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The effects of transplanted adipose-derived multipotent mesenchymal stromal cells from mice of different age or from aging donors in combination with melatonin at experimental parkinsonism



Labunets I.^{1,2*}, Utko N.^{1,2}, Panteleymonova T.^{1,2}, Kyryk V. M.^{1,2}, Kharkevych Yu.^{1,3}, Rodnichenko A.¹, Litoshenko Z.^{1,2}, Butenko G.^{1,2}

¹State Institute of Genetic and Regenerative Medicine of the National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine

²D. F. Chebotarev State Institute of Gerontology of the National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine

³National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

*Corresponding author's e-mail: irina_labunets@ukr.net

ABSTRACT

The transplantation of adipose-derived multipotent mesenchymal stromal cells (ADSCs) in Parkinson's disease/parkinsonism is a promising area in their therapy. The effects of such cells may be influenced by the age of the donor and biologically active factors.

THE PURPOSE of the study is to compare the effect of transplanted ADSCs of donor mice of different age on the parameters of behaviour, oxidative stress and neuroinflammation in the brain of mice with an experimental model of parkinsonism; to evaluate changes in the effects of cells from older donors under the influence of exogenous hormone melatonin.

MATERIALS AND METHODS. The object of the study was adult (5-6 months) and aging (15-17 months) 129/Sv mice. Adult mice were injected once with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), and after 17 days – ADSCs of adult or aging donor mice at a dose of 700 thousand cells in the tail vein. Some mice received ADSCs of aging donors in combination with melatonin. Behavioural parameters were assessed in open-field, rigidity and rotarod tests; the relative content of macrophages was measured in the brain, malondialdehyde (MDA), the activity of antioxidant enzymes.

RESULTS. Under the influence of MPTP, the number of squares, rearings, body length and step length are significantly less than in the intact group, and muscle tone is higher; in the brain the content of MDA and macrophages increases and the activity of superoxide dismutase (SOD) decreases. After the transplantation of adult donor ADSCs, the parameters of body and step length increase significantly, but not to the level of intact mice; the activity of SOD, glutathione reductase (GR) and the proportion of macrophages increase in the brain. After the administration of ADSCs of aging donors, the values of behavioural parameters and the proportion of macrophages in the brain correspond to the control group (only MPTP), and the activity of SOD corresponds to intact animals. In mice treated with ADSCs of aging donors in combination with melatonin, the direction of changes in behavioural parameters, SOD and GR activity, macrophage percentage was similar to that observed after the administration of adult donor ADSCs.

CONCLUSION. The effects of ADSCs transplantation in mice with the MPTP model of parkinsonism depend on the age of the donor and are more pronounced in transplanted cells derived from adult mice. The effects of ADSCs from aging donors in combination with melatonin are consistent with those observed after administration of cells from adult donors.

KEY WORDS: adipose-derived multipotent mesenchymal stromal cells; MPTP; parkinsonism; melatonin; behavioral reactions; oxidative stress; macrophages

Parkinson's disease (PD) is fairly common neurodegenerative pathology of the central nervous system (CNS), which tends to increase steadily [1]. Although this disease is found mainly in people over 60 years, today it is increasingly diagnosed in the age period of active work ability, namely 30-40 years. It has been proved that oxidative stress and neuroinflammation are of great importance in the development of morphofunctional disorders of the nervous system in PD/parkinsonism [2, 3].

Among the new promising approaches to the treatment of PD/parkinsonism is the transplantation of multipotent mesenchymal stromal cells (MMSCs) of different tissue origin (adipose tissue, bone marrow, umbilical cord, etc.) [4]. MMSCs have been shown to be capable of multilinear differentiation, synthesis and secretion of neurotrophic factors, trophic effects on damaged organs and tissues, and exhibit immunomodulatory, anti-inflammatory and antioxidant properties [5-8]. Researchers and clinicians are paying special attention to adipose-derived MMSCs (ADSCs) as one of the most accessible and safe sources.

However, the authors attribute the cases of insufficient effectiveness of transplanted ADSCs in CNS pathology to the age of these cell donors [9]. It has been established that with aging the proliferative, differentiating potential of MMSCs, their synthesis of growth, trophic factors and anti-inflammatory cytokines decreases [10].

There are approaches to influence both the biological properties of MMSCs and the survival of these cells after transplantation into organisms with various pathological conditions. In particular, it has been shown that the hormone melatonin *in vivo* alters the proliferation, migration and differentiation of MMSCs, promotes their survival after transplantation, and has anti-inflammatory, antioxidant and immunomodulatory properties [11, 12]. It is important that with age the synthesis of melatonin by the pineal gland decreases, while exogenous melatonin has a neuroprotective, antioxidant, anti-inflammatory effect in experimental parkinsonism [13, 14].

The **PURPOSE** of the study is to compare the effect of transplanted ADSCs of different age donor mice on the parameters of behaviour, oxidative stress and neuroinflammation in the brains of mice with an experimental model of parkinsonism; to evaluate changes in the effects of cells from older donors under the influence of exogenous hormone melatonin.

MATERIALS AND METHODS

Animals. The experiments were performed on 63 female 129/Sv (haplotype H-2b) mice of the age groups of 5-6 months (adults) and 15-17 months (aging) from the vivarium of State Institute of Genetic and Regenerative Medicine of the National Academy of Medical Sciences of Ukraine. The mice were kept in standard vivarium conditions at a fixed light regime of 12:12 and free access to food and water *ad libitum*. Biological material for the experiments was obtained by decapitation of mice under ether anesthesia in the morning. All experiments were performed in compliance with the Law of Ukraine "On Protection of Animals from Cruelty", "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

Experimental models. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which after systemic administration to mice damages dopaminergic neurons of the substantia nigra of the midbrain and leads to motor disorders similar to the symptoms of PD in humans, was used to reproduce the model of parkinsonism [15, 16]. In the experiment, MPTP (*Sigma*, USA) was administered to adult 129/Sv mice subcutaneously at a single dose of 30 mg/kg in 0.9 % saline. We previously found that MPTP at this dose damages more than 70 % of dopaminergic neurons of the substantia nigra of this strain of mice 17 days after administration [17, 18].

Isolation and cultivation of ADSCs. Isolation, cultivation and directed differentiation of ADSCs were performed according to standard protocols [19], as described in previous studies [20]. MMSCs from subcutaneous adipose tissue of adult (ADSCs-1) and aging (ADSCs-2) mice were obtained

by mincing their adipose tissue in 0.1 % collagenase 1A solution. The resulting mixture was resuspended, centrifuged, the supernatant was collected, and the cell pellet was resuspended in nutrient medium and seeded to culture flasks. Cultivation was performed in a nutrient medium containing 10 % fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin (all – *Sigma*, USA) at a temperature of +37 °C and 5 % CO₂.

ADSCs of the 2nd passage were used for the administration to experimental animals. The affiliation of these cells to MMSCs was determined by the appropriate immunophenotype, as well as the ability to differentiate in osteogenic and adipogenic directions, which meets the minimum criteria for MMSCs [21]. ADSCs of the 2nd passage expressed marker antigens CD44, CD73, Sca-1 and CD90 on the surface, but did not express CD45 and CD34 [20]. Cell culture phenotyping was performed using monoclonal antibodies to membrane antigens conjugated to fluorochromes, at a working concentration of 0.5 µg/mL (*Becton Dickinson*, USA). Immunophenotyping of cells was performed on a BD FACSAria sorter (*Becton Dickinson*, USA).

ADSCs-1 or ADSCs-2 of the 2nd passage were injected into the tail vein of mice once at a dose of 7·10⁶ cells in 50 µL of 0.9 % saline, 17 days after a single injection of MPTP. Considering mentioned before [18], mice develop pronounced morphofunctional changes in the CNS during this period. Control group got one injection of 0.9 % saline into the tail vein of mice with a model of parkinsonism.

Melatonin (*Sigma*, USA) was administered to mice with a model of parkinsonism intraperitoneally, daily at 6 pm, at a rate of 1 mg/kg, starting the day after injection of ADSCs-2 (a total of 13-14 injections).

Experimental groups of adult mice. 1 – intact group; 2 – mice injected with MPTP and 0.9 % saline (control group); 3 – mice injected with MPTP and ADSCs-1; 4 – mice injected with MPTP and ADSCs-2; 5 – mice injected with MPTP, ADSCs-2 and melatonin. We conducted earlier the studies in animals with a model of parkinsonism which received only exogenous melatonin, [13, 14]. There were 10 animals in each experimental group. Studies in all experimental groups of mice were performed in three weeks after transplantation of ADSCs.

The functional state of the CNS was studied in "open field" test, rigidity and the rotarod tests as we described earlier [17, 18]. The open field test assessed horizontal motor, vertical motor, emotional and explorative activities. Mice of all groups were tested for 3 minutes. The rotarod test allows you to examine the coordination, balance and muscle tone of mice. The results were presented as the total time (seconds) of holding on the shaft at 10 rpm and 20 rpm.

Rigidity in animals was assessed by changes in body length and step length (mm). To assess the gait, the animal feet were treated with non-toxic paints of different colours and the footprints were measured for the length of the step, the length and width of the foot (mm). The length of the step is one of the parameters of changes in the gait of animals and its decrease indicates a violation of muscle function [22].

Phenotyping of brain cells by markers CD3, CD11b was performed using monoclonal antibodies to mouse membrane antigens labelled with fluorochromes, at a working concentration of 0.5 µg/mL (*Becton Dickinson*, USA). 1·10⁶ brain homogenate cells in 50 µL staining buffer (phosphate buffer containing 0.1 % sodium azide and 1 % FBS) were added to 5 mL polystyrene tubes and monoclonal antibodies (1:50 dilution) was added to them. The cells were incubated for 20 min at 4 °C, and then washed in CellWash wash buffer, centrifuged at 200× g for 5 min while maintaining the temperature of 4 °C. Immediately before analysis, the suspension was passed through cellular filters with a pore diameter of 70 µm. Measurements were performed on a laser flow cytometer-sorter BD FACSAria (*Becton Dickinson*, USA) using the software BD FACS Diva 6.1.2.

Evaluation of oxidative stress and antioxidant protection of the brain. The content of malonic dialdehyde (MDA) was determined in brain homogenates by the Uchiyama method [23], with minor modifications. The principle of the method is to determine the colour intensity of the trime-

thine complex formed during the reaction between MDA and thiobarbituric acid and has a characteristic absorption spectrum with a maximum at a wavelength of 535 nm.

The activity of antioxidant enzymes was studied in the supernatants of brain homogenates by spectrophotometric method using μ Quant spectrophotometer (Bio-Tek, USA), as we described earlier [18]. The activity of superoxide dismutase (SOD) was evaluated in conventional units for its ability to inhibit the autooxidation of adrenaline to adrenochrome at pH 10.2 per 1 mg of protein per 1 min. Catalase activity was determined from the kinetics of H_2O_2 destruction and expressed in micromoles of utilized H_2O_2 per 1 mg of protein per 1 min. The activity of glutathione peroxidase (GP) and glutathione reductase (GR) was measured by the decrease in NADPH in the combined glutathione reductase reaction with the addition of appropriate reagents to the reaction mixture and expressed in nanomoles of oxidized NADPH per 1 mg of protein per 1 min. The protein content in the brain was measured by the Lowry method (all reagents – Riedel-deHaën, Fluka, Germany).

Statistical analysis of the results was performed using the Student's *t* test. The difference was considered reliable at a value of $p < 0.05$. Data are presented as arithmetic mean and standard deviations ($M \pm m$) (data are presented as mean \pm standard errors of the mean). Statistica 7.0 (StatSoft Inc., USA) was used for statistical analysis of the obtained results.

RESULTS AND DISCUSSION

The effects of ADSCs of adult or aging donor mice and the combination of the cells with melatonin on the behaviour of mice with MPTP-induced model of parkinsonism.

It was found that in mice after the administration of MPTP (control group) the number of crossed squares, rearings, explored holes, body and and step length is less than in intact animals, while the retention time on the shaft is higher (Table 1).

After transplantation of ADSCs-1 to mice with a model of parkin-

sonism, the values of body length and step length were significantly increased compared to the control group, and body length – to the values of intact animals (Table 1).

After ADSCs-2 administration to mice, most values corresponded to the values of the control group (Table 1). At the same time, mice treated with ADSCs-2 in combination with melatonin had a significant increase in the number of squares, body and step length, as well as a significant reduction in shaft retention time compared to the group that received only cells; the length of the body did not differ from the values of intact animals (Table 1).

Thus, motor and non-motor behavioural disorders were observed in mice under the influence of neurotoxin MPTP. After the transplantation of ADSCs from adult donor mice to such mice, some behavioural parameters (body and step length) improved significantly. A similar effect on CNS function was after the transplantation of ADSCs from aging donor mice. However, the administration of such cells in combination with melatonin led to positive changes in motor activity, the values of which did not differ from those after the administration of ADSCs from adult donor mice.

The effects of ADSCs of adult and aging donor mice, as well as the combination of the cells with melatonin on the parameters of oxidative stress and neuroinflammation in the brain of mice with MPTP-induced model of parkinsonism.

It was found that in mice under the influence of MPTP the content of MDA in the brain increased compared to intact animals and did not change after the administration of ADSCs-1 or ADSCs-2, as well as the combination of cells with melatonin (Table 2).

The activity of SOD in the brains of mice in the control group was significantly reduced compared to intact animals, whereas after the administration of ADSCs-1 it became higher than in animals of the intact and control groups. After ADSCs-2 transplantation the SOD activity in the brains of mice did not differ from that in intact animals, whereas after the use of cells in combination with melatonin it was higher than in the groups of intact, control mice and those animals that received only cells.

GR activity in the brain of mice injected with ADSCs-1 or ADSCs-2 in combination with melatonin is significantly higher than in intact animals.

Table 1. Behavioral parameters in mice of experimental groups, $M \pm m$

Parameter	Experimental group				
	Intact (n=10)	MPTP + saline (control) (n=10)	MPTP + ADSCs-1 (n=10)	MPTP + ADSCs-2 (n=10)	MPTP + ADSCs-2 + melatonin (n=10)
Number of crossings	57.2 \pm 5.1	19.3 \pm 3.2*	16.1 \pm 3.1*	15.2 \pm 2.1*	23.2 \pm 2.1* &
Number of rearings	2.2 \pm 0.7	0.2 \pm 0.02*	0.3 \pm 0.1*	0.2 \pm 0.05*	0.3 \pm 0.1*
Number of fecal boluses	2.0 \pm 0.4	1.3 \pm 0.3	1.5 \pm 0.4	1.3 \pm 0.3	1.5 \pm 0.3
Number of explored holes	2.8 \pm 0.8	0.3 \pm 0.1*	0.4 \pm 0.1*	0.3 \pm 0.1*	0.3 \pm 0.1*
Rotarod, sec	252.4 \pm 22.2	429.1 \pm 23.3*	428.4 \pm 25.1*	495.5 \pm 24.3*	393.4 \pm 32.1* &
Body length, mm	98.2 \pm 1.8	90.1 \pm 1.1*	95.2 \pm 1.3#	92.3 \pm 1.4*	98.5 \pm 1.8# &
Step length, mm	55.1 \pm 4.2	32.1 \pm 3.1*	42.3 \pm 4.1*#	34.4 \pm 2.1*	42.4 \pm 2.5*# &
Foot length, mm	13.8 \pm 0.4	13.7 \pm 0.5	13.8 \pm 0.6	14.0 \pm 0.4	13.9 \pm 0.4
Foot width, mm	8.0 \pm 0.2	8.1 \pm 0.1	8.0 \pm 0.2	8.2 \pm 0.3	8.0 \pm 0.2

Note: * – $p < 0.05$ compared to the intact group; # – $p < 0.05$ compared to the control group; & – $p < 0.05$ compared to the group receiving ADSCs-2

Table 2. Parameters of oxidative stress and neuroinflammation in the brain of experimental mice, $M \pm m$

Parameter	Experimental group				
	Intact (n=10)	MPTP + saline (control) (n=10)	MPTP + ADSCs-1 (n=10)	MPTP + ADSCs-2 (n=10)	MPTP + ADSCs-2 + melatonin (n=10)
Markers of oxidative stress					
Malondialdehyde, nM/mg	1.6 \pm 0.1	2.3 \pm 0.2*	2.5 \pm 0.3*	2.2 \pm 0.1*	2.4 \pm 0.2*
Superoxide dismutase, U/mg.min	13.5 \pm 0.3	12.5 \pm 0.2*	15.9 \pm 0.5*#	13.1 \pm 0.4^	15.3 \pm 0.5*# &
Catalase, μ M/mg.min	2.7 \pm 0.1	2.8 \pm 0.2	2.6 \pm 0.1	3.0 \pm 0.3	2.5 \pm 0.2
Glutathione peroxidase, nM/mg.min	7.5 \pm 0.5	7.4 \pm 0.4	6.9 \pm 0.3	7.2 \pm 0.4	7.7 \pm 0.5
Glutathione reductase, nM/mg.min	18.9 \pm 1.2	21.4 \pm 1.4	23.3 \pm 1.5*	22.4 \pm 1.3	23.0 \pm 1.4*
Markers of neuroinflammation					
CD3 ⁺ 11b ⁺ , %	1.4 \pm 0.2	1.9 \pm 0.03*	2.1 \pm 0.03*#	2.1 \pm 0.2*	3.6 \pm 0.2*# &

Note: * – $p < 0.05$ compared to the intact group; # – $p < 0.05$ compared to the control group; & – $p < 0.05$ compared to the group receiving ADSCs-2; ^ – $p < 0.05$ compared to the group receiving ADSCs-1

The proportion of CD3⁺11b⁺ cells in the brain increases significantly after the administration of MPTP compared to the intact group and remains increased in other experimental groups of animals (Table 2). In the group of animals receiving ADSCs-1 and ADSCs-2 in combination with melatonin, the values of the parameter exceeded those in the control group.

Thus, in the brain of mice with MPTP model of parkinsonism, an increase in MDA content was observed against the background of declining SOD activity. The proportion of macrophages in the organ also increased under the influence of MPTP. The transplantation of ADSCs-1 led to a significant activation of SOD and GR production and an increase in the content of macrophages in the brain of mice with parkinsonism. A similar effect on these parameters was exerted by transplanted ADSCs-2 in the combination with melatonin, whereas after self-administered cells, the activity of only SOD changed.

The effects of MPTP on behaviour, oxidative stress factors, antioxidant protection and neuroinflammation in the brain of mice.

In our experiment, the study was performed on a toxic MPTP model of parkinsonism. Other authors as well as we found that a single injection of neurotoxin MPTP at a dose of 30 mg/kg allows mice to reproduce the late stage of this pathology, which is characterized by significant damage to the structure of the substantia nigra and other parts of the brain, as well as motor and non-motor behaviour [15-18]. We have now confirmed that significant changes in motor, research activity, and muscle tone are observed in mice under the influence of MPTP.

An important pathogenetic link of morphofunctional brain damage in PD/parkinsonism is oxidative stress, which develops against the background of declining activity of antioxidant enzymes [2]. It is shown that one of the factors of oxidative stress that leads to such damage is MDA. The latter is formed as a result of peroxidation of polyunsaturated fatty acids and is able to react with nucleic acids, phospholipids and amino acids. We found that after the administration of MPTP, the content of MDA in the brain of mice increases significantly, which coincides with a significant decrease in the activity of SOD.

According to the literature, the toxic effects of MPTP on neurons of the substantia nigra and other parts of the brain may also be mediated by the products of neuroinflammatory cells such as activated microglia/macrophage cells and T-lymphocytes [15, 16]. In particular, there has been shown the damaging effect of pro-inflammatory cytokines (TNF- α , IL-1 β , IFN- γ), which are synthesized by cells of activated microglia/macrophages, on the neurons in CNS [3]. We found that in the brain of mice with MPTP model of parkinsonism, the proportion of CD3⁺11b⁺ cells, which are phenotypically related to activated macrophages [24], increases significantly.

Thus, after the administration of the neurotoxin MPTP in mice, pronounced behavioural changes are observed against the background of an imbalance of oxidative stress factors and antioxidant protection, as well as an increase in the proportion of activated macrophages in the brain.

Age features of ADSCs effects in experimental MPTP-induced parkinsonism.

At present, one of the promising approaches to the treatment of CNS functional disorders in PD/parkinsonism is ADSCs transplantation, the effectiveness of which may be influenced by the age of the donor [9, 25]. In order to comparatively assess the neuroprotective effect of ADSCs of adult or aging donor mice, the cells were administered to animals 17 days after MPTP injection, i.e. against the background of already significant morphofunctional CNS disorders.

We found some positive changes in the motor activity of mice with MPTP-induced model of parkinsonism after the transplantation of ADSCs from adult donor mice. These results are consistent with the data of other authors who showed a similar direction of behavioral changes in mice with MPTP model of parkinsonism after the administration of ADSCs [26, 27]. The authors found that in such animals the improvement of motor ac-

tivity coincided with an increase in the number of dopaminergic neurons in the substantia nigra, as well as the expression of neurotrophic (BDNF, GDNF) and homing (SDF) factors in the brain.

Among the ways of positive effect of ADSCs in CNS pathology, the antioxidant and anti-inflammatory effects of these cells are of great importance [7,28,29]. In particular, Wojtas and co-authors [7] believe that MMSC transplantation under such conditions can be an innovative approach to improving the balance of oxidative stress factors and antioxidant protection of the brain. There is evidence that the antioxidant effect of MMSCs in CNS pathology is associated with a decrease in the content of reactive radicals and increased expression of some antioxidant enzymes in the brain [7, 28]. In our work, it was found that in the brain of mice with MPTP model of parkinsonism, the activity of SOD and GR significantly increases after the transplantation of ADSCs of adult donor mice. However, in such animals, the content of MDA in the brain remained increased. Since neurodegenerative pathology in the brain also significantly increases the content of ROS (reactive oxygen species), which can be reduced by MMSC transplantation [30], it is possible that in our work the positive effect of ADSCs of adult donors on the activity of antioxidant enzymes was manifested against the background of decreasing ROS content.

We showed that after the administration of ADSCs of adult donor mice, the proportion of activated macrophages in the brains of mice with MPTP-induced parkinsonism became even higher compared to the control group. The discrepancy between these results and the authors' data on the anti-inflammatory effect of ADSCs can be partly explained by the different doses of transplanted cells, as well as the different timing of their administration after MPTP injection. Typically, the authors received a significant positive effect from the transplantation of such cells at a dose of 1·10⁶ and under conditions of their use in the early stages of MPTP effect, namely on the third day or a week after the injection of neurotoxin [26, 27].

At the same time, it is noteworthy that the increase in the proportion of CD3⁺11b⁺ cells with a proinflammatory phenotype [24, 31] in the brain of mice injected with ADSCs of adult donors coincides with positive changes in the functional state of the CNS. Therefore, we do not rule out that macrophages with an anti-inflammatory profile may appear in the brain of mice with a model of parkinsonism three weeks after the administration of cells. This assumption is based on the study of Praet et al [32], who showed that macrophages with an anti-inflammatory phenotype may appear due to prolonged exposure to neurotoxins, as well as under the influence of biologically active factors (including cytokines). However, this assumption requires further research using immunophenotyping of brain macrophages or their production of anti-inflammatory cytokines.

In the study of the neuroprotective effect of transplanted ADSCs of aging donors, we did not find their positive effect on the altered behaviour of mice with MPTP-model of parkinsonism. The lack of therapeutic effect of such cells compared to ADSCs of adult donors can be explained by age-related changes in their biological properties. Thus, with age there is a decrease in the proliferative and differentiating potential of MMSCs, changes in the spectrum of produced cytokines, the development of oxidative stress in these cells, a decrease in telomere length, etc. [10, 33, 34]. It is possible that age-related changes in the biological properties of ADSCs are also associated with a less pronounced effect of cells from aging donors on the activity of antioxidant enzymes in the brains of mice with parkinsonism.

Thus, the positive effect of ADSCs of adult donor mice on the motor activity of animals with the MPTP model of parkinsonism coincides with a significant activation of antioxidant enzymes (SOD and GR) in the brain. The lack of effect of ADSCs of aging donors on the behaviour of experimental animals is observed against the background of the restoration of SOD activity only. That is, the age of ADSCs donors is important for their influence on the behaviour and activity of antioxidant enzymes in the brains of mice with MPTP model of parkinsonism. The positive effects of ADSCs from adult donor mice are more pronounced than that of the cells of aging donors.

Effects of ADSCs of aging donor mice in combination with melatonin in experimental parkinsonism. It is known that in neurodegenerative pathology, the protective effect of MMSCs can be modified by using some means, in particular the hormone melatonin [11, 12]. This approach with the administration of MMSCs of the human umbilical cord in combination with melatonin has already shown its effectiveness in our previous studies on the model of multiple sclerosis [35]. The authors showed that in CNS pathology melatonin is able to penetrate the blood-brain barrier and enhance neuro-, myelogenesis and BDNF synthesis, increase the viability, proliferation and differentiation of neural stem cells, have an antiapoptotic effect on neurons and, as a result, improve the functional state of the CNS [36]. We also found positive changes in the behaviour of mice with parkinsonism after using the combination of ADSCs of aging donors with melatonin. At the same time, the values of the parameters did not differ from those in mice after the transplantation of ADSCs of adult donor mice.

The antioxidant and anti-inflammatory properties of melatonin in neurodegenerative pathology are also known [37, 38]. In particular, it was found that under these conditions melatonin acts as a direct and indirect antioxidant. We have shown a pronounced activation of antioxidant enzymes (SOD and GR) in the brains of mice with MPTP parkinsonism model, which were injected with ADSCs of aging donor mice in combination with melatonin. Characteristically, in such animals, the activity of antioxidant enzymes in the brain was similar to their activity after the administration of ADSCs of adult donor mice. The results suggest that the positive effect of the combination of ADSCs of aging donors and melatonin on the motor activity of mice with parkinsonism may be associated with increased antioxidant protection of the brain of such animals. We did not receive a decrease in the number of activated macrophages in the brains of experimental animals after using the combination of ADSCs of

aging donors and melatonin, although, according to our data, exogenous melatonin has anti-inflammatory effects in animals with experimental parkinsonism [13,14]. Moreover, the proportion of activated macrophages in the brains of mice with the MPTP model of parkinsonism even increased compared with the group of animals that received only ADSCs of aging donors.

This effect of the combination of transplanted cells of aging donors with melatonin requires further study, given the positive changes in CNS function in such animals, as well as literature data on the anti-inflammatory action of melatonin in neurodegenerative pathology [37].

In addition to the protective effect on the nervous system, the authors showed a positive effect of melatonin on the biological properties of transplanted MMSCs [11, 12, 39]. Thus, melatonin protects MMSCs from death, which can reach up to 90 % in the first 72 hours after transplantation into the body with pathology [39, 40]. In addition, under the influence of melatonin, the production of pro-inflammatory factors such as TNF- α and IL-6 is reduced in MMSCs of various tissue origins [12, 39]. This hormone regulates the expression of the NADPH oxidase gene in MMSCs, which generates ROS, and also activates the expression of genes for antioxidant enzymes in these cells [11].

Thus, we can assume that the above-mentioned properties of melatonin are important to ensure the neuroprotective effect of transplanted ADSCs of aging donor mice in experimental MTPP-parkinsonism. The obtained results may be the basis for the development of approaches involving melatonin to increase the regenerative potential of ADSCs of aging donors in the cellular therapy of parkinsonism. The results also deepen our understanding of the importance of melatonin for age-related changes in the biological properties of ADSCs in neurodegenerative pathology.

CONCLUSION

In mice with MPTP model of parkinsonism, there is a disorder of behavioural responses, an increase in the number of activated macrophages in the brain and the content of MDA as well as a decrease in the activity of antioxidant enzymes.

The transplantation of ADSCs of adult donor mice leads to positive changes in motor activity and pronounced activation of antioxidant enzymes (SOD, GR) in the brain of mice with a model of parkinsonism; the content of macrophages in the brain increases.

The effect of transplanted ADSCs of aging donors on the violated behaviour of mice with parkinsonism is absent, and, in the brain, there is a restoration of SOD activity.

The transplantation of ADSCs of aging donors in combination with melatonin leads to similar behavioural changes, antioxidant enzyme activity and the proportion of activated macrophages in the brain of mice with parkinsonism as the transplantation of ADSCs from adult donors.

REFERENCES:

1. Sulzev D, Surmeiter DJ. Neuronal vulnerability, pathogenests and Parkinson's disease. *Mov Disord.* 2013; 28:715-724. DOI: 10.1002/mds.25095
2. Guo J-D, Zhao X, Li Y., Li G-R, Liu X-L. Damage to dopaminergic neurons by oxidative stress in Parkinson's disease (Review). *Int J Mol Med.* 2018; 41:1817-1825. DOI: 10.3892/ijmm.2018.3406
3. Wang Q, Liu Y, Zhou J. Neuroinflammation in Parkinson's disease and its potential as therapeutic target. *Translat Neurodegenerat.* 2015; 4:19. DOI: 10.1186/s40035-015-0042-0
4. Li Zh, Cheung H-H. Stem cell-based therapies for Parkinson disease. *Int J Mol Sci.* 2020; 21: 8060. DOI: 10.3390/ijms21218060
5. Konala VB, Mamidi MK, Bhone R, Das AK, Pochampally R, Pal R. The current landscape of the mesenchymal stromal cell secretome. *Cytherapy.* 2016; 18:13-24. DOI: 10.1016/j.jcyt.2015.10.008
6. Zachar L, Bacenlova D, Rosocher I. Activation, homing and role of the mesenchymal stem cells in the inflammatory environment. *J Inflamm Res.* 2016; 9:231-240. DOI: 10.2147/JIR.S121994

7. *Wojtas E, Zachwieja A, Zwyrzykowska A, Kupczynski R, Marycz K.* The application of mesenchymal progenitor stem cells in the reduction of oxidative stress in animals. *Turk J Biol.* 2017; 41:12-19. DOI: 10.3906/biy-1603-13
8. *Laroni A, Kerlego de Rosbo N, Uccelli A.* Mesenchymal stem cells for the treatment of neurological diseases: immunoregulation beyond neuroprotection. *Immunology letter.* 2015; 168:183-190. DOI: 10.1016/j.imlet.2015.08.007
9. *Scruggs BA, Semon JA, Zhang X, Zhang Sh, Bowles A S, Pandey A C, et al.* Age of the donor reduces the ability of human adipose derived stem cells to alleviate symptoms in the experimental autoimmune encephalomyelitis mouse model. *Stem Cells Transl Med.* 2013; 2:797-807. DOI: 10.5966/sctm.2013-0026
10. *Li Yi, Wu Q, Wang Y, Li Li, Bu H, Bao J.* Senescence of mesenchymal stem cells (Review). *Int J Mol Med.* 2017; 39:775-782. DOI:10.3892/ijmm.2017.2012
11. *Hu Ch, Li L.* Melatonin plays critical role in mesenchymal stem cell-based regenerative medicine in vitro and in vivo. *Stem Cell Res Ther.* 2019; 10. DOI: 10.1186/s13287-018-1114-8
12. *Zhang S, Chen S, Li Y, Liu Y.* Melatonin as a promising agent of regulatory stem cell biology and its application in disease therapy. *Pharmacol Res.* 2017; 117:252-260. DOI: 10.1016/j.phrs.2016.12.035
13. *Labunets IF, Chaikovskiy YuB, Savosko SI, Butenko GM, Sagach VF, Kop'yak BS.* Effects of melatonin on the behavioral indices and structural characteristics of cerebral and spinal neurons of rats with experimental hemiparkinsonism. *Neurophysiology.* 2018; 1(50):11-22. DOI: 10.1007/s11062-018-9712-8
14. *Labunets IF.* Neuroprotective effects of the pineal hormone melatonin in animals with experimental model of neurodegenerative pathology. Conceptual options for the development of medical science and education. *Baltija Publishing.* 2020:355-370. DOI: 10.30525/978-9934-588-44-0/18
15. *Zeng XS, Geng WSh, Jia JJ.* Neurotoxin-induced animal models of Parkinson disease: pathogenic mechanism and assessment. *ASN Neuro.* 2018; 10:1-15. DOI: 10.1177/175909418777438
16. *Meredith GE, Rademacher DJ.* MPTP mouse models of Parkinson's disease: an update. *J Parkinsons Dis.* 2011;1(1):19-33. DOI: 10.3233/JPD-2011-11023
17. *Labunets IF.* Behavioral features in the mice of various strains and sex with model of parkinsonism. *Fiziol Zh.* 2020; 66(1):18-24. DOI: 10.15407/fz
18. *Labunets IF, Utko NA, Savosko SI, Panteleymonova TN, Butenko GM.* Changes in nigral neuronal structure, indices of antioxidant protection of the brain and behavior in mice of different age with MPTP parkinsonism model. *International neurological journal.* 2020; 3(16):7-15. DOI: 10.22141/2224-0713.16.3.2020.203444
19. *Prockop DJ, Phinney DG, Bunnell BA.* Mesenchymal stem cells: method and protocols.-Totowa, NJ: Humana Press, 2008. 192 p.
20. *Rodnichenko A.* Certain biological properties of multipotent mesenchymal stromal cells from bone marrow and adipose tissue of FVB/N mice. 2017; 5(2):194-199. DOI: 10.22494/COT.V5I2.77
21. *Dominici M, LeBlanc K, Mueller J.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International society for cellular therapy position statement. *Cytoterapy.* 2006; 8(4):315-317. DOI: 10.1080/14653240600855905
22. *Fernagut PO, Diguet E, Labattu B, Tison F.* A simple method to measure stride length as an index of nigrostriatal dysfunction in mice. *J Neurosci Methods.* 2002; 113(2):123-130. DOI: 10.1016/S0165-0270(01)00485-x
23. *Uchiyama M, Mihara M.* Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem.* 1978; 86(1):271-278. DOI: 10.1016/0003-2697(78)90342-1
24. *Rodriguez-Cruz A, Vesin D, Ramon-Luig L, Zuniga J, Quesniaux VFJ, Ryffel B, et al.* CD3⁺ macrophages deliver proinflammatory cytokines by a CD3⁺ and transmembrane TNF-dependent pathway and are increased at the BCG-infection site. *Front Immunol.* 2019; 10. DOI: 10.3389/fimmu.2019.02550
25. *Li K, Li X, Shi G, Lei X, Huang Y, Bai L, et al.* Effectiveness and mechanisms of adipose-derived stem cell therapy in animal models of Parkinson's disease: a systematic review and meta-analysis. *Translat Neurodegenerat.* 2021; 10:14. DOI: 10.1186/s40035-021-00238-1
26. *Park H, Chang KA.* Therapeutic Potential of Repeated Intravenous Transplantation of Human Adipose-Derived Stem Cells in Subchronic MPTP-induced Parkinson's Disease Mouse Model. *Int J Mol Sci.* 2020; 21:8129. DOI: 10.3390/ijms21218129
27. *Chi K, Fu R-H, Huang Yu-Ch, Chen Sh-Y, Hsu Ch-J, Lin Sh-Z, et al.* Adipose-derived Stem Cells Stimulated with n-Butylidenephthalide Exhibit Therapeutic Effects in a Mouse Model of Parkinson's Disease. *Cell Transplantation.* 2018; 27(3):456-470. DOI: 10.1177/0963689718757408
28. *Angeloni C, Gatti M, Prata C, Hrelia S, Maraldi T.* Role of mesenchymal stem cells in counteracting oxidative stress-related neurodegeneration. *Int J Mol Sci.* 2020; 21:3299. DOI: 10.3390/ijms21093299
29. *Munoz MF, Arguelles S, Medina R, Cano M, Ayala A.* Adipose-derived stem cells decreased microglia activation and protected dopaminergic loss in rat lipopolysaccharide model. *J Cell Physiol.* 2019; 234:13762-13772. DOI: 10.1002/jcp.28055
30. *Chierchia A, Chirico N, Boeri L, Raimondi I, Riva GA, Raimondi MT, et al.* Secretome released from hydrogel-embedded adipose mesenchymal stem cells protects against the Parkinson's disease related toxin 6-hydroxydopamine. *Eur J Pharm Biopharm.* 2017; 121:113-120. DOI: 10.1016/j.ejpb.2017.09.014
31. *Flaishon L, Hart G, Zelman E, Moussion Ch, Grabovsky V, Lapidot Tal G, et al.* Anti-inflammatory effects of an inflammatory chemokine: CCL2 inhibits lymphocyte homing by modulation of CCL21-triggered integrin-mediated adhesions. *Blood.* 2008; 112(13):5016-5025. DOI: 10.1182/blood-2007-12-129122
32. *Praet J, Guglielmetti C, Berneman Z.* Cellular and molecular neuropathology of the cuprizone mouse model: clinical relevance for multiple sclerosis. *J Neubiorev.* 2014; 47:485-505. DOI: 10.1016/j.neubiorev.2014.10.004
33. *Zhang D, He Sh, Wang Q, Pu Sh, Zhou Z, Wu Q.* Impact of aging on the characterization of brown and white adipose tissue-derived stem cells in mice. *Cells Tissues Organs.* 2020; DOI: 10.1159/000507434
34. *Fatian-Labora JA, Morente-Lopez M, Arufe MC.* Effect of aging on behaviour of mesenchymal stem cells. *World J Stem cells.* 2019; 11(6):337-346. DOI: 10.4252/wjsc.v11.16.337
35. *Labunets IF, Utko NA, Toporova OK.* Effects of multipotent mesenchymal stromal cells of the human umbilical cord and their combination with melatonin in adult and aging mice with a toxic cuprizone model of demyelination. *Adv Gerontol.* 2021; 11(2):173-180. DOI: 10.1134/S2079057021020077
36. *Yu X, Li Zh, Zheng H, Ho J, Chan M TV, Wu W K K.* Protective roles of melatonin in central nervous system disease by regulation of neural stem cells. *Cell prolifer.* 2017; 50(2):e12323. DOI: 10.1111/cpr.12323
37. *Chen D, Zhang T, Lee TH.* Cellular mechanisms of melatonin: insight from neurodegenerative diseases. *Biomolecules.* 2020; 10:1158. DOI: 10.3390/biom10081158

38. Heo JS, Pyo S, Lim JA-Y, Yoon DW, Kim BY, Kim J-H, et al. Biological effects of melatonin on human adipose-derived mesenchymal stem cells. *Int J Mol Med*. 2019; 44:2234-2244. DOI: 10.3892/ijmm.2019.4356
39. Luchetti F, Canonico B, Bartolini D, Arcangeletti M, Ciffolilli S, Murdolo G, et al. Melatonin regulates mesenchymal stem cell differentiation: a review. *J Pineal Res*. 2014; 56:382-397. DOI: 10.1111/jpi.12133
40. Tan ShS, Han X, Sivakumaran P, Lim ShY, Morrison WA. Melatonin protects human adipose-derived stem cells from oxidative stress and cell death. *APS*. 2016; 43(3):237-241. DOI: 10.5999/aps.2016.43.3.237



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Ефекти трансплантованих мультипотентних мезенхімальних стромальних клітин жирової тканини мишей різного віку або від старіючих донорів у комбінації з мелатоніном при експериментальному паркінсонізмі



Лабунець І. Ф.^{1,2}, Утко Н. О.^{1,2}, Пантелеймонова Т. М.^{1,2}, Кирик В. М.^{1,2}, Харкевич Ю. О.^{1,3}, Родніченко А. Є.¹, Літошенко З. Л.^{1,2}, Бутенко Г. М.^{1,2}

¹ДУ «Інститут генетичної та регенеративної медицини Національної академії медичних наук України», Київ, Україна;

²ДУ «Інститут геронтології ім. Д. Ф. Чеботарьова Національної академії медичних наук України», Київ, Україна

³Національний університет біоресурсів і природокористування України, Київ, Україна

РЕЗЮМЕ

Трансплантація мультипотентних мезенхімальних стромальних клітин жирової тканини (ММСК-ЖТ) при хворобі Паркінсона/паркінсонізмі є перспективним напрямком в їх терапії. На ефекти таких клітин можуть впливати вік донора і біологічно активні чинники.

МЕТА ДОСЛІДЖЕННЯ: порівняти дію трансплантованих ММСК-ЖТ мишей-донорів різного віку на показники поведінки, оксидативного стресу і нейрозапалення в головному мозку мишей із експериментальною моделлю паркінсонізму; оцінити зміни ефектів клітин від донорів старшого віку під впливом екзогенного гормону мелатоніну.

МАТЕРІАЛИ ТА МЕТОДИ: Об'єкт: миші лінії 129/Sv дорослі (5-6 міс) і старіючі (15-17 міс). Дорослим мишам одноразово вводили нейротоксин 1-метил-4-феніл-1,2,3,6-тетрагідропіридин (МФТП), а через 17 діб – у хвостову вену ММСК-ЖТ дорослих або старіючих мишей-донорів у дозі 700 тис. клітин. Частина мишей отримувала ММСК-ЖТ старіючих донорів у комбінації з мелатоніном. Оцінювали показники поведінки у тестах «відкрите поле», на ригідність і ротарод тесті; в головному мозку вимірювали відносний вміст макрофагів, малонового діальдегіду (МДА), активність антиоксидантних ферментів.

РЕЗУЛЬТАТИ. Під впливом МФТП число квадратів, стійок, довжина тіла і кроку суттєво менше, ніж в інтактній групі, а м'язовий тонус вище; в головному мозку зростає вміст МДА, макрофагів і падає активність супероксиддисмутази (СОД). Після трансплантації ММСК-ЖТ дорослих донорів показники довжини тіла і кроку суттєво підвищуються, проте не до рівня інтактних мишей; у головному мозку зростає активність СОД, глутатіонредуктази (ГР) і частка макрофагів. Після введення ММСК-ЖТ старіючих донорів значення показників поведінки і частки макрофагів у головному мозку відповідають групі контролю (тільки МФТП), а активність СОД – інтактним тваринам. У мишей, яким вводили ММСК-ЖТ старіючих донорів у комбінації з мелатоніном, спрямованість змін значень показників поведінки, активності СОД, ГР, частки макрофагів була аналогічною тій, що спостерігалась після введення ММСК-ЖТ дорослих донорів.

ВИСНОВКИ. Ефекти ММСК-ЖТ у мишей із МФТП-моделлю паркінсонізму залежать від віку донора і більш виразні у трансплантованих клітин, отриманих від дорослих мишей. Ефекти ММСК-ЖТ старіючих донорів у комбінації з мелатоніном практично відповідають тим, що спостерігались після введення клітин від дорослих донорів.

КЛЮЧОВІ СЛОВА: мультипотентні мезенхімальні стромальні клітини жирової тканини; МФТП; паркінсонізм; мелатонін; поведінкові реакції; оксидативний стрес; макрофаги