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STRUCTURAL CHANGES IN THE BRAIN OF RATS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS AFTER CRYOPRESERVED MESENCHYMAL STEM CELLS IMPACT

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In the chronic course of experimental allergic encephalomyelitis neurodegenerative changes in the brain tissue of rats exacerbate within 2 months. Suboccipital administration of mesenchymal stem cells obtained from human umbilical cord (1×10^6) and cryopreserved in the solution containing 3% dimethyl sulfoxide, 15% ethylene glycol, 10% sucrose, 12% trehalose, and 60% fetal calf serum inhibits these changes in hippocampus (day 35); administration of those cryopreserved in the solution containing 4% dimethyl sulfoxide, 6% trehalose, and 90% fetal calf serum inhibits these changes in both hippocampus (days 35-60) and brain cortex (day 60); administration of these cells cryopreserved in the solution containing 10% dimethyl sulfoxide and 90% fetal calf serum slows down the changes in hippocampus (days 35-60) and partly recover the neuronal component in the brain cortex (day 60).

Key words: mesenchymal stem cells from human umbilical cord, suboccipital administration, brain cortex, hippocampus, pathologically changed neurons, demyelination

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СТРУКТУРНІ ЗМІНИ У ГОЛОВНОМУ МОЗКУ ЩУРІВ З ЕКСПЕРИМЕНТАЛЬНИМ АЛЕРГІЧНИМ ЕНЦЕФАЛОМІЄЛІТОМ ЗА ВПЛИВУ КРІОКОНСЕРВОВАНИХ МЕЗЕНХІМАЛЬНИХ СТОВБУРОВИХ КЛІТИН

За хронічного перебігу експериментального алергічного енцефаломієліту у тканині головного мозку щурів упродовж 2 місяців наростають нейродегенеративні зміни. Мезенхімальні стовбурові клітини пуповини людини (1×10^6), кріоконсервовані у розчині з 3% диметилсульфоксиду, 15% етиленгліколю, 10% сахарози, 12% трегалози, 60% ембріональної телячої сироватки, після субокципітального введення гальмують ці зміни у гіпокампі (35-а доба); кріоконсервовані у розчині з 4% диметилсульфоксиду, 6% трегалози, 90% ембріональної телячої сироватки, – у гіпокампі (35-60-а доба) та корі головного мозку (60-а доба); кріоконсервовані у розчині з 10% диметилсульфоксиду, 90% ембріональної телячої сироватки, – сповільнюють зміни у гіпокампі (35-60-а доба) та частково відновлюють нейрональний компонент у корі головного мозку (60-а доба).

Ключові слова: мезенхімальні стовбурові клітини пуповини людини, субокципітальне введення, кора головного мозку, гіпокамп, патологічно змінені нейрони, демієлінізація

The work is a fragment of the research project "To study the biological properties and determine the regenerative potential of the cryopreserved mesenchymal stem cells of the human umbilical cord in the treatment of motor disorders in the experiment", state registration No.0116U001030.

Etiology and pathogenesis of multiple sclerosis (MS) has been actively studied given its global socio-economic significance, however, the outcomes of this pathology treatment remain unsatisfactory. MS is a chronic immune-mediated demyelinating disease of the central nervous system (CNS) characterized by inflammation, demyelination and axonal degeneration., which leads to the loss of sensory, motor, autonomic and cognitive functions depending on the location of the CNS damage [11]. The MS course is characterized by exacerbation of pathological changes in the white matter with the destruction of myelin sheaths surrounding the axons in different parts of the brain and spinal cord by autoreactive T cells [6]. Perifocal edema of the nerve tissue, which developed due to the important role of inflammatory mediators and vascular changes, is found around the foci of demyelination and axonal degeneration. These changes lead to the disruption of nerve impulses conduction in the axons, aggravating the pathological manifestations and symptoms of the disease course [2]. The model system most similar to human MS is experimental allergic encephalomyelitis (EAE) with a chronic remitting course [3], in which demyelination of nerve fibers prevails over inflammatory changes.

Given the etiology and pathogenesis of MS, the goal of treatment is to reduce inflammation and induce axonal regeneration. In this regard, methods of cell therapy are being tested, in particular those using mesenchymal stem cells (MSCs) -- a heterogeneous population of multipotent cells that can be obtained from various body tissues (such as, adipose tissue, bone marrow, placenta, chorionic villi, umbilical cord, umbilical cord blood) and have not only potential for recovery and differentiation and transdifferentiation (including neurons, oligodendrocytes, and Schwann cells), but also demonstrate pronounced immunomodulatory properties [1, 6].

In murine and rat EAE models it was found that the transplanted xenogeneic MSCs could migrate to the areas of inflammation and demyelination in the brain, express neuroglial markers, restore the number of endogenous oligodendrocytes, reduce the rate of infiltrating inflammatory and CD4+T cells, inhibit proliferation of autoreactive lymphocyte clones and promote repair of myelin sheath of the damaged nerve fibers axons with the recovery of neurological functions [6, 9, 10]. Autologous MSC transplantation in patients with MS helped to stabilize the neurological status and reduced the risk of emergence of active foci of demyelination, and provided an immunomodulatory effect without immunosuppressive therapy [5].

Given the prospects of clinical use of MSCs in demyelinating diseases, it seems relevant to develop optimal protocols for cryopreservation of these cells and to assess their therapeutic potential after the impact of cryopreservation in experimental models.

The purpose of the work is to study the effects of suboccipital administration of mesenchymal stem cells from human umbilical cord cryopreserved in preservatives of different composition on the brain tissue of animals with experimental allergic encephalomyelitis.

Materials and methods. The chronic EAE model was reproduced in outbred adult female rats (n = 32, age 3 months, weight (230±20) g) [9] in compliance with the principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes» (Strasbourg, 1986) and the Law of Ukraine №3447-IV "On protection of animals from cruel treatment" (2006). The animals were anethesized with intraperitoneal administration of a mixture of xylazine (15 mg/kg, "Sedazin", "Biovet", Poland) and ketamine (70 mg/kg, "Calypsol", "Gedeon Richter Ltd", Hungary). See the design of the experiment in the diagram (Fig. 1). On day 18 (the peak of clinical manifestations) experimental rats were suboccipitally injected with a suspension of mesenchymal stem cells from human umbilical cord (hUC-MSCs) (1×10^6/0.1 ml) cryopreserved in solutions of different composition: 1) cryo-1: 3% dimethyl sulfoxide (DMSO), 15% ethylene glycol, 10% sucrose, 12% trehalose, 60% fetal calf serum (FCS); 2) cryo-2: 4% DMSO, 6% trehalose, and 90% FCS; 3) cryo-3: 10% DMSO, 90% FCS. After thawing, the relative number of living cells was determined by trypan blue staining: hUC-MSCs (cryo-1) – 85.4±0.1%; hUC-MSCs (cryo-2) – 94.3±0.2%; hUC-MSCs (cryo-3) – 95.9±0.1%; more than 90% of cells were positive for CD -73, -90, -105 (specific surface markers of MSCs).

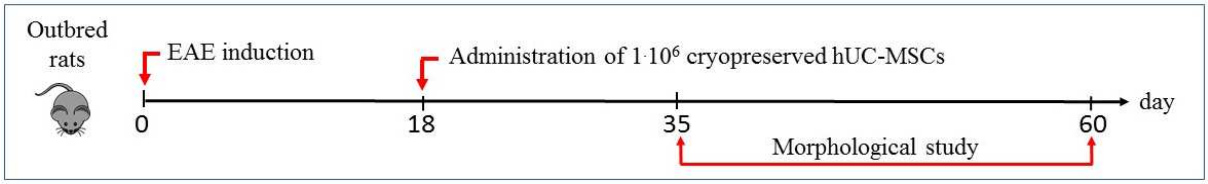

Fig.1. Design of the experimental study

The experimental groups of animals were formed accordingly: group 1 (EAE, n = 7); group 2 (EAE + hUC-MSCs (cryo-1), n = 7); group 3 (EAE + hUC-MSCs (cryo-2), n = 6); group 4 (EAE + hUC-MSCs (cryo-3), n = 8); control (intact animals, n = 4).

On day 35 and 60 after the induction of EAE (on day 17 and day 42 after the administration of hUC-MSCs, respectively) the brain (B) was removed from the rats cranial cavity, fixed in 10% formalin solution (pH 6.9, (Merck, Germany), paraffin tissue blocks were made, with their serial sections (5-7 μm) made with a microtome "Microm HM430" (Germany) and stained with hematoxylin-eosin, hematoxylin-picrofuxin and thionine ("Janssen Chimica"). Histological slides of B sections were studied on a light microscope "NIKON Eclipse E200" (Japan); morphometric analysis of digital images of samples was performed in 20 randomly selected fields of vision (x800) to estimate the quantitative changes of neurons (N) and gliocytes (G). According to morphological criteria the unchanged N (typical, normal structure) and the pathologically changed N (PCN) were tentatively distinguished: dystrophic (with signs of chromatolysis or inclusion of vacuoles) and irreversibly changed and dead N (the shadow cells).

Data were processed using the software package "Statistica 8.0" ("StatSoft, Inc.", 2007). Nonparametric methods of variation statistics (rank variance Kruskal-Wallis test for multiple and Mann-Whitney U-test for pairwise comparison of independent groups) were used. The normality of data distribution was determined by Shapiro-Wilk test. Differences at $p < 0.05$ were considered statistically significant. Data are presented as M (25%; 75%), where M is the median; (25%; 75%) – the quartile interval between the 25th and the 75th percentiles.

Results of the study and their discussion. *Group 1 (EAE).* On day 35 after EAE induction, significant changes of the rat brain tissue were found in the histological slides in comparison with the overall histological structure and architecture of the cortex, the brain white matter and hippocampus of intact animals (control, fig. 2a, b, c). In the brain cortex of rats with EAE clusters of hyperchromic deformed N with a dark pyknotized nucleus and a non-contoured nucleolus were detected (fig. 2d). Processes of some N were thickened, tortuous, which is a morphological manifestation of chronic irritation of these cells. It should be noted that the normal N has the following characteristic features: nuclei of regular rounded shape with a fine-

grained openwork pattern of chromatin substance, narrow cytoplasmic bodies extending into short conical processes, dense distribution of clusters of lumps and grains of basophilic (the so called tigroid or Nissl) substance in the bodies and at the base of dendrites, which is a structural manifestation of the RNA content in the endoplasmic reticulum ribosomes at the optical level. Morphological changes of this basophilic substance reflect the state of nucleic metabolism. On day 35 after induction the content of PCN (with reduced and shrunken cytoplasmic bodies, signs of tigrolysis of the Nissl substance, shortened or reduced processes, deformed nuclei with signs of pyknosis, hyperchromatosis or chromatolysis) in the brain cortex of rats with EAE is significantly higher than the control values (p= 0.001, table 1).

On day 60, the general architecture of the brain cortex of rats from group 1 was preserved (fig. 2e), but the proportion of PCN increased ($p = 3{,}0.10^{-6}$; $p = 5{,}0.10^{-7}$, compared with day 35 and control values; table 1); signs of perivascular edema, fibrosis of the vascular walls, stasis, endotheliocyte proliferation, small focal hemorrhages were detected.

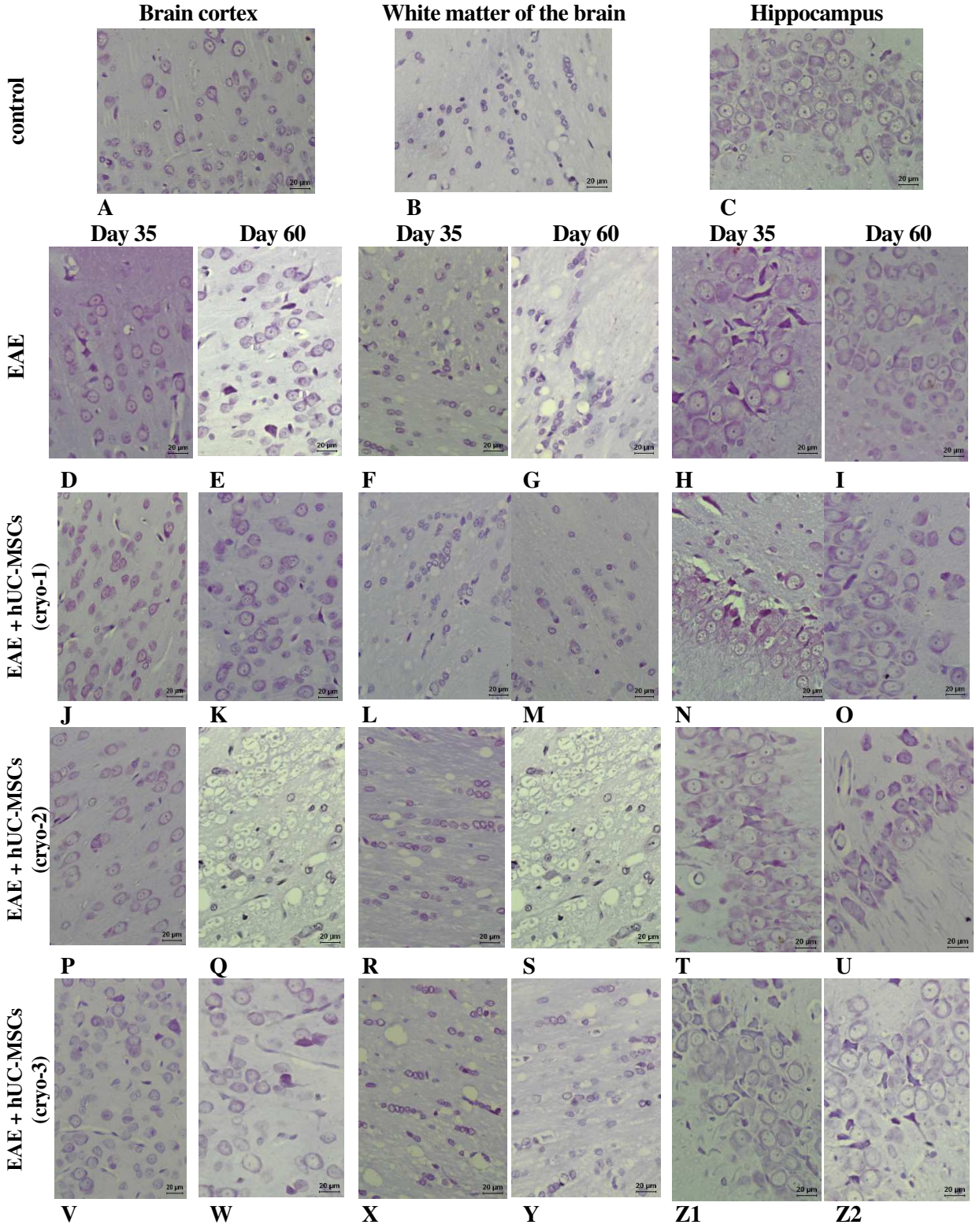

Fig.2. Morphological changes in brain tissue of rats with EAE after suboccipital injection of mesenchymal stem cells from human umbilical cord cryopreserved in solutions of different composition. Thionine staining.

The white matter of rat brain on day 35 after induction of EAE is moderately loose, with multiple rounded cavities (fig. 2f). Swelling of axons is noted in some areas. G mostly retain light nuclei of regular oval shape with a delicate uniform pattern of chromatin; individual cells are pyknotized, hyperchromically stained, the nuclei are vacuolated with signs of destruction. On day 60 in addition to these changes signs of demyelination appear in some areas and single shrunken G with hyperchromic staining (fig. 2g), fibrosis of the walls of individual vessels, the phenomena of stasis and focal hemorrhage are found.

On day 35 after EAE induction the cell layers in the rat hippocampus are loose and dispersed (fig. 2h), in contrast to the intact hippocampus which is characterized by a multilayered distribution of the monomorphic phenotype N with multiple processess (fig. 2c). About a third of N maintain a normal structure; PCN have varying degrees of the cytoplasm and nucleus contents homogenization, lost nucleolus, hyperchromic staining, and thickened tortuous processes. The content of PCN increases from day 35 up to day 60 of observation ($p = 2.0.10^{-10}$, $p = 1.8.10^{-8}$ respectively, in comparison with the control; table 1, fig.2i).

Table 1

Morphofunctional state of brain tissue neurons of rats with EAE under the influence of suboccipital injection of mesenchymal stem cells from human umbilical cord cryopreserved in solutions of different composition

No	Experimental conditions (groups of animals)	Number of pathologically changed neurons (M (25%; 75%))					
		brain cortex			hippocampus		
		Day 35	Day 60	P (Mann –Whitney U-test)	Day 35	Day 60	P (Mann –Whitney U-test)
1	Control (intact animals n = 7)	25 (15; 40) $*_1*_2*_3*_4$	25 (15; 40) $*_5*_6*_7$	$*_1$ □ $p_{1.2} = 0.001$ $*_2$ □ $p_{1.3} = 2.0{\cdot}10^{-5}$ $*_3$ □ $p_{1.4} = 2.0{\cdot}10^{-4}$ $*_4$ □ $p_{1.5} = 1.0{\cdot}10^{-6}$ $*_5$ □ $p_{1.2} = 5.0{\cdot}10^{-7}$ $*_6$ □ $p_{1.3} = 1.0{\cdot}10^{-6}$ $*_7$ □ $p_{1.4} = 4.0{\cdot}10^{-5}$ $\#_1$ □ $p_{2.5} = 4.0{\cdot}10^{-4}$ $\#_2$ □ $p_{3.5} = 6.0{\cdot}10^{-4}$ $\#_3$ □ $p_{4.5} = 0.03$ $\&_1$ □ $p_{2.2} = 3.0{\cdot}10^{-6}$ $\&_2$ □ $p_{3.3} = 2.0{\cdot}10^{-4}$ $\&_3$ □ $p_{4.4} = 0.04$ $\&_4$ □ $p_{5.5} = 0.009$	20 (10; 30) $*_1*_2*_3*_4$	20 (10; 30) $*_5*_6*_7*_8$	$*_1$ □ $p_{1.2} = 2.0{\cdot}10^{-10}$ $*_2$ □ $p_{1.3} = 0.006$ $*_3$ □ $p_{1.4} = 2.0{\cdot}10^{-6}$ $*_4$ □ $p_{1.5} = 1.0{\cdot}10^{-6}$ $*_5$ □ $p_{1.2} = 1.8{\cdot}10^{-8}$ $*_6$ □ $p_{1.3} = 1.8{\cdot}10^{-8}$ $*_7$ □ $p_{1.4} = 0.001$ $*_8$ □ $p_{1.5} = 7.0{\cdot}10^{-4}$ $\#_1$ □ $p_{2.3} = 3.0{\cdot}10^{-5}$ $\&_1$ □ $p_{3.3} = 0.001$ $\&_2$ □ $p_{4.4} = 0.02$
2	group 1 (EAE. n = 7)	40 (30; 50) $*_1\&_1$	60 (50; 70) $*_5\#_1\&_1$		60 (50; 70) $*_1\#_1$	70 (70; 85) $*_5$	
3	group 2 (EAE + hUC-MSCs (cryo-1). n = 7)	50 (40; 50) $*_2\ \&_2$	60 (50; 70) $*_6\ \#_2\&_2$		40 (30; 50) $*_2\#_1\ \&_1$	70 (70; 85) $*_6\ \&_1$	
4	group 3 (EAE + hUC-MSCs (cryo-2). n = 6)	45 (30; 50) $*_3\&_3$	50 (40; 70) $*_7\ \#_3\&_3$		50 (30; 60) $*_3\&_2$	50 (40; 75) $*_7\&_2$	
5	group 4 (EAE + hUC-MSCs (cryo-3. n = 8)	50 (40; 60) $*_4\ \&_4$	40 (30; 50) $\#_1\#_2\#_3\&_4$		50 (40; 60) $*_4$	65 (50; 70) $*_8$	

Note: M is the median; (25%; 75%) – interquartile range; differences are statistically significant: * – in comparison with control; # – between group values; & – between the values of one group on days 35 and day 60.

Group 2 (EAE + injection of hUC-MSCs (cryo-1). On day 35 of the experiment (the 17th day after the administration of hUC-MSCs) the general architecture of the brain was preserved in all parts. Proportion of PCN exceeded the control value ($p = 2{,}0{\cdot}10^{-5}$) and had been increasing up to day 60 of observation ($p = 2.0.10^{-4}$; table 1, fig.2j, k) not differing from the value of rats from the EAE group 1. The degenerated N in the form of shadow cells (12-20%) were found and there were signs of stasis in blood vessels.

On day 35 the structure in the white matter of brain of rats from group 2 was preserved, the nerve fibers were somewhat loose, and there were signs of demyelinating process in some areas: edema and deformation of axons, small round cavities in the deep parts of the brain. Some G were pyknotized, hyperchromically stained, with deformed homogenized nucleus (fig. 2l). On day 60 of the experiment, the state of the brain white matter was similar to that in the previous study period (fig. 2m).

On day 35 in the hippocampus of group 2 rats there were separate PCN with shrunken bodies and thickened processes, hyperchromically stained, with deformed nuclei and their homogenized content (fig. 2n). The proportion of PCN exceeded that of the intact animals, but was significantly lower than that of rats with EAE ($p = 0.006$, $p = 3.0.10^{-5}$, respectively; table 1). On day 60 of the experiment cell layers in some areas were dispersed; the proportion of PCN increased (fig. 2o), significantly exceeding the corresponding value of animals from this group on the day 35 ($p = 0.001$; table 1) and reaching the rate of rats with EAE.

Group 3 (EAE + injection of hUC-MSCs (cryo-2). On day 35 of the experiment (the 17th day after the injection of hUC-MSCs) both individual and quite significant accumulations of PCN were found in brain

cortex (fig. 2p), their proportion exceeded the control value ($p = 2.0.10^{-4}$) and had been increasing up to day 60 of observation ($p = 0.04$; table 1, fig.2q), however, it did not quite reach the value of rats from EAE group 1.

The white matter of rats from group 3 on day 35 was slightly loose with small rounded cavities, with individual axons having signs of edema. The vast majority of G had normal structure, only some of them were deformed, hyperchromically stained, and the content of the nucleus was homogenized (fig. 2r). On day 60 in some areas in the deep parts of the brain there were signs of demyelination with edema and axonal deformation (fig. 2s); individual G were shrunken and hyperchromically stained.

On day 35 in the hippocampus of rats from group 3 there were separate accumulations of PCN (fig. 2t); their proportion exceeded the values of the intact animals ($p = 2.0.10^{-6}$, table 1), however, it was slightly lower than the values of the EAE rats. On day 60 of the experiment, the proportion of PCN increased (fig. 2u), exceeding the corresponding value of animals in this group on day 35 ($p = 0.02$; table 1), but remained lower than the value of rats with EAE.

Group 4 (EAE + injection of hUC-MSCs) (cryo-3). On day 35 of the experiment (the 17th day after the injection of hUC-MSCs) the number of PCN in the brain cortex was higher than the control value ($p = 1.0.10^{-6}$; fig.2v). On day 60 (the 42nd day after the injection of hUC-MSCs) the proportion of PCN significantly decreased as compared to the day 35 value ($p = 0.009$; table 1, fig.2w) and relative to other groups on the 60th day of observation: rats with EAE ($p = 4.0.10^{-4}$) and subsequent administration of hUC-MSCs (cryo-1) ($p = 6.0.10^{-4}$) or hUC-MSCs (cryo-2) ($p = 0.03$; table 1).

On day 35 the white matter of group 4 rats' brain was spongy, with multiple rounded cavities having signs of focal demyelination of axons. Individual G were shrunken, hyperchromically stained, and the contents of the nucleus was homogenized (fig. 2x). Fibrosis of the walls of some vessels and focal hemorrhages on the border between the white and gray matter were found. On day 60 of the experiment, the brain white matter was loose in varying degrees, with multiple rounded cavities in some areas. G mainly retained a normal structure; shrunken, hyperchromically stained G were found in small quantities (fig. 2y). Endothelial proliferation of blood vessels and hemorrhages was observed.

On day 35 in the hippocampus of rats from group 4 the proportion of PCN (fig. $2z_1$) exceeded the values of intact animals ($p = 1.0.10^{-6}$, table 1), but was slightly less than the values of rats with EAE. On day 60 of the experiment, the proportion of PCN tended to increase in comparison with the previous term (table 1, fig. $2z_2$), but remained slightly lower than that of rats with EAE (table 1).

The performed study showed that neurodegenerative and demyelinating processes in the brain of rats with EAE can be corrected by suboccipital administration of hUC-MSCs cryopreserved in solutions of different composition, but it is the properties of cells after cryopreservation which become essential. Administration of hUC-MSCs (cryo-1) to rats at the peak of clinical manifestations of EAE did not change the state of the brain cortex and white matter but slowed down the exacerbation of pathological changes in the hippocampus 1 month after the induction of EAE, although this effect was offset after 2 months of experiment. The effect of hUC-MSCs (cryo-1) has lasted for at least 17 days after administration, so when using this variant of the solution for cryopreservation, it is obviously necessary to re-administer hUC-MSCs to prolong the therapeutic effect. The injection of hUC-MSCs (cryo-2) has inhibited the process of pathological changes exacerbation in the hippocampus for 2 months and showed a similar trend in the brain cortex on day 60 after the induction of EAE. The effect of hUC-MSCs (cryo-2) has lasted for 42 days after administration, so the use of this variant of the solution for cryopreservation is obviously more appropriate than the previous one (cryo-1). It is possible that repeated administration of hUC-MSCs, cryopreserved under these conditions, will increase the therapeutic effect. The injection of hUC-MSCs (cryo-3) revealed a tendency to restoring the content of normal N in the brain cortex on day 60 after induction of EAE, as well as inhibiting the process of exacerbation of pathological changes in the hippocampus within 2 months of follow-up. That is, the regenerative and/or modulating effect of hUC-MSCs (cryo-3) on brain N have lasted for 17- 42 days after administration; at the same time, no signs of inhibition of the demyelination process in the brain white matter were found during these observation periods. Obviously, to obtain a complex therapeutic effect on the brain of rats with EAE during cryopreservation of hUC-MSCs, it is advisable to give preference to solutions of another composition (cryo-2).

The mentioned differences in the effects of the injected cells are not related to their viability (at least 85% when using all 3 variants of solutions for cryopreservation), and obviously depend on the biological properties of hUC-MSCs (cryo-2) demonstrated *in vitro*: better ability of adhesion, expansion and monolayer formation, as compared with hUC-MSCs (cryo-1) and (cryo-3), which corresponds to the long-term therapeutic effect of hUC-MSCs (cryo-2) for 42 days after administration to rats with EAE.

Considered among the possible explanations for the phenomena of MSC impact are their properties, such as the ability to migrate and tropism to the damaged area, the potential for differentiation into CNS cells

under the influence of the brain microenvironment and integration into the nervous tissue of the recipient [4, 7, 12]. The immunomodulatory effect, aimed at reducing the inflammatory response and activation of reparative neuroregeneration and mediated by humoral and ligand-receptor interactions due to the secretion of soluble trophic factors is an important mechanism of the effects of transplanted MSCs [6, 8, 10].

In our study of brain tissues of rats with EAE after suboccipital administration of hUC-MSCs cryopreserved in solutions (cryo-1) and (cryo-2), inhibition of the neurodegenerative and demyelinating process was only noted. In this regard, further search for cryopreservation options to preserve the biological properties of hUC-MSCs and to develop modes and ways of their administration remains relevant.

Conclusion

In rats with simulated EAE of moderate severity with chronic remitting course signs of neurodegenerative and demyelinating process in brain tissue have exacerbated in the course of observation from the day 35 to day 60. hUC-MSCs cryopreserved in a solution of 3% DMSO, 15% ethylene glycol, 10% sucrose, 12% trehalose, 60% FCS, after suboccipital administration to rats with EAE at the peak of clinical manifestations (the 18th day) demonstrates a short-term effect, by slowing down the pathological progression in the hippocampus on day 35 of the experiment. The effect of hUC-MSCs cryopreserved in the solution of 4% DMSO, 6% trehalose, and 90% FCS lasts longer inhibiting the exacerbation of pathological changes in the brain cortex on day 60 and the hippocampus on day 35-60 of the experiment. hUC-MSCs cryopreserved in solution with 10% DMSO and 90% FCS, show a tendency to restore the neuronal component in the brain cortex on day 60 and slow down the exacerbation of pathological changes in the hippocampus on day 35-60 of observation; at the same time they do not affect the structural changes of the white matter of the experimental animals brain.

References

1. Zafranskaia MM, Fedulov AS, Demydchyk UE. Effect mezenkhimalnykh stvolovykh kletok pri kletochnoy terapii rasseyannogo skleroza. Minsk: Belarus. Navuka; 2016. 213 p. [in Russian]

2. Nefodov AA, Mamchur VI, Tverdokhleb IV. Osobennosti ultrastruktury frontalnoy kory i hippokampa krys v usloviyakh eksperimentalnogo allergicheskogo entsefalomielita. Morphologia. 2016; 10(1):54-61. [in Russian]

3. Nefodov AA, Mamchur VI, Kharchenko YV. Modelyuvannya ta otsinka perebihu eksperymentalnoho allergichnoho entsefalomiyelitu. Bulletin of problems in biology and medicine. 2014; 4(2):205-08. [in Ukrainian]

4. Pichkur LD, Kovalchuk MV, Deriabina OG, Verbovska SA, Akinola ST, Shuvalova NS, Kordium VA. Vyzhivannya transplantovanykh mezenkhimalnykh stovburovykh klityn vartonovykh dragliv pupovyny lyudyny v tsentralniy nervoviy systemi shyuriv pry eksperymentalnomu alergichnomu entsefalomiyeliti pislya yikh suboktsipitalnoho vvedennya. Ukrainian Neurosurgical Journal. 2017; 3: 30-35. doi:10.25305/unj.112102. [in Ukrainian]

5. Fedulov AS, Borisov AB, Moskovskikh YuV, Zafranskaia MM, Krivenko SI, Nizhegorodova DB et al. Autologichnaya transplantatsia mezenkhimalnykh stvolovykh kletok kak metod modifikatsii klinicheskogo techeniya rasseyannogo skleroza. Neurology and neurosurgery. Eastern Europe. 2016; 4: 516-22. [in Russian]

6. Abumaree MH, Al Saad K, Aldlamy M, Abomaray F, Jawdat D, Al Jumah M. Human placental mesenchymal stem cells (pMSCs) modulate the immunopathogenesis process in experimental autoimmune encephalomyelitis (EAE). ECTRIMS Online Library. 2015 Oct 9; 115237. Available from: https://onlinelibrary.ectrims-congress.eu/ectrims/2015/31st/115237/mohammed .abumaree.human.placental .mesenchymal. stem.cells. 28pmscs 29.modulate.the.html?f=m1.

7. Gennai S, Monsel A, Hao Q, Liu J, Gudapati V, Barbier EL, Lee JW. Cell-based therapy for traumatic brain injury. Br J Anaesth. 2015; 115(2):203-12.

8. Glenn JD, Smith MD, Kirby LA, Baxi EG, Whartenby KA. Disparate Effects of Mesenchymal Stem Cells in Experimental Autoimmune Encephalomyelitis and Cuprizone-Induced Demyelination. PLoS One. 2015 Sep 25; 10(9):e0139008. Available from: https://doi.org/10.1371/journal.pone.0139008

9. Jiang H, Zhang Yu, Tian K, Wang B, Han Sh. Amelioration of experimental autoimmune encephalomyelitis through transplantation of placental derived mesenchymal stem cells. Sci. Rep. 2017; 7:41837. Available from: https://doi.org/10.1038/srep41837.

10. Nasri F, Mohtasebi M, Hashemi E, Zarrabi M, Gholijani N, Sarvestani EK. Therapeutic Efficacy of Mesenchymal Stem Cells and Mesenchymal Stem Cells-derived Neural Progenitors in Experimental Autoimmune Encephalomyelitis**.** IJSC. 2018; 11:68-77. Available from: https://doi.org/10.15283/ijsc17052.

11. Silva BA, Leal MC, Farías MI, Avalos JC, Besada CH, Pitossi FJ, Carina Cintia Ferrari CC. A new focal model resembling features of cortical pathology of the progressive forms of multiple sclerosis: Influence of innate immunity. Brain, Behavior, and Immunity. 2018; 69:515–531. DOI: 10.1016/j.bbi.2018.01.010

12. Tajiri N, Duncan K, Antoine A, Pabon M, Acosta SA, de la Pena I, Hernadez-Ontiveros DG, Shinozuka K, Ishikawa H, Kaneko Y, Yankee E, McGrogan M, Case C, Borlongan CV. Stem cell-paved biobridge facilitates neural repair in traumatic brain injury. Front. Syst. Neurosci. 2014; 8:116. doi: 10.3389/fnsys.2014.00116

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