Journal of BioMed Research and Reports
2023 Volume 1, Issue 1
https://www.doi.org/brs/2023/jcssr/0013

BioRes Scientia
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Research Article

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Oxidative Stress Activity in Rats with Subtotal Brain Ischemia Under the Conditions of The Use of Modulators of the L-Arginine-No Pathway

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Abstract
Acute cerebrovascular accident is one of the most urgent problems in modern medicine. 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.
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 infarct, reduces vascular tone and causes a hypotensive effect, prevents and corrects ischemic and reperfusion damage to the brain and other organs.
The use of "L-arginine" in animals of the group with cerebral ischemia and the introduction of a non-selective NOS inhibitor – L-NAME did not have a significant positive effect. Thus, nitric oxide hyperproduction has been studied by stimulating synthesis by introducing the substrate L-arginine, and inhibition by using the non-selective NO synthase inhibitor N-nitro-L-arginine methyl ester.

Article History
Received: February 09, 2023;
Accepted: March 27, 2023;
Published: April 07, 2023

Keywords
NOS inhibitor; L-NAME; L-arginine; acute cerebrovascular accident

Introduction
Acute cerebrovascular accident is one of the most urgent problems in modern medicine. 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, 3, 8].
The search for new approaches to the treatment of acute ischemic stroke is one of the urgent problems of experimental and clinical neurology [2, 3, 8].
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 infarct, reduces vascular tone and causes a hypotensive effect, prevents and corrects ischemic and reperfusion damage to the brain and other organs [4, 5, 6, 7, 8, 9].
The aim of this work is to study oxidative stress in rats with subtotal cerebral ischemia under conditions of the use of modulators of the L-arginine-NO pathway.

Materials and methods of research
The control group (group 1) consisted of sham-operated rats receiving 0.5 ml of isotonic NaCl solution. 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). 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. Determination of the concentration of reduced glutathione (GSH) was carried out 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 malonic dialdehyde (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.

Research results

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. With the introduction of L-NAME, an aggravation of oxidative stress activity was noted. These changes indicate the inhibition of NO synthase and an increase in the degree of oxidative stress in animals with cerebral ischemia. In animals of the SCI + L-arginine group, compared with the IGM group, there is a slight increase in the concentration of reduced glutathione (GSH-) by 10% ($p<0.05$), total SH-groups of proteins and glutathione (TSH) – by 8% ($p <0.05$) and a slight decrease in glutathione peroxidase activity. In the group of animals with SCI + L-NAME + L-arginine, there were no significant changes in comparison with animals of the SCI + L-NAME group (table 1).

Table 1: Determination of indicators-markers of oxidative stress in rats with cerebral ischemia, as well as isolated and combined administration of L-NAME and L-arginine, 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+L-NAME	2,46*(2,11;2,57)	1,54*(1,38;1,63)	88,1*(86,7;92,3)
SCI+L-arginine	2,51* (2,48;2,58)	1,85(1,63;1,94)	75,6*(72,6;79,4)
SCI+L-NAME+ Larginine	2,50* (2,54;2,53)	1,55*(1,49;1,61)	87,2*(84,7;91,2)

Notes: * – $p<0.05$ – in relation to the values in the «control» group; SCI – subtotal cerebral ischemia; L-NAME – Nω-nitro-L-arginine; 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 a non-selective NOS inhibitor – L-NAME, increases the degree of oxidative stress. This effect is due to the resulting deficiency in the synthesis of nitric oxide through the use of its inhibitor (L-NAME). L-NAME irreversibly inhibits both isoforms of constitutional NO synthases and causes reversible inhibition of inducible NOS. The introduction of the drug «L-arginine» has a slight corrective effect on the indicators of oxidative stress in SCI. The use of «L-arginine» in animals of the group with cerebral ischemia and the introduction of a non-selective NOS inhibitor – L-NAME did not have a significant positive effect. Thus, nitric oxide hyperproduction has been studied by stimulating synthesis by introducing the substrate L-arginine, and inhibition by using the non-selective NO synthase inhibitor N-nitro-L-arginine methyl ester.

References

1. Bon, E.I. (2018). Modeling methods and morphofunctional markers of cerebral ischemia. *Biomedicine*, 2:59-71.
2. Maksimovich, N.Ye. (2023). Oxidative Stress and Methods of Its Determination in Experimental Brain Pathology. *Archives of Urology and Nephrology*, 2(1):1-11.
3. Bon, E.I. (2022). Characteristics of disorders of the prooxidant-oxidant balance in rats with cerebral ischemia. *Ulyanovsk biomedical journal*, 3:97-106.
4. Maksimovich N.E. (2004). The role of nitric oxide of neuronal and extraneuronal origin in endothelial damage and the formation of its dysfunction during cerebral ischemia-reperfusion in rats. *Regional blood circulation and microcirculation*, 2(10):63-68.
5. Maksimovich, N. E. (2003). Amino acid L-arginine and prospects for its use in the clinic. *Healthcare*, 5:35-37.
6. Maksimovich, N.E. (2003). Amino acid L-Arginine and prospects for its use in the clinic. *Healthcare*, 5: 35-37.
7. Maksimovich, N.E. (2012). Mitochondriotropic effects of succinate and thiamine in ischemia-

reperfusion of the brain. *Tauride Biomedical Bulletin*, 15(3):203-206.

8. Maksimovich, N.E. (2012). Prospects for the use of a complex of metabolites and antioxidants as neuroprotectors in ischemia-reperfusion of the brain. *Skolkovo*, 68.
9. Maksimovich, N.E. (2004). The role of nitric oxide in the pathogenesis of ischemic and reperfusion brain damage. *Grodno*, 184.

Cite this article: Maksimovich N.Ye., Troyan E.I., Bon E.I, Kokhan N.V. (2023). Oxidative Stress Activity in Rats with Subtotal Brain Ischemia Under the Conditions of The Use of Modulators of the L-Arginine-No Pathway. *Journal of BioMed Research and Reports*, BRS Publishers. 1(1); DOI: https://www.doi.org/brs/2023/jbrr/0013