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Influence of mesenchymal stromal cells of different origins on behavioural reactions of rats with cerebral ischemia-reperfusion

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Abstract. A new direction in cell therapy for ischemic stroke has been the use of mesenchymal stromal cells, which have shown a positive impact on functional changes in the central nervous system due to their neuroprotective effects, reduction of ischemia-reperfusion-induced injury, inhibition of ischemia-reperfusion-induced apoptosis, and restoration of motor function. This study aimed to investigate the effect of mesenchymal stromal cells of different origins, their lysate, and citicoline on the functional state of the central nervous system in rats with experimental brain ischemia-reperfusion. The study considered the effect of mesenchymal stromal cells derived from human umbilical cord Wharton's jelly, human and rat adipose tissue, rat embryonic fibroblasts, as well as mesenchymal stromal cell lysate and citicoline on the emotional and behavioural responses of sexually mature Wistar rats (3-4 months) weighing 160-190 g. The behavioural responses of rats were studied using the open field test on the 7th and 14th days of the experiment; the following behavioural acts were recorded: ambulation (locomotion), climbing, rearing, and grooming. The significance of differences was determined using the non-parametric Mann-Whitney U test. It was established that after ischemia-reperfusion, animals with control pathology showed a significant decrease in the duration of episodes of ambulation in peripheral and central squares, vertical locomotor activity, and exploratory activity compared to the sham-operated group. In rats that received citicoline and transplanted human umbilical cord Wharton's jelly mesenchymal stromal cells, a significant increase in the duration of episodes of horizontal locomotor activity was observed compared to other types of stem cells and the control. Intravenous administration of rat embryonic fibroblasts increased the emotional activity of the experimental animals. The least impact on locomotor and adaptive exploratory activity in rats with ischemia-reperfusion was registered in groups of animals that received mesenchymal stromal cells from human and

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rat adipose tissue, as well as mesenchymal stromal cell lysate. The practical significance of the study lies in the search for the most effective class of stem cells with neuroprotective properties for the creation of an injectable drug for intravenous transplantation in the treatment of patients with acute ischemic stroke

Keywords: stem cells; ischemia-reperfusion; adaptive behaviour; emotional activity; open field test

INTRODUCTION

Vascular pathology, and the associated ischemic stroke, according to the 2019 report from the American Heart Association's Statistics Committee, are leading causes of neural dysfunction, characterised by high morbidity, disability, and mortality [1]. The Stroke Expert Collaboration Group [2] has highlighted the substantial socioeconomic impact of stroke on households, underscoring the immense burden this disease places on families, communities, and nations. Stem cell transplantation has emerged as a promising avenue within regenerative strategies. Stem cells possess the unique ability to differentiate into various cell types and continuously self-renew [3]. Y. Zhang *et al*. [4] have demonstrated that stem cell transplantation can effectively treat neurodegenerative diseases, including stroke. Among stem cells, mesenchymal stromal cells (MSCs) are the most commonly employed cell type in advanced therapies for a wide range of diseases, many of which involve inflammation [5]. MSCs constitute a heterogeneous population of stem cells and exhibit neuroprotective properties when used to treat ischemic stroke. These properties manifest in reduced ischemic-reperfusion injury, inhibition of ischemia-reperfusion-induced apoptosis, and restoration of motor function [6]. Numerous preclinical studies have demonstrated the ability of MSCs to mitigate tissue damage, thereby promoting functional recovery through various mechanisms, including immunomodulation, pro-angiogenic signalling, secretion of neurotrophic factors, and neuronal differentiation [4, 7]. For instance, a research group led by J. Li *et al*. [7] discovered that MSCs can facilitate cell migration, angiogenesis, immunomodulation, neuroprotection, and the restoration of neural circuits. The paracrine action of MSCs can also provide neurotrophic effects and enhance functional recovery. In a few clinical studies, notably the STARTING-2 study conducted by a research team led by J.W. Chung *et al.* [8], autologous modified MSCs demonstrated significant improvements in lower limb motor function in patients with chronic ischaemic stroke. Furthermore, MSCs offer several advantages over other stem cell types due to easier procurement methods, a low risk of tumorigenicity, and the absence of ethical concerns [9]. However, several questions remain regarding the nature of the cell product, the delivery route, and the optimal cell dose and administration schedule [10]. MSCs can be derived from adipose tissue, dental buds and pulp, bone marrow, liver or umbilical cord, umbilical cord blood, and placenta [11]. Previous studies [12] have shown that different types of MSCs can influence mortality and neurological deficits in rats subjected to a model of brain ischaemia-reperfusion (IR).

Numerous studies investigating the effects of various factors on the behavioural responses of rats have not addressed how ischaemia-reperfusion injury of the brain in rats, or correction using MSCs, affects adaptive exploratory behaviour. Therefore, this study aimed to investigate the effects of MSCs from different sources, MSCs lysate, and citicoline on the functional state of the central nervous system (CNS) in rats with experimental brain IR.

MATERIALS AND METHODS

The study was conducted between 2021 and 2023 at the educational and research laboratory for preclinical evaluation of new drugs and biologically active compounds "Farmadar" of the National Pirogov Memorial Medical University, Vinnytsya (NPMMU) (technical competence certificate No. 031/18 valid until 31.10.2023), using sexually mature (3-4 months old) Wistar rats weighing 160-190 g, bred in the vivarium of NPMMU. Animals were kept in standard vivarium conditions with natural lighting and had free access to water and food. MSCs and MSCs lysate were obtained from the Institute of Molecular Biology and Genetics (IMBG) of the NAS of Ukraine. The transfer of cells was carried out based on the Agreement on Scientific Cooperation between IMBG NASU and NPMMU dated 22.09.2017. Cell viability was assessed by staining the cell sample for live-dead with trypan blue while counting the number of cells in the Goryaev chamber, with viability ranging from 95-98%. Cell quality was checked on a flow cytometer for the presence of minimal MSC markers – CD34, CD73, CD90, CD105. Occasionally, the ability of cells to differentiate into adipocytes and chondrocytes, which is characteristic of MSCs, was checked. Cell morphology was constantly monitored by assessing the shape (spindle-shaped).

Rats were used as experimental animals because the angioarchitecture and morphology of the rat cerebral cortex are similar to those of humans. An experimental model of IR was created by bilaterally ligating the internal carotid arteries (ICAs) for 20 minutes under propofol anaesthesia (Propofol-novo, LLC Novofarm-Biosyntez, Ukraine, 60 mg/kg). The chosen model reflects the clinical picture of cerebral infarction and is optimal for experimental studies of potential neuroprotective substances [13]. The distribution of animals into experimental groups is presented in Figure 1.

The test substances were administered intravenously into the femoral vein immediately after IR, as early transplantation of MSCs has been shown to promote better neurological recovery, reduce infarct volume, and require a smaller number (1×10^6) of donor cells to achieve a positive effect [14]. Adaptive behaviour and emotional reactivity of the animals were assessed using the open field test twice: on the 7th and 14th days after the experimental model of brain IR. Observations of animals in the open field were carried out using a standard setup – a rectangular chamber (100×100 cm) with transparent walls 40 cm high. The floor was divided into 25 (20×20 cm) equal squares [15]. Recording was performed using the EthoWatcher: A TOOL FOR BEHAVIORAL AND VIDEO-TRACKING ANALYSIS IN LABORATORY ANIMALS software-computer complex for studying the behavioural reactions of animals, for two days

at the same time of day under the same conditions in the laboratory, which was located in the vivarium of NPMMU. The exposure time for each animal in the open field was 3 minutes. The special room had a stable temperature (18-22°C), relative humidity (40-60%) and illumination of 250 lux. The first and second testing of the experimental animals was carried out to study the effect of treatment on the behavioural reactions of animals with brain IR. In the individual behaviour of rats, the following behavioural acts were recorded: ambulation (locomotion) – gradual movement of the animal in a horizontal plane (crossing central and peripheral squares); according to indicators of vertical locomotor activity – climbing (wall vertical stance on hind legs) and rearing (free vertical stance on hind legs) and emotional activity – grooming (number of washes and number of boluses during defecation).

Group 1 (n = 10): sham-operated animals + intravenous injection of 0.9% sodium chloride solution, 2 mL/kg

Group 2 (n = 40): ischemia-reperfusion + intravenous injection of 0.9% sodium chloride solution, 2 mL/kg

Group 3 (n = 20): ischemia-reperfusion + intravenous transplantation of human umbilical cord Wharton's jelly MSCs, 10^6 cells/animal

Group 4 (n = 20): ischemia-reperfusion + intravenous transplantation of rat embryonic fibroblasts, 10^6 cells/animal

Group 5 (n = 25): ischemia-reperfusion + intravenous transplantation of human adipose tissue MSCs, 10^6 cells/animal

Group 6 (n = 25): ischemia-reperfusion + intravenous transplantation of rat adipose tissue MSCs, 10^6 cells/animal

Group 7 (n = 25): ischemia-reperfusion + intravenous injection of cell lysate from human umbilical cord Wharton's jelly MSCs, 0.2 mL/animal

Group 8 (n = 25): ischemia-reperfusion + intravenous injection of citicoline ("Neuroxon", Corporation "Arteium", Ukraine), 250 mg/kg

Figure 1. Distribution of animals in the experiment

Source: compiled by the authors

Statistical analysis of the obtained data was performed using Microsoft Excel 2010 and Statistica 6.1 software. The significance of differences was assessed using the unpaired nonparametric Mann-Whitney U test. Differences between the measured parameters were considered statistically significant at $p < 0.05$. All manipulations with experimental animals were carried out following the International Rules and Standards of the Directive of the Council of the European Communities 86/609/EEC and the principles of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [16] and the Law of Ukraine dated 21.11.2006 No. 3447-IV "On the Protection of Animals from Cruelty" [17]. The research protocol was approved by the Bioethics Committee of NPMMU (protocol No. 2 dated January 31, 2024).

RESULTS

The open field test allows for the examination of rat behaviour by assessing levels of emotional and behavioural reactivity, the dynamics of individual behavioural elements, and locomotor stereotypy (Table 1).

Table 1. Comparison of behavioural response indicators in rats with a model of brain ischemia-reperfusion and amid a correction (M ± m)

Sham-operated						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	14 Days	7 Days	14 Days
Ambulation (peripheral)	2.26 ± 1.03	0.30 ± 0.18	18.00 ± 1.22	12.43 ± 1.68	115.27 ± 3.28	101.20 ± 9.48
Ambulation (centre)	5.05 ± 3.28	21.11 ± 19.17	3.14 ± 0.60	1.43 ± 0.52	11.97 ± 1.81	3.05 ± 1.61
Climbing	13.89 ± 3.07	34.07 ± 12.56	11.43 ± 0.88	5.86 ± 1.83	23.47 ± 2.87	14.82 ± 4.67
Rearing	33.41 ± 22.77	0 ± 0	0.86 ± 0.50	0 ± 0	1.52 ± 0.85	0 ± 0
Grooming	96.90 ± 14.06	41.02 ± 10.57	2.43 ± 0.88	3.29 ± 0.81	24.02 ± 4.55	40.26 ± 7.46
Ischaemia-reperfusion (IR)						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	0.16 ± 0.17	0.65 ± 0.71	16.00 ± 3.01	12.83 ± 2.14	74.05 ± 11.38*	91.66 ± 13.64
Ambulation (centre)	12.03 ± 6.49	3.35 ± 2.20	1.83 ± 0.59	1.50 ± 0.68	5.23 ± 1.91*	5.65 ± 2.26
Climbing	16.14 ± 8.12	20.41 ± 12.40	9.83 ± 2.76	5.50 ± 1.46	20.50 ± 4.29	12.11 ± 3.19
Rearing	5.02 ± 5.50	14.42 ± 15.79	0.17 ± 0.18	0.50 ± 0.55	0.28 ± 0.30	0.90 ± 0.99
Grooming	61.13 ± 12.59	61.51 ± 18.8	1.67 ± 0.23	2.17 ± 0.34	13.91 ± 4.36	16.36 ± 6.18*
IR + human Wharton's jelly MSCs						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	1.29 ± 0.94	5.72 ± 5.48	14.57 ± 2.18	12.57 ± 2.89	114.13 ± 12.58#	99.57 ± 21.15

Table 1. Continued

IR + human Wharton's jelly MSCs						
	Latent period		Number of episodes		Duration of episodes	
Ambulation (centre)	16.03 ± 10.58	5.16 ± 2.76	2.57 ± 0.88	2.00 ± 0.53	8.63 ± 3.10	6.98 ± 3.64
Climbing	10.99 ± 3.23	16.94 ± 4.76	7.14 ± 1.55	6.14 ± 1.79	11.02 ± 3.70*	12.52 ± 3.63
Rearing	16.36 ± 11.41	0 ± 0	0.43 ± 0.32	0 ± 0	0.43 ± 0.36	0 ± 0
Grooming	62.27 ± 22.98	40.09 ± 9.58	2.86 ± 1.01	3.00 ± 0.71	14.74 ± 4.70	19.18 ± 4.01*
IR + rat embryonic fibroblasts						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	1.50 ± 1.07	0.55 ± 0.44	14.50 ± 1.12	15.67 ± 3.17	87.10 ± 13.45*	76.94 ± 10.03
Ambulation (centre)	6.00 ± 6.04	3.24 ± 3.10	2.17 ± 0.34	1.83 ± 1.00	7.57 ± 2.49	4.13 ± 2.42
Climbing	8.83 ± 1.29	15.57 ± 7.12	7.50 ± 0.97	8.17 ± 2.67	13.98 ± 3.64	17.81 ± 6.64
Rearing	15.51 ± 17.00	30.16 ± 26.27	0.17 ± 0.18	0.50 ± 0.37	0.28 ± 0.30	0.55 ± 0.43
Grooming	35.84 ± 8.34*	13.41 ± 4.64#	4.17 ± 0.52#	4.33 ± 0.67#	39.12 ± 11.83#	25.66 ± 5.78
IR + human adipose MSCs						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	0.55 ± 0.39	3.05 ± 1.80	13.50 ± 2.03	6.83 ± 1.15*#	105.34 ± 14.84	53.99 ± 12.44*
Ambulation (centre)	12.12 ± 8.53	0 ± 0	1.83 ± 0.77	0.50 ± 0.24	4.95 ± 2.23	3.05 ± 1.80
Climbing	34.23 ± 23.13	0.39 ± 0.40*	6.67 ± 2.03	1.00 ± 0.75*	14.89 ± 4.28	2.24 ± 1.66*#
Rearing	8.70 ± 9.53#	0 ± 0	0.33 ± 0.37	0 ± 0	0.54 ± 0.59	0 ± 0
Grooming	52.65 ± 17.27	43.62 ± 31.31	2.83 ± 0.87	0.83 ± 0.44*	21.84 ± 8.32	4.22 ± 2.32*#
IR + rat adipose MSCs						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	0.01 ± 0.01	0.01 ± 0.01	14.50 ± 3.30	7.33 ± 1.08*	69.48 ± 14.09*$	63.47±10.50*
Ambulation (centre)	7.27 ± 5.68	0 ± 0	1.00 ± 0.40	0 ± 0	2.99 ± 1.57*	0 ± 0
Climbing	6.21 ± 3.12	13.73 ± 6.31	5.50 ± 2.80	3.00 ± 0.69	11.63 ± 6.65	7.92 ± 2.66
Rearing	11.84 ± 8.21	0 ± 0	0.67 ± 0.46	0 ± 0	1.17 ± 0.82	0 ± 0
Grooming	54.41 ± 23.71	25.10 ± 9.43	4.33 ± 0.88#	2.00 ± 0.69	29.14 ± 13.40	31.30 ± 18.22
IR + cell lysate from human Wharton's jelly MSCs						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	1.55 ± 1.70	0.52 ± 0.36	16.83 ± 4.19	9.83 ± 3.27	75.48 ± 13.34*$	69.36 ± 6.36*
Ambulation (centre)	8.38 ± 7.92	3.17 ± 3.48	1.67 ± 0.73	0.50 ± 0.24	6.06 ± 2.12	0.78 ± 0.38
Climbing	20.73 ± 14.87	8.79 ± 7.17	9.00 ± 3.49	3.33 ± 2.44	20.95 ± 7.94	6.50 ± 4.23
Rearing	27.63 ± 19.87	0 ± 0	0.50 ± 0.37	0 ± 0	0.74 ± 0.57	0 ± 0
Grooming	48.03 ± 17.59*	53.74 ± 29.40	3.00 ± 1.33	1.83 ± 0.96	16.12 ± 8.83	15.25 ± 8.06*
IR + Citicoline						
	Latent period		Number of episodes		Duration of episodes	
	7 Days	14 Days	7 Days	7 Days	14 Days	7 Days
Ambulation (peripheral)	3.10 ± 1.21	0 ± 0	14.17 ± 2.07	11.33 ± 2.11	125.90 ± 10.91#	79.66 ± 16.34
Ambulation (centre)	3.03 ± 3.32	11.36 ± 10.25	2.50 ± 0.68	0.83 ± 0.44	9.68 ± 2.34	1.42 ± 0.70
Climbing	13.95 ± 2.02	6.03 ± 2.55*	8.33 ± 2.01	5.83 ± 1.40	14.52 ± 4.24	10.91 ± 2.48
Rearing	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Grooming	47.40 ± 7.58*	43.19 ± 24.74	3.00 ± 0.75	2.33 ± 0.78	21.97 ± 7.34	19.28 ± 7.67

Notes: * – $p < 0.05$ compared to the sham-operated rats index; # – $p < 0.05$ compared to the control pathology index; $ – $p < 0.05$ compared to the citicoline group index

Source: compiled by the authors

The analysis of rat behaviour in the open field test, based on the "number of crossings" parameter, characterises the overall level of activation. As shown in Table 1, on the 7th day after IR, in animals with control pathology, the duration of ambulation episodes in peripheral and central squares decreased on average by 35.8% and 56.3% respectively ($p < 0.05$), compared to the sham-operated group. Therapeutic correction of IR injury using embryonic fibroblasts and MSCs from rat adipose tissue, as well as MSCs lysate, did not have a positive effect on the horizontal motor activity of the experimental animals, as observed in a significant decrease in the duration of episodes of horizontal locomotor activity on average by 24.4%, 39.7%, and 34.5%, respectively, compared to the sham-operated group. The use of citicoline, as well as the transplantation of human umbilical cord Wharton's jelly MSCs during the study period, proved to be the most effective methods of treating rats with IR brain injury when studying behavioural reactions in the open field, as manifested in an increase in the duration of episodes of horizontal locomotor activity on

average by 70.0% and 54.1% ($p < 0.05$), respectively, compared to the control.

On the 14th day of the experiment, there was a trend towards a decrease in horizontal motor activity in the experimental groups of rats during open field testing compared to the results obtained on the 7th day of observation (Table 1). A decrease in horizontal locomotor activity with crossings of peripheral squares was also observed in groups of animals that received MSCs from human and rat adipose tissue, as well as MSCs lysate, on average by 46.6%, 37.3%, and 31.5% respectively ($p < 0.05$), compared to the sham-operated group.

Vertical motor activity indicates the exploratory behaviour of the animals. A greater number of stances suggests a lower level of anxiety. Therefore, in the studied periods of the experiment, in groups of rats with control pathology and with its correction, compared to the sham-operated group, a tendency towards a decrease in vertical locomotor activity and exploratory behaviour was observed. In rats that received MSCs from human adipose tissue in the context of IR, on the 14th day of the experiment (Table 1), wall rearing was significantly less frequent compared to sham-operated animals and those with control pathology.

Emotional activity in the experimental animal groups on the 14th day of observation (Table 1) led to a decrease in the duration of grooming episodes by an average of 2-3 times compared to the sham-operated group. At the same time, a significant increase in the number of grooming episodes was observed on the 7th and 14th days of the experiment in the group of animals that received rat embryonic fibroblasts as a correction, on average 2.5 and 2 times, respectively, as well as on the 7th day of observation in the group of rats that were transplanted with MSCs from rat adipose tissue in the context of IR, on average 2.6 times, relative to the control pathology indicator. Acts of defecation and urination during the study in the open field conditions were absent in animals of all groups.

The 20-minute model of IR ICAs induced functional disturbances in the CNS of rats, manifested by a significant decrease in the number of vertical stances, squares crossed, and entries into the centre, indicating a suppression of locomotor and exploratory activity. There was also a tendency towards a decrease in emotional activity, and reduced feelings of anxiety and fear under stressful conditions, as represented by the open field test. The therapeutic use of citicoline and human umbilical cord Wharton's jelly MSCs in rats with IR brain injury led to a significant increase in the duration of episodes of spontaneous horizontal motor activity compared to the group of animals with control pathology. The use of rat embryonic fibroblasts as a therapeutic correction contributed to an increase in the emotional activity of the experimental animals with ischemic-reperfusion brain injury.

DISCUSSION

There is no consensus in the literature regarding the motivations that determine rat behaviour in the open field. Most authors believe that the factors determining this behaviour are exploratory motivation and so-called emotional reactivity [18]. The latter, in turn, is associated with such brain structures as the limbic system and hippocampus. For example, a study by Q. Lei *et al.* [19] using a mouse model with permanent middle cerebral artery occlusion found that transplantation of MSCs derived from the bone marrow of knockout ($SRC3^{-/-}$) mice had a minimal impact on reducing cognitive disorders, motor impairments, and anxiety, as assessed by the Morris water maze test and open field test. However, in another study conducted by M.K. Tobin *et al.* [20], it was shown that intravenous administration via the retro-orbital sinus of MSCs contributed to functional recovery in experimental animals with a 90-minute occlusion of the right middle cerebral artery according to the open field test.

Ischemia leads to progressive cerebral injury. Clinically, treating ischemic stroke remains challenging. Therefore, an increasing number of researchers are focusing on finding effective methods to reduce ischemic reperfusion injury in cerebral ischemia. MSC transplantation may be effective in slowing or stopping this process [21, 22]. Recent studies have shown that MSC transplantation therapy has a positive impact on the course of cerebral ischemia [23, 24]. In a study by J. He *et al.* [23], it was found that olfactory mucosa MSCs during cerebral ischemia-reperfusion attenuate apoptosis and oxidative stress in models of ischemic stroke, reduce infarct volume, and improve neurological deficits in rats. Many preclinical studies, as well as clinical trials, have demonstrated the efficacy of MSC therapy in preclinical stroke models and the safety of MSC treatment in clinical trials [14, 20, 25]. Researchers L. Zhou *et al.* [24] determined that MSC therapy is safe and effective for acute, subacute, and chronic ischemic stroke. In the acute phase of ischemic stroke in rats, MSC therapy enhanced neuronal plasticity and functional recovery by protecting mitochondria, suppressing neuronal pyroptosis and apoptosis, and reducing microglia activation in the penumbra. In the subacute phase of ischemic stroke, human umbilical cord MSC therapy effectively improved behavioural deficits, reduced infarct volume, and glial scar formation, and promoted angiogenesis in the ischemic penumbra. In a chronic stroke model in rats, transplantation of human umbilical cord MSCs maintained blood-brain barrier integrity, improved behavioural responses in animals, and promoted neurogenesis and angiogenesis.

In the study of Y. Chen *et al.* [14], it was noted that sensory and motor functions were significantly improved following the therapeutic application of human MSCs in rats that had undergone ischemic stroke. Additionally, a significant improvement in the recovery of behavioural responses and a reduction in infarct volume was observed in the group of animals that received an intravenous injection of $1x10^6$human MSCs per animal.

However, the extent of recovery following MSC treatment is not fully understood. Most clinical trials using stem cell therapy have applied this treatment to patients with subacute stroke [25-27]. Other experiments have focused on patients with chronic stroke [28, 29]. For instance, research by K.R. Nalamolu *et al.* [26] established that treatment with MSCs derived from human umbilical cord blood mitigates post-stroke brain damage and significantly improves neurological recovery in both male and female rats with induced stroke. In previous preclinical studies, scientists have demonstrated better neurological recovery after stroke in rats that received exosomes isolated from MSCs, as manifested by a reduction in infarct size and ipsilateral hemisphere swelling, preservation of neurological function, and facilitated recovery of rats [27]. Based on the

results obtained, the authors concluded that treatment with exosomes secreted from MSCs, under appropriate experimental conditions, attenuates post-stroke brain damage and improves neurological deficits. Y. Ogawa *et al.* [28, 29] found in their studies that intravenous transplantation of bone marrow mononuclear cells was insufficient for the treatment of chronic stroke, however, cell therapy-assisted training was effective.

The results of the few clinical trials have been inconsistent, particularly the intravenous transplantation of allogeneic adipose-derived MSCs (AMASCIS) for acute ischemic stroke (AMASCIS). E. de Celis-Ruiz *et al.* [30] conducted a randomised, double-blind, placebo-controlled, single-centre, pilot clinical trial that included elderly patients with moderate to severe acute stroke, as measured by the National Institutes of Health Stroke Scale (NIHSS) of 8-20 points. A two-week course of MSC treatment did not show significant differences in outcomes after 24 months of follow-up (median NIHSS scores were not significantly lower compared to the placebo group). For this reason, the authors of this study decided to determine whether MSCs of different origins and MSCs lysate could be beneficial for treating acute ischemic stroke. To do this, rats were subjected to 20 minutes of cerebral ischemia-reperfusion and intravenously transplanted with the studied MSCs immediately after reperfusion; the behavioural adaptation of the rats was assessed using the open field test.

J. Zheng *et al.* [31] aimed to investigate whether mesenchymal stem cells (MSCs) could improve their survival and alleviate cerebral ischemic injury in an ischemic microenvironment. The researchers used ischemic brain tissue to culture MSCs and evaluated the functional changes in rats after the administration of pre-treated MSCs with brain tissue following stroke. It was established that transplantation of MSCs promoted proliferation and the release of growth factors, enhanced neurogenesis, reduced behavioural changes, decreased infarct size, and suppressed apoptosis, representing an effective strategy for the treatment of cerebral ischemic injury.

J.-R. Chen e*t al.* [32] found that MSC transplantation significantly improved behavioural deficits in rats associated with induced ischemia-reperfusion. Behavioural improvements in the performance of elevated body swing test and forelimb stride length were achieved as early as 7 days after MSC implantation. The authors observed a sustained improvement in the elevated body swing test and stride length during locomotion, but only a slight improvement at one time point for the adhesive sensory stimulus-induced test. According to the authors, this suggests that MSC transplantation primarily improves motor functional recovery.

In the study by M.K. Tobin *et al.* [20], a significant improvement in functional recovery of the CNS was demonstrated in experimental animals with ischemic stroke that received treatment using interferon-γ-activated mesenchymal stem cells (aMSCγ). In animals that received aMSCγ, a significant reduction in infarct size and inhibition of microglial activation was observed. In another study, conducted by Y.S. Fu *et al.* [33], it was shown that MSCs derived from human umbilical cord Wharton's jelly, when transplanted into rats with middle cerebral artery occlusion, had therapeutic benefits for chronic ischemic stroke. The open field test enabled for the detection of changes in adaptive behaviour and emotional reactivity in animals, as well as disorders in locomotor stereotypy resulting from ischaemic reperfusion injury to the brain in rats. It also assessed the corrective effects of transplanting MSCs of various origins, MSCs lysate, and the reference drug citicoline. Thus, the analysed data are consistent with those of other researchers; however, the results of this study demonstrated an increase in the duration of episodes of spontaneous horizontal motor activity when using citicoline and MSCs from human umbilical cord Wharton's jelly. Intravenous transplantation of rat embryonic fibroblasts promoted increased emotional activity in experimental animals with IR brain injury. The least impact on locomotor and exploratory activity in rats with IR was recorded in groups of animals that received MSCs from human and rat adipose tissue, as well as MSCs lysate.

CONCLUSIONS

The results of the study revealed a positive therapeutic effect of MSCs of various origins, MSCs lysate, and the reference drug citicoline on the functional state of the CNS in rats with experimental brain IR. An experimental study of the effects of 20 minutes of brain ischemia-reperfusion on the behaviour of rats in the open field test revealed a significant decrease in the duration of episodes of ambulation of peripheral and central squares by an average of 35.8% and 56.3%, respectively, on day 7, and a tendency towards a decrease in vertical locomotor, exploratory and emotional activity, a decrease in feelings of anxiety and fear, compared to the sham-operated group of rats. The use of citicoline and transplantation of MSCs from human umbilical cord Wharton's jelly proved to be the most effective treatment methods, as evidenced by an increase in the duration of episodes of horizontal locomotor activity by 70.0% and 54.1% ($p < 0.05$), respectively, compared to the group of animals with control pathology. The least impact on locomotor and exploratory activity in rats with ischemia-reperfusion was recorded in groups of animals that received MSCs from human and rat adipose tissue, as well as MSCs lysate: a significant decrease in horizontal locomotor activity with the crossing of peripheral squares was observed on day 14 of the experiment, which averaged 46.6%, 37.3%, 31.5%, respectively, compared to the sham-operated group of rats. The use of rat embryonic fibroblasts as a therapeutic correction promoted an increase in the emotional activity of experimental animals with cerebral ischemia-reperfusion injury with an increase in the number of grooming episodes by 2.5 times; when MSCs from rat adipose tissue were administered – by 2.6 times, relative to the control pathology indicator ($p < 0.05$).

The data obtained from this research will be used to explore new avenues for treating brain ischemic reperfusion injury. These results will provide experimental evidence to justify clinical trials of an injectable drug based on the most effective class of MSCs for a new application, namely as a neuroprotectant in patients with ischemic stroke.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Вплив мезенхімальних стромальних клітин різного походження на поведінкові реакції щурів із церебральною ішемією-реперфузією

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Анотація. Новим напрямком клітинної терапії при ішемічному інсульті стало використання мезенхімальних стромальних клітин, що виявило позитивну динаміку на функціональні зміни в центральній нервовій системі, завдяки їхньому нейропротекторному ефекту, зменшенню ішемічно-реперфузійно-індукованого пошкодження, інгібуванню ішемічно-реперфузійно-індукованого апоптозу та відновленню рухової функції. Мета роботи полягала у вивченні впливу мезенхімальних стромальних клітин різного походження, їх лізату та цитиколіну на функціональний стан центральної нервової системи щурів з експериментальною ішемією-реперфузією головного мозку. Розглянуто вплив мезенхімальних стромальних клітин, отриманих із пуповинної тканини людини, жирової тканини людини та щура, ембріональних фібробластів щура, а також лізату мезенхімальних стромальних клітин та цитиколіну на стан емоційно-поведінкових реакцій статевозрілих щурів (3-4 міс) лінії Вістар із масою тіла 160-190 г. Поведінкові реакції щурів досліджували за тестом «відкрите поле» на 7-у та 14-у добу експерименту; реєстрували такі поведінкові акти як амбуляція (локомоція), кламбінг, рерінг і грумінг. Достовірність відмінностей визначали з використанням непараметричного U критерію Манна-Уітні. Встановлено, що після ішемії-реперфузії у тварин з контрольною патологією, тривалість епізодів амбуляції периферійних та центральних квадратів, вертикальної локомоторної активності та дослідницької активності достовірно знижувались, порівняно з групою псевдооперованих щурів. У щурів, яким вводили цитиколін і трансплантували мезенхімальні стромальні клітини Вартонових драглів пуповини людини, виявлено достовірне збільшення тривалості епізодів горизонтальної локомоторної активності, порівняно з іншими типами стовбурових клітин та контролем. Внутрішньовенне введення ембріональних фібробластів щура підвищувало емоційну активність піддослідних тварин. Найменший вплив на локомоторну та орієнтувально-дослідницьку активності у щурів з ішемією-реперфузією було зареєстровано в групах тварин, що отримали мезенхімальні стромальні клітини із жирової тканини людини та щура, а також лізат мезенхімальних стромальних клітин. Практична цінність дослідження полягає в пошуку найбільш ефективного за нейропротекторними властивостями класу стовбурових клітин з метою створення на його основі ін'єкційного препарату для внутрішньовенної трансплантації при лікуванні хворих із гострим ішемічним інсультом

Ключові слова: стовбурові клітини; ішемія-реперфузія; адаптивна поведінка; емоційна активність; тест «відкрите поле»