

Cell and Organ Transplantation. 2024; 12(2): 118-125.  
<https://doi.org/10.22494/cot.v12i2.169>

# Comparative effects of mesenchymal stromal cells of various origins and sources on biochemical parameters in the hippocampus of rats during cerebral ischemia-reperfusion



Konovalov S.<sup>1</sup>, Moroz V.<sup>1</sup>, Yoltukhivskiy M.<sup>1</sup>, Gadzhula N.<sup>1</sup>, Deryabina O.<sup>2,3</sup>, Kordium V.<sup>2,3</sup>

<sup>1</sup>National Pirogov Memorial Medical University, Vinnytsya, Ukraine

<sup>2</sup>State Institute of Genetic and Regenerative Medicine, National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine

<sup>3</sup>Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine

\*Corresponding author's e-mail: [gadzhula@vnmu.edu.ua](mailto:gadzhula@vnmu.edu.ua)

## ABSTRACT

*In neurodegenerative processes of the brain, the hippocampus is primarily affected, leading to subsequent cognitive impairments caused by increased generation of reactive oxygen and nitrogen species. These reactive species induce apoptosis and necrosis of neurons. Cell therapy using mesenchymal stromal cells (MSCs) has shown promising potential in activating endogenous mechanisms of neuroregeneration in response to ischemic injury of brain structures.*

**THE PURPOSE** of this study is to investigate the therapeutic potential of MSCs from various origins, MSC lysate, and the reference drug citicoline on the energy component of neuronal metabolism, as well as oxidative and nitrosative stress in the rat hippocampus under conditions of cerebral ischemia-reperfusion (IR).

**MATERIAL AND METHODS.** The experiment was conducted on 126 Wistar rats with a modeled pathology (20-minute IR of the internal carotid arteries). The animals were injected with human umbilical cord Wharton's jelly-derived MSCs, human and rat adipose-derived MSCs, rat embryonic fibroblasts, MSC lysate, or citicoline immediately after the removal of the ligatures. Biochemical parameters of carbohydrate metabolism (glucose, lactate), oxidative (NADPH oxidase activity) and nitrosative stress (NO synthase activity) in the rat hippocampus were determined on days 7 and 14 after IR under the conditions of cerebral IR and on the background of its correction.

**RESULTS.** It was established that during ischemia-reperfusion in the rat hippocampus, glucose and lactate levels increase, aerobic glucose oxidation is inhibited, anaerobic glycolysis intensifies, and lactic acidosis develops in hippocampal cells. Additionally, NADPH oxidase activity decreases, and an imbalance occurs in the nitric oxide system. A positive effect was observed from the transplantation of human umbilical cord Wharton's jelly-derived MSCs and rat embryonic fibroblasts, as well as from the use of citicoline, in stabilizing glucose, lactate, NADPH oxidase, and nitric oxide levels. In contrast, the transplantation of human and rat adipose-derived MSCs was significantly less effective than citicoline and demonstrated no statistically significant modulatory effect on biochemical parameters in the hippocampus of experimental animals with IR.

**CONCLUSIONS.** Transplantation of human umbilical cord Wharton's jelly-derived MSCs was not inferior to citicoline and, compared to other tested MSCs and their lysate, more effectively contributed to the recovery of disturbed energy processes (glucose levels) and the elimination of metabolic acidosis (lactate levels) in the hippocampus of rats. Moreover, it demonstrated a positive modulatory effect on the oxidant-antioxidant balance, as indicated by NADPH oxidase activity levels.

**KEY WORDS:** mesenchymal stromal cells; hippocampus; cerebral ischemia-reperfusion injury; carbohydrate metabolism; oxidative stress

In recent decades, vascular pathology occupies a leading position among the causes of disability and mortality of working-age people both in Ukraine and around the world. Among such diseases, the most severe, and sometimes fatal, is an acute violation of cerebral blood circulation [4]. The cause of 80 % of strokes is cerebral artery occlusion, which is accompanied by the development of cerebral infarction [1, 14, 23]. When the narrowing of the vessel lumen and the instability of the atherosclerotic plaque, a thrombus is formed, which causes ischemic occlusion. An embolic stroke is explained by a decrease in cerebral blood flow due to the inflow of an embolus [29].

According to modern ideas, neurodestruction by an ischemic mechanism is accompanied by the development of complex pathobiochemical cascades, first of all, by violation of energy metabolism and the formation of mitochondrial dysfunction [2, 4, 5]. As a result, the hyperproduction of reactive forms of oxygen, nitric oxide, excitotoxicity is triggered, the permeability of the blood-brain barrier (BBB), signs of inflammation and the expression of pro-apoptotic proteins increase, that eventually leads to neuronal cell death and impairment of sensory, motor and cognitive functions [17, 26, 33].

The use of thrombolytic therapy or endovascular thrombectomy, as the most effective method of ischemic stroke treatment, is performed within 3-6 hours [15, 21, 36]. However, recovery of perfusion of ischemic tissue contributes to the deepening of disorders of metabolic processes in the brain, which leads to reperfusion injuries [6, 27]. The destruction of desmosomes and an increase in the distance between individual neurons promotes the spread of free radicals and secondary messengers, which causes damage to intact cells and thus increases the focus of the lesion [6, 32]. During IR, there is significant damage to the hippocampus, the main structure of the brain associated with learning and memory, which leads to cognitive impairment [20]. The result of ischemic-reperfusion damage is increased generation of reactive oxygen species (ROS) and reactive nitrogen species, which lead to apoptosis and necrosis of neurons [18]. It is worth noting that the hippocampus is primarily damaged in neurodegenerative processes [34].

Encouraging results regarding endogenous mechanisms of neuroregeneration in response to ischemic damage to brain structures were demonstrated by cell therapy using MSCs [9, 40]. Many preclinical studies have demonstrated the ability of MSCs to reduce tissue damage, promoting functional recovery through multiple mechanisms, including immunomodulation, pro-angiogenic signal transmission, neurotrophic factor secretion, and neuronal differentiation [9, 24, 40]. Along with that, MSCs have several advantages over other stem cells due to easier methods of obtaining, low risk of tumorigenicity and absence of ethical problems [10].

Among MSCs, attention is drawn to those derived from embryonic tissue or Wharton's jelly (WJ-MSCs). MSCs obtained from bone marrow or adipose tissue, unlike those from perinatal organs, have certain limitations, such as an invasive harvesting procedure, a higher risk of transmitting infectious diseases, donor age, and limited proliferative potential [35]. WJ-MSCs exhibit a good proliferative potential, a faster growth rate, and the ability to maintain their multipotency for a greater number of passages in vitro compared to MSCs derived from bone marrow or adipose tissue [25]. Thus, in the study by E. Semenova et al., it was shown that MSCs derived from different parts of the umbilical cord varied in their properties. WJ-MSCs, compared to other types of umbilical cord-derived MSCs, demonstrated a high and stable proliferative potential and phenotype, which, according to the authors, can be considered a promising source of stem cells for further clinical applications [35].

In view of this, **THE PURPOSE OF THE STUDY** is to evaluate the therapeutic effect of MSCs of various origins, MSC lysate, and the reference drug Citicoline on the energy component of neuronal metabolism, oxidative and nitrosative stress in the hippocampus of rats under conditions of cerebral IR.

## MATERIALS AND METHODS

The experiment was performed on 126 male Wistar rats aged 4 months and weighing 160-190 g on the basis of National Pirogov Memorial Medical University, Vinnytsya, Ukraine. Animals were kept in standard vivarium conditions and had access ad libitum to food and water. All manipulations with experimental animals were carried out in accordance with the International rules and standards of European Communities Council Directives 86/609/EEC (1986), and according to the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes" and the Law of Ukraine "On the protection of animals from cruelty". The research protocol was approved by the Committee on Bioethics of our university (protocol No. 2 dated January 31, 2024).

MSCs and MSC lysate were obtained from the Institute of Molecular Biology and Genetics (IMBG) of the National Academy of Sciences of Ukraine. The transfer of cells was carried out on the basis of the Agreement on scientific cooperation between the IMBG and National Pirogov Memorial Medical University, Vinnytsya (from September 22, 2017). Methods for obtaining cell cultures from various sources, with adherence to relevant ethical standards and obtaining informed consent from donors for the use of biomaterial in the study, as well as the validation of MSCs according to appropriate compliance criteria, were published in our previous work [22].

Rats were used as experimental animals because the angioarchitectonics and morphology of the cerebral cortex of rats and humans are similar. The experimental IR model was made by bilateral occlusion of internal carotid arteries (ICA) lasting 20 minutes under propofol anesthesia using "Propofol-Novo" (*Novofarm-Biosintez LLC*, Ukraine) at a dose of 60 mg/kg intraperitoneally. The selected model reflects the clinical manifestation of cerebral infarction and is optimal for experimental studies of potential neuroprotective substances.

During the experiment, the animals were divided into nine groups (14 in each). Group 1 included intact animals. Group 2 consisted of pseudo/sham-operated rats, which underwent skin incision and vascular preparation after anesthesia without ligation of the ICA (to eliminate the impact of the traumatic experimental conditions) and with a single injection of 0.9 % saline solution into the femoral vein at a dose of 2 mL/kg. Group 3 included animals with control pathology: rats in this group were subjected to 20-minute cerebral ischemia by ligatures placing on the ICA, which were removed in 20 minutes (reperfusion) and by injection a 0.9 % saline solution in the same dose as to group 2. MSCs of various origins were transplanted into other groups of animals once into the femoral vein immediately after IR at a dose of  $1 \times 10^6$  cells/animal in 0.2 mL of 0.9 % saline solution: group 4 of rats were transplanted with human umbilical cord Wharton's jelly-derived MSCs (hUC-MSCs), group 5 – MSCs derived from rat embryonic fibroblasts (REF), group 6 – MSCs derived from human adipose tissue (hAT-MSCs), group 7 – MSCs derived from rat adipose tissue (RAT-MSCs), group 8 – 0.2 mL/animal of lysate derived from hUC-MSCs (MSC lysate). Group 9 of rats was administered intravenously with a single dose of the reference drug citicoline "Neuroxon" (*Arterium Corporation*, Ukraine) at 250 mg/kg. To achieve a positive effect, the injection of the studied substances was performed once immediately after IR, since early transplantation of MSCs leads to rapid neurological recovery, a decrease in the infarct volume and requires a smaller number ( $1 \times 10^6$ ) of donor cells [7, 31].

On the 7<sup>th</sup> and 14<sup>th</sup> day, 126 animals (14 rats from each group) were euthanized under propofol anesthesia (60 mg/kg intraperitoneally) with rapid extraction of rat brains immediately after decapitation. The hippocampal tissues were washed with a cold 1.15 % KCl solution and homogenized at 3000 rpm (Teflon-glass) in a medium containing 1.15 % KCl (ratio 1:3). The post-nuclear fraction was obtained from the homogenates by centrifugation (30 min, 600 ×g at -40 °C) and stored at -20 °C until further analysis. Parameters of carbohydrate metabolism (glucose,

lactate), oxidative (NADPH oxidase activity level) and nitrosative stress (NO synthase activity) in the hippocampus were determined under the conditions of cerebral IR and on the background of its correction.

Biochemical studies were carried out in the scientific-research and clinical-diagnostic laboratory of National Pirogov Memorial Medical University, certified by the Ministry of Health of Ukraine. Glucose concentration was determined by the glucose oxidase method using standard kits (*Filiclit-Diagnostics*, Ukraine). The content of lactate was determined by the colorimetric method, NADPH-oxidase – by the degree of absorption of NADPH at 340 nm according to T. Fukui et al. (1997), the total activity of NO-synthase was measured by the number of formed nitrite anion NO<sub>2</sub><sup>-</sup> after incubation of the post-nuclear supernatant for 60 min in 1 mL of medium, contained 50 mM KH<sub>2</sub>PO<sub>4</sub>-NaOH-buffer (pH = 7.0), 1 mM MgCl<sub>2</sub>, 2 mM CaCl<sub>2</sub>, 1 mM NADPH, 2.2 mM L-arginine (*Sigma*, USA) [16]. The reduction of NADPH was equimolar to the amount of NO produced, which was recorded spectrophotometrically at a wavelength of 340 nm. A spectrophotometric method was used to determine the total activity of NOS and the activity of NADPH oxidase. Optical density measurements were carried out using the spectrophotometer PD-303 (*Apel*, Japan).

Statistical analysis of the obtained data was performed with the use of Statistica 6.0 (*StatSoft® Inc.*, USA). Significance of differences was assessed using the parametric Student’s t-test and the non-parametric Mann-Whitney U-test. The results for each experimental group are presented as the arithmetic mean ± standard error of the mean (Mean ± SEM). The difference between the studied parameters was considered statistically significant at a value p < 0.05.

## RESULTS AND DISCUSSION

In the study of intact and sham-operated animals, no differences were found between the parameters of metabolic processes in the brain tissues, therefore, we used a group of sham-operated rats as a control (**Table 1**).

On the model of IR in rats, we found that as a result of ischemia, the hippocampus undergoes significant changes in stable parameters of homeostasis, namely glucose imbalance. Significantly higher glucose content was noted on the 7<sup>th</sup> day of the experiment – up to 63.0 % of the baseline (p < 0.05). Transplantation of MSCs of various origins, as well as the use of MSC lysate and citicoline contributed to the stabilization of carbohydrate metabolism in hippocampal cells after cerebral ischemia, as evidenced by a lower glucose level. However, quantitative changes in glucose were more clearly revealed in hUC-MSCs, REF and citicoline, which significantly reduced glucose content in the hippocampus by an average of 25.6 %, 16.8 %, and 28.4 %, respectively, compared to the control pathology group (**Table 1**).

On the 14<sup>th</sup> day of the experiment, the increase in glucose content in the hippocampus was less extensive – up to 42.9 % of the baseline (p < 0.05). A positive effect of hUC-MSCs and REF transplantation, and the use of citicoline on the glucose level in hippocampal cells was also found, with levels being significantly lower by an average of 21.7 %, 11.8 %, and 22.9 %, respectively, compared to the group with control pathology. When compared with the parameters of group 9 of rats which were administered citicoline on the background of IR, it was revealed that therapy with the use of hUC-MSCs was not inferior to this reference drug.

The study of lactate content showed that under the conditions of an ischemic stroke, the process of aerobic oxidation of glucose was inhibited, anaerobic glycolysis increased, and lactic acidosis developed in hippocampal cells (**Table 1**). It can be assumed that the activation of anaerobic glucose catabolism is a consequence of adenosine diphosphate (ADP) accumulation – an allosteric activator of regulatory enzymes of glycolysis.

On the 7<sup>th</sup> day of the study, the lactate level was higher in the hippocampus of groups 3, 4, 5, 6, 7, 8, 9 of experimental animals, which on average amounted to 352.4 %, 184.3 %, 240.4 %, 320.5 %, 327.7 %, 354.2 % and 177.1 % respectively, compared to sham-operated animals (p < 0.05). Correction of IR injury of rat brain with the use hUC-MSCs, REF and citicoline resulted in significantly lower lactate content in hippocampal tissues compared to the control pathology by an average of 37.2 %, 24.8 % and 38.7 %, respectively.

On the 14<sup>th</sup> day of the experiment, the increase in lactate levels in the hippocampus was less pronounced in experimental groups 3, 4, 5, 6, 7, 8, and 9 compared to the sham-operated group, averaging 338.7 %, 141.9 %, 194.2 %, 303.2 %, 309.0 %, 336.8 %, and 129.7 %, respectively (p < 0.05). It should also be noted that the positive effect of transplantation of hUC-MSCs and REF, and using citicoline on the lactate levels in the hippocampus, resulting in significantly lower levels by an average of 44.8 %, 32.9 %, and 47.6 %, respectively, compared to the control pathology group. Therefore, hUC-MSCs, better than other studied MSCs and MSC lysate, at the same level as the reference drug citicoline, contributed to the recovery of lactate content in hippocampal tissues on the background of cerebral IR in rats.

The positive effect of therapy with the studied stem cells and citicoline on the state of pro-oxidant enzymes in the hippocampus was also noted. In groups 4, 5, 6, 7, 8, and 9, the activity of NADPH oxidase was significantly higher than the similar parameters in sham-operated animals on the 7<sup>th</sup> day of the experiment, by an average of 19.5 %, 29.6 %, 61.0 %, 67.9 %, 70.4 %, and 7.5 %, respectively, and in the group of animals with control pathology, the activity of this enzyme was higher by 80.5 % (**Table 1**).

**Table 1.** Indicators of carbohydrate metabolism, oxidative, and nitrosative stress in the rat hippocampus during cerebral ischemia-reperfusion and in response to its correction

Periods of follow-up	Biochemical parameters (M ± m, n=14)			
	Glucose, µmol / g of dry tissue	Lactate, µmol / g of dry tissue	NADPH oxidase, nmol / min•mg of protein	NO-synthase, pmol / min•mg of protein
<b>Group 1 - Intact rats</b>				
7 <sup>th</sup> day	2.3 ± 0.09	1.6 ± 0.06	1.5 ± 0.07	95.5 ± 3.11
14 <sup>th</sup> day	2.2 ± 0.07	1.5 ± 0.05	1.5 ± 0.09	93.3 ± 1.89
<b>Group 2 - Sham-operated rats</b>				
7 <sup>th</sup> day	2.2 ± 0.05	1.7 ± 0.05	1.6 ± 0.04	98.1 ± 3.79
14 <sup>th</sup> day	2.3 ± 0.09	1.6 ± 0.04	1.6 ± 0.03	97.0 ± 3.09
<b>Group 3 - IR (control pathology)</b>				
7 <sup>th</sup> day	3.5 ± 0.15* (+63.0 %)	7.5 ± 0.16* (+352.4 %)	2.9 ± 0.08* (+80.5 %)	210.0 ± 5.31* (+114.1 %)
14 <sup>th</sup> day	3.2 ± 0.05* (+42.9 %)	6.8 ± 0.16* (+338.7 %)	2.6 ± 0.09* (+69.0 %)	200.3 ± 6.64* (+106.4 %)

Periods of follow-up	Biochemical parameters (M ± m, n=14)			
	Glucose, μmol / g of dry tissue	Lactate, μmol / g of dry tissue	NADPH oxidase, nmol / min*mg of protein	NO-synthase, pmol / min*mg of protein
<b>Group 4 – IR + human umbilical cord Wharton's jelly-derived MSCs</b>				
7 <sup>th</sup> day	2.6 ± 0.08*# (+21.3 %) [-25.6 %]	4.7 ± 0.07*# (+184.3 %) [-37.2 %]	1.9 ± 0.06*#S (+19.5 %) [-33.8 %]	122.7 ± 4.13*#S (+25.1 %) [-41.6 %]
14 <sup>th</sup> day	2.5 ± 0.07*# (+11.9 %) [-21.7 %]	3.8 ± 0.14*# (+141.9 %) [-44.8 %]	1.9 ± 0.06*#S (+19.4 %) [-29.4 %]	122.4 ± 2.29*#S (+26.2 %) [-38.9 %]
<b>Group 5 – IR + rat embryonic fibroblasts</b>				
7 <sup>th</sup> day	2.9 ± 0.06*#S (+35.6 %) [-16.8 %]	5.7 ± 0.18*#S (+240.4 %) [-24.8 %]	2.1 ± 0.10*#S (+29.6 %) [-28.2 %]	152.9 ± 3.54*#S (+55.8 %) [-27.2 %]
14 <sup>th</sup> day	2.9 ± 0.11*#S (+26.1 %) [-11.8 %]	4.6 ± 0.22*#S (+194.2 %) [-32.9 %]	2.1 ± 0.09*#S (+33.6 %) [-21.0 %]	148.3 ± 5.37*#S (+52.8 %) [-26.0 %]
<b>Group 6 – IR + human adipose-derived tissue MSCs</b>				
7 <sup>th</sup> day	3.3 ± 0.07*#S (+52.8 %)	7.0 ± 0.25*#S (+320.5 %)	2.6 ± 0.15*#S (+61.0 %)	196.0 ± 4.69*#S (+99.8 %)
14 <sup>th</sup> day	3.1 ± 0.10*#S (+37.2 %)	6.3 ± 0.28*#S (+303.2 %)	2.4 ± 0.08*#S (+56.8 %)	183.7 ± 6.05*#S (+89.3 %)
<b>Group 7 – IR + rat adipose-derived MSCs</b>				
7 <sup>th</sup> day	3.4 ± 0.15*#S (+55.6 %)	7.1 ± 0.27*#S (+327.7 %)	2.7 ± 0.15*#S (+67.9 %)	201.1 ± 7.49*#S (+105.0 %)
14 <sup>th</sup> day	3.1 ± 0.08*#S (+35.8 %)	6.3 ± 0.20*#S (+309.0 %)	2.5 ± 0.12*#S (+61.3 %)	187.6 ± 10.29*#S (+93.3 %)
<b>Group 8 – IR + lysate of Wharton's jelly-derived MSCs</b>				
7 <sup>th</sup> day	3.4 ± 0.13*#S (+56.5 %)	7.5 ± 0.25*#S (+354.2 %)	2.7 ± 0.06*#S (+70.4 %)	206.0 ± 6.72*#S (+110.0 %)
14 <sup>th</sup> day	3.3 ± 0.04*#S (+43.8 %)	6.8 ± 0.14*#S (+336.8 %)	2.5 ± 0.12*#S (+60.0 %)	193.4 ± 7.98*#S (+99.3 %)
<b>Group 9 – IR + citicoline</b>				
7 <sup>th</sup> day	2.5 ± 0.11*# (+16.7 %) [-28.4 %]	4.6 ± 0.15*# (+177.1 %) [-38.7 %]	1.7 ± 0.04*# (+7.5 %) [-40.4 %]	111.6 ± 2.42*# (+13.7 %) [-46.9 %]
14 <sup>th</sup> day	2.5 ± 0.06*# (+10.2 %) [-22.9 %]	3.6 ± 0.14*# (+129.7 %) [-47.6 %]	1.7 ± 0.05*# (+8.4 %) [-35.9 %]	109.6 ± 3.48*# (+12.9 %) [-45.3 %]

Note: \* –  $p < 0.05$  compared to the corresponding time group of sham-operated animals;

# –  $p < 0.05$  compared to the corresponding time group of animals with control pathology;

\$ –  $p < 0.05$  compared to the corresponding time group of animals treated with citicoline.

In round brackets – changes of the parameter compared to its level in sham-operated animals; in square brackets – changes compared to the parameter of the control pathology group.

IR therapy of hippocampus injury with the use of hUC-MSCs, REF and citicoline had a depriving effect on the activity of NADPH oxidase, the activity level was significantly lower compared to the similar parameters in control animals by an average of 33.8 %, 28.2 %, and 40.4 %, respectively. On the 14<sup>th</sup> day of the experiment, the activity of NADPH oxidase was significantly less elevated in experimental groups 4, 5, 6, 7, 8, and 9 compared to the sham-operated group of rats, averaging 19.4 %, 33.6 %, 56.8 %, 61.3 %, 60.0 %, and 8.4 %, respectively. In the group of animals with the control pathology, the enzyme activity level was 69.0 % higher ( $p < 0.05$ ). Intravenous transplantation (IV) of hUC-MSCs, REF and administration of citicoline in cerebral IR contributed to a lower NADPH oxidase activity level in the hippocampus, averaging 29.4 %, 21.0 %, and 35.9 % respectively, compared to the group of animals with the control pathology ( $p < 0.05$ ). At the same time, therapy with the use of citicoline had a more pronounced depriving effect on the activity of NADPH oxidase in the hippocampus of rats with modelling IR, and IV transplantation of hUC-MSCs was not inferior to the reference drug and was better than the other tested MSCs and MSC lysate.

The next stage of our research was the identification of the corrective effect on the total activity of NO-synthases of the tested MSCs, MSC lysate and citicoline, in particular, on the development of nitrosative stress

in the hippocampus during modelling IR of the brain in rats (Table 1). Thus, the subacute period of a stroke is characterized by an imbalance in the functioning of the nitric oxide system in the hippocampus, which is associated with more than twofold activation of NO synthase. Similar changes were observed in the recovery period of acute cerebral IR. The corrective effect of the studied MSCs and citicoline on NO metabolism was manifested in a decrease in the total activity of NO synthases in the hippocampus. Thus, on the 7<sup>th</sup> day of the experiment, significantly lower total NO-synthase activity was observed in the groups of rats that were IV injected with hUC-MSCs, REF and citicoline on the background of IR, averaging 41.6 %, 27.2 %, and 46.9 %, respectively, compared to the group with the control pathology. On the 14<sup>th</sup> day of follow-up, the total activity of NO-synthase in these studied groups remained lower, averaging 38.9 %, 26.0 %, and 45.3 %, respectively ( $p < 0.05$ ). The effect of hUC-MSCs, similar to the reference drug citicoline, may be one of the key mechanisms of their protective effect on hippocampal neurons in model IR of the brain in rats.

According to many authors, one of the main causes of IR injury in the hippocampus is the increased production of ROS and energy deficiency caused by changes in mitochondrial metabolism [2, 39]. Ischemia causes the accumulation of succinate in hippocampal cells and changes in

mitochondrial NADPH, which leads to the formation of excessive ROS [28, 37]. Microglia and astrocytes are the main producers of ROS and reactive nitrogen species, which together affect synaptic transmission, playing a crucial role in neuron-glia interaction in the hippocampus [8, 42]. NO synthase is involved in the production of nitrogen monoxide (NO), which plays an important role in the immune response, and during ischemia-reperfusion of brain injury affects synaptic transmission and non-synaptic communication between neurons and glia [6, 13]. When oxygen combines with NO, it creates the extremely harmful peroxynitrite (ONOO<sup>-</sup>). ROS and reactive nitrogen species, diffusing to myelin membranes of oligodendrocytes during periods of increased neuronal activity, activate protein kinase C and post-translationally modify the main myelin protein [8, 42]. Indeed, a number of studies have demonstrated that mitochondrial dysfunction deepens postischemic injury resulting from abnormal hyperproduction of ROS, accumulation of calcium ions, defective mitochondrial biogenesis, which is accompanied by a violation of the ADP ratio and a decrease in NAD<sup>+</sup> levels, which subsequently activates apoptosis [12, 28].

Also, some researchers have found that the pathology of regulatory mechanisms in the endocrine and autonomic nervous systems during IR, as well as the release of pro-inflammatory mediators from the brain, activate the immune response and systemic inflammation. At the same time, the liver, as the main metabolic organ, contributes not only to immunosuppression after a stroke, but also to stress-induced hyperglycemia [19]. Systemic hyperglycemia associated with IR injury can contribute to the inflow of glucose into ischemic brain tissues due to BBB damage [33, 37]. At the stage of energy shifts, this compensation activates the anaerobic pathway of glucose metabolism, increasing lactate and hydrogen ion production, which leads to the development of metabolic acidosis [3].

Regenerative medicine based on the use of MSCs may be a new treatment option for cerebral IR injury, as it was shown in preclinical studies. Animal models have shown the promising use of MSCs for ischemic stroke treatment due to their regenerative and immunomodula-

tory properties [30, 41]. Recent reports indicate that MSCs isolated from bone marrow may exhibit different functional and molecular phenotypes depending on the methods and agents used for their isolation [38]. This highlights the challenges in maintaining consistent quality during cell culture. Additionally, each type of stem cell has its own advantages and disadvantages, and it remains unclear which cell type is the most effective for the treatment of IR in acute cerebrovascular accident. Therefore, the search for an effective MSC population in the clinic has intensified [11].

Therefore, as a result of the performed research, we found that the therapeutic IV transplantation of hUC-MSCs at a dose of  $1 \times 10^6$  cells/animal was better at normalizing parameters of carbohydrate metabolism in the rat hippocampus with cerebral IR than the other tested MSCs and MSC lysate. At the same time, hUC-MSCs compared with citicoline (250 mg/kg), were not inferior in their effect on the processes of aerobic and anaerobic oxidation of carbohydrates and, as a result, more effectively increased the energy fund of neurons.

Also, the use of hUC-MSCs had a depriving effect on the activity of NADPH-oxidase and NO-synthase in the hippocampus of rats with cerebral IR and was better than the other studied cells and MSC lysate and was not inferior to the reference drug. Accordingly, MSCs derived from fetal and perinatal tissues exert a more pronounced modulatory effect on biochemical changes in the hippocampus of rats with acute IR compared to MSCs obtained from human and rat adipose tissue, as well as lysates of human Wharton's jelly. We attribute this to the higher regenerative potential of these cells, which is likely related to their cytological origin. Mechanisms underlying the favorable results of stromal cell transplantation include bystander effects, paracrine mechanisms, or regenerative effects mediated with extracellular vesicles. However, at present, there are factors contributing to differences between results obtained in animal models and clinical data, including, in particular, differences in the preparation, efficiency and functionality of MSCs in terms of tissue origin, culture and reproduction.

## CONCLUSION

1. ***A 20-minute cerebral ischemia-reperfusion of the internal carotid artery caused disorders of carbohydrate metabolism, and also leads to a noticeable development of oxidative and nitrosative stress in the hippocampus of the rat brain.***
2. ***Intravenous transplantation of human umbilical cord-derived MSCs, better than other tested cells and MSC lysate, contributed to the recovery of disturbed energy processes and eliminated metabolic acidosis in the rat hippocampus, i.e. had a positive modulating effect on the oxidant-antioxidant balance, which is probably one of the mechanisms of their neuroprotective action.***
3. ***Human umbilical cord-derived MSCs were not inferior to the reference drug citicoline in the results of protecting neurons from oxidative stress by NADPH oxidase activity in the post-ischemic rat hippocampus.***

## FUNDING

The study is a fragment of the initiative research "Pathogenetic substantiation of the expediency of use stem cells of various origins in the treatment of acute cerebral ischemia (experimental study)", state registration No. 0120U101861.

## REFERENCES:

1. Akinyemi RO, Norrving B, Brainin M, Feigin VL. Stroke Experts Collaboration Group Primary stroke prevention worldwide: translating evidence into action. *The Lancet. Public health.* 2022; 7(1), e74-e85. [https://doi.org/10.1016/S2468-2667\(21\)00230-9](https://doi.org/10.1016/S2468-2667(21)00230-9)
2. Andrabi SS, Parvez S, Tabassum H. Ischemic stroke and mitochondria: mechanisms and targets. *Protoplasma.* 2020; 257(2):335-343. <https://doi.org/10.1007/s00709-019-01439-2>
3. Arnberg F, Grafström J, Lundberg J, Nikkhou-Aski S, Little P, Damberg P, et al. Imaging of a clinically relevant stroke model: glucose hypermetabolism revisited. *Stroke.* 2015; 46(3):835-842. <https://doi.org/10.1161/STROKEAHA.114.008407>
4. Brooks B, Ebedes D, Usmani A, Gonzales-Portillo JV, Gonzales-Portillo D, Borlongan CV. Mesenchymal Stromal Cells in Ischemic Brain Injury. *Cells.* 2022; 11(6):1013. <https://doi.org/10.3390/cells11061013>
5. Campbell BCV, De Silva DA, Macleod MR, Coutts SB, Schwamm LH, Davis SM, et al. Ischaemic stroke. *Nature reviews. Disease primers.* 2019; 5(1):70. <https://doi.org/10.1038/s41572-019-0118-8>
6. Chavda V, Chaurasia B, Garg K, Deora H, Umana GE, Palmisciano P, et al. Molecular mechanisms of oxidative stress in stroke and cancer. *Brain Disord.* 2022; 5:100029. <https://doi.org/10.1016/j.dscb.2021.100029>
7. Chen Y, Peng D, Li J, Zhang L, Chen J, Wang L, et al. A comparative study of different doses of bone marrow-derived mesenchymal stem cells improve post-stroke neurological outcomes via intravenous transplantation. *Brain research.* 2023; 1798:148161. <https://doi.org/10.1016/j.brainres.2022.148161>
8. Chen Y, Qin C, Huang J, Tang X, Liu C, Huang K, et al. The role of astrocytes in oxidative stress of central nervous system: A mixed blessing. *Cell proliferation.* 2020; 53(3):e12781. <https://doi.org/10.1111/cpr.12781>
9. Chung JW, Chang WH, Bang OY, Moon GJ, Kim SJ, Kim SK, et al. Efficacy and Safety of Intravenous Mesenchymal Stem Cells for Ischemic Stroke. *Neurology.* 2021; 96(7):e1012-e1023. <https://doi.org/10.1212/WNL.00000000000011440>
10. Cui LL, Golubczyk D, Tolppanen AM, Boltze J, Jolkkonen J. Cell therapy for ischemic stroke: Are differences in preclinical and clinical study design responsible for the translational loss of efficacy? *Ann Neurol.* 2019; 86(1):5-16. <https://doi.org/10.1002/ana.25493>
11. Galipeau J, Sensébé L. Mesenchymal Stromal Cells: Clinical Challenges and Therapeutic Opportunities. *Cell stem cell.* 2018; 22(6):824-833. <https://doi.org/10.1016/j.stem.2018.05.004>
12. Galluzzi L, Kepp O, Trojel-Hansen C, Kroemer G Mitochondrial control of cellular life, stress, and death. *Circulation research.* 2012; 111(9):1198-1207. <https://doi.org/10.1161/CIRCRESAHA.112.268946>
13. García-Sánchez A, Miranda-Díaz AG, Cardona-Muñoz EG. The Role of Oxidative Stress in Physiopathology and Pharmacological Treatment with Pro- and Antioxidant Properties in Chronic Diseases. *Oxidative medicine and cellular longevity.* 2020; 2082145. <https://doi.org/10.1155/2020/2082145>
14. GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet. Neurology.* (2019)18(5), 459-480. [https://doi.org/10.1016/S1474-4422\(18\)30499-X](https://doi.org/10.1016/S1474-4422(18)30499-X)
15. Grossberg JA, Rebello LC, Haussen DC, Bousslama M, Bowen M, Barreira CM, et al. Beyond Large Vessel Occlusion Strokes: Distal Occlusion Thrombectomy. *Stroke.* 2018; 49(7):1662-1668. <https://doi.org/10.1161/STROKEAHA.118.020567>
16. Gula NM, Kosyakova GV, Berdyshev AG. The effect of N-stearoyl ethanolamine on the NO-synthase pathway of nitric oxide generation in the aorta and heart of rats with streptozotocin-induced diabetes. *The Ukrainian Biochemical Journal.* 2007; 79(5):153-158.
17. He Z, Ning N, Zhou Q, Khoshnam SE, Farzaneh M. Mitochondria as a therapeutic target for ischemic stroke. *Free Radic Biol Med.* 2020; 146:45-58. <https://doi.org/10.1016/j.freeradbiomed.2019.11.005>
18. Higashi Y, Aratake T, Shimizu T, Shimizu S, Saito M. Protective Role of Glutathione in the Hippocampus after Brain Ischemia. *International journal of molecular sciences.* 2021; 22(15):7765. <https://doi.org/10.3390/ijms22157765>
19. Inderhees J, Schwaninger M. Liver Metabolism in Ischemic Stroke. *Neuroscience.* 2024; 550:62-68. <https://doi.org/10.1016/j.neuroscience.2023.12.013>
20. Jacobs LC, Arntz RM, Schoonderwaldt HC, Dorresteijn LD, de Leeuw FE, Kessels RP. Ipsilateral hippocampal atrophy is associated with long-term memory dysfunction after ischemic stroke in young adults. *Human brain mapping.* 2015; 36(7):2432-2442. <https://doi.org/10.1002/hbm.22782>
21. Jadhav AP, Desai SM, Kenmuir CL, Rocha M, Starr MT, Molyneaux BJ, et al. Eligibility for Endovascular Trial Enrollment in the 6- to 24-Hour Time Window: Analysis of a Single Comprehensive Stroke Center. *Stroke.* 2018; 49(4):1015-1017. <https://doi.org/10.1161/STROKEAHA.117.020273>
22. Konovalov S, Moroz V, Konovalova N, Deryabina O, Shuvalova N, Toporova O, et al. The effect of mesenchymal stromal cells of various origins on mortality and neurologic deficit in acute cerebral ischemia-reperfusion in rats. *Cell Organ Transplant.* 2021; 9(2):104-108. <https://doi.org/10.22494/cot.v9i2.132>
23. Kuriakose D, Xiao Z. Pathophysiology and Treatment of Stroke: Present Status and Future Perspectives. *International journal of molecular sciences.* 2020; 21(20):7609. <https://doi.org/10.3390/ijms21207609>
24. Li J, Zhang Q, Wang W, Lin F, Wang S, Zhao J. Mesenchymal stem cell therapy for ischemic stroke: A look into treatment mechanism and therapeutic potential. *Journal of neurology.* 2021; 268(11):4095-4107. <https://doi.org/10.1007/s00415-020-10138-5>
25. Liao LL, Ruzsyzmah BHI, Ng MH, Law JX. Characteristics and clinical applications of Wharton's jelly-derived mesenchymal stromal cells. *Current research in translational medicine.* 2020; 68(1):5-16. <https://doi.org/10.1016/j.retram.2019.09.001>
26. Liu, L., Keams, K. N., Eli, I., Sharifi, K. A., Soldo, S., Carlson, E. W., Scott, K. W., Sluzewski, M. F., Acton, S. T., Stauderman, K. A., Kalani, M. Y. S., Park, M., & Tvrdik, P. (2021). Microglial Calcium Waves During the Hyperacute Phase of Ischemic Stroke. *Stroke*, 52(1), 274-283. <https://doi.org/10.1161/STROKEAHA.120.032766>
27. Martynov MY, Zhuravleva MV, Vasyukova NS, Kuznetsova EV, Kameneva TR. Oxidative stress in the pathogenesis of stroke and its correction. *Zhurnal neurologii i psikiatrii imeni S. S. Korsakova.* 2023; 123(1):16-27. <https://doi.org/10.17116/jnevro202312301116>
28. Murphy MP, Hartley RC. Mitochondria as a therapeutic target for common pathologies. *Nature reviews. Drug discovery.* 2018; 17(12):865-886. <https://doi.org/10.1038/nrd.2018.174>
29. Musuka TD, Wilton SB, Traboulsi M, Hill MD. Diagnosis and management of acute ischemic stroke: speed is critical. *CMAJ.* 2015; 187(12):887-893. <https://doi.org/10.1503/cmaj.140355>
30. Ntege EH, Sunami H, Shimizu Y. Advances in regenerative therapy: A review of the literature and future directions. *Regenerative therapy.* 2020; 14:136-153. <https://doi.org/10.1016/j.reth.2020.01.004>
31. Oh SH, Choi C, Noh JE, Lee N, Jeong YW, Jeon I, et al. Interleukin-1 receptor antagonist-mediated neuroprotection by umbilical cord-derived mesenchymal stromal cells following transplantation into a rodent stroke model. *Exp Mol Med.* 2018; 50(4):1-12. <https://doi.org/10.1038/s12276-018-0041-1>
32. Olufunmilayo EO, Gerke-Duncan MB, Holsinger RMD. Oxidative Stress and Antioxidants in Neurodegenerative Disorders. *Antioxidants (Basel, Switzerland).* 2023; 12(2):517. <https://doi.org/10.3390/antiox12020517>
33. Owolabi MO, Thrift AG, Mahal A, Ishida M, Martins S, Johnson WD, et al. Primary stroke prevention worldwide: translating evidence into action. *The Lancet. Public health.* 2022; 7(1):e74-e85. [https://doi.org/10.1016/S2468-2667\(21\)00230-9](https://doi.org/10.1016/S2468-2667(21)00230-9)

34. Pérez-Corredor PA, Gutiérrez-Vargas JA, Ciro-Ramírez L, Balcazar N, Cardona-Gómez GP. High fructose diet-induced obesity worsens post-ischemic brain injury in the hippocampus of female rats. *Nutritional neuroscience*. 2022; 25(1):122-136. <https://doi.org/10.1080/1028415X.2020.1724453>
35. Semenova E, Grudniak MP, Machaj EK, Bocian K, Chroscinska-Krawczyk M, Trochonowicz M, et al. Mesenchymal Stromal Cells from Different Parts of Umbilical Cord: Approach to Comparison & Characteristics. *Stem cell reviews and reports*. 2021; 17(5):1780-1795. <https://doi.org/10.1007/s12015-021-10157-3>
36. Simão F, Ustunkaya T, Clermont AC, Feener EP. Plasma kallikrein mediates brain hemorrhage and edema caused by tissue plasminogen activator therapy in mice after stroke. *Blood*. 2017; 129(16):2280-2290. <https://doi.org/10.1182/blood-2016-09-740670>
37. Song K, Li Y, Zhang H, An N, Wei Y, Wang L, et al. Oxidative stress-mediated blood-brain barrier (BBB) disruption in neurological diseases. *Oxid Med Cell Longev*. 2020. <https://doi.org/10.1155/2020/4356386>
38. Stroncek DF, Jin P, McKenna DH, Takanashi M, Fontaine MJ, Pati S, et al. Human Mesenchymal Stromal Cell (MSC) Characteristics Vary Among Laboratories When Manufactured From the Same Source Material: A Report by the Cellular Therapy Team of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative. *Front Cell Dev Biol*. 2020; 8:458. <https://doi.org/10.3389/fcell.2020.00458>
39. Tao J, Chen B, Gao Y, Yang S, Huang J, Jiang X, et al. Electroacupuncture enhances hippocampal NSCs proliferation in cerebral ischemia-reperfusion injured rats via activation of notch signaling pathway. *The International journal of neuroscience*. 2014; 124(3):204-212. <https://doi.org/10.3109/00207454.2013.840781>
40. Wang F, Tang H, Zhu J, Zhang JH. Transplanting mesenchymal stem cells for treatment of ischemic stroke. *Cell transplantation*. 2018; 27(12):1825-1834. <https://doi.org/10.1177/0963689718795424>
41. Wu X, Jiang J, Gu Z, Zhang J, Chen Y, Liu X. Mesenchymal stromal cell therapies: immunomodulatory properties and clinical progress. *Stem Cell Res Ther*. 2020; 11(1):345. <https://doi.org/10.1186/s13287-020-01855-9>
42. Zhao SC, Ma LS, Chu ZH, Xu H, Wu WQ, Liu F. Regulation of microglial activation in stroke. *Acta pharmacologica Sinica*. 2017; 38(4):445-458. <https://doi.org/10.1038/aps.2016.162>



ARTICLE ON THE SITE  
[TRANSPLANTOLOGY.ORG](https://www.transplantology.org)

*The authors declare that there is no potential conflict of interest regarding the research, authorship and/or publication of this article.*

УДК 612.82:616.12:615.3:59.084+569.323.4

# Порівняльний вплив мезенхімальних стромальних клітин різного походження та джерел на біохімічні показники в гіпокампі щурів при ішемії-реперфузії головного мозку



Коновалов С. В.<sup>1</sup>, Мороз В. М.<sup>1</sup>, Йолтухівський М. В.<sup>1</sup>, Гаджула Н. Г.<sup>1</sup>, Дерябіна О. Г.<sup>2,3</sup>, Кордюм В. А.<sup>2,3</sup>

<sup>1</sup>Вінницький національний медичний університет ім. М. І. Пирогова, Вінниця, Україна

<sup>2</sup>Інститут генетичної та регенеративної медицини, ДУ "Національний науковий центр "Інститут кардіології, клінічної та регенеративної медицини імені академіка М. Д. Стражеска Національної академії медичних наук України", Київ, Україна

<sup>3</sup>Інститут молекулярної біології і генетики Національної академії наук України, Київ, Україна

## РЕЗЮМЕ

При нейродегенеративних процесах головного мозку першочергово відбуваються значні пошкодження гіпокампа з наступними когнітивними порушеннями унаслідок підвищеної генерації активних форм кисню й активних форм азоту, які призводять до апоптозу та некрозу нейронів. Обнадійливі результати щодо ендогенних механізмів нейровідновлення у відповідь на ішемічне пошкодження структур головного мозку продемонструвала клітинна терапія з використанням мезенхімальних стромальних клітин (МСК).

**МЕТОЮ ДОСЛІДЖЕННЯ** стало вивчення терапевтичного потенціалу МСК різного походження та джерел, лізату МСК і референс-препарату цитиколіну на енергетичну складову нейронального метаболізму, оксидативного та нітрозативного стресу в гіпокампі щурів за умов ішемії-реперфузії (ІР) головного мозку.

**МАТЕРІАЛИ ТА МЕТОДИ.** Експеримент проведено на 126 щурах лінії Вістар із модельованою патологією (20-хвилинна ІР внутрішніх сонних артерій), яким відразу після зняття лігатур трансплантували МСК з Вартонових драглів пуповини людини, МСК з жирової тканини людини та щура, ембріональні фібробласти щура, лізат МСК, цитиколін. Визначено біохімічні показники вуглеводного обміну (глюкоза, лактат), оксидативного (активність НАДФН-оксидази) та нітрозативного стресу (активність NO-синтази) в гіпокампі щурів на 7-му та 14-ту добу після ІР головного мозку та на тлі її корекції.

**РЕЗУЛЬТАТИ.** Встановлено, що при ІР в гіпокампі головного мозку щурів підвищувався вміст глюкози та лактату, пригнічувався процес аеробного окиснення глюкози, посилювався анаеробний гліколіз, розвивався лактацидоз у клітинах, знижувався рівень активності НАДФН-оксидази, виникав дисбаланс у функціонуванні системи монооксиду азоту. Відзначено позитивний вплив трансплантації МСК Вартонових драглів пуповини людини, ембріональних фібробластів щура та застосування цитиколіну на стабілізацію рівнів глюкози, лактату, активності НАДФН-оксидази та монооксиду азоту. Трансплантації МСК із жирової тканини людини та щура виявились вірогідно менш ефективними за цитиколін і не демонстрували модулюючий вплив на біохімічні показники в гіпокампі піддослідних тварин із ІР.

**ВИСНОВКИ.** Трансплантація МСК з Вартонових драглів пуповини людини не поступалася цитиколіну, краще за решту досліджуваних клітин та їх лізату сприяла відновленню порушених енергетичних процесів (за впливом на рівень глюкози) та усувала метаболічний ацидоз (за зміною рівня лактату) у гіпокампі щурів, а також мала позитивний модулюючий вплив на оксидантно-антиоксидантний баланс (за рівнем активності НАДФН-оксидази).

**КЛЮЧОВІ СЛОВА:** мезенхімальні стромальні клітини; гіпокамп; ішемія-реперфузія головного мозку; вуглеводний обмін; оксидативний стрес