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ORIGINAL PAPER

Changes of the Nitric Oxide and Copper Content in the Olfactory Bulbs of Rat Brain After Modeling of Brain Stroke and Intranasal Administration of Mesenchymal Stem Cells

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Abstract

A comparative experimental analysis by EPR spectroscopy of the intensity of nitric oxide (NO) production and the content of the copper in the tissues of olfactory bulb of the brain of male Wistar rats were performed after modeling of ischemic stroke and treatment with immediate intranasal injection of mesenchymal stem cells (MSCs). Brain ischemia was simulated by ligation at the level of bifurcation of the common carotid arteries. It was found a significant reduction of NO content in the olfactory bulb of the brain of rats on the 1st and 2nd days after modeling of ischemia. The level of NO production was also reduced on the 1st and 2nd days after ischemia with MSCs' administration as compared to intact animals. It was not found the significant difference of the NO content in rats after ischemia with MSCs' administration relative to ischemic rats. The copper content in the olfactory bulb of the rat, which corresponds to the level of superoxide dismutase 1 and 3, tended to increase after modeling ischemia and remained for 2 days. The MSCs' administration was accompanied by a significant increase in copper content on the 1st day after modeling of ischemia and by decrease of its content on 2nd day after ischemia. The experiments showed that MSCs' administration did not affect the intensity of NO production on the 1st and 2nd days after the modeling of brain ischemia, but was accompanied by an increase in the antioxidant protection of the nervous tissue 1 day after ischemia.

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1 Introduction

Nitric oxide is chemically highly reactive free radical, which can act as an oxidizing agent as well as a reducing agent [1]. Therefore, there is an assumption about the diverse effects of NO in biological tissues. And this notion has been confirmed many times in experiments and applied studies. Consider the functions of NO in the nervous system. NO is known as one of the most vital signal molecules, which regulates the physiological functions of the organism and the metabolism of cells [2, 3]. The research of the role of NO in the transference of signals in the nervous system had begun shortly after its discovery [4–6]. Ever since then, it has been demonstrated that NO participates in different functions of the nervous system and regulates the proliferation and differentiation of nerve cells [3, 7]. It has also been discovered that NO acts as a neurotransmitter, which provides the relaxation for the smooth muscles [8, 9]. The role of NO in gastrointestinal, respiratory, and genitourinary tracts in the functions of the cardiovascular system has been proven [9–12].

In recent years, there are many facts, indicating that biosynthesis of NO is one of the key factors in the pathophysiological response of the brain to hypoxia–ischemia [5, 13–15]. One of the reasons of the involvement of NO into the pathological process is the prolonged lack of oxygen, which leads to brain hypoxia. Hypoxia is accompanied by the increase in a tissue ischemia, which always arises when the supply of body tissues with oxygen does not match the real requirement [16]. NO activates soluble heme-containing guanylate cyclase and ADP-ribosyl transferase [4, 8]. As a result of hydrolysis of cyclic guanosine monophosphate (cGMP) forms the type 5 phosphodiesterase [5, 17, 18]. In addition, NO is also involved in the regulation of the intracellular concentration of Ca^{2+} ions and in pH control against the background of cerebral ischemia [1, 3, 19]. Existing controversial data suggest that nowadays, there is no consensus on the role of endogenous NO in processes occurring with damages of the nervous system [20, 21]. In experiments with ischemia by the method of occlusion of the middle cerebral artery, there was found a sharp temporary increase in the activity of NO synthases (NOS) during the first hour of cerebral ischemia [22]. An immunohistochemistry analysis of citrulline, a marker of the activity of NO synthase, showed that ischemia caused a marked increase in the immunoreactivity of citrulline, and to a greater extent in the perifocal zone of the heart attack than in the tissue undergoing a stroke. This increase is due to the activation of a large population of nNOS, which is catalytically inactive under basal conditions, which indicates tight regulation of the physiological production of NO in the brain [23].

Cerebral ischemia causes multiple and multidirectional changes of NO in the brain and in signal transferring [15, 24]. In experiments with measuring of the activity of NO synthases, it was found an increase in the productivity of nNOS in the beginning of the ischemia [25], as well as at the beginning of the expression of inducible NOS (iNOS) a day after ischemia [26]. The increase of the production of NO within 60 min after the ischemia was demonstrated by EPR spectroscopy [27]. These data contradict to the results of other researchers [28, 29], which

showed the absence of the effect of NOS blockade on the brain after ischemia or even on extension of the injured site [30]. There are data containing information about successful usage of NO donors as neuroprotective agents after ischemic brain damage [31–33].

The authors of the article had conducted the previous research directed to the study of presence of NO in the locus of cerebral ischemia (left brain) by EPR spectroscopy, which showed that in the ischemic part of the left hemisphere of cortex, the presence of NO in the spinal trap decreased by five times in 5 h after the modeling of the ischemic stroke, and this decrease was maintained within a day after the stroke [14]. Another research demonstrated, that in 5 days after the occurrence of the signs as both ischemic and hemorrhagic stroke, the production of NO in the area of the hippocampus decreased by 2–3 times and this decrease was maintained within 24 and 72 h [34]. It was shown that ATP-dependent K-channels participate in these processes [35, 36].

In the past few decades, evidence has emerged that physiological renewal and tissue regeneration throughout the life of an animal and a human occur through stem cells [37–40]. The most important adult stem cell population is mesenchymal stem cells (MSCs) [41]. Brain MSCs are the focus of the great interest [18, 42]. Under physiological conditions, endogenous stem cells in these areas of the brain generate neuroblasts that migrate to those parts of the brain where an intensive formation of new neural networks is required [42]. The authors of the article in experiments on rodents found that intranasal administration of MSCs in the acute period of brain ischemia was accompanied by a faster restoration of the control of motor activity [36, 43]. According to this, the aim of this work was to study the intensity of NO production and the presence of copper (as an indicator of superoxide dismutase) in the olfactory bulbs of the brain of the rat by EPR spectroscopy after modeling ischemic stroke of the brain, as well as the effect of intranasal administration of MSCs in the acute period after obstruction of the common carotid arteries.

2 The Methods

2.1 The Protocol of the Experiment: Modeling the Ischemic Stroke in Rats and Administration Using a Trap for Nitrogen Oxide

The modeling of the ischemic stroke was carried out in accordance with the approved protocol of the Ethics Commission of the Institute of Physiology of the National Academy of Sciences of Belarus (NAS of Belarus), Minsk. The animals were kept under standard vivarium conditions (maintaining a 12/12-h rhythm of lighting and darkness, air temperature at 23 ± 1 °C, and a stable supply and exhaust ventilation) with free access to water and food (ad libitum) and the same diet in accordance with the standards for the maintenance of laboratory animals. Brain ischemia was modeling by ligating the common carotid arteries at the bifurcation level in male Wistar rats ($n = 20$) under ketamine–xylazine–acepromazine anesthesia [22, 44, 45]. The experimental animals were divided into two groups (ten animals in each). The collection of tissues of the olfactory bulb was performed 24 and 48 h after modeling of ischemia. The first

group represented animals after ischemic damage. Animals of the 2nd group were injected 50 µl of a suspension containing 400 thousand MSCs (intranasal administration) under the mucous membrane of the rat nasal cavity 10 min after the modeling of brain ischemia [44]. In both series, on the day of the experiment, rats were anesthetized by intraperitoneal administration of a mixture of ketamine–xylazine–acepromazine (55.6 mg, 5.5 mg and 1.1 mg/kg, accordingly). The third group of Wistar rats was intact animals ($n=10$), which were not subjected to surgical manipulations in the brain area. The components of the spinal trap for nitric oxide (DETC-Na, $FeSO_4$, sodium citrate) were administrated to the animals 30 min before the extraction of the studied tissues. After guillotine and brain isolation from the cranial cavity, rat brain fragments 1.5×1.5 mm in size were immediately isolated and frozen at the temperature of liquid nitrogen. Tissue fragments were stored and transferred in plastic containers with dry ice for measurements by EPR spectroscopy.

2.2 Formation of a NO Complex with a Spin Trap

The intensity of NO production by EPR spectroscopy was measured using the spin trap technique [19, 46], at the Zavoisky Physical-Technical Institute of KazSC RAS. Spin trap components: DETC-Na was administered intraperitoneally at a dose of 500 mg/kg in 2.5 ml of water, a mixture of solutions of iron sulfate ($FeSO_4{\cdot}7\ H_2O$, Sigma, USA) at a dose of 37.5 mg/kg and sodium citrate at a dose of 187.5 mg/kg solved in a volume of 1 ml of water per 300 g of the weight of the animal prepared immediately before the injection was injected subcutaneously at the right and left thigh and the rostral part of the interscapular region [47]. As a result, the compound DETC-Fe^{2+} is formed, which forms the stable radical $(DETC)_2$–Fe^{2+}–NO with NO. This $(DETC)_2$–Fe^{2+}–NO complex is characterized by an easily recognizable EPR spectrum with g-factor $g=2.038$ and a triplet hyperfine structure [19, 48]. In addition, the spin trap interacts with Cu to form the $Cu(DETC)_2$ complex, which can also be detected by the method of EPR spectroscopy [48]. The spectra of the complex $(DETC)_2$–Fe^{2+}–NO and $Cu(DETC)_2$ were measured on Brucker X spectrometers (9.50 GHz) EMX/plus with a temperature module ER 4112HV and ER 200 SRC with a magnetic field modulation of 100 kHz and a modulation amplitude of 2 G, with a microwave power of 30 mW, a time constant of 200 ms, and a temperature of 77 K in a finger Dewar of the Brucker company. In all experiments, the modulation amplitude, amplification, and microwave power were selected with the condition that there was no overmodulation and saturation of the EPR signal and remained the same throughout all measurements. The mass of the samples was about 100 mg. The amplitude of the EPR spectra was always normalized to the weight of the sample and to the amplitude of the EPR signal of the reference sample (the details of the method for measuring EPR signals were described by us earlier) [14].

2.3 Statistical Processing of the Result

The result is presented as $M\pm m$ (mean ± standard error of the mean). Statistical data processing was performed using t-student criterion. Differences were considered significant at $p<0.05$.

3 Results

Figure 1 shows the EPR spectrum of the tissues of the olfactory bulb of a healthy (intact) rat. A rectangle selection showed the characteristic triplet signal from the complex $(DETC)_2$–Fe^{2+}–NO with a *g*-factor equal to 2.038 [14, 46]. The signal from the complex $Cu(DETC)_2$ was also well represented in Fig. 1. Figure 2 shows the EPR spectrum of the tissues of the olfactory bulb of the rat 1 day after the modeling of ischemia caused by ligation of the carotid arteries. Figure 3 shows the EPR spectrum of the tissues of the olfactory bulb of the rat 1 day after the modeling of ischemia with simultaneous intranasal administration of MSCs. The solid bold line represents the spectrum of the sample, the thin line shows the signal from NO bound to the spin trap in the spectrum of the $(DETC)_2$–Fe^{2+}–NO complex, and the dashed line represents the signal from the $Cu(DETC)_2$ complex. The relative change in the amount of NO-containing complexes was evaluated by the integrated signal intensity from the spin trap $(DETC)_2$–Fe^{2+}–NO. The relative change in the content of copper was also evaluated by the integrated signal intensity of the complex Cu(D ETC)$_2$.

Figure 4 shows data on the average values of NO production intensities. The results show a significant ($p < 0.05$) decrease in the NO content in the olfactory bulb 1 day after the modeling of ischemia caused by ligation of the carotid arteries ($n = 10$). No difference in the content of NO in the olfactory bulb between ischemic rats and rats with the simultaneous intranasal administration of MSCs ($n = 10$) after modeling of ischemia was found. After 2 days, content of NO in the olfactory bulb of ischemic rats tended to decrease even more. In the olfactory bulb of rats, in which ischemia was modeled simultaneously with the intranasal administration of MSCs, there was no significant difference in the content of NO relative to ischemic rats 2 days after modeling of ischemia (Fig. 4). Thus, an analysis of the results showed a significant decrease in the content of NO after

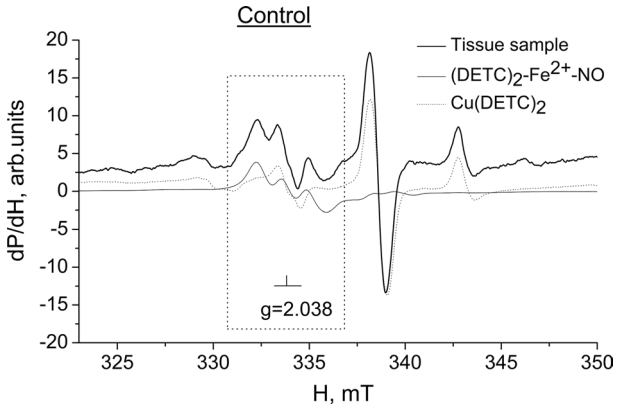

Fig. 1 EPR spectra of olfactory bulb of healthy rat. The signals from: (a) tissue sample, (b) complex $(DETC)_2$–Fe^{2+}–NO, and (c) complex $Cu(DETC)_2$. Temperature is 77 K. The rats were injected with (DETC)–Fe^{2+}–citrate. $g = 2.038$

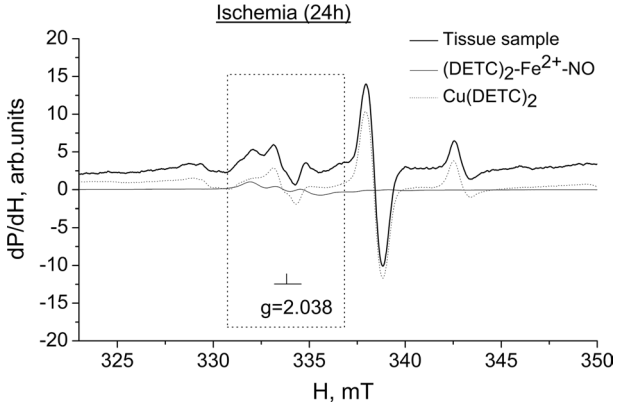

Fig. 2 EPR spectra of olfactory bulb of rat after modeling of ischemia. The signals from: (a) tissue sample, (b) complex $(DETC)_2$–Fe^{2+}–NO, and (c) complex $Cu(DETC)_2$. Temperature is 77 K. The rats were injected with (DETC)–Fe^{2+}–citrate. $g = 2.038$

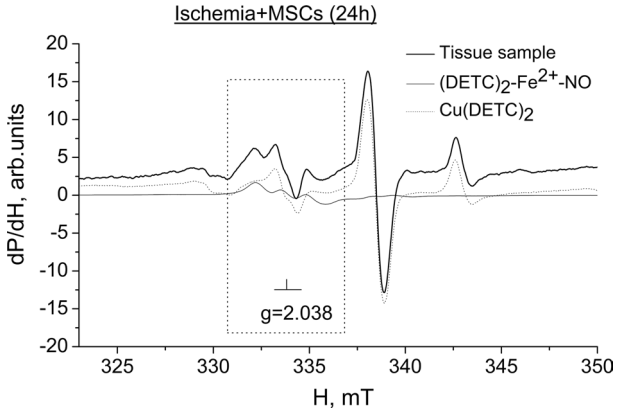

Fig. 3 EPR spectra of olfactory bulb of rat after modeling of ischemia with simultaneously intranasal administration of MSC. The signals from: (a) tissue sample, (b) complex $(DETC)_2$–Fe^{2+}–NO, and (c) complex $Cu(DETC)_2$. Temperature is 77 K. The rats were injected with (DETC)–Fe^{2+}–citrate. $g = 2.038$

ischemia; however, intranasal administration of MSCs does not change the content of NO relative to ischemic rats despite a significant restoration of the motor activity of animals [36]. The content of copper, which corresponds to the level of superoxide dismutases 1 and 3 increased in the olfactory bulb of the rat but not significantly 1 day after the modeling of ischemia caused by ligation of the carotid arteries and persisted after 2 days (Fig. 5). Intranasal administration of MSCs was accompanied by a significant ($p < 0.05$) increase in the content of Cu 1 day after the modeling of ischemia, and decreased 2 days after.

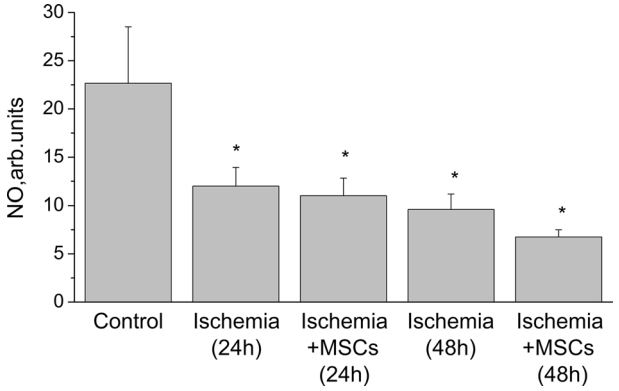

Fig. 4 Relative content of NO in the olfactory bulb of healthy rats (Control) and rats after 1 (Ischemia, 24 h) and 2 days (Ischemia, 48 h) after modeling of ischemia and rats after 1 (Ischemia, 24 h + MSC) and 2 days (Ischemia, 48 h + MSC) after modeling of ischemia with simultaneously intranasal administration of MSC. The ordinates axis is the average integral intensity of the signal

4 Discussion

4.1 Discussion of the Design of Experiment

Traumatic and ischemic brain injuries continue to be one of the most difficult problems of modern medicine [49]. The study of the mechanisms of reparative processes in the nervous tissue, and the development of new methods for the restoration of neuronal structures are one of the relevant areas in physiology and medicine and have a great importance for the development of new therapeutic and rehabilitation strategies [1, 32, 50]. Brain ischemia, which fatally ends with an ischemic

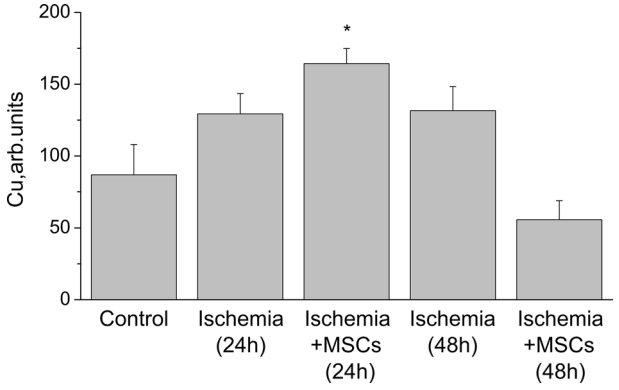

Fig. 5 Relative content of NO in the olfactory bulb of healthy rats (Control) and rats after 2 days after modeling of ischemia (Ischemia, 24 h) and rats after 2 days after modeling of ischemia with simultaneously intranasal administration of MSC (Ischemia, 24 h + MSC). The ordinates axis is the average integral intensity of the signal

stroke, often occurs with cerebral blood flow disorders, which is accompanied by an in sufficient supply of oxygen in the brain, and the development of hypoxia of varying severity [18, 32]. For experimental modeling of cerebral ischemia, various approaches are used, among which the authors focused on the model of ischemia, which is achieved after rapid ligation of the common carotid arteries at the level of their bifurcation. This technique takes a few minutes, which is important to minimize side effects [22, 45]. The use of a spin trap for binding NO imposes certain restrictions on the experimental protocol associated with the toxicity of the drug and the need for signal accumulation within 30 min [19, 46, 48, 51]. In this regard, in all experiments, a spin trap for NO was introduced 30 min before euthanasia.

4.2 Discussion of the Results of Experiment

In present time, there is a conception that the primary brain injuries and secondary injuries caused by stroke are the complex pathophysiological processes, consisting of the inflammatory reaction, apoptosis of neurons, ischemic reperfusion injury, producing of free radicals, etc. [16, 18]. On one hand, the development of cerebral ischemia and the subsequent occurrence of stroke are associated with impaired cerebral blood flow, as well as impaired regulation of the blood supply to brain tissue by the NO system [5, 13–15, 50, 52]. On the other hand, hypoxia resulting from ischemic stroke itself is accompanied by early cell death in various parts of the brain and subsequent programmed late death of other cells by apoptosis [16, 53].

According to the results of studies, the role of NO in these hypoxia–ischemia processes is contradictory, as NO can perform both neurotoxic and neuroprotective functions [20, 21]. There are many reasons for this diversity of NO functions. First, in addition to the main synthesis of NO by NO synthases [5, 7, 15], there is also a nitroreductase-dependent component of the NO cycle, when NO is formed from nitrites and nitrates [52, 54]. Second, there are a significant number of depots for NO, which interacts with iron-containing complexes (e.g., heme structures), with thiols and other compounds [12, 45, 55, 56]. Third, it is shown that NO causes a change in the length and width of the synaptic active zone [57]. Fourth, there is a dependence of NO content from age. The authors found that the concentration of NO in the tissues of the heart and liver of rats changed with age. In addition, the signal from the T-conformer disappeared with age [11, 58].

Fifth, it should be noted that, depending on the objectives of the study, experimenters use different models of ischemia, which develop according to different mechanisms. First of all, this concerns the expression of various types of NOS. Most authors agree that the main functions of NO, as a signaling mechanism that regulates almost all critical cellular functions, are provided by endothelial NOS (eNOS) [53, 59]. Activation of eNOS facilitates recovery from ischemic damage [60]. Evidence on the role of neuronal NOS (nNOS) in the development of brain ischemia is controversial [3, 15]. For example, it is considered that the production of NO by neurons exacerbates acute ischemic damage, while vascular NOS (eNOS) protects after occlusion of the middle cerebral artery. Other authors indicate that overexpression of NO begins with the activation of a large population

of nNOS [23]. The NO generated by inducible NOS (iNOS) has potential as a pathological source, also leading to overexpression of the transmitter and disruption of normal physiological processes [7, 61]. For this reason, it is advantageous to block the activity of nNOS and iNOS during the development of hypoxia to prevent brain ischemia. However, these recommendations are some relative and archaic, because the authors forget about the presence in the brain of many neurotransmitter systems and trophic factors that are involved in the stabilization of brain functions during hypoxia and ischemia.

Our experiments showed a significant decrease in the intensity of NO production by about 2 times during the first 2 days after modeling of ischemia. This demonstrated that the conditions for activation, for example, of iNOS were not formed in the model of brain ischemia chosen by the authors. Previously, an increase of the NOS activity during the first hour of cerebral ischemia was found in the same model of ischemia [22]. However, 7 days after, the level of NO in the brain tissue decreased below the level that can be detected, which indicated a long-term deficiency of NO in the ischemic brain. These results related not only with a number of data from other authors, but also with our previous results, where we used other methods for modeling ischemia [14, 34, 36, 45]. The literature data show that the cytotoxic effects of NO are significantly associated with peroxynitrite formed in the reaction between NO and another free radical, superoxide anion [1, 7, 21, 59]. The dismutation of superoxide with the help of the cytosolic enzyme Cu,Zn-COD (superoxide dismutase) is the primary and main protection against free radical oxidation processes; however, peroxynitrite formed during excessive NO production can inactivate the COD enzyme and accelerate free radical oxidation processes [7, 56]. In this study, it was shown that the copper content (as an indicator of COD level) in the olfactory bulb increases in the first day after the modeling of ischemic stroke. These results demonstrated a clear increase in the antioxidant defense of the brain in the first day after modeling of ischemia. Based on such data, a therapy protocol that focuses on enhancing the antioxidant system can be proposed.

It is recognized that the difference of the effects of NO depends on its concentration in the tissue [1, 7]. Precision determination of the amount of NO in tissues under various functional and pathological conditions is important not only for experimental studies, but also is relevant for practicing physicians [62]. This is about the fact that donors, precursors of the NO, and modulators of the functional activity of NO receptors are widely used in clinical practice.

There is another aspect of the problem in addition to the above. Currently, the use of stem cells in regenerative medicine as a leading technique of cell therapy is a main trend [43, 63]. Researchers and clinicians are attracted by the therapeutic properties of MSCs [41, 64], including brain MSCs [42, 65]. It was shown that intranasal administration of MSCs after modeling ischemia is accompanied by a rapid restoration of motor activity in experimental animals [36]. Therefore, we decided to verify the role of NO in this process. The experiments showed that the administration of MSCs did not affect the intensity of NO production on the 1st and 2nd days after modeling of ischemia, but increased the antioxidant defense of the brain after ischemia.

5 Conclusion

Thus, the experiments revealed a significant decrease in the intensity of NO production by two times during the first 2 days after modeling of ischemia. This demonstrated that the conditions for the activation of iNOS were not formed in our model of brain ischemia. These results related not only with a number of data of other authors [23, 60, 66], but also with our previous results, where we used the other methods of ischemia modeling [14, 34, 36, 45]. We suggested that it was found the time period of the development of processes associated with cerebral ischemia which allowed to propose the effective time period for therapy based on involving of the NO donors. Also it was found an increase in the copper content as an indicator of the antioxidant defense of the brain in the first day after the modeling of ischemia and its amplification (24 h after the modeling of ischemia) when using MSCs. Therefore, a therapy protocol based on the activation of the antioxidant system of the body can be assumed.

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References

1. A.F. Vanin, Nitric Oxide **54**, 15–29 (2016). https://doi.org/10.1016/j.niox.2016.01.006
2. A.F. Vanin, Biohimia **63**, 924–938 (1998). **(in Russian)**
3. J. Garthwaite, Eur. J. Neurosci. **27**, 2783–2802 (2008). https://doi.org/10.1111/j.1460-9568.2008.06285.x
4. J. Garthwaite, C.L. Boulton, Annu. Rev. Physiol. **57**, 683–706 (1995)
5. J.P. Bolanos, A. Almeida, Biochim. Biophys. Acta **1411**, 415–436 (1999)
6. D. Boehning, S.H. Snyder, Annu. Rev. Neurosci. **26**, 105–131 (2003). https://doi.org/10.1146/annurev.neuro.26.041002.131047
7. J.R. Steinert, T. Chernova, I.D. Forsythe, Neuroscientist **16**, 435–452 (2010). https://doi.org/10.1177/1073858410366481
8. G.F. Sitdikova, A.L. Zefirov, Rossijskii fiziologicheskii zhurnal im. I. M. Sechenova **92**, 872–882 (2006). **(in Russian)**
9. V.L. Lakomkin, A.F. Vanin, A.A. Timoshin, V.I. Kapelko, E.I. Chazov, Nitric Oxide Biol. Chem. **16**(4), 413–418 (2007). https://doi.org/10.1016/j.niox.2007.03.002
10. V.P. Reutov, V.E. Okhotin, A.V. Shuklin, E.G. Sorokina, N.S. Kosicin, V.N. Gurin, Uspehi fiziologicheskih nauk **38**, 39–58 (2007). **(in Russian)**
11. V.V. Andrianov, F.G. Sitdikov, Kh.L. Gainutdinov, S.V. Yurtaeva, L.N. Muranova, A.A. Obynochnyi, F.K. Karimov, V.M. Chiglintsev, V.S. Iyudin, J. Dev. Biol. **38**, 352–356 (2008)
12. M.I. Remizova, N.I. Kochetygov, K.A. Gerbout, V.L. Lakomkin, A.A. Timoshin, E.N. Burgova, A.F. Vanin, Eur. J. Pharmacol. **662**(1–3), 40–46 (2011). https://doi.org/10.1016/j.ejphar.2011.04.046
13. E.B. Manukhina, I.Y. Malyshev, B.V. Smirin, S.Y. Mashina, V.A. Saltykova, A.F. Vanin, Nitric Oxide **3**, 393–401 (1999). https://doi.org/10.1006/niox.1999.0244

14. Kh.L. Gainutdinov, S.A. Gavrilova, V.S. Iyudin, A.V. Golubeva, M.P. Davydova, G.G. Jafarova, V.V. Andrianov, V.B. Koshelev, Appl. Magn. Reson. **40**, 267–278 (2011)
15. N.A. Terpolilli, M.A. Moskowitz, N. Plesnila, J. Cereb. Blood Flow Metab. **32**, 1332–1346 (2012). https://doi.org/10.1038/jcbfm.2012.12
16. Z.Q. Chen, R.T. Mou, D.X. Feng, Z. Wang, G. Chen, Med. Gas Res. **7**(3), 194–203 (2017). https://doi.org/10.4103/2045-9912.215750
17. J.D. Corbin, S.H. Francis, J. Biol. Chem. **274**, 13729–13732 (1999). https://doi.org/10.1074/jbc.274.20.13729
18. R.L. Zhang, Z.G. Zhang, M. Chopp, Expert Opin. Investig. Drugs **22**(7), 843–851 (2013). https://doi.org/10.1517/13543784.2013.793672
19. A.F. Vanin, A. Huisman, E.E. Van Faassen, Pitfalls Successes **359**, 27–42 (2003). https://doi.org/10.1016/s0076-6879(02)59169-2
20. A. Godecke, J. Schrader, Circ. Res. **94**, e55–e57 (2004)
21. V. Calabrese, C. Mancuso, M. Calvani, E. Rizzarelli, D.A. Butterfield, A.M.G. Stella, Nat. Rev. Neurosci. **8**, 767–775 (2007). https://doi.org/10.1038/nrn2214
22. A. Kader, V.I. Frazzini, R.A. Solomon, R.R. Trifiletti, Stroke **24**, 1709–1716 (1993)
23. M.J.L. Eliasson, Z. Huang, R.J. Ferrante, M. Sasamata, M.E. Molliver, S.H. Snyder, M.A. Moskowitz, J. Neurosci. **19**(14), 5910–5918 (1999)
24. M.A. Salykina, E.G. Sorokina, I.A. Krasilnikova, V.P. Reutov, V.G. Pinelis, Bull. Exp. Biol. Med. **155**, 40–43 (2013). https://doi.org/10.1007/s10517-013-2075-7
25. A.F. Samdani, T.M. Dawson, V.L. Dawson, Stroke **28**, 1283–1288 (1997)
26. C. Iadecola, F. Zhang, R. Casey, M. Nagayama, M.E. Ross, J. Neurosci. **17**, 9157–9164 (1997)
27. T. Tominaga, S. Sato, T. Ohnishi, S.T. Ohnishi, J. Cereb. Blood Flow Metab. **14**, 715–722 (1994)
28. D.A. Dawson, K. Kusumoto, D.I. Graham, J. McCulloch, I.M. Macrae, Neurosci. Lett. **142**, 151–154 (1992)
29. G. Sancesario, M. Iannone, M. Morello, G. Nistico, G. Bernardi, Stroke **25**, 436–443 (1994)
30. S. Yamamoto, E.V. Golanov, S.B. Berger, D.J. Reis, J. Cereb. Blood Flow Metab. **12**, 717–726 (1992)
31. M. Willmot, L. Gray, C. Gibson, S. Murphy, P.M. Bath, Nitric Oxide **12**, 141–149 (2005). https://doi.org/10.1016/j.niox.2005.01.003
32. M. Godinez-Rubi, A.E. Rojas-Mayorquin, D. Ortuno-Sahagun, Oxid. Med. Cell. Longev. **13**, 1 (2013)
33. V.S. Kuzenkov, A.L. Krushinskii, V.P. Reutov, Bull. Exp. Biol. Med. **155**, 748–751 (2013)
34. V.V. Andrianov, S.G. Pashkevich, G.G. Yafarova, A.A. Denisov, V.S. Iyudin, T.Kh. Bogodvid, M.O. Dosina, V.A. Kulchitsky, Kh.L. Gainutdinov, Appl. Magn. Reson. **47**(9), 965–976 (2016)
35. O.G. Deryagin, S.A. Gavrilova, S.V. Buravkov, V.V. Andrianov, G.G. Yafarova, Kh.L. Gainutdinov, V.B. Koshelev, Neurosci. Behav. Physiol. **48**, 58–63 (2018). https://doi.org/10.1007/s11055-017-0530-z
36. T. Bogodvid, O. Deryagin, S. Gavrilova, S. Buravkov, V. Andrianov, G. Yafarova, K. Gainutdinov, V. Koshelev, Eur. J. Clin. Investig. **49**(Suppl 1), 161 (2019). https://doi.org/10.1111/eci.13109
37. U. Reincke, H. Burlington, E.P. Cronkite, J. Laissue, Fed. Proc. **34**, 71–75 (1975)
38. Y. Kong, R. Xu, M.A. Darabi, W. Zhong, G. Luo, M.M. Xing, J. Wu, Int. J. Nanomed. **11**, 2543–2555 (2016). https://doi.org/10.2147/IJN.S102861
39. L. Harris, O. Zalucki, S. Oishi, T.H. Burne, D.J. Jhaveri, M. Piper, Dev. Dyn. **247**, 194–200 (2018). https://doi.org/10.1002/dvdy.24545
40. B. Lukomska, L. Stanaszek, E. Zuba-Surma, P. Legosz, S. Sarzynska, K. Drela, Stem Cells Int. **19**, 9628536 (2019). https://doi.org/10.1155/2019/9628536
41. E. Eggenhofer, F. Luk, M.H. Dahlke, M.J. Hoogduijn, Front. Immunol. **5**, 148 (2014). https://doi.org/10.3389/fimmu.2014.00148
42. C.R. Harrell, C. Fellabaum, N. Jovicic, V. Djonov, N. Arsenijevic, V. Volarevic, Cells **8**(5), 467 (2019). https://doi.org/10.3390/cells8050467
43. V. Kulchitsky, A. Zamaro, Y. Shanko, S. Koulchitsky, J. Neurol. Stroke **8**(2), 87–88 (2018). https://doi.org/10.15406/jnsk.2018.08.00286
44. T. Bogodvid, S. Pashkevich, M. Dosina, A. Zamaro, Y. Takalchik, G. Yafarova, V. Andrianov, A. Denisov, D. Loiko, K. Gainutdinov, V. Kulchitsky, Eur. J. Clin. Investig. **49**(Suppl 1), 161 (2019). https://doi.org/10.1111/eci.13109

45. Y. Shanko, A. Zamaro, S.Y. Takalchik, S. Koulchitsky, S. Pashkevich, E. Panahova, V. Navitskaya, M. Dosina, A. Denisov, S. Bushuk, V. Kulchitsky, J. Sci. Tech. Res. **7**(5), 1–2 (2018). https://doi.org/10.26717/bjstr.2018.07.001567
46. O.G. Deryagin, S.A. Gavrilova, Kh.L. Gainutdinov, A.V. Golubeva, V.V. Andrianov, G.G. Yafarova, S.V. Buravkov, V.B. Koshelev, Front. Neurosci. **11**, 427 (2017). https://doi.org/10.3389/fnins.2017.00427
47. V.D. Mikoyan, L.N. Kubrina, V.A. Serezhenkov, R.A. Stukan, A.F. Vanin, Biochim. Biophys. Acta **1336**, 225–234 (1997). https://doi.org/10.1016/S0304-4165(97)00032-9
48. Kh.L. Gainutdinov, V.V. Andrianov, V.S. Iyudin, S.V. Yurtaeva, G.G. Jafarova, R.I. Faisullina, F.G. Sitdikov, Biophysics **58**, 203–205 (2013)
49. E.E. van Faassen, M.P. Koeners, J.A. Joles, A.F. Vanin, Nitric Oxide **18**, 279–286 (2008). https://doi.org/10.1016/j.niox.2008.02.003
50. G.A. Donnan, M. Fisher, M. Macieod, S.M. Davis, Stroke Lancet **371**, 1612–1623 (2008)
51. S.E. Lakhan, A. Kirchgessner, M. Hofer, J. Transl. Med. **7**, 97 (2009). https://doi.org/10.1186/1479-5876-7-97
52. A.I. Ismailova, O.I. Gnezdilov, L.N. Muranova, A.A. Obynochny, V.V. Andrianov, Kh.L. Gainutdinov, A.G. Nasyrova, R.R. Nigmatullina, F.F. Rahmatullina, A.L. Zefirov, Appl. Magn. Reson. **28**, 421–430 (2005)
53. P.S. Garry, M. Ezra, M.J. Rowland, J. Westbrook, K.T.S. Pattinson, Exp. Neurol. **263**, 235–243 (2015). https://doi.org/10.1016/j.expneurol.2014.10.017
54. M.H.K. Ansari, P. Karimi, N. Shakib, S.M. Beyrami, Crescent J. Med. Biol. Sci. **5**(1), 50–56 (2018)
55. V.P. Reutov, Biochemistry **67**(3), 293–311 (2002)
56. S.V. Yurtaeva, V.N. Efimov, G.G. Yafarova, A.A. Eremeev, V.S. Iyudin, A.A. Rodionov, Kh.L. Gainutdinov, I.V. Yatsyk, Appl. Magn. Reson. **47**(6), 555–565 (2016)
57. V.E. Prusakov, Y.V. Maksimov, D.Sh. Burbaev, V.A. Serezhenkov, R.R. Borodulin, N.A. Tkachev, V.D. Mikoyan, A.F. Vanin, Appl. Magn. Reson. **50**(7), 861–881 (2019)
58. V.P. Reutov, N.V. Samosudova, E.G. Sorokina, Biophysics **64**(2), 233–250 (2019)
59. V. Andrianov, F. Sitdikov, R. Zaripova, G. Yafarova, L. Muranova, S. Yurtaeva, V. Iyudin, M. Sungatullina, T. Zefirov, K. Gainutdinov, Eur. J. Clin. Investig. **49**(Suppl 1), 95–96 (2019). https://doi.org/10.1186/1471-2202-7-81
60. P. Pacher, J.S. Beckman, L. Liaudet, Physiol. Rev. **87**, 315–427 (2007). https://doi.org/10.1152/physrev.00029.2006
61. K. Gertz, M. Endres, Future Neurol. **3**(5), 537–550 (2008)
62. K. Fassbender, M. Fatar, A. Ragoschke, M. Picard, T. Bertsch, S. Kuehl, M. Hennerici, Stroke **31**, 2208–2211 (2000)
63. C.L. Hawkins, M.J. Davies, Biochim. Biophys. Acta **1840**, 708–721 (2014). https://doi.org/10.1016/j.bbagen.2013.03.034
64. P.P. Nimiritsky, R.Y. Eremichev, N.A. Alexandrushkina, A.Y. Efimenko, V.A. Tkachuk, P.I. Makarevich, Int. J. Mol. Sci. **20**(4), 823 (2019). https://doi.org/10.3390/ijms20040823
65. N. Kim, S.-G. Cho, Korean J. Intern. Med. **28**, 387–402 (2013). https://doi.org/10.1186/1756-8722-5-19
66. Y. Stukach, Kh. Gainutdinov, M. Dosina, S. Pashkevich, V. Andrianov, A. Denisov, T. Bogodvid, G. Yafarova, S. Bushuk, T. Kuznetsova, V. Kulchitsky, J. Stem Cells Regen. Ther. **1**, 1–8 (2016)
67. Z. Jiang, C. Li, D.M. Arrick, S. Yang, A.E. Baluna, PLoS ONE **9**(3), e93134 (2014). https://doi.org/10.1371/journal.pone.0093134

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