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Comparative effects of human umbilical cord-derived mesenchymal stromal cells and their extracellular vesicles in a mouse model of parkinsonism



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ABSTRACT

Numerous studies have demonstrated the therapeutic potential of multipotent mesenchymal stromal cells (MMSCs) in neurodegenerative diseases due to their trophic properties, suppression of inflammation at the lesion site, reduction of apoptosis, and stimulation of endogenous neurogenesis via the secretion of bioactive factors. Similar to the cells from which they originate, extracellular vesicles (EVs) exert therapeutic effects, including stimulation of cell migration and extracellular matrix synthesis, as well as anti-apoptotic, immunomodulatory, and anti-inflammatory activities. Given their improved safety profile, EVs are considered a promising alternative to cell therapy for nervous system disorders.

THE AIM of study was to compare the effects of human umbilical cord-derived MMSCs (hUC-MMSCs) and their EVs on behavioral parameters, immune cell populations, and antioxidant defense in the brains of mice with an experimental model of parkinsonism.

MATERIALS AND METHODS. Parkinsonism was modelled in 6-7-month-old male 129/Sv mice by a single subcutaneous injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at a dose of 30 mg/kg (control group). To assess therapeutic efficacy, either hUC-MMSCs (5×10^5 cells) or EVs derived from an equivalent number of cells were administered via tail vein injection 7 days post-neurotoxin injection. Flow cytometry was used to determine the percentages of CD3⁺ T lymphocytes and CD11b⁺ macrophages in brain cell suspensions. Biochemical analysis of brain homogenates was performed to assess malondialdehyde (MDA) levels and the activities of antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GP) and glutathione reductase (GR). Motor and non-motor behavior indicators were evaluated using the open field, rigidity, memory, and rotarod tests.

RESULTS. MPTP administration led to reduced motor, exploratory, and cognitive activity, and increased emotional activity compared to intact animals. An increase in brain macrophage content and MDA levels, along with a reduction in GP and GR activities, was also observed. hUC-MMSC transplantation partially restored emotional and motor functions, reduced macrophage numbers and MDA levels, and increased GP activity. However, it was associated with further suppression of some cognitive parameters, potentially related to the treatment regimen. EV administration similarly improved motor and emotional functions, but unlike hUC-MMSCs, did not impair cognitive performance. Moreover, EVs more effectively enhanced GP and GR activities and reduced brain macrophage levels compared to cell therapy.

CONCLUSIONS. Both hUC-MMSCs and their EVs improve CNS function in experimental parkinsonism by reducing macrophage infiltration and oxidative stress in the brain. The more pronounced beneficial effects observed with EVs suggest they may represent a promising and safer alternative to cell-based therapies for Parkinson's disease.

KEY WORDS: umbilical cord-derived multipotent mesenchymal stromal cells; extracellular vesicles; MPTP; parkinsonism; behavioral reactions; T-lymphocytes; macrophages; oxidative stress.

Parkinson's disease is one of the most common progressive neurodegenerative pathologies, in which motor and non-motor disorders of the functional state of the central nervous system (CNS) develop [1, 2]. Oxidative stress and neuroinflammation factors are of great importance in the pathogenesis of nervous system damage in Parkinson's disease [3, 4]. To date, the effectiveness of using multipotent mesenchymal stromal/stem cells (MMSCs) of various tissue origins (adipose tissue, umbilical cord, bone marrow, etc.) in the treatment of Parkinson's disease/parkinsonism has been proven [5]. These cells are capable of multilineage differentiation, trophic effects on damaged organs and tissues, and exhibit anti-inflammatory, antioxidant, and immunomodulatory properties. It has been shown that the therapeutic potential of MMSCs is largely mediated by paracrine factors that they produce and secrete. In particular, growth factors, cytokines, chemokines, extracellular matrix components, and extracellular vesicles (EVs) determine the positive effects of MMSCs on tissue regeneration and repair [6, 7].

Among the paracrine factors, EVs attract attention, which may be an alternative to the use of native MMSCs in pathological processes in the nervous system. The advantages of using EVs compared to MMSCs include a higher safety profile, mainly due to their nanosize. Thus, unlike MMSCs, which have a diameter of 30-60 μm , nanosized EVs can be efficiently transported to specific tissues after administration without aggregation in the pulmonary microcirculation [8, 9], avoiding the possibility of pulmonary embolism caused by the injected cells [10]. Although EVs exhibit properties similar to the MMSCs from which they are derived, they are less immunogenic, contain enzymes, signaling molecules, and cytokines, as well as microRNAs involved in maintaining cellular homeostasis [11, 12]. EVs have been shown to cross the blood-brain barrier [13, 14] and stimulate neurogenesis and angiogenesis in the brain [15]. We have previously shown that human umbilical cord-derived MMSCs (hUC-MMSCs) and EVs derived from them effectively restore cognitive function in mice with an experimental model of neuroinflammation [16].

THE PURPOSE is to compare the effect of human umbilical cord-derived MMSCs and EVs derived from them on behavioral indicators, immune system cells, and the state of antioxidant protection in the brain of mice with an experimental model of Parkinson's disease.

MATERIALS AND METHODS

Animals. The experiments were performed on male mice of the 129/Sv strain (genotype H-2b, $n = 34$) aged 6-7 months (adults) from the nursery of the Institute of Genetic and Regenerative Medicine of M. D. Strazhesko National Scientific Center "Institute of Cardiology, Clinical and Regenerative Medicine of the National Academy of Medical Sciences of Ukraine. Experimental animals were kept in standard vivarium conditions with a fixed light regime of 12:12 and free access to food and water ad libitum.

Biological material for experiments (brain) was obtained by decapitation of mice in the morning hours (9.00-10.00) under ether anesthesia. All works on experimental animals was performed in compliance with the Law of Ukraine "On the Protection of Animals from Cruelty to Animals", "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

To reproduce the parkinsonism model in mice, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, Sigma, USA) was used. After systemic administration to mice, this neurotoxin not only damages dopaminergic neurons in the substantia nigra of the midbrain, leading to motor behavioral disorders, but also reduces dopamine levels and the expression of dopaminergic markers (tyrosine hydroxylase) in the hippocampus, which coincides with the development of non-motor behavioral changes [17-19].

Isolation and cultivation of hUC-MMSCs. Cells were isolated by the method of explants from the umbilical cord of a male fetus born as a result of natural childbirth from a healthy woman [20]. The mother signed an

informed consent to provide material for scientific research. hUC-MMSCs of the second passage were used for injection into experimental mice. To assess the condition of the cultures, an inverted DMIL microscope (*Leica*, Germany) was used. The culture of cells obtained from umbilical cord tissue after the 2nd passage contained mainly small mitotically active spindle-shaped cells that expressed marker antigens CD105, CD73 and CD90 on the surface (more than 96 %) and did not express CD45 and CD34 [20]. Immunophenotyping of cells was performed on a BD FACSAria cell sorter (*Becton Dickinson*, USA) using the BD FACS Diva 6.1 program. hUC-MMSCs were directed to differentiate *in vitro* into osteoblasts, adipocytes and chondrocytes, which meets the minimum criteria for MMSCs [21].

Preparation of extracellular vesicles/exosomes from hUC-MMSCs.

Cells were cultured in DMEM/F12 medium supplemented with 10 % FCS, 100 U/mL penicillin and 100 mg/mL streptomycin (all from *Biowest*, France) and incubated at 37 °C in 5 % CO₂. Extracellular vesicles were isolated from second-passage MMSCs by ultracentrifugation at the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine [22]. When the monolayer reached 70-80 % confluence, the cells were washed three times with phosphate-buffered saline and cultured in serum-free medium. After 48 hours, the serum-free conditioned medium was collected for exosomes isolation. Cells were detached from the surface of the flask by treatment with 0.2 % trypsin solution (*Biowest*, France) at 37 °C for 2 minutes and counted on an automated cell counter Cell Counter (*Corning*, USA). The conditioned medium with 1.2×10^7 MMSCs in a volume of 32 mL was centrifuged at 300 $\times g$ for 10 minutes to remove detached cells from the surface. The supernatant was centrifuged again for 10 minutes at 2000 $\times g$ to remove cell debris. The supernatant was collected and filtered through 0.45 μm filters (*Merck Millipore*, Germany). The filtered conditioned medium was centrifuged for 2 h at 100,000 $\times g$ and 4 °C in a Sorvall WX 80 high-speed ultracentrifuge (*Thermo Electron*, Germany) using an AN-629 bucket rotor. The supernatant was carefully removed and the obtained extracellular vesicle pellet was resuspended in chilled phosphate-buffered saline. The sizes of the obtained EVs were analyzed by nanoparticle tracking (NTA) using a Zetasizer Nano ZS (*Malvern Instruments*, UK), and the presence of specific extracellular vesicle surface marker proteins CD63 and CD81 was determined by western blot analysis. Identification of EV morphology was performed using transmission electron microscopy.

Experimental groups of mice: 1) intact (8 animals); 2) mice that were injected once with MPTP at a dose of 30 mg/kg (subcutaneously, in the neck area) and after 7 days into the tail vein with 50 μl of 0.9 % NaCl (control group, 9 animals); 3) mice that were injected once with MPTP at a dose of 30 mg/kg and after 7 days into the tail vein with native hUC-MMSCs of the 2nd passage at a dose of 500 thousand cells in 50 μl of 0.9 % NaCl solution (9 animals); 4) mice that were injected once with MPTP at a dose of 30 mg/kg and after 7 days into the tail vein with EVs obtained from 5×10^5 hUC-MMSCs of the 2nd passage in 50 μl of 0.9 % NaCl solution (8 animals). The choice of the specified term of administration of hUC-MMSCs or EVs to mice is explained by the development of changes in the functional state of brain neurons already 7 days after the administration of MPTP to animals [23]. Studies in all experimental groups of mice were carried out at a time corresponding to three weeks after transplantation of hUC-MMSCs or EVs.

The functional state of the CNS was determined by indicators of motor and non-motor behavior in the "open field" tests, rigidity, the conditioned passive avoidance reaction (CPAR) and rotarod test [17, 24, 25]. It has been shown that mice are an adequate model object for studying behavior and its changes under the influence of factors of various nature, since the neurochemical and molecular processes in their brain are similar to those occurring in humans [26, 27].

The "open field" test allows us to assess not only the motor behavior of animals, but also the orientation-exploratory activity number of rearings and hole-peeking, exits to the center and emotional activity (the number of fecal boluses, urinations). Mice of all groups were tested for 3 minutes.

The rotating shaft method (rotarod test) allows you to assess coordination, muscle tone and endurance to physical exertion. The study used one of the test variants, according to which the shaft rotation speed was gradually increased every 30 s from the initial 4 rpm at the start to 40 rpm. The total time spent on the shaft (sec) and the critical (maximum) speed (rpm) at which the animal refused to move (hanged) or fell from the shaft were recorded.

Rigidity in mice was studied by step length (mm), which is one of the indicators of changes in the animals' gait and its decrease indicates impaired muscle function. Before assessing the gait of the animals, their feet were treated with non-toxic solutions of different colors.

The study of cognitive function in mice was carried out using the CPAR method in a standardized setup, which structurally has the form of a rectangular chamber placed at a height of 1 m from the floor, illuminated by a 50 W lamp and connected by a partition to the same dark chamber, in which an electric current (0.45 mA) is applied to the floor. The latency time of the first entry into the dark chamber (initial) and 24 h after training (the influence of damaging factors) was assessed. Animals that did not enter the dark chamber for 5 min after 24 h were considered to have reached the learning criterion.

The activity of antioxidant enzymes was assessed in the supernatants of brain hemisphere homogenates of animals of the experimental groups by the spectrophotometric method [28] on a Synergy HT spectrophotometer (BioTek, USA). To study the activity of superoxide dismutase (SOD), a method based on the enzyme's ability to inhibit the autoxidation reaction of adrenaline to adrenochrome (*Darnytsia*, Ukraine) at pH 10.2 was used [29]. SOD activity was expressed in conventional units per 1 mg of protein per 1 min. Catalase activity was determined from the kinetics of H₂O₂ destruction and expressed in micromoles of utilized H₂O₂ per 1 mg of protein per minute [30]. Glutathione peroxidase (GP) and glutathione reductase (GR) activities were measured by the reduction of NADPH in a coupled glutathione reductase reaction with the addition of the appropriate reagents to the reaction mixture in Tris-HCl buffer with EDTA (*Sigma*, USA) and expressed in nanomoles of oxidized NADPH (*Chimlaborreaktiv*, Ukraine) per 1 mg of protein per minute [31]. Protein content in the brain was measured by the Bradford method using Bradford Dye Reagent (*Sigma-Aldrich*, USA) [32].

The content of malondialdehyde (MDA) was determined in brain homogenates by the Uchiyama method [33]. The principle of the method is to determine the intensity of the color formed during the reaction between MDA and thiobarbituric acid (*Organica*, Germany), which occurs in an acidic environment at high temperature. As a result of the reaction,

a trimethine complex is formed, containing one molecule of MDA and two molecules of thiobarbituric acid and having a characteristic absorption spectrum with a maximum at a wavelength of 535 nm.

Immunophenotyping of brain cells for CD3, CD11b (Mas-1) markers was performed using monoclonal antibodies to mouse membrane antigens conjugated with fluorochromes: CD3 PE-conjugated antibodies (cat. no. 555275), CD11b FITC-conjugated antibodies (cat. no. 557396) (*BD Bioscience*, USA). The working concentration of monoclonal antibodies was 0.5 µg/mL. 1×10⁶ cells of brain homogenate were added to 5 mL tubes in 50 µl of staining buffer (phosphate buffer containing 0.1 % sodium azide and 1 % fetal bovine serum) and monoclonal antibodies were then added at a dilution of 1:50. Incubation was carried out for 20 min at 4 °C, after which the cells were washed in CellWash (*BD Bioscience*, USA) buffer, centrifuged at 200 ×g for 5 min at 4 °C. Immediately before analysis, the cell suspension was passed through cell filters with a pore diameter of 70 µm. Measurements were performed on a BD FACSAria laser flow cytometer-sorter (*Becton Dickinson*, USA) using the BD FACS Diva 6.1 program.

Statistical analysis of the results was performed using the Student's t-test. The difference between the values of the indicators in the experimental groups of animals was considered significant at p < 0.05. For statistical processing of the obtained results, the Statistica 7.0 program (*StatSoft Inc.*, USA) was used. The results were presented in the form of arithmetic mean and standard error of the mean (M ± m).

RESULTS AND DISCUSSION

Effect of hUC-MMSCs or EVs administration on behavioral indicators of mice with an experimental model of parkinsonism. When studying the behavior of mice in the "open field" test, it was found that under the influence of MPTP, the number of crossed squares, rearings, hole-peeking, exits to the center and step length were less, and the number of boluses was higher than in intact animals (**Table 1**). After hUC-MMSCs transplantation, the number of boluses in mice becomes less, and the step length is longer compared to the control group. After EV administration, the number of boluses decreased even further compared to the cell transplantation group. The stride length in the group of mice that received EVs exceeded the value in the control group but, as in the group with hUC-MMSCs transplantation, remained lower than in intact animals. The rotarod test performance did not differ between mice in the experimental groups.

Table 1. Behavioral indicators in mice of experimental groups in the "open field" test, M ± m

Parameter	Experimental group			
	Intact (n = 8)	MPTP + 0,9 % NaCl (control) (n = 9)	MPTP + hUC-MMSCs (n = 9)	MPTP +EVs (n = 8)
Number of crossed squares	53.7 ± 5.0	34.7 ± 6.9*	30.5 ± 7.7*	22.8 ± 5.3*
Number of rearings	1.9 ± 0.4	0.5 ± 0.1*	0.5 ± 0.1*	0.4 ± 0.1*
Number of holes	2.0 ± 0.5	0.2 ± 0.1*	0.3 ± 0.1*	0.3 ± 0.1*
Number of exits to the center	0.7 ± 0.1	0	0	0
Number of boluses	1.2 ± 0.2	2.0 ± 0.2*	1.5 ± 0.1#	0.8 ± 0.04#&
Number of urinations	0	0	0	0
Step length, mm	51.0 ± 2.1	29.8 ± 1.7*	45.0 ± 2.4*#	43.3 ± 1.9*#
Rotarod, rpm	27.3 ± 1.7	23.0 ± 3.3	24.0 ± 3.3	24.0 ± 2.1
Rotarod, s	166.6 ± 16.7	136.2 ± 26.6	140.5 ± 25.6	138.3 ± 10.6

Note: * – p < 0.05 compared to the intact group; # – p < 0.05 compared to the control group (MPTP); & – p < 0.05 compared to the group of mice injected with hUC-MMSCs.

When studying cognitive function in the "CPAR" test, it was found that after the administration of MPTP, the initial values of the latent period of mice entering the chamber, as well as the percentage of animals that reached the learning criterion, become less than in the intact group (**Table 2**). After hUC-MMSCs transplantation, the values

of the latent period of mice entering the chamber have lower values compared to control animals (**Table 2**). After the administration of EVs, the values of the mentioned indicators do not differ from the control group and, in addition, the percentage of mice that reached the learning criterion increases.

Table 2. Cognitive function indicators in mice from experimental groups in the conditioned passive avoidance reaction test, M ± m.

Parameter	Experimental group			
	Intact (n = 8)	MPTP + 0.9 % NaCl (control) (n = 9)	MPTP + hUC-MMSCs (n = 9)	MPTP +EVs (n = 8)
Latency period of entry into the chamber (initial), s	66.5 ± 8.9	25.9 ± 1.8*	15.9 ± 1.1*#	26.8 ± 7.4*
Latency period of entry into the chamber (in 24 h), s	170.5 ± 11.4	167.0 ± 10.2	114.0 ± 8.9*#	164.2 ± 20.2&
Number of mice that reached the learning criterion, %	37.5 % (3/8)	33.3 % (3/9)	33.3 % (3/9)	50.0 % (4/8)

Note: * – $p < 0.05$ compared to the intact group; # – $p < 0.05$ compared to the control group (MPTP); & – $p < 0.05$ compared to the group of mice injected with hUC-MMSCs.

Thus, the administration of MPTP leads to the suppression of motor, exploratory and cognitive activity of mice, as well as to an increase in their emotional activity. Transplantation of hUC-MMSCs has a positive effect on the altered emotional and motor activity (step length) of mice with a model of Parkinsonism, but the values of some indicators of their cognitive activity become even smaller. In mice after the administration of EVs, similarly to the group with hUC-MMSCs, motor activity becomes greater, but emotional activity is even lower and there is no suppression of cognitive function compared to control animals.

Effect of hUC-MMSCs or EVs administration on immune system cells and oxidative stress indices in the brain of mice with an experimental model of parkinsonism. It was found that after MPTP administration in the brain of mice, the MDA content has higher values, and the GP and GR activity has lower values, than in the group of intact mice (Table 3). After hUC-MMSCs transplantation, the MDA content and SOD activity in the brain become lower, while the GP activity is higher compared to the control group (Table 3). After EVs administration, the MDA content in the brain does not differ from the intact group; the SOD activity becomes lower than in control animals, and the GP and GR activity exceeds the values of the control group (Table 3). At the same time, the GP activity in the group with EVs is higher than in the group with cells.

Table 3. Indicators of oxidative stress and neuroinflammation in the brain of mice from experimental groups, M ± m.

Parameter	Experimental group			
	Intact (n = 7)	MPTP + 0.9 % NaCl (control) (n = 9)	MPTP + hUC-MMSCs (n = 9)	MPTP +EVs (n = 8)
Oxidative stress indicators				
Malondialdehyde, nmol/mg	280.8 ± 4.8	309.2 ± 6.6*	278.5 ± 9.5#	282.0 ± 12.7
Superoxide dismutase, U/mg·min	3.5 ± 0.1	3.4 ± 0.04	3.18 ± 0.06*#	3.0 ± 0.09*#
Catalase, μmol/mg·min	3.0 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	2.8 ± 0.2
Glutathione peroxidase, nmol/mg·min	22.8 ± 1.2	17.5 ± 1.2*	27.4 ± 1.0*#	33.4 ± 2.8*#&
Glutathione reductase, nmol/mg·min	33.5 ± 1.1	26.9 ± 0.7*	25.5 ± 1.3*	31.2 ± 3.5
Neuroinflammation indicators				
CD3 ⁺ , %	1.5 ± 0.2	1.8 ± 0.2	1.4 ± 0.1	1.5 ± 0.1
CD11b ⁺ , %	0.10 ± 0.01	0.22 ± 0.03*	0.14 ± 0.01*#	0.12 ± 0.02#

Note: * – $p < 0.05$ compared to the intact group; # – $p < 0.05$ compared to the control group (MPTP); & – $p < 0.05$ compared to the group of mice injected with hUC-MMSCs.

Thus, in the brain of mice with an experimental model of parkinsonism an increase in the content of one of the factors of oxidative stress (MDA) is observed against the background of inhibition of the activity of antioxidant enzymes. Transplantation of hUC-MMSCs into mice with a model of parkinsonism leads to an improvement in the balance between factors of oxidative stress and antioxidant protection (GP activity). After the administration of EVs, a decrease in the content of MDA in the brain of mice with a model of parkinsonism is also observed, but the activation of antioxidant enzymes is more pronounced than in the group with cells. At the same time, SOD activity decreases in the brain of experimental mice after the administration of hUC-MMSCs or EVs.

When studying the content of immune system cells (neuroinflammation markers) in the brain, it was found that under the influence of MPTP, the proportion of CD11b⁺ (macrophages) has higher values compared to the indicators of the intact group (Table 3). After the administration of hUC-MMSCs or EVs, the values of the indicator become lower than in the

control group; at the same time, in the group with EVs they do not differ from the group of intact animals. The indicators of the content of CD3⁺ cells (T-lymphocytes) in the brain of mice of the experimental groups do not differ from each other.

Therefore, in mice with an experimental model of parkinsonism, the content of macrophages in the brain becomes greater, while after the administration of hUC-MMSCs or EVs it is less, especially in the EV group.

Effects of the neurotoxin MPTP in mice. We have established that after the administration of MPTP, significant disorders in motor, emotional, orientation-exploratory and cognitive activity are observed in mice. Changes in the functional state of the CNS in experimental mice can be explained by the damaging effect of this neurotoxin on the structure of neurons of the substantia nigra, cortex, hippocampus, thalamus, as well as the suppression of the expression of neurotrophic factor (BDNF) in the brain [25, 34-36]. This effect of MPTP on nerve cells can be mediated by oxidative stress factors [4] and pro-inflammatory cytokines (TNF-alpha, IL-1beta, IFN-gamma), which are produced by activated microglia/macrophages and

T-lymphocytes of the brain [3, 37, 38]. Indeed, in this experiment we have shown that the content of MDA becomes higher against the background of lower activity of antioxidant enzymes in the brain of mice with MPTP-model of parkinsonism. In addition, we have established a higher number of macrophages in this organ after the administration of MPTP. At the same time, we did not observe a change in the number of T-lymphocytes in the brain of experimental mice of the 129/Sv strain, which is probably due to the linear features of the response of these cells to the administration of the neurotoxin MPTP [20].

Effects of transplanted hUC-MMSCs in mice with an experimental model of parkinsonism. We have found positive changes in the functional state of the CNS (motor and emotional activity) after transplantation of hUC-MMSCs into mice with a model of parkinsonism. The results obtained confirm our previous data on the positive effect of a similar dose of hUC-MMSCs on the impaired behavior of mice of the 129/Sv strain with the MPTP model of parkinsonism [20]. The improvement of behavioral reactions in such animals may be associated with positive changes in the structure of CNS neurons. Thus, we and other authors have established a decrease under the influence of hUC-MMSCs in the number of pathologically altered neurons in the sensorimotor cortex, CA1 zone of the hippocampus of animals with models of neurodegenerative pathology (parkinsonism, cerebral ischemia) [25, 39]. In addition, according to our data, after transplantation of hUC-MMSCs into mice of the 129/Sv strain with the MPTP model of parkinsonism, the number of pathologically altered neurons in the dentate gyrus of the hippocampus, which is one of the sources of neural stem cells, decreases [40]. It is believed that increased proliferation of neural progenitors and their secretion of growth and trophic factors, activation of neurogenesis and angiogenesis are of great importance in the mechanism of the neuroprotective effect of hUC-MMSCs in experimental CNS pathology [41].

It is known that for the implementation of the protective effect of hUC-MMSCs in CNS pathology, their antioxidant effect is also important, namely, a decrease in the content of reactive radicals and an increase in the expression of a number of antioxidant enzymes [42]. We have established that after transplantation of hUC-MMSCs in the brain of mice with an experimental model of parkinsonism, the content of MDA decreases and the activity of glutathione peroxidase increases. In addition, the number of macrophages in the brain decreases in such animals, which may be a manifestation of the anti-inflammatory effect of transplanted hUC-MMSCs. It has

been proven that the anti-inflammatory effect of these cells is an important mechanism of their protective effect in neurodegenerative pathology, which is associated with the activation of IL-10 production, a decrease in IL-1 beta production and manifestations of active gliosis in the brain [43, 44].

Effects of EVs in mice with a model of parkinsonism. Dabrowski F et al. found that the paracrine effect of hUC-MMSCs is important in the implementation of their protective properties in neurodegenerative pathology of the CNS [45]. At the same time, other researchers have shown that not only transplanted hUC-MMSCs, but also EVs obtained from them, are able to stimulate neurogenesis in the brain of animals with CNS pathology [15, 44]. In our work, we compared the effects of hUC-MMSCs and EVs obtained from them on the behavior of mice with the MPTP model of parkinsonism. We found that the administration of EVs, like hUC-MMSCs, has a positive effect on changes in the emotional and motor activity of experimental animals, but, unlike cells, does not lead to a decrease in the values of cognitive function indicators. The literature reports the existence of side effects of cell therapy for Parkinson's disease, related, in particular, to the dose of MMSCs, the nature of the cytokines they produce, the duration of use, etc. [5]. An experimental model of neuroinflammation is also important for reproducing cognitive impairment [16].

The possibility of implementing the anti-inflammatory effect of the administered EVs has been shown, namely, reducing the content of pro-inflammatory cytokines in the brain of mice with an LPS model of neuroinflammation [16]. It is known about the antioxidant effect of EVs in various pathologies (in particular, the nervous system), accompanied by the development of oxidative stress, which is associated with such bioactive molecules as mRNA and microRNA [46]. We have established that after the administration of EVs, not only does the activity of some antioxidant enzymes increase and the number of macrophages in the brain of mice with a model of parkinsonism decrease, but such changes are even more significant compared to hUC-MMSCs.

It is worth noting that the unidirectionality of positive changes in behavioral indicators, the content of neuroinflammatory cells, and oxidative stress factors in the brain of animals after the administration of hUC-MMSCs or EVs may indicate the involvement of the latter in the mechanism of the therapeutic effect of cells in parkinsonism. At the same time, the schemes for using both hUC-MMSCs and EVs obtained from them require further development.

CONCLUSION

- 1. In adult male mice of the 129/Sv strain with an experimental model of parkinsonism, motor, orientation-exploratory, and cognitive activities become less, and emotional activity is greater than in intact animals; in the brain, the content of macrophages and malondialdehyde has higher values, while the activity of antioxidant enzymes (glutathione peroxidase and glutathione reductase) has lower values compared to intact animals.**
- 2. hUC-MMSC transplantation positively affects emotional and motor activity, the content of macrophages and malondialdehyde, and the activity of glutathione peroxidase in the brain of mice with a model of parkinsonism. However, in such mice, cognitive function indicators change even more compared to the control and intact groups of animals.**
- 3. The administration of extracellular vesicles, like hUC-MMSCs, has a positive effect on changes in emotional and motor activity, but, unlike cells, does not have a negative effect on the values of some indicators of cognitive function compared to the control group. In addition, under the influence of extracellular vesicles, positive changes in the activity of antioxidant enzymes and the number of macrophages in the brain are more pronounced than after the transplantation of cells.**
- 4. In general, hUC-MMSCs and extracellular vesicles derived from them improve the functional state of the CNS in experimental parkinsonism, affecting such pathogenetic links of this pathology as manifestations of neuroinflammation and oxidative stress in the brain. At the same time, the positive effects of extracellular vesicles on macrophages and the activity of antioxidant enzymes are more pronounced compared to hUC-MMSCs, which indicates the prospect of their use as an alternative to cell therapy for parkinsonism.**

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Порівняльні ефекти мультипотентних мезенхімальних стромальних клітин пуповини людини та отриманих із них позаклітинних везикул у мишей з експериментальною моделлю паркінсонізму

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РЕЗЮМЕ

Численні дослідження продемонстрували ефективність застосування мультипотентних мезенхімальних стромальних клітин (ММСК) для лікування нейродегенеративних захворювань завдяки їхнім трофічним властивостям, пригніченню запалення в осередку uszkodження, зменшенню апоптозу і посиленню ендогенного нейрогенезу за допомогою секреції біоактивних факторів. Подібно до клітин, з яких вони походять, позаклітинні везикули (ПВ) реалізують такі ж терапевтичні ефекти, включно зі стимуляцією міграції клітин та синтезу позаклітинного матриксу, антиапоптотичними, імуномодуючими та протизапальними проявами. Враховуючи вищий профіль безпеки, їх розглядають як альтернативу клітинній терапії при патології нервової системи.

МЕТА – порівняти вплив ММСК з пуповини людини та отриманих із них ПВ на показники поведінки, клітини імунної системи та стан антиоксидантного захисту головного мозку мишей із експериментальною моделлю паркінсонізму.

МАТЕРІАЛИ І МЕТОДИ. Для моделювання паркінсонізму дорослим (6–7 міс) самцям мишей лінії 129/Sv (генотип Н-2b) одноразово підшкірно вводили нейротоксин 1-метил-4-феніл-1,2,3,6-тетрагідропіридин (МФТП) у дозі 30 мг/кг (контрольна група). Для порівняння терапевтичних ефектів через 7 діб у хвостову вену вводили ММСК у дозі 5×10^6 клітин або ПВ, отримані з тієї ж кількості клітин. В суспензії клітин головному мозку методом проточної цитометрії визначали вміст CD3⁺ Т-лімфоцитів, CD11b⁺ макрофагів, та в гомогенаті біохімічними методами – вміст малонового діальдегіду (МДА), активність антиоксидантних ферментів супероксиддисмутази, каталази, глутатіонпероксидази (ГП) і глутатіонредуктази (ГР). Показники моторної та немоторної поведінки тварин оцінювали в тестах "відкрите поле", на ригідність, пам'ять і в ротород-тесті.

РЕЗУЛЬТАТИ. Показано, що під впливом МФТП у мишей показники рухової, орієнтовно-дослідницької, когнітивної активності були менші, а емоційної – більші, ніж у інтактних тварин. У головному мозку вміст макрофагів і МДА був більшим, тоді як активність глутатіонпероксидази і глутатіонредуктази мали менші значення порівняно з інтактними тваринами. Трансплантація ММСК мишам із моделлю паркінсонізму позитивно впливала на змінені показники емоційної і рухової активності, вмісту в головному мозку макрофагів, МДА, активності ГП, але призводила до подальшого пригнічення деяких показників когнітивної функції, що може бути пов'язано зі схемою їх застосування. Введення ПВ аналогічним чином впливало на порушену емоційну та рухову активність, але, на відміну від ММСК, не зменшувало значення деяких показників когнітивної активності. Крім того, у мишей після введення ПВ більш виразно, ніж в групі з клітинами, підвищувалась активність ГП і ГР та зменшувалась частка макрофагів у головному мозку.

ВИСНОВКИ. ММСК з пуповини та отримані з них позаклітинні везикули поліпшують функціональний стан ЦНС при експериментальному паркінсонізмі шляхом зменшення кількості макрофагів і проявів оксидативного стресу в головному мозку. Більша виразність деяких позитивних ефектів позаклітинних везикул, ніж ММСК, свідчить про перспективність їх застосування, як альтернативи клітинної терапії паркінсонізму.

КЛЮЧОВІ СЛОВА: мультипотентні мезенхімальні стромальні клітини; позаклітинні везикули; МФТП; паркінсонізм; поведінкові реакції; Т-лімфоцити; макрофаги; оксидативний стрес.