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The effect of mesenchymal stromal cells of different origin on morphological parameters in the somatosensory cortex of rats with acute cerebral ischemia

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ABSTRACT

Ischemic stroke is the second leading cause of death and the leading cause of disability worldwide. So far, promising experimental data have been obtained regarding the elimination of neurological dysfunction and the reduction of the area of ischemic damage when using mesenchymal stromal cells (MSCs).

***THE PURPOSE** is to characterize the effects of MSCs of different origin, lysate of human Wharton's jelly-derived MSCs and citicoline on the dynamics of destructive changes in the somatosensory cortex of rats with acute cerebrovascular accident according to light microscopy and micromorphometry data.*

***MATERIALS AND METHODS.** An experiment was performed using 4-month-old male Wistar rats weighing 160-190 g, which were subjected to transient bilateral 20-minute ischemia-reperfusion (IR) of the internal carotid arteries. After modeling the pathology, the animals were injected into the femoral vein with obtained from human umbilical cord Wharton's jelly-derived MSCs, human and rat adipose tissue-derived MSCs at a dose of 10^6 cells/animal. Other groups of experimental animals were intravenously injected with fetal rat fibroblasts at a dose of 10^6 cells/animal in 0.2 mL of saline and lysate of human umbilical cord Wharton's jelly-derived MSCs at a dose of 0.2 mL/animal. Control animals were injected IV with 0.2 ml of physiological solution. The last group of rats received a single dose of the reference drug citicoline at a dose of 250 mg/kg. The morphometric studies were conducted on the 7^{th} and 14^{th} day. In the somatosensory cortex, 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 pathological changes (karyorrhexis and karyopyknosis) was also determined.*

***RESULTS.** The transplantation of stem cells, lysate of human umbilical cord Wharton's jelly-derived MSCs, or citicoline contributed to an increase in the number of neurons with nuclei in the somatosensory cortex, as well as an increase in the number of nuclei that did not undergo pathological changes. The transplantation of human umbilical cord Wharton's jelly-derived MSCs had the most positive effect. The number of neuron nuclei in 1 mm^2 that did not undergo pathological changes in the somatosensory cortex in this group of animals approached the number of nuclei in the group of pseudo-operated animals, while 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 injection of various types of studied mesenchymal stromal cells, lysate or citicoline. The most positive result in the somatosensory cortex was achieved after the transplantation of human umbilical cord Wharton's jelly-derived MSCs.*

***KEY WORDS:** somatosensory cortex; ischemic stroke; mesenchymal stromal cells; cell transplantation*

For many years, vascular pathology has been a leading cause of disability and mortality among people of working age around the world. Stroke is the second leading cause of death and the leading cause of disability worldwide [1]. Indeed, in 2017, 1.12 million cases of stroke were registered in the European Union, of which 0.46 million people died and 7.06 million patients remained disabled [2]. The updated 2021 heart disease and stroke statistics from the American Public Health Association state that in 2019, 6.6 million deaths were attributable to cerebrovascular disease worldwide, and a total of 3.3 million people died from ischemic stroke [3]. According to official statistics in Ukraine, 100,000-110,000 Ukrainians develop an acute cerebrovascular accident every year. A third of such patients are of working age, up to 50 % will have a disability, and only one in ten will return to a full life [4].

The formation of an ischemic focus in acute cerebrovascular accident (ACVA) is accompanied by the formation of an infarct core, where cells rapidly necrotize, and a surrounding penumbra zone, where cells are exposed to lower levels of ischemia and remain viable for a limited time, although they are functionally suppressed [5]. In the nucleus, which is severely deprived of oxygen and glucose, cell death occurs through necrosis affecting neurons, glial cells, and endothelial cells; while in penumbra the process is slower and occurs mainly through apoptosis [6].

In the treatment of ischemic stroke, it is important to restore the perfusion of the ischemic area as soon as possible. This can be done in the narrow time window after ischemic stroke by administration of recombinant tissue plasminogen activator (tPA) or thrombectomy. Since tPA must be administered within 4.5 h of the onset of ischemic stroke symptoms, only about 7 % of ischemic stroke patients will be able to receive tPA [7]. This prompts the search for effective methods of neuroprotection.

There is currently promising experimental evidence that cell therapy with mesenchymal stromal cells (MSCs) can promote recovery after stroke. A variety of stem cells have been tested in animal models of ischemic stroke. In recent works on this problem, the effect of MSCs from one specific source was studied, while we compared the effect of both xenogeneic and allogeneic cells from two sources. The dose of cells used by the researchers was 10^5-10^6 cells per animal, which is consistent with our method [8-11]. In the study, we administered the substance into the femoral vein once and immediately after IR, because earlier transplantation of MSCs led to greater neurological recovery and reduced infarct volume, and also required a smaller number ($1 \cdot 10^6$) of donor cells for a beneficial effect. The aim of our study was the histological assessment of the influence of MSCs of different origin, lysate of human umbilical cord Wharton's jelly-derived MSCs and citicoline on the dynamics of destructive changes in the somatosensory cortex of rats with acute cerebrovascular accident. The obtained results are a continuation of our earlier studies [12].

MATERIALS AND METHODS

An experiment was performed using 190 4-month-old male Wistar rats weighing 160-190 g, which underwent transient bilateral 20-minute ischemia-reperfusion of the internal carotid arteries (IR ICA). The animals of the vivarium of the Vinnytsia National Medical University named after M. Pyrogov, were kept in standard conditions with access to water and food ad libitum. The study was conducted according to the methodological recommendations of the Ministry of Health of Ukraine and 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. An experimental model of ischemia-reperfusion (IR) was created by placing ligatures on the internal carotid arteries on both sides under propofol anesthesia (Propofol-novo, Novofarm-Biosintez LLC, Ukraine, 60 mg/kg) for 20 minutes. The selected model reflects the clinical picture of brain infarction and is adequate for the experimental study of potential neuroprotective substances [13]. Experimental animals were divided into 8 groups (**Table 1**).

Table 1. Distribution of animals in the experimental groups.

Group of animals	Number of animals	Treatment
Group 1	10	sham-operated animals + 0.9 % solution of NaCl at a dose of 2 mL/kg
Group 2	40	IR + 0.9 % solution of NaCl in a dose of 2 mL/kg
Group 3	20	IR + human umbilical cord Wharton's jelly-derived MSCs at a dose of 10^6 cells/animal
Group 4	20	IR + fetal rat fibroblasts at a dose of 10^6 cells/animal
Group 5	25	IR + human adipose tissue-derived MSCs at a dose of 10^6 cells/animal
Group 6	25	IR + rat adipose tissue-derived MSCs at a dose of 10^6 cells/animal
Group 7	25	IR + lysate of human umbilical cord Wharton's jelly-derived MSCs at a dose of 0.2 mL/animal
Group 8	25	IR + citicoline 250 mg/kg

Group 1 is sham-operated rats sequentially subjected to the following interventions (anesthesia, skin incision, vessel preparation) except for the ligation of the internal carotid artery (ICA), which reproduced the effect of the traumatic conditions of the experiment. Group 2 is a group with control pathology. Rats of this group underwent 20-minute brain ischemia (I) by applying ligatures to the ICA. After 20 minutes, the ligatures from the ICA were removed (reperfusion(R)) and 0.9 % NaCl solution at the rate of 2 mL/kg was injected once into the femoral vein. A similar dose was admi-nistered to Group 1 rats. Group 3 were transplanted with 10^6 cells/animal human Wharton's jelly-derived MSCs immediately after IR. Group 4 underwent a single transplantation of rat fetal fibroblasts at a dose of 10^6 cells/animal immediately after IR. Group 5 received 10^6 cells/animal of human adipose tissue-derived MSCs. Group 6 were injected with rat adipose tissue-derived MSCs at a dose of 10^6 cells/animal immediately after IR. Group 7 were injected with lysate of human umbilical cord Wharton's jelly-derived MSCs at a dose of 0.2 mL/animal immediately after IR. Group 8 received a single dose of the reference drug citicoline (*Neuroxon, Arterium Corporation*, Ukraine) immediately after IR at a dose of 250 mg/kg. Citicoline was chosen among all available drugs due to its ability to enhance neuroregenerative processes in an experiment on rats and to improve cognitive and memory functions in patients with cerebral ischemia [14-17].

Obtaining all types of cells used in the experiment was described earlier [12]. The research was conducted on the 7th day (subacute period of ischemia) and 14th day (recovery period) [18].To analyze the influence of MSCs of different origin, lysate of human umbilical cord Wharton's jelly-derived MSCs, and citicoline on the dynamics of destructive changes in the somatosensory cortex, rat brains were quickly removed after decapitation using anesthesia with 100 mg/ kg pentobarbital ("Penbital", *Bioveta a.s.*, Czech Republic) after 7 days (subacute period of ischemia) and 14 days after IR (recovery period) [18-20].

The brains of the experimental animals were fixed with a 4 % formaldehyde solution for 24 hours. After fixation, the brains were washed in running water, passed through alcohols of increasing concentration and xylene, and after standard histological processing, embedded in Paraplast Plus© (*Leica Scientific*, Germany). Sections 5 μm thick were made on a rotary microtome. Deparaffinized sections were stained with hematoxylin-eosin according to standard methods.

Digital images of frontal brain slices of experimental animals obtained with a BX-51 microscope (*Olympus*, Japan) were analyzed using the ImageJ 1.48v software (*free license*, Rasband, USA). In the somatosensory cortex of the large cerebral hemispheres of rats, 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 pathological changes (karyorrhexis and karyopyknosis) was also determined. To evaluate the obtained results, statistical processing were performed using Excel© 2010 software (*Microsoft*©, USA). The nonparametric Kruskel-Wallis method was used to analyze the reliability of differences between groups. Means, standard errors, and significance of differences were calculated and considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Histological analysis of frontal paraffin sections of the rat brain showed no differences between intact and sham-operated animals, so the group of sham-operated animals was used as a control.

Microscopic examination of the somatosensory cortex of the large cerebral hemispheres of sham-operated animals revealed the normal structure of the cortex, which consisted of the following cell layers: I – molecular (lamina molecularis), II – external granular (lamina granularis externa), III – external pyramidal (lamina pyramidalis externa), IV – internal granular (lamina granularis interna), V – internal pyramidal (lamina pyramidalis interna), VI – polymorphic (lamina multiformis) (**Fig. 1**).

In the molecular layer, which was located directly under the soft meninges, only a few cells, usually glial, were observed. This layer consisted mainly of vertically oriented apical dendrites and horizontally oriented axons of neurons localized in deeper layers.

The relatively thin outer granular layer consisted of numerous small (5-15 μm) stellate neurons, as well as small pyramidal neurons. The outer pyramidal layer contained small and medium-sized pyramidal neurons. The surface cells of this layer were smaller than those located deeper. The inner granular layer consisted of small stellate neurons and various types of pyramidal neurons. The inner pyramidal layer contained large pyramidal neurons. The polymorphic layer consisted mainly of spindle cells and triangular pyramidal cells. Pyramidal neurons in these layers varied in size, usually having one apical dendrite directed toward the cortical surface and several basal dendrites; had a euchromatin nucleus and basophilic Nissl granules (**Fig. 1 B**). The stellate cells were smaller in size with a euchromatin nucleus and clearly visualized nucleolus, had processes diverging in different planes. Stellate neurons were localized in all layers except the external one. Endotheliocytes with heterochromic nuclei were well visualized in the wall of blood vessels.

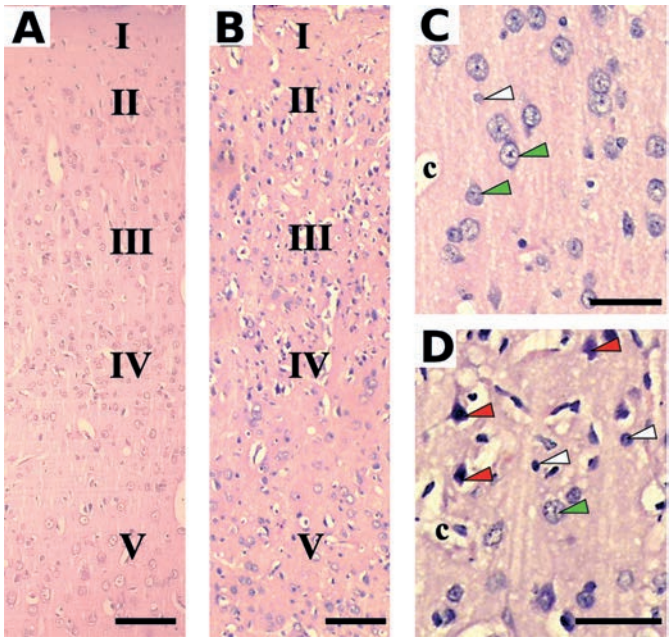

Fig. 1. Micrographs of somatosensory cortex of the rat brain sections. A, B – sham-operated animal; B, D – 14 days after ischemia-reperfusion. Cell layers are indicated by numbers: I – molecular, II – outer granular, III – outer pyramidal, IV – inner granular, V – inner pyramidal. Green triangles indicate intact neurons, red triangles – damaged pyramidal neurons, white – glial cells, c – the lumen of a blood vessel. Light microscopy, hematoxylin-eosin staining; scale bars:
A, B – 100 μm, B, D – 50 μm.

After ischemia-reperfusion, pyramidal neurons had shrunken hyperchromic bodies with acidophilic cytoplasm without visualization of nuclei and nucleoli (**Figs. 1 B, D; 2**).

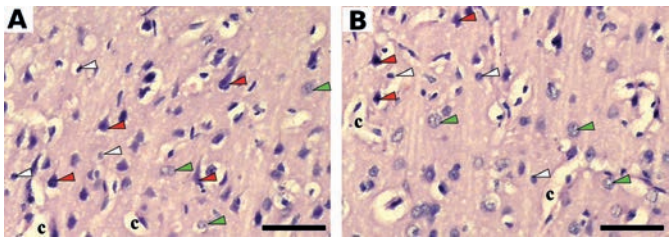

Fig. 2. Micrographs of the somatosensory cortex of the rat brain sections on the 7th (A) and 14th days (B) after ischemia-reperfusion. Green triangles indicate intact neurons, red triangles – damaged pyramidal neurons, white – glial cells, c – the lumen of a blood vessel. Light microscopy, hematoxylin-eosin staining; scale bar – 50 μm.

Pronounced pericellular edema was observed, which formed light "halos" around pyramidal neurons, as well as vacuolation of neuropil (**Fig. 2**). Perivascular edema was also detected, the lumen of blood vessels was narrowed, and the accumulation of blood plasma proteins was observed. Glial cells acquired dark color, reactive gliosis was observed. Stellate neurons almost did not undergo any structural changes and had the appearance of neurons of the control group. On the 14th day after ischemia-reperfusion, the nature of destructive changes in the somatosensory cortex was similar to that on the 7th day, but there was a slight decrease in the number of damaged pyramidal neurons, a decrease in reactive gliosis (**Fig. 2 B**).

The transplantation of human umbilical cord Wharton's jelly-derived MSCs had the most positive effect on the structure of the somatosensory cortex after ischemia-reperfusion. In this group of animals, on the 7th day after transplantation, only single shrunken hyperchromic pyramidal neurons were observed, the cytoarchitectonics of the somatosensory cortex was similar to the group of sham-operated animals (**Fig. 3 A**).

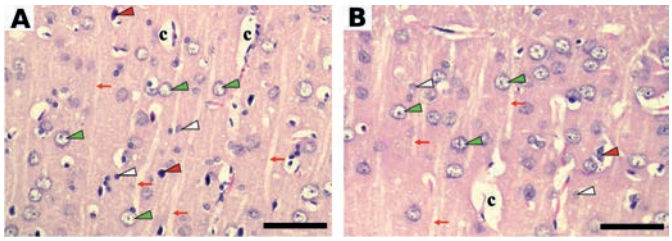

Fig. 3. Micrographs of the somatosensory cortex of the rat brain sections on the 7th (A) and 14th days (B) after ischemia-reperfusion and transplantation of human umbilical cord Wharton's jelly-derived MSCs. Green triangles indicate intact neurons, red triangles – damaged neurons, white – glial cells, red arrows – apical dendrites, c – blood vessel lumen Light microscopy, hematoxylin-eosin staining; scale bar – 50 μm.

Most pyramidal neurons had a euchromatin nucleus, stellate cells had an unchanged morphology. A characteristic parallelism of apical dendrites was observed in the neuropil. The wall of blood vessels was formed by endotheliocytes with nuclei rich in heterochromatin. Some glial cells had a dark color. On the 14th day after the transplantation of human MSCs, the cytoarchitectonics of the somatosensory cortex was similar to that on the 7th day: single hyperchromic pyramidal neurons, most pyramidal neurons had a euchromatin nucleus, unchanged stellate cells, a small number of dark glial cells (**Fig. 3 B**).

On the 7th day after the modeling of ischemia-reperfusion and transplantation of rat fetal fibroblasts, both pyramidal neurons with a euchromatin

nucleus and damaged neurons with signs of karyopyknosis and acidophilic cytoplasm were visualized (**Fig. 4 A**). In the neuropil, the swelling of the intercellular space, perivascular edema, and constriction of the vessel lumen were observed.

On the 14th day after the modeling of ischemia-reperfusion and transplantation of rat fetal fibroblasts, heterochromic and pyknotic nuclei with pronounced perinuclear edema were also observed among the pathologically unchanged euchromic nuclei of pyramidal neurons(**Fig. 4 B**). Cytoarchitectonics was similar to that on the 7th day.

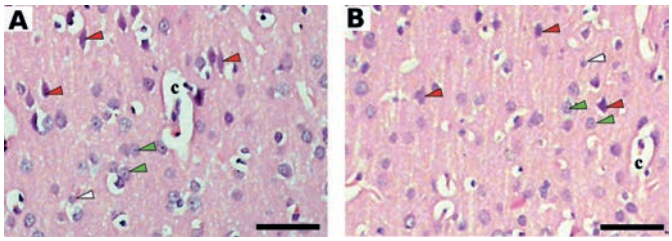

Fig. 4. Micrographs of the somatosensory cortex of the rat brain sections on the 7th (A) and 14th days (B) after ischemia-reperfusion and transplantation of rat fetal fibroblasts. Green triangles indicate undamaged neurons, red triangles – damaged pyramidal neurons, white – glial cells with – the lumen of a blood vessel. Light microscopy, hematoxylin-eosin staining; scale bar – 50 μm.

On the 7th day after the modeling of ischemia-reperfusion and transplantation of human adipose tissue-derived MSCs, morphologically normal pyramidal neurons with a euchromatin nucleus and only a small number of hyperchromic pyramidal neurons were observed in the somatosensory cortex (**Fig. 5 A**). The characteristic parallelism of the apical dendrites was also visualized. Endotheliocytes with heterochromic nuclei are clearly visible in the walls of blood vessels. Microglial infiltration was not observed.

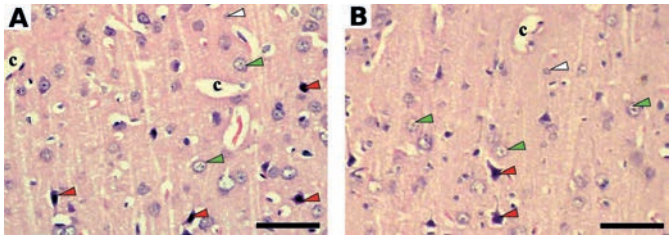

Fig. 5. Micrographs of the somatosensory cortex of the rat brain sections on the 7th (A) and 14th days (B) after ischemia-reperfusion and transplantation of human adipose tissue-derived MSCs. Green triangles indicate intact neurons, red triangles – damaged neurons, white – glial cells, c – the lumen of a blood vessel. Light microscopy, Light microscopy, hematoxylin-eosin staining; scale bar – 50 μm.

On the 14th day after the modeling of ischemia-reperfusion and transplantation of human adipose tissue-derived MSCs, the cytoarchitectonics of the somatosensory cortex was similar to that on the 7th day: mostly pyramidal neurons had a pathologically unchanged euchromic nucleus and one nucleolus, but single heterochromic and pyknotic neurons as well as nuclei with pronounced perinuclear edema were also observed. (**Fig. 5 B**).

On the 7th day after the modeling of ischemia-reperfusion and transplantation of rat adipose tissue-derived MSCs, cells with unchanged morphology were mostly observed in the somatosensory cortex (**Fig. 6 A**). A small number of pyramidal neurons were hyperchromic with pericellular edema with compaction of their nucleus. Areas of microvacuolization and perivascular edema were isolated, microglial infiltration was insignificant.

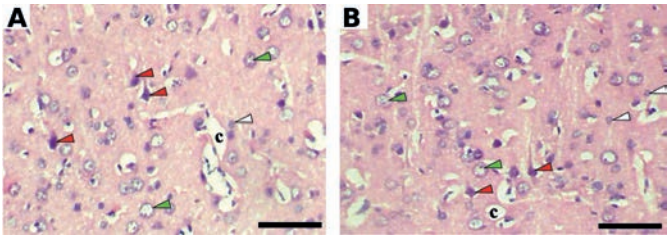

Fig. 6. Micrographs of the somatosensory cortex of the rat brain sections on the 7th (A) and 14th days (B) after ischemia-reperfusion and transplantation of rat adipose tissue-derived MSCs. Green triangles indicate intact neurons, red triangles – damaged pyramidal neurons, white – glial cells, c – the lumen of a blood vessel. Light microscopy, hematoxylin-eosin staining; scale bar – 50 μm.

On the 14th day after the modeling of ischemia-reperfusion and transplantation of stem cells from rat adipose tissue, the nature of changes in the somatosensory cortex was similar to that on the 7th day, a decrease in the number of damaged pyramidal neurons was observed (**Fig. 6 B**). Areas with well-defined strands of apical dendrites were visualized. Stellate cells on both the 7th and 14th days almost did not undergo structural changes.

On the 7th day after the modeling of ischemia-reperfusion and the application of lysate of human umbilical cord Wharton's jelly-derived MSCs, both pathologically unchanged euchromic nuclei of pyramidal neurons containing one nucleolus and heterochromic and pyknotic nuclei with pronounced perinuclear edema were observed in the somatosensory cortex (**Fig. 7 A**). Apical dendrites were almost not visualized. Glial cells had a dark color. The stellate cells did not undergo any structural changes and looked like neurons of the control group. Edema of the intercellular space and perivascular edema were observed.

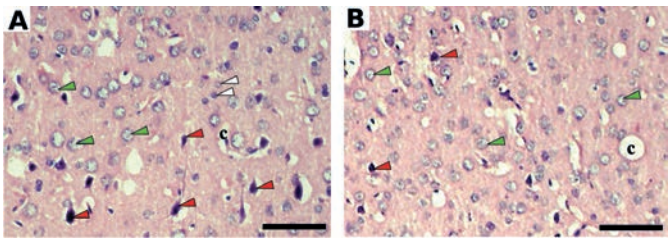

Fig. 7. Micrographs of the somatosensory cortex of the rat brain sections on the 7th (A) and the 14th days (B) after ischemia-reperfusion and the use of lysate. Green triangles indicate intact neurons, red triangles – damaged pyramidal neurons, white – glial cells, c – the lumen of a blood vessel. Light microscopy, hematoxylin-eosin staining; scale bar – 50 μm.

On the 14th day after the modeling of ischemia-reperfusion and the use of lysate, the nature of structural changes in the somatosensory cortex was similar to that on the 7th day, but a decrease in the number of damaged pyramidal neurons, a decrease in the swelling of the intercellular space and perivascular edema were observed (**Fig. 7 B**).

On the 7th day after the modeling of ischemia-reperfusion and the application of citicoline, the vacuolation of the neuropil of the somatosensory cortex was observed, pyramidal neurons were shrunken and hyperchromic, but the number of damaged neurons was significantly less than in the ischemia-reperfusion group (**Fig. 8 A**). Glial cells had a dark color.

A characteristic pericellular edema was also observed, which formed light "halos" around the pyramidal neurons, as in the ischemia-reperfusion group (**Fig. 8 A**). Apical dendrites were almost not visualized. On the 14th day, the nature of destructive changes in the somatosensory cortex was similar to that on the 7th day, but a slight decrease in the number of damaged pyramidal

neurons was observed (**Fig. 8 B**). Both on the 7th and 14th days, the stellate neurons almost did not undergo any structural changes.

As shown by morphometric analysis, the transplantation of stem cells or the use of their lysate or citicoline contributed to the improvement of neuroprotection by increasing the number of nucleus neurons that did not undergo pathological changes in 1 mm^2 in the somatosensory cortex compared to the group of animals with ischemia-reperfusion, in which 58.3 ± 3.8 % of intact neuron nuclei were observed on the 7th day, and 60.4 ± 4.6 % - on the 14th day (**Table 2**).

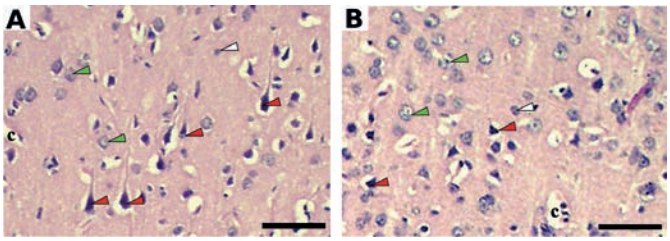

Fig. 8. Micrographs of the somatosensory cortex of the rat brain sections on the 7th (A) and 14th (B) days after the ischemia-reperfusion modeling and the use of citicoline. Green triangles indicate neurons with a preserved structure, red – damaged pyramidal neurons, white – glial cells, c – the lumen of a blood vessel. Light microscopy, hematoxylin-eosin staining; scale bar – 50 μm.

Table 2. The ratio of the number of intact and damaged neuronal nuclei (karyorrhexis and karyopyknosis) in 1 mm^2 in the somatosensory cortex of rats on the 7th and 14th days after ischemia-reperfusion modeling and treatment.

Group	Intact nuclei, %	
	day 7	day 14
Sham-operated animals	100.0 ± 2.0*	100 ± 2.0*
Ischemia-reperfusion (control pathology)	58.3 ± 3.8	60.4 ± 4.6
IR + human umbilical cord Wharton's jelly-derived MSCs	89.0 ± 2.9*	93.1 ± 1.1*
IR + rat fetal fibroblasts	84.4 ± 3.0*#	86.1 ± 1.9*#
IR + human adipose tissue-derived MSCs	82.1 ± 1.1*#	84.1 ± 3.3*#
IR + rat adipose tissue-derived MSCs	83.3 ± 1.0*#	85.3 ± 1.6*#
IR + lysate	80.7 ± 1.2*#	82.3 ± 4.7*#
IR + citicoline	75.7 ± 4.8*#	77.0 ± 6.7*#

*Note: * – $p < 0.05$ compared to the control group; # – $p < 0.05$ compared to the group of hUC-MSCs.*

As can be seen from **Tab. 2**, the transplantation of human umbilical cord Wharton's jelly-derived MSCs had the most positive effect on the cytoarchitectonics of the somatosensory cortex after the modeling ischemia-reperfusion of the brain in rats. This group of animals had the highest percentage of intact neuronal nuclei (89.0 ± 2.9 % on the 7th day and 93.3 ± 1.1 % on the 14th day) compared to the untreated control (58.3 ± 3.8 % on the 7th day and 60.4 ± 4.6 % on the 14th day, $p < 0.05$) and all other experimental groups (at $p < 0.05$).

The lowest percentage of intact neuron nuclei among the experimental groups of animals transplanted with stem cells or treated with their lysate or citicoline was in IR + citicoline group (75.7 ± 4.8 % on the 7th day and 77.0 ± 6.7 % on the 14th day).

This study demonstrated that intravenous allogeneic and xenogeneic transplantation of adipose-derived MSCs, human umbilical cord Wharton's jelly-derived MSCs and their lysate, rat fetal fibroblasts, and the reference drug citicoline in a rat model of ischemic stroke reduced the volume of ischemic damage to the somatosensory cortex of the brain. The conducted therapy contributed to the reduction of neuro- and astrocytic apoptosis, cerebral edema, which helped to maintain the integrity of the blood-brain barrier after cerebral infarction. Various authors have shown that the transplantation of MSCs after ischemic stroke improves brain function, effectively protects ischemic neurons and restores brain damage [21, 22]. In histopathological analysis, the authors showed that MSC treatment did not reduce the size of the lesion, but increased the number of neurons that survived after stroke in rats [23]. Therefore, the main direction of cytoprotection can be considered the development and clinical use of drugs based on MSCs, which are able to prevent and inhibit the very phenomenon of apoptosis.

This is due to the fact that MSCs secrete a wide array of trophic and immunomodulatory cytokines, commonly referred to as the MSC secretome, which has significant potential for the treatment of ischemic brain injury through the induction of endogenous neuroprotection, neurogenesis, and angiogenesis [24, 25]. We believe that the transplantation of MSCs in our experiment directly after ischemia-reperfusion contributed to neuroprotection both by activating the proliferation of neural progenitors and by preventing their damage and death. MSCs of human Wharton's cells provided better efficacy of neuroprotection, as they enhanced the growth of neurons and reduced the apoptotic death of primary cortical cells in acute cerebral ischemia [26].

CONCLUSION

1. The modeling of ischemia-reperfusion on the 7th and 14th days leads to almost complete degeneration of the somatosensory cortex of the brain in rats.

2. In all experimental groups, after the injection of various types of the studied stem cells, lysate or citicoline, a significant increase in the number of neurons, the nuclei of which did not undergo pathological changes, was observed.

3. The most positive result was achieved after the transplantation of human umbilical cord Wharton's jelly-derived MSCs after the modeling of ischemia-reperfusion of the rat brain.

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Вплив мезенхімальних стромальних клітин різного походження на морфологічні показники у соматосенсорній корі щурів із гострою церебральною ішемією

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РЕЗЮМЕ

Ішемічний інсульт є другою за поширеністю причиною смерті та основною причиною інвалідності в усьому світі. Наразі отримано багатообіцяючі експериментальні дані щодо усунення неврологічної дисфункції та зменшенні зони ішемічного ураження при використанні мезенхімальних стромальних клітин (МСК).

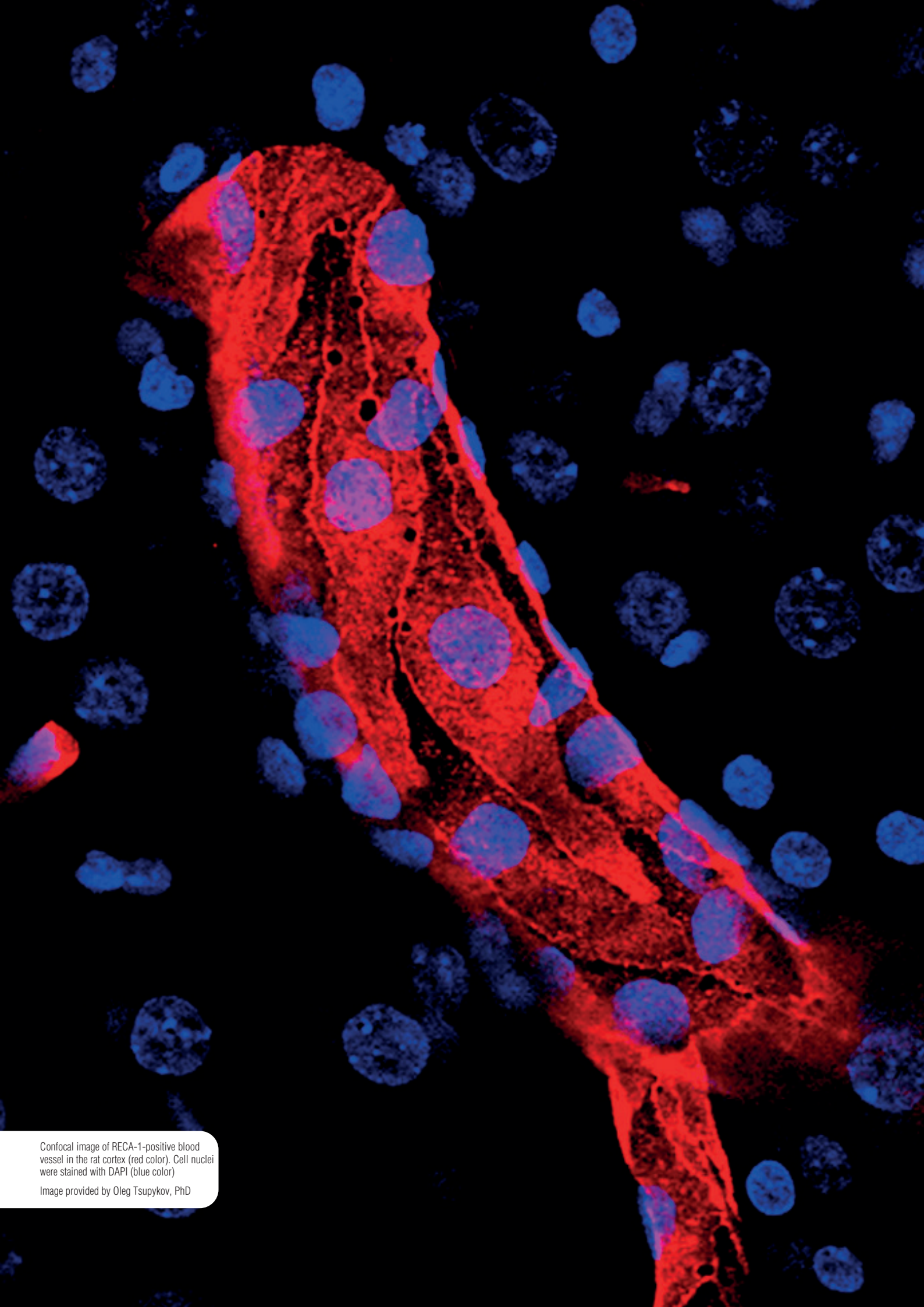
***МЕТА ДОСЛІДЖЕННЯ**: охарактеризувати вплив МСК різного походження, лізату МСК Вартонових драглів людини та цитиколіну на динаміку деструктивних змін у соматосенсорній корі щурів із гострим порушенням мозкового кровообігу за даними світлової мікроскопії та мікроморфометрії.*

***МАТЕРІАЛИ ТА МЕТОДИ**. Виконано експеримент з використанням 4-х місячних самців щурів Вістар масою 160-190 г, яким проведена транзиторна двобічна 20-хвилинна ішемія-реперфузія (ІР) внутрішніх сонних артерій. Після моделювання патології тваринам в стегнову вену вводили МСК, отримані з Вартонових драглів пуповини людини, з жирової тканини людини та щура в кількості 10^6 клітин/тварину. Іншим групам піддослідних тварин в/в вводили фетальні фібробласти щура 10^6 клітин/тварину в 0,2 мл фізіологічного розчину 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. Морфометричне дослідження проводили на 7-у та 14-у добу. У соматосенсорній корі підраховували загальну кількість ядер нейронів у 1 мм^2, 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 сприяли збільшенню кількості нейронів із ядрами у соматосенсорній корі, також зростала кількість ядер, які не зазнавали патологічних змін. Найбільш позитивний ефект мала трансплантація МСК із Вартонових драглів людини. Кількість ядер нейронів, які не зазнавали патологічних змін у соматосенсорній корі на 1 мм^2 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 соматосенсорній корі було досягнуто після трансплантації МСК із Вартонових драглів пуповини людини.*

***КЛЮЧОВІ СЛОВА**: соматосенсорна кора; ішемічний інсульт; мезенхімальні стромальні клітини; трансплантація клітин*



Confocal image of RECA-1-positive blood vessel in the rat cortex (red color). Cell nuclei were stained with DAPI (blue color)

Image provided by Oleg Tsupykov, PhD