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Prooxidant-Antioxidant Balance of The Brain of Rats with Cerebral Ischemia with The Introduction Of L-Name, L-Arginine, And Omega-3 Polyunsaturated Fatty Acids

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Abstract

The search for new approaches to the treatment of acute ischemic stroke is one of the urgent problems of experimental and clinical neurology. This is due to the fact that cerebrovascular diseases of ischemic origin tend to grow, rejuvenate, are associated with a severe clinical course, high rates of disability and mortality. One of the promising neuroprotective amino acids is L-arginine. Most of the effects caused by this amino acid are associated with its ability to increase the formation of NO, acting as a source for its formation. An important role of ω-3 polyunsaturated fatty acids (Omega-3 PUFAs) is to ensure the functioning of cell membranes, transmembrane ion channels and the regulation of physiological processes through the synthesis of lipid mediators, which, lining up in the phospholipid layer of cell membranes, affect their fluidity. Cerebral ischemia is characterized by the activation of prooxidant mechanisms, as well as the inhibition of energy metabolism in the brain tissue. The introduction of the drug «Omega-3 PUFA» has a corrective effect on the indicators of oxidative stress in SCI. The use of «Omega-3 PUFA» in animals of the group with cerebral ischemia and the introduction of a non-selective NOS inhibitor – L-NAME did not have a positive effect.

Keywords: *prooxidant-antioxidant balance; cerebral ischemia; L-NAME; L-arginine; Omega-3 polyunsaturated fatty acids*

Introduction

Cerebrovascular diseases of ischemic origin tend to grow, rejuvenate, are associated with a severe clinical course, high rates of disability and mortality. The urgency of the problem of cerebrovascular diseases can rightfully be defined as extraordinary, requiring the concentration of efforts of specialists of different profiles to solve it [1, 2, 5].

The search for new approaches to the treatment of acute ischemic stroke is one of the urgent problems of experimental and clinical neurology [1, 4].

One of the promising neuroprotective amino acids is L-arginine. Most of the effects caused by this amino acid are associated with its ability to increase the formation of NO, acting as a source for its formation. It has been shown that the use of L-arginine reduces the size of the infarction, reduces vascular tone and causes a hypotensive effect, prevents and corrects ischemic and reperfusion injuries of the brain and other organs [1–6].

An important role of ω-3 polyunsaturated fatty acids (Omega-3 PUFAs) is to ensure the functioning of cell membranes, transmembrane ion channels and the regulation of physiological processes through the synthesis of lipid mediators, which, lining up in the phospholipid layer of cell membranes, affect their fluidity. Omega-3 PUFAs control the functioning of the immune and reproductive systems, being precursors of the biosynthesis of prostaglandins, leukotrienes and thromboxanes and other cytokines [7].

Brain neurons, being electrically active cells rich in ion channels, are most sensitive to deficiency of polyunsaturated fatty acids [7, 8].

Omega-3 PUFAs are involved in the implementation of the main functions of neurons, such as the transmission of impulses and the functioning of

receptors [8].

In this regard, it is of interest to study the morpho functional features of brain neurons in rats with subtotal cerebral ischemia against the background of the administration of Omega-3 PUFAs and L-NAME [1, 4, 7, 8].

The aim of the work is to study the activity of oxidative stress in rats with subtotal cerebral ischemia under conditions of the use of modulators of the L-arginine-NO pathway and against the background of the administration of Omega-3 polyunsaturated fatty acids.

Materials and methods of research

Subtotal cerebral ischemia (SCI) was modeled by ligation of both common carotid arteries (CCA) under conditions of intravenous thiopental anesthesia (40-50 mg/kg) – group 2. Rats of the 3rd group immediately before CCA ligation were injected intramuscularly with L-NAME at a dose of 5 mg/kg, animals of the 4th group were additionally injected with L-arginine at a dose of 200 mg/kg of body weight (SCI + L-NAME + L-arginine), and rats of the 5th group received only L-arginine at a similar dose before surgery (SCI + L-arginine), animals of the 6th group were additionally intragastrically given Omega-3 PUFA at a dose of 5 mg/kg of body weight (SCI + L-NAME + Omega-3 PUFA) intragastrically for a week, rats of group 7 were injected before surgery only Omega-3 PUFAs in the same dose (SCI + Omega-3 PUFAs), while the rats of the 8th group were given L-NAME, L-arginine and Omega-3 PUFAs in the above doses (SCI + L-NAME + L-arginine + Omega-3 PUFA). The control group consisted of sham-operated rats treated with 0.5 ml of isotonic NaCl solution.

The duration of SCI was 60 minutes, after which the rats were decapitated.

In rats, indicators of oxidative stress were studied.

For the subsequent study of the prooxidant-antioxidant state, the brain was frozen and stored in liquid nitrogen. Changes in indicators-markers of oxidative stress were determined: reduced glutathione (GSH), total sulfo groups (TSH), glutathione peroxidase activity, products that react with thiobarbituric acid (TBARS). Reduced glutathione (GSH) is known to have antioxidant activity. Glutathione peroxidase reduces oxidized hydrogen molecules, as well as lipid and other organic molecules oxidized by oxygen radicals.

When studying ischemia-reperfusion of the liver, malondialdehyde (MDA) and diene conjugates (DC) were determined.

The concentration of reduced glutathione (GSH) was determined spectrophotometrically according to the Ellman method using the molar extinction coefficient ε_{412}=13600 M^{-1} cm^{-1}. 1 ml of 15% brain homogenate was poured into a clean tube, 0.2 ml of 25% TCA was added, shaken and centrifuged at 5000 rpm for 5 minutes. To the resulting supernatant (0.2 ml) was added 1.2 ml of 0.5 M phosphate buffer (pH 7.8) and 50 µl of Ellman's reagent. The GSH concentration was calculated taking into account the molar extinction coefficient by determining the optical density of the samples under study at λ= 412 nm on a «PV 1251C» spectrophotometer.

Determination of TSH concentration was carried out by adding 30 µl of 3% dodecyl sulfate sodium salt solution to 60 µl of brain homogenate. Then, 1.2 ml of 0.5 M phosphate buffer (pH 7.8) and 50 µl of Ellman's reagent were added to 25 µl of the resulting mixture taken into a clean test tube (epindorf). The concentration of TSH was calculated after determining the optical density on a «PV 1251C» spectrophotometer at λ=412 nm, taking into account the molar extinction coefficient after a 10-minute incubation of the mixture at room temperature.

Measurement of glutathione peroxidase activity. Take 0.8 ml of Tris-HCl buffer (pH 7.25) containing 0.012 M sodium azide, 0.001 M EDTA and 4.8 mM GSH. After 3 minutes warming at 37°C, 0.1 ml of 20 mM tert-butyl hydroperoxide (t-BHP) was added and incubated for 10 minutes at 37°C. After that, 0.1 ml of a sample was taken and 0.02 ml of 25% trichloroacetic acid (TCA) was added. A similar procedure was followed to obtain a zero point immediately after the addition of t-BHP. After adding TCA, the samples were centrifuged at 5000 rpm for 5 minutes. For spectrophotometry, 30 µl of the resulting supernatant and 30 µl of Ellman's reagent were added to 1 ml of phosphate buffer (pH 7.8). Enzyme activity was expressed in mmol GSH/l^{-1}x min^{-1}.

The method for determining the content of TBARS is based on the reaction of aldehyde products of lipid peroxidation, primarily malondialdehyde (MDA) with thiobarbituric acid, which forms a trimethyl complex at high temperature and low pH, consisting of two molecules of thiobarbituric acid and one MDA molecule. According to the method, 2.4 ml of 0.07 N solution of sulfuric acid and 0.3 ml of 10% solution of phosphotungstic acid were sequentially added to the test sample of 10% brain homogenate (0.3 ml). To the precipitate washed twice in bidistilled water, dissolved in 3.0 ml of bidistilled water, was added 1 ml of a 0.85% aqueous solution of thiobarbituric acid dissolved in acetic acid. The color reaction proceeded

in hermetically sealed tubes at a temperature of 96°C for 60 minutes. After cooling them in water for 5 minutes, the optical density of the centrifuged supernatant was determined on a «PV 1251C» spectrophotometer at wavelengths of 532 nm and 580 nm.

Determination of indicators-markers of oxidative stress revealed a change in the concentration of indicators characterizing it. In the SCI group, there was a decrease in the indicators of non-enzymatic defense mechanisms: the concentration of reduced glutathione and total SH-groups. A decrease in the concentration of reduced glutathione (SH-) by 11% ($p<0.05$), total SH-groups of proteins and glutathione (TSH) – by 16% ($p<0.05$) and an increase in the activity of glutathione peroxidase – by 24% ($p<0.05$), reflecting the high intensity of the enzymatic mechanisms of antioxidant protection. In animals of the SCI + Omega-3 group, compared with the SCI group, an increase in the concentration of reduced glutathione (SH-) by 15% ($p<0.05$), total SH-groups of proteins and glutathione (TSH) – by 15% ($p< 0.05$) and a slight decrease in the activity of glutathione peroxidase. In the SCI + L-NAME + Omega 3 and SCI + L-NAME + L-arginine + Omega-3 groups, there were no significant changes in comparison with the animals of the SCI + L-NAME group (table 1).

Table 1: Determination of indicators-markers of oxidative stress in rats of the control group, with SCI, SCI + Omega-3 PUFA, SCI + L-NAME + Omega-3 PUFA and SCI + L-NAME + L-arginine + Omega-3, Me (LQ; UQ)

Groups	TSH, mmol/l	GSH, mmol/l	GP, mmol GSH/ min.×l
Control	2,78(2,63; 2,92)	1,89 (1,76; 2,01)	62,8(59,2; 64,3)
SCI	2,34*(2,09; 2,58)	1,68*(1,43; 1,92)	78,2*(75,6 ;81,3)
SCI+Omega 3	2,68#(2,62 ;2,2,71)	1,75√(1,69; 1,81)	75,3*(67,6 ;82,4)
SCI+L-NAME+Omega 3	2,53*(2,45; 2,63)	1,59*(1,48; 1,65)	85,2*(78,7 ;90,1)
SCI + L-NAME + L-arginine + Omega-3	2,47* (2,33;2,51)	1,79*(1,75; 1,83)	74,8*(72,1 ;77,9)

Notes

* – $p<0.05$ – in relation to the values in the «control» group

– $p<0.05$ – in relation to the values in the «SCI» group

√ – $p<0.05$ – in relation to the values in the «SCI+L-NAME» group

SCI – subtotal cerebral ischemia

L-NAME – Nω-nitro-L-arginine

Omega-3 – Omega-3 PUFA

GSH – reduced glutathione

TSH – common sulfo groups

GP – glutathione peroxidase activity

Cerebral ischemia is characterized by the activation of prooxidant mechanisms, as well as the inhibition of energy metabolism in the brain tissue. The introduction of the drug «Omega-3 PUFA» has a corrective effect on the indicators of oxidative stress in SCI. The use of «Omega-3 PUFA» in animals of the group with cerebral ischemia and the introduction of a non-selective NOS inhibitor – L-NAME did not have a positive effect.

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