ISSN: 03875547
Volume 44, Issue 03, June, 2021

# Mesenchymal Stem Cells Ameliorate Scopolamine-Induced Dementia Of Alzheimer's Type

Yevgeniya M. Zorenko[1*], Olena O. Pavlova[1], Elena A. Shchegelskaya[2], Elena A. Omelchenko[3], Tatyana V. Gorbach[4], Iryna M. Vasylyeva[4]

Kharkiv National Medical University, Department of Physiological Pathology, Kharkiv, Ukraine[1]
Kharkiv National Medical University, Department of Neurosurgery, Kharkiv, Ukraine[2]
Kharkiv Medical Academy of Postgraduate Education, Kharkiv, Ukraine[3]
Kharkiv National Medical University, Department of Biochemistry, Kharkiv, Ukraine[4]

Corresponding Authors: 1*

**Keywords:**
Alzheimer's disease, scopolamine, stem cells, endothelium, redox state.

**ABSTRACT**

Alzheimer's disease (AD) is considered to be multifactorial. AD is associated with loss of neurons, stem cell regenerative therapy seems to be promising in AD. The aim of our study was to assess the effectiveness of stem cells in rats with scopolamine-induced model of dementia of Alzheimer's type. The Extrapolation Escape Task and Passive Avoidance Test were used for evaluating the cognitive functions in rats with scopolamine-induced AD model at different period of disease and after mesenchymal stem cells intravenous introduction. The concentration of parameters of endothelial dysfunction, redox state and ROS generation was measured by using the spectrophotometric, photometric, immuno-enzymatic method and flow cytometry. Scopolamine injections in rats resulted in the lipid peroxidation - antioxidant system imbalance and vascular damage. At later stages of the disease, lipid peroxidation was enhanced, the endothelial dysfunction was developing and progressing. The most part of animals with developing endothelial dysfunction in cerebral vessels suffered from cognitive impairment. The regenerative effect of stem cells is more pronounced in animals with a 4-week scopolamine-induced dementia than in animals with a 2-week experimental model. The mesenchymal stem cells derived from bone-marrow had positive effects on cognitive functions, endothelial regeneration and redox state in rats with scopolamine-induced dementia of Alzheimer's type.

This work is licensed under a Creative Commons Attribution Non-Commercial 4.0 International License.

## 1. INTRODUCTION

Analysis of population ageing trends shows that life expectancy has been increasing for decades. This results in an increase in the prevalence of age-related diseases. It is worth mentioning that dementia is one of the most common age-related pathologies. According to the Alzheimer's Disease International, approximately 50 million people are suffering from dementia worldwide and this amount will reach 152 million by 2050. Alzheimer's disease (AD) is the most common cause of dementia in population aged over

60 years and may account for 60–70% of cases [1]. According to the most accepted amyloid cascade hypothesis, excessive production of amyloid plaques with their subsequent accumulation and apoptosis of nerve cells is observed in AD. It is obvious that this theory cannot explain all steps of neurodegeneration. Thus, other mechanisms for progressive injury and loss of neurons have been suggested. Along with the previously discussed hypothesis, the role of hyperphosphorylated microtubule-associated tau protein and p53 protein accumulation in neurodegeneration should be emphasized [2]. However, most of the theories of AD sporadic early onset focus on explaining the genetic predisposition attributed to the presence of apolipoprotein E4 allele, presenilin-1,2, Klotho gene mutations, etc. [3]. In addition, new genome- wide association studies (GWAS) significantly contributed to the elucidation of the role of genetic risk AD factors. GWAS studies have demonstrated that single nucleotide polymorphisms (SNPs) in ABCA7, BIN1, CASS4, CD2AP, CD33, CELF1, CLU, CR1, EPHA1, FERMT2, HLA-cluster, INPP5D, MEF2C, MS4A6A, NME8, PICALM, PTK2B, SLC24A4/RIN3, SORL1, CELF1, NME8, FERMT2, CASS4, DGS2, and ZCWPW1 loci are associated with higher risks for late onset AD [4].

In contrast to the genetic factors mentioned above, other triggers such as hypertension, diabetes mellitus, dyslipidemia, and smoking promote the accumulation of senile plaques due to the damage of brain blood-barrier vessels [5]. Furthermore, there is some evidence that AD is accompanied by mitochondrial dysfunction and energy deficit in neurons, local cerebral hypothyroidism, overproduction of hypoxia-inducible factor-1 (HIF-1), etc. [6]. Several studies have demonstrated an important role of brain neuroinflammation in which microglia and astrocytes are activated by β-amyloid. Microglia can be transformed into inflammatory (M1) and alternatively activated (M2) phenotypes. As a result, M1 microglial cells release pro- inflammatory cytokines, namely tumor necrosis factor (TNF)-α, IL-6, IL-23, IL-1β, IL-12, nitric oxide (NO), and chemokines [7]. There is strong evidence that neuroinflammation and accompanying oxidative stress can promote damage to nerve cells. In particular, oxidative stress is considered to contribute significantly to the development of AD. Furthermore, it has been reported that oxidative stress as a multifactorial process can be an early sign of AD. On the other hand, it is not clear whether excessive ROS (reactive oxygen species) generation is a cause or consequence of AD development [8]. Accumulation of metal ions such as copper, zinc, iron in amyloid plaques, alterations of tau protein, defects in mitochondria, as well as neuroinflammation may contribute to the increase in ROS production [9]. Therefore, the oxidative damage to biomolecules can give rise to neurodegeneration, endothelial dysfunction and blood-barrier impairment. Thus, the vicious circle can be formed. It is crucial to mention that mitochondria, which are the major sources of ROS, are abundant in endothelial cells of the brain [10]. Thus, the impaired mitochondrial function may closely relate to endothelial and ROS dysfunction causing the damage to neurons. Since AD is associated with loss of neurons, stem cell regenerative therapy seems to be promising in AD.

***The aim of our study*** was to assess the effectiveness of stem cells in rats with scopolamine- induced model of dementia of Alzheimer's type.

## 2. Materials and methods

### *2.1 Animals and groups*

A total of 48 male WAG rats weighing 200±20 g were divided into 5 groups. Rats from group 1 (scopolamine 2 weeks, n=8) and group 3 (scopolamine 4 weeks, n=8) were subjected to 2-week-long and 4-week-long scopolamine injections, respectively, resulted in the development of dementia of Alzheimer's type. Group 2 (scopolamine 2 weeks + stem cells, n=8) and group 4 (scopolamine 4 weeks + stem cells, n=8) included animals treated with stem cells against the background of experimental scopolamine-induced

AD. Group 5 (control group, n=16) was composed of control animals. The animals were housed in four cages (4 rats in each cage) in standard laboratory conditions at room temperature (24 ± 2 °C) and relative humidity of 60±10 %. Water and food were given ad libitum. When the animals were sacrificed, their blood was collected into sterile EDTA VACUTAINER tubes and brain was extracted to prepare homogenates. All institutional and national guidelines for the care and use of laboratory animals were strictly followed. The Ethics and Bioethics Commission of the Kharkiv National Medical University (October 10, 2018, minutes of the meeting №8) confirmed that the design and manipulations during this experiment were compliant with bioethical requirements of EU Directive 2010/63/EU on the protection of animals used for scientific purposes and the Council of Europe Convection for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS123).

***2.2 AD induction and stem cells injections***

In our experiment the scopolamine model of Alzheimer's type dementia was used. It is well known that the chronic scopolamine administration to experimental animals in dose of 1 mg/kg of body mass during 27-28 days induced cholinergic deficit and cognitive disorders that were proved in previous works [6], [11], [12]. It was interesting to learn the endothelium and redox state in brain of rats with experimental dementia both after 28 days injections and after 14 days injections. Therefore, in our experiment rats from groups 1-4 were injected with scopolamine at a dose of 1 mg/kg of body mass intraperitoneally during 14 and 28 days (2 and 4 weeks). Control animals were injected intraperitoneally and intravenously with 0.1 ml isotonic saline during the same period of time. Every rat from groups 2 and 4 received 500,000 mesenchymal stem cells (MSCs) intravenously. All rats were tested and sacrificed on day 14 after injections of MSCs. This scheme of MSCs introduction was proposed and used in rats with Parkinson's disease and neurotraumatic disorders by [14].

***2.3 Cognitive assessment***

Cognitive functions were evaluated using the Extrapolation Escape Task (EET) and Passive Avoidance Test (PAT). In EET, the escaping reaction of rats was evaluated: if a rat tries to jump on the wall of cylinder after water immersion or does nothing during 120 secs, the test is considered failed (0). On the contrary, if a rat dives under the lower edge of cylinder, the test is considered passed (1). In PAT, the formation of the conditioned reflex was fixed during 180 sec. If an animal crosses from the light to the dark compartment with mild foot shock next day after training, the passive avoidance response or conditioned reflex is not formed (0). If a rat avoids the entry to the dark compartment and stays at the light compartment, the passive avoidance response is formed (1) [15;16]. To estimate more significant results of the study of the effect of stem cells on cognitive functions, a general comparison of the groups (1+3) without and groups (2+4) with stem cells administration was carried out. 4. The latency period of PAT was not included because it was more significant for us to assess the presence of the conditioned reflex.

***2.4 Description of stem cells***

Primary culture of MSCs was obtained from femoral bone marrow. The suspensions were washed in Hanks' balanced salt solution, centrifuged at 450g for 10 min and plated in 75-cm2 culture flasks at a density of 4x105 cells/cm2 in Dulbecco′s Modified Eagle′s Medium DMEM/F12 (1/1) containing 2mM L-glutamine, 10% Fetal Bovine Serum (FBS) (SIGMA-ALDRICH, cat.n. F7524) and 2 µl/ml, Antibiotic Antimycotic Solution (SIGMA-ALDRICH, cat.n. A5955). The medium with nonadherent cells was discarded after 24 hours of the culture and the fresh medium was added to the adherent fibroblast-like MSCs. They were cultured at 37ºC and 5% $CO_2$ in air in a $CO_2$ - incubator for 14 days in the medium changed every 3 days [13]. All reagents were purchased from SIGMA- ALDRICH (USA).

***2.5 Preparation of homogenates***

The isolated brain was placed on ice. The brain cortex was extracted, weighed, cut into small pieces, and put in a Potter homogenizer with the glass-teflon gap of 0.2 mm. The samples were homogenized in an isolation medium (1:10) during 30 sec on ice; the medium consisted of 0.25 M sucrose, 0.2 M Tris-HCl, and 1.0 mM EDTA (pH 7.4). The suspension obtained was centrifuged at 3,000 rpm for 10 min, isolated by supernatant centrifugation at 14,000 rpm during 20 min using a vacuum refrigerator centrifuge [6].

***2.6 Evaluation of the activity of superoxide dismutases (SOD) and catalase, as well as thiobarbituric acid-reactive products (TBARs) concentration, in brain homogenates***

SOD activity was determined according to the method described by Kostiuk et al., based on the ability of SOD found in homogenates to inhibit the reaction of spontaneous oxidation of quercetin [17]. Catalase activity was detected by measuring the rate of utilization of hydrogen peroxide from the incubation medium in a color reaction with ammonium molybdate. The ability of hydrogen peroxide to form a stable colored complex with molybdenum salts underlies the method. The optical density of the experimental, control and standard samples was measured against blank tubes on a spectrophotometer Solar PV12521 (Belorussia) at a wavelength of 410 nm. The content of TBARs was analyzed according to the Gavrilov's method. The concentration of TBARs was measured during heating and interaction with 2-thiobarbituric acid that results in the formation of a colored complex with an absorption maximum at $\lambda = 533$ nm [18].

***2.7 Evaluation of endothelin-1 (ET-1), vascular endothelial growth factors A (VEGF-A), endothelial nitric oxide synthase (eNOS) and von Willebrand factor (vWF) levels in blood serum***

ET-1, VEGF-A and eNOS concentrations in blood serum were determined by enzyme-linked immunosorbent assay (ELISA) using the standard reagent kits on a semi-automatic enzyme immunoassay STAT FAX 303+ (Elabscience, Wuhan, Hubei, China, 2019). The vWF determination was based on ristomycin-aggregation of formalin-fixed platelets obtained from rats photometrically using a spectrophotometer Solar PV12521 (Belorussia).

***2.8 ROS generation analysis***

ROS generation in leukocytes was evaluated utilizing 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA). Samples of blood collected in K2EDTA Vacutainer test tubes were collected and then aliquots of 100 µl stained were lysed with Pharmlyse solution (BD, USA) and washed twice with phosphate-buffered saline (PBS). Then leukocyte suspensions were incubated with a H2DCFDA working solution (Invitrogen™) with 5 µM of the dye prepared from 10mM solution in Dimethyl Sulfoxide (DMSO) and 5 µl of 7-aminoactinomycin D (7AAD, Pharmingen™, material no 559926, USA) in the dark for 30 min [19]. After incubation and further PBS addition, the leukocyte suspensions were instantly analyzed by flow cytometry using a BD FACSCanto™ II flow cytometer (Becton Dickinson, USA). To analyze results, the BD FACS Diva™ software was used. H2DCFDA is known to be converted into 2', 7'–dichlorofluorescein (DCF) in a ROS-mediated way. DCF fluorescence is proportional to ROS concentrations. The mean fluorescence intensity (MFI) of DCF was analyzed in granulocytes whose region was gated as available in Figure 1. MFI values of DCF were registered for rats in groups 3, 4, 5 (Figure 2, 3).

BD FACSDiva™ software was used to assess the results.

ISSN: 03875547
Volume 44, Issue 03, June, 2021

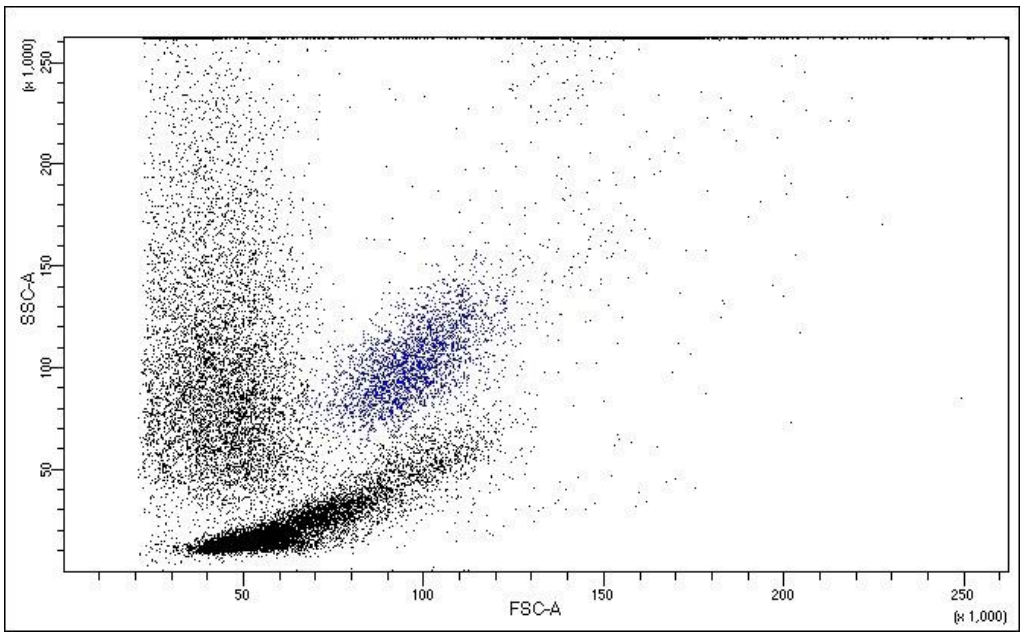


**Fig. 1.** The gating strategy for granulocytes. The region of granulocytes is highlighted in blue.

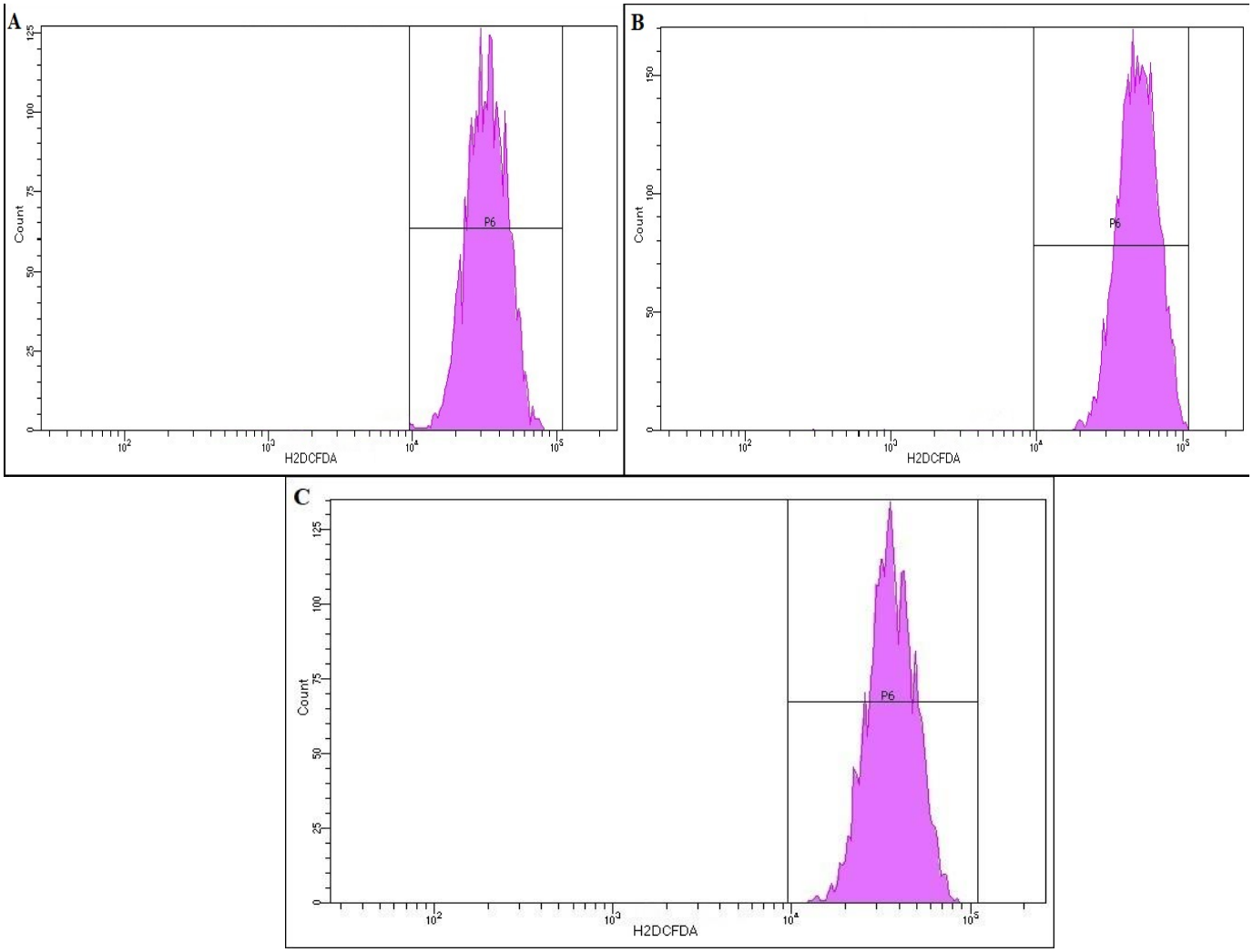


**Fig.2.** Representative histograms of 2', 7' –dichlorofluorescein (DCF) fluorescence reflecting reactive oxygen species (ROS) generation in gated granulocytes of animals from the control (A), group 3

(scopolamine 4 weeks) (B), group 4 (scopolamine 4 weeks+stem cells) (C) are shown.

### *2.9 Statistical analysis*

An assessment of the normality of data was performed by Shapiro-Wilk test. Based on its results, non-parametric tests were used to compare independent groups of variables. To evaluate differences between five independent groups in study, the Kruskal–Wallis one-way analysis of variance was selected. It was followed by the Dunn's multiple comparisons test. To estimate the effects of stem cells on cognitive functions, the Pearson's chi-squared test was used. If p values were below 0.05, the difference was statistically significant. All numerical data were analyzed using GraphPadPrism 5.0 (GraphPad Software Inc., CA, USA) and Statistical Package for the Social Sciences (SSPS).

## 3. Results

EET showed that 81.25% rats receiving stem cells dived under the low edge of cylinder and passed the test. In contrast to that, 62.5% rats without stem cells injections failed this test. The significant differences between all results confirmed the positive effect of stem cells on cognitive functions (Table 1).

**Table 1** Extrapolation Escape Task (EET): cognitive function comparison between groups with stem cell injections (group 2+group 4) and groups without stem cells injections (group 1+group 3)

| Test | Scopolamine 1, 3 weeks groups without stem cells (n=16) | Scopolamine 2, 4 weeks groups with stem cells (n=16) | Totally |
|---|---|---|---|
| Failed | 10 | 3 | 13 |
| Passed | 6 | 13 | 19 |
| Totally | 16 | 16 | 32 |

Obtained results are statistically different (Pearson's chi-squared test, p=0.012).

The percentage of rats who passed PAT was 87.5% from groups 2, 4 and 31.25% in groups 1, 3. These results supplemented the conclusion on the effectiveness of stem cells (Table 2).

**Table 2** Passive Avoidance Test (PAT): cognitive function comparison between groups with stem cell injections (group 2+group 4) and groups without stem cells injections (group 1+group 3)

| Test | Scopolamine 1, 3 weeks groups without stem cells (n=16) | Scopolamine 2, 4 weeks groups with stem cells (n=16) | Totally |
|---|---|---|---|
| Failed | 11 | 2 | 13 |
| Passed | 5 | 14 | 19 |
| Totally | 16 | 16 | 32 |

Obtained results are statistically different (Pearson's chi-squared test, p=0.001).

Despite the fact that there were no changes in values for parameters that characterize the redox state between groups 1 and 2, 3 and groups 2 and 1, 4, 5, the catalase activity and levels of TBARs were significantly different between group 1 and control group. In group 3, the significant decrease in SOD and

catalase activities and an increase in TBARs level were found compared with groups 2, 5. Against the background of stem cell therapy (in rats from group 2), the activity of SOD and the concentration of TBARs did not have differences from the control group. At the same time, catalase activity in group 2 was slightly higher than in group 1, but did not reach the level of group 5. After administration of stem cells in rats with a 28-day (4-week) scopolamine model, the levels of TBARs, SOD and catalase activity improved and almost reached control values (Table 3).

**Table 3** Parameters of the redox state

| Parameter | Group 1 (gr.1) (Scopolamine-2 weeks) | Group 2 (gr.2) (Scopolamine-2 weeks +stem cells) | Group 3 (gr.3) (Scopolamine-4 weeks) | Group 4 (gr.4) (Scopolamine- 4 weeks +stem cells) | Group 5 (gr.5) (Control group) |
|---|---|---|---|---|---|
| Superoxide dismutase, U/mgprotein | 91.84±0.30, $p < 0.05$ – gr. 1 vs 4, $p > 0.05$ – gr. 1 vs 2, 3, 5 | 98.30±0.24, $p < 0.05$ – gr. 2 vs 3, $p > 0.05$ – gr. 2 vs 1, 4, 5 | 79.37±0.46, $p < 0.05$ – gr. 3 vs 2, 4, 5, $p > 0.05$ – gr. 3 vs 1 | 99.93±0.46, $p < 0.05$ – gr.4 vs 3, 1, $p > 0.05$ – gr. 4 vs 2, 5 | 98.31±0.3, $p < 0.05$ – gr. 5 vs 3, $p > 0.05$ – gr. 5 vs 1, 2, 4 |
| Catalase, µkat/g protein | 200.31±2.94, $p < 0.05$ – gr. 1 vs 5, $p > 0.05$ – gr. 1 vs 2, 3, 4 | 208.93±2.60, $p < 0.05$ – gr. 2 vs 3, $p > 0.05$ – gr. 2 vs 1, 4, 5 | 180.70±0.44, $p < 0.05$ – gr. 3 vs 2, 5, $p > 0.05$ – gr. 3 vs 1, 4 | 206.97±2.42, $p < 0.05$ – gr. 4 vs 3, 5, $p > 0.05$ – gr. 4 vs 1, 2 | 224.68±7.90, $p < 0.05$ – gr. 5 vs 1, 3, 4 $p > 0.05$ – gr. 5 vs 2 |
| Thiobarbituric acid-reactive products, nM/g protein | 2.20±0.14, $p < 0.05$ – gr. 1 vs 5, $p > 0.05$ – gr. 1 vs 2, 3, 4 | 1.86±0.04, $p < 0.05$ – gr. 2 vs 3, $p > 0.05$ – gr. 2 vs 1, 4, 5 | 4.03±0.056, $p < 0.05$ – gr. 3 vs 2, 5, $p > 0.05$ – gr. 3 vs 1, 4 | 2.06±0.07, $p < 0.05$ – gr. 4 vs 5, $p > 0.05$ – gr. 4 vs 1, 2, 3 | 1.82±0.05, $p < 0.05$ – gr. 5 vs 1, 3, 4 $p > 0.05$ – gr. 5 vs 2 |

Values are mean±confidence interval (CI) for the mean. $p < 0.05$ - the results are significantly different (Kruskal–Wallis test and Dunn's multiple comparisons test).

The MFI of DCF in granulocytes in group 3 was 17% significantly higher than in the control group, whereas in rats from group 4, the MFI values were 15% statistically significantly lower than in group 3. There were no noticeable significant changes in MFI values of DCF in granulocytes between the control group and group 4 (Fig. 3).

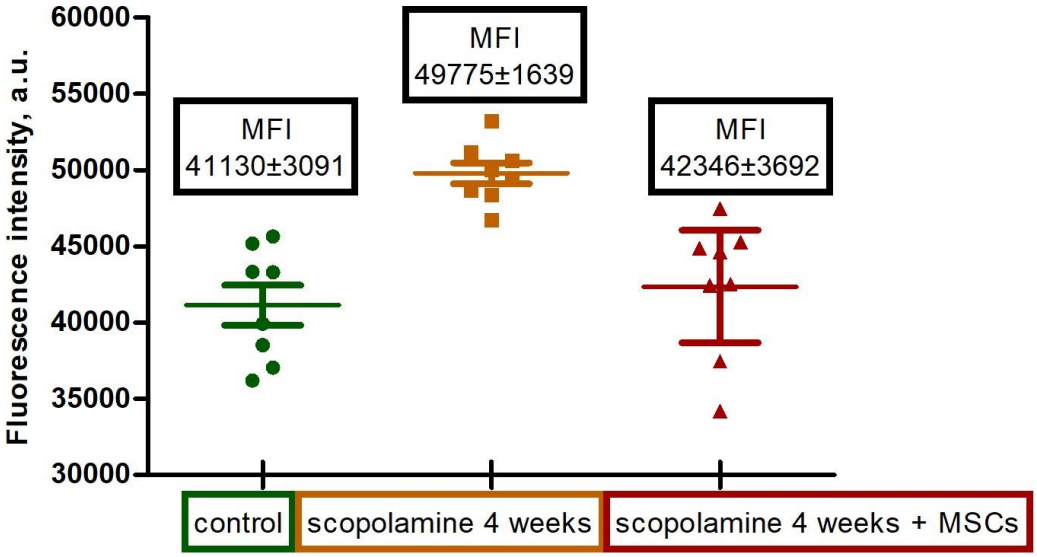


**Fig.3** Mean fluorescence intensity (MFI) of 2, 7' –dichlorofluorescein (DCF) fluorescence reflecting reactive oxygen species (ROS) generation in granulocytes (mean±confidence interval (CI)) in groups 3 (scopolamine 4 weeks), 4 (scopolamine 4 weeks + mesenchymal stem cells (MSCs), 5 (control group).

Significant elevation of ET-1 concentration in blood serum was observed in each experimental group against the control group. Additionally, the eNOS level in groups 1, 2 was higher compared with group 5. The VEGF-A and vWF concentrations were higher in all groups compared with group 5, except for group 2. The highest levels of these parameters were found in group 3.

In group 4, the levels of ET-1, vWF were higher than in the control group, they were but lower than in group 3 and approximately reached levels of groups 1, 2. The concentration of VEGF-A was significantly greater than in the control group but almost 2.2-fold lower than in group 3 (Table 4).

**Table 4** Endothelial function parameter

| Parameter | Group 1 (gr.1) (Scopolamine-2 weeks) | Group 2 (gr.2) (Scopolamine-2 weeks +stem cells) | Group 3 (gr.3) (Scopolamine-4 weeks) | Group 4 (gr.4) (Scopolamine- 4 weeks +stem cells) | Group 5 (gr.5) (Control group) |
|---|---|---|---|---|---|
| Endotheli n-1,pg/ml | 1.37±0.30, $p < 0.05$ – gr. 1 vs 5, $p > 0.05$ – gr. 1 vs 2, 3, 4 | 1.24±0.07, $p < 0.05$ – gr. 2 vs 5, $p > 0.05$ – gr. 2 vs 1, 3, 4 | 4.05±0.25, $p < 0.05$ – gr.3 vs 5, $p > 0.05$ – gr.3 vs 1, 2, 4 | 1.26±0.06, $p < 0.05$ – gr. 4 vs 5, $p > 0.05$ – gr. 4 vs 1, 2, 3 | 0.87±0.07, $p < 0.05$ – gr. 5 vs 1, 2, 3, 4 |
| Endothelial nitricoxide synthase, pg/ml | 107.23±1.86, $p < 0.05$ – gr. 1 vs 3, 5, $p > 0.05$ – gr. 1 vs 2, 4 | 113.05±3.19, $p < 0.05$ – gr. 2 vs 3, 5, $p > 0.05$ – gr.2 vs 1, 4 | 80.04±0.77, $p < 0.05$ – gr. 3 vs 1, 2, 4, $p > 0.05$ – gr. 3 vs 5 | 100.65±1.42, $p < 0.05$ – gr. 4 vs 3, $p > 0.05$ – gr. 4 vs 1, 2, 5 | 95.96±1.34, $p < 0.05$-gr. 5 vs 1, 2 $p > 0.05$ – gr. 5 vs 3, 4 |

| | | | | | |
|---|---|---|---|---|---|
| Vascular endothelial growth factor A,pg/ml | 63.66±0.81,<br>p < 0.05 – gr. 1 vs 5,<br>p > 0.05 – gr. 1 vs 2, 3, 4 | 58.42±0.51,<br>p < 0.05 – gr. 2 vs 3,<br>p > 0.05 – gr. 2 vs 1, 4, 5 | 259.35±0.93,<br>p < 0.05 – gr. 3 vs 2, 5,<br>p > 0.05 – gr. 3 vs 1, 4 | 116.54±0.70,<br>p < 0.05 – gr. 4 vs 5,<br>p > 0.05 – gr. 4 vs 1, 2, 3 | 39.46±0.95,<br>p < 0.05-gr. 5 vs 1, 3, 4<br>p > 0.05 – gr. 5 vs 2 |
| Von Willebrand factor , % | 90.63±1.15,<br>p < 0.05 – gr. 1 vs 5,<br>p > 0.05 – gr. 1 vs 2, 3, 4 | 85.15±1.58,<br>p < 0.05 – gr. 2 vs 3,<br>p > 0.05 – gr. 2 vs 1, 4, 5 | 115.42±8.81,<br>p < 0.05 – gr. 3 vs 2, 5,<br>p > 0.05 – gr. 3 vs 1, 4 | 89.96±0.79,<br>p < 0.05 – gr. 4 vs 5,<br>p > 0.05 – gr. 4 vs 1, 2, 3 | 84.37±0.79,<br>p < 0.05-gr. 5 vs 1, 3, 4<br>p > 0.05 – gr. 5 vs 2 |

Values are mean±confidence interval (CI) for the mean. $p < 0.05$ - the results are significantly different (Kruskal–Wallis test and Dunn's multiple comparisons test).

**4. Findings and discussion**

In our experiment, the scopolamine model of Alzheimer's type dementia was used, with the emergence of the cholinergic deficiency, amyloid deposition in nerve cells (after 20–27 days of injections) and cognitive deficiency [11]. In this study, the functional role of endothelial cells was examined using markers of vascular development, regulation and inflammation. VEGF-A is produced in response to hypoxia and activates not only angiogenesis, but also increases vascular permeability, stimulates eNOS in endothelium and can inhibit apoptosis [20]. The hemodynamic findings, inflammation, hypoxia lead to changes in calcium metabolism in vessels and disturbance between production of a powerful vasoconstrictor ET-1 and eNOS (an endothelial enzyme that activates the NO production). Furthermore, damage to endothelial cells causes the imbalance in vWF production that plays a role in blood coagulation, as well as angiogenesis and inflammation [21]. In 2 weeks after scopolamine injection, vascular damage was triggered, as evidenced by VEGF-A level elevation. The significant increase in ET-1 and eNOS concentration indicated the onset of damage and functional impairment of the endothelium. Whereas the simultaneous increase in the concentration of vWF together with changes of the abovementioned parameters, confirmed the onset of the endothelial dysfunction development. Moreover, at a later stage of disease (in 4 weeks after scopolamine injections), the endothelial dysfunction was developing and progressing. Note, that the most part of animals with developing endothelial dysfunction in cerebral vessels suffered from cognitive impairment, evidenced by EET and PAT. A similar pattern of results was obtained in a study where the vascular endothelial dysfunction was found in animals with scopolamine-induced cognitive impairment [22]. However, when comparing results of Safar M. to our studies, it should be pointed out that the animals with scopolamine-induced dementia after intravenously injections of endothelial progenitor cells from bone marrow had improvement of cognitive functions via boosting vascular and nerve growth factors and decreasing proinflammatory factors. Contrary to these findings, our animals were received MSCs from bone barrow at different stages of disease and had diverse effect. In early stages, after 2 weeks-scopolamine injections, the changes in the studied markers of the endothelium functional state (ET-1, vWF) were not observed. Thus, no corrective effect was found. It is probably due to the beginning of the pathological process development in the vessels, where an external activator of cell regeneration was not required. In the advanced stages, after the administration of stem cells, the regenerative effect on blood vessels was revealed. The EET and PAT confirmed these results by increasing the number of rats passed it after stem cells injections. It can be assumed that cognitive functions partially returned. To sum up, these findings are in accordance with findings where the endothelial dysfunction improving and synaptic plasticity enhancing were reached in a murine model of AD after MSCs administration [23].

In most cases, endothelial dysfunction and oxidative stress act jointly in amyloid formation. It has been shown that the activation of the transient receptor potential melastatin 2 (TRPM2) by amyloid protein, which regulates the mitochondria and the endoplasmic reticulum Ca 2+ channels of endotheliocytes, results in the intracellular Ca 2+ overload, imbalance of the lipid peroxidation - antioxidant system in endotheliocytes and vasomotor dysfunction [24]. The prevalence of products of lipid peroxidation such as TBARS and others over the enzymes (catalase, SOD) that eliminate free radicals cause the oxidative stress. In our experiment, scopolamine injections in rats resulted in the lipid peroxidation - antioxidant system imbalance, which was accompanied by SOD and catalase reduction and elevation of TBARs concentration (prior to the morphologically recorded formation of amyloid). At later stages of the disease, lipid peroxidation was enhanced. Moreover, overgeneration of ROS was found in granulocytes of rats with experimental AD, evidenced by higher MFI values of DCF compared with controls. The same conclusion was reached in previous investigations, where male Kunming mice were received intraperitoneal injections of scopolamine in dosage 3 mg/kg for 1 week and had increased ROS, such as the redox-sensitive fluorescent dye (DCFH-DA) and malondialdehyde (MDA) levels and suppressed the activity of SOD in the homogenized hippocampus and cortex tissues. After correction by sodium Tanshinone IIA sulfonate (STS) it was observed the protective functions against the cholinergic system dysfunction and imbalance between ROS generation and their elimination [25]. Consistent with other studies, our results showed that after intravenous stem cells injection the antioxidant system was activated, which prevents the development of oxidative stress and thereby slows down the progression of the disease.

AD is considered to be multifactorial. Our findings show that endothelial dysfunction of cerebral blood vessels and ROS overproduction play a significant role in vascular damage and cognitive impairment. Accordingly, it has been shown that the ROS formation can occur both in nerve cells and in endothelial cells. Under the influence of pro-inflammatory cytokines and ROS, the permeability of the vascular endothelium increases and its dysfunction develops [26]. Furthermore, it is generally believed that prolonged stimulation of microglia by amyloid leads to the activation of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase and an increase in ROS synthesis [27]. However, many authors refer to the fact that lipid peroxidation occurs before the amyloid plaques formation in AD models in transgenic mice and in patients with Down syndrome and is stimulated predominantly by pro-inflammatory cytokines [28]. The development of oxidative stress prior to the amyloid deposition in a 2-week scopolamine model is in agreement with this claim. The sharp deficit of cognitive functions as well as SOD, catalase and eNOS in animals with a 4-week scopolamine model confirmed the hypothesis that amyloid (its accumulation during this period of the experiment was demonstrated morphologically by many researchers) directly binds to catalase and promotes the accumulation of hydrogen peroxide in hippocampal neurons [29]. Analysis of available data on stem cell correction of neurodegenerative diseases has focused on embryonic stem cells. Using MSCs intracerebrally from umbilical cord blood in a transgenic mice model of AD resulted in reduction of Aβ plaques and improvement in cognition [30]. The positive influence of neural and adipose-derived stem cells on memory in mice with transgenic model of AD has been demonstrated [31]. The limitations of the present study include no comparing the effectiveness between stem cells and other pharmacological substances, such as acetylcholinesterase inhibitors, STS on AD progression at different experimental models. Moreover, further research is needed to investigate the possibility of MSCs intravenous injections in clinical studies enrolling patients with early stages of AD.

**5. Conclusion**

The mesenchymal stem cells derived from bone-marrow had positive effects on cognitive functions, endothelial regeneration and redox state in rats with scopolamine-induced dementia of Alzheimer's type. Indeed, these findings provided additional information about stem cells influence at different stages of

disease. Our findings indicate that the regenerative effect of stem cells is more pronounced in animals with a 4-week scopolamine-induced dementia than in animals with a 2-week experimental model. However, the side effect of intravenous stem cells injection remains unclear, which should be studied in the future.

**6. Acknowledgements**
Authors would like to express their gratitude to Anton Tkachenko and Anatolii Onishchenko for their indispensable assistance in flow cytometry analysis.

**7. References**

[1] Garre Olmo, J. (2018). Epidemiología de la enfermedad de Alzheimer y otras demencias. Revista de Neurología, 66(11), p.377.

[2] Maphis, N., Xu, G., Kokiko-Cochran, O. N., Jiang, S., Cardona, A., Ransohoff, R. M., Lamb, B. T., & Bhaskar, K. (2015). Reactive microglia drive tau pathology and contribute to the spreading of pathological tau in the brain. Brain, 138(6), pp.1738–1755.

[3] Moos, W. H., Faller, D. V., Glavas, I. P., Harpp, D. N., Kanara, I., Mavrakis, A. N., Pernokas, J., Pernokas, M., Pinkert, C. A., Powers, W. R., Sampani, K., Steliou, K., Vavvas, D. G., Zamboni, R. J., Kodukula, K., & Chen, X. (2020). Klotho Pathways, Myelination Disorders, Neurodegenerative Diseases, and Epigenetic Drugs. BioResearch Open Access, 9(1), pp.94–105.

[4] Verheijen, J., & Sleegers, K. (2018). Understanding Alzheimer Disease at the Interface between Genetics and Transcriptomics. Trends in Genetics, 34(6), pp.434–447.

[5] Raz, L., Knoefel, J., & Bhaskar, K. (2015b). The neuropathology and cerebrovascular mechanisms of dementia. Journal of Cerebral Blood Flow & Metabolism, 36(1), pp.172–186.

[6] Gorbach, T. V., Nakonechna, O. A., Tkachenko, A. S., Shcholok, T. S., & Onikova, A. O. (2018). Levels of Thyroid Hormones and Indices of Energy Metabolism in the Cerebral Cortex of Rats with Experimental Alzheimer's Disease. Neurophysiology, 50(3), pp.159–165.

[7] Bolós, M., Perea, J. R., & Avila, J. (2017b). Alzheimer's disease as an inflammatory disease. Biomolecular Concepts, 8(1), pp.37–43.

[8] Cheignon, C., Tomas, M., Bonnefont-Rousselot, D., Faller, P., Hureau, C., & Collin, F. (2018). Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox Biology, 14, pp.450–464.

[9] Yang, S., & Lian, G. (2019). ROS and diseases: role in metabolism and energy supply. Molecular and Cellular Biochemistry, 467(1–2), pp.1–12.

[10] Khalin, I., Alyautdin, R., Ismail, N. M., Haron, M. H., & Kuznetsov, D. (2014). Nanoscale drug delivery systems and the blood-brain barrier. International Journal of Nanomedicine, 9, pp.795-811.

[11] Deiko, R. D., Shtrygol', S. Y., & Laryanovskaya, Y. B. (2017). Chronic blockade of central muscarinic receptors in rats reproduces primary pathogenetic links of Alzheimer's disease. Actual Problems of Modern Medicine, 17(3), pp.13–25.

[12] ICD-10-CM Diagnosis Code G30.1 (2021). Alzheimer's disease with late onset. [online] Available at: https://www.icd10data.com/ICD10CM/Codes/G00-G99/G30-G32/G30-/G30.1/ [Accessed 10 May. 2021].

[13] Pyatikop, V., Msallam Jr, M. A., Shchegelskaya, E., Kutovoy, I., & Gubina-Vakulik, G. (2014). Migration features of labeled bone marrow mesenchymal stem cells in rats with modeled Parkinson-like syndrome. Ukrainian Neurosurgical Journal, 0(3), pp.42–48.

[14] Piatykop, V., Kaliuzhka, V., Shchegelska, O., & Markevych, M. (2019). Efficacy of fibrin matrix with neuroinduced mesenchymal stem cells transplantation for restoration sciatic nerve function after its complete rupture in rats. Inter Collegas, 6(3), pp.168–173.

[15] Eagle, A., Wang, H., & Robison, A. (2016). Sensitive Assessment of Hippocampal Learning Using Temporally Dissociated Passive Avoidance Task. BIO-PROTOCOL, 6(11), e1821.

[16] Bondarenko, N. (2017). Anxiety and the problem of “inattentive” animals in water maze tests. The Russian Journal of Cognitive Science, 4(4), pp.45–51.

[17] Kostiuk, V., Potapovich, A., & Kovaleva, Z. (1990). Prostoĭ i chuvstvitel’nyĭ metod opredeleniia aktivnosti superoksid- dismutazy, osnovannyĭ na reaktsii okisleniia kvertsetina [A simple and sensitive method of determination of superoxide dismutase activity based on the reaction of quercetin oxidation]. Vopr Med Khim, 36(2), pp.88–91.

[18] Gavrilov, V. B., & Mishkorudnaya, M. I. (1983). Spectrophotometric Determination of the Content of Lipid Hydroperoxides in Blood Plasma. Lab. Delo, 3, pp.33–36.

[19] Tkachenko, A. S., Klochkov, V. K., Lesovoy, V. N., Myasoedov, V. V., Kavok, N. S., Onishchenko, A. I., Yefimova, S. L., & Posokhov, Y. O. (2020). Orally administered gadolinium orthovanadate GdVO4:Eu3+ nanoparticles do not affect the hydrophobic region of cell membranes of leukocytes. Wiener Medizinische Wochenschrift, 170(7–8), pp.189–195.

[20] Goncharov, N. V., Nadeev, A. D., Jenkins, R. O., & Avdonin, P. V. (2017). Markers and Biomarkers of Endothelium: When Something Is Rotten in the State. Oxidative Medicine and Cellular Longevity, 2017, pp.1– 27.

[21] Randi, A. M., & Laffan, M. A. (2017). Von Willebrand factor and angiogenesis: basic and applied issues. Journal of Thrombosis and Haemostasis, 15(1), pp.13–20.

[22] Safar, M. M., Arab, H. H., Rizk, S. M., & El-Maraghy, S. A. (2014). Bone Marrow-Derived Endothelial Progenitor Cells Protect Against Scopolamine-Induced Alzheimer-Like Pathological Aberrations. Molecular Neurobiology, 53(3), pp.1403–1418.

[23] Garcia, K. O., Ornellas, F. L. M., Martin, P. K. M., Patti, C. L., Mello, L. E., Frussa-Filho, R., Han, S. W., & Longo, B. M. (2014). Therapeutic effects of the transplantation of VEGF overexpressing bone marrow mesenchymal stem cells in the hippocampus of murine model of Alzheimer's disease. Frontiers in Aging Neuroscience, 6, p.30.

[24] Koizumi, K., Wang, G., & Park, L. (2015b). Endothelial Dysfunction and Amyloid-β-Induced Neurovascular Alterations. Cellular and Molecular Neurobiology, 36(2), pp.155–165.

[25] Xu, Q. Q., Xu, Y. J., Yang, C., Tang, Y., Li, L., Cai, H. B., Hou, B. N., Chen, H. F., Wang, Q., Shi, X. G., & Zhang, S. J. (2016). Sodium Tanshinone IIA Sulfonate Attenuates Scopolamine-Induced Cognitive Dysfunctions via Improving Cholinergic System. BioMed Research International, 2016, pp.1–9.

[26] Abbott, N. J., Rönnbäck, L., & Hansson, E. (2006). Astrocyte–endothelial interactions at the blood–brain barrier. Nature Reviews Neuroscience, 7(1), pp.41–53.

[27] Shelat, P. B., Chalimoniuk, M., & Wang, J. H. (2008). Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A2 in cortical neurons. J Neurochem, 106(1), pp.45–55.

[28] Nunomura, A., Perry, G., Pappolla, M. A., Friedland, R. P., Hirai, K., Chiba, S., & Smith, M. A. (2000). Neuronal Oxidative Stress Precedes Amyloid-β Deposition in Down Syndrome. Journal of Neuropathology & Experimental Neurology, 59(11), pp.1011–1017.

[29] Habib, L. K., Lee, M. T., & Yang, J. (2010). Inhibitors of Catalase-Amyloid Interactions Protect Cells from β-Amyloid-Induced Oxidative Stress and Toxicity. Journal of Biological Chemistry, 285(50), pp.38933– 38943.

[30] Lee, H. J., Lee, J. K., Lee, H., Carter, J. E., Chang, J. W., Oh, W., Yang, Y. S., Suh, J. G., Lee, B. H., Jin, H. K., & Bae, J. S. (2012). Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation. Neurobiology of Aging, 33(3), pp.588–602.

[31] Bali, P., Bammidi, S., Banik, A., Nehru, B., & Anand, A. (2018). CD34 and CD117 Stemness of Lineage-Negative Cells Reverses Memory Loss Induced by Amyloid Beta in Mouse Model. Front Behav Neurosci, 12, p.222.