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The Effects of Intranasal Implantation of Mesenchymal Stem Cells on Nitric Monoxide Levels in the Hippocampus, Control of Cognitive Functions, and Motor Activity in a Model of Cerebral Ischemia in Rats

Guzel Yafarova[1] · Yulia Tokalchik[2] · Tatiana Filipovich[2] · Vyacheslav Andrianov[1,3] · Lyeh Bazan[3] · Tatiana Bogodvid[1,4] · Chihab Abdulla[1] · Aleksandra Zamaro[2] · Vladimir Kulchitsky[1] · Khalil Gainutdinov[1,3]

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Abstract

Hypoxia occurs in situations of disbalance between metabolic needs and the supply of oxygen to organs and tissues of the body. In this regard, tissue hypoxia and ischemia are essential components of the pathogenesis of many diseases. One of the promising areas of research into the mechanisms of ischemia is attempting to weaken the negative effect of hypoxia and ischemia in the brain by using a variety of techniques that activate neuroprotective mechanisms. Here, we aimed to assess the dynamics of restoration of motor activity control in an experimental model of ischemic stroke in rats (cerebral ischemia, CI) after intranasal perineural implantation of mesenchymal stem cells into the receptive field of the olfactory nerve. It was found that the perineural administration of MSCs to rats in the acute period of cerebral ischemia was accompanied by clear signs of recovery of cognitive and motor functions within 1 and 3 days after the operation. On the seventh day after ischemia modeling, rats with the introduction of MSCs had no distinctive features in the control of motor activity compared to the period before the operation in the same rats. In the hippocampus of rats after modeling ischemia, a significant decrease in the content of NO by about 50% relative to the initial level is observed after 1 day. In the hippocampus of rats in which ischemia was modeled with simultaneous intranasal administration of MSC, a significant decrease in NO content by 39% relative to the initial level was also observed after 1 day. The content of NO increases slightly, but the difference in the level of NO relative to ischemic rats was not significant. The copper content in the hippocampus in the rats of these two groups did not change. There was a tendency to increase the efficiency of the antioxidant system 1 day after ischemia in both studied groups, and this effect was more pronounced with intranasal administration of MSC.

Keywords Mesenchymal stem cells · Ischemic brain stroke · Nitric oxide · Copper · Electron paramagnetic resonance

✉ Khalil Gainutdinov
kh_gainutdinov@mail.ru

Guzel Yafarova
gusadila@mail.ru

Yulia Tokalchik
stukachyulya@gmail.com

Tatiana Filipovich
semionik88@mail.ru

Vyacheslav Andrianov
slava_snail@yahoo.com

Lyeh Bazan
l_v_bazan@mail.ru

Tatiana Bogodvid
tat-gain@mail.ru

Chihab Abdulla
abdallash95@gmail.com

Aleksandra Zamaro
zamaroal@gmail.com

Vladimir Kulchitsky
vladi@fizio.bas-net.by

[1] Department of Human and Animals, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russian Federation

[2] Brain Center, Institute of Physiology, National Academy of Sciences, Minsk, Belarus

[3] Zavoisky Physical-Technical Institute of the Russian Academy of Sciences, Kazan, Russian Federation

[4] Volga Region State University of Physical Culture, Sport and Tourism, Kazan, Russian Federation

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1 Introduction

The issues of an effective and sustainable increase in the resistance of the human body to the development of hypoxia and ischemia have not yet been resolved. Hypoxia occurs in situations of discrepancies between the metabolic needs of the whole organism and/or various organs and tissues and their oxygen supply [1–3]. In this regard, tissue hypoxia and ischemia are key components of the pathogenesis of many diseases [4–6]. The problem of hypoxia and ischemia is especially relevant for the neural networks of the brain, which control the somatic and visceral functions of the body and can function optimally only with a stable supply of oxygen. A significant number of studies have been devoted to seeking mechanisms and effective approaches to adapting to hypoxia in the whole organism and various organs and tissues [2, 7–10]. One of the directions in this aspect is an attempt to reduce the negative effects of hypoxia and ischemia on the brain using a variety of techniques that activate endogenous neuroprotective mechanisms [11–14]. For this purpose, it seems promising to use mesenchymal stem cells (MSCs), which have a high reparative and immunosuppressive potential, as well as a directed migratory ability to reach damaged tissues to activate proliferative processes in damaged areas [15–18]. The positive effects of MSCs are hypothetically explained by the inhibition of lipid peroxidation, an increase in the antioxidant properties of cells and tissues, which is accompanied by an active release of pro-angiogenic and anti-apoptotic cytokines from MSCs, control of inflammation and immune responses, activation of reparative processes in cells and tissues, accompanied by an increase in resistance to hypoxia [10, 14, 16, 18, 19].

To rationally use MSCs, methods for their targeted delivery to damaged areas of the brain are being actively developed [20–25]. For example, systemic administration of stem cells into venous or arterial vessels of the bloodstream of patients has been used in cases of developing destructive processes in the CNS [26, 27]. The disadvantage of this technique is the diffuse distribution of MSCs throughout the circulatory system of the whole organism, so a relatively small number of cells reach the blood–brain barrier, and an even smaller number passes it (about 1–3%) [28]. The introduction of MSCs into the cerebrospinal fluid pathways during brain injuries was tested, most often by lumbar puncture [29], without taking into account the need for MSCs to overcome the intense cerebrospinal fluid flow from the brain to the spinal cord. Another method of MSC implantation is based on the local injection of cells during neurosurgical operations into the damaged area of the brain [22–25, 30].

The indicated shortcomings of the abovementioned methods of treating brain injuries and stroke using MSCs are pushing the search for more effective ways to implant MSCs in the brain. In previous studies, it was found by many authors that within no more than 1 hour after implanting MSCs into the submucosal space of the nasal cavity of rats in the region of the terminals of the olfactory nerve, MSCs begin to accumulate in damaged areas of the brain and continue to accumulate throughout the day [31, 32, 33, 34, 35, 36, 37]. Nevertheless, there is work that denies the possibility of migration of stem cells from the nasal cavity to the brain [38]. It has been shown that MSC migration is not carried out randomly throughout the brain, but rather in somatotopic dependence from the endings of the olfactory nerve, in the nasal cavity, to the olfactory bulb into the anterior cranial fossa and from the terminals of the trigeminal nerve to its nuclei in the brainstem (in the posterior cranial fossa) due to their perineural migration [21, 25, 32, 33, 38, 39]. Suggestively, it is promising to further investigate the possibility of using intranasal stem cell implantation with the aim of their subsequent migration to the brain for the treatment of various brain diseases [34–38, 40].

Thus, here, we have studied and established the conditions for experimental substantiation of the selective methods of perineurally implanting stem cells for their subsequent migration into the anterior or posterior cranial fossa to activate reparative and metabolic processes in the brain aiming at restoring damaged neural networks and controlling impaired brain functions after a stroke [25, 33, 39].

Studies of stroke models reveal that restoration of brain function involves many mechanisms, including cerebral angiogenesis, axonal and dendritic growth, oligodendrogenesis, neurogenesis, and restoration of the balance of neurotransmitters and signaling molecules. At present, a group of diseases has been experimentally and clinically identified, in the development of which pronounced changes in the exchange of the gaseous mediator nitric oxide (NO) are observed. Among them, first of all, diseases of the cardiovascular system, as well as hypertension, myocardial infarction, pulmonary hypertension and atherosclerosis, in addition, stroke occupies a special place in this list [41–44]. NO is capable of exerting both activating and inhibitory effects on various metabolic processes in the human body [45, 46]. NO plays an essential role in the functioning of neurons in the brain and the potentiation of long-term memory [44, 47].

Elucidation of the role of NO in the physiology and pathology of the cell makes it possible to modulate the NO generation systems to enhance the protective and physiological action of NO and simultaneously limit its damaging effects. It should be noted that, according to scientific literature, the effects of NO depend not only on its concentration, but also on the site of its production by isoforms of NO synthase (NOS), diffusion in cells and tissues, interaction with reactive oxygen species, and many other factors [45, 46].

Therefore, both the concentration and the site of NO generation in body tissues from substrates or pharmacological donors of NO should be taken into account when predicting the results of its biological action.

Recently, electron paramagnetic resonance (EPR) has been considered one of the most effective methods for the detection and quantitative determination of NO in biological tissues [48, 49]. This is due to the development of the technique by Vanin et al. [48] using the spin capture method, which is based on the reaction of a radical (in this case, NO) with a spin trap. As a result of this reaction, an adduct with a characteristic EPR spectrum is formed. The authors used the Fe^{2+} complex with diethyldithiocarbamate (DETC) to capture NO and form a stable ternary complex $(DETC)_2$-Fe^{2+}-NO in various animal tissues [48]. This complex is characterized by an easily recognizable EPR spectrum with a *g*-factor, $g = 2.035–2.040$, and a triplet hyperfine structure. This method has a sensitivity of 0.04–0.4 nM allowing direct measurements and is highly sensitive due to the use of spin traps [48, 49].

The purpose of the study was to evaluate the dynamics of restoring the control of cognitive and motor functions as well as NO production in the hippocampus of rats in an experimental model of ischemic stroke in the hippocampus after intranasal implantation of MSCs into the receptive field of the olfactory nerve for subsequent perineural migration of MSCs to damaged areas of the brain located in the anterior and middle cranial fossae.

2 Materials and Methods of Research

The experiments were carried out on male rats ($n = 90$), weighing 270–300 g aged 7–8 months. The animals were kept under standard vivarium conditions with access to food and water ad libitum. All surgical procedures were performed on anaesthetized animals (55.6 mg/kg ketamine, 5.5 mg/kg xylazine, 1.1 mg/kg acepromazine, intraperitoneally).

Cerebral ischemia (CI) was modeled by ligation of both common carotid arteries at the bifurcation level (in the Brain Center, Institute of Physiology of the National Academy of Sciences of Belarus, Minsk) [17, 50, 51]. Three groups of animals were formed: (1) group 1, CI (in this group, rats were anaesthetized and surgical access to the area of the carotid arteries was established, with further bilateral ligation of the common carotid arteries; $n = 30$); (2) group 2, CI + MSC (included animals after ligation of the common carotid arteries with simultaneous intranasal perineural administration of MSCs; $n = 30$); (3) the third group is a control group (anesthesia and surgical access to the carotid arteries area is used for this, but without ligation of the common carotid arteries) (control, $n = 30$). In each group, 10 animals underwent behavioral tests, and the hippocampus was extracted from 10 animals after decapitation for subsequent measurements of NO and Cu by EPR spectroscopy. Another 10 animals in each group did not participate in the experiments; they served as a survival control during the entire time of the experiments.

For implantation of stem cells, primary culture of MSCs obtained from the adipose tissue of female rats ($n = 13$), weighing 250–300 g, was used according to the method approved by the Scientific Council of the Institute of Physiology of the National Academy of Sciences of Belarus (protocol No. 8, 26.08.2010). MSCs were obtained according to the recommendation of experts in cell technology from the adipose tissue of females, due to special features such as the size and blood supply of adipose tissue in females [22, 25, 52–54]. Detailed protocol for the primary isolation and cultivation of adipose tissue-derived stem cells, their phenotypic profile, and comparison with the characterization of MSCs from rat bone marrow are given in the available publications [18, 55, 56]. MSCs were cultured for 7 days. The concentration of living cells in the suspension ready for implantation was 50,000 cells in 50 µl of phosphate buffer solution (counting was carried out in a Goryaev chamber). To initiate the movement of implanted cells along the olfactory nerve, a suspension of MSCs was injected using an insulin syringe under the mucous membrane of the upper nasal cavity of rats [32, 50, 57]. Unilateral injection of a suspension of MSCs (50 µl) into the receptive area of the olfactory nerve was performed 10 min after stroke modeling [52–55].

Using the computerized certified technique elevated plus maze (a neurophysiological method for assessing the effectiveness of cognitive and motor function control of rodents), we studied the features of the regulation of motor activity and maintaining habitual body positions in space for rodents at rest and during movement within several days after modeling CI and intranasal administration of MSCs. This test was chosen due to the features of the setup, which combines both open ("dangerous") and closed "mink" areas, as well as a large number of various parameters (more than 200) that the ANY-Maze program allows to calculate (distance traveled, average speed, number of visits, time spent in sectors, etc.). Each animal was tested for 5 min. At the same time, using a Logitech Webcam 905 video camera (Logitech, China), located at a height of 2.4 m above the installation, the behavior of rats in the maze and the following parameters were constantly recorded: the distance traveled; the average speed of movement of animals; the number of visits and the time spent in open, closed, and central sectors; the time of verticalization (racks) in closed sectors; the nature of grooming; and several other indicators. The obtained data were converted from analogue to digital form using the ANY-Maze licensed software package for animal behavior visualization (Stoelting Inc., USA) (version 4.82, serial number:

DBUT-VNJJ-JD8S-SFGU; license number: PK8W-NBWV-HTHT-BYHB dated February 28, 2013). The ANY-Maze software package allows automatic statistical processing of the received data.

Using EPR spectroscopy, NO production was assessed in intact animals along with rats on the first day after stroke [58, 59]. To form the complex $(DETC)_2$-Fe^{2+}-NO in the tissues of rats, DETC-Na was injected intraperitoneally at a dose of 500 mg/kg in 0.5 ml of water (per animal); a mixture of solutions, ferrous sulfate ($FeSO_4 \times 7\ H_2O$, Sigma, USA) at a dose of 37.5 mg/kg and sodium citrate at a dose of 187.5 mg/kg (in a volume of 1 ml of water per 300 g of animal weight), prepared immediately before introduction, were injected intramuscularly at three points—the right and left thighs and at the withers. DETC-Na and iron citrate are distributed throughout the body and upon interaction form a water-insoluble DETC-Fe^{2+} complex, which can interact with NO to form a stable radical $(DETC)_2$-Fe^{2+}-NO [48, 60]. Typical EPR spectra of frozen rat organ samples containing $(DETC)_2$-Fe^{2+}-NO represent an EPR signal with $g_{\perp} = 2.038$ and $g_{||} = 2.01$ and a triplet hyperfine structure at $g_{\perp}$. The results obtained make it possible to quantify the NO content in biological tissues. Tissues were harvested 40 min after the introduction of the spin trap components. Due to their small volume, the left and right parts of the hippocampus were combined into one sample (100 mg).

The spectra of the $(DETC)_2$-Fe^{2+}-NO complex were measured using an EMX/plus X-band (9.50 GHz) spectrometer with the temperature controller Bruker ER 4112HV at a temperature of 77 K with a magnetic field modulation of 100 kHz, modulation amplitude 2G, microwave power 30 mW, and the time constant 200 ms. The modulation amplitude, amplification, and microwave power in all experiments were selected so that there was no overmodulation and saturation of the EPR signal and they remained the same throughout all measurements. The ready samples, truncated in the shape of the measurement cuvette, were immediately weighed before the measurement. The sample weights were about 100 mg. The amplitude of the EPR spectra was normalized to the sample weight and the amplitude of the EPR signal of a reference sample with a known concentration [42].

The results are presented as the mean and error of the mean ($M \pm m$). To assess the significance of differences between the samples, the Mann–Whitney *U*-test and the Wilcoxon *T*-test were used. Differences were considered significant at a significance level of $p < 0.05$.

3 Results

The results of testing the studied groups of animals on the 1st day after CI are shown in Fig. 1. In the CI group without MSC implantation, on the first day after modeling cerebral ischemia, a significant decrease was observed in the total time of mobility of rats to reach on average up to 39% of the total time of mobility before ligation of common carotid arteries (control). On the other hand, in the CI + MSC group, the animals in which were perineurally implanted with MSCs, the decrease of the total mobility time on the first day after CI was less pronounced and averaged 67% of the control level (Fig. 1). When assessing the average distance traveled for 5 min on the first day in rats of the CI group, a statistically significant decrease was observed (on average, up to 28% of the distance traveled by intact animals). In the CI + MSC group, which were injected with MSCs after CI, the total distance traveled significantly differed from that of animals without therapy and averaged 65% of the control level (Fig. 1). Similar changes were observed for the total number of intersections of maze lines by rats: in the CI group, on day 1 after CI, a decrease by more than 6 times, on average, in this parameter was recorded, to reach a level of 16% of the initial one, while in the CI + MSC group with the use of MSCs, the decrease in this parameter was insignificant—by an average of 7%. The average speed of movement of animals before surgery and 24 h after modeling CI is a kind of resulting general motor activity of animals. A day after CI, a progressive recovery of the average speed of movement through the maze was observed precisely in those rats that were injected with MSCs. In particular, the average speed of movement of animals in the maze on the first day after CI simulation decreased by 73% in rats that

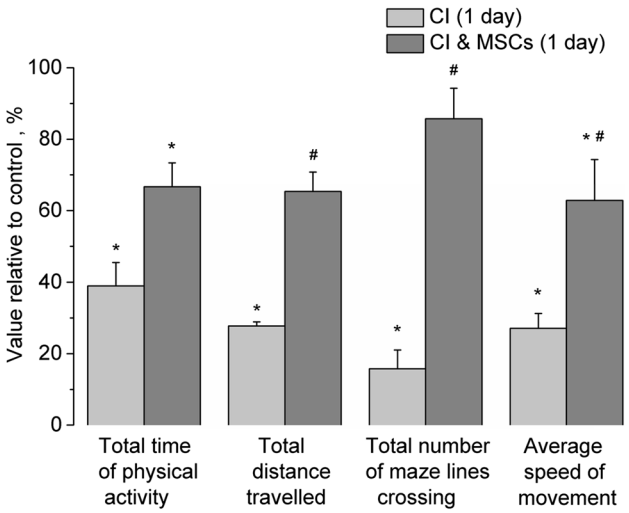

Fig. 1 The total time of motor activity; the total distance traveled; the total number of intersections of the maze lines; and the average speed of movement 1 day after the operation of modeling cerebral ischemia in rats with modeling of occlusion of the common carotid arteries (CI) and in rats with modeling occlusion of the common carotid arteries and the introduction of MSCs (CI & MSCs). *Significantly differ from the values before the operation; #significant differences between the groups after surgery without and with the introduction of MSCs

were not injected with MSCs and by 37% in rats from the CI + MSC group, which were injected with MSCs (Fig. 1). The total number of "freezes" of animals on the first day after CI modeling increased significantly in both groups: the increase was on average 83 ± 10% in rats that did not receive MSCs, and in the group of rats with MSCs, the increase was less pronounced (average by 63 ± 8%, $p < 0.05$).

The results of motor and cognitive analysis of CI and CI + MSC are presented in the figure (% on day 1) and in the table (numbers on day 3). On the 3rd day after CI, in the CI + MSC group, there was a tendency for a more rapid recovery of control of orienting motor activity, which is presented in Table 1. In the CI group of rats (without the introduction of MSCs), decrease in the distance traveled over 5 min of observation was found at this time from 7.63 ± 0.85 to 3.27 ± 0.53 m (by 57%) compared to control values. The animals also decreased the distance traveled in the closed zone, the number of episodes of mobility, the total distance traveled in the closed zone, the average speed of movement, and the total time of mobility in the closed zone, and there was no visit to the open areas of the maze. A similar picture was observed in animals of this group (group CI) on the seventh day after the operation. This pattern reflects a decrease in orienting motor activity in laboratory animals without the introduction of MSCs after ligation of the common carotid arteries; moreover, these changes persisted both in the initial period (third day) and in a longer period (seventh day) after modeling ischemic stroke.

In comparison, in the group of rats that were perineurally implanted with MSCs during the acute period of stroke, it was found that this therapy is accompanied by a faster recovery of the time of motor activity in animals. On the third day of observation, the distance covered in 5 min decreased only by 32%, while in the group without therapy by 57%. In animals without treatment, on the third day of observation, a decrease in the time of motor activity by 59% was noted, and in the group of rats that were injected with MSCs, the decrease in this indicator reached only 36%. A similar pattern was noted when comparing the number and time of verticalization in rodents in the pre- and postoperative periods. Thus, on the third day of observation, the number of upright stances of rats for 5 min in the CI group decreased in comparison with the preoperative period by an average of 62%, and in the CI + MSC group only by 30%. The comparison of the verticalization time in rats of the two groups with CI modeling revealed a more rapid recovery of this indicator in those animals that were intranasally implanted with MSCs after occlusion of the common carotid arteries (Table 1). In the CI group, on the third day of observation, a decrease in the time spent by animals in the central zone of the plus maze by an average of 59% compared with the same indicator in the same rats in the preoperative period (control) was found. For comparison, for the same periods in patients with intranasal implantation of MSCs after CI, a less pronounced decrease in the time spent by animals in the central zone of the plus maze was found—by an average of 42% compared with control indicators. It should also be noted that on the seventh day after the simulation of ischemia in rats of the CI + MSC group, there were actually no obvious distinguishing signs in the control of orienting motor activity in comparison with the preoperative period in the same rats.

It should be noted that 10 animals in each group that did not participate in the experiments and served as a survival control did not experience discomfort during the entire time of the experiments.

Thus, the introduction of MSCs in the acute phase of stroke was accompanied by a significant protective effect on the preservation and/or restoration of control of cognitive functions and motor activity of animals. Considering that hippocampal neural networks play a significant role in the processes of memorization and in the process of controlling the approximate motor activity of animals [44], it was decided to emphasize the detailing of metabolic events in CI at the NO system level in the hippocampus.

To study the dynamics of NO content in the hippocampus by EPR spectroscopy using the spin trap technique, spectra were recorded before and after modeling of CI and simultaneous intranasal administration of MSCs. Figure 2 shows

Table 1 Evaluation of the effectiveness of cognitive and motor function control in rodents on the 3rd day after modeling cerebral ischemia with simultaneous intranasal perineural administration of MSCs

	CI		CI + MSC	
	Before	3 days	Before	3 days
Distance (m)	7.63 ± 0.85	3.27 ± 0.53	7.20 ± 0.66	4.94 ± 0.63
Time mobile (s)	143.9 ± 8.8	58.2 ± 9.8	138.9 ± 9.8	88.3 ± 16.4
Number of verticalizations	13.6 ± 1.7	5.2 ± 1.9	10.7 ± 1.7	7.5 ± 2.5
Time of verticalization (s)	19.9 ± 4.1	6.2 ± 2.6	17.1 ± 1.7	9.9 ± 3.2
Time in the center zone (s)	65.9 ± 17.1	27.6 ± 11.6	70.6 ± 12.0	42.4 ± 6.9

Shown are the values before and after cerebral ischemia (CI), as well as before and after cerebral ischemia with intranasal perineural administration of MSCs (CI + MSC). mean ± SEM values of the distance traveled (distance), the total time of mobility of rats (time mobile), and the number (number of verticalization) and time (time of verticalization) of verticalization in closed sections, as well as the time of stays in the central sector, are given

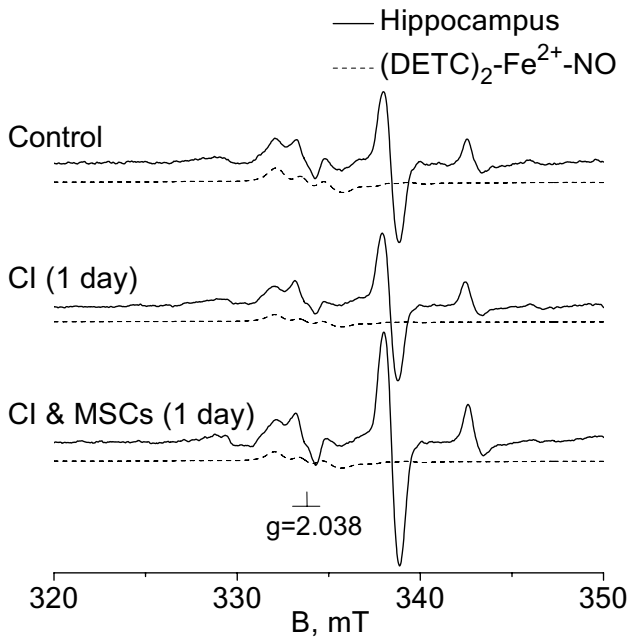

Fig. 2 EPR spectra of the hippocampus of intact rats (control), 1 day after the modeling ischemia without therapy (CI) and 1 day after modeling ischemia with simultaneous intranasal administration of MSCs (CI & MSCs). The signals are from (a) tissue sample, (b) complex $(DETC)_2$-Fe^{2+}-NO, and (c) complex $Cu(DETC)_2$. We see a characteristic triplet signal of the entire complex $DETC)_2$-Fe^{2+}- NO with a *g*-factor value of 2.038. The abscissa is the magnetic field H, mT. Temperature 77 K

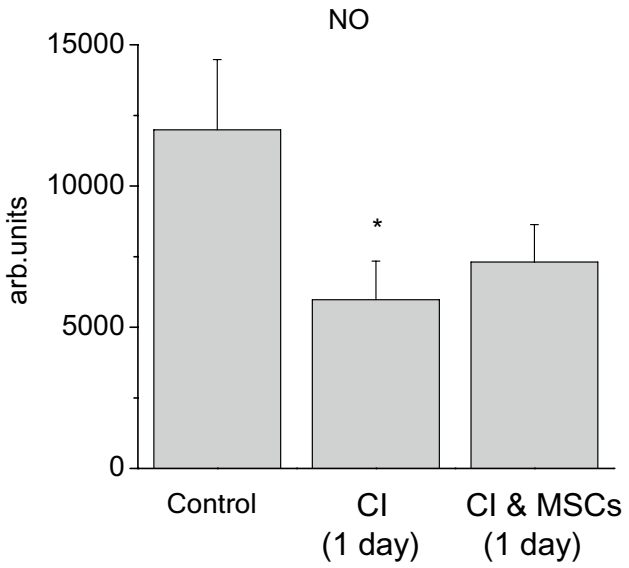

Fig. 3 NO content in the hippocampus of intact rats (control), 1 day after modeling ischemia(CI) as well as 1 day after modeling ischemia with simultaneous intranasal administration of MSCs (CI & MSCs). Shown are the average values and standard errors of the mean and the difference from the control (*t*-test, $p < 0.05$). The ordinate axis is the average intensity of the integral signal of the complex $(DETC)_2$-Fe^{2+}-NO. *Significantly differ from the values before the operation

examples of EPR spectra of the hippocampus of a control rat compared to rats after 1 day of ischemia caused by ligation of the carotid arteries both with simultaneous intranasal administration of MSCs and without administration of MSCs. The spectra show a characteristic triplet signal from the $(DETC)_2$-Fe^{2+}-NO complex. Additionally, in the same region, a signal from the $(DETC)_2$-Cu complex can also be detected by EPR spectroscopy [61]. Figure 3 shows statistical data on the integral signal intensities of $(DETC)_2$-Fe^{2+}-NO in the spectra of the studied samples (hippocampus). The results show a significant reduction, around 49.8% ($p < 0.05$), in NO content 1 day after modeling CI caused by ligation of the carotid arteries compared to intact (control) snails. Furthermore, in the hippocampus of rats in which ischemia was modeled with simultaneous intranasal administration of MSCs, the NO content was also reduced by 39% compared to intact snails after 1 day after ischemia. Although the reduction in the NO content in this group seems to be less than the reduction in their ischemic rats, it is no statistically significant. Nonetheless, this result shows that the use of MSCs may have some therapeutic potential.

As already mentioned, in the EPR spectra of hippocampal tissues, in addition to the characteristic triplet signal from the $(DETC)_2$-Fe^{2+}-NO complex, signals from the $(DETC)_2$-Cu complex were analyzed. The copper content in the rat hippocampus 1 day after modeling CI caused by ligation of the carotid arteries tended to decrease (around 24% compared to intact snails) (Fig. 4). In the group with the MSC implantation, there was a tendency to restore copper concentrations to levels that are characteristic of intact animals. This result shows that the use of MSC enhances the antioxidant properties of the tissue, i.e., there is some therapeutic effect. Interesting results were obtained by calculating the Cu/NO ratio in the hippocampus of rats, which indicates the characteristics of correlation between the states of the NO system and antioxidant system (Fig. 5): a tendency to increase in the efficiency of the antioxidant system 1 day after ischemia in both studied groups was observed. This effect was more pronounced with intranasal administration of MSCs.

4 Discussion

The problem of restoring lost brain functions after circulatory disorders is of great relevance in the clinical aspect [29]. And since technologies for effectively restoring control of impaired somatic and autonomic brain functions after circulatory disorders have not yet been developed, experimental developments in this direction are being carried out intensively in many countries across the globe [28, 62].

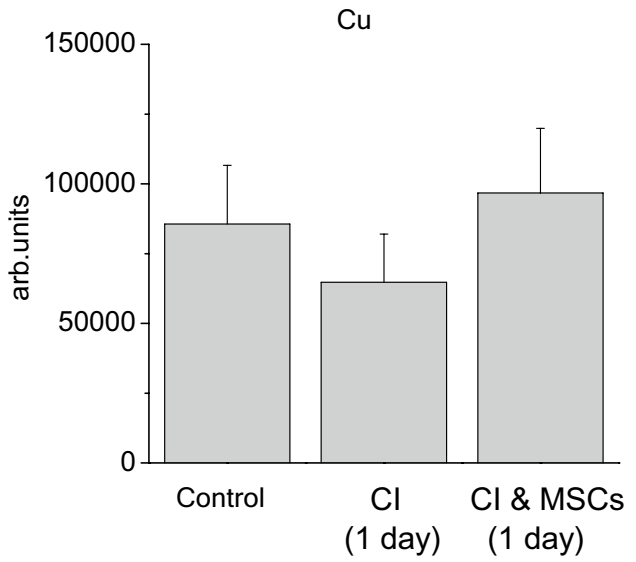

Fig. 4 The copper content in the hippocampus of intact rats (control), in rats 1 day after modeling ischemia (CI), as well as in 1 day after modeling ischemia with simultaneous intranasal administration of MSCs (CI & MSCs). Shown are the average values and standard errors of the mean. The ordinate axis is the average intensity of the integral signal of the complex $Cu(DETC)_2$

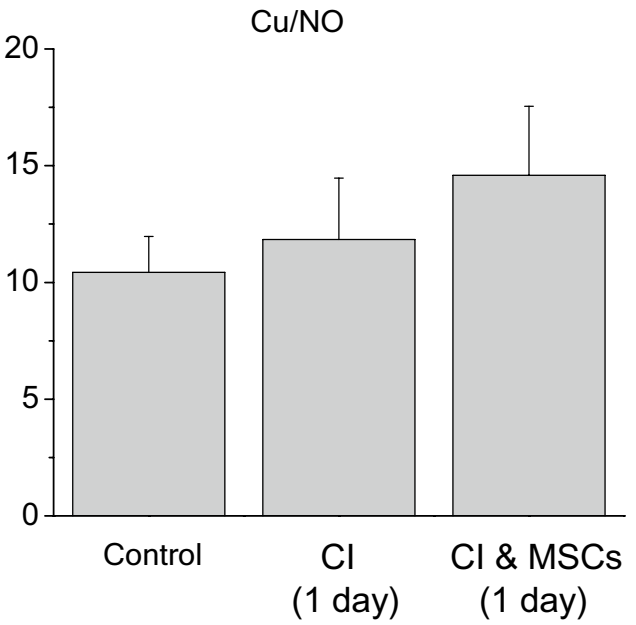

Fig. 5 Cu/NO ratio in the hippocampus of intact rats (control) and rats 1 day after modeling ischemia (CI), as well as after modeling ischemia with simultaneous intranasal administration of MSCs (CI & MSCs). Shown are the means and standard errors of the mean

Our study showed that the central control of the orienting motor activity of rats is recovered faster in the group of animals in which MSCs migrate to the ischemic areas of the brain after implanting them intranasally in the region of the olfactory nerve terminals. The data obtained in the plus maze showed that perineural implantation of MSCs attenuates the negative consequences of ischemia on the brain systems that control the motor activity of animals, which manifests as a faster recovery of animal activity, an increase in the average speed of movement and the distance traveled during the implementation of orienting behavior in the maze, and a series of other indicators of tentative motor activity of rats. The fact that after perineural implantation MSCs migrate in the direction of damaged areas of the nervous tissue is well established [33, 39, 57]. Previously, it was shown that when modeling local neurodestruction in the sensorimotor zone of the brain and subsequent injection of MSCs into the submucosal region of the nasal cavity of rats, MSCs move along the fibers of n. olfactorius into the central structures of the olfactory system and then get distributed in areas of brain destruction in the anterior and middle cranial fossae [32, 33, 39]. MSCs contain and secrete many biologically active substances. The reparative potential of MSCs is manifested through the secretion of highly active molecules and the formation and release of exosomes containing miRNAs, as well as factors that have an immunosuppressive effect. With intranasal administration of MSCs, even without the subsequent migration of MSCs into the cranial cavity, biologically active substances released upon the destruction of the MSCs in the nasal cavity might act on the endings of the olfactory and trigeminal nerves. Therefore, information is transmitted through these nerves to the brain and changes its functional state, which affects the control of somatic and visceral functions [16, 20, 56, 62–64]. The data obtained form a basis for analyzing the mechanisms of the revealed MSC reparative phenomenon in the nervous tissue of the brain [50, 56, 64].

Under hypoxic conditions, the impaired control of metabolic processes is accompanied by neurodestruction in various parts of the brain. The occlusion of the common carotid arteries is a common method for modeling ischemia [10, 14, 50, 56, 57, 64, 65]. With occlusion of the external carotid arteries, the structures of the neocortex and hippocampus are primarily affected due to the weakening of cerebral blood flow and deterioration of conditions for adequate oxygenation [66–68]. When modeling chronic cerebral hypoperfusion by ligation of the common carotid arteries on both sides, histological signs of damage were assessed after ligation of the carotid arteries [51]. Inflammatory reactions, loss of neurons, and a number of other morphological disorders were identified. Brain damage was then reduced by the use of propofol. We also found lesions upon modeling ischemia [50, 57]. It is self-evident that neurons are more sensitive to a drop in tissue oxygen saturation compared to glial components [46, 51]. In the process of evolution, protective processes have evolved in the nervous tissue of the brain

to ensure plasticity under different conditions, including disruption of the redox potential and energy processes, which in brain cells are controlled by signaling molecules such as the gaseous mediators NO [7, 9, 13, 69]. One of the mechanisms contributing to the positive effects of MSCs in the brain tissue is an adaptive change in the NO system, which promotes the activation of reparative processes in the brain tissue during hypoxia induced by occlusion of the common carotid arteries. This is evidenced by the relatively lower decrease in the content of NO in cerebral ischemia after the use of MSC.

The role of gaseous neurotransmitters during conditions of normoxia, hypoxia, and cerebral ischemia is traditionally regarded as a topic of highest priority in experimental research and clinical observations [12, 45, 46]. Taking into account the importance of the neural networks of the brain and specifically the hippocampus in the processes of memorization [44] and the properties of the control of locomotor behavior patterns in the process of orienting motor activity of animals, it was decided to focus on detailing the metabolic events during CI in terms of the NO levels in the hippocampus.

The results obtained by us earlier using EPR spectroscopy in a model of ischemia caused by ligation of the external carotid arteries demonstrate that brain hypoxia is accompanied by a significant decrease in NO production by 36% in the hippocampus within the first 3 days after ischemic stroke modeling. We also showed a decrease in copper content by an average of 24%. In the same model of ischemia, a significant decrease in the content of NO in the olfactory bulb of the brain of rats was found two times 1 and 2 days after modeling the cerebral ischemia [42, 48, 49, 70]. The level of NO production in rats in which ischemia was modeled with a simultaneous intranasal administration of MSCs was also reduced 1 and 2 days after brain ischemia to 50%. No significant difference was found in the NO content in rats that underwent modeling of ischemia with simultaneous intranasal administration of MSCs relative to ischemic rats. The copper content, which corresponds to the level of superoxide dismutases 1 and 3, in the olfactory bulb of rats tended to increase after modeling ischemia and persisted for 2 days of observation [71].

Previously, we also showed that after the onset of signs of stroke induced by 5 min in hypobaric hypoxic conditions (conditional rise to a height of 4500 m above sea level), already after 5 h of ischemia, the formation of NO in the hippocampus decreases by 2–3 times and this decrease persists within 24 and 72 h [72]. It is believed that during transient ischemic attacks and ischemic stroke, not only neuronal and glial elements are damaged, but the structure of the endothelium of blood vessels is also disturbed. Disruption of the endothelial structure is accompanied by a decrease in the production of endothelial NO, which leads to vasoconstriction and, consequently, impairs brain oxygen saturation [46, 73].

Earlier, we have shown that changes in the NO system in the brain during hypoxia have a complex nature—on the one hand, the NO level decreases due to a decrease in the activity and content of endothelial NO synthase, and on the other hand, there is a compensatory NO increase in the structures of the nervous tissue, mainly due to the expression of neuronal and inducible NO synthase. This last aspect in cerebral ischemia is one of the key incentives to seek methods to control the level of NO in the brain tissue by correcting the activity of NO synthase, since under different metabolic conditions, pathological processes in tissues are enhanced both with a lack of NO and with an excess of its formation [41, 47, 71, 73].

Therapeutic strategies that reduce energy consumption and reduce the severity of oxidative stress are currently one of the most developed methods of primary and secondary neuroprotection of postischemic cerebral disorders [51, 74]. These strategies are primarily based on the activation of the body's own antioxidant resources under conditions of hypoxia and ischemia, where Zn-Cu-superoxide dismutase is of the greatest importance. The effectiveness of the neuroprotective action of superoxide dismutases increases during hypoxia and glutamate cytotoxicity; therefore, in the culture of cortical neurons and transgenic mice genetically predisposed to hypersecretion of superoxide dismutases, there is practically an absolute resistance of cells to NMDA toxicity and focal ischemia [5, 75]. It has been established that the number of dead neurons is higher in mice with a genetically determined deficiency of superoxide dismutases during ligation of the middle cerebral artery since it has been demonstrated that they are less resistant to elevated concentrations of glutamate, hydrogen peroxide, and NO donors in experiments in vitro [76]. It is important to note that the degree of activity and protective properties of superoxide dismutase are predetermined depending on the level of NO production, the formation of superoxides, and the concentration of Ca^{2+} ions. So, in animals with the receiving MSCs, the activity of the antioxidant system after modeling cerebral ischemia was more pronounced [77]. The results obtained indicate that even a single perineural administration of MSCs into the receptive fields of the cranial nerves is sufficient for achieving selective accumulation of MSCs in areas of brain damage and, as a result, activating the restoration of functions impaired due to brain damage. The basis for the activation of reparative processes in the nervous tissue of the brain after CI modeling is the secretion of numerous neurotrophic factors by MSCs in the areas of neurodestruction after disrupting the blood supply to the brain [77].

All in all, taking into account the literary information [7, 9, 13, 66–69] and our results published earlier [17, 42, 70, 72], after modeling CI, a decrease in NO production

was observed, which was accompanied by a disruption of the control of motor activity in rats. However, in the group that received MSCs, the decrease in NO was less pronounced; this group was also characterized by better parameters of orienting motor reactions.

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Author Contribution Guzel Yafarova carried out the interpretation of the results of measuring the behavioral changes and compared them with the results of NO level; prepared Fig. 1; and helped in drafting the manuscript.

Yulia Tokalchik participated in the simulation of ischemia and in measuring behavioral changes after this operation and participated in its interpretation and in extraction of brain samples for further measurements by EPR spectroscopy.

Tatiana Filipovich participated in the simulation of ischemia and in measuring behavioral changes after this operation and participated in its interpretation and in extraction of brain samples for further measurements by EPR spectroscopy.

Vyacheslav Andrianov carried out calculations and analysis of nitric oxide (NO) and copper signals in EPR spectra of the samples' intensity; performed the statistical analysis; prepared Figs. 2, 3, 4, and 5; participated in interpretation of the results; and helped in drafting the manuscript.

Lyeh Bazan carried out measurements of EPR spectra of samples and participated in their interpretation.

Tatiana Bogodvid did the literature search, participated in the analysis of results and interpretation the role of NO in brain ischemia and protective actions at the NO level, and helped in drafting the manuscript.

Abdulla Chihab helped in drafting the manuscript and was responsible for its editing.

Aleksandra Zamaro participated in the simulation of ischemia and in measuring behavioral changes after this operation and participated in its interpretation and in extraction of brain samples for further measurements by EPR spectroscopy.

Vladimir Kulchitsky conceived the study, participated in its design and coordination, helped in drafting the manuscript, and was responsible for final approval.

Khalil Gainutdinov conceived the study, was responsible for the conception of its design and coordination, helped in drafting the manuscript, and did the literature search and final approval.

All authors read and approved the final manuscript.

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Data Availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

Ethics Approval and Consent to Participate The experimental procedures (using anesthesia methods) are in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985) and the UK Animals Scientific Procedures Act 1986 or the European Communities Council Directive of 24 November 1986 (86/609/EEC). The methods are approved by the Scientific Council of the Institute of Physiology of the National Academy of Sciences of Belarus (protocol No. 8, 26.08.2010).

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