ЕКСПЕРИМЕНТАЛЬНА МЕДИЦИНА ТА БІОЛОГІЯ

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CHANGES OF BIOMETALS IN THE RAT FOREBRAIN IN THE EARLY PERIOD OF BLAST-INDUCED TRAUMATIC BRAIN INJURY

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Blast-induced traumatic brain injury is a complex of impairments caused by the rapid transfer of energy from the explosion to the brain. The main pathogenic factor is the blast wave, which leads to primary diffuse damage. The question of the biometals participation in the pathogenesis of secondary damage in various structures of the brain, in particular in the forebrain in the early period of mild blast-induced traumatic brain injury, is attracting considerable interest at present. The experiment carried out on 36 sexually mature male Wistar rats weighing 220-270 g in the laboratory of the Department of Pathological Anatomy, Forensic Medicine and Pathological Physiology of the Dnipro State Medical University in compliance with current legislation on ethical treatment of animals. Rats were kept in standard vivarium conditions and were randomly divided into 2 groups: experimental (subjected to anaesthesia and exposure to a baroacoustic wave of 26-36 kPa) and intact groups. On the 14th, 21st, and 28th days of the post-traumatic period, the animals were euthanized with halothane followed by removal of the brain and separation of the forebrain. Spectral research was carried out using energy-dispersive X-ray fluorescence analysis. The analysis of the results showed an intragroup increase in the level of Fe by 3% and Cu by 36% and a decrease in Zn by 36%, but all these biometals and their ratios (Cu/Fe, Cu/Zn, Zn/Fe) were more in the experimental rats. We consider that these biometals lead to oxidative stress, damage to the neurons and mitochondria membranes, development of energy deficit, excitation of neurons, disruption of axonal conduction and synaptic transmission. The obtained data can be suggested as factors of secondary damage and used as diagnostic and prognostic markers of this injury type; they can also be used as the foundation for developing pathogenetically validated neuroprotection.

Key words: biometals, explosion, trauma, brain, early period.

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Introduction

Blast-induced traumatic brain injury (bTBI) is a group of impairments resulting from the quick transfer of energy from an explosion to the brain [1]. Currently, experts are actively investigating the pathogenesis of this complex and striving to comprehend the specific mechanisms by which the explosion and related forces impact brain tissue and its aftermath. The blast wave (BW) is now established as the primary pathogenic factor. The impact of BW results in an immediate and vigorous surge in atmospheric pressure, promptly trailed by a decrease, causing a compressed air wave that radiates outward from the explosion's epicentre. When the BW interacts with the head, there is an abrupt surge in pressure, causing an impact on the surface of the skull. As a result, the head accelerates, intracranial pressure changes, fluids are displaced, and a cavitation effect occurs, leading to primary diffuse brain tissue injury [2, 3].

The initial damage caused by BW results in blood-brain barrier violation and neuron injury [4, 5], contributing to the onset of a secondary cascade of pathological and biochemical reactions [6]. The study reveals potential links between pro-inflammatory mediators, oxidative stress, and changes in biometals homeostasis [7, 6]. However, further investigation is needed to comprehend the extent of these changes across varying brain regions and stages of the post-traumatic period. Given that Fe is involved in synthesis of neurotransmitters and ATP formation in mitochondria of neurons and is present in enzymes involved in the antioxidant system [8], Cu is involved in promoting neurotransmission in synapses and the myelination of nerve fibres. As Fe is a component of antioxidant enzymes and regulates homeostasis [9], and Zn is a regulator of redox reactions with anti-inflammatory effects [10], changes in these biometals are believed to significantly contribute to bTBI pathogenesis.

Therefore, the objective of this study is to determine the changes in biometals (Fe, Cu, Zn) in the forebrain of rats during the early stages of bTBI.

Materials and methods

The study was carried out on 36 sexually mature, healthy male Wistar rats, body mass 220-270 g and aged 6-7 months at the laboratory of the Department of Pathological Anatomy, Forensic Medicine and Pathological Physiology of the Dnipro State Medical University (DSMU). The procedures adhered to the "General ethical principles of animal experiments" endorsed by the Fifth National Congress on Bioethics (Kyiv, 2013) and guided by the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental Research and other Scientific Purposes (Strasbourg, 1986). Every effort was made to minimize both the number of animals used and their suffering, as evidenced by an extract from the minutes of the biomedical ethics commission DSMU № 3 meeting on 02.11.2021.

The rats were kept in rectangular plastic cages (floor S=1500 cm^2) with a wire mesh cover, and were marked with a permanent marker. Wood shavings measuring 2-3 cm in depth were used as bedding. The temperature in the room was maintained between 20-25^o C, with a humidity level of 50-60%. The rats were provided with food and tap water ad libitum and subjected to a daily 12:12 light-dark cycle.

The selected rats were randomly divided into 2 groups: group I consisted of experimental animals (n=18) anaesthetised with Halothan Hoechst AG (Germany), gently immobilised in a horizontal position on their abdomens, with their muzzles positioned 5 cm from the device opening and exposed to a baroacoustic wave with an excess pressure of 26-36 кPa [11]. Group II consisted of intact rats (n=18). On days 14, 21 and 28 of the post-traumatic period, the subjects were humanely euthanized using halothane and their brains were removed. The forebrain was extracted for spectral analysis through the use of energy-dispersive X-ray fluorescence analysis (EDRFA) on the EXPERT 3 XL analyser [12].

Samples (forebrain) of native unfixed brain were placed in the measurement chamber and irradiated for 10 minutes (600 s). Then, by using software manufactured by LLC "Scientific and Production Enterprise Institute of Analytical Control Methods ("INAM" LLC), Kyiv, Ukraine, at the laboratory of the Dnipropetrovsk Regional Bureau of Forensic Medical Examination, calculations were made in automated mode. The results are presented in the form of peak spectra and tables for each sample with the corresponding mass concentrations. After obtaining the quantitative mass fractions of biometals, the ratios of Cu/Fe, Cu/Zn, Zn/Fe were calculated on the basis of the mass fractions of each element in percent (ω) and the data were compared between the two groups.

Numerical results were performed using Microsoft Office Excel-2003® (№ 74017-641-9475201-57075) (Microsoft Corporation, USA) and Statistica v6.1 (Statsoft Inc., USA) (ser. № AGAR909E415822FA). Mathematical processing included calculations of arithmetic means (M) and standard deviations (SD). To determine the degree and nature of the relationship between the research parameters of the experimental and intact groups for each observation period, a comparative analysis (Student's t-test) was used between the obtained results, which were considered statistically reliable if $p<0.01$ or $p<0.05$ [13].

Results and Discussion

The analysis of the mass fractions in percentage (ω) of biometals in the forebrain of the test rats in the early period of bTBI (Tab. 1) by comparing with the indicators of the intact rats showed an increase in Fe on 14th and 21st days by 9%, on 28th day by 12% (p≤0.01), and the total intragroup increase was 3%.

Table 1.
The level of biometals in the forebrain, presented as mass fractions in percent (ω)

Biometals	Fe (ω)	Cu (ω)	Zn (ω)
Intact / Experiment	1.66±0.02	0.125±0.01	0.6±0.01
14th day	1.84±0.03	0.25±0.02*	1.16±0.02*
21st day	1.84±0.04	0.364±0.02*	1.12±0.02*
28th day	1.9±0.1*	0.391±0.02*	0.74±0.01**

*Note: results were considered statistically reliable * - p≤0.01, * - p≤0.05.*

At the same time, Cu increased significantly by 50% (p≤0.01) on day 14, by 65% (p≤0.01) on day 21 and by 68% (p≤0.01) on day 28 in the test rats. The total intra-group increase was 36%. A gradual decrease to 36% was observed in the analysis of Zn indicators within the test group by 64%, but this biometric was constantly higher compared to the indicators in the intact group: on day 14 by 48%, on day 21 by 46% (p≤0.01) and on day 28 by 19% (p≤0.05).

In order to evaluate the effect of changes in the investigated biometals on the forebrain state in the early period of bTBI, the ratios of Cu/Fe, Cu/Zn and Zn/Fe were calculated (Table 2).

A comparison of the biometal ratios in the forebrain showed a significant increase in all indicators of the test group compared to the intact group. The Cu/Fe ratio gradually increased by 42% (p≤0.05) on the 14th day, by 60% (p≤0.01) on the 21st day, and by 62% (p≤0.01) on the 28th day, and total intra-group value increased by 33%. The Cu/Zn ratio increased by 33% (p≤0.05) on 21st day and by 59% (p≤0.01) on 28th day, and intra-group value increased by 58%. The Zn/Fe ratio grew by 44% on 14th day, by 39% on 21st day and only by 6% on 28th day, and intra-group decrease by 40% was observed.

Table 2.
Ratios of biometals in the forebrain

Experiment \ Intact	Ratios		
	Cu/Fe	***Cu/Zn***	***Zn/Fe***
	0.08±0.02	0.22±0.02	0.37±0.01
14th day	0.14±0.01**	0.22±0.02	0.66±0.02*
21st day	0.20±0.01*	0.33±0.02**	0.61±0.01*
28th day	0.21±0.02*	0.53±0.03*	0.395±0.02

*Note: results were considered statistically reliable * - p≤0.01, * - p≤0.05.*

The literature search showed great interest in the issue of the state of biometals in various parts and structures of the brain in various diseases of the central nervous system (Alzheimer's disease, Parkinson's disease, autism, head trauma of other genesis) [10, 14, 15]. The analysis of modern sources showed different changes in Fe, Cu and Zn in separate structures, but their influence on the functional indicators for which these structures are responsible, including impairment of cognitive and behavioural indicators, was detected [6, 16, 17]. Therefore, in the course of our own research, we decided to determine the presence of changes in these biometals in separate brain structures responsible for behavioural and cognitive activity. And considering that the forebrain is directly involved in the processes of memory formation and emotional reactions [18], which, according to clinical and experimental data, are disturbed even in mild bTBI, it was necessary to carry out this study.

Pathomorphological studies have revealed that even mild bTBI can cause diffuse damage to both brain tissues and the blood-brain barrier (BBB). The main factor of cell disruption by a blast wave is injury to the mitochondria [7]. Damage to the BBB leads to increased vascular permeability and haemorrhages through diapedesis. Consequently, pro- and inflammatory mediators can enter the brain tissue along with the release of iron from erythrocytes. These factors combine and lead to secondary brain damage, including secondary mitochondrial imbalance. Numerous studies on trace elements in the brain suggest that there are distinct types of changes in biometals associated with various brain pathologies, such as Alzheimer's disease, Parkinson's disease, traumatic brain injury, and amyotrophic lateral sclerosis. Specifically, there may be a simultaneous increase in Fe, Cu, and Zn [19]. We observed changes in the levels of Fe and Cu, which increased by 3% and 36% respectively, as well as a 36% decrease in Zn amongst the studied group. However, all biometals analyzed in the forebrain of rats during the early period of mild bTBI were higher than those in the intact group. These alterations suggest that metabolic and regulatory processes involving biometals in the brain are disrupted. The development of oxidative stress and activation of the inflammatory reaction in the early stages lead to the initiation of a "vicious circle" because biometals participate in the regulation of each other [20]. A decrease in the membrane potential leads to a disruption of mitochondrial functions caused by the dysfunction of Fe-, Cu-, and Zn-containing enzymes. This leads to ATP deficiency and metabolic process violations. Along with an imbalance of biometals, it causes intracellular accumulation of neurotoxic substances from the Fenton reaction (for instance, β-amyloid and Lewy bodies) [7, 10, 21], which in turn leads to synaptic dysfunction and impaired axonal transport.

The alterations in ratios that we observed, specifically the rise in Cu/Fe, Cu/Zn and Zn/Fe over time, suggest that Cu plays a significant part in the generation of biometal imbalance during the early stages of mild bTBI. Numerous studies suggest that copper, a heavy metal, contributes to the development of oxidative stress, membrane polyunsaturated acid oxidation and peroxidized lipid formation. This increases mitochondrial membrane permeability and triggers apoptosis [22, 23]. Additionally, copper has been shown to impair glutamate regulation and activate the excitotoxic cascade of cell damage [23]. Considering that Zn is involved in synaptic transmission, modifies neuronal excitability, and controls redox homeostasis of DNA, an augmentation in its presence in mild bTBI can result in intensifying these procedures and augmenting oxidative harm to brain cells [24]. These stated processes are considered factors in the advancement of neurological ailments.

Consequently, deeming the well-known physiological role of Fe, Cu and Zn [8-10] and the disturbances in the central nervous system established by scientists in the event of their imbalance, we suppose that the results obtained by us reveal important links in the pathogenesis of secondary brain damage, namely, the features of their changes in the forebrain in the early period of mild bTBI with primary exposure to BW.

The obtained results can be used as potential diagnostic and prognostic markers of bTBI. And therefore, this knowledge must be applied to develop effective prevention strategies and protective measures for people with bTBI in the early post-traumatic period, such as military personnel, first responders, and civilians in high-risk environments. We assume that therapeutic measures should be aimed at neuroprotection against the effects of free radicals, stabilization of the BBB, as well as restoration of the biometals balance, which will interrupt the "vicious circle" of secondary damage.

Considering the established physiological func-

tions of Fe, Cu and Zn [8-10], as well as the known disturbances in the central nervous system resulting from their imbalance, our results suggest significant connections in the pathogenesis of secondary brain injury. Specifically, we have identified distinctive changes in the forebrain during the early stage of mild bTBI with primary exposure to BW.

The results obtained could be used as potential markers for bTBI diagnosis and prognosis. Therefore, this knowledge should be applied to develop effective prevention strategies and protective measures for people with bTBI in the early post-traumatic period, such as military personnel, rescuer, and civilians in high-risk environments. We assume that therapeutic measures should be aimed at neuroprotection against the effects of free radicals, stabilization of the BBB, as well as restoration of the biometals balance, which will interrupt the "vicious circle" of secondary damage.

Conclusions

1. In the early period of mild bTBI, we observed an intragroup increase in the level of Fe by 3% and Cu by 36% and a decrease in Zn by 36%, however, all these biometals and their ratios (Cu/Fe, Cu/Zn, Zn/Fe) were higher in the forebrain of rats in the early period of mild bTBI when compared with the indicators of the intact rats.

2. According the physiological function of Fe, Cu, and Zn, and data on their role in brain damage in other diseases, we assume that these biometals lead to oxidative stress, neuronal and mitochondrial membrane damage, energy deficits, neuronal excitability, impaired axonal conduction, and synaptic transmission.

3. The obtained data of the Fe, Cu and Zn changes in the forebrain of rats in the early period of mild bTBI can be deemed as factors of secondary damage and used as diagnostic and prognostic markers of this type of injuries as well as the foundation for elaborating pathogenetically justified neuroprotection.

Prospects for further research

Henceforth, it is planned to carry out a correlation analysis between the obtained results of the biometals composition and indicators of behavioral and cognitive activity using various tests to establish the presence or absence of connections between them.

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Реферат

ЗМІНИ БІОМЕТАЛІВ У ПЕРЕДНЬОМУ МОЗКУ ЩУРІВ У РАННЬОМУ ПЕРІОДІ ВИБУХО-ІНДУКОВАНОЇ ТРАВМИ ГОЛОВНОГО МОЗКУ

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Ключові слова: біометали, вибух, травма, головний мозок, ранній період.

Вибухо-індукована травма головного мозку – це складне ураження, спричинене швидкою передачею енергії від вибуху до мозку. Головним патогенним фактором є вибухова хвиля, що призводить до первинного дифузного пошкодження. Цікавим стало питання участі біометалів в патогенезі вторинного ушкодження в різних структурах головного мозку, зокрема в передньому мозку в ранньому періоді легкої вибухо-індукованої травми. Експеримент проведено на 36 статевозрілих щурах самцях лінії Wistar масою 220-270г в лабораторії кафедри Патологічної анатомії, судової медицини та патологічної фізіології Дніпровського державного медичного університету із дотриманням чинного законодавства щодо етичного поводження із тваринами. Щури утримувались в стандартних умовах віварію і були рандомно розділені на 2 групи: експериментальна (піддавались наркотизації і впливу бароакустичній хвилі 26-36кПа) та інтактна групи. На 14, 21 та 28 добу пост-травматичного періоду тварин піддавали евтаназії галотаном з наступним вилученням головного мозку і відокремленням переднього мозку. Спектральне дослідження проводили за допомогою енергодисперсного рентгенівського флуоресцентного аналізу. Аналіз результатів показав внутрішньогрупові збільшення рівня Fe на 3% та Cu на 36% і зменшення Zn на 36%, проте всі ці біометали і їх співвідношення (Cu/Fe, Cu/Zn, Zn/Fe) були більше у експериментальних щурів. Вважаємо, що ці біометали призводять до окисного стресу, ушкодження мембрани нейронів та мітохондрій, розвитку енергетичного дефіциту, збудження нейронів, порушення аксональної провідності та синаптичної передачі. Отримані дані можна вважати факторами вторинного ушкодження і використовувати їх в якості діагностичних та прогностичних маркерів такого виду травм. А також для розробки патогенетично обґрунтованої нейропротекції.