be explained by its strong antioxidant potential – emoxypine, a derivate of 3-hydroxypyridine which thanks to its antiradical properties makes stabilizing effect on cell membranes restoring the functional activity of cells (Deryugina & Shumilova, 2017). Besides, in the case of hypoxia mexicor is capable to restore NO producing function of vascular endothelium thereby making a positive effect on angiogenesis (Boyarinov, 2016).

Cytoflavin makes influence predominantly on the processes of energy production in the cell. Amber acid, which is a component of the preparation, activates the succinate dehydrogenase oxidation and stimulates the ETC (electron transport chain) activity that provides its antihypoxant effect (Cherny et al., 2015). The antihypoxant effect of amber acid complements with riboflavin which is capable thanks to its coenzyme properties to increase the activity of dehydrogenase and to restore oxidized glutathione (Novikov & Levchenkova, 2013). Inosine deactivates xanthine oxidase reducing the production of high-reactive oxygen species and compounds (Okovity et al., 2012).

So, cytoprotectors improve the state of endothelium and microcirculatory flow. It normalizes qualitative and quantitative changes in brain tissue. Consequently, it's pathogenetically reasonable to use cytoprotectors as a remedy that improves microcirculation and has an effect of endothelium protection.

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