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MORPHOLOGICAL CHARACTERISTICS OF THE BLOOD-BRAIN BARRIER IN 1 DAY OF EXPERIMENTAL BLAST-INDUCED TRAUMATIC BRAIN INJURY

Kozlova Yu.V. ✉, Tryasak N.S., Klopotskyi G.A., Kozlova K.S. Morphological characteristics of the blood-brain barrier in 1 day of experimental blast-induced traumatic brain injury.
Dnipro State Medical University, Dnipro, Ukraine.
ABSTRACT. Background. Blast-induced traumatic brain injury is becoming widespread in connection with the use of explosives in military conflicts all over the world and today in Ukraine, which needs the elaboration of modern pathogenetically based treatment methods of the acute period and prevention of remote complications. Scientists have established that one of the significant primary injuries, which is the basis for the realization of secondary ones, in the case of a mild blast-induced traumatic brain injury, is a violation not only of neurons themselves, but also of the blood-brain barrier. **Aim.** Morphological determination of changes in the blood-brain barrier in 1st day of experimental blast-induced traumatic brain injury using a proprietary device for simulating blast injury. **Methods.** Brain sections of 12 albino male Wistar rats (body mass 220-270 g, age 6-7 months) were examined using light microscopy. Rats were randomly divided into 2 groups: I – Experimental group (n=6), the animals of which were anesthetized with Halothane, fixed with their heads to the muzzle end of a self-made and patented device at a distance of 5 cm and subjected to the action of a blast wave of 26-36 kPa; II - Sham (n=6). After 1 day, the rats of both groups were euthanized and brains were removed, which were then fixed and sections were prepared and stained with hematoxylin and eosin according to standard methods. **Results.** Established credible signs of blood-brain barrier disruption using our own device to reproduce a blast-induced traumatic brain injury, which are primary damages as a result of the action of the blast wave and are the basis for triggering secondary damage mechanisms and lead to neurodegeneration processes. **Conclusion.** The mild blast-induced traumatic brain injury after using a proprietary device was confirmed by morphological changes in the blood-brain barrier in the 1st day of the post-traumatic period in the form of ruptured and paretically dilated capillaries and desquamation of the vascular endothelium, the presence of erythrocyte aggregates. Increased permeability of blood vessels led to swelling of brain tissue and neurocytes.
Key words: blood-brain barrier, explosion, brain, trauma.

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Background

Blast-induced traumatic brain injury (bTBI) is becoming widespread in connection with the use of explosives in military conflicts all over the world and today in Ukraine [1, 2]. This leads to an increase in the interest of scientists and doctors in the mechanisms of bTBI development and the elaboration of modern pathogenetically based treatment methods of the acute period and prevention of remote complications [3].

Blast-induced traumatic brain injury is one type of traumatic brain injury (TBI). However, the main difference between bTBI and classical TBI is the specific damage caused by the blast wave, what is currently subject to definitive research, and for what we have developed and patented a device [4, 5]. It is known from previous studies that the brain under the influence of an explosive wave even a small force

undergoes significant microscopic damage as a result of displacement of the brain and impact on the walls of the skull, changes in intracranial pressure and the pressure of extracellular and intracellular fluids, the formation of bubbles due to cavitation, which also damage brain cells [6, 7]. After the primary damage, the cascades of secondary damage reactions are activated [8, 9]. Scientists have established that one of the significant primary injuries that underlies the realization of secondary ones in mild bTBI is a violation not only of neurons themselves, but also of the blood-brain barrier (BBB) [10].

In this regard, the **Aim** of the study was the morphological determination of changes in the blood-brain barrier in 1st day of experimental blast-induced traumatic brain injury using a proprietary device for simulating blast injury.

Materials and methods

The experiment was carried out on 12 albino male Wistar rats (body mass 220-270 g, age 6-7 months). The animals were kept in standard conditions and on the standard diet of the Dnipro state medical university (DSMU) vivarium [11], all researches were conducted in accordance with modern international requirements and norms of humane attitude of animals (European Convention, 18.03.1986 (Strasbourg); Declaration of Helsinki, 1975, revised and supplemented in 2000, Law of Ukraine №3447-IV, 21.02.2006), what is attested by an extract from the minutes of the commission on biomedical ethics meeting of DSMU № 3, 2.11.2021. Randomly selected rats were divided into two groups: group I – experimental, Exp (n=6), animals were subjected to inhalation anesthesia with halothane (Halothan Hoechst AG , Germany), fixed in a horizontal position on the abdomen at a distance of 5 cm head to muzzle end and simulated an explosion-induced brain injury by generating a shock wave with an overpressure of 26-36 kPa on a self-made device [5], II group – sham, Sh (n=6).

The 1 day after bTBI simulation, rats of both groups were euthanized with halothane, after which they were decapitated and the brain was removed and then was fixed in a 10% solution of neutral formalin with exposure for 24 hours. After fixation, paraffin blocks were prepared, from which sections with a thickness of 3-5 μm were made on a Thermo HM 355S microtome (Thermo Scientific, Germany). Before staining, sections were deparaffinized in xylene, rehydrated in decreasing concentrations of isopropanol. Brain sections were stained with hematoxylin and eosin according to generally accepted standards of pathogistological procedures [12]. Microscopic examination was carried out using a trinocular light-optical microscope "Primo Star Carl Zeiss" with photo output and using a lens.

Results and discussion

For any experimental research, it is important to choose an adequate model, which, in addition to ensuring the reproduction of damage as close as possible to the actual pathology, also meets the modern requirements of bioethical treatment of experimental animals. Therefore, before carrying out the planned research, each model, including ours, needs morphological confirmation. Thus, we obtained verifiable morphological signs of brain damage as a result of the blast wave action, namely the presence of various diameters capillaries ruptures and desquamation of the vascular endothelium (Fig. 1-3), extravasal aggregation of unchanged erythrocytes. These phenomena were diffuse in different parts of the brain.

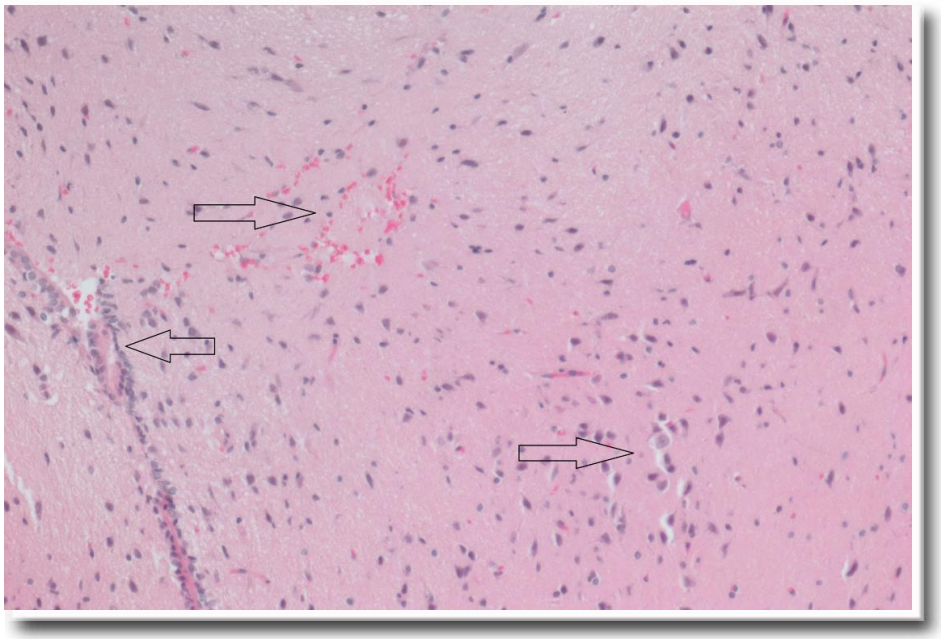

Fig. 1. Histological section of the brain of a rat of the experimental group (1st day of post-traumatic period). Staining with hematoxylin and eosin. ×200.

Hemorrhagic diapedesis, paretic dilated vessels due to impaired autoregulation and loosening of brain tissue due to edema were also detected. In addition to ruptures of capillaries, there is a violation of contacts between vessels and glial cells, which are also part of the blood-brain barrier complex.

Along with BBB disruption, heterogeneously colored neurons, as well as their swelling and pericellular edema attract attention (Fig. 3).

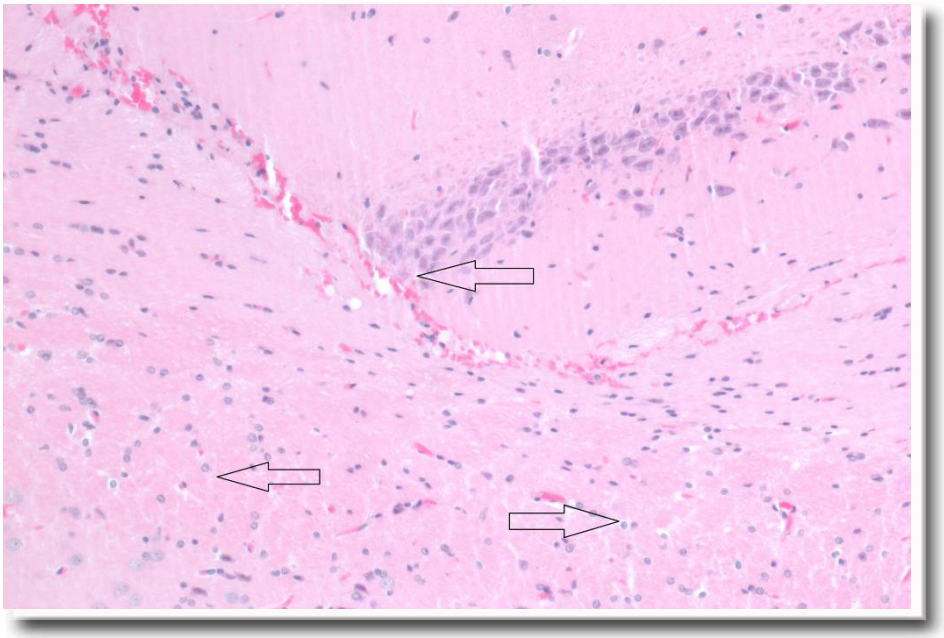

Fig. 2. Histological section of the brain of a rat of the experimental group (1st day of post-traumatic period). Staining with hematoxylin and eosin. ×200.

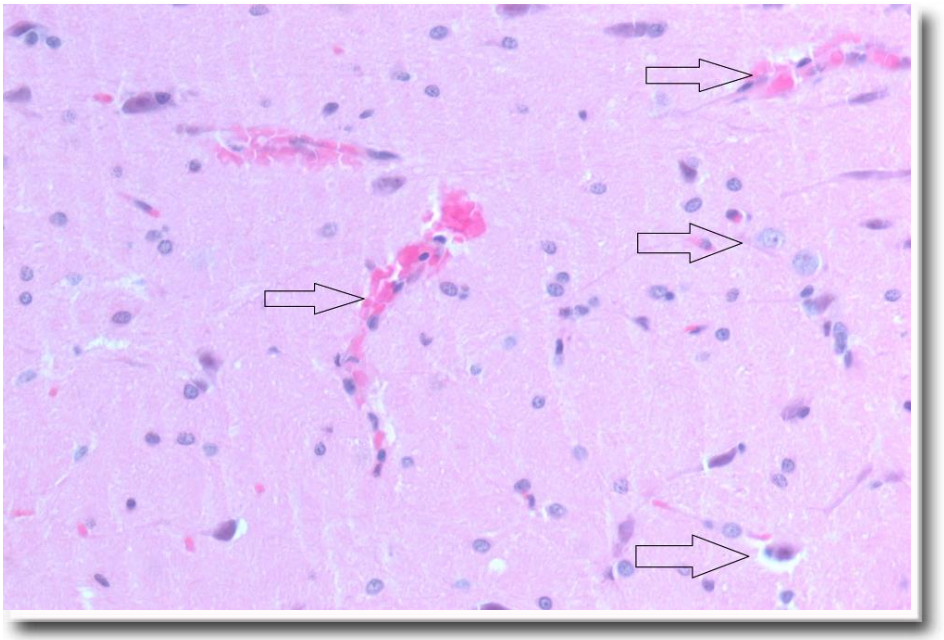

Fig. 3. Histological section of the brain of a rat of the experimental group (1st day of post-traumatic period). Staining with hematoxylin and eosin. ×400.

At the same time, there were no massive hemorrhages as a result of damage to larger diameter vessels, and there were no foci of neuronal necrosis. This testifies to the correct operation of the own device and to the correctly selected pressure of the explosive wave, which leads to a mild blast injury.

The results obtained by us are coincide with the data of modern studies on the violation of the BBB during the pathogenic action of the blast wave [10, 13]. And knowledge about the functions of the BBB and the consequences of its violation help to reveal the pathogenesis of both early and long-term com-

plications of bTBI. After all, the BBB is a physiological internal histological barrier between nervous tissue and blood, represented by endothelial cells of vessels of the central nervous system, which together with pericytes, astrocytes, neurons and microglia form a neurovascular unit [14]. The BBB itself and the neurovascular unit provide a delay in the passage of neurotoxic substances, infections, maintain water balance, biochemical and bioelemental homeostasis, and are responsible for an adequate immune response [15].

Thus, BBB disruption in the acute post-traumatic period (1 day) due to increased permeability of the vascular wall leads to accumulation of fluid and swelling of brain tissues, as well as to the ingress of biologically active pro-inflammatory substances (cytokines) and causes neuroinflammation [16]. Violation of microcirculation due to paresis of the vascular wall in combination with increased utilization of glucose in mitochondria leads to hypoxia and oxidative stress [17]. The start of anaerobic processes of ATP formation leads to acidosis [18]. The release of erythrocytes contributes to the accumulation of Fe, which, in turn, increases oxidative stress [19].

This complex of disturbances further increases neuroinflammation, which is a secondary damage to brain tissues and triggers apoptosis, unregulated necrosis and necroptosis [20]. Also, as a result of primary damage by a blast wave, a primary immune response is implemented, which further activates the cells of the adaptive immune system by releasing chemokines, inducing adhesion molecules on the BBB, and expressing molecules on microglia. And damage to the BBB itself is a trigger for autoimmune damage to neurons, which leads to gradual neurodegeneration in the long term [21].

Conclusion

The mild blast-induced traumatic brain injury using a proprietary device was confirmed by morphological changes in the blood-brain barrier in the 1st day of the post-traumatic period in the form of ruptured and paretically dilated capillaries and desquamation of the vascular endothelium, the presence of erythrocyte aggregates. Increased permeability of blood vessels led to swelling of brain tissue and neurocytes.

Prospects for further development are to establish the impairment of cognitive functions of the brain in rats with experimental blast-induced trauma, as well as to reveal the biochemical and bioelemental features of brain damage.

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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Козлова Ю.В., Трясак Н.С., Клопоцький Г.А., Козлова К.С. Морфологічна характеристика гематоенцефалічного бар'єру у 1 добу експериментальної вибухо-індукованої травми головного мозку.

РЕФЕРАТ. Актуальність. Все більш поширеною стає вибухо-індукована травма головного мозку у зв'язку із застосуванням вибухівки у воєнних конфліктах в усьому світі і на сьогодні в Україні, що потребує розробки сучасних патогенетично обгрунтованих засобів лікування гострого періоду і профілактики віддалених ускладнень. Науковці встановили, що одним із вагомих первинних ушкоджень, що лежить в основі реалізації вторинних, при легкій вибухо-індукованій травмі головного мозку є порушення не тільки власне нейронів, а й гематоенцефалічного бар'єру. **Мета.** Морфологічне визначення змін гематоенцефалічного бар'єру в 1 добу експериментальної вибухо-індукованої травми головного мозку при використанні власного пристрою для моделювання вибухової травми. **Методи.** За допомогою світлової мікроскопії дослідили зрізи головного мозку 12 білих щурів-самців лінії Wistar (вагою 220-270 г, віком 6-7 місяців). Щури були рандомно розділені на 2 групи: I – експериментальна (n=6), тварин якої наркотизували Галотаном, фіксували головою до дульного кінця власно виготовленого і запатентованого пристрою на відстані 5 см та піддавали дії вибухової хвилі 26-36 kPa; II – контрольна (n=6). Через 1 добу щурам обох груп проводили евтаназію та вилучення головного мозку, який надалі фіксували та готували зрізи і зафарбовували гематоксиліном та еозином за стандартними методиками. **Результати.** Встановили достеменні ознаки порушення гематоенцефалічного бар'єру при застосуванні власного пристрою для відтворення вибухо-індукованої травми головного мозку, що є первинними пошкодженнями в результаті дії вибухової хвилі і лежать в основі запуску вторинних механізмів пошкодження, які запускають процеси нейродегенерації. **Висновки.** Вибухо-індукована травма головного мозку легкого ступеня при використанні власного пристрою підтверджено морфологічно наявними змінами гематоенцефалічного бар'єру в 1 добу посттравматичного періоду у вигляді розірваних і паретично дилатованих капілярів та десквамації ендотелію судин, наявних агрегантів еритроцитів. Підвищення проникності судин призвело до набряку тканини мозку і набухання нейроцитів.

Ключові слова: гематоенцефалічний бар'єр, вибух, головний мозок, травма.