SECTION V. MEDICINE

Chebotarova A.¹, Shypilova I.², Khmelevskaya I.³**Nitric Oxide-dependent mechanism of adaptation to the ischemic damage of lower extremities against the background of mesenchymal stem cells administration**¹*American University of Integrative Sciences
(Barbados)*²*All Saints University college of Medicine
(St. Vincent and Grenadines)*³*Kursk State Medical University
(Russia, Kursk)*

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Abstract

The article analyzes the level of an active Nitric Oxide (NO) metabolite – Nitrite ion – in ischemic muscle tissue of lower extremities of laboratory animals, against the background of administration of allogenic mesenchymal stem cells (MSC). It was indicated, that the administration of mesenchymal stem cells was accompanied with decreased level of Nitrite ion in muscle tissue during first days after ischemic process modeling, and a significant increase in the level of this metabolite in 21 days after the injection. This may indicate the activation of adaptation processes in the damaged tissue as a backdrop of application of mesenchymal stem cell therapy.

Keywords: Nitric Oxide, mesenchymal stem cells, ischemia.

In recent years, an active search for new directions for the therapy of ischemic diseases of the lower extremities is done by scientists and physicians of many countries. These new methods must be safe, efficient and should serve as an alternative to surgical and pharmacological methods of treatment. Usage of allogenic or heterogeneous mesenchymal stem cells (MSC) is considered as one of possible alternative methods of ischemic pathology therapy. Stem cells biological actions are related to properties of an extensive proliferation, tissue specific differentiation, and the ability to synthesize a complex of trophic (growth) factors and other biologically active molecules. The specificity of the action of stem cells is determined by their phenotypic affiliation to some tissue and the molecular-cellular characteristics of the microenvironment. Any ischemic pathological process always involves a vascular wall with development of endothelial dysfunction, and synthesis of a large number of biologically active regulatory factors [10]. The majority of authors recognize nitric oxide as the main active metabolite determining the functional state of the endothelium [12]. NO is a local tissue hormone with multiple effects, including anti-inflammatory and vascular effects, and stimulation of angiogenesis [11]. Of considerable interest are studies of the participation of NO in damage mechanisms and compensation for ischemia [7]. There is evidence of an increase in the activity of inducible nitric oxide synthase in cells of various organs in chronic hypoxia. This indicates the important role of this enzyme in adaptation to cellular hypoxia [2, 4]. According to other studies, NO in large amounts contributes to the development of oxidative stress, mitochondrial dysfunction, inhibiting cytochrome oxidase and reducing oxygen consumption [1, 8]. In addition, the interaction of NO and myoglobin can play a key role in maintaining a fine balance in the reaction of free radical oxidation, which largely

determines the fate of the cell under conditions of hypoxia in ischemic lesions of muscle tissue [5,9]. Also, according to several authors, one of the possible mechanisms of adaptation to the state of ischemia is the stimulation of angiogenesis by nitric oxide by regulation of endothelial growth factors [3].

The idea of stem cell usage for treatment of the lower extremities ischemia has been successfully realized in recent years. This became possible, due to the intensive study of the cellular mechanisms of angiogenesis and the isolation of precursor cells of the vascular endothelium and angioblasts. These cells directly affect erythropoiesis and angiogenesis, releasing various bioactive substances that, when implanted, induce repair processes at a site of injury [6]. However, insufficient attention is paid to studying of the biochemical processes occurring in a body and the reaction of a tissue to a similar kind of action.

Thus, the purpose of our work was to determine the content of nitrite ion in ischemic muscle tissue of the lower limb after the introduction of mesenchymal stem cells.

Materials and methods. As the object of the study, 60 male white mongrel rats weighing 200-250 grams were used. Experimental ischemia of the lower extremities was studied on the model of reduced blood circulation, which was formed by ligation with further crossing of the femoral artery under thiopental anesthesia. The bone marrow mesenchymal stem cells were collected by washing the femoral cavity of adult laboratory rats. The obtained cells were placed in IGLA-MEM medium enriched with L-glutamine, 10% calf embryonic serum and antibiotics. The cells were cultured for 14 days at 37°C with medium change once a week. Evaluation of the viability and number of cells was carried out in a test with a nuclear dye Trypan blue. The injection of mesenchymal stem cell was performed one week after the operation. The number of cells administered to one animal was 5 million. The cells were administered intramuscularly along the course of the neurovascular bundle. The animals were kept in standard vivarium conditions.

The animals were recombined into 3 groups: 1st group - intact rats (animals, who didn't receive any surgery - 21 rats), 2nd group - control (rats who were under development of the lower extremities ischemia, but no injection of MSCs - 20 rats), and 3rd group - experimental (animals were injected with MSC in the simulated pathological state - 19 rats).

The rats of the experimental group were decapitated under ether anesthesia on the 7th, 14th, 21st days after the injection of mesenchymal stem cells, and the control ones were decapitated correspondingly to the day of observation in the experimental group.

For the study, the calf muscle of the operated limb was taken. The Griess test was used to determine the concentration of stable nitrite ion in the tissue. The result was calculated from the calibration curve using standard solutions of sodium nitrite.

The results were processed using the statistical t-Student test using Statistica 6.0 software package. The results were considered reliable at a value of $p < 0,05$.

Results and discussion. As a result of the conducted studies, it was found that the content of nitrite ion in muscle tissue depends on the duration of ischemia and significantly changes after the administration of MSCs (Table 1).

Table 1

Dynamics of the content of nitrite ion in muscle tissue of lower limb under experimental ischemia and after administration of MSCs.

Groups	Days of the experiment		
	7 days	14 days	21 days
1 st group - intact animals	0,008 ± 0,0003 mmol/mg protein	0,008 ± 0,0003 mmol/mg protein	0,008 ± 0,0003 mmol/mg protein

2 nd group -control	0,005 ±0,0002 mmol/mg protein*	0,01 ±0,0004 mmol/mg protein*	0,022 ±0,0008 mmol/mg protein*
3 rd group –experimental animals	0,002 ±0,0001 mmol/mg protein**	0,009 ±0,0003 mmol/mg protein**	0,048 ±0,009 mmol/mg protein**

Notes: *-theresultsarereliablewithrespectto intactgroup, $p < 0.05$; ** -theresultsarereliable relativeto the control group, $p < 0.05$.

Thus, on the 7th day of observation, the content of the nitrite ion was minimal in the group of animals injected with mesenchymal stem cells, and was 2,5 times lower than that of the control animals, and 4 times lower compared to the intact rats. In 14 days after the administration of MSC, the level of the metabolite did not differ significantly from the group of intact animals, and was slightly lower than in the animals of the control group (in 10%). By the 21st day of the experiment, there was a significant increase in content of nitrite ion in the muscle tissue of the experimental group of animals, reliably exceeding ($p < 0.05$) in the control and intact groups – 6 times and 2,2 times respectively. An increase in the level of a stable metabolite of nitric oxide may testify to the functioning of NO as an intracellular superoxide trap by changing the activated AFK-dependent signaling mechanisms to alternative ones that are activated by NO. The decrease in the content of nitrite anion on the 7th day of observation may be due to the possibility of myoglobin to reduce it to NO under conditions of ischemic damage to muscle tissue. Thus, the combination of the oxygen-storing and nitrite-reductase ability of myoglobin can consist in providing energy systems of muscle cells with oxygen.

Conclusions. 1. Administration of MSC leads to an increase in the level of nitrite ion in muscle tissue of the lower extremities under conditions of experimental ischemia, which may indicate the importance of NO-dependent compensatory adaptation reactions in the ischemic process.

2. One of possible ways of endothelial dysfunction correction in ischemic tissue damage, is the activation of the nitric oxide system with following implementation of its angioprotective, vasodilating, anticoagulant and stress-adaptive properties.

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Morozov A.N., Nikoghosyan S. M., Vecherkina Zh. V., Chirkova N.V., Koretskaya I.V.
Antinociceptive protection in endodontic procedures

Voronezh state medical university n. N.N. Burdenko
(Russia, Voronezh)

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Abstract

The implementation of the model of preventive and multimodal antinociceptive protection of the pain syndrome associated with endodontic interventions in acute complicated forms of carious lesions is a promising task for modern dentistry.

Keywords: endodontic intervention, pain syndrome, local anesthesia, antinociceptive protection.

Research methods.

The study involved 200 patients with acute complicated forms of carious lesions. The stratification of the participants into 2 groups of 100 people was carried out in accordance with the traditional approach and the antinociceptive protection technique. The traditional approach for endodontic intervention was used in the control group (n=100 people): local anesthesia with articaine hydrochloride 40 mg/ml + adrenaline hydrochloride 10 µg/ml 1.7–3.4 ml; the use of ketorolac tromethamine 10–40 per os during the first day of the postoperative period. The developed approach was applied to the main group (n=100 человек): ketorolac tromethamine 30 mg in 10–20 ml of 0.9% NaCl solution iv bolus shortly before the endodontic intervention and local anesthesia (articaine hydrochloride 40 mg/ml + adrenaline hydrochloride 10 µg/ml 1.7–3.4 ml). The standardization of the compared groups was achieved by ensuring the identity of the intra-group distribution of the participants in accordance with gender and age, forms of acute carious complications, local anesthesia techniques. The pain syndrome and the level of situational and personal anxiety were assessed during the follow-up period.

Research result.

The developed program of antinociceptive protection for endodontic interventions is an optimal solution of the problem due to the following functional characteristics in the form of statistically significant advantages in comparison with the traditional approach ($p < 0.05$): a decrease in the level of intraoperative "stressority" by 25.8–48.14%; the actual prevention of the development of postoperative pain syndrome of any intensity in 96% of cases; the prevention of an increase in the level of situational anxiety due to the absence of postoperative pain syndrome in 77% of cases.

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