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EXPERIMENTAL ARTICLES

Efficiency of Cortexin under the Conditions of Experimental Chronic Brain Ischemia

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Abstract—Chronic brain ischemia was accompanied by decreased activities of glutathione-dependent enzymes and an increased content of nitrotyrosine in the rat brain, which indicates the development of oxidative and nitrosative stress and a decline of cognitive functions. Treatment with cortexin normalized the activities of glutathione-dependent enzymes, increased the contents of intermediates of the thiol–disulfide system, decreased the level of nitrotyrosine, which is a marker of nitrosative stress, and improved memory.

Keywords: chronic brain ischemia, thiol–disulfide system, glutathione, nitrotyrosine, cognitive functions, cortexin

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INTRODUCTION

One of the main problems of modern neuropharmacology is the medical treatment of brain ischemia on the basis of data on the mechanisms of neuronal death. According to the modern concepts, ischemia-induced neuronal damage is accompanied by the development of complex pathobiochemical cascades in neurons, including inhibition of bioenergetic processes, glutamate excitotoxicity, overproduction of active oxygen species, a decrease in the activities of antioxidant systems, and activation of apoptosis [1, 2].

The antioxidant system, in which low- and high-molecular-weight thiol-containing compounds play a leading role, is a promising field of neuropharmacology [3]. Thiol-containing compounds are molecules that have SH-groups in their composition. They are widely distributed in the cell in the form of the tripeptide glutathione and multiple proteins [4]. Glutathione plays the most important role in the functioning of the cell and the entire body and serves as a key intracellular antioxidant [5]. Under ischemic conditions, inhibition of glutathione-dependent enzymes, such as glutathione reductase and glutathione transferase, is followed by oxidative modification of low-molecular-weight thiols, the formation of homocysteine, and thus, impairment of nitric oxide transport and formation of its cytotoxic derivatives, which additionally intensify thiol oxidation. The presence of an active thiol antioxidant system in neurons may regulate nitric oxide transport and provide the resistance of a cell to nitrosative stress, which is the earliest neurodamaging mechanism under ischemia conditions [6].

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Studies on pathobiochemical mechanisms of brain ischemia/hypoxia that are responsible for secondary damage to the brain and the development of cognitive and neurological deficits are important for both theory and practice because they establish new trends in pathogenetically directed therapy of cerebral ischemia.

The discovery of neurotrophic peptide factors motivated researchers to form a new strategy of pharmacotherapy; specifically, peptidergic or neurotrophic therapy of diseases of the central nervous system. Several drugs have been developed and are used for the treatment of neurological disorders. These drugs are known as neurotrophic cerebroprotectors and combine nootropic, vasoactive, and neuroprotective effects. Cortexin is a drug from this group [7]. Cortexin is a complex of polypeptides and L-amino acids with a molecular weight of 1–10 kDa, which is extracted from the calf cerebral cortex. Cortexin contains microelements that play an important role in the mechanisms of neuroprotection and the maintenance of the activities of more than 1000 intracellular enzymes and proteins, which regulate the processes of cellular dynamics and apoptosis. Thus, lithium inhibits the release of excitotoxic amino acids, such as aspartate and glutamate. Selenium inhibits apoptosis and stimulates angiogenesis. Manganese promotes the synthesis of superoxide dismutase. Zinc stabilizes the functions of the NMDA, GABA, acetylcholine, and DOPA receptors. Cortexin is composed of 15 amino acids, which are in their L-forms, i.e., levo isomers, in contrast to dextroisomers, which are chemically synthesized. The spatial distribution of amino acids in cortexin promotes their active involvement in neuronal metabolism. The effect of cortexin on the func-

tional–biochemical state of the CNS is mediated by both the recovery of the balance between excitatory (aspartate, glutamine, and gluatamic acid) and inhibitory (GABA, serine, and glycine) amino-acid neurotransmitters and mineral substances from cortexin, which activate enzymes and regulate apoptosis, the antioxidant system, and the functional states of dopamine and acetylcholine receptors [8, 9].

Experimental studies have demonstrated that the neuroprotective and nootropic effects of cortexin are associated with its capability to attenuate mitochondrial dysfunction and neuroapoptosis, which are complex pathological processes that result in persistent cognitive impairments [1].

Therefore, studies on the neuroprotective effects of cortexin under the conditions of experimental chronic brain ischemia are important.

The aim of the present study was to examine the efficiency of the effects of cortexin on the activities of antioxidant glutathione-dependent enzymes, the contents of reduced forms of the thiol–disulfide system, the level of a marker of nitrosative stress, nitrotyrosine, and the cognitive functions of animals with chronic brain ischemia.

MATERIALS AND METHODS

We used 23 male and 22 female white rats that weighed 180–200 g, which were supplied by the breeding center of the Institute of Pharmacology and Toxicology of the Ukrainian Academy of Medical Sciences (Kiev, Ukraine). The animals were acclimated for 14 days. The animals were housed under the standard vivarium conditions with free access to water and standard granulated food.

All experiments were performed in accordance with the Guide for the use of animals in biomedical studies. Because we studied the neuroprotective efficacy of cortexin for the treatment of cerebral ischemia, we had to use experimental models that are relevant to the clinical signs of chronic brain ischemia.

For this purpose, we used irreversible bilatereal occlusion of common carotid arteries taking the anatomical and physiological features of the blood supply in the brain of white rats into account. Following occlusion, neurological deficit, cognitive impairments, and biochemical alterations in the brain tissue occurred [10].

The experiments were performed in an experimental operating room after UV sterilization and antiseptic treatment at a temperature of 19–20°C. The coat was shaved and the surgical field was treated with brilliant-green solution. Bilateral occlusion of common carotid arteries was performed under thiopental anesthesia (40 mg/kg). After surgical incision, the carotid arteries were separated and occluded by silk ligatures. After the occlusion of the carotid arteries, the incision was sutured layer-by-layer and treated with brilliant green. In sham-operated animals, the skin was cut and sutured. Due to the high mortality in this model, we performed occlusion on many animals in order to have 15 rats at day 21 in each experimental group.

In order to assay the efficacy of the neuroprotective action of cortexin we estimated the activities of antioxidant glutathione-dependent enzymes, the level of reduced intermediates of the thiol–disulfide system, and the marker of nitrosative stress, nitrotyrosine, in the cerebral cortex of white rats and cognitive functions during the recovery period after experimental impairment of brain circulation.

We studied three experimental groups: 1, the main group consisted of animals with chronic brain ischemia that were treated intraperitoneally with cortexin at a dose of 0.5 mg/kg for 21 day ($n = 15$; eight males and seven females); 2, the control group consisted of animals with chronic brain ischemia that were treated intraperitoneally with isotonic saline at a volume of 2 mL/kg during the entire observation period ($n = 15$; eight males and seven females); 3, sham-operated animals ($n = 15$; eight males and seven females).

On day 21, the animals were anesthetized with thiopental at a dose of 40 mg/kg and killed.

We studied the activities of glutathione-dependent enzymes, viz., glutathione reductase (GR), glutathione peroxidase (GPO), and glutathione transferase (GT), as well as the contents of reduced glutathione and reduced (SH)-groups of thiols [10].

Brain tissue homogenate prepared in the cold was used for biochemical studies. Brain tissue was homogenized in 0.25 M sucrose buffer using a Silent Crusher S homogenizer (Heidolph). The protein content was measured by direct spectrophotometry at $\lambda = 280$ nm [11]. The activities of glutathione-dependent enzymes of the thiol–disulfide system and the thiol content were calculated per gram of protein.

We also measured the content of a marker of nitrosative stress, total nitrotyrosine, in the cytosol fraction of brain homogenate using the enzyme-linked immunosorbent sandwich assay with an ELISA kit (Hycult Biotech, cat. no. HK 501–02).

Cognitive functions were studied using passive-avoidance conditioning. Passive-avoidance training was performed on day 20 of the recovery period after the impairment of brain circulation [12]. Testing of passive avoidance was performed 24 h after the training. The latency of the entry into the dark compartment of an experimental chamber was used as an index of the memory of electroshock application during passive-avoidance training.

Statistical analysis was performed using STATISTICA for Windows 6.0 (StatSoftInc., United States; no. AXXR712D833214FAN5). The statistical significance of the differences between the groups was estimated using the nonparametrical Mann–Whitney *U*-test. The data are presented as the median values and 25–75% interquartile interval (Me [Q1–Q3]). A paired

Table 1. The activities of the enzymes of the glutathione part of the thiol–disulfide system in the cerebral cortex of the animals that were subjected to chronic brain ischemia

Groups of animals	GR, μmol/min/mg protein	GPO, μmol/min/mg protein	GT, μmol/min/mg protein
Ischemia + cortexin, 0.5 mg/kg ($n = 15$)	10.28 (9.48–12.31)*	3.93 (2.38–4.72)*	9.06 (8.18–11.97)*
Ischemia (control), ($n = 15$)	4.29 (3.30–5.36)#	1.26 (0.77–1.74)#	5.64 (4.39–6.44)#
Sham-operated ($n = 15$)	14.85 (11.54–18.88)	6.06 (4.38–8.01)	10.91 (10.06–13.61)

*, $p < 0.0001$ as compared to the animals subjected to chronic brain ischemia; #, $p < 0.0001$ as compared to the sham-operated animals.

Table 2. The indices of the thiol–disulfide system and nitrotyrosine in the cerebral cortex of animals that were subjected to chronic brain ischemia

Groups of animals	SH-groups, μmol/g protein	Reduced glutathione, μmol/g protein	Nitrotyrosine, nmol/g protein
Ischemia + cortexin, 0.5 mg/kg ($n = 15$)	21.47 (18.39–24.89)*	2.81 (2.46–3.12)*	9.56 (8.07–11.88)*
Ischemia (control), ($n = 15$)	6.46 (4.72–8.00)#	0.68 (0.56–0.74)#	23.56 (17.77–31.67)#
Sham-operated ($n = 15$)	29.85 (27.17–35.79)	3.91 (3.72–4.15)	7.02 (5.81–8.22)

*, $p < 0.0001$ as compared to the animals that were subjected to chronic brain ischemia; #, $p < 0.0001$ as compared to the sham-operated animals.

comparison of dependent samples was performed using the non-parametric Wilcoxon *T*-test ($p < 0.05$).

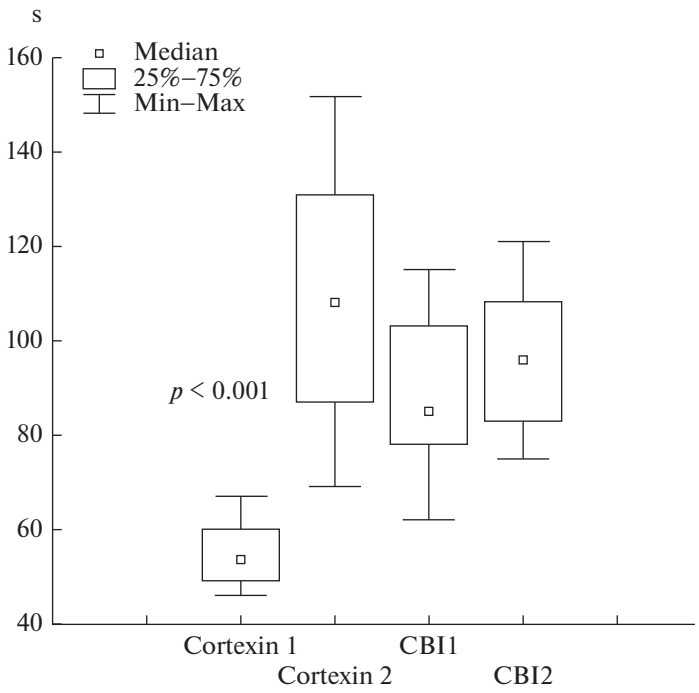
RESULTS AND DISCUSSION

Our data show that chronic brain ischemia resulted in a decrease in the activities of antioxidant glutathione-dependent enzymes and shifted the thiol–disulfide balance to the decreased content of the reduced forms (Tables 1 and 2). The activities of glutathione-dependent enzymes, such as GR, GPO, and GT, decreased by 71.1, 79.2, and 48.3%, respectively. The content of the marker of the nitrosative stress, nitrotyrosine, increased by a factor of 3.36. These data indicate the development of oxidative and nitrosative stress in the brain tissue of experimental animals after chronic ischemia.

Nitrosative stress, which develops early after the initiation of ischemic brain damage, results in thiol nitrosylation. The development of oxidative stress then substantially shifts the thiol–disulfide balance to the prevalence of oxidized thiols and stable mitochondrial dysfunction with a deficit of energy substrates develops [1]. Alterations of the thiol component of the antioxidant system, which include a decrease in the reduced form and an increase in the oxidized form, are the earliest signs of impairments of cell defense under ischemia.

Damage to the nervous tissue induces significant modifications in the expression of neuropeptides, which induce apoptosis [13]. Presently, nearly 100 neuropeptides are known that are synthesized by various neuronal populations of the mammalian brain, whose physiological activities are much higher than the activities of non-peptide compounds [14]. However, the role of peptidergic impairments in the development of brain-tissue destruction has not been completely studied.

Today, pharmacological groups of drugs with potential neuroprotective effects are developed; neuropeptides are among them. Cortexin and cerebrolysin are drugs with neuromodulatory activities. The antioxidant and antiapoptotic effects of cerebrolysin, its neurotrophic activity, effects on microelement homeostasis, elevation of MAP2 protein content, and normalization of neurotransmitter metabolism have been observed in many experimental and clinical studies [15, 16]. The efficacy of cerebrolysin for glutathione-dependent enzymes of the brain and reduced glutathione has been demonstrated in models of denervation of the hippocampus [17] and moderate hypoglycemia [18], respectively. However, the effects of cerebrolysin on the thiol–disulfide balance in neurons and glutathione-dependent enzymes under the conditions of chronic brain ischemia have not been studied.



Changes in the latency of entries of rats into the dark compartment in the passive-avoidance test. 1, passive-avoidance training; 2, passive-avoidance testing 24 h after training. CBI, chronic brain ischemia.

Our experimental study shows that administration of cortexin to the animals significantly increased the activities of antioxidant glutathione-dependent enzymes, such as GR, GT, and GPO, by factors of 1.7, 1.3, and 1.8, respectively. We observed a decrease in the content of the marker of nitrosative stress, nitrotyrosine, after cortexin treatment (Table 1). Cortexin treatment significantly increased the level of SH-groups and reduced glutathione in the animals (Table 2). These data indicated the positive effect of cortexin on the glutathione component of the thiol–disulfide system.

Because the glial tissue is a target for neuropeptide action, neuropeptides may be used not only during the acute period of impairment of brain circulation, but also in the recovery period, when they may improve brain plasticity and the formation of new associative connections [14].

In the control group of animals with chronic brain ischemia, we observed a cognitive deficit in the passive-avoidance test because the latency of the transition of a rat to the dark compartment decreased by 92.2% ($p < 0.001$) as compared to that found in the sham-operated animals.

After neuroprotective therapy with cortexin (figure), the latency of the transition of a rat to the dark compartment in the passive-avoidance test increased by two times, which indicates the improvement of cognitive functions in the experimental animals, specifically, their memory ($p < 0.001$).

The data of the present study show that cortexin efficiently increased the activities of glutathione-dependent enzymes and reduced intermediates of the thiol–disulfide system, decreased the content of nitrotyrosine in the cerebral cortex, and improved memory processes in experimental animals that were subjected to chronic brain ischemia.

Thus, experimental neuroprotective therapy in white rats with chronic brain ischemia decreased the intensity of oxidative and nitrosative stress in the brain tissue and attenuated a cognitive deficit.

CONCLUSIONS

(1) Modeling of chronic brain ischemia in white rats was associated with a decrease in the activities of glutathione-dependent enzymes and contents of reduced glutathione and SH-groups, an increase in the level of nitrotyrosine, which indicated the development of oxidative and nitrosative stress in the brain, and deterioration of memory processes.

(2) Treatment of animals with chronic brain ischemia with cortexin increased the activities of glutathione-dependent enzymes and the contents of reduced intermediates of the thiol–disulfide system and decreased the level of nitrotyrosine.

(3) Cortexin improved cognitive functions in experimental animals that were subjected to chronic brain ischemia.

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