DOI 10.1007/s10517-015-2964-z
Bulletin of Experimental Biology and Medicine, Vol. 159, No. 3, July, 2015

PHARMACOLOGY AND TOXICOLOGY

Neuroprotective and Antioxidant Effects of Neuroglutam in Cerebral Ischemia

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 159, No. 3, pp. 344-347, March, 2015
Original article submitted January 21, 2014

We studied *in vitro* and *in vivo* neuroprotective and antioxidant properties of neuroglutam, a new glutamic acid derivative. In experiments on immortalized mouse hippocampal cell line HT22, neuroglutam exhibited a neuroprotective effect in the model of oxidative stress after its introduction, both before and after H_2O_2. *In vivo* study on animals treated with neuroglutam against the background of cerebral ischemia modeled by irreversible occlusion of the common carotid arteries showed that plasma level of TBA-active products was significantly lower and activities of cell antioxidant enzymes (superoxide dismutase and catalase) were higher than in control animals receiving saline under the same conditions.

Key Words: *neuroprotective properties; antioxidant system; neuroglutam*

Pathogenetic therapy of ischemic stroke is the key problem of clinical neurology due to high fatality rate, significant disability, and social maladjustment of the patients after stroke. The dynamics of nervous tissue damage during cerebral ischemia is characterized by cascades of metabolic changes. Ischemic brain damage is accompanied by a series of pathobiochemical reactions of the glutamate-calcium cascade and each stage of this cascade is associated with activation of free radical processes manifested in ROS overproduction; under ischemic conditions, these disturbances are exacerbated by progressive glucose metabolism disorders and development of acidosis, which leads to damage of cellular membranes. In addition, increased production of free radicals is one of the major reasons for prolonged spasm of cerebral vessels, progression of post-ischemic edema and neuronal degeneration due to membrane disintegration during acute and chronic cerebral circulatory disorders [1]. Drugs with antioxidant activity are of particular interest [9,10], because they protect neurons from the impact of universal damaging factors underlying most clinical forms of CNS pathology.

In previous studies [4], we have studied the neuroprotective effects of neuroglutam, a new derivative of glutamic acid, a compound with antidepressant and anxiolytic properties [8]. Under conditions of cerebrovascular insufficiency in rats caused by irreversible simultaneous bilateral occlusion of the common carotid arteries, preventive administration of neuroglutam reduced animal mortality and severity of neurological, cognitive, and behavioral deficits in comparison with animals receiving saline.

Here we studied the neuroprotective and antioxidant effects of neuroglutam in the model of oxidative stress *in vitro* and during partial cerebral ischemia in rats caused by irreversible occlusion of the common carotid arteries (*in vivo*).

MATERIALS AND METHODS

The study was performed in two stages. At the first stage, we studied neuroprotective activity of neuroglutam *in vitro* on the model of oxidative stress using

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immortalized mouse hippocampal cell line HT-22. To simulate oxidative stress, H_2O_2 in a final concentration of 1.5 mM was used [11]. HT22 cells with H_2O_2 were incubated at 37°C and 5% CO_2 for 30 min. Then, the culture medium containing H_2O_2 was replaced with normal medium and cell viability was measured after 4 h by MTT assay.

The tested drug was dissolved in sterile deionized water. The final concentration was calculated during the study in accordance with the amount of the drug added to the culture medium. The solutions were prepared every day. Neuroglutam was added in final concentrations of 10^{-5} to 10^{-8} M 24 h before H_2O_2 injury or immediately after washing. The examined neuroglutam concentrations were prepared by successive dilution of the stock solution (10^{-3} M) with sterile deionized water.

At the second stage we studied neuroprotective activity of neuroglutam in cerebral ischemia in rats. The work carried out on adult Wistar male rats primarily demonstrating medium behavioral activity in the open-field test. The animals were selected in accordance with the requirements for building representative samples by randomization. The rats were divided into groups (10 animals each) except for ischemic control group that consisted of 20 rats. Group 1 included sham-operated animals, group 2 comprised rats with cerebral ischemia simulated by irreversible occlusion of the common carotid artery (CCA) [3] (control group), group 3 included animals with cerebral ischemia treated with neuroglutam (26 mg/kg) intraperitoneally once a day for 3 days after surgery; the first injection was performed after recovery from anesthesia (~30-60 min after occlusion). In sham-operated and control group, the animals received saline according to the same schedule as in the experimental group.

Biochemical blood tests were taken from femoral artery 72 h after occlusion. LPO intensity was assessed by measuring the content of TBA-reactive substances (TBA-RS, primarily MDA) [7] using an Agat-Med standard kit; a colored complex was formed as a result of interaction between LPO products and TBA. To characterize the antioxidant status, superoxide dismutase (SOD) and catalase activities were measured spectrophotometrically. SOD activity was measured by a method based on quercetin oxidation [6], catalase activity by the method based on H_2O_2 ability to form stable yellow complex with molybdenum salts [5].

The data were analyzed statistically using Microsoft Excel and BioStat 2008 Professional 5.2.5.0 softwares using Kruskal–Wallis test (ANOVA) followed by Dunn post-hoc test.

RESULTS

Using neuronal HT22 cells, we showed that neuroglutam added 24 h before H_2O_2 showed a neuroprotective effect in the entire range of tested concentrations; the maximum effect was observed in a concentration of 10^{-6} M, and its reduction, from 10^{-7} to 10^{-8} M (Fig. 1).

Neuroglutam administered after H_2O_2 produced a protective effect only in concentrations of 10^{-5} and 10^{-6} M (Fig. 1).

Thus, neuroglutam exhibited protective properties in *in vitro* model of oxidative stress; therefore, it is expedient to study neuroprotective and antioxidant action of this drug in *in vivo* experiments.

Brain ischemia was accompanied by LPO activation, which was seen from accumulation of TBA-RS in blood plasma in comparison with the group of sham-operated animals (Fig. 2). In control rats with brain ischemia, TBA-RS level after 72 h was higher by 75.6% than in sham-operated animals. In animals treated with neuroglutam, TBA-RS level was significantly lower ($p<0.05$) than in the control group by ~33%, which attests to a decrease in LPO intensity (Fig. 2, *a*).

Against the background of LPO intensification, activity of antioxidant enzymes in rats with experi-

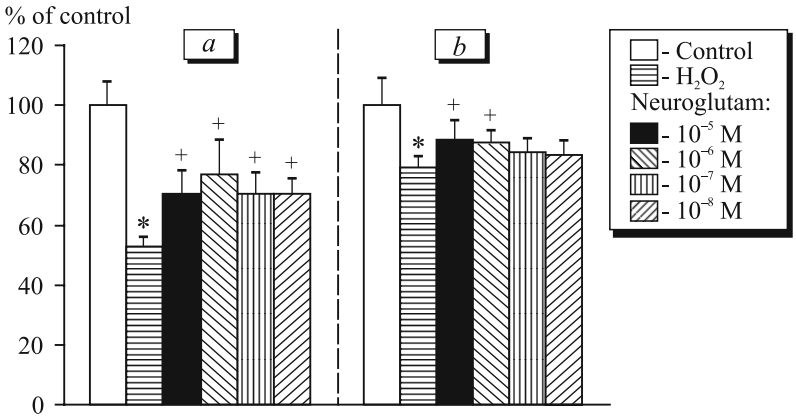

Fig. 1. Effect of different concentrations of neuroglutam on the survival of neuronal HT22 cells in the simulation of oxidative stress. *a*) 24 h before H_2O_2, *b*) after H_2O_2. $p<0.05$ in comparison with *controls, $^+H_2O_2$.

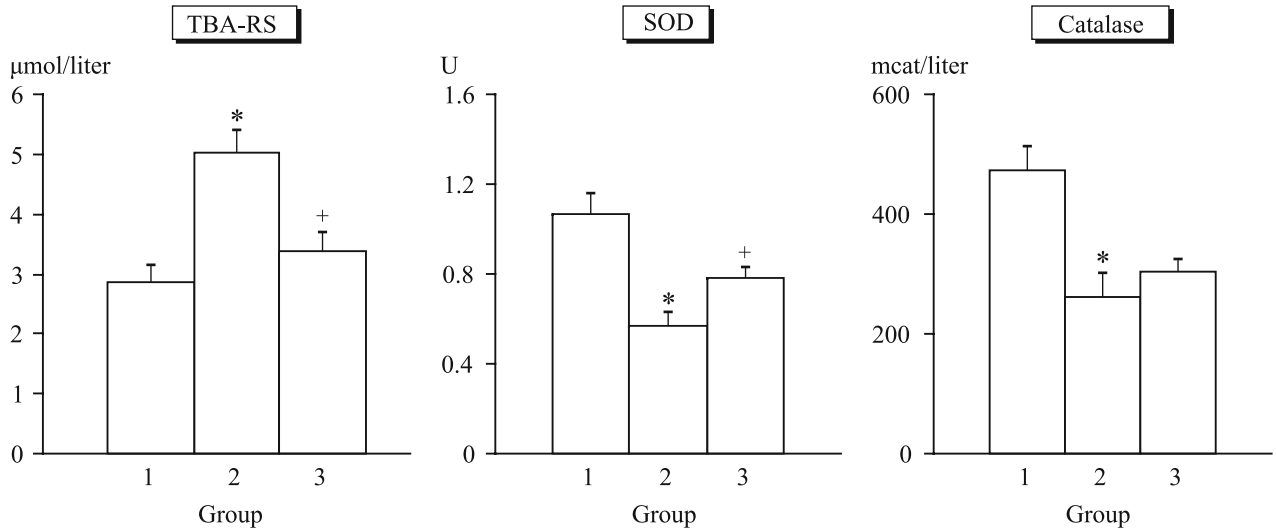

Fig. 2. Plasma levels of TBA-RS and SOD and catalase activities in 72 h after irreversible occlusion of the CCA. $p<0.05$ in comparison with *group 1, +group 2.

mental ischemia was reduced. Plasma activity of SOD and catalase in control rats with cerebral ischemia was significantly reduced in comparison with sham-operated rats by 46.4 and 45%, respectively (at $p<0.05$; Fig. 2, *b*, *c*). Administration of neuroglutam increased plasma SOD activity in comparison with control group by on average 36% (Fig. 2, *b*). Catalase activity in animals treated with neuroglutam was also higher than in the control group (Fig. 2, *c*).

Significant effect of the test compound neuroglutam on SOD activity and plasma levels of LPO products in animals with brain ischemia suggests its effect on cell antioxidant defense system, which that may be one of the molecular mechanisms of its neuroprotective effect.

The development new methods for the therapy of cerebrovascular insufficiency remains a pressing problem for many decades. Different strategies were proposed at different stages of exploration of the etiopathogenesis of this disease. One of such strategies is administration of neuroprotective drugs that affect different stages of the pathogenesis of ischemic damage to the nervous system including the development of oxidative stress, which, in turn, enhances the effects cerebrovascular spasm, formation of perivascular edema, impairment of neuronal membrane permeability, *etc.* [2]. Oxidative stress is regarded as a universal pathophysiological mechanism coupled with cerebral circulation disorders and other nervous system pathologies. The need for directed pharmacological modulation of free radical formation, that is, development of drugs with antioxidant activity for the use in clinical practice including neurology, is beyond doubt [2]. In the course of the study, it was shown that neuroglutam exhibits a neuroprotective effect in the model of oxidative stress both *in vitro* and *in vivo*. Moreover, neuroprotective effect of neuroglutam in the survival test with neuronal HT22 cells is more pronounced after its introduction to the culture medium 24 h after H_2O_2 addition, which can be explained by its effect on the synthesis of cytoprotective proteins.

Further study of the molecular mechanisms of neuroprotective action of neuroglutam in the experiments *in vitro* and *in vivo* are required.

REFERENCES

1. S. G. Burchinskii, *Zdorov'ye Ukrainy*, No. 19, 70-71 (2008).
2. S. G. Burchinskii, *Mezhdunarodnyi Nevrologicheskii Zh.*, No. 1 [URL: www.mif-ua.com/archive/article-print/8106] (2009).
3. E. V. Volotova, D. V. Kurkin, I. N. Tyurenkov, and A. A. Litvinov, *Vestn. Volgograd. Gos. Med. Univer.*, No. 2, 72-75 (2011).
4. E. V. Volotova, N. V. Mazina, D. V. Kurkin, and I. N. Tyurenkov, *Vestn. Volgograd. Gos. Med. Univer.*, No. 1, 40-42 (2013).
5. M. A. Korolyuk, L. I. Ivanova, N. O. Maiorova, *et al.*, *Lab. Delo*, No. 1, 16-19 (1988).
6. V. A. Kostyuk, A. I. Potapovich, and Zh. V. Kovaleva, *Vopr. Med. Khimii*, **36**, No. 2, 88-91 (1990).
7. T. V. Sologub, M. G. Romantsov, I. V. Kremen', *et al.*, *Free Radical Processes and Inflammation (Pathogenic, Clinical and Therapeutic Aspects)* [in Russian], Moscow (2008).
8. V. I. Petrov, I. N. Tyurenkov, V. V. Bagmetova, *et al.*, *Patent RF No. 2429834. Agent Exhibiting Antidepressant, Anxiolytic, Neuroprotective and Immunostimulatory Effects*, *Byull.*, No. 27, September 27, 2011.
9. I. A. Tregubova, V. A. Kosolapov, and A. A. Spasov, *Uspekhi Fiziol. Nauk*, **43**, No. 1, 75-94 (2012).
10. A. I. Fedin, *Atmosfera: Nervnye Bolezni*, No. 1, 15-18 (2002).
11. G. R. Jackson, K. Werrbach-Perez, E. L. Ezell, *et al.*, *Brain Res.*, **592**, No. 1-2, 239-248 (1992).