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# The effect of mesenchymal stromal cells of various origins on mortality and neurologic deficit in acute cerebral ischemia-reperfusion in rats



Konovalov S.<sup>1</sup>, Moroz V.<sup>1</sup>, Konovalova N.<sup>1</sup>, Deryabina O.<sup>2,3</sup>, Shuvalova N.<sup>2</sup>, Toporova O.<sup>2,3</sup>, Tochylovsky A.<sup>4</sup>, Kordium V.<sup>2,3</sup>

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

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

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

<sup>4</sup>BioTexCom LLC, Kyiv, Ukraine

Corresponding author's e-mail: [oderyabina@gmail.com](mailto:oderyabina@gmail.com)

## ABSTRACT

Stroke is a global epidemic issue and the second leading cause of death in the world and in Ukraine. According to official statistics, every year 100-110 thousand Ukrainians suffer acute cerebrovascular disorders. One third of such patients are of working age, up to 50 % will have a disability, and only one in ten will fully return to full life. So far, promising experimental data on the treatment of neurological dysfunction using mesenchymal stromal cells (MSCs) have been obtained.

**THE AIM OF STUDY** is to compare the effect of MSCs of different origins on mortality and neurologic deficit in rats with acute cerebral ischemia-reperfusion injury (CIRI).

**MATERIALS AND METHODS.** Acute cerebral ischemia-reperfusion was modeled by transient bilateral 20-minute occlusion of internal carotid arteries in male Wistar rats aged 4 months. Animals were injected intravenously with  $1 \cdot 10^6$  MSCs derived from human umbilical cord Wharton's jelly (hWJ-MSC), human and rat adipose tissue. Other groups of experimental animals were injected intravenously with rat fetal fibroblasts and cell lysate from hWJ-MSC. The last group of rats received Citicoline at a dose of 250 mg/kg as a reference drug. Control animals were injected intravenously with normal saline. The cerebroprotective effect of therapy was assessed by mortality and neurologic deficit in rats on the McGraw's stroke index score.

**RESULTS.** After 12 hours of observation in the crucial period in the development of experimental acute cerebrovascular disorders with the administration of hWJ-MSC, mortality was only 10 % against 45 % of animals in the control group. The use of rat fetal fibroblasts reduced the mortality of animals compared to the control group by an average of 25 %. CIRI in rats caused severe neurologic deficits: paralysis, paresis, ptosis, circling behavior. On the 7<sup>th</sup> day of observation in the control group of animals, the mean score on the McGraw's stroke index indicated severe neurological disorders. On the 14<sup>th</sup> day of observation in this group of animals there was no complete recovery of lost central nervous system functions. Compared with the control group of animals, all the treatment agents for acute CIRI (MSCs of various origins, MSC's lysate and Citicoline) contributed to a significant regression of neurologic deficit.

**CONCLUSIONS.** Thus, transplantation of human Wharton's jelly-derived MSCs and rat fetal fibroblasts reduced mortality and alleviated neurological symptoms in rats with experimental ischemic stroke. hWJ-MSC, rat fetal fibroblasts, and rat adipose-derived MSCs reduced the incidence of neurological disorders better than Citicoline, which was accompanied by a regression of neurologic deficit dynamics on the 14<sup>th</sup> day of follow-up. The ability of stem cells of different origins to reduce neurologic deficit indicates the feasibility of their use in experimental acute cerebral ischemia.

**KEY WORDS:** ischemic stroke; cerebral ischemia-reperfusion injury; neurologic deficit; mesenchymal stromal cells; cell lysate; Citicoline

Stroke is a global epidemic and the second leading cause of death in the world and Ukraine [1, 2]. According to official statistics, every year

100-110 thousand Ukrainians develop acute cerebrovascular disorders. One third of such patients are of working age, up to 50 % will have a dis-

ability, and only one in ten will fully return to normal life [2]. Therefore, medical care in acute cerebrovascular disorders is a priority in the Program of medical guarantees with funding at a higher rate [3].

In the treatment of stroke, it is important to restore perfusion of the ischemic area as soon as possible [4, 5]. However, the search for effective and safe means to reduce the manifestations of acute cerebrovascular disorders is still ongoing.

So far, promising experimental data on the treatment of neurological dysfunction using mesenchymal stromal cells (MSCs) have been obtained [6-9]. It should be noted that MSCs are multipotent cells that have unlimited ability to grow, divide and even differentiate *in vitro* into neurons, astrocytes and oligodendrocytes [10-12]. *In vivo* MSCs in the cerebral infarction model in rats have also been shown to improve neurological function [12]. However, comparative studies for different types of MSCs in cerebrovascular diseases model have been described little.

The **AIM** of our study was to compare the effect of MSCs of different origins on mortality and neurologic deficits in rats with acute cerebral ischemia-reperfusion injury (CIRI).

## MATERIALS AND METHODS

For the experiment, we used 200 adult 4-month-old albino Wistar rats weighting 160-190 g. Animals were kept under standard conditions with water and food ad libitum in the vivarium of M. I. Pirogov Vinnytsia National Medical University. The study followed the guidelines of the State Expert Center of the Ministry of Health of Ukraine and national requirements "General Ethical Principles of Animal Experiments", approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001) as well as the Law of Ukraine "On Protection of Animals from Cruelty" (dated 26.02.2006).

**Cerebral ischemia-reperfusion injury model.** An experimental CIRI was modeled by bilateral ligation of the internal carotid arteries for 20 minutes under anesthesia with Propofol-Novo 60 mg/kg (*Novopharm-Biosynthesis LLC*, Ukraine). The selected model reflects the clinical picture of cerebral stroke and is adequate for the experimental study of potential neuroprotective agents [13]. MSCs of various origins in the amount of  $1 \cdot 10^6$  cells per animal in 0.2 mL of 0.9 % NaCl solution were injected once intravenously into the catheterized femoral vein immediately after CIRI. Immediate administration was performed to assess the potential of MSCs to prevent the progression of acute ischemic injury, namely the area of ischemic tissue "penumbra", which is the main target of ischemic stroke therapy in the first hours and days after the onset of the disease. The effectiveness of the treatment of acute cerebral ischemia was determined by the dynamics of rat mortality and neurologic deficit.

**The isolation of human MSCs from umbilical cord-derived Wharton's jelly.** After an informed consent, umbilical cord was obtained in aseptic conditions from a healthy woman during a normal delivery at 39 weeks of pregnancy. All experiments were performed on a sample from one umbilical cord.

The tissue was delivered to the laboratory in DMEM medium (*BioWest*, Austria), with antibiotics (penicillin 100 IU/mL, streptomycin 100 µg/mL) and processed no later than 8 hours after the labor. The umbilical cord was washed from the blood as much as possible, placed in a new medium for 20-30 minutes with a 10-fold concentration of penicillin 1000 IU/mL, streptomycin 1000 µg/mL (*Kyivmedpreparat*, Ukraine) and antifungal amphotericin B 50 IU/mL (*Biowest*, France). All manipulations were performed under sterile conditions. Vessels (a vein and two arteries) were removed from the umbilical cord. Umbilical cord Wharton's jelly was isolated, minced with scissors to the pieces up to 0.1-0.5 cm and resuspended in warm (37 °C) growth medium alpha-MEM (*BioWest*, Austria), supplemented with 10 % fetal bovine serum (*HyClone*, USA) 2 mM L-glutamine (*Sigma*, USA) and 10 nM fibroblast growth factor-1 (*Interpharm Biotek*, Ukraine). The cultivation was performed in a 25 cm<sup>2</sup> or 75 cm<sup>2</sup> culture flask (*Bioswisstec*, Switzerland) in a CO<sub>2</sub> incubator at 37 °C and 5 % CO<sub>2</sub> by explant technique. After 7-14 days, the first clones were obtained from the explants, which were grown to 80 % monolayer.

The medium was changed every 3 days. Solutions of 0.02 % Versen and 0.25 % trypsin (*Biowest*, France) in a ratio of 1:1 were used for cell subculture according to standard methods. Cells were seeded at  $5 \cdot 10^3$  cells per cm<sup>2</sup>, expanded to the 2<sup>nd</sup> passage and characterized by morphology and surface markers [14] before further administration to experimental animals.

**The isolation of human adipose-derived MSCs.** We use standard method with modification to obtain adipose-derived MSCs [15]. After signing an informed consent, adipose tissue was obtained from 23-33 year-old women during cosmetic liposuction from the abdominal fat. All experiments were performed with the sample of one liposuction.

Within 2 hours after the surgery, the material was delivered to the laboratory and immediately processed to isolate MSCs. 25 mL of fat was centrifuged for 10 min at 800 xg at room temperature to separate from blood. Then it was washed twice by centrifugation with phosphate buffer saline (PBS) (*Sigma*, USA) in a ratio 1:1. An equal volume of 0.2 % solution of collagenase type I (*Gibco*, USA) in PBS heated to 37 °C was added to the washed adipose tissue. The resulting mixture was shaken on a shaker for 1 h at 37 °C, and then centrifuged at room temperature for 10 min at 800 xg. Mature adipose cells on top and most of the fluid were discarded, leaving about 5 mL of cell pellet. The obtained pellet was resuspended in 20 mL of nutrient medium DMEM/F12 (*BioWest*, Austria), supplemented with 10 % fetal calf serum with antibiotics, and seeded into 25 cm<sup>2</sup> culture flasks in a volume of 5 mL. After 24 h, the nutrient medium was changed discarding non-adherent cells. Subsequently, the medium was changed every 3 days. When 70-80 % of the monolayer was reached, the first cell subculture was performed using a mixture of 0.25 % trypsin solution and 0.02 % Versene solution in a ratio of 1:1 according to standard methods. The seeding density was  $5 \cdot 10^3$  cells/cm<sup>2</sup>. Cells were expanded to the 2<sup>nd</sup> passage.

Umbilical cord and adipose-derived MSCs at the second passage were stained with hematoxylin and eosin to check their morphology, and also analyzed by flow cytometry using cell sorter FACSaria (*BD Biosciences*, USA). Cell phenotyping for the surface markers CD73, CD90, CD105, CD34 and CD45 were performed according to the standard procedure using anti-CD105 PerCP-Cy5.5 (cat. #560819), anti-CD90 PerCP-Cy5.5 (cat. #561557), anti-CD73 PerCP-Cy5.5 (cat. #561260), anti-CD34 APC (cat. #345804), anti-CD45 FITC (cat. #345808) mouse anti-human monoclonal antibodies (all – *BD Bioscience*, USA) [14]. The multipotent potential of cells was confirmed by directed differentiation into adipogenic, chondrogenic and osteogenic direction using the STEMPRO<sup>®</sup> Adipogenesis Differentiation Kit, STEMPRO<sup>®</sup> Chondrogenesis Differentiation Kit and STEMPRO<sup>®</sup> Osteogenesis Differentiation Kit (*Thermo Fisher Scientific*, USA), according to the instructions of manufacturer (data not presented). These analyses confirmed belonging of isolated cells to MSCs according to minimal criteria.

**The isolation of rat adipose-derived MSCs.** MSCs from rat adipose tissue were isolated according to standard protocol with some modifications [16]. Abdominal adipose tissue of rats was obtained in the amount of 2 g. The experiment used sample from one animal euthanized with a 10-fold overdose of sodium thiopental at 50 mg/kg (*Brovarpharma*, Ukraine). The adipose tissue was washed twice by centrifugation with PBS, and then added an equal volume of 0.2 % solution of collagenase type I heated to 37 °C in PBS. Subsequent phenotyping were performed as described for human adipose-derived MSCs, using mouse anti-rat antibodies anti-CD45 FITC (cat. #554883, *BD Pharmingen*<sup>™</sup>, USA), anti-CD90 PE (cat. #551401, *BD Pharmingen*<sup>™</sup>, USA), anti-CD34 FITC (cat. #11-0341-82, *Invitrogen*, USA), anti-CD105 FITC (cat. #MA1-19594, *Invitrogen*, USA).

**The isolation of rat embryonic fibroblasts.** The study used the muscle tissue of four 15-day-old rat embryos; the gestation period was determined by the copulation plug. The female was euthanized with sodium thiopental 50 mg/kg. The tissue was kept for 20 min in DMEM medium with 10-fold concentration of antibiotics (penicillin 1000 IU/mL and streptomycin 1000 µg/mL), then placed in 0.25 % solution of a mixture of trypsin and 0.02 % Versen in a ratio of 1:1 and kept at 37 °C overnight. In the morning, the fluid was discarded, 10 mL of serum-free DMEM

medium was added to the tissue and pipetted to obtain a homogeneous cell suspension. Then fetal bovine serum up to 10 % was added and the suspension was seeded in Petri dishes. After 24-48 h, a monolayer of cells was formed and detached from the plastic substrate using a mixture of trypsin 0.25 % and Versen 0.02 % in a ratio of 1:1 for further use in the experiment [17]. Cells were identified by spindle-shaped morphology and hematoxylin-eosin staining.

**The obtaining of MSC's lysate.** Wharton's jelly-derived cells at the 80-90 % monolayer density were detached from substrate, centrifuged, counted, diluted with DMEM medium to the concentration of  $1 \cdot 10^6$ /mL and aliquoted in 1 mL. On an ultrasonic disintegrator Ultrasound-2T (Summy, Ukraine), the cells were subjected to ultrasound disintegration for 10 s with a power of 22 kHz, then centrifuged for 10 min at 12,000 xg. The supernatant was collected and used in further experiments.

**Study design.** Experimental animals were divided into 8 groups (Table 1).

Group 1 is sham-operated rats, which were successively subjected to the following interventions (anesthesia, skin incision, vascular isolation) except for internal carotid artery ligation, which reproduced the effects of traumatic experimental conditions. Group 2 is a control group. The rats in this group underwent 20-minute cerebral ischemia by internal carotid artery ligation. After 20 min, the ligatures were removed from the arteries (reperfusion) and a single dose of 0.9 % normal saline was injected into the femoral vein at a dose of 2 mL/kg. A similar dose was administered to rats of Group 1. Group 3 animals were once transplanted with human Wharton's jelly-derived MSCs at a dose of  $1 \cdot 10^6$  cells per animal. Group 4 underwent a single transplantation of human adipose-derived MSCs at a dose of  $1 \cdot 10^6$  cells/animal. Group 5 received  $1 \cdot 10^6$  cells/animal of rat fetal fibroblasts. Group 6 received  $1 \cdot 10^6$  cells/animal of rat adipose-derived stem cells. Group 7 was administered a single dose of human Wharton's jelly-derived MSC's lysate (0.2 mL/animal). Group 8 received a single dose of 250 mg/kg of reference drug Citicoline (*Neuroxon, Artemium Corporation, Ukraine*). Citicoline was chosen because of its ability to positively affect the regression of neurologic deficit in the experiment and improve cognitive functions in patients with cerebral ischemia [18-21].

**The modified McGraw's stroke index score** was chosen for the analysis of neurologic deficit in rats (Table 2) [21]. Studies have been performed on the 7<sup>th</sup> (subacute period of ischemia) and 14<sup>th</sup> days (recovery period) [22, 23]. The severity of the neurologic deficit was assessed by the sum of the corresponding points: up to 2.5 points – mild symptoms (paucity of movements, weakness of the extremities, unilateral semiptosis, tremor, circling behavior); from 3 to 10 points – severe manifestations of neurological disorders (paresis and paralysis of the limbs, lateral position).

Statistical processing of the obtained data was performed using MS Excel 2010 (*Microsoft, USA*) and Statistics 6.1 software (*StatSoft Inc., USA*). The reliability of differences was assessed using the nonparametric Mann-Whitney U-test. Data are presented as mean and standard deviation ( $M \pm \sigma$ ). The difference between the studied parameters was considered statistically significant at a value of  $p < 0.05$ .

## RESULTS AND DISCUSSION

Experimental cell therapy of acute transient cerebral ischemia-reperfusion in rats using stem cell transplantation of various origins provided the reduce of the mortality rate of experimental animals (Fig. 1).

The greatest protective effect in rats with CIRI was observed when using human Wharton's jelly-derived MSCs (Group 3). At 12 hours of follow-up as a critical period in the development of experimental acute cerebrovascular disorders the mortality was only 10 % vs. 45 % of shamoperated animals ( $p < 0.05$ ). The use of rat fetal fibroblasts reduced the mortality of animals relative to the control group by an average of 25 % ( $p < 0.05$ ). Compared with the reference drug Citicoline at a dose of 250 mg/kg, the use of MSCs of various origins had no significant differences.

Table 1. The distribution of animals in the experiment.

GROUP	NUMBER OF ANIMALS	TREATMENT
1	10	sham-operated animals + normal saline at a dose of 2 mL/kg
2	40	CIRI + normal saline at a dose of 2 mL/kg
3	20	CIRI + human Wharton's jelly-derived MSCs at a dose of $1 \cdot 10^6$ cells/animal
4	25	CIRI + human adipose-derived MSCs at a dose of $1 \cdot 10^6$ cells/animal
5	20	CIRI + fetal rat fibroblasts at a dose of $1 \cdot 10^6$ cells/animal
6	25	CIRI + rat adipose-derived MSCs at a dose of $1 \cdot 10^6$ cells/animal
7	25	CIRI + lysate of human Wharton's jelly-derived MSCs at a dose 0.2 mL/animal
8	25	CIRI + Citicoline at a dose 250 mg/kg

SYMPTOMS	POINTS
Paucity of movements	0.5
Tremor	1
Unilateral semiptosis	1
Bilateral semiptosis	1.5
Leg weakness	1.5
Unilateral ptosis	1.5
Bilateral ptosis	1.5
Circling behavior	2.0
Paresis	2-5
Paralysis	3-6
Comatose	7.0
Fatal case	10.0

Table 2. Modified McGraw's stroke index score for neurologic deficit assessment.

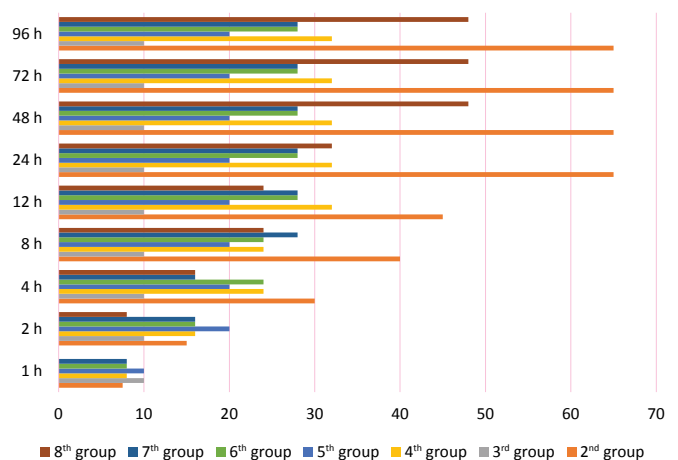


Fig. 1. The mortality rate of rats with transient cerebral ischemia-reperfusion in experimental groups, %. Data for Group 1 are not presented because all animals survived.

During the observation period in Group 1 of sham-operated animals no fatalities were recorded. One day after the start of the observation, the mortality of rats with CIRI was significantly lower both in groups with administration of different types of MSCs, cell lysate and Citicoline compared with the control group (Fig. 1). During the following observation (up to the 14<sup>th</sup> day of the experiment), there was no mortality of rats with CIRI in these groups.

According to the degree of the survival effect in the critical period of CIRI (12 h), the studied products can be arranged in the following order: human Wharton's jelly-derived MSCs > rat fetal fibroblasts > Citicoline > rat adipose-derived stem cells = lysate > human adipose-derived stem cells >.

For statistical reliability of neurologic deficit assessment, 7 animals of each group were analyzed on the 7<sup>th</sup> and 14<sup>th</sup> day of observation. CIRI in rats caused severe neurological disorders: paralysis, paresis, ptosis, circling behavior – (Table 3).

On the 7<sup>th</sup> day of observation in the control group of animals, the mean McGraw's stroke index score indicated severe neurological disorders. On the 14<sup>th</sup> day of observation in this group of animals there was no complete recovery of lost CNS functions.

Compared to the control Group 2 all treatments for acute CIRI (MSCs of various origins, cell lysate and Citicoline) led to a significant regression of neurologic deficit. The best result was obtained with the use of human Wharton's jelly-derived MSCs (Group 3). Also, the result of the use of human Wharton's jelly-derived MSCs was significantly better compared to the reference drug Citicoline on both the 7<sup>th</sup> and 14<sup>th</sup> day of the study.

In general, there are a small number of *in vivo* studies of human Wharton's jelly-derived MSCs in ischemia models in rats. Wu C. et al. found that intracerebral and intravenous transplantation of human Wharton's jelly-derived MSCs improved neurological function in experimental animals [24]. The study of Toyoshima A. et al. confirmed a significant reduction in rat cerebral infarction, improved neurological function and reduced neuroinflammation in 3 and 5 days after the surgery [25]. Similar results were obtained by Zhang L. et al. on the 7<sup>th</sup> day after the transplantation of human Wharton's jelly-derived MSCs. The authors confirmed the localization of donor cells in the infarct area histologically, and some cells differentiated into neurons after 35 days [12]. That is, exogenous human Wharton's jelly-derived MSCs transplanted to rats are able to migrate to the ischemic area, survive there and even differentiate into neurons to partially restore the affected motor function.

Thus, untreated acute cerebral ischemia rats had severe neurologic deficit at the corresponding time. Human Wharton's jelly-derived MSCs, rat fetal fibroblasts and rat adipose-derived MSCs better contributed to the regression of neurological disorders than Citicoline and cells from other sources, accompanied by a reduction in neurologic deficit on the 14<sup>th</sup> day of follow-up.

The ability of stem cells of different origins to reduce neurologic deficit indicates the feasibility of their use in acute cerebral ischemia. According to the literature, the study of the effects of MSCs in the treatment of stroke has been conducted for almost two decades. In preclinical trials,

Table 3. The dynamics of neurologic deficit in rats of experimental groups on the 7<sup>th</sup> and 14<sup>th</sup> days after acute cerebral ischemia-reperfusion (n = 7).

GROUP	NEUROLOGIC DEFICITS, MCGRAW'S STROKE INDEX SCORE	
	DAY 7	DAY 14
Group 1 (sham-operated animals)	0.00 ± 0.00	0.00 ± 0.00
Group 2 (CIRI + normal saline)	11.79 ± 0.48*	9.14 ± 0.30*
Group 3 (CIRI + human Wharton's jelly-derived MSCs)	7.14 ± 0.19**^	4.86 ± 0.15**^
Group 4 (CIRI + human adipose-derived MSCs)	8.86 ± 0.23**	5.71 ± 0.23**
Group 5 (CIRI + fetal rat fibroblasts)	7.71 ± 0.28**^	5.14 ± 0.19**^
Group 6 (CIRI + rat adipose-derived MSCs)	8.50 ± 0.26**^	5.43 ± 0.14**^
Group 7 (CIRI + lysate of human Wharton's jelly-derived MSCs)	8.57 ± 0.22**^	5.57 ± 0.22**
Group 8 (CIRI + Citicoline)	9.21 ± 0.16**	6.14 ± 0.15**

Notes:

\* –  $p < 0.05$  compared to the sham-operated rats (Group 1);

# –  $p < 0.05$  compared to the rats with CIRI (Group 2);

^ –  $p < 0.05$  compared to the treatment with Citicoline (Group 8).

rats are the most widely used animals to model the cerebral ischemia. At the same time, even in recent studies, the main source of MSCs was bone marrow and adipose tissue, and only one study was performed with placental cells, and, as in our case, both allogeneic [25] and xenogeneic [26] cells were used in the studies. No reports on the use of MSC's lysate were found. Interestingly, in recent researches on this issue the effects of MSCs from one source were studied, while we compared the impact 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 experiment design.

Various authors have shown that MSCs transplantation after ischemic stroke improves brain function [25-27], effectively protects ischemic neurons and restores brain injury [28]. Our data on the survival and development of neurological disorders in animals using MSCs immediately after ischemia/reperfusion are only the first stage of study, the results of biochemical, histological and immunochemical studies will be published later.

## CONCLUSION

1. Human Wharton's jelly-derived MSCs and fetal rat fibroblasts significantly better than Citicoline contributed to the regression of the neurologic deficit on the 14<sup>th</sup> day after acute cerebral ischemia-reperfusion.

2. The potential of stem cells of different origins to reduce neurologic deficit indicates the feasibility of their use in experimental acute cerebral ischemia for preclinical studies of cell therapy.

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# Вплив мезенхімальних стромальних клітин різного походження на летальність та неврологічний дефіцит при гострій ішемії-реперфузії головного мозку у щурів



Мороз В. М.<sup>1</sup>, Коновалов С. В.<sup>1</sup>, Коновалова Н. В.<sup>1</sup>, Дерябіна О. Г.<sup>2,3</sup>, Шувалова Н. С.<sup>2</sup>, Топорова О. К.<sup>2,3</sup>, Точилловський А. А.<sup>4</sup>, Кордюм В. А.<sup>2,3</sup>

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

<sup>2</sup>ДУ «Інститут генетичної та регенеративної медицини Національної академії медичних наук України», Київ, Україна

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

<sup>4</sup>ТОВ «ВіоТехСом», Київ, Україна

## РЕЗЮМЕ

Інсульт – це глобальна епідемія і друга причина смертності населення у світі та в Україні. За офіційною статистикою щорічно у 100-110 тис. українців розвивається гостре порушення мозкового кровообігу. Третина таких хворих – це особи працездатного віку, до 50 % матимуть інвалідність, і лише кожен десятий повністю повернеться до повноцінного життя. Наразі отримано багатообіцяючі експериментальні дані щодо усунення неврологічної дисфункції при використанні мезенхімальних стромальних клітин (МСК).

**МЕТА** – порівняти вплив МСК різного походження на летальність та неврологічний дефіцит у щурів при гострій ішемії-реперфузії (ІР) головного мозку.

**МАТЕРІАЛИ ТА МЕТОДИ.** Самцям щурів Вістар віком 4 міс. моделювали гостру ішемію-реперфузію головного мозку шляхом двобічної 20-хвилинної оклюзії внутрішніх сонних артерій та внутрішньовенно вводили МСК, отримані з Вартонових драглів пуповини людини, з жирової тканини людини та щура в дозі  $1 \cdot 10^6$  клітин. Іншим групам піддослідних тварин в/в вводили фетальні фібробласти щура та лізат з культури клітин Вартонових драглів пуповини людини. Остання група щурів отримувала однократне референс-препарат цитиколін у дозі 250 мг/кг. Контрольним тваринам в/в вводили фізіологічний розчин. Оцінку церебропротекторного ефекту терапії проводили за показниками летальності та неврологічного дефіциту у щурів за шкалою McGraw's stroke-index.

**РЕЗУЛЬТАТИ.** Через 12 годин спостереження в критичний період у розвитку експериментального гострого порушення мозкового кровообігу при введенні МСК з Вартонових драглів пуповини людини смертність склала лише 10 % проти 45 % тварин контрольної групи. Застосування фетальних фібробластів щура зменшувало летальність тварин відносно контрольної групи в середньому на 25 %. ІР у щурів викликала тяжкі неврологічні зміни: паралічі, парези, птоз, манежні рухи. На 7-му добу спостереження у контрольній групі тварин середній бал за шкалою McGraw's stroke-index свідчив про важкі неврологічні порушення. На 14-ту добу спостереження в цій групі тварин не відбулося повного відновлення втрачених функцій ЦНС. В порівнянні з контрольною групою тварин усі обрані для дослідження варіанти лікування гострої ІР (МСК різного походження, лізат та цитиколін) сприяли достовірному регресу неврологічного дефіциту.

**ВИСНОВКИ.** Таким чином, трансплантація МСК, отриманих із Вартонових драглів пуповини людини, та фетальних фібробластів щура знижувала летальність, полегшувала неврологічну симптоматику у щурів із експериментальним ішемічним інсультом. МСК Вартонових драглів пуповини людини, фетальні фібробласти та МСК жирової тканини щура краще за цитиколін сприяли зменшенню проявів неврологічних порушень, що супроводжувалось регресом динаміки неврологічного дефіциту на 14-ту добу спостереження. Спроможність стовбурових клітин різного походження зменшувати неврологічний дефіцит свідчить про доцільність їх застосування в умовах експериментальної гострої ішемії головного мозку при доклінічних дослідженнях ефективності клітинної терапії.

**КЛЮЧОВІ СЛОВА:** ішемічний інсульт; церебральна ішемія-реперфузія; неврологічний дефіцит; мезенхімальні стромальні клітини; лізат клітин; цитиколін