April 30, 2021

PharmacologyOnLine

Archives • 2021 • vol.1 • 6-11

THE INVOLVEMENT OF BRAIN SPECIFIC PROTEINS IN THE PATHOGENESIS OF THE CONSEQUENCES OF CLOSED PULMONARY CRANIAL INJURY

[1]Gozhenko, A. I.; [2]Korshnyak, V. A.; [3]Nasibullin, B. A.; [4]Savytskyi, I. V.; [3]Gushcha, S. G.; [1]Badiuk, N. S.*

[1]State Enterprise Ukrainian Research Institute of Transport Medicine of the "Ministry of Health of Ukraine", Odesa, Ukraine

[2]State Enterprise "Research Institute of Neurology, Psychiatry and Narcology of the National Academy of Medical Sciences of Ukraine", Kharkiv, Ukraine

[3]State Institution "Ukrainian Research Institute of Medical Rehabilitation Therapy of Ministry of Health of Ukraine", Odesa, Ukraine

[4]International European University, Kyiv, Ukraine

*badiuk_ns@ukr.net

Abstract

The authors carried out a complex experimental and clinical study using 30 white Wistar rats of autobred breeding, body weight 180-200 g and data obtained in the study of 28 patients with MILD TBI at the age of 25-43 years and MILD TBI 2-5 years old. the aim of the work was to assess the relationship between structural changes in brain matter, clinical manifestations and the content of some regulatory molecules in MILD TBI. Studies have revealed similar changes in cognitive functions and indicators of autonomic status in experimental animals and patients with MILD TBI. At the same time, structural changes in the cortex, thalamic and hypothalamic regions occurred in the brain of the experimental animals, similar to the descriptions found in individuals with TBI.

The examined patients showed changes in the content of regulatory molecules (brain-specific proteins, BDNF, melatonin).

The authors believe that damage to neurons and neuronal populations in TBI leads to dysregulation of the functional systems of the brain, including humoral mechanisms, which determines the formation of clinical manifestations. At the same time, the disorder of humoral regulatory mechanisms through which the functions of controlling the vital activity of the cellular elements of the brain are realized complicates the restoration of functional systems, which contributes to the chronicity of the clinical manifestations of MILD TBI.

All human studies were conducted in compliance with the rules of the Helsinki Declaration of the World Medical Association "Ethical principles of medical research with human participation as an object of study". Informed consent was obtained from all participants.

Key words: *traumatic brain injury, brain-specific proteins, brain*

Introduction

According to WHO, from 5 to 7 thousand craniocerebral injuries are diagnosed in the world per 1 million population with a predominance of mild traumatic brain injury (mild TBI) - 85-90%. [1, 2]. The incidence of TBI in Ukraine is about 26 cases per 1000 population [3]. Generally accepted ideas about the pathogenesis of CHMT associate changes in the structure of the functional organization of brain regions and disruption of the activity of regulatory systems with the formation of dysregulatory pathology. The latter, in combination with a psychological reaction to trauma, determines the formation of pathological functional systems of the brain [1, 4, 5].

Since there is a combination of changes in the structural and functional organization of the brain with the appearance of unusual functional ones in the central nervous system, there is a depleting load on the autonomic regulatory centers of the autonomic nervous system, which aggravates dysregulation in the central (cerebral) regulatory centers. In particular, brain neurotrophic factor (BDNF) can be distinguished among the latter. This compound is a 27 kDa polypeptide. Similar to other nonspecific growth factors (NGF, NT-3, NT-4), it modulates the activity of different types of neurons [6]. It should be especially noted that the source of BDNF is platelets, which release, deposit and bind it in response to external stimulation. Penetrate from the blood through the blood-brain barrier, it protects neuronal structures from traumatic, hypoxic and other influences [7]. In other words, somatic humoral, regulatory mechanisms are actively involved in the regulation of the vital processes of brain cells. However, in the available literature, we did not find works showing the dependence of changes in the brain matter with the features of humoral regulation and clinical manifestations of MILD TBI. One of the main reasons for this situation, I is the impossibility of conducting morphological studies of the brain in patients with closed traumatic brain injury (ethical rules). In view of the above, we decided to conduct an experimental clinical study, the purpose of which was to assess the relationship between structural changes in the brain substance in MILD TBI, and clinical manifestations and changes in the content of regulatory molecules (brain-specific proteins, BDNF, melatonin).

Methods

The proposed study consisted of two stages. At the first stage, MILD TBI was simulated in rats with the study of changes in the functional activity of the central nervous system and morphological changes in the brain substance. In case of coincidence or similarity of changes in the functional activity of the central nervous system of experimental rats with the clinical manifestations of MILD TBI in humans, we proceeded to the second stage of the work - assessment of clinical manifestations and changes in the content of antibodies to S-100; BDNF, hyaline fibrillar protein (HFP); encephalogenic protein (EP); myelin basic protein (MBP) and melatonin.

MILD TBI was struck by animals with a free falling weight of 85 g and a fall height of 850 mm. the severity of injury was assessed by changes in motor function and by changes in the functional activity of the central nervous system (open field test). changes in motor function were assessed by countering lateral shocks to the right and left and by the ability to stand on an inclined surface to the left and right and vertical positions. During the "open field" test, the number of exits to the center, the number of squares crossed, the number of vertical posts, and peeping into holes were recorded.

Emotional activity was assessed by the number and duration of grueling and the number of urinations and deformations. Examination of histological preparations frontal brain sections taken at a point relatively allowed to identify and evaluate changes in the structural and functional organization of the brain substance.

In the work, 30 white rats of the Wistar line of autobred breeding weighing 180-200 g were used. The content of work with animals was carried out in accordance with the requirements of Directive 2010/631 EU of the European Parliament and Council of September 22, 2010 on the protection of animals used for scientific purposes and the order of the Ministry of Education and Science of Ukraine No. 249 dated 01.03. 2012 [8, 9]. The duration of the experiment was 10 days after the application of MILD TBI. During the day of bringing the animals and the west, the "open field" test was carried out and the ability of the animals to stay on an inclined

surface, as well as the ability to resist lateral shocks, were evaluated. To study the ability of animals to stay on an inclined surface, they were placed in a chamber with an area of 22 × 18 cm, the bottom of which can be tilted by a rotary mechanism to an easily taken into account angle. Shock resistance was tested by placing the rats in an open field setup prior to starting the test. When conducting the "open field" test, the number of exits to the center, the number of squares crossed, the number of vertical racks, the number of peeking into holes, the number and duration of grooming, the number of boluses and urinations were taken into account. The animals were removed from the experiment by decapitation under ether anesthesia. From the extracted brain, a frontal layer up to 4 mm thick was isolated (from the caudal direction. The obtained material was fixed in 4% paraformaldehyde solution for 48 hours. Then it was passed through alcohols of increasing concentration and embedded in celloidin. From the obtained blocks, histological sections with a thickness of 7 - 9 μm, which were stained with hematoxylin-eosin and gentian violet according to Nissl. The obtained preparations were studied under a light microscope.

For the second stage of work, we recruited 28 men aged 25-43 years old with MILD TBI 2-3 years. Their comprehensive examination included an assessment of their clinical condition (subjective and objective examination). 5ml of venous blood was taken from patients in which the presence of antibodies to brain-specific proteins HFP, EP, MBP was determined by the ECLIA method (immunochemical with electrochemiluminescence detection). The same method was used to determine the content of S-100 and BDNF (analyzer and test system cobas 6000 Roche Diagnostics - Switzerland). The content of melatonin in the blood was determined by radioimmunoassay using an analyzer and a Yammamfter test system, Pharmacia LKB biotechnology AB (Sweden), labor diagnostical Nord Ct MBH (Germany). An elevated melatonin content was considered if it was ≥ 24 pk / ml. And reduced if it was ≤ 6-8 pk / ml. The rate of melatonin in the blood is 8-20 pk / ml. Statistical processing of the results was carried out using the Statistica 6.0 software taking into account the Student's coefficient of reliability $p < 0.05$.

Results

We began assessing the functional state of the central nervous system with a shock resistance test. in healthy rats, pushes to the left or to the right were accompanied by displacement of the animals by 12 cm along the horizontal surface and the absence of overturning on their side. Among the rats that underwent MILD TBI, in 12 cases out of 20, overturning took place on the side with pushing from the right and left, and in three more cases, overturning took place only with pushing from the left. Holding test on an inclined surface showed that among the control animals all of them were held on an inclined surface at an angle of 38 to 40 degrees. That is, the test result can be estimated at 4 points. Among the animals that underwent MILD TBI, retention at large angles of inclination (38-40 degrees) was not observed in any one. The rats were kept from stalling at the angles of inclination 33-35.5 °. It should be noted that repeated tests in one session were accompanied by a slight increase in the retention angle. In general, it can be argued that the injury reduced the stability of the rats to 2-2.5 points. The results of the "open field" test are shown in Table 1.

According to the data in Table 1, in animals that underwent MILD TBI, there is a decrease in locator activity - a decrease in the number of crossed squares by more than two times for and the number of exits to the center.

The latter can still be associated with the presence of fear in animals. Orientation and exploratory activity in rats after MILD TBI weakens, as evidenced by a significant decrease in the number of upright stands and looking into burrows. in general, we can talk about the weakening of analogs of cognitive functions in humans.

As for the state of the emotional sphere, there are also negative changes. This is evidenced by the increase in the number of grooming, while reducing the duration of each act, that is, the animals are confused and alarmed emotionally. At the same time, the number of boluses and urinations increases, which also reflects increased anxiety and fear of the animals.

Histological studies of the cerebral cortex first of all revealed the discharge fields of the neural population. Since the fields are large enough and

are located on several layers, there is a visual blurring of the division of the neural population into layers. The fields of depression mainly concern the middle layers of the cortex, in the deep layers small foci of ganglionic cell prolapse and the presence of individual cases of neuronophagy in the halo are determined. The vessels inside the hemispheres are moderately full-blooded, some of them are spasmodic. Chromatophilic small-lumpy substance, the nuclei of neurons are average with homogeneous contents. In a histological study of thalamic structures, neurons also with small-lumpy chromatophilic substance, and in some, the cytoplasm is homogeneous. a visual comparative assessment of the density of distribution of neurons (intact and experimental rats), one can note a rarefaction of their distribution in the latter rats. In the hypothalamic region, the discharge of the neuronal population and the homogeneity of the cytoplasm of some neurons are also determined. In general, we can talk about the similarity of the revealed changes in the structural and functional organization of different parts of the brain of experimental rats and the description in the literature of changes in the brain in humans in the remote period of trauma.

Moving on to the second stage of work, we began with a subjective and objective assessment of the condition of patients with MILD TBI 2-3 years ago. When assessing the subjective manifestations of pathology (complaints of patients), it was found that 94% of them have persistent headache, which in 75% of patients is complemented by pain in the eyes. The majority of patients complained of: fatigue (90%), lethargy (88%), irritability (81%), decreased ability to work (80%), sleep disturbance (79%), hyperhidrosis (72%).

An objective study revealed that the most common manifestations of the long-term MILD TBI period were: convergence disorder (79%), facial asymmetry 89%, tendon anisoreflexia (80%), tremors of the eyelids and fingers (88%), autonomic disorders.

In general, the most common disorders identified in the examined patients are associated with the activity of the regulatory centers of the basal parts of the brain. Following the assessment of neurogenic regulation, we investigated the states of neurohumoral regulation. The results of assessing the content of a number of regulatory humoral factors by the amount of antibodies are shown in Table 2.

According to the data in Table 2, after MILD TBI, the content of s-100, HFP and EP in the body of the victims significantly and very significantly increases, and the content of melatonin also significantly increases. The MBP content practically does not change in relation to the control data, and the BDNF content can be regarded as a weakening of modulation of the activity of neurons of different types, which can provide dysregulation of neurons of different types [6]. A significant (several times) increase in the content of antibodies S-100, HFP and EP can be regarded as evidence of increased activity of dystrophic processes in neurons and their processes, which may create additional conditions for the formation of dysregulatory pathology - an important factor in the disorder of cognitive and autonomic functions. At the same time, the content of melatonin increases, which, being a regulatory factor of plasticity and vital processes, has a restraining effect on the consequences of dysregulation and autoimmune processes in the brain substance.

Conclusions

The results of our studies have shown that mild closed craniocerebral trauma is accompanied by disorder and impairment of cognitive and autonomic processes in the victims, which coincides with changes in the functional activity of the central nervous system in experimental traumatic brain injury. In cases of experimental MILD TBI, changes in the substance of the brain in the form of rarefaction of the neural population of the cortex; the appearance of foci of gliocellular precipitation in the deep layers of the cortex and individual cases of neuronophagy, the phenomenon of neuronal dystrophy and a decrease in the density of their distribution take place in the thalamic and hypothalamic structures. It can be assumed that with the similarity of physiological disorders in the activity of the central nervous system in traumatic brain injury in the clinic and in the experiment, there will be similarities or even coincidence in changes in the structural and functional organization of brain structures. the formation of DS regulatory pathologies as a result of this is accompanied by

violations of humoral regulation in the form of changes in the content of brain-specific proteins, BDNF and melatonin, which leads to the preservation and, possibly, aggravation of the violation of the structural and functional organization of the brain substance, a disorder in the activity of the functional systems of the brain (dysregulation) and the preservation of clinical and neurological manifestations of MILD TBI. In other words, trauma to the brain matter, even a light one, triggers a cascade of structural, regulatory, autoimmune processes that form a vicious circle, ensuring the chronicity of the clinical manifestations of this pathological process.

Acknowledgments (optional)

The authors declare that there are no conflicts of interest.

References

1. Taitslin V.I. Closed craniocerebral injury and its consequences // International Medical Journal. - 2002. - No. 1-2. - S. 58-63.
2. Likhterman LB - Traumatic brain injury. M.-2003 - 365p.
3. Traumatic brain injury: modern principles of emergency care: Uch.-method. allowance / E.G. Pedachenko, I.P. Shlapak, A.P. Hooke, M.N. Pilipenko. - K.: CJSC "Vipol", 2009. - 216 p.
4. Dysregulation pathology / under the editorship of G. N. Kryzhanovsky. - M., Medicine.-2002.-492 p.
5. Dysregulatory pathology of the nervous system / ed., Gusev EI, Kryzhanovsky GN / - M., LLC "MIA" - 2009-512 p.
6. Hironobu Fujimura - Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation - C Anthony Altar, Ruoyan Chen, Takashi Nakamura, Thromb Haemost. 2002 Apr; 87 (4): 728-34.
7. Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry JM. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res. 2002; 109 (2): 143-8.
8. Directive 2010/63 / EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific - official journal L 276- 20.10.2010 - p.0033-0079.
9. Order of the Ministry of Education and Science; to the youth and sports of Ukraine "About the hardened order of scientific installations of preliminaries, experiments on creatures" dated 01.03.2012 N 249 - official newsletter of Ukraine dated 06.04.2012 - 2012 - N24, p. 82, article 942, pid act 60909/2012.

Table 1. The state of functional activity of the central nervous system and the emotional sphere in rats after MILD TBI

Group/ index	The control	Rat with MILD TBI 10 days	P
number of exits to the center, n	1,0±0,2	0,31±0,07	<0,05
Stops, n	2,33±0,43	2,89±0,47	>0,05
Stops, S	81,0±14,0	93,15±15,52	>0,05
number of squares crossed, n	44,67±4,64	17,86±1,41	<0,05
number of racks	9,17±6,5	5,6±0,51	<0,05
number of peeps in mink, n	7,17±1,4	4,6±0,93	<0,05
Grooming, n	2,67±0,37	3,17±0,26	<0,05
Grooming, S	9,70±1,66	7,76±1,03	<0,05
number of boluses, n	2,17±0,70	2,63±0,90	>0,05
number of urinations, n	6,67±1,31	8,0±1,05	>0,05

Table 2. Dynamics of the content of antibodies to brain-specific proteins and melatonin in persons undergoing MILD TBI

Group/ Index	The control	MILD TBI	P
S-100 г/л	0,115±0,02	0,566±0,03	<0,05
MBP г/л	2,516±0,25	2,458±0,12	<0,1
HFP г/л	1,26±0,13	2,716±0,30	<0,05
ЗДТ-9-Д6 г/л	0,872±0,09	1,240±0,5	<0,1
BDNF г/л	459,2±21,6	253,2±12,4	<0,05
EP г/л	0,688±0,10	2,218±0,20	<0,05
Melatonin pc / ml	14,7±4,3	23,1±3,3	<0,05