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DYNAMICS OF IMMUNOHISTOCHEMICAL CHANGES IN THE LUMBAR SPINAL CORD UNDER THE CONDITIONS OF IMPACT-WAVE INFLUENCE

Kitova I.V., Kosharny V.V. ✉, Abdul-Ogly L.V., Kozlovska G.O., Demchenko O.M. Dynamics of immunohistochemical changes in the lumbar spinal cord under the conditions of impact-wave influence.
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ABSTRACT. Background. In connection with the current realities in Ukraine, combat surgical trauma became one of the most urgent problems of 2014 and increased many times. Injuries of the spine and spinal cord in the general structure of combat surgical trauma do not exceed 2%, but are accompanied by high mortality (from 19.1 to 52.9%) and permanent loss of working capacity in most cases of injuries. **The purpose** of our scientific work was to investigate the dynamics of microscopic changes that occur in the lumbar spinal cord during shock wave exposure using immunohistological research methods. **Results**. At the microscopic level, after exposure to a shock wave for 7 days, an increase in the size of neurons and interstitial space was found, which indicated the effects of the shock wave, which were more clearly accompanied by patency, hyperemia of vessels and swelling of their endothelium, which immunohistochemically confirmed more accumulation of the marker of endothelial NO-synthase in the first experimental group and a decrease in the accumulation of HIF-1α, a hypoxia marker, in this experimental group, but an increase in the eNos marker in this group compared to the second and third groups, due to the activation of adaptive processes of the microcirculatory bed and the cell energy supply apparatus that occur in neurocytes after the action of the shock wave after 14 days. **Conclusion.** Thus, the impact of the shock wave has more morphological vascular ulcerative consequences in the acute and early periods, which have a slow but reversible nature of manifestations on the structural elements of the nervous tissue of the spinal cord, but in the late period, as a result of clearly hypoxic processes, these consequences have a more destructively progressive nature , which confirmed at the microscopic level the expression of HIF-1α - a marker of hypoxia in the third experimental group.
Key words: spinal cord, lumbar spinal cord, immunohistochemistry, neurocyte, spinal node, neuroglia, barotrauma, blast injury.

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Introduction

Due to the current realities in Ukraine, combat surgical trauma has become one of the most pressing problems in 2014 and has increased in relevance many times over. Injuries to the spine and spinal cord in the overall structure of combat surgical trauma do not exceed 2% but are accompanied by high mortality (from 19.1 to 52.9%) and permanent disability in most cases of injury [1,2] . The main cause of most deaths is the severity of the injuries [3]. Up to 25% of fatal complications occur immediately after the injury, and more than a third of victims with spinal cord injuries die before admission to the clinic, but those who are hospitalised account

for 2-3% of all patients admitted to neurosurgical departments. In 40-60% of patients with spinal cord injury, there are injuries to other organs and tissues and, in general, damage to the musculoskeletal system [4,5]. However, closed blast-induced traumatic injuries of other internal organs are difficult to diagnose at the stages of the diagnostic process [6,7]. Delayed detection of explosion-induced spinal cord injury, accompanied by ruptures of neurovascular bundles, spinal cord membranes and damage to internal organs, such as the intestines, leads to severe consequences, namely peritonitis, intra-abdominal bleeding, as a result of traumatic perforations and ruptures of intracranial vessels [8,13]. This is especially true of low-intensity explosions when a person does not pay attention to it and does not seek medical attention or does not have the opportunity to do so [9]. Various diagnostic algorithms are used to recognise spinal cord injuries using ultrasound, X-ray tomography and magnetic resonance therapy, as well as special medical equipment (diagnostic laparoscopy). However, this list, in the absence of a pathomorphological picture, does not allow the detection of spinal cord injuries that form at the ultra-, cellular and tissue levels. In recent years, various immune markers of pathological processes have been actively introduced into the diagnostic programme [14]. Therefore, there is a need for an in-depth study of spinal cord injury, its possible complications, as well as the impact of the shock wave and the adaptive mechanisms of the nervous system under such conditions at the cellular level [10]. However, it should be remembered that the morphological substrate of the injury is not only the spine, but also the spinal cord, its membranes and roots. A similar analogy for traumatic brain injury (TBI) would look like a fracture of the skull bones complicated by brain damage [11]. But, given the terminology and rules for formulating a diagnosis, this approach is not entirely correct. Reducing mortality and complications in victims of shock wave exposure largely depends on timely diagnosis of injuries and skilled actions of medical personnel at the prehospital and early hospital stages [12]. Experimental models, combined with clinical and morphological studies, allow us to assess the adaptive capabilities of the spinal cord and the neurotrophic and neuroprotective effects of the nervous system after spinal cord injury. These data may provide important perspectives for the treatment of patients not only with spinal cord injuries but also with neurological diseases, including post-traumatic diseases, in the mechanism of development in which neuroglia cells are involved [15].

The objective

To establish the dynamics of microscopic changes in the lumbar spinal cord under conditions of shock wave exposure using immunohistological methods of research.

Material and methods

The study involved 111 white outbred mature rats weighing 180-200 g. During the entire period of preparation for the experiment and its conduct, the rats were kept in the vivarium of Dnipro State Medical University, at a temperature of 20-25 C, a humidity of at least 50%, in a well-ventilated room and day/night light, in standard plastic cages with no more than five individuals in each with a standard diet: the daily requirement of an adult animal is on average 30-32 g (25 g of sour cream feed, 5-7 g of vegetables. All rats involved in the experiment were healthy and active. The experimental animals were divided into 4 groups of 30 animals each in three experimental groups, which accounted for 21.0%, and 21 animals in the control group. Rats in the control group were put under thiopental anaesthesia and fixed. The experimental group consisted of rats that underwent modelling of spinal cord barotrauma under thiopental anaesthesia (patent Pat. 146858 Ukraine, IPC G09B23/28). The rats were subjected to shockwave exposure to the lumbar spinal cord and were divided into 3 experimental groups and a control group, which were withdrawn from the experiment in the first two hours, 7 and 14 days after receiving spinal barotrauma. The experiment was performed by the rules for experimental animals, in compliance with the principles of humanity outlined in the European Community Directives and the Helsinki Declaration. After withdrawal from the experiment, pathological, immunohistochemical and morphometric studies of the rat spinal cord were performed. The expression of the hypoxia marker HIF-1a and endothelial dysfunction eNos was studied. Immunohistochemical studies were performed to determine the modification of individual receptors of structural components of the rat spinal cord wall to detect endothelial dysfunction antigen with a specific marker (eNOS) and hypoxia antigen (HIF, with specific markers (HIF - hypoxia-inducible factor) HIF-1a after exposure to spinal barotrauma in the acute, early and late periods, with the aim of possible regulation of adaptive processes, which provides new opportunities to correct and help the possible reparative process after spinal cord injury.

Results

In our experiment, we studied macroscopic and microscopic changes after exposure to a shock wave after 2 hours, 7 days, and 14 days. Compared to the phases that were identified earlier, it can be understood that the period of exposure, after which we conducted the morphological study, does not reach the chronic phase. However, the changes we observed in the acute and partially subacute phases, in which macroscopic and microscopic damage to the structures of the nervous system occurs, are more clearly indicated. This is a direct result of the trauma, which is very aggressive, especially in the first hours and the first week after the impact, characterised by a clear localisation of the focus, limited to the area of the shock wave. However, it should be

noted and understood that macroscopic and microscopic changes are morphologically different in different periods of exposure after barotrauma. Thus, microscopically, in the acute period of spinal cord injury, two hours later, pronounced edema, vascular fullness and intracerebral haemorrhage were noted, partially with rupture of small vessels, but with clear localisation. However, seven days later, external signs of edema were present, but practically invisible, they were less localised and more widespread over the area of the lesion. There was compression and haemorrhage at the site of injury in the lumbar spinal cord, which decreased and was practically not noticeable after fourteen days after exposure. Microscopically, grey matter contains: neuronal bodies, neuroglia and myelin-free nerve fibres, while white matter contains most myelin fibres, a small number of myelin fibres and neuroglia. Functionally, the nervous system's divisions form nerve centres of the nuclear type, which are accumulations of neuronal bodies around which white matter is located to control individual functions. But in the first two hours after exposure to barotrauma, microscopically, the injuries were the most severe with damage to structural elements. In particular, it was with the rupture of small vessels and capillaries, especially in the grey matter of the lumbar spinal cord, where there are many capillaries. This was followed by haemorrhages, which was confirmed by the expression of markers of vascular dysfunction - endothelial NO synthase, which accumulated more in the nervous tissue of the spinal cord in the acute period, in the first experimental group, but more localised (Fig. 1). The expression of eNos in the cells of the lumbar spinal cord is detected in the early stages after exposure to shock wave action; during this period, it is most active and is one of the main local vasodilators. NO synthase is involved in the synthesis of NO by the endothelium and regulates vascular tone, blood pressure and blood flow. During the immunohistochemical study, a positive reaction with antibodies was detected in the endothelial lining of the vessels of the haemo microcirculatory system, especially capillaries, which ensures the maintenance of low vascular resistance.

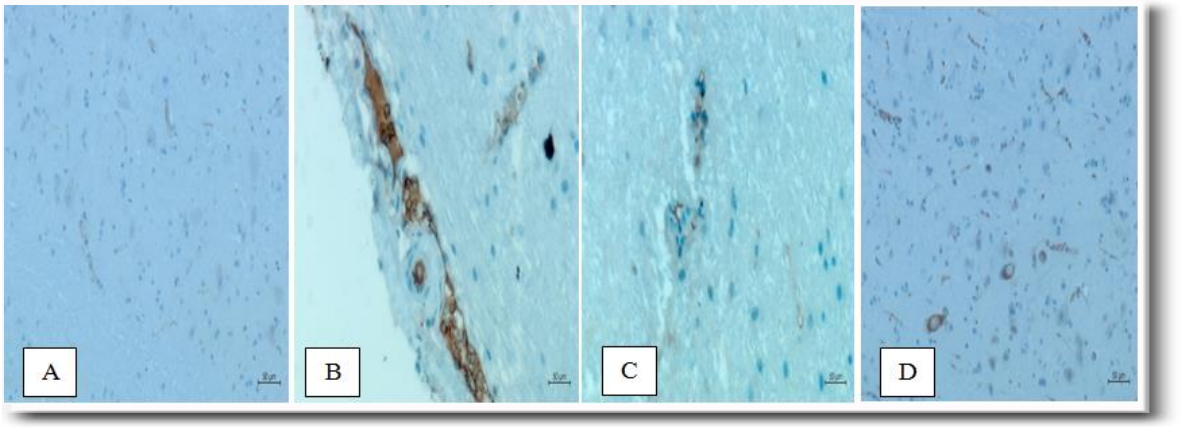

Fig. 1. Spinal cord with expression of e-Nos (endothelial dysfunction) marker. H&E staining. ×200. A - control group, B - acute period, C - 7 days after shock wave exposure, D - 14 days after shock wave exposure.

In the second experimental group, after seven days of exposure to barotrauma, histological findings revealed an effect directly on the vascular endothelium with perivascular edema, accompanied by erythrocyte leakage into the tissue and local cessation of flow in the microcirculatory bed, mainly in capillaries, postcapillaries and venules. However, this was accompanied by an inflammatory process, and thus, additionally, an increase in the size and number of neurons with degenerative changes, more saturated transparent nuclei, and cytoplasmic vacuolation was observed, indicating the effects of the shock wave, which are more widespread. Because of this, the accumulation of the hypoxia marker HIF-1α increased in the second and even more in the third experimental group, due to the progression and sharp increase in destructive and necrotic processes and, as a result, hypoxic processes, which was confirmed by the expression of the hypoxia marker HIF-1α (Fig. 2 - A, B, C).

On the contrary, in the second experimental group, after seven days of exposure to barotrauma, it can be said that there was no more quantitative accumulation of endothelial NO-synthase, but the expression was more sparse, indicating a decrease in vascular disorders during this period. In the third experimental group, after exposure to the shock wave fourteen days later, a sharp decrease in the accumulation of endothelial NO-synthase was observed due to the disappearance or sharp reduction of vascular disorders (Fig. 3 - A, B, C).

Thus, the impact of a shock wave in the first two hours after barotrauma primarily causes severe disorders of the hemocirculatory system, which was confirmed by the expression of the e-Nos marker, but it should be noted that these changes have a slow but reversible effect on the structural elements of the nervous tissue of the spinal cord. However, on the

contrary, in the later period, after exposure to barotrauma on the lumbar spinal cord, due to progressive, destructive-necrotic, partially irreversible processes, hypoxic-traumatic effects are more ulcerative, which is characterised by a significant progressive accumulation of the hypoxia marker HIF-1a after 14 days of shock wave exposure (Fig. 4 - A, B, C). In our study, we analysed the accumulation of markers of endothelial dysfunction and hypoxia in the spinal nodes of rats with a well-developed connective tissue stroma after 2 hours, 7 and 14 days after shock wave exposure. However, first of all, the structure of neurocytes in the spinal node was analysed histologically. Thus, neurocyte perikaryons, most of which are rounded, have different sizes and are divided into small, medium and large. It should be noted that their main accumulation is located along the connective tissue and capsule, where they are arranged in groups, but there are also single locations of nerve cells (Fig. 5 - A, B).

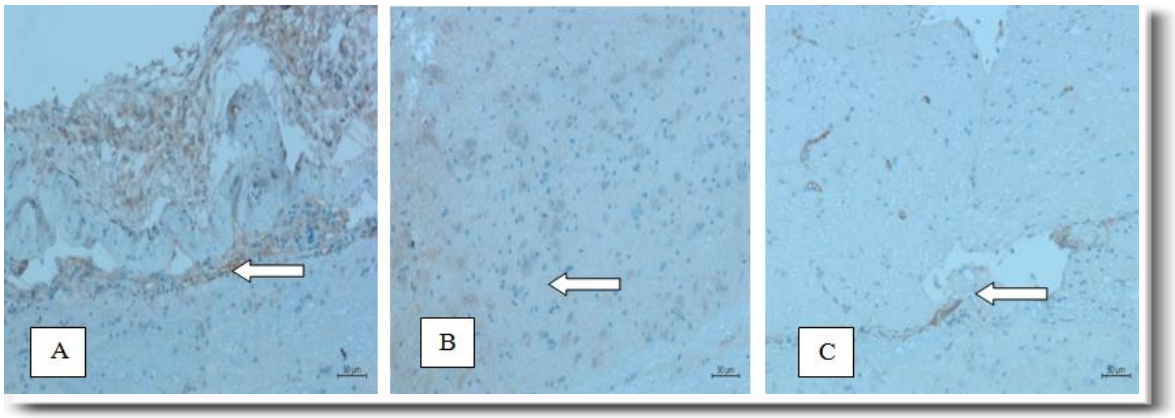

Fig. 2. Spinal cord with expression of HIF-1a marker (hypoxia). H&E staining. ×200. A - acute period, B - 7 days after shock wave exposure, C - 14 days after shock wave exposure. Arrows indicate neurocytes.

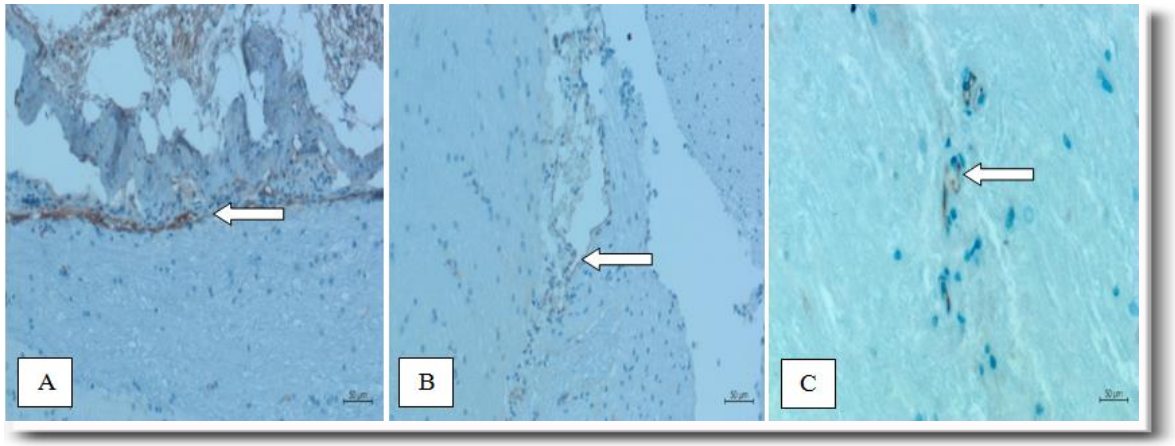

Fig. 3. Spinal cord with expression of e-Nos (endothelial dysfunction) marker. Arrows indicate the highest accumulation of the marker. ×200. A - acute period, B - 7 days after shock wave exposure, C - 14 days after shock wave exposure.

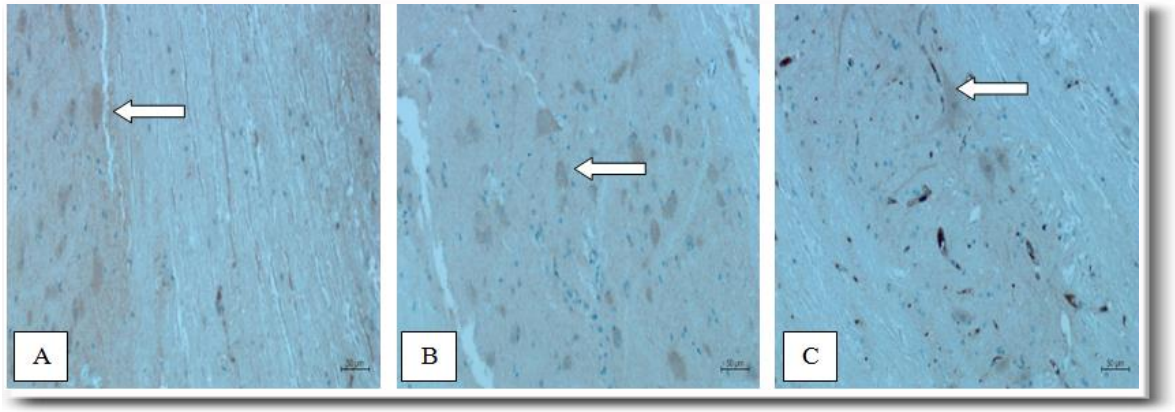

Fig. 4. Spinal cord wall marker HIF-1a (hypoxia). H&E staining. ×200. A - acute period, B - 7 days after shock wave exposure, C - 14 days after shock wave exposure. Arrows indicate neurocytes.

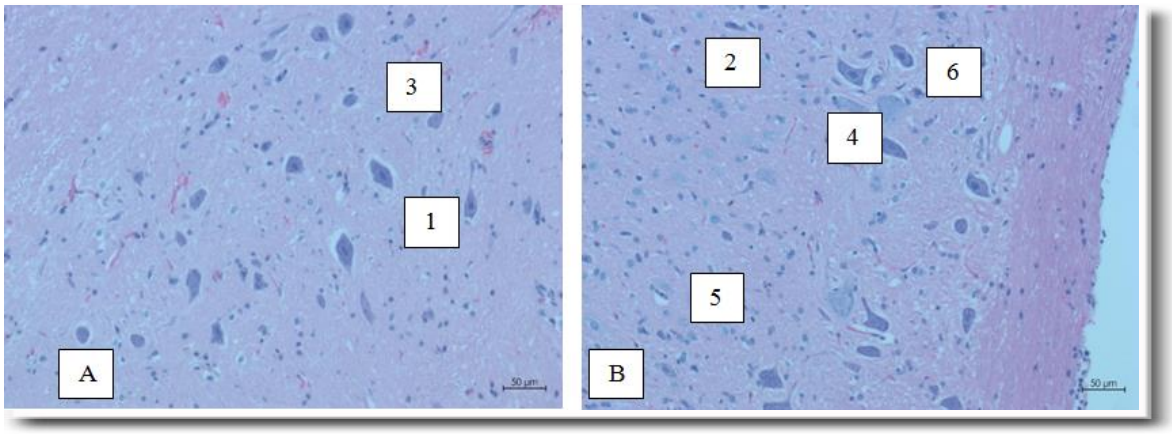

Fig. 5. Chromatophilic substance in neurocytes of the spinal cord. H&E staining. ×400. 1 - perikaryon, 2 - dendrites, 3 - axon, 4 - neurocyte nucleus, 5 - chromatophilic substance, 6 - gliocyte nuclei.

Further, they are visualised and the processes, especially the dendrites, are very clearly distinguished, with the nucleus and nucleoli located away from the centre. The rounded nucleus of the cell appears light, and the nucleus is brightly coloured. The cytoplasm has a blue tint, and the Golgi apparatus is clearly visible around the nucleus. In nerve cells, it forms a basket covering the entire nucleus (Fig. 6 - A, B).

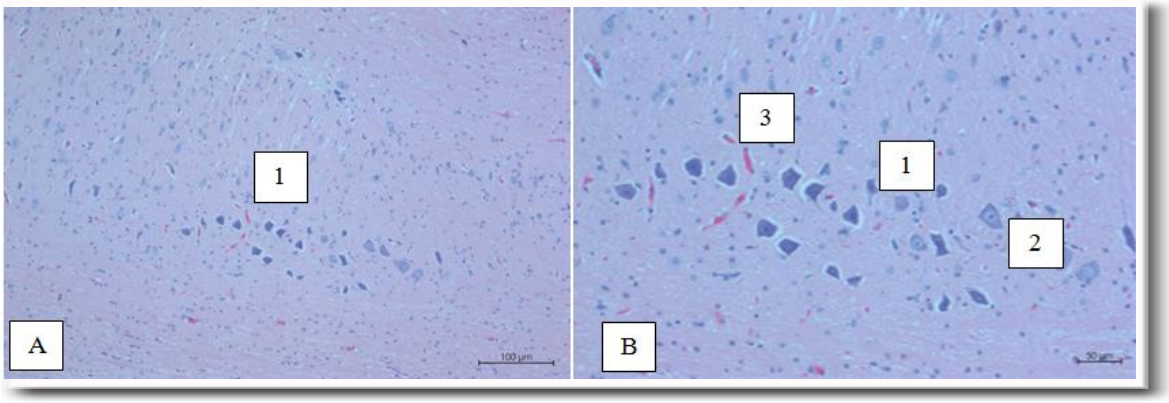

Fig. 6. Microscopic structure of the rat spinal node: 1 - neurocytes; 2 - neurocyte nucleus and nucleolus; 3 - neuroglial cells. The lamellar Golgi complex in neurocytes of the spinal ganglion is indicated by an arrow. Hematoxylin and eosin staining, A - ×4; H&E - ×10, B - magnified Fig. 6 A, ×40.

In the cytoplasm of the nerve cell body and dendrites, the chromatophilic substance is evenly distributed in the form of grains or lumps, which are stained basophilic. Small blue neuroglia nuclei are observed between the nerve cells, but there is no granularity in the axon and axonal tubercle (Fig. 7).

Conclusion

Thus, using immunohistochemical methods of studying the lumbar spinal cord, we found minimal expression of endothelial NO synthase in the third group, in the late period after 14 days of shock wave exposure. The obtained results indicate a decrease in the processes of endothelial dysfunction in the late period, which progress in the first two hours after exposure when they are more localised. On the contrary, the greatest increase in the expression of the hypoxia marker HIF-1α in the third group compared to the second and first groups indicates a deeper hypoxic damage in the late period. In addition, hypoxia has a vasoconstrictor effect, which can cause complications. These changes indicate dysfunction and imbalance between vasodilator and vasoconstrictor mechanisms, with an increase in vasoconstrictor effects. In particular, there is a violation of tissue antioxidant mechanisms. Increased expression of (HIF-1α) indicates hypoxia, which can lead to vascular dysfunction in the future. In general, we studied and analysed the accumulation of markers of general pathology. This made it possible to establish the main mechanisms of delayed effects of shock wave exposure and their prediction.

At the microscopic level, after exposure to the shock wave for 7 days, an increase in the size of neurons and interstitial space was detected, which indicated the effects of the shock wave. They were clearly accompanied by pastiness, hyperaemia of the vessels and edema of their endothelium, which was immunohistochemically confirmed by a greater ac-

cumulation of the endothelial NO synthase marker in the first experimental group and a decrease in the accumulation of HIF-1α, a marker of hypoxia in this experimental group, but an increase in the eNos marker in this group compared to the second and third groups.

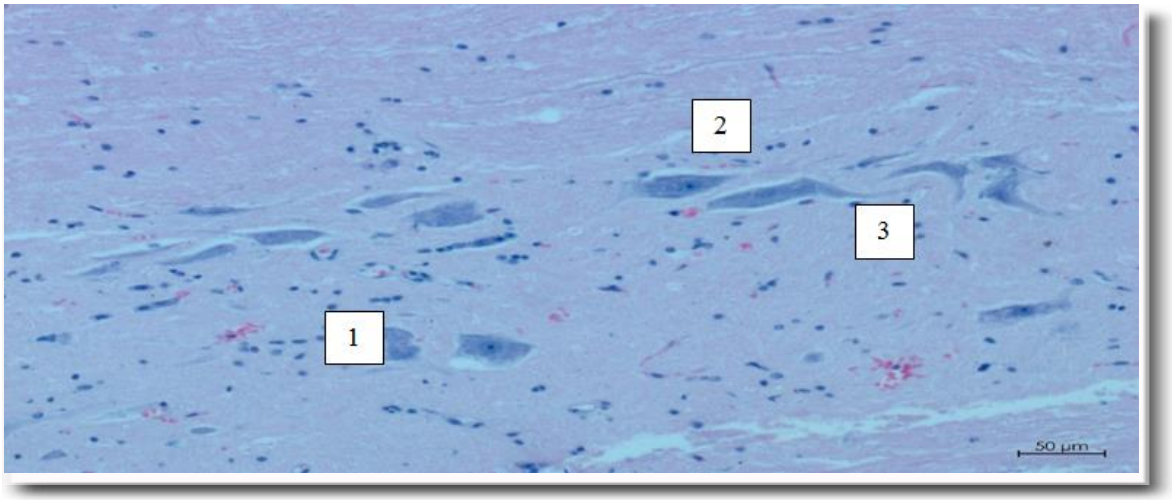

Fig. 7. Microscopic structure of the rat spinal node. Hematoxylin and eosin stain, ×400. 1 - neurocytes; 2 - neurocyte nucleus and nucleolus; 3 - neuroglial cells. The arrow indicates neurocytes.

This was due to the activation of adaptive processes of the microcirculatory bed and the cellular energy supply apparatus that occur in neurocytes after exposure to a shock wave for 14 days. Thus, the impact of a shock wave has more morphological vascular ulcerative effects in the acute and early periods, which have a slow but reversible effect on the structural elements of the nervous tissue of the spinal cord. However, in the late period, due to distinctly hypoxic processes, these effects are more destructively progressive, which was confirmed at the microscopic level by the expression of HIF-1α, a marker of hypoxia in the third experimental group.

Prospects for further research

It is planned to investigate morphometric changes in the spinal cord under conditions of barotrauma at the early and late stages of exposure.

Information on conflict of interest

There are no potential or apparent conflicts of interest related to this manuscript at the time of publication and are not anticipated.

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Китова І.В., Кошарний В.В., Абдул-Огли Л.В., Козловська Г.О., Демченко О.М. Динаміка імуногістохімічних змін поперекового відділу спинного мозку за умов ударно-хвильового впливу.

РЕФЕРАТ. Актуальність. У зв'язку з нинішніми реаліями в Україні, бойова хірургічна травма стала однією з найактуальніших проблем 2014 року та зросла в рази. Поранення хребта і спинного мозку в загальній структурі бойової хірургічної травми не перевищують 2%, але супроводжуються високою летальністю (від 19,1 до 52,9%) і стійкою втратою працездатності в більшості випадків поранень. **Метою** нашої наукової роботи стало дослідити динаміку мікроскопічних змін, які відбуваються у поперековому відділі спинного мозку при ударно - хвильовому впливі за допомогою імуногістологічних методів дослідження. **Результати.** На мікроскопічному рівні після дії ударної хвилі з терміном впливу 7-ми діб було виявлено збільшення розмірів нейронів, інтерстиціального простору, що свідчило про наслідки впливу ударної хвилі, які більш виразно супроводжувалися пастозністю, гіперемією судин і набряк їх ендотелію, що імуногістохімічно підтверджувало більш накопичення маркеру ендотеліальної NO-синтази у першої експериментальної групі і зменшення накопичення HIF-1α маркеру гіпоксії у даної експериментальної групі, але підвищення у цій групі маркеру eNos у зрівнянні з другою та третьої групою, внаслідок активації адаптивних процесів мікроциркуляторного русла та апарату енергозабезпечення клітин, що виникають в нейроцитах після дії ударної хвилі через 14-ть діб. **Підсумок.** Вплив ударної хвилі більш має морфологічні судинні виразкові наслідки в гострий та ранній періоди, які мають повільний, але зворотній характер проявів на структурні елементи нервової тканини спинного мозку, але у пізньому періоді, внаслідок виразно гіпоксичних процесів, ці наслідки мають більш деструктивно прогресуючий характер, що і підтвердило на мікроскопічному рівні експресію HIF-1α - маркеру гіпоксії у третьої експериментальної групи.

Ключові слова: спинний мозок, поперековий відділ спинного мозку, імуногістохімія, нейроцит, спинальний вузол, нейроглія.