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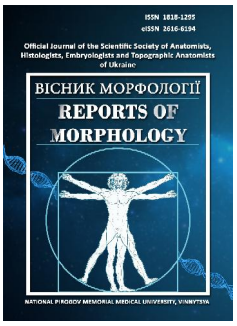


# Therapeutic potential of mesenchymal stromal cells on morphological parameters in the hippocampus of rats with brain ischemia-reperfusion modeling

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*Ischemic stroke is an extremely important pathology with high mortality, in which more than 50 % of patients with occlusion of the main vessels remain disabled, despite early reperfusion therapy by thrombolysis or thrombectomy. As part of the regenerative strategy, stem cell transplantation in ischemic stroke became a new impetus. Cell therapy with the use of mesenchymal stromal cells demonstrated encouraging results regarding endogenous mechanisms of neuroregeneration in response to ischemic damage to brain structures. The aim of the research is to study the influence of mesenchymal stromal cells of various genesis, lysate of mesenchymal stromal cells obtained from Wharton's jelly umbilical cord and citicoline on the dynamics of morphological changes in the hippocampal CA1 region of rats with acute cerebral ischemia-reperfusion according to light microscopy and micromorphometry data. The experiment was carried out using 200 male Wistar rats, which were subjected to ischemia-reperfusion by reversible 20-minute bilateral occlusion of the internal carotid arteries. Animals with modeled pathology were intravenously transplanted with mesenchymal stromal cells of various genesis (from Wharton's jelly of the human umbilical cord, human and rat adipose tissue), and rat embryonic fibroblasts, lysate of mesenchymal stromal cells and citicoline were injected. Histological analysis of rat brain sections was performed on the 7th and 14th day of the experiment. Statistical analysis was performed using "Statistica 6.0" (StatSoft® Snc, USA). The significance of differences was assessed using the Student's t-test and the nonparametric Mann-Whitney U test. During the study, it was found that modeled ischemia-reperfusion in rats caused almost complete degeneration of the structure of the pyramidal layer of hippocampal CA1 region, gave uniformity to the structure of the radiant layer, infiltration of microglia, contributed to the disruption of the arrangement of apical dendrite bundles and narrowing of blood vessels as a result of perivascular edema. Also, the modeled pathology reduced the total number of neuronal nuclei in the hippocampal CA1 area, the overwhelming majority of which had signs of pathological changes. Transplantation of mesenchymal stromal cells of various origins, lysate of mesenchymal stromal cells and citicoline contributed to a significant increase in the number of neuronal nuclei in the hippocampal CA1 zone and nuclei that did not undergo pathological changes. The most positive effect was found in the transplantation of mesenchymal stromal cells from human Wharton's jelly-derived cells. Thus, both in the subacute and recovery periods of ischemic stroke in rats, the transplantation of human Wharton's jelly-derived mesenchymal stromal cells was significantly surpassed the reference drug citicoline in its ability to reduce the number of pathologically changed nuclei by 1.5 times ($p<0.05$). At the same time, the number of pathologically unchanged nuclei significantly exceeded the number of nuclei with signs of karyorrhexis and karyopyknosis, so it would be advisable to use mesenchymal stromal cells of various genesis, lysate or citicoline in conditions of acute cerebral ischemia-reperfusion, taking into account their ability to*

*reduce the volume of the infarct. In the future, an injectable drug will be created from the most effective culture of mesenchymal stromal cells in terms of cerebroprotective properties for cell therapy of patients with acute ischemic stroke.*
**Keywords:** *mesenchymal stromal cells, neurons, hippocampus, ischemia-reperfusion, rats.*

## Introduction

Ischemic stroke is a global problem of humanity, because it leads to a high mortality, in which more than 50 % of patients with occlusion of the main vessels remain disabled, despite the earliest possible thrombolytic therapy [13, 14, 21]. Every year, about 150,000 cases of stroke occur in our country, among which more than 100,000 cases end in death of patients from stroke or other cerebral circulation disorders. The majority of patients (80%) who have survived after the stroke later suffer from physical disorders, cognitive and psychological disorders, and socio-economic challenges, which imposes a considerable burden of this pathology on the state budget [17]. Among patients with ischemic stroke, every third person is of working age, and every tenth patient will be able to recover and fully return to normal life. Of the ischemic stroke patients, every third is of working age, and every tenth patient will be able to recover and completely return to normal life [21]. Therefore, assistance with acute cerebrovascular accident (ACVA) is the main focus of the Medical Guarantee Program with financing at an increased tariff [16, 17].

A sign of destructive-degenerative injury of cellular structure in central nervous system's tissue in the formation of a focus of ischemia as a result of ACVA is a decrease in the density of neurons, as well as their nucleic acid content. A decrease in brain blood supply results in insufficient supply of oxygen ($O_2$) and glucose to it. At the same time, a decrease in the level of glucose contributes to the activation of glycolysis and, accordingly, suppression of the formation of adenosine triphosphate in the brain. As a result of the loss of potassium ions (K+) by nerve cells and the accumulation of sodium ions (Na+) and water ($H_2O$), brain tissue inflammation occurs [26]. In emergency stroke therapy, there is a need quickly restore the perfusion of the ischemic area [1]. It should be noted that even early reperfusion does not always live up to doctors' expectations, as reperfusion injury of neurons often occurs after recanalization of the occluded artery. Therefore, the development of modern drugs to promote cerebroprotection is of primary importance in order to improve the results of prevention and treatment of ischemic stroke. At present, encouraging results have been obtained from recent experiments on the ability of mesenchymal stromal cells (MSCs) to prevent the appearance of plastic exchange disorders and the loss of their structural components by neurons, which may be a sign of their cerebroprotective effect in acute brain ischemia [6, 10, 28]. However, such researches are limited, and rarely comparative. From literary sources, it is known that the study of the influence of mesenchymal stromal cells in the treatment of ischemic stroke has been conducted for the past two decades. MSCs are derived from adipose tissue, tooth buds and tooth pulp, bone marrow, liver or umbilical cord, umbilical cord blood and placenta. Various stem cells have been tested in animal models of ischemic stroke as monotherapy [19]. Bone marrow-derived cells have been well studied in recent years, as have adipose-derived MSCs, and only a few studies have been carried out with placental cells [3, 20]. Therefore, in our study, as in other experiments, researchers used both allogeneic [27] and xenogeneic [18] MSCs, but we compared the cerebroprotective effect of both allogeneic and xenogeneic MSCs from several sources. The optimal dose of MSC transplantation in ischemic stroke in rats was determined by Chen Y. et al. [5] and was 105-106 cells per animal, which corresponded to the number of cells intravenously transplanted into rodents in our study. MSCs obtained from Wharton's human umbilical cord blood cells (HUC-MSCs), due to their unique beneficial properties, have the best clinical perspectives in use. They do not have ethical and legal concerns, do not induce oncogenesis, they are easy to obtain in large quantities (multipotent, proliferative and hypoimmunogenic).

The above-mentioned data regarding the presence of cerebroprotective effect of MSCs in case of cerebral ischemia-reperfusion injury became the basis for carrying out this experimental study.

*The purpose of the research* is to study the influence of mesenchymal stromal cells of various genesis, lysate of mesenchymal stromal cells obtained from Wharton's jelly umbilical cord and citicoline on the dynamics of morphological changes in the hippocampal CA1 region of rats with acute cerebral ischemia-reperfusion according to light microscopy and micromorphometry data.

## Materials and methods

The experiment was carried out in 200 4-month-old male Wistar rats weighing between 160-190 g, which were subjected to transient bilateral 20-minute ischemia-reperfusion of the internal carotid arteries. The animals were a brood of the vivarium of National Pirogov Memorial Medical University (Vinnytsya, Ukraine) and were kept in standard conditions with free access to feeder. An experimental model of ischemia-reperfusion was made by placing ligatures on the internal carotid arteries (ICA) bilaterally under propofol "Propofol-Novo" anesthesia (Novofarm-Biosintez LLC, Ukraine) at the dose of 60 mg/kg lasting 20 minutes. The chosen model reflects the clinical manifestations of cerebral infarction and is adequate for experimental study of potential neuroprotective

**Table 1.** Distribution of rats by experimental groups during therapeutic administration of the studied substances.

| Group of animals | Number of animals | Treatment |
|---|---|---|
| Group 1 | 10 | intact rats |
| Group 2 | 10 | sham-operated rats + 0.9 % sodium chloride solution, 2 ml/kg |
| Group 3 | 40 | ischemia-reperfusion + 0.9 % sodium chloride solution, 2 ml/kg |
| Group 4 | 20 | ischemia-reperfusion + HUC-MSCs, 106 cells/animal |
| Group 5 | 20 | ischemia-reperfusion + rat embryonic fibroblasts, 106 cells/animal |
| Group 6 | 25 | ischemia-reperfusion + MSCs from human adipose tissue, 106 cells/animal |
| Group 7 | 25 | ischemia-reperfusion + MSCs from rat adipose tissue, 106 cells/animal |
| Group 8 | 25 | ischemia-reperfusion + cell lysate from HUC-MSCs, 0.2 ml/animal |
| Group 9 | 25 | ischemia-reperfusion + Citicoline, 250 mg/kg |

substances [9]. Rats were separated into 9 groups (Table 1).

Group 1 included intact animals. Group 2 consisted of pseudo/sham-operated rats, which were sequentially subjected to the following interventions (anesthesia, skin incision and vessel preparation) without subsequent ligation of ICA to reduce the impact of the traumatic experimental conditions. Group 3 included rats with control pathology; they were subjected to 20-minute cerebral ischemia by ligatures placing on the ICA. In 20 minutes the ligatures were removed from the ICA (reperfusion) and a 0.9 % saline solution was injected into the femoral vein (2 ml/kg). The same dose of physiological solution was administered to group 2 rats. In the group 4 the rats were transplanted with 106 HUC-MSCs immediately after ischemia-reperfusion. In the group 5 of animals with ischemia-reperfusion a single transplantation with 106 cells/animal of rat embryonic fibroblasts was used. Group 6 of rats with ischemia-reperfusion were transplanted with 106 cells/animal derived from human adipose tissue MSCs. Group 7 of rats were injected with 106 cells/animal derived from rat adipose MSCs after ischemia-reperfusion. Group 8 of animals with ischemia-reperfusion was injected with 0.2 ml/animal of lysate derived from human Wharton's jelly MSCs. Group 9 of rats immediately after ischemia-reperfusion administered a single dose (250 mg/kg) of the reference drug citicoline "Neuroxon" (Arterium Corporation, Ukraine). Citicoline was chosen among all available drugs due to its ability to enhance neuroregenerative processes in the rat experiment and to improve cognitive and memory functions in patients with cerebral ischemia [4, 15, 24].

To analyze the influence of mesenchymal stromal cells of different origin, cell lysate from HUC-MSCs and citicoline on the dynamics of destructive changes in the CA1 area of the hippocampus, the brain of rats was removed immediately after decapitation using propofol anesthesia with an overdose of pentobarbital ("Penbital", Bioveta JSC, Czech Republic, 100 mg/kg) in 7 days (subacute period of ischemia) and 14 days (recovery period) after ischemia-reperfusion [12, 27]. The brains of rats were fixed with a 4% solution of formaldehyde within 24 hours. After fixation, the brains were washed in water, passed through ethanol of ascending concentration and xylene, and after standard histological processing, embedded in Paraplast Plus© (Leica Scientific (McCormick©), USA). The sections at 5 μm thickness were made on a rotary microtome. Deparaffinized sections were stained according to the Nissl staining method. Digital images of frontal brain sections of experimental animals obtained with a BX-51 microscope (Olympus, Japan) were analyzed using the ImageJ computer program (1.48 v software, freeware license, Rasband, USA, 2015). In hippocampal CA1 region, the total neuronal nuclei numbers per 1 $mm^2$ was estimated, and the ratio of the number of unchanged neuronal nuclei and nuclei with pathological changes (karyopyknosis and karyorrhexis) was also determined.

When carrying out the study, the methodological recommendations of SEC of Ministry of Health of Ukraine and bioethics requirements in relation to the National "General Ethical Principles of Experiments on Animals" approved by the First National Congress of Bioethics (Kyiv, Ukraine, 2001) and the Law of Ukraine dated 21.11.2006 No. 3447-IV "On the Protection of Animals from Cruelty" were taken into account.

Bioethics Committee of National Pirogov Memorial Medical University, Vinnytsya (protocol No. 2 dated 31.01.2024) established that the research materials do not contradict the basic moral and ethical standards of the Helsinki Declaration, the Council of Europe Convention on the Protection of Vertebrate Animals Used in the Experiment dated 18.03.1986, standards of the Directive of the Council of the European Communities 86/609/EEC dated 24.11.1986. The study is a fragment of the initiative research work "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.

Statistical analysis of the obtained data was performed using Microsoft© Exel©-2010 and "Statistica 6.0" (StatSoft® Snc, USA) software. The significance of differences was assessed using the Student's t-test and unpaired nonparametric Mann-Whitney U test. Differences between the measured parameters were considered statistically significant at $p<0.05$.

## Results

The lack of differences and clear visualization of all zones and layers in the hippocampus of intact and sham-operated animals during histological examination of frontal paraffin sections of rat brains identified the latter as the control group (Fig. 1).

Basal dendrites of pyramidal neurons formed the ascending layer of the CA1 zone in rat hippocampus, and pyramidal neurons' bodies - a dense layer made of 3-5 rows of pyramidal cells. Pyramidal neurons had a nucleus containing a single nucleolus and euchromatin in enough amount (see Fig. 1).

Unbranched apical dendrites of pyramidal neurons were radially oriented in the radiant layer. Their thin endings formed a lacunose-molecular layer, in which a large number of blood vessels were visualized. Blood vessels wall was formed by endotheliocytes with heterochromic nuclei.

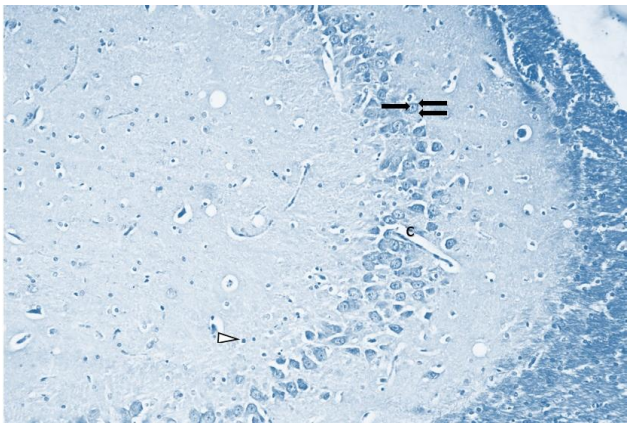


**Fig. 1.** The figure shows the fragment of CA1 zone in rat hippocampus in the group of pseudo-operated animals. Pyramidal neuronal nucleus is pointed out with a double arrow, while its nucleolus - with a single one. The astrocyte nucleus is shown by a white triangle. C letter indicates a blood vessel lumen. Staining according to Nissl. x200.

On day 7 after ischemia-reperfusion in the control group of rats, a visible devolution in the pyramidal layer took place in the part of the hippocampal slices made in the CA1 zone, in particular puckered hyperchromic neuronal bodies with acidophilic cytoplasm and also karyopyknosis and karyorrhexis were well visible (Fig. 2A).

It should be emphasized that intense infiltration with microglial cells took place in the pyramidal and radiant layers of the CA1 zone in the hippocampus. While the structure of the radiant layer became homogeneous, the architecture of the apical dendrites forming beams was affected (Fig. 2A). There was edema of the intercellular space, as well as pronounced perivascular edema and narrowing of the vessel lumen. In some rats with ischemia-reperfusion modeling hemorrhage and hyaline masses formation, sludge of red blood cells (RBCs) and destruction were visualized.

In fourteen days after ischemia-reperfusion modeling, degeneration of the CA1 pyramidal layer also was clear in the hippocampus (Fig. 2B). The destructive changes manifestations in the CA1 zone were analogous as that in seven days after ischemia-reperfusion injury, i.e., the structure of the radiant layer of the CA1 hippocampal zone also was connatural, intercellular edema and pronounced perivascular edema persisted, the arrangement of apical dendrites strands was affected (see Fig. 2B).

On the 7th day after HUC-MSCs transplantation to the rats with ischemia-reperfusion modeling (it's the study group 4) against the background of the above-mentioned changes found in the pyramidal layer of the CA1 zone, lots of unharmed pyramidal neurons was detected (Fig. 3A). Microglial infiltration of the radiant and pyramidal layers was almost not observed. 3-5 ordered rows of pyramidal neuronal bodies were noticed in the pyramidal layer. Midst pathologically unchanged euchromatic nuclei of pyramidal neurons having one nucleolus, there were also

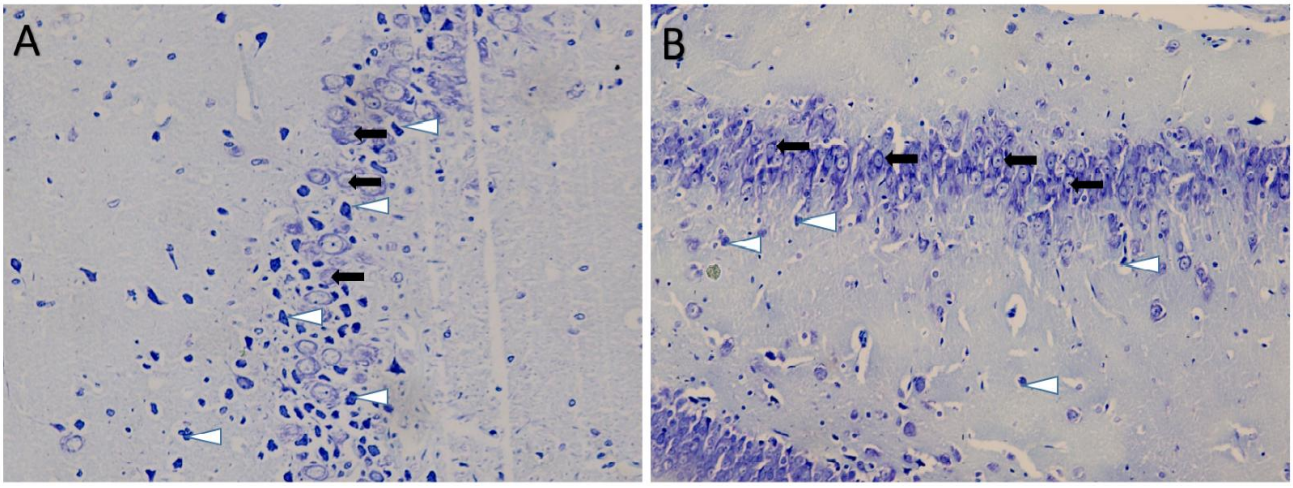


**Fig. 2.** The figure shows the degeneration of the pyramidal layer in the CA1 hippocampal zone on day 7 (**A**) and day 14 (**B**) after ischemia-reperfusion modeling. In neurons, their bodies are puckered and hyperchromic, their cytoplasm is acidophilic (pointed out with triangles). Separate neurons, the structure of which is preserved, are pointed out with arrows. Intercellular edema is visible. Staining according to Nissl x200.

heterochromatic and pyknotic nuclei with pronounced perinuclear edema. Apical dendrites shaped ordered strands. The vascular wall in capillaries was lined with endotheliocytes having heterochromatic nuclei. Next to some capillaries perivascular edema was visible.

On the 14th day after ischemia-reperfusion and subsequent transplantation of HUC-MSCs, in the CA 1 zone of the rat hippocampus the cytoarchitectonics was analogous to investigated on the 7th day: in particular, pyramidal neurons were orderly oriented and formed unceasing beams, euchromatin nucleoli were obvious in their nuclei (Fig. 3B). We could also see damaged hyperchromic neuronal bodies containing acidophilic cytoplasm. Apical dendrites of neurons in this layer formed radially directed unbranched structured beams and endothelial cells with heterochromatic nuclei were very noticeable in the vascular wall.

In the group of rats with ischemia-reperfusion and consequent transplantation of rat embryonic fibroblasts on the day 7 after ischemia-reperfusion modeling, both pyramidal neurons with euchromatin nuclei and one nucleolus and harmed hyperchromic neurons having karyopyknosis features and acidophilic cytoplasm took place in the hippocampal CA1 zone (Fig. 4A). The cytoarchitectonics of the radiant layer in the CA1 zone of the rat hippocampus was disturbed: the apical dendrites of neurons did not form orderly unceasing beams, narrowing

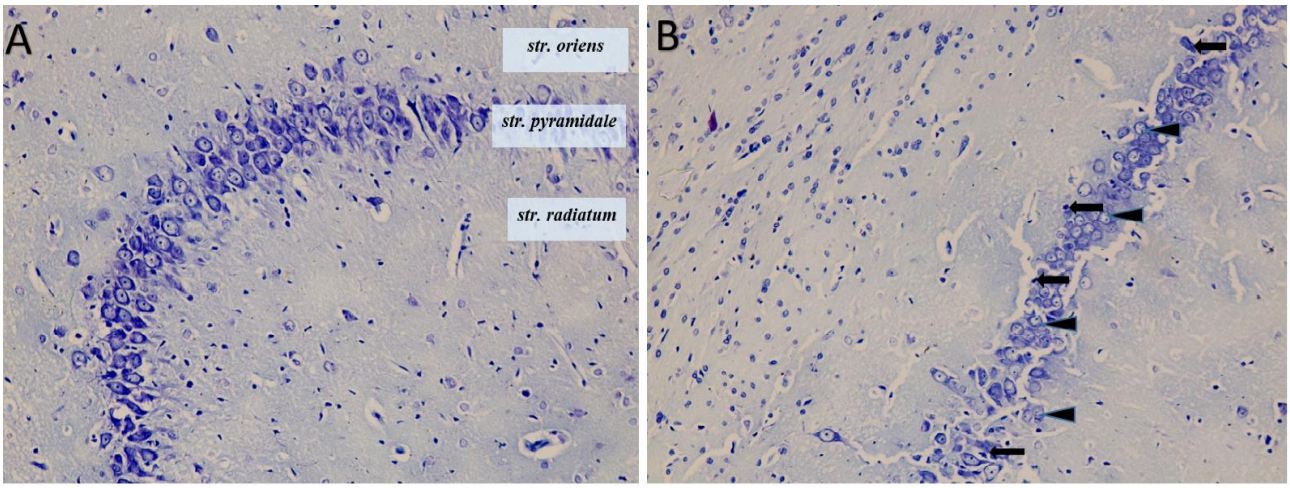


**Fig. 3. A** - the figure shows the pyramidal layer in the CA1 area of rat hippocampus on the day 7 after ischemia-reperfusion and consequent Wharton's jelly-derived MSCs transplantation. A considerable number of unharmed neurons is in the pyramidal layer, while radially oriented apical dendrites arrange straight beams in the radiant layer. **B** - on the figure 3B the pyramidal layer in the CA1 area on the day 14 after ischemia-reperfusion and consequent transplantation of human MSCs is given. It is clearly visible that the neuronal nuclei contain large euchromatin amount, one or two nucleoli and are well ordered forming beams. Neurons which have a preserved structure are pointed out with triangles, while the damaged hyperchromic neurons having acidophilic cytoplasm - with arrows. Staining according to Nissl. x200.

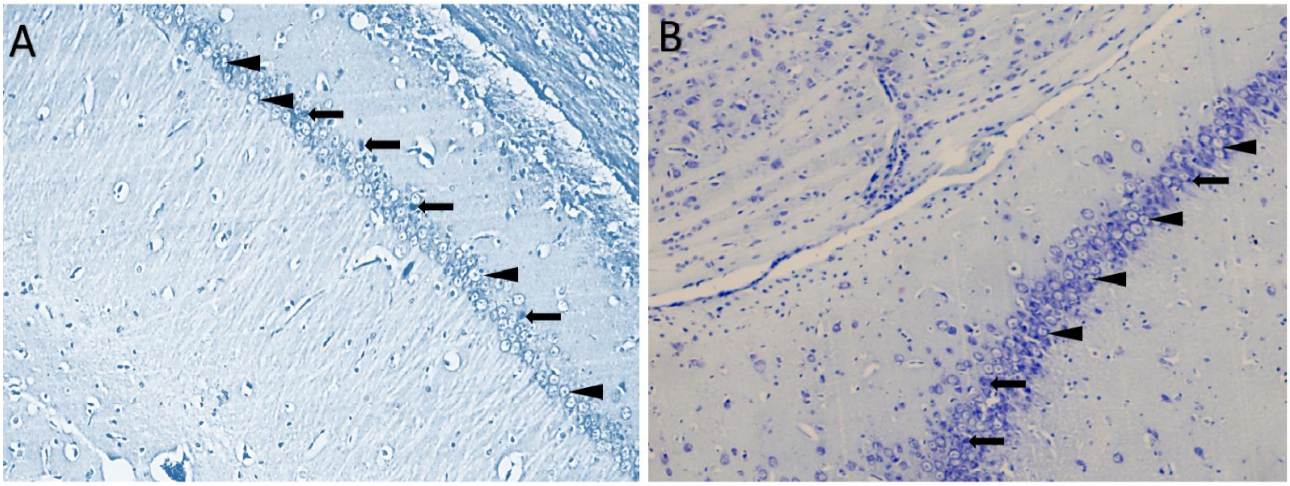


**Fig. 4. A** - the figure shows the pyramidal layer in the CA1 zone of the rat hippocampus on the day 7 after ischemia-reperfusion and consequent transplantation of rat embryonic fibroblasts. Pyramidal neurons containing euchromatin nuclei are pointed with triangles, and damaged hyperchromic neurons with karyopyknosis features and acidophilic cytoplasm are marked with arrows. Perivascular edema is observed. **B** - the pyramidal layer of the CA1 hippocampal zone on the day 14 after ischemia-reperfusion and consequent transplantation of rat embryonic fibroblasts. Disorganization of the radiant layer takes place. Perivascular edema persists. Stasis and RBCs sludge are observed in blood capillaries. Endotheliocytes of the capillary wall are characterized by heterochromatic nuclei. Staining according to Nissl. x200.

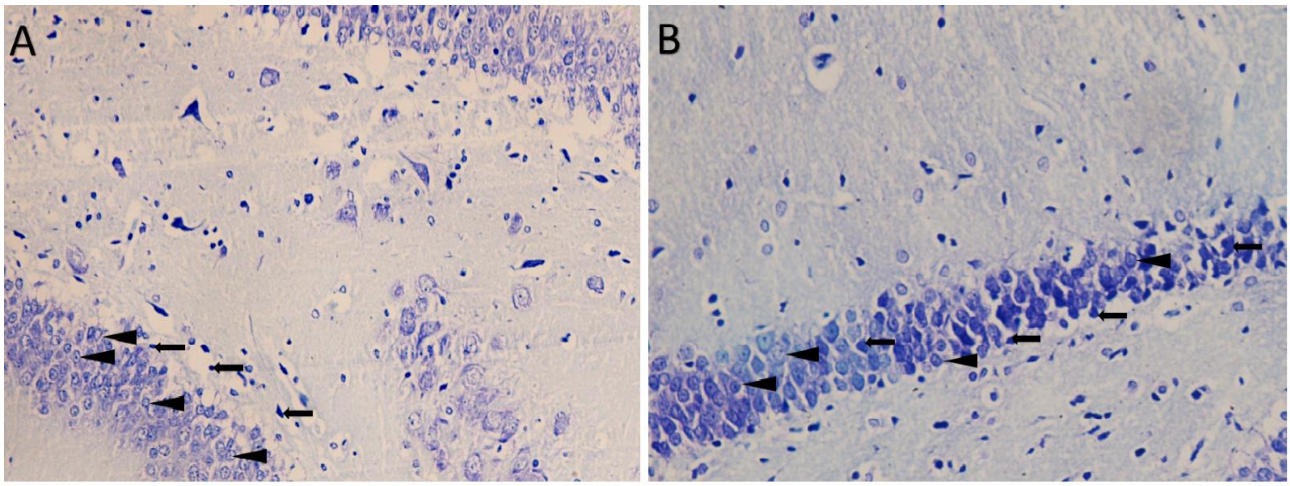


**Fig. 5.** The picture shows the pyramidal layer of the CA1 hippocampal zone on day 7 (**A**) and day 14 (**B**) after ischemia-reperfusion modeling and consequent MSCs transplantation of the cells obtained from human adipose tissue. Neurons with a preserved structure are pointed with triangles, while damaged ones - with arrows. Staining according to Nissl. A - x200, B - x400.

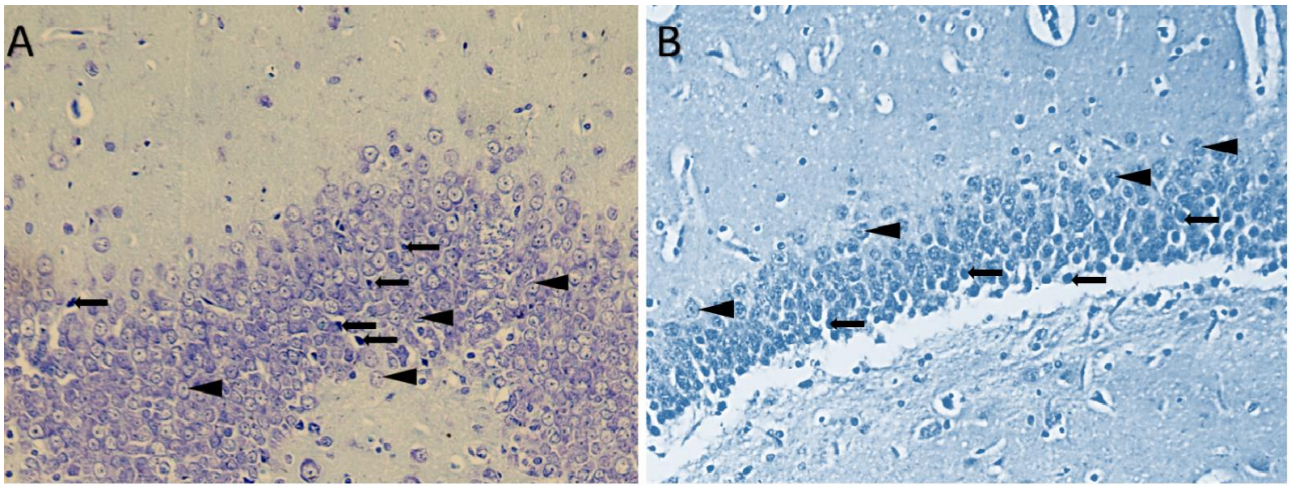


**Fig. 6.** The figure shows the pyramidal layer of the CA1 hippocampal zone in rats on day 7 (**A**) and day 14 (**B**) after ischemia-reperfusion followed by transplantation of stem cells obtained from rat adipose tissue. **A** - the neuronal nuclei are saturated in euchromatin and have a well-visible nucleolus. Infiltration of the pyramidal layer with microglia does not occur. Unharmed neurons are pointed out with triangles, while the damaged ones - with arrows. **B** - pyramidal neurons nuclei are saturated with euchromatin and have a well-visible nucleolus. Apical dendrites are well oriented. Intercellular swelling takes place. Unchanged pyramidal neurons are pointed out by triangles, damaged neurons - by arrows. Staining according to Nissl. x200.

of blood vessels was observed, in the lumen of which visible RBCs were, perivascular edema occurred also.

On the 14th day after ischemia-reperfusion modeling followed by rat embryonic fibroblasts transplantation in the CA1 zone of rat hippocampus pyramidal neurons were ordered forming straight beams made of some layers of pyramidal cells (Fig. 4B). Their nuclei were abundant with euchromatin, with one nucleolus. Solitary heterochromatic or bulging nuclei were between such the nuclei. The radiant layer, although it looked disorganized, but, unlike the 7th day, it showed straight beams built from apical dendrites. In a part of the blood vessels, the lumen was narrowed, perivascular edema took place.

In the group of rats with ischemia-reperfusion modeling and the subsequent transplantation of MSCs obtained from human adipose tissue on the 7th day in the CA1 hippocampal zone pyramidal neurons fit tightly to each other, forming straight beams (Fig. 5A). Neuronal nuclei with excess euchromatin and one or two nucleoli could be seen. Only solitary nuclei of pyramidal neurons demonstrated characteristics of karyopyknosis and swelling. Although the apical dendrites showed an organized structure, it was different from that of the sham-operated animals. Endothelial cells of the vascular wall had heterochromatic nuclei.

In the animals of this group, on the 14th day after ischemia-reperfusion modeling and transplantation of MSCs from human adipose tissue, pathologically unchanged nuclei of neurons were observed in the CA1 hippocampal zone, they were situated in several rows. Howsoever, considerable heterochromatization of pyramidal neuronal nuclei and nuclear swelling were occasionally (Fig. 5B).

In the radiant layer the apical dendrites formed straight beams. Intercellular swelling took place. Simultaneously, blood vessels were narrowed, inside the vessels RBC sludge was as well as accumulation of blood plasma proteins, perivascular edema also present.

In seven days after ischemia-reperfusion modeling followed by transplantation of stem cells from rat adipose tissue pyramidal neurons were arranged in a dense layer of 3-5 ordered cellular rows in the CA1 area of the rat hippocampus (Fig. 6A). Their nuclei contained a large amount of euchromatin with a well-defined nucleolus. Among pathologically unchanged euchromatic nuclei of pyramidal neurons we also could observed single heterochromatic and pyknotic ones. There was no microglial infiltration in the pyramidal layer. The capillary wall from the inside was covered with endotheliocytes with heterochromatic nuclei. In some of the capillaries perivascular edema occurred.

In fourteen days after ischemia-reperfusion modeling followed by transplantation of MSCs from rat adipose tissue pyramidal neurons in the CA1 hippocampal zone formed straight, well-ordered beams, their nuclei were euchromatic, with one nucleolus (Fig. 6B). Unitary harmed hyperchromic bodies of pyramidal cells having acidophilic cytoplasm were well visible. Most of apical dendrites were ordered, slight swelling was noticeable between them. Blood capillaries were filled with blood, endotheliocytes in the vascular wall contained heterochromatic nuclei. Perivascular edema was less intensive (compared to the day 7).

When analyzing results obtained during the examination of the rat group that received cell lysate of HUC-MSCs after ischemia-reperfusion, it was found that in seven days after treatment pyramidal neurons (in the CA1 area of rat

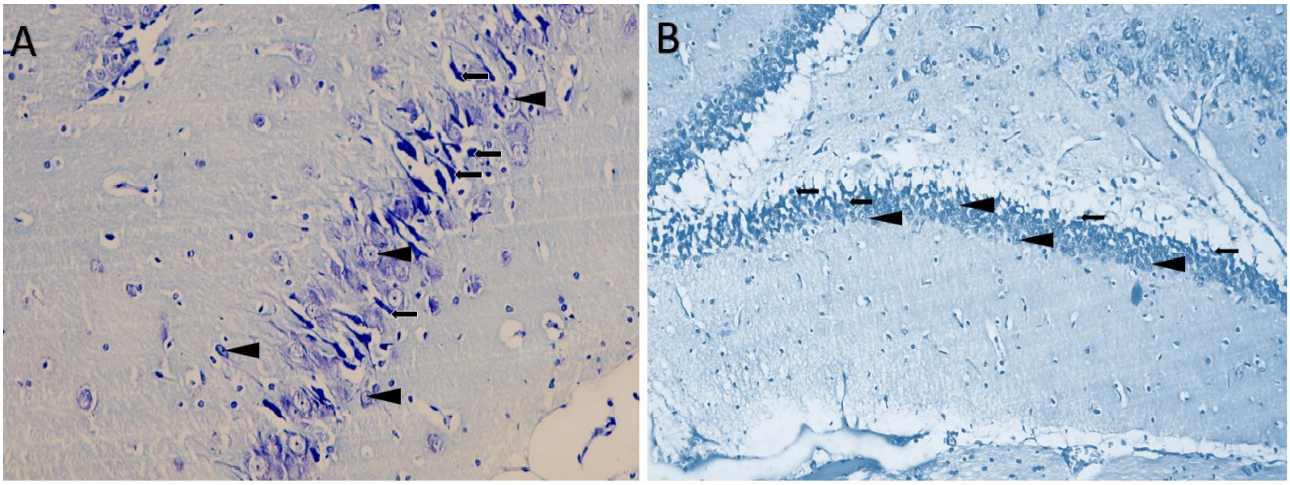


**Fig. 7.** The figure shows pyramidal layer in the CA1 zone of rat hippocampus on the day 7 (**A**) and day 14 (**B**) after ischemia-reperfusion modeling and subsequent HUC- MSCs lysate injection. Pyramidal neurons in the hippocampus are not situated compactly and are placed enough chaotically; unharmed neurons are pointed out with triangles, while affected hyperchromic neuronal bodies - with arrows. Staining according to Nissl. A - x400, B - x100.

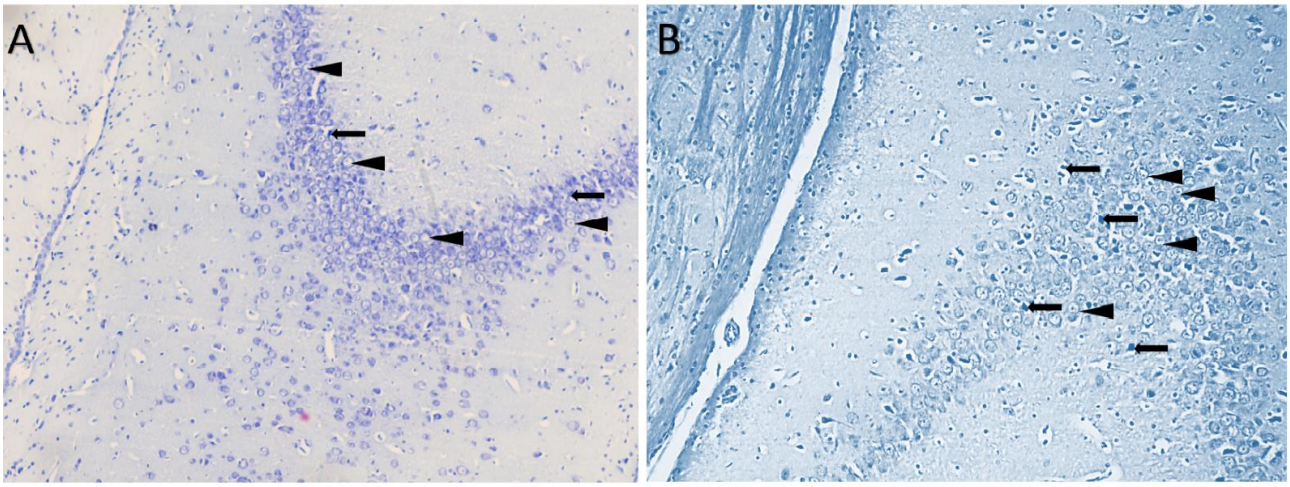


**Fig. 8.** The figure shows changes of the pyramidal layer in the CA1 zone of the rat hippocampus on the day 7 (**A**) and day 14 (**B**) after ischemia-reperfusion and subsequent citicoline administration. **A** - lots of neurons are affected. Homogenization as well as microvacuolation in the radiant layer is. Unharmed neurons are pointed out with triangles, while the affected hyperchromic neuronal bodies - with arrows. **B** - pyramidal neurons are situated loosely. Neurons with non-changed structure are pointed out by triangles, damaged hyperchromic neuronal bodies - by arrows. Staining according to Nissl. x200.

hippocampus) were located chaotically, in two-three layers significantly different from those in the pyramidal layer in sham-operated rats, pyramidal neurons were both hyperchromic and intact (Fig. 7A). In the radiating layer, parallelism of apical dendrites was not. The radiant layer was visualized neatly. Pyramidal and radiating layers were infiltrated with microglia. Perivascular edema was visible in some areas of the lacunose-molecular layer, the blood vessels lumen was narrowed, blood plasma proteins were accumulated in blood vessels, RBCs sludge was observed. The vascular wall contained endotheliocytes, nuclei of which were enriching in heterochromatin.

In fourteen days after ischemia-reperfusion modeling followed by administration of cell lysate of HUC-MSCs, we could observe disarrangement in the pyramidal layer of the CA1 area; it lost its density, the nervous cells were lying loosely (Fig. 7B). Hyperchromic neurons also met between the unharmed ones. Apical dendrites sent their collaterals into the radiant layer.

In seven days after ischemia-reperfusion and immediately subsequent citicoline administration in the radiant layer of the CA1 hippocampal area, alternation of homogenization with microvacuolation and normal fibrous structure were. We could see such the picture next to the layer of pyramidal cells (Fig. 8A), last one contained a lot of harmed hyperchromic neurons having acidophilic cytoplasm. Infiltration with microglia was insignificant.

In fourteen days after ischemia-reperfusion modeling and treatment with citicoline in the hippocampal CA1 region of rats, pyramidal neurons were loosely located, affected hyperchromic neurons having acidophilic cytoplasm were found enough (Fig. 8B). The rats with citicoline administration on day 14 after treatment had greater number of unharmed neurons in the pyramidal layer compared to such the group on day 7. Homogenization in the radiant layer was preserved, perivascular edema also.

When analyzing the morphometric parameters, it was established that the total number of neuronal nuclei in 1 $mm^2$ of the CA1 hippocampal zone in the rats with modeled ischemia-reperfusion (without treatment) compared to sham-operated animals decreased by more than 2 times and amounted to an average of 1627±179 nuclei on the 7th day and 1740±254 nuclei on the 14th day (Table 2). Most of these nuclei were subject to destruction: in the subacute follow-up period, 95.8% of the nuclei were pathologically altered (karyorrhexis and karyopyknosis), in the recovery period - 91.5%.

The use of MSCs, cell lysate of HUC-MSCs, and citicoline as a therapy for ischemia-reperfusion brain injury in rats contributed to a significant reduction of pathological damage to the nuclei of neurons in the hippocampal CA1 region, which in turn increased the number of intact neurons compared to a group of animals with control pathology. These results demonstrate the neuroprotective properties of the studied substances, among which the best protective effect on neurons during cerebral ischemia-reperfusion in rats was MSC transplantation derived from human Wharton's jelly. The number of neuronal nuclei in the hippocampal CA1 zone in this group of rats approached the number of nuclei in the group of sham-operated animals and on the 7th day after transplantation was on average 3226±260 nuclei, and on the 14th day - 3329±213 nuclei, while the number of pathologically unchanged nuclei significantly exceeded the number of nuclei with signs of destruction: on the 7th day - 90.20±12.01 and 15.60±3.51; on the 14th day - 105.20±7.40 and 16.40±7.40, respectively ($p<0.05$), compared to the control.

HUC-MSCs demonstrated a significantly better neuroprotective effect than the reference drug citicoline. Thus, in the research periods in the group of rats treated with citicoline (250 mg/kg, intravenously), significantly lower values of the intact neuronal nuclei number and higher

**Table 2.** Morphometric analysis of hippocampal CA1 region in rats with modeled ischemia-reperfusion.

| Period | Group 2 | Group 3 | Group 4 | Group 5 | Group 6 | Group 7 | Group 8 | Group 9 |
|---|---|---|---|---|---|---|---|---|
| The total number of neuronal nuclei in 1 $mm^2$ of hippocampal CA1 region | | | | | | | | |
| 7th day | 3977±234 | 1627±179*$ | 3226±260*#$ | 2773±300*# | 2802±199*# | 3123±242*#$ | 2673±182*# | 2456±201*# |
| 14th day | | 1740±254*$ | 3329±213*#$ | 2875±204*# | 2929±204*# | 3230±318*#$ | 2720±226*# | 2553±180*# |
| The total number of nuclei | | | | | | | | |
| 7th day | 128.3±9.40 | 23.67±7.79* | 105.80±14.0*# | 83.60±6.99*#$ | 95.40±15.45*#$ | 91.50±17.35*#$ | 89.00±9.25*#$ | 68.50±10.97*# |
| 14th day | | 37.33±6.28* | 121.60±12.30#$ | 88.67±3.39*#$ | 99.80±3.90*#$ | 96.83±19.59*#$ | 89.83±8.30*#$ | 79.33±4.84*# |
| The number of intact neuronal nuclei | | | | | | | | |
| 7th day | 128.3±9.40 | 1.00±0.89*# | 90.20±12.01*#$ | 60.00±4.30*# | 70.20±1.92*#$ | 70.50±12.44*#$ | 55.33±8.59*#$ | 37.67±6.41*# |
| 14th day | | 3.170±1.17* | 105.20±7.40*#$ | 66.00±6.10*# | 75.00±1.87*#$ | 76.50±14.01*#$ | 58.67±4.89*#$ | 45.50±3.27*# |
| The number of damaged neuronal nuclei (karyorrhexis and karyopyknosis) | | | | | | | | |
| 7th day | 0 | 22.67±7.28* | 15.60±3.51*$ | 23.60±2.88*$ | 25.20±13.70* | 21.00±5.83*$ | 33.67±1.97*# | 30.83±5.74*# |
| 14th day | | 34.17±5.42* | 16.40±7.40*#$ | 22.67±3.67*#$ | 24.80±2.77*#$ | 20.33±6.02*#$ | 31.17±5.67* | 33.83±2.86* |

**Notes:** * - $p<0.05$ relative to group 2 (sham-operated rats); # - $p<0.05$ relative to group 3 (control pathology); $ - $p<0.05$ relative to group 9 (citicoline).

pathologically changed nuclei values were observed - 30.83±5.74 on day 7 and 45.50±3.27 on day 14, compared to the group of rats that HUC-MSCs were injected (37.67±6.41 and 33.83±2.86 respectively, $p<0.05$). From the given data, it can be seen that both in the subacute and recovery periods of acute ischemic stroke in rats, transplantation of HUC-MSCs significantly exceeded the reference drug citicoline in its ability to reduce the number of pathologically altered nuclei by 1.5 times ($p<0.05$). An analogous pattern was observed when using lysate of HUC-MSCs: the ratio of the intact nuclei number to pathologically changed ones was 55.33±8.59 and 33.67±1.97 on the 7th day and 58.67±4.89 and 31.17±5.67 on the 14th day, which was significantly different from the group of animals that were injected with HUC-MSCs.

## Discussion

Thus, the results of the conducted research demonstrate the presence of expressive cerebroprotective properties in MSCs (106 cells/animal, intravenously) in conditions of ischemia-reperfusion caused by acute brain ischemia. The best result among MSCs of various origins was found in HUC-MSCs. In our opinion, in the acute period of ischemia-reperfusion, MSCs contributed to preserving the area and density of intact neurons of hippocampal CA1 region due to the improvement of brain perfusion. This may occur due to the presence of a modulating effect of MSCs on the processes of necrosis and neuroapoptosis in the ischemic penumbra zone.

Cerebral ischemia-reperfusion involves many complex pathological processes, including oxidative stress, blood-brain barrier disruption, mitochondrial dysfunction, inflammation, and apoptosis, leading to neuronal cell death and impairment of sensory, motor, and cognitive functions [13].

The study demonstrated that intravenous xenogeneic and allogeneic transplantation of MSCs obtained from adipose tissue, HUC-MSCs and cell lysate of HUC-MSCs, rat embryonic fibroblasts, and citicoline in modeled ischemia-reperfusion in rats reduced the extent of ischemia-reperfusion injury of the brain.

Researchers proved that transplantation of MSCs after cerebral ischemia in animals improved brain function, effectively protected and restored neurons in ischemic-reperfusion brain damage [2, 18, 20]. This is caused by the fact that MSCs release a wide range of trophic and immunomodulatory cytokines, which are called the MSC secretome, which has significant potential for treating degenerative brain diseases through the induction of neurogenesis, angiogenesis, and endogenous neuroprotection [2, 11, 25].

MSCs derived from adipose tissue are available and easily obtained in large quantities. The effectiveness and safety of MSCs obtained from human adipose tissue in stroke treatment has been proven in many experimental studies [8]. It has been shown that MSC transplantation reduced apoptosis and loss of neurons due to their death, thereby demonstrating a significant protective effect on them through inactivation of KDM6B/BMP2/BMF in rats with cerebral ischemia [30].

In many researches, the effectiveness of HUC-MSCs in ischemic stroke has been studied. It has been reported that transplantation of HUC-MSCs into rats with ischemic stroke reduced neuroinflammation, enhanced neuroregeneration and protection of vascular endothelium, which resulted in a reduction in infarct volume [7, 23, 28, 29].

In the above-mentioned studies, the effects of MSCs obtained from a single cell culture were studied, but we compared the action of both allogeneic and xenogeneic cells obtained from two sources. Paracrine factor variations of different classes of MSCs promote various possibilities for nerve tissue recovery. MSCs derived from human Wharton's jelly or adipose tissue act differently on the population of cells of the central nervous system, in which HUC-MSCs demonstrate a more pronounced positive effect on metabolism and neurons' density in the hippocampal CA1 zone [22]. We also revealed a neuroprotective effect of MSCs lysate obtained from HUC-MSCs in animals with modeled ischemia-reperfusion of ICA. These data suggest that HUC-MSCs are an effective and safe source of therapy for experimental reversible cerebral ischemia.

In further studies, it is planned to prove the cerebroprotective effect of MSCs in ischemia-reperfusion model of the brain in rats. To identify the most effective class of stem cells in terms of neuroprotective properties among the studied MSCs, in order to subsequently create an injectable drug for intravenous transplantation in the treatment of patients with acute ischemic stroke.

## Conclusions

1. Acute cerebral ischemia-reperfusion in rats in the subacute and recovery periods led to almost complete degeneration of the pyramidal layer of hippocampal CA1 region; caused the infiltration of microglia in the pyramidal and radiate layers, that disrupted the arrangement of apical dendrites bundles; formed edema surround the blood vessels with their subsequent narrowing. Also, in the studied periods of modeled ischemia-reperfusion in rats, the total number of neuronal nuclei in 1 $mm^2$ of hippocampal CA1 zone was significantly reduced, of which the vast majority had signs of pathological changes.

2. Mesenchymal stromal cells, cell lysate of HUC-MSCs, and citicoline have pronounced neuroprotective properties, due to a significant increase in the number of neuronal nuclei in the hippocampal CA1 region in rats and nuclei that were not pathologically altered, compared to a group of animals with ischemia-reperfusion without administered treatment. HUC-MSCs had the best neuroprotective effect among the studied stem cells and substances.

3. The use of MSCs of various genesis, lysate of HUC-MSCs, or citicoline in conditions of acute cerebral ischemia in rats caused a decrease in the volume of ischemic-reperfusion injury in the hippocampus, which may indicate the feasibility of their use in the therapy of ischemic stroke.

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**ТЕРАПЕВТИЧНИЙ ПОТЕНЦІАЛ МЕЗЕНХІМАЛЬНИХ СТРОМАЛЬНИХ КЛІТИН НА МОРФОЛОГІЧНІ ПОКАЗНИКИ У ГІПОКАМПІ ЩУРІВ ІЗ МОДЕЛЬНОЮ ІШЕМІЄЮ-РЕПЕРФУЗІЄЮ ГОЛОВНОГО МОЗКУ**

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*Ішемічний інсульт є вкрай важливою патологією з високою летальністю, при якій понад 50 % хворих з оклюзією магістральних судин залишаються інвалідами, незважаючи на якомога ранню реперфузійну терапію шляхом тромболізису чи тромбектомією. У рамках регенеративної стратегії новим поштовхом стала трансплантація стовбурових клітин при ішемічному інсульті. Обнадійливі результати щодо ендогенних механізмів нейровідновлення у відповідь на ішемічне пошкодження структур головного мозку продемонструвала клітинна терапія з використанням мезенхімальних стромальних клітин. Мета дослідження - вивчення впливу МСК різного походження, лізату МСК отриманих із Вартонових драглів пуповини людини та цитиколіну на динаміку морфологічних змін у зоні СА1 гіпокампа щурів із гострою церебральною ішемією-реперфузією за даними світлової мікроскопії та мікроморфометрії. Експеримент проведено з використанням 200 самців щурів лінії Вістар, яким здійснено ішемію-реперфузію шляхом оборотної 20-и хвилинної білатеральної оклюзії внутрішніх сонних артерій. Тваринам із модельованою патологією внутрішньовенно трансплантували МСК різного походження (з Вартонових драглів пуповини людини, з жирової тканини людини та щура), вводили ембріональні фібробласти щура, лізат МСК із Вартонових драглів людини, цитиколін. Гістологічний аналіз зрізів головного мозку щурів проводили на 7 та 14 добу експерименту. Статистичну обробку виконували за допомогою "Statistica 6.0" (StatSoft® Snc, США). Достовірність відмінностей визначали з використання t-критерію Ст'юдента, непараметричного U критерію Манна-Уітні. Під час дослідження встановлено, що модельна ішемія-реперфузія у щурів викликала майже повну дегенерацію пірамідного шару СА1 зони гіпокампу та надала однорідності структури променистому, інфільтрацію мікроглії, порушення розташування тяжів апікальних дендритів, звуження кровоносних судин у наслідок периваскулярного набряку. Також, змодельована патологія зменшувала загальну кількість ядер нейронів СА1 зони гіпокампа, переважна більшість з яких мали ознаки патологічних змін. Трансплантація мезенхімальних стромальних клітин різного походження, лізату мезенхімальних стромальних клітин та цитиколіну сприяли достовірному збільшенню кількості ядер нейронів у СА1 зоні гіпокампа та ядер, які не зазнавали патологічних змін. Найкращий позитивний ефект виявлено при трансплантації мезенхімальних стромальних клітин із Вартонових драглів людини. Таким чином, як у підгострому, так і відновлювальному періодах ішемічного інсульту в щурів, трансплантація мезенхімальних стромальних клітин Вартонових драглів людини вірогідно перевершувала референс-препарат цитиколін за здатністю зменшувати кількість патологічно змінених ядер у 1,5 рази (p<0,05). При цьому кількість патологічно неушкоджених ядер значно перевищувала кількість ядер з ознаками каріорексису та каріопікнозу, тому доцільно було б застосовувати мезенхімальні стромальні клітини різного походження, лізат або цитиколін в умовах гострої ішемії-реперфузії головного мозку, враховуючи їх здатність зменшувати обсяг інфаркту. У подальшому буде створений ін'єкційний препарат, із найбільш ефективної за церебропротекторними властивостями культури мезенхімальних стромальних клітин для клітинної терапії хворих на гострий ішемічний інсульт.*

**Ключові слова:** *мезенхімальні стромальні клітини, нейрони, гіпокамп, ішемія-реперфузія, щури.*

**Author's contribution**

*Konovalov S. V.* - conceptualization, research, methodology and writing of the original draft, data visualization, resources, software.
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*Gadzhula N. G.* - formal analysis and validation, writing the article, editing.
*Gusakova I. V.* - writing the article.
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*Kordium V. A.* - conceptualization, formal analysis and validation.