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Original Article

Structural changes in the myocardium under conditions of traumatic brain injury on the background of hyperglycemia

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Abstract

Traumatic brain injury (TBI) is a life-threatening injury and the leading cause of death and major disability across all ages. Another important modern medical problem is diabetes mellitus (DM). The study aimed to investigate the hearts of rats under the influence of traumatic brain injury with streptozotocin-induced hyperglycemia. The study was conducted on 40 male rats weighing 180–220 g. DM was induced by a single intraperitoneal injection of streptozotocin (Sigma, USA). TBI was caused by the Impact Acceleration Model-free weight loss in the parieto-occipital area of the rat, according to the developed patent. The animals were removed from the experiment after 3 hours, 1 and 5 days after the inflicted TBI. Heart tissue was taken for histological studies. The sections obtained on a sled microtome were stained with hematoxylin and eosin, as well as Heidenhain. Dystrophic, destructive, and infiltrative processes occurred in the heart when simulating closed brain injury and brain injury on the background of diabetes. Under conditions of craniocerebral injury, they reached a maximum on the first day of the experiment and had stable positive dynamics on the fifth day. In the case of craniocerebral trauma in combination with diabetes, the progression of structural disorders was observed up to the fifth day from the beginning of the experiment. Non-specific morphological changes occur in the myocardium of animals with TBI, which are the morphological substrate of organ failure. The degree of structural changes in the heart against the background of diabetes has a more severe progressive nature due to the development of diabetic microangiopathy.

Keywords: traumatic brain injury, diabetes mellitus, hyperglycemia, heart remodeling.

Introduction

Traumatic brain injury (TBI) is one of the most common injuries and accounts for about 40% of all types of injuries. Brain damage is more common than any other disease, including breast cancer, AIDS, Parkinson's disease, and multiple sclerosis [1, 2]. In connection with the war on the territory of Ukraine, the number of military personnel and civilians with craniocerebral trauma caused by the action of the blast wave has increased. Brain injury is accompanied by high mortality (mortality from TBI is 1% of total mortality), leading to significant disability of victims in the post-traumatic period [3, 4]. Factors that determine high mortality in severe TBI are the severity of primary and secondary brain damage, severe metabolic disorders, and extracranial complications [3, 5–7].

According to the currently dominant concept, progressive disturbances in intracellular calcium homeostasis, glutamatergic signal transduction, and oxidative stress are central to brain neuronal damage in severe traumatic brain injury [8]. The above-mentioned pathogenetic factors are activated immediately after the injury, as well as due to the development of secondary

post-traumatic disorders of the microcirculation of the brain, caused by a complex of various intra- and extracerebral patho- and sanogenetic factors, mainly of an ischemic nature [8–12].

The problem of cerebrovisceral interactions in TBI deserves special attention. It is known that in acute brain injury, visceral homeostasis is disturbed in direct proportion to the severity of brain damage. At the same time, neurological symptoms somewhat mask the manifestations of vegetative-visceral pathology. In turn, the morpho-functional changes of internal organs contribute to the disruption of neurohumoral and enzyme-metabolic functions, which further burdens the general condition of patients. It should also be noted that the clinical course of TBI is, without a doubt, largely determined by the background against which it occurred, first of all, by the presence of chronic somatic diseases in the patient [13, 14].

Diabetes mellitus belongs to the top three diseases that are the most common cause of early disability and mortality among the population in almost all countries of the world. According to the World Health Organization, about 537 million (10.5%) of the world's population suffer from diabetes, and the number of patients with diabetes will reach 643 million by 2030 [15].

It is known that when a brain injury and diabetes are combined, the course of each pathology worsens [16, 17]. In order to study the nature of morphological changes in the tissues of internal organs under the influence of traumatic impact, a study of the histological structure of the heart as a result of TBI against the background of induced hyperglycemia was conducted.

Material and methods

Study design

The study was conducted on 40 sexually mature outbred male rats weighing 180–220 g. 8 intact animals served as controls. The animals were kept in standard vivarium conditions on a complete food diet with free access to water. All experimental procedures were carried out in accordance with the provisions of the European Convention for the Protection of Vertebrates Used for Experimental and Other Purposes (Strasbourg, 18 March 1986), the resolution of the First National Congress of Ukraine on bioethics (2001) and the Order of the Minister of Public Health of Ukraine No. 690 of 23/09/2009 [18, 19]. Diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (Sigma, USA) at a dose of 60 mg/kg [20]. Animals with a glucose level of more than 16 mmol/l were considered diabetic. Traumatic brain injury was caused by the Impact

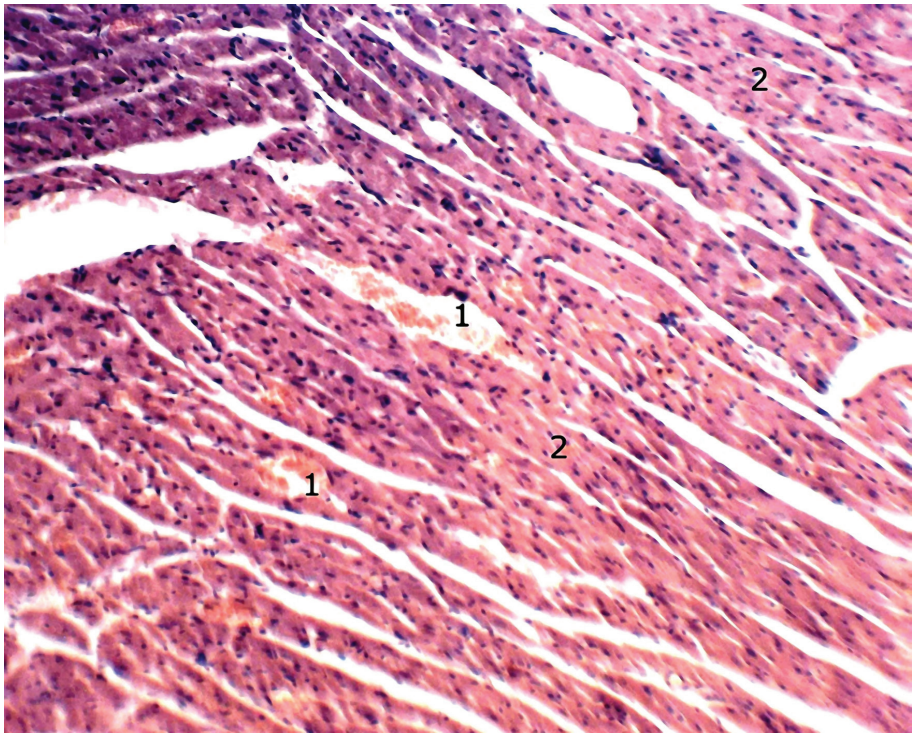

Figure 1: A fragment of a rat myocardium 3 hours after a craniocerebral injury. Full blood of dilated vessels of the venous link sludge phenomenon (1). Cardiomyocytes are unevenly stained, sometimes fragmented (2). Staining with hematoxylin and eosin. ×80.

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Acceleration Model-free weight loss in the parieto-occipital area of the rat, according to the developed patent [21]. After 3 hours, 1 and 5 days after the inflicted TBI, the animals were removed from the experiment under thiopental-sodium anesthesia (40 mg/kg) by total bleeding from the heart. Heart tissue was taken for histological studies. The obtained pieces of organs were fixed in 10% neutral formalin solution and Lilly's fixative, followed by embedding in paraffin. The sections obtained on a sled microtome were stained with hematoxylin and eosin, as well as Heidenhain. The nature and depth of morphological changes were determined using an Olimpus microscope and a system for displaying images of histological preparations.

The study was conducted following the guidelines of the Helsinki Declaration and in accordance with the regulations on the care of animals. All experimental procedures were approved by the Bioethics Commission of I. Horbachevsky Ternopil National Medical University.

Results

Histological examination of the internal organs of rats 3 hours after receiving a craniocerebral injury showed, first of all, the presence of blood circulation disorders. In the myocardium, the redistribution of blood with its distinct accumulation in the vessels of the microcirculatory channel attracted attention. Dilation of capillaries and venules, gluing of erythrocytes (sludge phenomenon) was observed (Figure 1). The perivasal stroma looked moderately distended due to edema. The intercellular interstitial tissue had a normal appearance. The arrangement of cardiomyocytes is unidirectional and compact. However, in many cells transverse banding was not visualized, and the cytoplasm unevenly absorbed the dye. Fragmentation of cells was observed in some small areas.

Heidenhain staining revealed small focal necrosis of cardiomyocytes and clearly visualized stasis in capillaries (Figure 2).

After 1 day of observation, blood circulation disorders were increasing in the myocardium, manifested by total dilatation of vessels, mainly of the venous link of the microcirculatory bed and capillaries. The lumens of the vessels were filled with blood with a tendency to sludge and hemolysis. Intramural plasmorrhages occurred in some places. Such stasis created conditions for active transudation of plasma outside the vessels and the development of perivascular edema, which became widespread at this time of the study.

The swelling contributed to the loosening of the intercellular connective tissue. At the same time, cardiomyocytes were located scattered, and their orientation and integrity were often violated – areas of chaotic and wave-like arrangement of cell fragmentation were observed. The latter acquired a much more widespread character compared to the previous term. Transverse

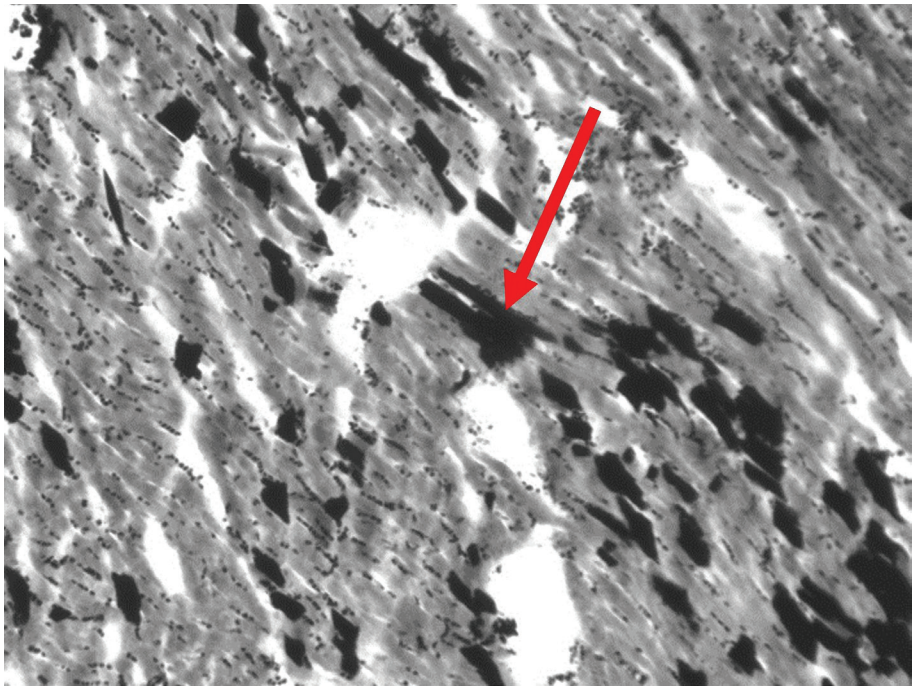

Figure 2: A fragment of a rat myocardium 3 hours after a craniocerebral injury. Small focal necrosis of cardiomyocytes. Clearly expressed stasis in the capillaries. Staining according to Heidenhain. ×100.

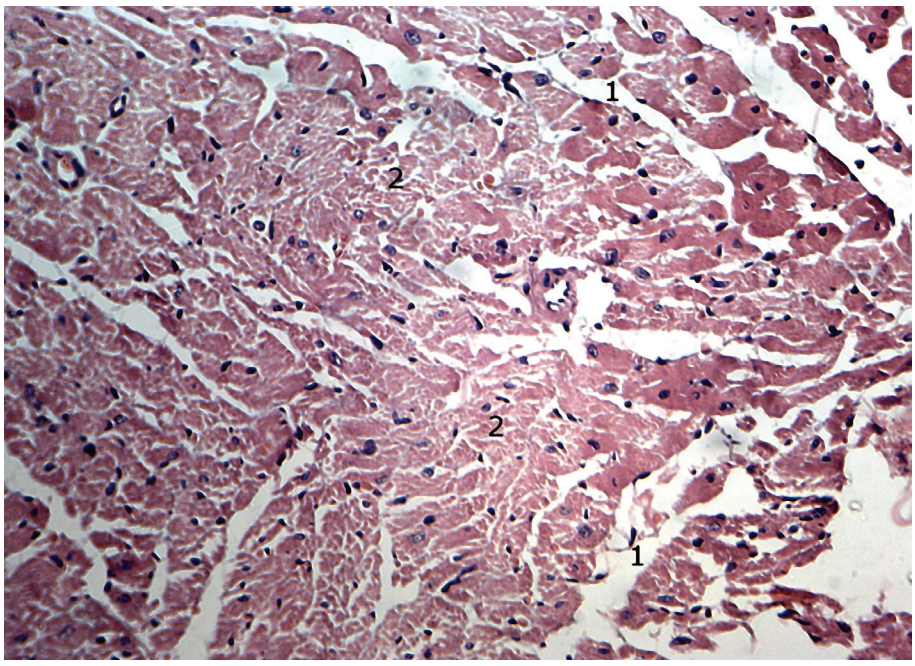

Figure 3: Histological structure of the myocardium of a rat with TBI on the first day of the experiment. Pronounced stromal edema (1), dystrophic changes, fragmentation and focal disintegration of cardiomyocytes (2). Staining with hematoxylin and eosin. ×100.

striations disappeared in cardiomyocytes. Intracellular components underwent qualitative changes: the cytoplasm poorly and unevenly absorbed the dye, becoming hypochromic. In addition, it revealed foci of deep disintegration of the homogeneous cytoplasmic matrix, occasionally – vacuolization. The nuclei in such cells were heterogeneous – from pyknotic to weakly contoured, swollen. In part of the cardiomyocytes, nuclei were not visualized (Figure 3).

In the next period of the experiment, on the fifth day, the study of the heart showed stabilization and positive shifts in the dynamics of structural changes and

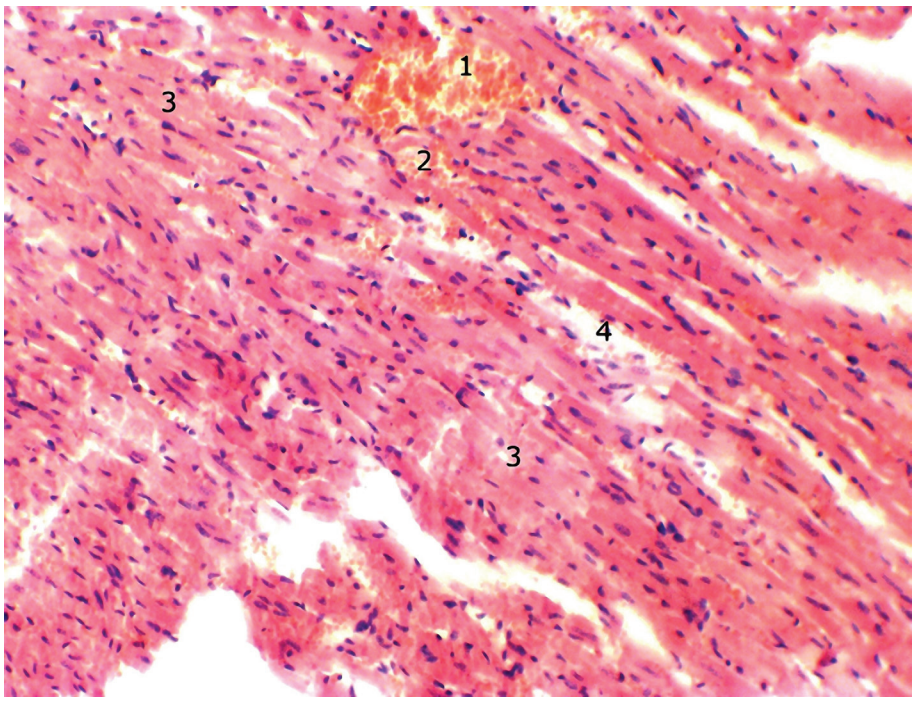

Figure 4: A fragment of the myocardium of a diabetic rat 3 hours after a brain injury. Venous congestion (1), perivasal hemorrhage (2), cardiomyocyte dystrophy and fragmentation (3). Stromal edema (4). Staining with hematoxylin and eosin. ×100.

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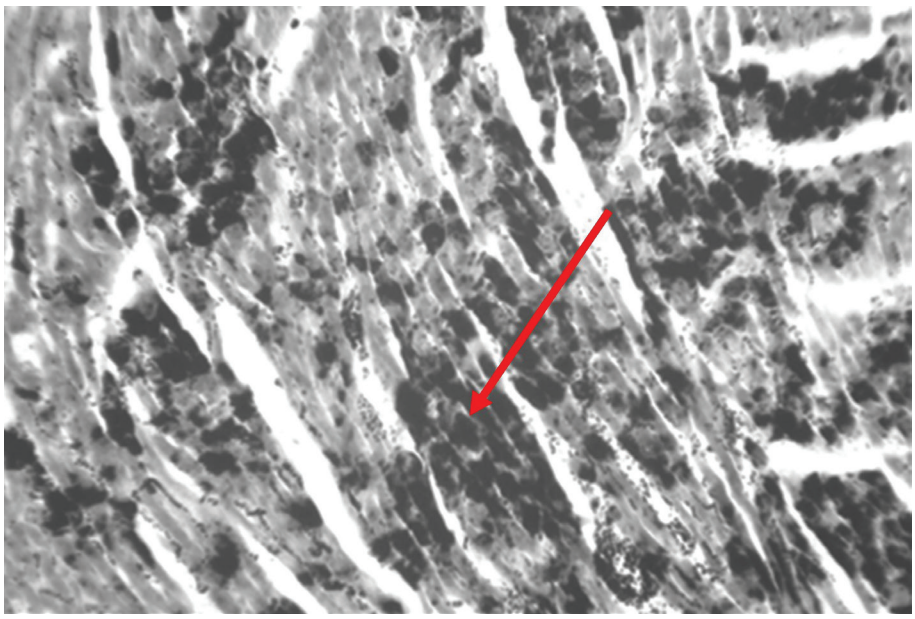

Figure 5: A fragment of the myocardium of a diabetic rat 3 hours after a brain injury. Multiple necrosis of cardiomyocytes. Staining according to Heidenhain. ×100.

the appearance of individual morphological signs of adaptive and compensatory changes in rats with isolated brain injury. The general regularity of the structural changes described above was preserved, but the degree of their expression was somewhat less than in the previous terms.

A completely different histological picture was observed in the specified period in rats with diabetes. Dystrophic-destructive changes in the structural components of internal organs increased and were associated with prolonged hemodynamic disorders.

Under the conditions of a combination of TBI and hyperglycemia, 3 hours after the injury in the myocardium, edema became diffuse – perivascular areas and intercellular stroma were expanded, with signs of edema. In some cases, perivasal hemorrhages were observed.

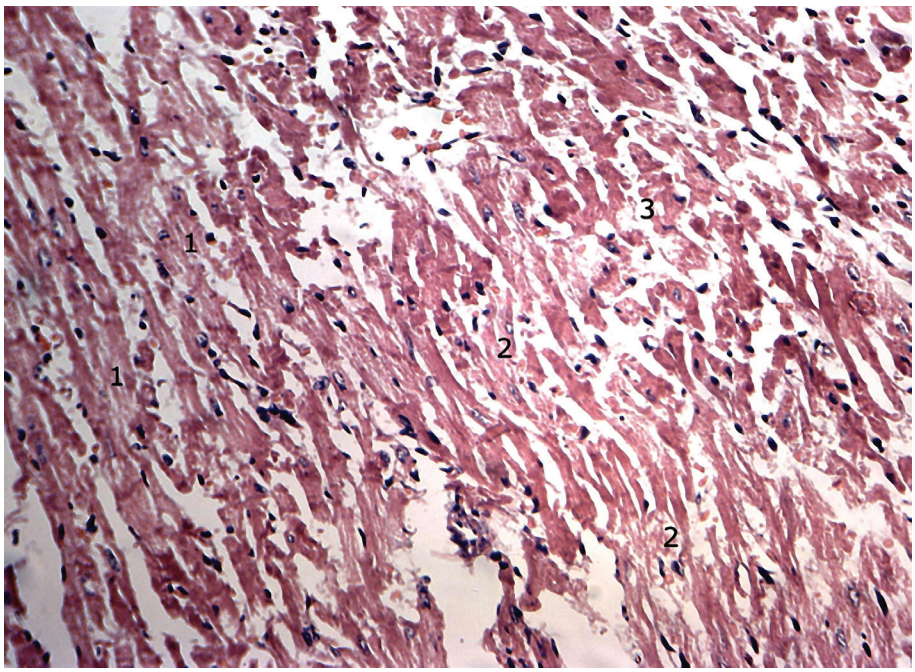

Figure 6: Histological structure of a rat myocardium under the conditions of TBI against the background of streptozotocin-induced diabetes at 24 hours of the experiment. Diffuse dystrophic changes, subtotal fragmentation (1), focal deep decay of cardiomyocytes (2) and disintegration of the cell layer (3). Staining with hematoxylin and eosin. ×100.

Fragmentation covered wide fields of cardiomyocytes; the cells had predominantly dim homogeneous or unevenly stained cytoplasm with indistinct nuclear contours (Figure 4).

Nuclei were not identified in many cells. Heidenhain staining also revealed a greater number of necrotized cardiomyocytes compared to injured non-diabetic animals (Figure 5).

Edema of the connective tissue of the myocardium of rats with a craniocerebral injury against the background of hyperglycemia 24 hours after the injury acquired a systemic character, disintegrating the cellular layer. Separated cardiomyocytes also lost their correct placement. Such changes, combined with fragmentation and signs of necrobiotic and necrotic changes, indicated a sharp decrease in the contractile capacity of the myocardium of experimental animals (Figure 6). In some places, polymorphic cellular infiltrates with a predominance of lymphocytes were found in the stroma.

Heidenhain staining showed increasing necrotic changes in the myocardium of animals with combined pathology 24 hours after inflicting a craniocerebral injury (Figure 7).

After 5 days in the animals, after brain injury against the background of induced diabetes, small focal necrosis in the myocardium changed to sufficiently wide fields of necrosis (Figure 8).

However, changes in the walls of vessels of small and medium caliber became more pronounced. This is applied to both veins, arterioles and arteries. Their walls thickened not only due to plasma seepage but also the proliferation of connective tissue fibers. Homogeneous eosinophilic foci of hyalinosis appeared. Proliferating thin connective tissue fibers and small cellular infiltrates appeared in the stroma of the examined organ, along with edema (Figure 9).

Discussion

Histological examination of the myocardium of rats 3 hours after TBI showed the presence of blood circulation disorders in the form of full blood, dilated vessels of the venous link, sludge phenomenon and the development of perivascular edema.

Microscopic changes in the heart of animals with brain injury combined with diabetes were similar to those described in the previous group of rats, but more profound structural changes were observed. In the myocardium, dystrophy and fragmentation of cardiomyocytes, venous congestion, diffuse edema and a greater number of necrotized cardiomyocytes, compared to injured animals without diabetes, were determined.

On the first day of the experiment, in the heart of rats with the combined pathology, we observed negative trends in changes in structural components compared to those that were detected at the 3rd hour. In particular, in the myocardium against the background

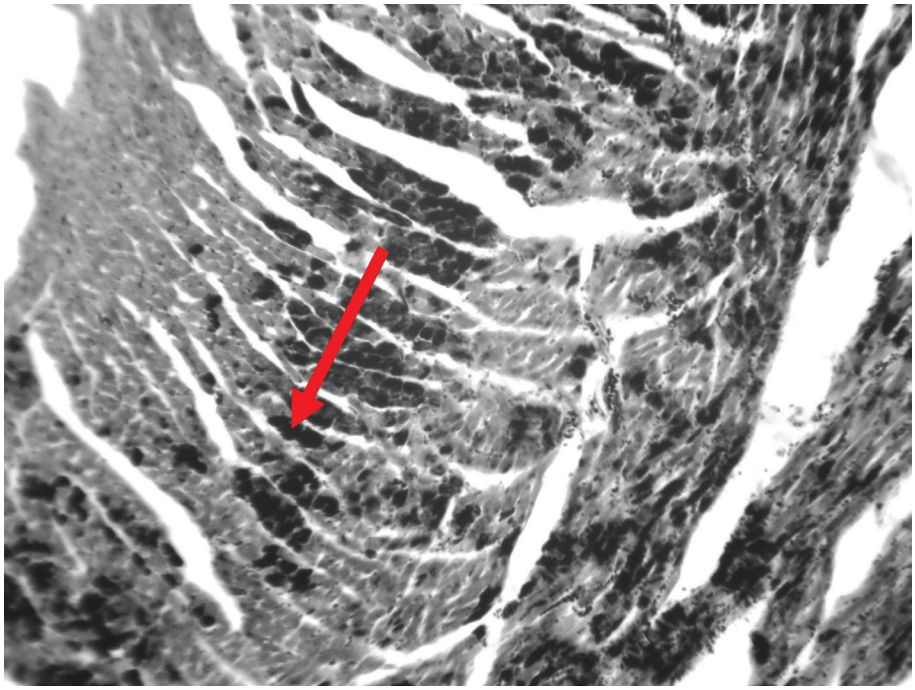

Figure 7: Necrotic changes in rat cardiomyocytes under conditions of TBI against the background of streptozotocin-induced diabetes for 24 hours of the experiment. Staining according to Heidenhain. ×100.

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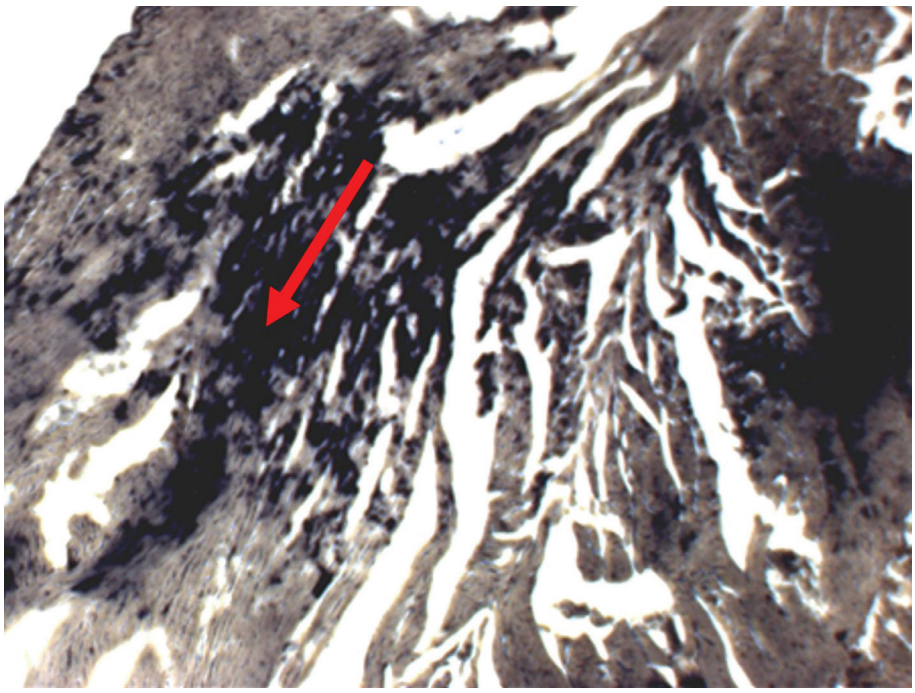

Figure 8: Histological structure of the myocardium of a rat with streptozotocin-induced diabetes 5 days after TBI. Widespread necrosis of cardiomyocytes. Staining according to Heidenhain. ×80.

of pronounced stromal edema, dystrophic changes, widespread fragmentation and focal disintegration of cardiomyocytes were noted.

It should be emphasized that the structural changes described in animals with TBI after 1 day almost completely corresponded to those in injured rats with diabetes 3 hours after TBI. In general, on the first day of the study, the morphological changes after the injury against the background of hyperglycemia were accelerated, deeper and continued to progress.

On the fifth day of the post-traumatic period, stabilization and positive shifts in the dynamics of structural changes in the myocardium and the appearance of individual morphological signs of adaptive and

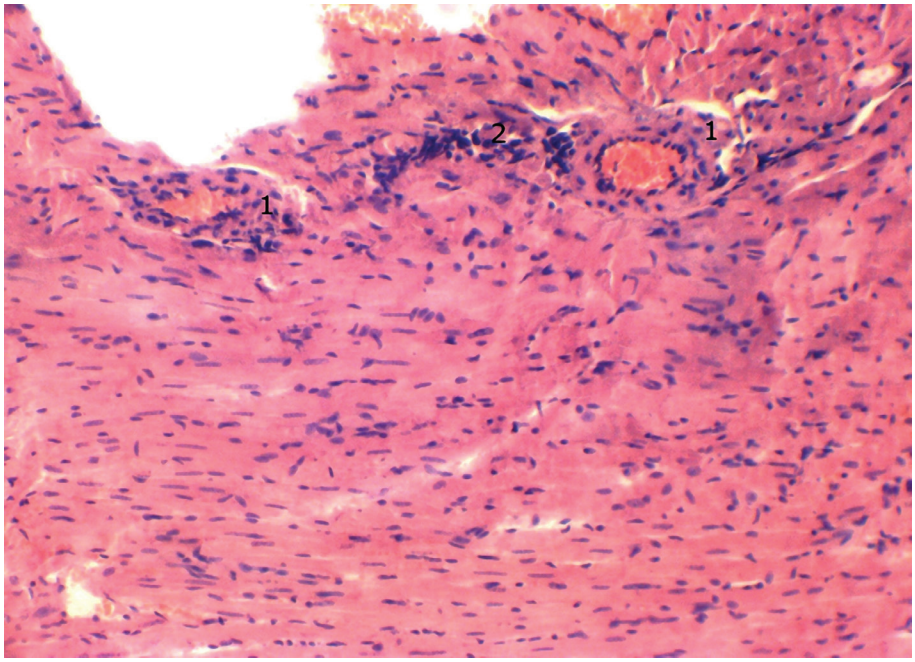

Figure 9: Histological structure of rat myocardium with streptozotocin-induced diabetes 5 days after TBI. Thickening of vascular walls, intramural (1) and stromal round cell infiltrates (2). Staining with hematoxylin and eosin. ×80.

compensatory changes in rats with craniocerebral trauma were detected. The general regularity of the structural changes described above remained, but their degree of expression was somewhat less than in the previous terms. However, dystrophic-destructive changes in the structural components of the heart of injured animals with diabetes increased and were associated with the prolonged hemodynamic disorders described in the earlier study periods. Structural changes in the myocardium of animals during this period were similar to the changes that occurred in injured animals without diabetes on the first day of the post-traumatic period. We noted sufficiently wide fields of necrosis in the myocardium, thickening of the walls of small and medium-sized vessels, and the appearance of hyalinosis foci.

The worse morpho-functional state of the myocardium under conditions of TBI and diabetes may be caused by diabetic cardiomyopathy, the pathogenesis of which is obviously multifactorial. Inhibition of glucose utilization in the "diabetic" heart, slowing down its transport through the sarcolemma membrane, is probably mediated by impaired functioning of glucose transporters (GLUT-1, GLUT-4). Activation of the processes of glucose oxidation and, accordingly, an increase in the concentration of free fatty acids causes an inhibitory effect on the functioning of the pyruvate dehydrogenase complex, which is one of the mechanisms of inhibition of glucose oxidation [3, 10, 22]. These changes lead to a decrease in ATP reserves in the myocardium, a violation of its contractile function. They are accompanied by pronounced ultrastructural changes and are the leading link in carbohydrate metabolism disorders in the "diabetic" heart. In addition, hyperglycemia causes the formation of active forms of O2 and NO, which increases oxidative stress, disrupts gene expression signal transduction, and activates metabolic pathways of programmed myocardial cell death.

Conclusion

During histological examination, non-specific morphological changes in the form of edema, dystrophic and destructive changes occur in the myocardium of animals with craniocerebral trauma, which can be considered as a morphological substrate of organ failure.

The degree of structural changes in the heart in rats with TBI in combination with diabetes is significantly higher and is deepened by manifestations of concomitant pathology. In rats with TBI, morphological changes reach a maximum on the first day of the experiment and have stable positive dynamics on the fifth day. In traumatized animals with diabetes, the progression of structural changes is observed up to 5 days after the start of the experiment.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Carney N, Totten AM, O'Reilly C, Ullman JS, Hawryluk GW, Bell MJ, *et al*. Guidelines for the Management of Severe Traumatic Brain Injury, Fourth Edition. Neurosurgery 80(1):6-15, 2017. doi: 10.1227/NEU.0000000000001432. PMID: 27654000.
2. Mayumi P, Tiffany G, Daya A, Christopher CG. The pathophysiology of traumatic brain injury at a glance. Disease Models & Mechanisms 6: 1307-1315, 2013.
3. Ghajar J. Traumatic brain injury. Lancet 356 (9233):923-929, 2000.
4. Capizzi A, Woo J, Verduzco-Gutierrez M. Traumatic brain injury: an overview of epidemiology, pathophysiology, and medical management. Medical Clinics of North America 104(2): 213-238, 2020. https://doi.org/10.1016/j.mcna.2019.11.001.
5. Tagliaferri F, Compagnone C, Korsic M, Servadei F, Kraus J. A systematic review of brain injury epidemiology in Europe. Acta Neurochir 148(3):255-268, 2006.
6. McDonald SJ, Sharkey JM, Sun M, Kaukas LM, Shultz SR, Turner RJ, *et al*. Beyond the brain: peripheral interactions after traumatic brain injury. Journal of Neurotrauma 37:770–781, 2020. DOI: 10.1089/neu.2019.6885
7. Lele AV, To-adithep P, Chanthima P, Lakireddy V, Vavilala MS. Correlation between brain trauma foundation guidelines and published severe traumatic brain injury research. Journal of Neurosurgical Anesthesiology 33(4): 323-328, 2021 https://doi.org/10.1097/ANA.0000000000000684
8. Shohami E, Cohen-Yeshurun A, Magid L, Algali M, Mechoulam R. Endocannabinoids and traumatic brain injury. British Journal of Pharmacology 163(7): 1402–1410, 2011.
9. Maas A, Murray GD, Henney H, Kassem N, Legrand V, Mangelus M, *et al*. Efficacy and safety of dexanabinol in severe traumatic brain injury: results of a phase III randomized, placebo-controlled clinical trial. Lancet Neurol 5: 38-45, 2006.
10. Ng SY, Lee AYW. Traumatic brain injuries: pathophysiology and potential therapeutic targets. Front. Cell. Neurosc 13:528, 2019. doi: 10.3389/fncel.2019.00528
11. Jassam YN, Izzy S, Whalen M, McGavern DB, El Khoury J. Neuroimmunology of traumatic brain injury: time for a paradigm shift. Neuron 95(6):1246-1265, 2017. https://doi.org/10.1016/j.neuron.2017.07.010.
12. Dixon K. Pathophysiology of traumatic brain injury. Phys Med Rehabil Clin N Am 28: 215-225, 2017.
13. Bruns JJr, Hauser WA. The epidemiology of traumatic brain injury: a review. Epilepsia 44(10): 2-10, 2003.
14. Wendong You, Yuanrun Zhu, Anqi Wei, Juan Du, Yadong Wang, Peidong Zheng, *et al*. Traumatic brain injury induces gastroin-

 https://doi.org/10.46389/rjd-2023-1360

testinal dysfunction and dysbiosis of gut microbiota accompanied by alterations of bile acid profile. Journal of Neurotrauma 227-237, Jan 2022. http://doi.org/10.1089/neu.2020.7526
15. Boyko EJ, Magliano DJ, Karuranga S, Piemonte L, Riley P, Saeedi P, Sun H. IDF Diabetes Atlas. International Diabetes Federation 135, 2021. https://diabetesatlas.org/atlas/tenth-edition/
16. Yuki Tatara, Ryo Shimada, Kazuhiko Kibayashi. Effects of preexisting diabetes mellitus on the severity of traumatic brain injury. Journal of Neurotrauma 886-902, Apr 2021. http://doi.org/10.1089/neu.2020.7118
17. Ting-Ting Li, Wan-Chao Yang, Yue-Zhen Wang, Tian Sun, Hong-Ling Cao, Jian-Feng Chen, Wen-Zhi Li. Effects of a high concentration of hydrogen on neurological function after traumatic brain injury in diabetic rats. Brain Research 1730: 146651, 2020. https://doi.org/10.1016/j.brainres.2020.146651.
18. Council of Europe Strasbourg. European convention for the protection of vertebrate animals used for experimental and other scientific purposes 123: 52, 1986.
19. Reznikov OG. General ethical principles of experiments on animals. Endocrinology 8(1):142-145, 2003.
20. Stefanov OV. Doklinichni doslidzhennia likarskykh zasobiv. Metodychni rekomendatsii. Kyiv: Avitsena. 528 p. 2001 [in Ukrainian].
21. Meretskyi VM. Method for Simulation of Traumatic Brain Injury. Ukrainian Patent No. 74935, 12 November 2012, N 21. Available online: http://uapatents.com/5-74935-sposib-modelyuvannya-cherepno-mozkovo-travmi.html.
22. Clausen F, Lundqvist H, Ekmark S. Oxygen free radical-dependent activation of extracellular signal-regulated kinase mediates apoptosis-like cell death after traumatic brain injury. Journal of neurotrauma 21(9):1168-1182, 2004.