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INFLUENCE OF CARBACETAM ON NEUROLOGIC DESTRUCTION PROCESSES UNDER THE EXPERIMENTAL TRAUMATIC BRAIN INJURY

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Abstract

Objective of the research. Provide the research of the influence of Carbacetam on the neurologic destruction processes in paraventricular and supraoptic nuclei of the hypothalamus under the experimental traumatic brain injury (TBI).

Materials and methods of the research. The research was held by means of white outbred male rats weighing 200±10 g. The modulation of the traumatic brain injury was based on the method of V.M. Eslki and S.V. Ziablitsev (2008), where the TBI was caused due to gravity load on animals with the strike energy of 0.52 J. The lethal outcome during the first 5 days after the TBI was 84%. The control group (n=10) was administered 1 ml of saline intraperitoneally within the 10 days after TBI. The rats of experimental group (n=10) were provided Carbacetam (5 mg per 1 kg) in 1 ml of saline. After the experiment, the animals were decapitated followed by the removal of the brain, and the histological medicines were produced by means of Microtome after the appropriate histological processing. Some sections were stained with hematoxylin and eosin, others - properly prepared before applying the neuromarkers NSE, S-100 and GFAP. The morphological and immune histochemical

evaluation of neurodegenerative changes in the nerve tissue were done by the produced medicines.

Outcomes and discussion of them. The outcomes of the research show that Carbacetam influences the decrease of the degenerative processes in the neural tissue of paraventricular and supraoptic nuclei of the hypothalamus. The neurons of the animals after TBI being administered with the Carbacetam, are characterized by the restoration of the normal morphological features unlike the rats that did not receive the medicine. Immune histochemical research of the brain neuronal markers confirms the functional recovery of the neurons and astrocytes in the researched areas of the hypothalamus of the rats after administration of Carbacetam. The decrease in the expression of glial markers GFAP and S-100 has been observed, showing the reduction of degenerative changes in the neural tissue. Meanwhile, the expression of neuronal marker NSE has increased, showing high metabolic activity of the nerve cells. However, changes in the expression of the neural markers and glia feature the restoring of normal neuronal activity due to the administration of Carbacetam.

Therefore, further research of Carbacetam effects is promising in terms of restoring the neuronal destruction under TBI.

Keywords: traumatic brain injury, neurospecific protein S-100B, NSE, GFAP, Carbacetam.

Introduction

The issue of medical treatment of the consequences of traumatic brain injury (TBI) is the high relevance in modern medicine as this particular type of brain damage is characterized by high mortality and advocates the disability cause of people of working age among all traumatic injuries (dangerous injury of medium and severe grade). Furthermore, even after mild trauma the final phenomena may form. The hazard of TBI manifests itself in a wide range of negative consequences, i.e. from physical damage tissues to psychological and cognitive problems [5].

A key role in the pathogenesis of TBI is played by destructive changes in the neural tissue of the brain, situated in damaged neurons and glial cells. Morphological disorders of neural tissue elements are conjugated with functional disorders, including dysfunction of intercellular interactions being the basis for various diseases [6]. Given the high danger of the TBI consequences, various drugs that are considered in the perspective correction and restoration of the functional state of the brain is extensively developed and researched today.

One of these drugs is Carbatsetam exerting the antiamnestic, antigypoksytic, anxiolytic and antishoking effects. Therefore, the drug is seen as a promising tool to prevent neurogenic dysfunction under TBI [4].

Because of that, there exists the emerging interest of Carbacetam influence on morphological and immune histochemical peculiarities of the nerve tissue of the various sites of damaged brain under TBI. Hence, it shows the importance of study of nerve tissue morphology as well as neuronal markers expression (NSE) and astrocytes (GFAP and S-100) in the hypothalamic nuclei of rats under Carbacetam administration. It is worth of noting that the studied neuronal markers are very informative under the diseases studies, followed by the neuronal degenerating processes caused by TBI. Moreover, the above mentioned neuronal markers and astrocytes suggest the objective evaluation of the therapeutic effect of the studied drugs, identifying the functional modification in the nerve tissue [10].

Objective of the research

Provide the research of the influence of Carbacetam on the neurologic destruction processes in paraventricular and supraoptic nuclei of the hypothalamus under the experimental traumatic brain injury (TBI).

Materials and methods of the research

The research was held by means of 20 white outbred male rats weighing 200±10 g struck by medium-severe grade TBI based on the method of V.M. Eslki and S.V. Ziablitsev (2005) [3]. The postmortem macroscopic study has shown that TBI was characterized by the presence of skin and "shell" bruising around the strike; cranial vault fractures of moderate degree without displacement; crushing bark parietal and temporal lobes (in the strike area) and the basis of the frontal and temporal lobes (in anti-shock area). Epy ымall spot hemorrhage were available in the brain tissue.

The domestic drug Carbacetam was developed at the Institute of Physical Organic Chemistry and Coal Chemistry of National Academy of Sciences of Ukraine (Donetsk, UA) [2]. Carbacetam belongs to endogenous modulators of the GABA-benzodiazepine receptor complex, derivatives of β-carboline being the carboline isostere (1-oxo-3,3,6-trimethyl-1,2,3,4-tetrahidroindolo [2,3-c] quinoline) β-carboline structure is the basis for alkaloids (β-carbolines), which are marked from the flower Peganum harmala.

The brain received on the 7th days of decapitation under general anesthesia, was placed in neutral buffered formaldehyde solution (pH 7,4) and fixed within 24 hours. After dehydration pieces were embedded in paraffin by standard methods. Sections of 3-4 microns width were obtained on rotary microtomes MPS-2 and microtome Microm HM 335 E. Further

sections were examined by light microscopy. There was applied the light-optical microscope "Olimpus BX 40" with digital camera "Olimpus C3030-ADU" and C200o ZOOM, "Olimpus BX 43" with the digital camera "Olimpus SC100" and software "Olimpus DP-Soft", as well as light-optical microscope Axio Imager.A2 "Carl Zeiss" (Germany) with the data processing system "Axiovision" featuring the zooming lens ×5, ×10, ×20, ×40, binocular tips ×1,5 and eyepieces ×10.

Sections were stained with hematoxylin and eosin. Slices were placed on glass slides coated with adhesive Super Frost Plus (Menzel, Germany) for immune histochemical study. The rehydrated sections were thermally treated in Target Retrieval Solution (DAKO, Denmark) using a microwave oven to unmask the antigens. Then the sections were enzymatically treated with proteinase K (DAKO) for 5 minutes. The blocking of endogenous peroxidase activity was performed eventually by means of peroxidase block and unspecific binding block, i.e. the protein block (DAKO). Then primary antibody was applied, i.e. protein S-100 (multifunctional, code z0311), neurospecific enolase (NSE; code N1557); glial fibrillar acidic protein (GFAP; code IS 524). The visualization of the primary antibodies was performed by highly sensitive detection polymer system DAKO Poly Vue HRP/DAB.

The outcomes of the research and their discussion

Paraventricular nucleus of the hypothalamus (PVN). It belongs to major cellular nuclei of the hypothalamus. PVN of the control group of rats is performed by the neurons and glial cells, being in a close contact to each other. Neurons are inherent in large light round or oval nuclei. Some nuclei show notable small chromatin structures diffusely distributed with certain concentrations under nuclear membrane. Glial cells are characterized by a smaller size compared to neurons. They are located around the neurons and are essentially dominated by their number (Fig. 1A).

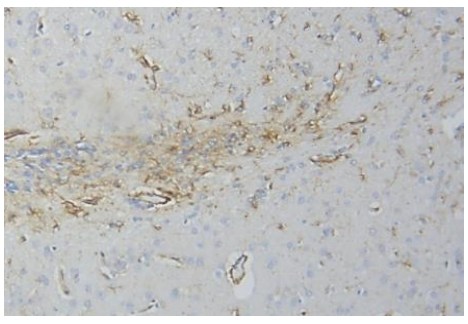
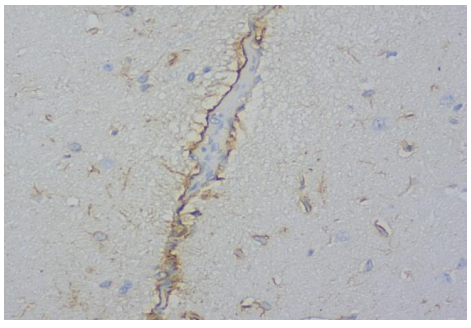
A  B 

Fig. 1. Photomicrograph of rat hypothalamus PVN under TBI. Immune histochemical detection of GFAP. ×400:

A) Control group;

B) Group under Carbacetam

Similar morphological of PVN characteristics may be found in the researches of other authors as well [7].

The most common type of glial cells are astrocytes being well defined by means of immune histochemical methods GFAP is a marker of these cells. The degree of GFAP expression for this group of animals is not sufficiently expressed, indicating the presence of minor damage to nerve tissue. Morphologically it is proved by overwhelming number of normal neurons with no degenerative signs. Only a few nerve cells has hyperchromic nucleus of irregular shape (Fig. 1), reflecting the functional activity of the violation until the complete function loss. Overall PVN of rats in control groups featured the morphological and immune histochemical features of destructive changes of the neural tissue.

PVN of rats under TBI followed by Carbacetam administration generally retains the same morphologic features as the animals in the control group. The heterogeneous neurons are found in this group of rats, both oval shaped with light nuclei and irregularly shaped cells with hyperchromic nuclei. Indicative astrocytes feature of rats in this group has a much smaller degree of GFAP expression, than animals in the control group (Fig. 1B). Such changes may be related to the corrective action of Carbacetam onto destructive changes in neural tissue under TBI, particularly onto the glial component [12].

The low expression level of neural marker was observed under NSE study of PVN in the control group of rats (Fig. 2A). Since NSE is considered as the marker of metabolically active neurons, the insignificant level of expression can be seen as a sign of low energy exchange in neural tissue [11].

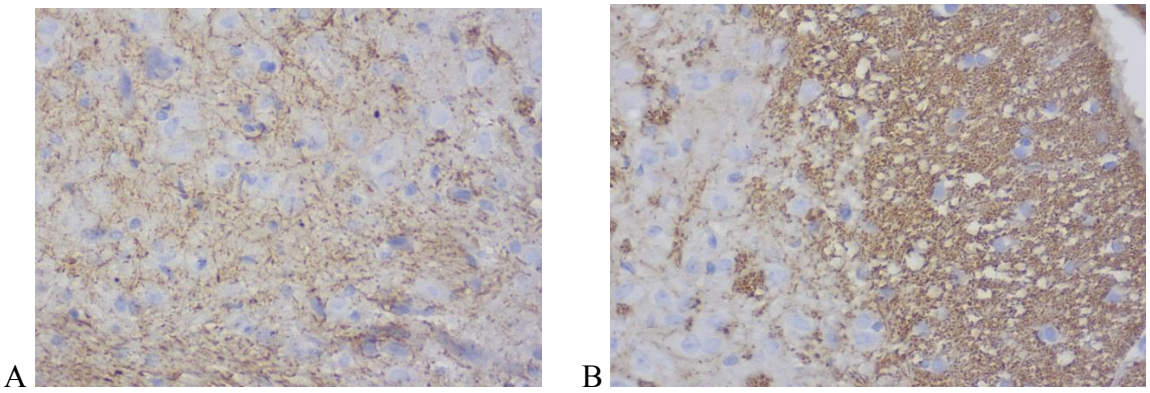


Fig. 2. Photomicrograph of rat hypothalamus PVN under TBI. Immune histochemical detection of NSE. ×400:

A) Control group;

B) Group under Carbacetam

High level of NSE expression is observed under administration of Carbacetam into rat hypothalamus PVN in comparison to control group (Fig. 2B), showing the increase of the neuron metabolic activity as a result of the mentioned drug activity.

The astrocytes producing this protein were defined while the immune histochemical study of S-100 expression in hypothalamus PVN of rats (Fig. 3A). A relatively small number of such cells indicates a slight degree of neural tissue after TBI, preserving the ability of neurons and glial cells to regenerate.

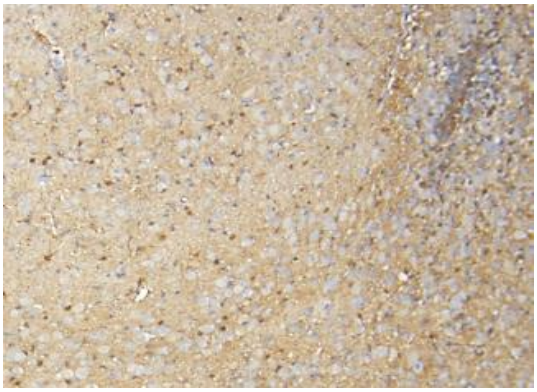
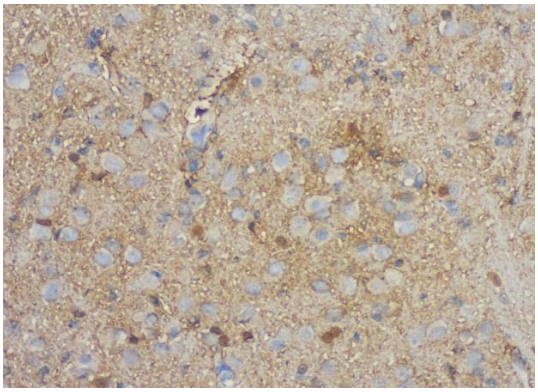
A  B 

Fig. 3. Photomicrograph of rat hypothalamus PVN under TBI. Immune histochemical detection of S-100. ×400:

A) Control group;

B) Group under Carbacetam

The rats, treated by Carbacetam, had had detected cells expressing a protein S-100 in hypothalamus PVN as well (Fig. 3B). The degree of expression of S-100 in the group of rats was significantly decreased in comparison to the control group, i.e. Carbacetam caused some reduction in expression of the neural marker. Hence, there occurred the restoration of the astrocyte function under the influence of Carbacetam as a result of TBI.

Hypothalamus supraoptic nucleus (SON). SON as well as PVN belongs to major cellular nuclei of the hypothalamus. SON of the control group of rats is represented by neurons and smaller glial cells surrounding the neurons. This description is consistent with the data from other researchers [7]. Part of neurons observed feature large round light nuclei with small sites of chromatin, which shifted mainly to the periphery of the nucleus, reflecting the high functional activity of the cells. However, the major part of the neural cells is dominated by heterochromatin, and the nucleus gets the dark color (Fig. 4A). Therefore, the lower functional activity of cells is observed. Moreover, the nuclei of neurons are characterized by a certain rugosity (deviation from the typical circular or oval) shape, indicating degenerative

processes in the cell. Apart from that, the group of rats showed the noticeable high level of GFAP expression around neurons, which is inherent to cell death and confirmed by morphological data. Intensive selection of GFAP is explained that in case of the damaged neurons, the astrocytes increase their maximum functional activity of compensation for the lost neuronal function necessary for normal cellular activity in the affected area of the brain.

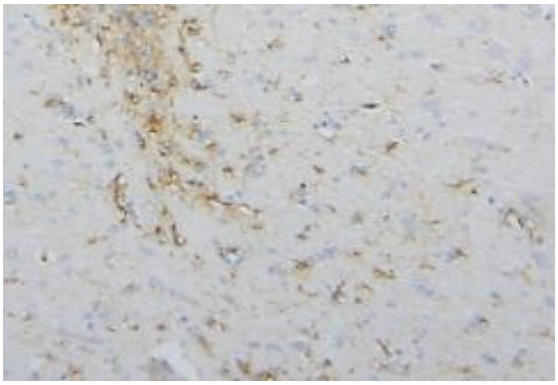
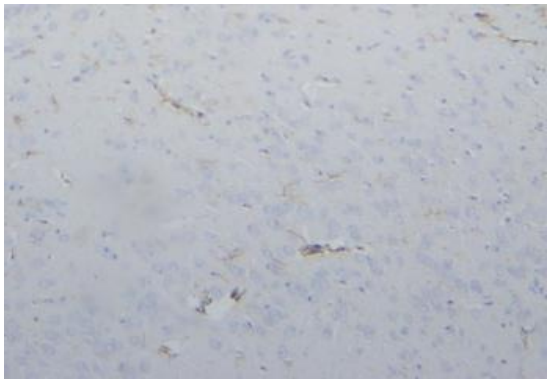
A  B 

Fig. 4. Photomicrograph of rat hypothalamus SON under TBI. Immune histochemical detection by GFAP. ×400:

A) Control group;

B) Group under Carbacetam

The decrease of GFAP expression is observed under Carbacetam administration into the hypothalamus SON of rats compared to the control, being a sign of decrease of the degenerative damages of the neural tissue under TBI. Moreover, the normal functional state of neurons in the animals of this group is morphologically confirmed. The of most of nerve cell nuclei feature the rounded shape and a lot of euchromatin (a sign of synthetic processes intensity) (Fig. 4B). Thus, the hypothalamus SON of rats influenced by Carbacetam shows the recovery of the degenerative changes in the neural tissue under TBI manifested both morphologically and modification of the level of GFAP expression.

The results of NSE expression in hypothalamus SON of rats of the control group showed lower grade of the mentioned neural marker expression (Fig. 5A), explained by a low metabolic activity of most the neurons.

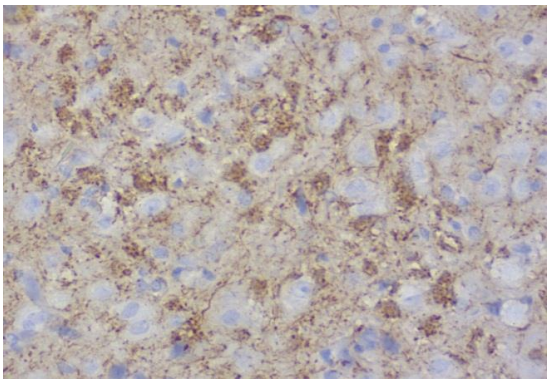
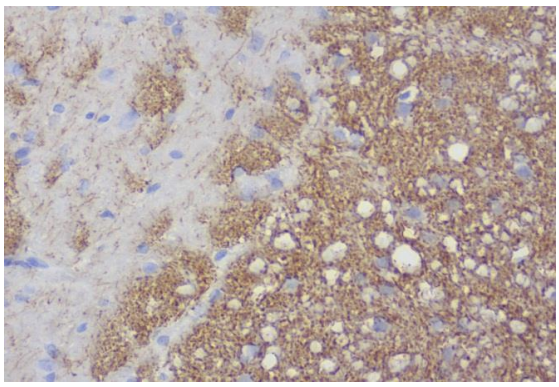
A  B 

Fig. 5. Photomicrograph of rat hypothalamus SON under TBI. Immune histochemical detection of NSE. ×400:

a) Control group;

b) Group under Carbacetam

The NSE expression in all the neurons of the particular brain site is observed by all rats treated with Carbacetam, i.e. its administration caused NSE expression increase in hypothalamus SON of rats compared to control (Fig. 5B). Such changes in expression grade of the mentioned neural marker can be explained by the enhanced influence of Carbacetam onto metabolism in the neurons [8].

The immune histochemical detection of S-100 in the hypothalamus SON of rats in control group showed that this neural marker is expressed in many astrocytes of this hypothalamus area (Fig. 6A), indicating a significant level of damage to neural tissue due to TBI.

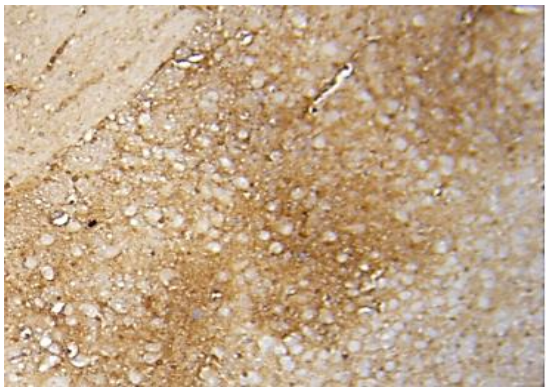
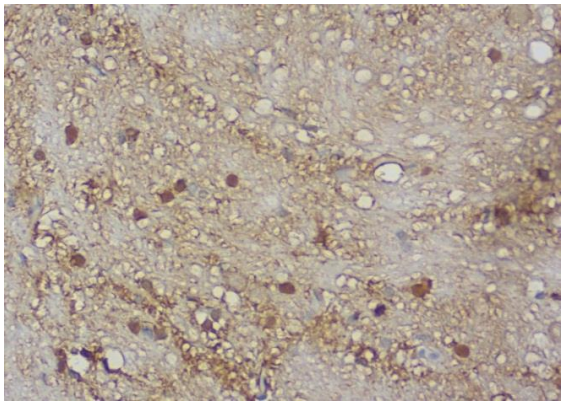
A  B 

Fig. 6. Photomicrograph of rat hypothalamus SON under TBI. Immune histochemical detection of S-100. ×400:

a) Control group;

b) Group under Carbacetam

The rats being administered Carbacetam, showed S-100 expression grade in hypothalamus SON slightly varying compared to control group downwards (Fig. 6B). Therefore, it might be noted that the drug inhibits the intensity of the degenerative processes in this site of hypothalamus under TBI.

The overall study of the neural tissue morphology of SON and PVN in hypothalamus showed that the part of neurons of these brain sites is expressed in different signs of damage under TBI, i.e. irregular shape and dark cores, accumulation of heterochromatin. Immune histochemical detection of neural markers GFAP, NSE and S-100 confirms the presence of degenerative changes in the nervous tissue in TBI. GFAP and S-100 is expressed in the neural tissue by astrocytes, while S-100 is expressed by neurons. Meanwhile, SON and PVN of hypothalamus of the animals under TBI showed the high GFAP and S-100 expressions, representing the disorder of neuron-glial interactions and all the processes, dependent on coordinated functioning of the neurons and astrocytes. Apart from that, there was observed a slight NSE expressions, connected to metabolism disorders in the neurons under TBI. Hence, the studied hypothalamus sites of rats under TBI showed the morphological and immune histochemical features of the neural tissue damage. It is reflected under the damage of the functional opportunities of the neural and glial tissues.

The rats treated by Carbacetam, had had SON and PVN neurons of the hypothalamus featuring the morphological characteristics of cells with normal functionality, round or oval nuclei shape, high content of euchromatin, no degenerative signs.

The decrease of GFAP and S-100 expression is observed under the neural markers expression study, showing the decrease or disappearance of the degenerative modification in the neural tissue. Moreover, the increase of the expression of the mentioned markers, especially GFAP, reflects the activation of the astrocytes, performing a number of important functions, connected to neural activity provision. The increase of astrocyte markers expression is connected to the certain disorders in the neural tissue, which is TBI in this research, resulting in the neural functionality disorder. Meanwhile, the astrocytes are activated to provide the maximal compensation of the damaged neurons functions occurring under TBI [1]. NSE marker expression was observed in all the neurons of SON and PVN of hypothalamus of rats, showing the high metabolic activity in the neural cells [9].

Conclusions. Thus, the comprehensive morphological and immune histochemical study of SON and PVN in hypothalamus of rats under TBI showed that Carbacetam corrects the degenerative changes in the neural tissue as a result of brain damage. Moreover, the drug influences both the neurons and the glia, improving the metabolism and the neuronal function

by activating the astrocyte function, providing the key role in maintaining the normal functional status of the neural tissue. Therefore, Carbacetam effect is manifested as morphological changes in neurons and in terms of immune histochemical expression of the neural markers, reflecting the state of the nervous tissue. In general, the drug reduces the degenerative changes in the neural tissues under TBI.

Further scientific and research perspectives. It is planned to hold the morphological and immune histochemical study of the other sites of rat brain, including the hippocampus and cortex, by administering Carbacetam under experimental TBI.

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