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CARBACETAM EFFECT ON PROTEIN AND LIPID PEROXIDE OXIDATION, MORPHOLOGICAL STATE OF THE CEREBRAL CORTEX AND HIPPOCAMPUS OF RATS WITH MODELED NEURODEGENERATION

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Abstract

Objective of the study was examination of carbacetam effect on protein and lipid peroxide oxidation, and evaluation of the morphological state of the cerebral cortex of rats with modeled neurodegeneration.

The experiments were conducted on nonlinear albino male rats 0,18-0,20 kg of the body weight. Modeled neurodegeneration was simulated by intraperitoneal introduction of scopolamine hydrochloride (Sigma, USA) during 27 days in the dose of 1 mg/kg. Beginning with the 28th day of the experiment, carbacetam was introduced intraperitoneal in the dose of 5 mg/kg in 1 ml of physiological solution – once a day during 14 days. In rats with modeled scopolamine-induced neurodegeneration in the cerebral cortex and hippocampus under carbacetam effect, the content of products, reacting with 2-thiobarbituric acid and proteins of a neutral and major character, decreases, which is indicative of a reduced intensity of lipid and protein peroxide oxidation. Morphologic the number of cells with karyopyknosis signs decreases and a relative staining density of the neuron tigroid substance increases, which is indicative of inhibition of the progress of the cerebral neurodestructive processes under conditions of scopolamine-induced damage.

Keywords: *scopolamine-induced neurodegeneration, carbacetam, protein and lipid peroxide oxidation*

Introduction

Neurodegenerative diseases (NDD) take one of the leading places in the structure of neurological pathology. They are the main cause of progressing motor disorders, dementia, and are characterized by restricted possibilities of existing pathogenic therapy. In spite of considerable variety of clinical symptoms, certain common regularities of the course and likelihood of typical mechanisms promoting development of NDD should be admitted. In particular, a pathogenic basis of many NDD (Alzheimer disease, Parkinson disease, Huntington disease, lateral amyotrophic sclerosis, tauopathy, etc.) is alternation of cellular protein conformation with their further deposition and aggregation in the target neurons [1]. In its turn, it results in a number of pathologic reactions such as disorders of oxidation phosphorylation and glycosylation, activation of lipid and protein peroxide oxidation and apoptosis development.

Nowadays peroxide oxidation under conditions of neurodegenerative processes is known to involve not only lipids but proteins of the plasmatic membranes as well [2]. A negative effect of oxidation-modification proteins (OMP) in the cells is considered to be associated with the fact that they are a source of free radicals exhausting the deposits of cellular antioxidants [3]. The products of free radical protein oxidation result in DNA oxidation lesion. At the same time, protein peroxide oxidation is not only a triggering mechanism of pathologic cascade, but the earliest marker of neurodegeneration [4, 5]. In its turn it leads to functional disorders of a number of neuromediator systems of the brain.

Gamma-amino butyric acid (GABA) is a universal neuromediator stipulating balance between stimulation and inhibition, metabolic processes, energy supply and resistance of the brain to hypoxia. GABA-containing agents possess a wide pharmacological spectrum; they are actively indicated to increase integrative activity of the central nervous system in case of many psycho-neurological diseases. In this respect investigation of pharmacodynamics of a new modulator of GABA-ergic system, original derivative of β-carbolineum – carbacetam, is rather topical. It was synthesized at L.M. Lytvynenko Institute of Physical Organic Chemistry and Carbon Chemistry, the National Academy of Sciences of Ukraine under the supervision of Doctor of Chemical Sciences S.L. Bogza [6]. The results of the investigations performed are indicative of pronounced anti-hypoxic, anti-ischemic, anti-inflammatory properties and corrective effect of carbocetam on cognitive functions under conditions of experimental craniocerebral injury [7].

Objective of the study was examination of carbacetam effect on protein and lipid peroxide oxidation, and evaluation of the morphological state of the cerebral cortex of rats with modeled neurodegeneration.

Materials and methods

The experiments were conducted on nonlinear albino male rats 0,18-0,20 kg of the body weight, kept under standard vivarium conditions at the temperature of 18-22 °C and relative humidity 40-60 %, fed on balanced food allowance and free access to water. All the experiments with animals were conducted according the main principles of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasburg, 1986). All the rats were divided into two groups: 1 – control (intact) rats; 2 – rats with modeled neurodegeneration. Taking into account the general acceptance of cholinergic hypothesis in NDD pathogenesis, the study was conducted on rats with scopolamine-induced neurodegeneration [8]. To create the model scopolamine hydrochloride (Sigma, USA) was injected intraperitoneally (i/p) in the dose of 1 mg/kg of the body weight in the form of 0,01 % water solution (0,5 ml/100 g), once a day during 27 days. On the 28th day the rats with neurodegeneration (3rd group) were administered to i/p carbacetam injection in the dose of 5 mg/kg of the body weight in 1 ml of physiological solution (saline). The chosen dose was administered by other scientists to investigate nootropic effects of carbacetam under other experimental conditions [9]. An equivalent amount of the solution was administered to rats of 1, 2 groups in a similar way. Euthanasia of the animals was performed under light ether narcosis. At a cold temperature the brain was removed, carefully washed with cool 0,9 % NaCl solution, and the cerebral cortex and hippocampus were isolated

according to the coordinates of the stereotaxic atlas [10], since it is these parts of the brain that participate in the mechanisms of emotion formation and memory consolidation [11, 12]. Cytoplasmic fraction was isolated by means of the method of homogenate differentiation centrifugation of the examined structures on the refrigerator centrifuge at 1000 g 10 min., later – 1400 g 10 min., at a temperature of 4 °C.

OMP content in homogenates was determined by the amount of their oxidation modification products by means of spectrophotometry method with the wave length of 370 and 430 nm. The method is based on the reaction of interaction of oxidized amino acid protein residues with 2,4-dinitrophenylhydrazine with the formation of its derivatives, which optic density was determined by means of spectrophotometry. Aldehyde- or ketone-derivatives of a neutral or the main character possessing different ranges of absorption spectrum are known to be formed resulting from protein oxidation depending on amino acids of a neutral (valine, leucine, isoleucine, etc.) or main (lysine, arginine, etc.) character prevailing in their molecules. With λ=370 nm ketone dinitrophenylhydrazones of a neutral character are determined, with λ=430 nm – aldehyde dinitrophenylhydrazones of the main character [13]. OMP content was expressed in the units per gram of the tissue. Intensity of lipid peroxide oxidation (LPO) was evaluated by the content of products reacting with 2-thiobarbituric acid, which amount was calculated in mcmol per 1 gram of the tissue [14].

The brain samples for histological examination were fixed in 10 % neutral formalin solution, and after the standard histologic processing the tissue was embedded in paraffin. Paraffin histological sections of the cerebral cortex tissues 5 mcm thick were prepared by means of a sliding microtome MC-2. After deparaffinization certain specimens were stained with hematoxylin and eosin, others – with neutral red by Nisl method in order to find tigroid substance [15]. In order to find amyloid the brain tissue specimens were stained with Congo red with control in the polarizing system [15, 16]. Microslides were examined under the light microscope. Digital copies of optic images were obtained by means of the digital camera Olympus SP550UZ and analyzed by means of the specialized computer program ImageJ for histologic studies (1.48v, free License, W.Rasband, National Institute of Health, USA, 2015) [17].

The results of the study were statistically processed by means of Student t-criterion. Distribution of values in samples was preliminary checked in order to prove an adequate method of statistical assessment of a mean difference between the groups of the study. According to Shapiro-Wilk criterion the data concerning distribution deviation in samples from that of the norm were not obtained ($p>0,05$). Taking into account the above mentioned application of Student t-criterion was considered to be sufficient to obtain valid conclusions. At the same time, to prove reliability of conclusions Mann-Whitney non-parametric comparison criterion was applied, which showed similar results of calculations by means of Student t-criterion concerning p value. Therefore, $p\leq0,05$ was considered to be a sufficient level of discrepancy probability.

Results and discussion

Compared with the control group, rats with modeled neurodegeneration demonstrated increased content of proteins of a neutral character in the cerebral cortex and hippocampus 25,1 and 33,6 % respectively, and the main character – 16,3 and 22,5 % as much. At the same time, the proteins of a neutral character were more subjected to peroxide oxidation. Increased degree of OMP of the examined homogenates is indicative of cerebral protein damage under conditions of neurodegeneration.

After carbacetam administration protein peroxide oxidation in the cerebral cortex and hippocampus registered at λ=370 nm became 16,1 and 22,7 % higher than that of the control. OMP registration at λ=430 nm detected on an average 15,4% increase of the content of neutral and main character proteins in both structures of the brain under carbacetam effect. It should be noted that in rats with scopolamine-induced neurodegeneration after carbacetam administration during 14 days the degree of protein damage decreased.

One of the causes promoting increase of OMP concentration in the brain homogenates under conditions of NDD can be decreased activity of the antioxidant protection system, especially enzymes

able to neutralize active forms of oxygen first of all, which is the major cause of protein peroxide oxidation.

The content of GABA-containing agents is known to be used as an indicator of lipid oxidation degree of the biological systems, which increase in the body, occurs due to breaking down of polyunsaturated fats by oxygen forms able to high reaction and serves as a marker of damage. Analysis of the obtained results (Table) in rats with scopolamine-induced neurodegeneration determined increased amount of GABA-containing agents in the cerebral cortex and hippocampus as 47,6 and 46,9 % respectively compared with the control group. In rats administered to carbacetam the content of GABA-containing agents became 10,5 % lower in the cerebral cortex and – 27,2 % in the hippocampus compared with rats with modeled pathology. Meanwhile, this index remains higher than that of the control group.

In histological specimens of the control group of rats the neurons with karyopyknosis signs were not found (Fig.1a, 1b, 1c). Against the ground of scopolamine-induced neurodegeneration the neurons with karyopyknosis signs were found compared with the control rats (in 6,9±0,18%). In addition, a relative staining density of the neuron tigroid substance in the cerebral cortex was twice decreased as much (Fig. 2a, 2b). Plaque-like Congo-rot-positive formations of homogenous or fibrous structure of various sizes are found in the white substance. Separate fine calcifications are seen near by (Fig. 2c). After carbacetam administration the amount of cells with karyopyknosis decreased twice as much, and relative staining density of the neuron tigroid substance 39,3 % increased (Fig. 3a, 3b). At the same time the amount of plaque-like formations did not decrease (Fig. 3c).

Thus, the results of examination of protein and lipid peroxide oxidation in the cerebral cortex and hippocampus, as well as morphological state of the cerebral cortex under conditions of inhibition of the central cholinergic effects produced by scopolamine, are indicative of the development of cerebral degeneration. After carbacetam administration OMP degree decreases, and the content of GABA-containing agents reduced considerably. Due to the less intensity of peroxide oxidation processes, first of all, lipids, the intensity of structural changes in the cerebral cortex decreases under carbacetam effect. The results obtained are indicative of the ability of carbacetam to inhibit neurodegeneration development in case of scopolamine-induced cerebral injury.

Conclusions

1. In rats with modeled scopolamine-induced neurodegeneration in the cerebral cortex and hippocampus under carbacetam effect, the content of products, reacting with 2-thiobarbituric acid and proteins of a neutral and major character, decreases, which is indicative of a reduced intensity of lipid and protein peroxide oxidation.

2. Under carbacetam effect the number of cells with karyopyknosis signs decreases and a relative staining density of the neuron tigroid substance increases, which is indicative of inhibition of the progress of the cerebral neurodestructive processes under conditions of scopolamine-induced damage.

3. Comprehensive biochemical and morphological investigation is indicative of carbacetam correcting effect on protein and lipid peroxide oxidation in the cerebral cortex and hippocampus of rats with scopolamine-induced neurodegeneration, which is the evidence of neuroprotective effect under conditions of central cholinergic blockade.

Prospects of further studies

Biochemical and morphological examinations of other portions of the brain are planned after carbacetam administration under conditions of experimental neurodegeneration.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Table: Carbacetam effect on protein and lipid peroxide oxidation in the cytosolic fraction of the cerebral cortex and hippocampus of rats with scopolamine-induced neurodegeneration (M±m, n=7)

Indices	Examined structures	Control	Neurodegeneration model	Neurodegeneration model + carbacetam
OMP λ=370, units/g of tissue	Cerebral cortex	30,486±1,162	40,7±1,888*	36,357±0,925*
	Hippocampus	20,357±1,649	30,643±1,860*	26,329±0,916*
OMP λ=430, units/g of tissue	Cerebral cortex	30,514±0,724	36,457±0,577*	34,843±0,648*
	Hippocampus	20,514±0,724	26,457±0,577*	24,843±0,648*
GABA, mcmol/g of tissue	Cerebral cortex	43,002±2,367	82,048±1,662*	74,246±2,067*#
	Hippocampus	39,961±3,107	75,198±5,327*	59,118±3,273*#

Notes: * – reliability compared with the control, # – reliability compared with neurodegeneration.

Figure 1. Cerebral cortex of the control group of rats (x200):a – hematoxylin-eosin, b – neutral red by Nisl; c – Congo red

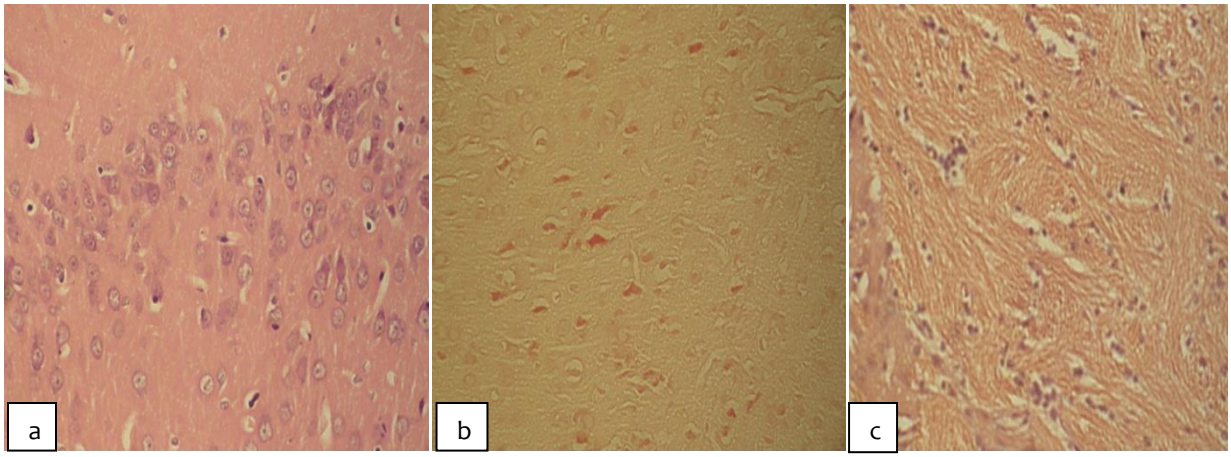

Figure 2: Cerebral cortex of rats with scopolamine-induced neurodegeneration (x200):a – hematoxylin-eosin, b – neutral red by Nisl; c – Congo red

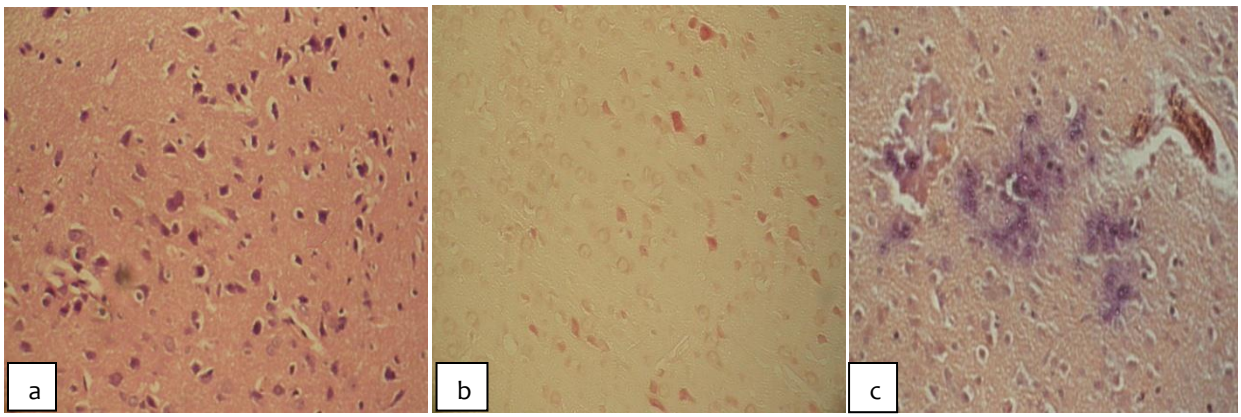

Figure 3: Cerebral cortex of rats with scopolamine-induced neurodegeneration under conditions of carbacetam administration (x200): a – hematoxylin-eosin, b – neutral red by Nisl; c – Congo red.