The effect of mesenchymal stromal cells of various origins on morphology of hippocampal CA1 area of rats with acute cerebral ischemia

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ABSTRACT

Every year, about 150,000 strokes occur in Ukraine, and more than 100,000 people die from the consequences of stroke and other circulatory disorders in the brain. So far, promising experimental data on the treatment of neurological dysfunction using mesenchymal stromal cells (MSCs) have been obtained.

PURPOSE: to characterize the impact of MSCs of various origins, lysate from Wharton's jelly-derived MSCs and citicoline on the dynamics of destructive changes in the hippocampal CA1 area of rats with the model of acute cerebral ischemia according to morphometric data.

MATERIALS AND METHODS. An experiment was performed using 4-month-old male Wistar rats, which were subjected to transient bilateral 20-minute ischemia-reperfusion (IR) of the internal carotid arteries. After modeling, the animals were injected intravenously with Wharton's jelly-derived MSCs, human and rat adipose-derived MSCs at the dose of 10^6 cells/animal. Other groups were intravenously injected with rat fetal fibroblasts at the dose of 10^6 cells/animal and lysate from Wharton's umbilical cord MSCs at the dose of 0.2 mL/animal. Control animals were injected with 0.2 mL of saline. The last group of rats received a single dose of the reference drug citicoline at the dose of 250 mg/kg. On the 7th and 14th day, the total number of neuron nuclei per 1 mm^2 brain section was counted in the hippocampal CA1 area, and the ratio of the number of intact neuron nuclei and nuclei with changes (karyorrhexis and karyopyknosis) was determined.

RESULTS. The transplantation of MSCs, lysate from Wharton's jelly-derived MSCs, or citicoline contributed to the greater number of nuclei in the hippocampal CA1 area, and the number of nuclei that did not undergo pathological changes also increased. The transplantation of Wharton's jelly-derived MSCs had the most positive effect. The number of neuron nuclei per 1 mm^2 in the hippocampal CA1 area in this group of animals approached the number of nuclei in the group of sham-operated animals. At the same time, the number of nuclei that did not undergo pathological changes significantly exceeded the number of nuclei with signs of destruction.

CONCLUSION. A significant increase in the number of neurons without signs of pathological changes was observed in all experimental groups of rats during the modeling of ischemic brain injury after the administration of various types of studied mesenchymal stromal cells, lysate or citicoline. The most positive result in the hippocampal CA1 area was achieved after the administration of Wharton's jelly-derived MSCs.

KEY WORDS: ischemic stroke; mesenchymal stromal cells; hippocampal CA1 area; cell transplantation

Acute cerebrovascular accident (ACVA) accompanied by cerebral infarction is a global epidemic and the second leading cause of death in the world as well as in Ukraine [1, 2]. About 150,000 strokes occur in Ukraine every year, and more than 100,000 people die from strokes and other vascular disorders in the brain. Most people who have survived a stroke suffer from its physical, cognitive, mental, and socioeconomic conse-

quences throughout their lives, which cause a huge burden of this disease on families, communities, and the state [3]. A third of such patients are people of working age, up to 50 % will have a disability, and only one in ten will return to a full life in all respects [2]. Therefore, treatment of acute cerebrovascular disorders is a priority direction in the Program of Medical Guarantees in Ukraine with funding at an increased rate [4].

The formation of an ischemic foci in ACVA is accompanied by destructive-degenerative changes in the cytoarchitectonics of nervous tissue, which sign is a decrease in the area and density of neurons and the content of nucleic acids in them. Decreased blood supply to the brain leads to a limited supply of O_2 and glucose to the brain. Hypoglycemia causes the activation of glycolysis and, accordingly, a decrease in the formation of ATP in the brain. Neural cells lose K^+ ions, accumulate Na^+ and H_2O ions leading to cytotoxic swelling of the brain tissue [5]. In the treatment of stroke, it is important to restore the perfusion of the ischemic area as soon as possible [6, 7]. However, the search for effective and safe means to reduce the manifestations of ACVA is still ongoing.

So far, promising experimental data have been obtained regarding the ability of mesenchymal stromal cells (MSCs) to prevent the occurrence of metabolic disorders and depletion of structural components of neurons, which is one of the signs of their protective effect in conditions of cerebral ischemia [8-11]. However, such studies are limited, and even less often comparative. The study of the impact of MSCs in the treatment of stroke has been carried out for the last two decades. MSCs can be obtained from bone marrow, umbilical cord, adipose tissue, placenta, neural crest-derived tissues, and others. To date, bone marrow-derived MSCs are the most studied and best characterized MSCs [12]. However, even in recent studies, the main source of MSCs was bone marrow and adipose tissue, and only one study was performed with placental cells [13]. At the same time, as in our case, both allogeneic [14] and xenogeneic [15] cells were used in the research. As for the use of MSC lysate, we could not find any references. It is interesting that in recent studies of this problem, the action of MSCs from one specific source was studied, while we compared the effects of both xenogeneic and allogeneic cells from two sources. Wharton's jelly-derived MSCs have the best clinical prospects due to their unique beneficial properties. They do not cause ethical and legal controversies; they are easy and painless to obtain in large number, multipotent, high proliferative, hypoimmunogenic and do not induce malignant tumors.

The above data on the neuroprotective effects of MSCs on the ischemic brain served as the basis for conducting this study, the **PURPOSE** of which was to characterize the impact of MSCs of various origins, lysate from Wharton's jelly-derived MSCs, and citicoline on the dynamics of destructive changes in the hippocampal CA1 area of rats with ACVA according to morphometric data.

MATERIALS AND METHODS

An experiment was performed using 200 4-month-old male Wistar rats with a body weight of 160-190 g, which were subjected to transient bilateral 20-minute ischemia-reperfusion (IR) of the internal carotid arteries. Animals from the vivarium of National Pirogov Memorial Medical University (Vinnytsya, Ukraine) were kept in standard conditions with free access to water and food *ad libitum*. When conducting the study, the recommendations of the State Expert Center of the Ministry of Health of Ukraine, the requirements of bioethics regarding the National "General Ethical Principles of Animal Experiments" approved by the 1st National Congress on Bioethics (Kyiv, Ukraine, 2001) and the Law of Ukraine "On the Protection of Animals from Cruelty" dated February 26, 2006 were respected. The experimental model of ischemia-reperfusion was performed by occlusion of the internal carotid arteries on both sides for 20 minutes (ischemia) followed by removal of ligatures (reperfusion) and suturing of the skin. The surgery was performed under anesthesia with 60 mg/kg "Propofol-novo" (*Novofarm-Biosyntez LLC*, Ukraine). The selected model

reflects the clinical picture of brain infarction and is adequate for the experimental study of potential neuroprotective agents [17]. Experimental animals were divided into 9 groups (**Table 1**).

Table 1. Distribution of animals by groups in the experiment

Group	Number of animals	Treatment
1	10	Intact animals
2	10	Sham-operated animals + 0.9 % saline solution at the dose of 2 mL/kg
3	40	IR + 0,9% saline solution at the dose of 2 mL/kg
4	20	IR + human Wharton's jelly-derived MSCs at the dose of 10^6 cells/animal
5	20	IR + rat fetal fibroblasts at the dose of 10^6 cells/animal
6	25	IR + human adipose-derived MSCs at the dose of 10^6 cells/animal
7	25	IR+ rat adipose-derived MSCs at the dose of 10^6 cells/animal
8	25	IR + lysate of Wharton's jelly-derived MSCs at the dose of 0.2 mL/animal
9	25	IR + citicoline 250 mg/kg

The obtaining of cells from human Wharton's jelly and adipose tissue, rat fetuses and adipose tissue, as well as cell lysate were described in our previous study [16].

The 1st group is intact animals. The 2nd group is sham-operated rats under anesthesia, which were sequentially subjected to the standard surgery (skin incision, vessel preparation) without the ligation of the internal carotid artery, which reproduced the effect of the traumatic conditions of the experiment. The 3rd group is a control group subjected to 20-minute IR model and injected intravenously with a 0.9 % saline solution at 2 mL/kg. The same dose was administered to rats of the 2nd group. The 4th group of animals immediately after IR was transplanted with human Wharton's jelly-derived MSCs at the dose of 10^6 cells/animal. The 5th group of animals underwent a single transplantation of rat fetal fibroblasts at the dose of 10^6 cells/animal immediately after IR. The 6th group of animals with IR received human adipose-derived MSCs at the dose of 10^6 cells/animal. The 7th group of animals were injected with rat adipose-derived MSCs at the dose of 10^6 cells/animal immediately after IR. The 9th group of rats received a single dose of citicoline "Neuroxon" (*Arterium Corporation*, Ukraine) at the dose of 250 mg/kg immediately after IR. Citicoline was chosen as a reference drug due to its ability to enhance neuroregeneration in an experiment on rats and to improve cognitive and memory functions in patients with cerebral ischemia [18-21]. The cell dose used by other researchers was 10^5-10^6 cells per animal, which is consistent with our methodology.

To analyze the effect of MSCs of various origin, lysate of human Wharton's jelly-derived MSCs and citicoline on the dynamics of destructive changes in the hippocampal CA1 area in 7 days (subacute ischemia) and 14 days (recovery period) after IR, rats were euthanized by decapitation with a previous overdose of pentobarbital (*Bioveta JSC*, Czech Republic) at the dose of 100 mg/kg [13, 14, 22].

The brains of experimental animals were prepared and fixed with a 4 % formaldehyde solution for 24 hours. After fixation, the brains were washed in water, processed with ethanol of increasing concentration and xylene, and embedded in Paraplast Plus© (*McCormick©*, USA). 5 μm thick sections were made on a rotary microtome. Deparaffinized sections were stained with hematoxylin-eosin according to standard methods.

Digital images of frontal brain sections obtained by BX-51 microscope (*Olympus*, Japan) were analyzed using ImageJ 1.48v software (freeware license, Rasband, USA). In the hippocampal CA1 area, the total

number of neuron nuclei per 1 mm^2 was counted, and the ratio of the number of intact neuron nuclei and nuclei with changes (karyorrhexis and karyopyknosis) was also determined. To evaluate the obtained results, statistical processing using Excel 2010 (*Microsoft*, USA) and STATISTICA 6 software (*Statsoft Inc.*, USA) was performed. The hypothesis regarding the normality of the distribution of groups of experimental animals was tested by the Shapiro-Wilk test. The non-parametric Kruskal-Wallis test was used to analyze differences between groups of animals. Means and standard errors were calculated; differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Histological analysis of frontal sections of rat brain showed no differences between intact and sham-operated animals, so the group of sham-operated animals was used as a control group. In the hippocampus of sham-operated animals, all zones and layers were clearly visualized, no pathological changes were observed (**Fig. 1**).
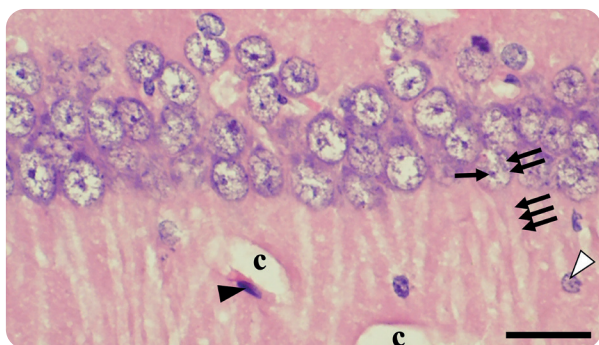

Fig. 1. Photomicrograph of a rat brain section. CA1 pyramidal layer of the hippocampus of a sham-operated animal. The nucleus of a pyramidal neuron (marked by two arrows) contains a nucleolus (marked by an arrow) and a large amount of euchromatin. The apical dendrite (indicated by three arrows) of the pyramidal neuron is directed into the stratum radiatum. The nucleus of an endotheliocyte is marked with a black triangle, the nucleus of an astrocyte is marked with a white triangle; c – the lumen of a blood vessel. Scale bar – 20 µm. Light microscopy, hematoxylin-eosin staining.

In the hippocampal CA1 area, the *stratum oriens* was formed by basal dendrites of pyramidal cells. In the *stratum pyramidale*, the bodies of pyramidal neurons formed a dense layer of cells that were compactly arranged in 3-5 rows. The nuclei of pyramidal neurons contained single nucleoli and a large amount of euchromatin (**Fig. 1**). Radially oriented unbranched apical dendrites of pyramidal neurons were observed in the *stratum radiatum*. In the *stratum lacunosum-moleculare*, which was formed by the thin ends of the apical dendrites of pyramidal cells, a significant number of blood vessels were visualized, in which single erythrocytes were observed. The wall of small vessels was lined by endotheliocytes with nuclei rich in heterochromatin.

In the group of control ischemic animals, on the 7th day after the modeling of ischemia-reperfusion, almost complete degeneration of the pyramidal layer was observed in a part of the hippocampal CA1 area slices: shrunken hyperchromic bodies of neurons with acidophilic cytoplasm and karyopyknosis and karyorhexis of the nuclei (**Fig. 2 A**). Intensive infiltration of microglial cells was observed in the *stratum pyramidal and stratum radiatum*.
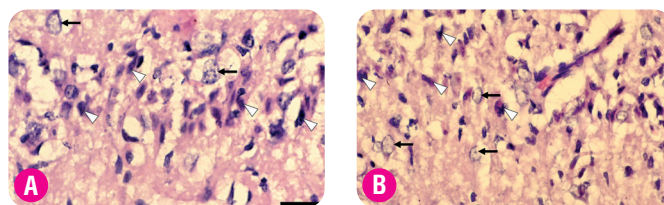

Fig. 2. Photomicrograph of rat brain sections. Degeneration of the pyramidal layer of the hippocampal CA1 area on the 7th day (A) and on the 14th day (B) after IR modeling. Shrunken hyperchromic bodies of neurons with acidophilic cytoplasm are indicated by triangles. Single neurons with normal structure are indicated by arrows. Swelling of the intercellular space. Scale bar – 20 µm. Light microscopy, hematoxylin-eosin staining.

The structure of the stratum radiatum in the hippocampal CA1 area became homogeneous, and the architecture of the apical dendrites bundles was disrupted (**Fig. 2 A**). Swelling of the intercellular space, marked perivascular edema and constriction of the vessel lumen developed. Hemorrhage with the formation of hyaline masses, sludge and destruction of erythrocytes was observed in some animals of this group (**Fig. 3**).
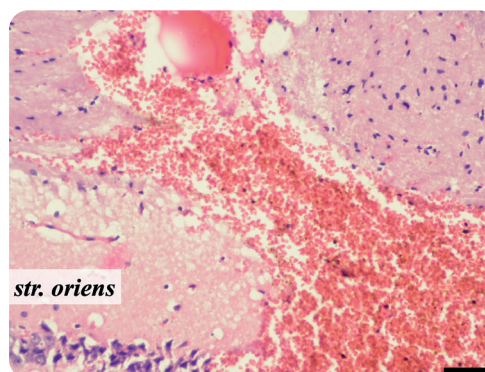

Fig. 3. Photomicrograph of a rat brain section. Hippocampal CA1 area on the 7th day after the modeling of ischemia-reperfusion. Hemorrhage with the formation of hyaline masses. Scale bar – 50 µm. Light microscopy, hematoxylin-eosin staining.

On the 14th day after the modeling of ischemia-reperfusion, degeneration of the pyramidal layer of the hippocampal CA1 area was also observed. The nature of destructive changes was similar to that on the 7th day after IR. The structure of the *stratum radiatum* of hippocampal CA1 area was also homogeneous, swelling of the intercellular space and marked perivascular edema were observed, and the architecture of the apical dendrites bundles was disturbed (**Fig. 2 B**).

Against the background of the above-described changes in the pyramidal layer of hippocampal CA1 area after ischemic injury, a significant number of intact pyramidal neurons was observed in the rats of the group of IR + human Wharton's jelly-derived MSCs on the 7th day after the transplantation (**Fig. 4 A**). Microglial infiltration in the hippocampal stratum radiatum and stratum pyramidal was almost not detected. In the pyramidal layer, the bodies of pyramidal neurons were observed in the form of 3-5 ordered cell layers. Among the normal euchromic nuclei of pyramidal neurons containing one nucleolus, heterochromic and pyknotic nuclei with marked perinuclear swelling were also observed. Apical dendrites formed ordered bundles. The capillary wall consisted of endotheliocytes with heterochromic nuclei. Perivascular edema was observed in some of the capillaries.
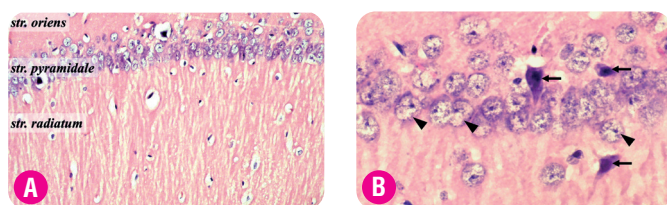
Fig. 4. Photomicrograph of rat brain sections. The pyramidal layer of the hippocampal CA1 area on the 7th (A) and 14th (B) days after modeling of ischemia-reperfusion and transplantation of human Wharton's jelly-derived MSC. A – a significant number of intact neurons in the pyramidal layer; radially oriented apical dendrites form equal bundles in the stratum radiatum. Scale bar – 50 μm. B – nuclei of neurons with a large amount of euchromatin and one or two nucleoli form structured bundles; neurons with normal structure are indicated by triangles; damaged hyperchromic neurons with acidophilic cytoplasm are indicated by arrows. Scale bar – 20 μm. Light microscopy, hematoxylin-eosin staining.

On the 14th day after transplantation of human Wharton's jelly-derived MSCs, the cytoarchitectonics of the hippocampal CA1 area was similar to that on the 7th day. Pyramidal neurons formed continuous, ordered bundles, and nucleoli and euchromatin were clearly visible in the nuclei of these neurons (**Fig. 4 B**). Damaged hyperchromic bodies of neurons with acidophilic cytoplasm were also observed. The apical dendrites formed radially oriented unbranched structured bundles. Endotheliocytes with heterochromic nuclei are clearly visible in the wall of blood vessels.

On the 7th day after the modeling of ischemia-reperfusion and transplantation of rat fetal fibroblasts, both pyramidal neurons with a euchromatin nucleus and one nucleolus, as well as damaged hyperchromic neurons with signs of karyopyknosis and acidophilic cytoplasm, were observed in the hippocampal CA1 area (**Fig. 5 A**). The *stratum radiatum* was disorganized, the apical dendrites did not form ordered bundles. The lumen of blood vessels was narrowed, blood plasma proteins and erythrocytes were clearly visible, and perivascular edema was also observed.
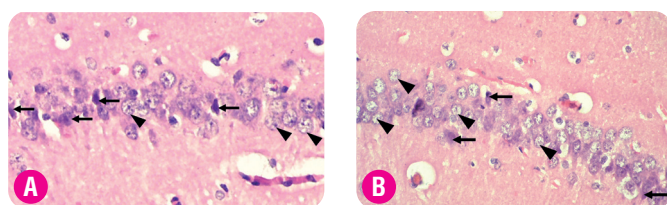

Fig. 5. Photomicrograph of rat brain sections. Pyramidal layer of the hippocampal CA1 area on the 7th (A) and 14th (B) days after IR modeling and transplantation of rat fetal fibroblast. A – pyramidal neurons with a euchromatin nucleus are indicated by triangles, damaged hyperchromic neurons with signs of karyopyknosis and acidophilic cytoplasm are indicated by arrows; perivascular edema. Scale bar – 20 μm. B – disorganization of the stratum radiatum; perivascular edema; erythrocyte stasis and sludge in the lumen of blood capillaries; endotheliocytes of capillary wall with heterochromic nuclei. Scale bar - 50 μm. Light microscopy, hematoxylin-eosin staining.

On the 14th day after the modeling of ischemia-reperfusion and transplantation of rat fetal fibroblasts in the hippocampal CA1 area, pyramidal neurons formed an even bundle of several cell layers (**Fig. 5 B**). The nuclei of the neurons were rich in euchromatin and had a single nucleolus. Single heterochromic or swollen nuclei were observed between these nuclei. The stratum radiatum was disorganized, but, in contrast to the 7th day, the apical dendrites in some places formed equal bundles. The lumen of the part of the blood vessels was narrowed, perivascular edema was observed.

On the 7th day after the IR modeling and transplantation of human adipose-derived MSCs in the hippocampal CA1 area, pyramidal neurons closely adjoined each other, forming equal tracts (**Fig. 6 A**). Nuclei of neurons with a large amount of euchromatin and one or two nucleoli were observed. Single nuclei of pyramidal neurons expressed signs of karyopyknosis and edema. The apical dendrites had a more or less ordered structure, but not the same as in sham-operated animals. In the wall of blood vessels, there were endothelial cells with heterochromic nuclei.
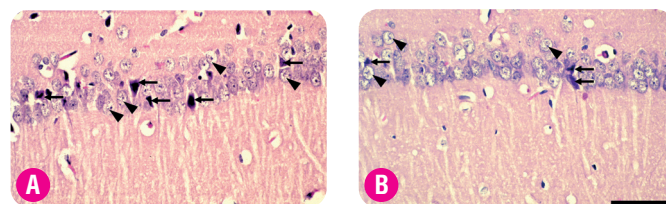

Fig. 6. Photomicrograph of rat brain sections. Pyramidal layer of the hippocampal CA1 area on the 7th day (A) and 14th day (B) after IR modeling and transplantation of human adipose-derived MSCs. Neurons with normal structure are indicated by triangles; damaged neurons are indicated by arrows. Scale bar – 50 μm. Light microscopy, hematoxylin-eosin staining.

On the 14th day after the modeling of ischemia-reperfusion and transplantation of human adipose-derived MSCs, unchanged nuclei of neurons forming several rows were observed in the hippocampal CA1 area. However, significant heterochromatization of the nuclei of pyramidal neurons and swelling of the nuclei were sometimes observed (**Fig. 6 B**). In the stratum radiatum in some areas, the apical dendrites formed ordered bundles. Swelling of the intercellular space was detected. The lumen of the blood vessels is narrowed; erythrocyte sludge and the accumulation of blood plasma proteins were observed, as well as perivascular edema was detected.

On the 7th day after IR modeling and transplantation of rat adipose-derived MSCs in the hippocampal CA1 area, pyramidal neurons formed a dense layer of 3-5 rows of ordered cells (**Fig. 7 A**). The nuclei of neurons were rich in euchromatin and with well-defined nucleoli. Single heterochromic and pyknotic nuclei were also observed among unchanged euchromic nuclei of pyramidal neurons. Microglial infiltration of the pyramidal layer was not detected. The capillary wall consisted of endotheliocytes with heterochromic nuclei. Perivascular edema was observed in some of the capillaries.
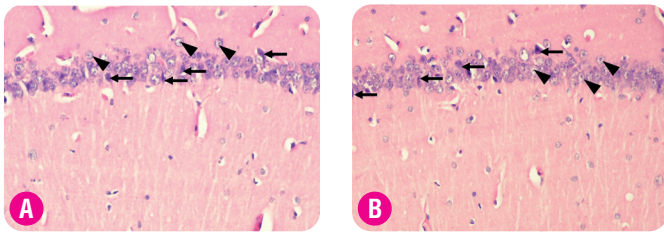
Fig. 7. Photomicrograph of rat brain sections. Pyramidal layer of the hippocampal CA1 area on the 7th (A) and 14th day (B) after IR modeling and transplantation of rat adipose-derived MSCs. A – the nuclei of neurons are rich in euchromatin, with a well-defined nucleolus: microglial infiltration of the pyramidal layer is not detected; neurons with normal structure are indicated by triangles; damaged neurons are indicated by arrows. Scale bar – 50 µm. B – the nuclei of pyramidal neurons are rich in euchromatin, well-defined nucleoli; apical dendrites form ordered structures; a slight swelling of the intercellular space; pyramidal neurons with normal structure are indicated by triangles; damaged neurons are indicated by arrows. Scale bar – 50 µm. Light microscopy, hematoxylin-eosin staining.

On the 14th day after the modeling of ischemia-reperfusion and the transplantation of rat adipose-derived MSCs in the hippocampal CA1 area, pyramidal neurons formed continuous, ordered bundles, most of the nuclei of hippocampal pyramidal neurons had a large amount of euchromatin and one nucleolus (**Fig. 7 B**). Rare damaged hyperchromic bodies of neurons with acidophilic cytoplasm were observed. A significant number of apical dendrites formed ordered structures, between which slight swelling was detected. Blood filling was found in the blood capillaries, the capillary wall was lined with endotheliocytes with heterochromic nuclei. A decrease in perivascular edema was observed compared to the 7th day.

On the 7th day after the modeling of IR and the application of the lysate of human Wharton's jelly-derived MSCs, both hyperchromic and intact pyramidal neurons were observed in the pyramidal layer of the hippocampal CA1 area (**Fig. 8 A**). Pyramidal neurons were arranged rather chaotically, sometimes in two, and only in some places in three layers, which differed significantly from the pyramidal layer in sham-operated control animals.
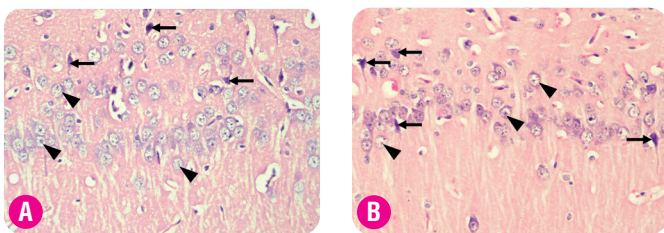

Fig. 8. Photomicrograph of rat brain sections. Pyramidal layer of the hippocampal CA1 area on the 7th (A) and 14th day (B) after IR modeling and injection of lysate from umbilical cord-derived MSCs. Pyramidal neurons are located rather chaotically and loosely; neurons with normal structure are indicated by triangles; damaged hyperchromic bodies of neurons are indicated by arrows. Scale bar – 50 µm. Light microscopy, hematoxylin-eosin staining.

Parallelism of apical dendrites in the stratum radiatum was rarely observed. The *stratum radiatum* was visualized much more clearly than in the animals of the ischemia-reperfusion group, but compared to the

sham-operated animals, it was significantly rarefied. Microglial infiltration of the radiating and pyramidal layers of the hippocampus was also much smaller. In some areas of the lacunar-molecular layer, perivascular edema was observed, in the lumens of blood capillaries, stasis and sludge of erythrocytes were detected (**Fig. 9**). The wall of blood vessels was formed by endotheliocytes with nuclei rich in heterochromatin.
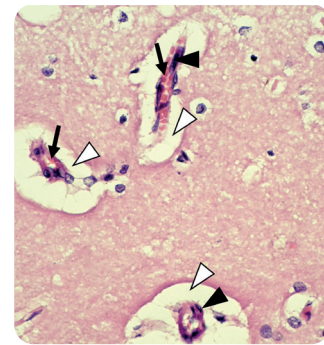

Fig. 9. Photomicrograph of a rat brain section. Hippocampal CA1 area on the 7th day after IR modeling and injection of the lysate from umbilical cord-derived MSCs. Perivascular edema is indicated by white triangles; the lumen of blood vessels contains erythrocytes (indicated by arrows); the vessel wall is lined by endotheliocytes with nuclei rich in heterochromatin (indicated by black triangles). Scale bar – 20 µm. Light microscopy, hematoxylin-eosin staining.

On the 14th day after the modeling of IR and the application of the lysate, the disorganization of the pyramidal layer was also observed in the hippocampal CA1 area, and the neurons were not located densely, but at some distance from each other (**Fig. 8 B**). Among the intact pyramidal neurons, hyperchromic ones were also found. Collaterals of apical dendrites were observed in some areas of the stratum radiatum.

On the 7th day after modeling of IR and application of citicoline in the *stratum radiatum* of the hippocampal CA1 area, single areas of normal fibrous structure were observed against the background of homogenization and microvacuolation. Such areas were located next to the pyramidal layer (**Fig. 10 A**).
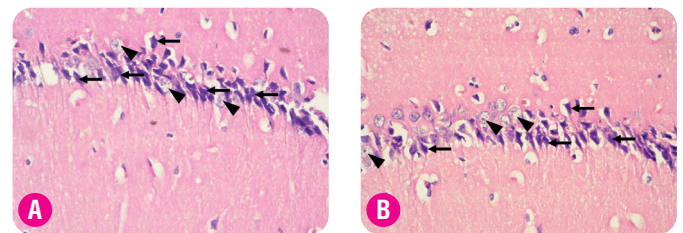

Fig. 10. Photomicrograph of rat brain sections. Pyramidal layer of the hippocampal CA1 area on the 7th (A) and 14th (B) day after IR modeling and citicoline injection. A – a significant number of neurons are damaged in the pyramidal layer; homogenization and microvacuolation of the stratum radiatum; neurons with normal structure are indicated by triangles; damaged hyperchromic bodies of neurons are indicated by arrows. B – pyramidal neurons are loosely located; neurons with normal structure are indicated by triangles; damaged hyperchromic bodies of neurons are indicated by arrows. Scale bar – 50 µm. Light microscopy, hematoxylin-eosin staining.

A significant number of damaged hyperchromic neurons with acidophilic cytoplasm were observed in the pyramidal layer. Microglial infiltration of the pyramidal layer was moderate. Perivascular edema was detected in the lacunar-molecular layer, the lumen of blood vessels was narrowed, accumulation of blood plasma proteins and erythrocyte sludge was observed (**Fig. 11**). The wall of blood vessels was formed by endotheliocytes with nuclei rich in heterochromatin.
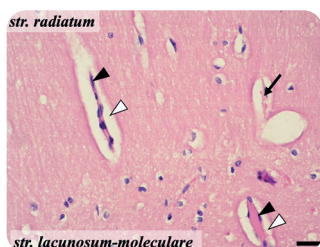

Fig. 11. Photomicrograph of a rat brain section. Hippocampal CA1 area on the 7th day after IR modeling and citicoline injection. The lumen of blood vessels is narrowed, there is an accumulation of blood plasma proteins; perivascular edema was detected (indicated by white triangles); the lumen of blood vessels contains erythrocytes (indicated by arrows); the vessel wall is lined by endotheliocytes with nuclei rich in heterochromatin (indicated by black triangles). Scale bar – 20 µm. Light microscopy, hematoxylin-eosin staining.

On the 14th day after IR modeling and citicoline application, pyramidal neurons were incompactly located in the hippocampal CA1 area, and a significant number of damaged hyperchromic neurons with acidophilic cytoplasm were observed (**Fig. 10 B**). Compared to the same group of animals on the 7th day, these animals had a greater number of intact neurons in the pyramidal layer. Homogenization of the stratum radiatum was preserved. Perivascular edema was also observed.

Morphometric analysis showed that the number of neuron nuclei in 1 mm² of the hippocampal CA1 area of animals with modeled IR was 2.3 times lower than in sham-operated animals. On the 7th day, their number decreased to 1627.3 ± 179.0 nuclei, and on the 14th – to 1739.8 ± 254.4 nuclei, compared to sham-operated animals (3977.4 ± 233.7 nuclei) (**Fig. 12**).
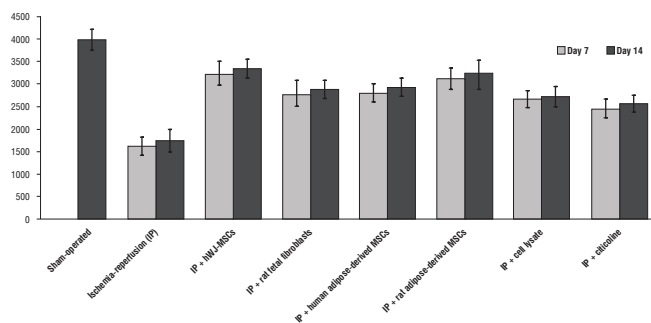

Fig. 12. The number of neuron nuclei per 1 mm² of the hippocampal CA1 area on the 7th and 14th days after IR modeling and treatment.

Notes: * – p < 0.05 compared to sham-operated animals;
– p < 0.05 compared to animals with modeled IR (group 2);
@ – p < 0.05 compared to the citicoline group.

A significant number (more than 90 %) of these nuclei were altered with the signs of karyorrhexis and karyopyknosis (**Fig. 13**). Transplantation of MSCs of various origins, lysate from human Wharton's jelly-derived MSCs or citicoline contributed to an increase in the number of nuclei in the hippocampal CA1 area, as well as an increase in the number of normal unchanged nuclei (**Figs. 12, 13**).
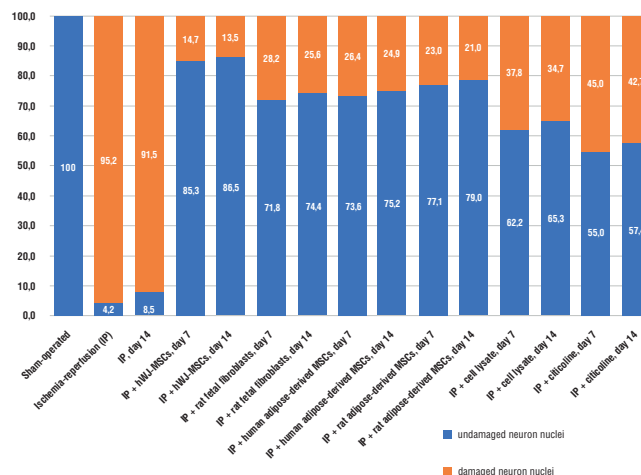

Fig. 13. Ratio of the number of intact and altered nuclei of neurons with signs of karyorrhexis and karyopyknosis per 1 mm² hippocampal CA1 area on the 7th and 14th day after IR modeling and treatment.

The most positive effect after modeling of ischemia-reperfusion of the rat brain had the transplantation of human Wharton's jelly-derived MSCs. The number of nuclei of neurons in the hippocampal CA1 area in this group of animals approached the number of nuclei in the group of sham-operated animals and on the 7th day after transplantation was 3226.0 ± 259.7 nuclei, on the 14th day – 3329.3 ± 212.8 nuclei, while the number of normal nuclei significantly exceeds the number of nuclei with the signs of destruction (**Fig. 13**).

This study demonstrated that intravenous allogeneic or xenogeneic transplantation of adipose-derived MSCs, human Wharton's jelly-derived MSCs and their lysate, rat fetal fibroblasts, and the reference drug citicoline in a rat ischemic stroke model reduced the volume of ischemic brain injury.

Various authors have shown that transplantation of MSCs after ischemic stroke improves brain function, effectively protects ischemic neurons and restores brain injury [12-15]. This is due to the fact that MSCs secrete a numerous trophic and immunomodulatory cytokines, commonly referred to as the MSC secretome, which has significant potential for the treatment of various diseases and brain injury through the induction of endogenous neuroprotection, neurogenesis, and angiogenesis [23, 24].

Adipose tissue is an available source of MSCs, from which they can be easily obtained in significant quantities. Their effectiveness and safety in the treatment of stroke was confirmed in animal models [25]. In rats with middle cerebral artery occlusion, the transplantation of adipose-derived MSCs has been shown to attenuate apoptosis and neuronal death, exhibiting a significant neuroprotective effect through inhibition of the KDM6B/BMP2/BMF axis [26].

Many researches have studied the efficacy of human Wharton's jelly-derived MSCs in ischemic stroke. It was reported that in rats with occlusion of the middle cerebral artery, transplantation of human Wharton's jelly-derived MSCs reduced inflammation, enhanced neurogenesis and angiogenesis, as a result of which the infarct volume decreased [27-30].

In the above studies, the effect of MSCs from one specific source was studied, while we compared the effect of both xenogeneic and allogeneic

cells from two sources. Variations in paracrine factors of various MSC populations contribute to different levels of repair activity. MSCs from human umbilical cord or adipose tissue act differently on cell populations of central nervous system, in which Wharton's jelly-derived MSCs have a better effect on the metabolic viability and cell density of primary hippocampal neurons [31]. We also found a neuroprotective effect of lysate from Wharton's jelly-derived MSCs in rats with a brain ischemia-reperfusion model. These data suggest that human Wharton's jelly-derived MSCs are a promising agent for experimental therapy of ischemic stroke. Existing evidence suggests that MSC therapy in ischemic stroke is safe and promising.

CONCLUSION

1. *The modeling of brain ischemia-reperfusion in rats on the 7th and 14th day leads to almost complete degeneration of the pyramidal layer of the hippocampal CA1 area, microglial infiltration of the stratum pyramidal and stratum radiatum, homogenization of the stratum radiatum, disruption of the architecture of apical dendritic bundles, perivascular edema and constriction of the lumen of blood vessels. The number of neuronal nuclei in the hippocampal CA1 area significantly decreases, the vast majority of which have signs of alteration.*

2. *In all experimental groups, after the administration of various types of studied MSCs, lysate from MSCs or citicoline, a decrease in the number of neurons with altered nuclei and a significant increase in the number of neurons whose nuclei did not undergo pathological changes were observed, compared to the control group with only ischemia-reperfusion injury, demonstrating neuroprotective properties of cellular therapy.*

3. *The ability of MSCs of various origins, lysate from MSCs or citicoline to reduce the volume of the infarct in the brain indicates the expediency of their application in conditions of acute brain ischemia.*

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Вплив мезенхімальних стромальних клітин різного походження на морфологічні показники у СА1 зоні гіпокампа щурів із гострою церебральною ішемією

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РЕЗЮМЕ

Щороку в Україні трапляється близько 150 тис. інсультів і понад 100 тис. людей помирають від наслідків інсульту та інших порушень кровотоку в мозку. Наразі отримано багатообіцяючі експериментальні дані щодо усунення неврологічної дисфункції при використанні мезенхімальних стромальних клітин (МСК).

МЕТА: охарактеризувати вплив МСК різного походження, лізату МСК Вартонових драглів людини та цитиколіну на динаміку деструктивних змін у ділянці СА1 зони гіпокампа щурів із гострим порушенням мозкового кровообігу за даними світлової мікроскопії та мікроморфометрії.

МАТЕРІАЛИ ТА МЕТОДИ. Виконано експеримент з використанням 4-х місячних самців щурів Вістар, яким проведена перехідна двобічна 20-хвилинна ішемія-реперфузія (ІР) внутрішніх сонних артерій. Після моделювання патології тваринам в/в вводили МСК, отримані з Вартонових драглів пуповини людини, з жирової тканини людини та щура в кількості 10⁶ клітин/тварину. Іншим групам піддослідних тварин в/в вводили фетальні фібробласти щура 10⁶ клітин/тварину та лізат з Вартонових драглів пуповини людини в дозі 0,2 мл/тварину. Контрольним тваринам в/в вводили 0,2 мл фізіологічного розчину. Остання група щурів отримувала однократно референс-препарат цитиколін у дозі 250 мг/кг. На 7-у та 14-у добу у СА1 зоні гіпокампа підраховували загальну кількість ядер нейронів на 1 мм² зрізу, а також визначали співвідношення кількості неушкоджених ядер нейронів та ядер із патологічними змінами (каріорексис та каріопікноз).

РЕЗУЛЬТАТИ. Трансплантація стовбурових клітин, лізату МСК Вартонових драглів людини чи цитиколіну сприяла більшому значенню кількості ядер у СА1 зоні гіпокампа, також зростала кількість ядер, які не зазнавали патологічних змін. Найбільш позитивний ефект мала трансплантація МСК із Вартонових драглів людини. Кількість ядер нейронів на 1 мм² у СА1 зоні гіпокампа в цій групі тварин наближалася до кількості ядер у групі псевдооперованих тварин. При цьому кількість ядер, які не зазнавали патологічних змін значно перевищувала кількість ядер з ознаками деструкції.

ВИСНОВКИ. В усіх експериментальних групах щурів при моделюванні ішемічного пошкодження головного мозку після введення різних типів досліджуваних мезенхімальних стромальних клітин, лізату або цитиколіну спостерігається значне зростання кількості нейронів без ознак патологічних змін. Найбільш позитивний результат у СА1 зоні гіпокампа було досягнуто після введення МСК із Вартонових драглів пуповини людини.

КЛЮЧОВІ СЛОВА: ішемічний інсульт; мезенхімальні стромальні клітини; СА1 зона гіпокампа; трансплантація клітин