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DYNAMICS OF CHANGES IN S-100 PROTEIN AND NEURON-SPECIFIC ENOLASE IN RABBITS WITH OCULAR CONTUSION AND ITS CORRECTION WITH L-LYSINE – (S)-2,6-DIAMINOHEXANOIC ACID 3-METHYL-1,2,4-TRIAZOLYL-5-THIOACETATE

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Eye injuries are a serious medical and social problem, the relevance of which is significantly increasing in conditions of military conflicts. Damage to the visual organs can cause temporary or permanent vision loss, significantly affecting the quality of life of those affected and their ability to work. In peacetime, the main causes are domestic, industrial, and sporting incidents. At the same time, in military conditions, up to 16 % of all combat injuries occur to the organs of vision, of which about 80 % are associated with the effects of blast waves. Biochemical markers are an important aspect of understanding the mechanisms of such damage. In particular, the enzyme neuron-specific enolase and the S100 protein are indicators of nerve tissue damage. neuron-specific enolase, an intracellular enzyme, indicates damage to neuronal membranes, while increased levels of S100 protein indicate an inflammatory reaction in glial tissue, active division of neuroglial cells, and neuroapoptosis. The presence of these markers in the blood allows us to assess the severity of damage and the effectiveness of therapeutic measures. The relevance of neuroprotection in the treatment of eye injuries is due to the need to protect and restore the nervous structures of the eye, particularly the retina and optic nerve. The study found that therapy with Angiolin eye drops significantly reduced the markers of neurodestruction in the serum of rabbits with ocular contusion. Namely, neuron-specific enolase decreased by 87.2 % and S100 by 89.9 %. The results of the study confirm the pronounced neuroretinoprotective effect of the drug Angiolin.

Key words: ocular contusion, optic nerve, retina, neuron-specific enolase, S100 protein, neuroprotection.

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ДИНАМІКА ЗМІН БІЛКУ S-100 ТА НЕЙРОН-СПЕЦИФІЧНОЇ ЕНОЛАЗИ У КРОЛІВ ПРИ КОНТУЗІЙНІЙ ТРАВМІ ОКА ТА ЇЇ КОРЕКЦІЯ L-ЛІЗИНОМ – (S)-2,6-ДІАМІНОГЕКСАНОВОЇ КИСЛОТИ 3-МЕТИЛ-1,2,4-ТРІАЗОЛІЛ-5-ТІОАЦЕТАТОМ

Очні травми є серйозною медичною та соціальною проблемою, актуальність особливо зростає в умовах військових конфліктів. Ушкодження зорових органів можуть спричиняти тимчасову або стійку втрату зору, що суттєво впливає на якість життя постраждалих та їхню працездатність. У мирний час основними причинами є побутові, виробничі та спортивні інциденти, тоді як у військових умовах до 16 % всіх бойових ушкоджень припадає на органи зору, з яких близько 80 % пов'язані з дією вибухових хвиль. Важливим аспектом у розумінні механізмів таких ушкоджень є біохімічні маркери. Зокрема, фермент нейрон-специфічна енолаза (NSE) та білок S100 є показниками ушкодження нервової тканини. NSE, що є внутрішньоклітинним ферментом, свідчить про пошкодження мембран нейронів, тоді як підвищення рівня білка S100 вказує на запальну реакцію в гліальній тканині, активний поділ клітин нейроглії та нейроапоптоз. Наявність цих маркерів у крові дозволяє оцінити тяжкість ушкоджень та ефективність терапевтичних заходів. Актуальність нейропротекції в лікуванні очних травм обумовлена необхідністю захисту та відновлення нервових структур ока, зокрема сітківки та зорового нерва. Проведене дослідження виявило, що терапія очними краплями Ангіолін сприяла значному зниженню рівнів маркерів нейродеструкції в сироватці крові кроликів з контузійною травмою ока, а саме: NSE зменшився на 87,2 %, а S100 – на 89,9 %. Результати підтверджують виражену нейроретинопротективну дію препарату Ангіолін.

Ключові слова: контузійна травма ока, зоровий нерв, сітківка нейрон-специфічна енолаза, білок S100, нейропротекція.

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Eye trauma remains one of the most pressing problems of modern medicine, as its consequences often lead to a significant deterioration in the quality of life, reduced working capacity, and social isolation of victims. It is one of the leading causes of unilateral blindness worldwide, and its incidence is a concern: approximately 55 million cases of eye injuries are recorded each year, resulting in 19 million cases of low vision or complete loss of vision [10].

Eye injuries become a particularly significant problem during hostilities, which, unfortunately, further emphasizes its relevance for Ukraine. Since the full-scale invasion began, there has been a significant increase in the number of people with visual impairments. According to the National Health Service of Ukraine (NHSU), in 2021, 17,478 individuals were registered, and by 2022, the number had

increased to 19,551. In the first seven months of 2023 alone, doctors reported more than 19,000 new cases, surpassing the number from the previous year [3, 10]. About 16 % of all military injuries are eye injuries, with about 80 % of them caused by blast waves. With minor eye injuries caused by a blast wave (BW), there may be no initial decrease in visual function. However, microstructural damage resulting from trauma can eventually lead to visual system dysfunction, manifesting itself weeks, months, and even years after the traumatic event [2, 4, 10]. During an eye contusion, after a short-term mechanical impact on the eyeball, a complex chain of pathophysiological processes occurs, which causes damage to various eye structures. The retina and optic nerve are particularly affected, which, depending on the severity of the injury, leads to impaired visual functions, from minor temporary changes to a persistent decrease in visual acuity and, in some cases, to blindness [4]. Experimental studies of traumatic optic nerve and retinal injuries aim to explore new approaches that may improve treatment outcomes for this complex condition. Particular attention is paid to neuroprotection, axon regeneration, and the use of neurotrophic factors. The release of neuron-specific enolase (NSE) and S100 protein into the bloodstream is an indicator of nerve tissue damage. NSE, an intracellular enzyme, indicates damage to neuronal membranes when its level increases. The S100 protein, which is specific to astrocytic glia, demonstrates an inflammatory reaction in glial tissue, and an increase in its concentration indicates active division of neuroglial cells and increased neuronal apoptosis activity [5, 7]. Neuroprotection involves using drugs that reduce neuronal apoptosis and limit secondary damage after injury, making it one of the primary methods of treating this condition. Based on the conducted studies, the compound L-lysine – (S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate (Angiolin) with the most pronounced neuroprotective effect in conditions of traumatic brain injury was identified. It also significantly reduces neuronal death in ischemic and hemorrhagic strokes, normalizes the functioning of the compensatory GABA shunt, and increases the level of ATP in brain tissues [7, 8]. These properties of Angiolin potentially justify its use as an effective neuroretinoprotector in contusion injury of the eye.

The purpose of the study was to investigate the effect of Angiolin and the reference drug (citicoline, OMK-1 eye drops) on the levels of markers of neurodestruction (neuron-specific enolase and S-100 protein) using enzyme-linked immunosorbent assay as a potential mechanism of their neuroretinoprotective action.

Materials and methods. The experiments were performed on 20 rabbits of both sexes weighing 3.0–3.5 kg, Californian rabbit breed, obtained from a farm in Zaporizhia district. Before the start of the study, animals that met the inclusion criteria for the experiment were divided into groups using the randomization method. When working with laboratory animals, we followed the methodological recommendations of the "State Expert Center of the Ministry of Health of Ukraine" and the requirements of bioethics by the national "General Ethical Principles of Animal Experiments" (2001), which comply with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes."

Experimental ocular contusion in rabbits, caused by the action of a flow of carbon dioxide gas under pressure, was modeled using a self-developed method using a gas cylinder pneumatic pistol of the Baikal MR-654K brand with a mass of liquefied CO2 of 12 g under pressure (Crosman, USA, serial number 456739). Immediately after the contusion injury, 0.2 ml of the test drug was instilled into the affected eye 3 times a day – Angiolin-1 % eye drops (made extemporally at the Department of Pharmaceutical, Organic and Bioorganic Chemistry of the ZSMFU from a substance obtained from the Scientific and Technological Complex "Institute of Single Crystals" of the National Academy of Sciences), as well as a reference drug – OMK-1 eye drops (Citicoline – 2 %) (Omicron Italy S. R. L., Viale Bruno Buozzi, Rome, Italy). On the 10th day, in rabbits with ocular contusion (OC), 1 hour after the last instillation of the studied eye drops under anesthesia (propofol, 40 mg/kg, Fresenius Kabi, Austria), blood was taken from the superior ophthalmic vein for enzyme-linked immunosorbent assay (0.5 ml). The main criteria for assessing the neuroprotective effect of the studied drugs in the acute post-concussion period were the levels of neuron-specific enolase (NSE) and S100 protein in the blood serum of rabbits. NSE activity (ng/ml) and S100 content (ng/ml) were determined by solid-phase enzyme immunoassay using NSE ELISA KIT # MBS2024030 (MyBioSource.Inc, USA) and S-100 ELISA KIT # LS-F25201 (LifeSpan Biosciences.Inc, USA). Statistical processing of the obtained results was performed using standard methods using the program "Statistica SPSS 10.0 for Windows" (license number 305147890). The results obtained were presented as M±m. The Shapiro-Wilk test was used to test the normality of the distribution. The significance of the difference in mean values was assessed using the Student's t-test (for normal distribution) and the Mann-Whitney U-test (if the distribution deviates from normal). Differences were considered statistically significant at $p<0.05$.

Results of the study and their discussion. When we assess the severity of an injury, including a craniocerebral injury, primary clinical manifestations in the form of contusion, hemorrhage, and edema are mandatory. Speaking of the visual analyzer, this is no exception, and the strength of the impact of the traumatic agent and the speed of assisting in the early stages will play a massive role in restoring the function of the damaged organ. In areas of tissue adjacent to the zone of action of the traumatic factor, damage and subsequent cell death occur.

Even though, in parallel with the damage and death of cells, including nerve cells, neurotrophic endogenous factors are also produced, which indirectly affect the cell genome, their activity in the event of significant damage is insufficient to stop the processes of neurodestruction and cell functions continue to be impaired.

To determine the fact of neuronal death and assess the effectiveness of neuroprotective therapy, the activity of such neuro markers as neuron-specific enolase and S 100 protein is determined, the level of which, in turn, may indicate the depth and severity of neurodestructive processes in the cells of the nervous tissue [3].

As shown in the data presented in Table 1, when a blank shot from an air pistol was fired close to the center of the cornea of the rabbit eye 10 days after pathology modeling, the activity of the membrane integrity marker of neurons NSE in the control pathology group significantly increased by 36.8 times compared to intact values, which indicates the development of significant destruction of neurons and the prevalence of the necrotic type of their death. On the 10th day of the experiment, the level of another marker, protein S 100, which reflects the activity of astrocytic glia, the change which is a natural response of nervous tissue to necrotic and necrobiotic processes, increased by 11.5 times compared to intact values. The results indicate significant primary damage to the neuronal array and intensification of neuroglioproliferative processes on the 10th day after injury.

Table 1

Concentration of markers of neurodestruction – neuron-specific enolase and S-100 protein in the blood of rabbits after ocular contusion and 10-day experimental therapy

Experimental groups	NSE, ng/mL	S-100, ng/mL
Intact	0.266±0.025	0.082±0.007
OC (control)	9.57±0.16[1]	0.92±0.013[1]
OC + OMK-1 drops (Citicoline)	5.92±0.18*[1]	0.58±0.023*[1]
OC + Angiolin drops	1.22±0.22*[1.2]	0.093±0.010*[2]

Note: * – p <0.05 relative to the control group, [1] – p <0.05 relative to the intact group, [2-] – p <0.05 relative to the OMK-1 group.

An endothelioprotective effect is predicted for thiotriazoline and L-lysine aescinate. The drug “Angiolin” combines thiotriazoline and L-lysine aescinate molecules in its structure. “Angiolin” has endothelioprotective properties due to its ability to regulate NO formation, reduce peroxynitrite and homocysteine production, increase the activity of superoxide dismutase and NO synthase, and increase the preservation of reduced thiol groups and L-arginine [9]. The drug increases the bioavailability of NO, improves its transport to brain and myocardium cells, increases the density of endothelial cells of the capillary network of the cerebral cortex and the vascular wall of the brain vessels, the content of RNA in the nuclei of endothelial cells, the density of proliferating endothelial cells in these vessels, against the background of an increase in the concentration of vascular endothelial growth factor (VEGF), activates the glutathione link of the thiol-disulfide system and increases the activity of glutathione peroxidase and glutathione transferase, and reduces the accumulation of markers of oxidative and nitrosative stress [9]. Based on these properties of the drug, we used “Angiolin” eye drops to study the neuroretinoprotective effect in contusion eye injury.

Course therapy with Angiolin eye drops contributed to a significant reduction in the levels of neurodestruction markers in rabbits' blood serum with OC: NSE decreased by 87.2 % and S-100 by 89.9 %. Regarding effectiveness in reducing these indicators, Angiolin eye drops significantly outperformed the reference drug OMK-1. Angiolin eye drops exhibit a significant neuroretinoprotective effect in OC, reducing damage to retinal neurons. Regarding the strength of the neuroretinoprotective effect, Angiolin eye drops significantly outperform the reference drug OMK-1 eye drops.

Analyzing eye trauma resulting from blast injuries, the following types of injuries can be distinguished: primary (level I) blast injuries develop when explosive shock waves cause a pressure difference with the subsequent implosion effect in the eyeball. The maximum IOP during the explosion can reach a peak of 0.29 MPa (2175 mmHg) in 1.63 ms, which is twice the physiological IOP in healthy eyes [10]. Crushing the eye with blunt force can lead to contusion with subsequent eyeball rupture when

its pressure is exceeded. Secondary (Level II) blast injuries develop when explosive fragments or exogenous fragments ejected by the explosion rupture the cornea or sclera (OGI rupture, including IOFB, penetration, and perforation), cut the surface and wall of the eyeball (lamellar rupture or superficial foreign body, CGI types), or cause blunt trauma or laceration. As for the time of action, the speed of the primary blast wave was much higher and could reach the eyeball before the fragments moved along it. Thus, level II blast injuries are secondary to level I in mechanism, order, or both [10]. Based on the above-described types of blast trauma in our study, it is possible to assume that eye trauma was classified as the first type of blast injury, which led to contusion damage to the eye. Therefore, Angiolin eye drops have demonstrated their effectiveness in treating this contusion eye injury, as confirmed by a decrease in the neuromarkers neuron-specific enolase and S-100 protein.

Conclusion

The study's results confirm the pronounced neuroretinoprotective effect of the drug Angiolin. OMK-1 eye drops also demonstrated a neuroretinoprotective effect in OC, reducing NSE levels by 38.1 % and S-100 by 36.9 %, but were significantly less effective than Angiolin eye drops. Therapeutic use of all studied drugs led to a decrease in the level of markers of neurodestruction. However, Angiolin proved to be the most effective, inhibiting damaging processes within 10 days after contusion. This creates a scientific basis for the potential use of the drug in ophthalmology to treat injuries to the visual analyzer.

References

1. Kilkist lyudey z porushennyam zoru v Ukrayini zrostaye: yak initsiatyvy PROON spryyayut yakisniy reabilitatsiyi. UNDP. URL: https://www.undp.org/uk/ukraine/news/kilkist-lyudey-z-porushennyam-zoru-v-ukrayini-zrostaye-yak-initsiatyvy-undp-spryyayut-yakisniy-reabilitatsiyi. [in Ukrainian].
2. Krasnovid TA, Aslanova VS, Bondar NI. Osnovni aspekty travmatychnykh ushkodzhen oka pid chas voyen i viyskovykh konfliktiv. Archive of Ukrainian Ophthalmology. 2020. Vol. 8, no. 1. P. 78–85. doi: 10.22141/2309-8147.8.1.2020.200741. [in Ukrainian].
3. Semenenko A, Semenenko S, Solomonchuk A, Semenenko N. Doslidzhennya vplyvu 1-adamantyletyloksy-3-morfolino-2-propanolu hidrokhlorydu na dynamiku neyron-spetsyfichnoyi enolazy pry cherepno-mozkoviy travmi u shchuriv. In ANESTHESIA AND ANALGESIA. Vol. 2021. 133, No. 3 S_ SUPPL, pp. 1002-1002. [in Ukrainian].
4. DeMar J, Sharrow K, Hill M, Berman J, Oliver T and Long J. Effects of Primary Blast Overpressure on Retina and Optic Tract in Rats. Front. Neurol. 2016; 59: 1-12. doi: 10.3389/fneur.2016.00059.
5. Eye injury in conflict zones: the unseen epidemic of war. LSHTM. URL: https://www.lshtm.ac.uk/research/centres/health-humanitarian-crises-centre/news/427156/eye-injury-conflict-zones-unseen-epidemic-war.
6. Global, national and regional prevalence, and associated factors of ocular trauma. Medicine. 2020. Vol. 99, no. 35. P. e21870. doi: 10.1097/md.0000000000021870.
7. Hicks C, Dhiman A, Barrymore C, Goswami T. Traumatic Brain Injury Biomarkers, Simulations and Kinetics. Bioengineering 2022, 9, 612. doi: 10.3390/ bioengineering9110612.
8. Jing C, Jun-Chun T, Meng-Xian P. L-lysine confers neuroprotection by suppressing inflammatory response via microRNA-575/PTEN signaling after mouse intracerebral hemorrhage injury. Experimental Neurology 327. 2020. 113214. doi: 10.1016/j.expneurol.2020.113214.
9. New original metabolitotropic endothelioprotector “Angiolin”: quantum-chemical parameters and peculiarities of pharmacological action. Reports of the National Academy of Sciences of Ukraine. 2017. No. 8. P. 86–93. doi: 10.15407/dopovidi2017.08.086.
10. Zhang Y, Kang X, Wu Q. Explosive eye injuries: characteristics, traumatic mechanisms, and prognostic factors for poor visual outcomes. Military Med Res 10, 3 (2023). doi: 10.1186/s40779-022-00438-4.

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