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Correlation peculiarities between morphometric and flow cytometric indexes in the rat hippocampus and somatosensory cortex afterward cerebral ischemia-reperfusion and its correction

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Abstract. The increase in the ischaemic stroke incidence has become one of the main problems in the world due to high its disablement and mortality. The purpose of the study was to establish and analyse the correlations between the number of affected neuronal nuclei in the somatosensory cortex and hippocampus and flow cytometric indexes in rats having model cerebral ischaemia-reperfusion. The effects of mesenchymal stromal cells (acquired from human umbilical cord, rat and human adipose tissue, and rat embryonic fibroblasts and their lysate) on morphometric and flow cytometric parameters in the hippocampus and somatosensory cortex of adult Wistar rats (at the age – 3-4 months old, with a body weight – 160-190 g, after model cerebral ischaemia-reperfusion) were explored. The neuronal nuclei total numbers per 1 mm and the ratio of the intact neuronal nuclei number to the pathologically affected neuronal nuclei number (having karyopyknosis or karyorrhexis) were counted in the somatosensory cortex and hippocampal CA1 area. Nonparametric Spearman's correlation rank analysis was used to determine relationships between individual parameters. When using mesenchymal stromal cells having different ancestry and their lysate as therapy for ischaemia-reperfusion damage to brain structures, multidirectional correlations (both direct and inverse) were found between flow cytometry parameters and the affected neuronal nuclei number on day 7 and 14 after the ischaemia-reperfusion modelling, both in the somatosensory cortex and in the hippocampus. Thus, the results of the correlation analysis demonstrated that mesenchymal stromal cells of different ancestry have a distinct neuroprotective effect aimed at restoring neurogenesis in brain structures and suppressing the intensity of neuroapoptosis in post-perfusion injuries. The data obtained from the correlation analysis will be used to determine the most effective stem cells' class as a neuroprotectant and further develop an injectable drug based on it for the ischaemic stroke therapy

Keywords: mesenchymal stromal cells; cerebral ischaemia-reperfusion; morphometry; neuroapoptosis; correlation analysis

INTRODUCTION

The progressive increase in the number of brain disease cases accompanied by cerebrovascular disorders makes this problem of modem medicine increasingly relevant around the world. The most common of these is a stroke, a neurological disease with high mortality and disablement [1]. Ischaemic stroke amounts for about 70% of strokes, and this figure is growing every year due to ageing of a population and other causes [2]. An acute ischaemic stroke is a pathological process evoked by an abrupt cessation of cerebral blood circulation leading to the neuronal death and causing an irreversible damage to brain tissue. Up to 2025, the main treatment for ischaemic stroke was thrombolysis and mechanical thrombectomy. However, C.T. Primiani *et al*. [3] showed that problems with accessibility due to the narrow time interval and the bleeding risk make these treatments possible in only 5%

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of patients. M. Li *et al*. [4] emphasised that that perfusion restoration in the ischaemic zone contributes to the aggravation of metabolic disorders in the brain and development of reperfusion injury.

Moreover, as W.J. Powers *et al*. [5] showed, further search for effective and safe treatments is still relevant. As for 2022, according to M. Kawabori *et al*. [6] therapy with stem cells has shown encouraging results in the ischaemic stroke treatment. N.G. Toman [7] analysed that numerous preclinical studies in animal models have shown that therapy by stem cells transplantation reduces ischaemic brain injury through targeting nerve cells proliferation and differentiation. Amongst different stem cells types, mesenchymal stromal cells (MSCs) were proven by J. Li *et al*. [8] to be the best choice for ischaemic stroke treatment due to such their characteristics as availability, ease of isolation and cultivation, sufficient immune tolerance, few complications during treatment. The study by J.W. Chung *et al*. [9] showed that autologous MSCs administration with autologous serum intravenously was permissible and safe in persons having a chronic stroke.

B. Brooks *et al*. [10] discovered that they also possess plasticity and the ability to undergo multidirectional differentiation, have anti-inflammatory and immunomodulatory actions, which may contribute to regeneration in the ischaemic brain tissue. Thus, the study by Y. Li *et al*. [11] demonstrated that human umbilical cord MSC therapy in combination with curcumin had anti-inflammatory and antioxidant efficacy and contributed to the neurological function improvement after an acute ischaemic stroke. H. Cao *et al*. [12] found that the combined therapy with umbilical cord MSCs and tetramethylpyrazine improved neurogenesis, suppressed inflammation, and reduced histological damage after cerebral ischaemia in rats. Therefore, the purpose of this study was to find out and analyse correlations between the affected neuronal nuclei number in the somatosensory cortex and hippocampus and flow cytometry indexes in rats subjected to model cerebral ischaemia-reperfusion (IR) and therapeutic MSCs transplantation (of different genesis) or MSCs transplantation of cell lysate from human Wharton's jelly (hWJ).

MATERIALS AND METHODS

The research was conducted at the Educational and Research Laboratory for Preclinical Evaluation of New Medicinal Products and Biologically Active Compounds "Farmadar" (Certificate for Technic Competence No. 031/18 suitable for 31.10.2023) of Vinnytsya National Pirogov Memorial Medical University (for 2021-2023 years) on rats (adult Wistar rats at the age – 3-4 months old, with a body weight – 160-190 g). The rats were held on the standard water-food diet under normal vivarium conditions with controlled lighting (12-hour light cycle), 20-26°C room temperature, 40-70% air humidity, with unlimited food and water access. The study protocol was confirmed by the Bioethics Committee of Vinnytsya National Pirogov Memorial Medical University (protocol No. 2 of January 31, 2024). All experimental studies were carried out in accordance with modern methodological approaches and in compliance with relevant requirements and standards, in particular, European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [13]. The animals were kept and all manipulations were carried out in accordance with the provisions of the Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" [14].

MSCs and cell lysate from hWJ-MSCs were obtained from the Institute of Molecular Biology and Genetics at the Ukrainian National Academy of Sciences. The cells were transferred on the basis of the Agreement on Scientific Cooperation between the Institute of Molecular Biology and Genetics and Vinnytsya National Pirogov Memorial Medical University of September 22, 2017. Brain cortex morphology of rats and humans is similar, making rats ideal experimental animals. The therapeutic effect and cytoprotective properties of MSCs (of different ancestry) and cell lysate from hWJ-MSCs were studied in rats right after the model transient ischaemia-reperfusion of a rat brain (the internal carotid arteries were legated for 20 minutes bilaterally, Propofol-Novo medication was used for the central anaesthesia, produced by Novopharm-Biosynthesis, Ukraine, at the dose 60 mg/kg). The distribution of rats into experimental groups in the study was as follows (Fig. 1).

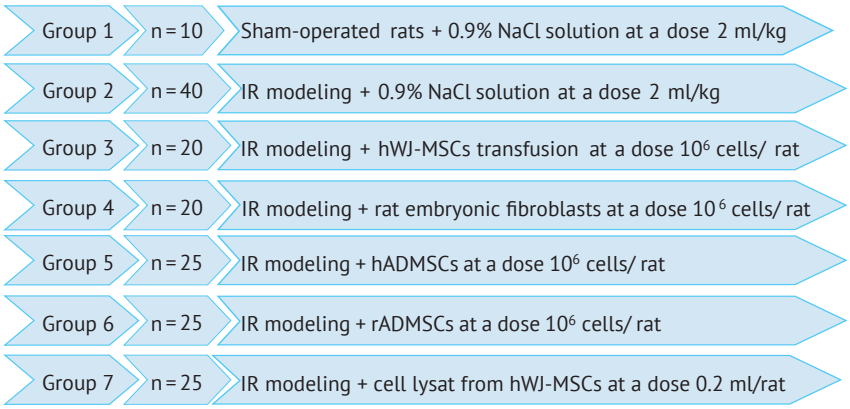

Figure 1. Distribution of rats into experimental groups in the study
Source: compiled by the author

MSCs and cell lysate from hWJ-MSCs were administered into the femoral vein right after IR, as an early transplantation had a better cerebroprotective effect and needed smaller donor MSCs number (1×10^6) [15]. On Day 7

(subacute ischaemic period) and Day 14 (recovery ischaemic period) days after the IR, animals were euthanised by the humane method (decapitation) using central pentobarbital anaesthesia (Penbital, produced by Bioveta, Czech Republic, 100 mg/kg) [16]. Rat brains were extracted, morphological studies were performed, and the DNA fragmentation level in the neuronal nuclei of the hippocampus and somatosensory cortex was determined using by flow cytometry method [17]. Nuclear suspensions of rat sensorimotor cortex and hippocampus biopsies, made according to SyStain DNA Step 1 (Partec apparatus, Germany) were prepared immediately after sampling and washing with cold (temperature +4 to +8°C) phosphate-salt buffer solution having pH 7.4 (Sigma). Flow cytometry was performed on the Partec PAS multifunctional flow cytometer (Partec, Germany). UV rays were used to excite DAPI fluorescence. 20,000 events were analysed from each nuclear suspension sample. Cyclic cell analysis was performed using the FloMax software suite (Partec, Germany) in a full digital accordance to the mathematic pattern, which determined: G0G1% as the ratio (in %) of the G0G1 phase cells to all the cells in the cell cycle (DNA content – 2s); S% as the ratio (in %) of the DNA synthesis phase cells to all the cells in the cell cycle (DNA content – between 2s and 4s); G2+M% as the ratio (in %) of the G2+M phase cells to all the cells in the cell cycle (DNA content – 4s);

DNA fragmentation (i.e. apoptosis) was determined through SUB-GOGI region highlighting in DNA histograms – RN1 prior to the G0G1 peak, which indicates the cell nuclei having DNA content less 2s. For morphological studies, the extracted rat brains were fixed in 4% formaldehyde solution for 24 hours at the appropriate time points. After fixation, the brains were washed in running water, passed through ascending alcohols and xylenes, and after the standard histological wiring, embedded in Paraplast Plus (Leica Scientific (McCormicke), USA). 5 μm thick sections were made in a rotary microtome. Deparaffinised sections were dyed with haematoxylin-eosin (ordinarily) as well as by the Nissl method. Morphometric analysis was performed in an automatic mode.

Digital images of the frontal brain slices obtained on the BX-51 microscope (Olympus, Japan) were analysed using the ImageJ computer programme (1.48v, free license, Rasband, USA, 2015). The total number of neuronal nuclei in 1 mm of the somatosensory cortex and hippocampal CAl area, and the ratio of the intact neuronal nuclei number to the pathologically changed neuronal nuclei number (i.e. with karyorrhexis and karyopyknosis) were counted. Statistical processing of the research results was carried out using the Statistica 7.0 software suite (StatSoft Inc., USA) by the methods of non-parametric (Wilcoxon-Mann-Whitney U-test) statistics. Differences in the average values of indicators between comparison groups were considered significant at $p \leqslant 0.05$. Spearman's non-parametric correlation rank analysis was used to determine the relationship between individual parameters. Correlation dependence reflected the statistical relationship of two (or more) random variables, while changes in the values of one (or more) of these variables accompany a systematic change in the values of another (or other) variables.

RESULTS

A correlation relationship reflects a statistical relationship between two (or more) random variables, whereby changes in the values of one (or more) of these variables accompany a systematic change in the values of another (or other) variables. When analysing the significant correlations between flow cytometry parameters and the damaged neuronal nuclei number in the hippocampus and somatosensory cortex of rats with IR (Group 2), a single strong inverse ($r = -0.90$, $p < 0.05$) relationship between the G0G1% in the hippocampus and the damaged nuclei number in the hippocampus on Day 7 after IR was discovered; including a direct ($r = 0.90$, $p < 0.05$) relationship between the G2 + M% in the hippocampus on Day 7 and the damaged nuclei number (with karyopyknosis and karyorrhexis) in the hippocampus on Day 14 after IR (Table 1).

Table 1. Correlation between the damaged neuronal nuclei number in the hippocampus and somatosensory cortex and flow cytometry indexes in rats with model brain IR and in the background of correction

Parameters	The number of damaged nuclei (karyopyknosis, karyorrhexis) in the somatosensory cortex				The number of damaged nuclei (karyopyknosis, karyorrhexis) in the hippocampus			
	Day 7		Day 14		Day 7		Day 14	
	r	p	r	p	r	p	r	p
IR (control pathology)								
G0G1	-0.21	0.7406	0.30	0.6238	**-0.90**	**0.0374**	-0.60	0.2848
S	0.21	0.7406	-0.30	0.6238	1.00		0.30	0.6238
G2+M	0.82	0.0886	0.50	0.3910	0.10	0.8729	**0.90**	**0.0374**
Sub-G0G1	0.82	0.0886	-0.10	0.8729	0.10	0.8729	-0.60	0.2848
IR + human umbilical cord Wharton's jelly MSCs								
G0G1	0.50	0.3910	0.00	1.0000	0.60	0.2848	-0.50	0.3910
S	-0.40	0.5046	0.30	0.6238	0.10	0.8729	**0.90**	**0.0374**
G2+M	-0.30	0.6238	-0.40	0.5046	**-0.90**	**0.0374**	-0.50	0.3910
Sub-G0G1	-0.10	0.8729	0.20	0.7471	-0.10	0.8729	**0.90**	**0.0374**
IR + rat embryonic fibroblasts								
G0G1	0.10	0.8696	**-0.90**	**0.0374**	0.53	0.3615	-0.82	0.0886
S	-0.36	0.5528	0.40	0.5046	-0.37	0.5411	0.67	0.2189
G2+M	-0.10	0.8696	**0.90**	**0.0374**	-0.53	0.3615	**0.97**	**0.0048**

Table 1. Continued

Parameters	The number of damaged nuclei (karyopyknosis, karyorrhexis) in the somatosensory cortex				The number of damaged nuclei (karyopyknosis, karyorrhexis) in the hippocampus			
	Day 7		Day 14		Day 7		Day 14	
	r	p	r	p	r	p	r	p
Sub-G0G1	-0.82	0.0886	0.70	0.1881	-0.53	0.3615	0.15	0.8048
IR + human adipose tissue MSCs								
G0G1	0.30	0.6238	-0.21	0.7406	-0.80	0.1041	0.20	0.7471
S	-0.20	0.7471	-0.21	0.7406	**0.90**	**0.0374**	-0.10	0.8729
G2+M	-0.70	0.1881	0.87	0.0539	0.30	0.6238	-0.20	0.7471
Sub-G0G1	0.40	0.5046	-0.21	0.7406	0.50	0.3910	-0.50	0.3910
IR + rat adipose tissue MSCs								
G0G1	0.10	0.8696	**0.90**	**0.0374**	0.10	0.8729	0.20	0.7471
S	-0.21	0.7406	-0.80	0.1041	0.00	1.0000	0.10	0.8729
G2+M	0.21	0.7406	-0.50	0.3910	-0.60	0.2848	-0.80	0.1041
Sub-G0G1	0.15	0.8048	-0.30	0.6238	-0.20	0.7471	0.50	0.3910
IR + cell lysate from hWJ-MSCs								
G0G1	-0.30	0.6238	**0.90**	**0.0374**	**-0.95**	**0.0138**	-0.60	0.2848
S	0.30	0.6238	**-0.90**	**0.0374**	0.32	0.6042	0.70	0.1881
G2+M	-0.10	0.8729	-0.30	0.6238	**0.95**	**0.0138**	0.60	0.2848
Sub-G0G1	0.30	0.6238	**-0.90**	**0.0374**	0.53	0.3615	**0.90**	**0.0374**

Notes: r – rank correlation coefficient; p – probability of the expected result
Source: compiled by the author

Correlations analysis made between flow cytometry parameters and the damaged neuronal nuclei number in the hippocampus and somatosensory cortex in the rats with IR and consequent immediate intravenous transplantation of human umbilical cord MSCs (Group 3) revealed strong direct ($r = 0.90$, $p < 0.05$ in both cases) relationships between the S% and the number of damaged nuclei (having karyopyknosis and karyorrhexis) in the hippocampus on Day 14 after IR and corresponding therapy, as between the SUB-G0G1 areas in the DNA histograms – RNI before the G0G1 peak, which indicates cell nuclei with DNA content less 2s and the damaged nuclei number in the hippocampus on Day 14 after IR and corresponding therapy. An inverse ($r = -0.90$, $p < 0.05$) relationship was found between the G2 + M% and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus on Day 7 after IR and corresponding therapy (Table 1).

In rats of the Group 4 (i.e. in the rats having IR followed by rat embryonic fibroblasts transplantation) a strong direct ($r = 0.90$, $p < 0.05$) relationship between the G2 + M% on Day 7 and the damaged nuclei number (with karyopyknosis and karyorrhexis) in the somatosensory cortex on Day 14 after IR and corresponding therapy was found. In addition, an inverse ($r = -0.90$, $p < 0.05$) relationship between the G0G1% on Day 7 and the damaged nuclei number in the somatosensory cortex on Day 14 after IR and corresponding therapy was observed. A direct ($r = 0.97$, $p < 0.01$) relationship between the G2 + M% in the hippocampus on Day 7 and the damaged nuclei number also in the hippocampus on Day 14 after IR and corresponding therapy was found (Table 1).

In the Group 5 (where rats were undergone IR and subsequent MSCs administration having human adipose tissue origin) a strong direct ($r = 0.90$, $p < 0.05$) relationship between the S% in the hippocampus and the damaged nuclei number (with karyopyknosis and karyorrhexis) in the hippocampus on Day 7 after IR and corresponding therapy was (Table 1) was found. The correlations analysis made in the Group 6 (rats with IR and consequent MSCs of the rat adipose tissue origin administration) showed a significant strong direct correlation ($r = 0.90$, $p < 0.05$) between the G0G1% in the somatosensory cortex on Day 7 and the damaged nuclei number (karyopyknosis, karyorrhexis) in the somatosensory cortex on day 14 after IR and corresponding therapy (Table 1).

Last group (Group 7) of rats (which had IR and immediate cell lysate from hWJ-MSCs transplantation) demonstrated significant correlations between flow cytometry parameters and the damaged neuronal nuclei number, such as strong direct ($r = 0.90$ to 0.95, $p < 0.05$) relationships between the GOG1% in the somatosensory cortex on Day 7 and the damaged nuclei number (with karyopyknosis and karyorrhexis) in the somatosensory cortex on Day 14 after IR and corresponding therapy, between the G2 + M% and the damaged nuclei number in the hippocampus on Day 7 after IR corresponding MSCs therapy, between the SUB-G0G1 region highlighting in DNA histograms – RN1 prior to the G0G1 peak, which indicates the cell nuclei having DNA content less 2s in the hippocampus and the damaged nuclei number also in the hippocampus on Day 14 after IR and corresponding therapy. Strong inverse ($r = -0.90$ to -0.95, $p < 0.05$) relationships were found in this group between the S% in the somatosensory cortex and DNA fragmentation ibid on Day 14; between the SUB-G0G1 region highlighting in DNA histograms – RN1 prior to the G0G1 peak, which indicates the cell nuclei having DNA content less 2s in the somatosensory cortex and the damaged nuclei number also in the somatosensory cortex on Day 14 after IR and corresponding therapy; between the G0G1% in the hippocampus on Day 7 and the damaged nuclei number in the hippocampus on Day 7 after IR and corresponding therapy (Table 1).

When analysing the correlations between flow cytometry parameters and the damaged neuronal nuclei number in the hippocampus and somatosensory cortex in rats with experimental acute IR lesions and their MSC therapy, the principle of data independence was strictly adhered, which implies that the values of variables in one sample are not related to the values of variables in another sample with which the comparison is made. The study found that intravenous allogeneic and xenogeneic transplantations of MSCs having different origins and the cell lysate from hWJ-MSCs introduction at once after IR modelling in rats reduce the volume of ischaemic brain damage. The demonstrated cerebroprotective properties of MSCs transplantations may indicate the prospect of their use in cell therapy of acute cerebral ischaemia.

DISCUSSION

Numerous preclinical studies in animal models have shown that stem cell transplantation therapy reduces ischaemic brain damage and neurological deficits through directed proliferation and differentiation of nerve cells [7, 18]. In the group of rats with model IR (without treatment), when analysing the correlations between flow cytometry parameters and the damaged neuronal nuclei number (both in the hippocampus and somatosensory cortex) during the studied periods, there were no correlations in the somatosensory cortex. The author found mainly single linear relationships in the hippocampus between the damaged nuclei number (with karyopyknosis and karyorrhexis) and the G0G1% in the hippocampus (on Day 7 after IR, $r = -0.90$, $p < 0.05$), indicating an increase in neuroapoptosis intensity in the rat hippocampus. There is a direct correlation between the G2 + M% in the hippocampus and the damaged nuclei number in the hippocampus (on Day 14 after IR, $r = 0.90$, $p < 0.05$), which may indicate neuronal recovery beginning in the hippocampus on Day 14 after IR.

In rats from the Group 3 (i.e., rats with a model of acute subtotal cerebral IR injury and subsequent therapeutic MSCs transplantation of umbilical cord hWJ), there was no correlation in the somatosensory cortex between the flow cytometry parameters and the number of damaged neuronal nuclei, both on Day 7 and Day 14 after IR. Attention should be drawn to the reliable linear multiple dependencies between the G2 + M% in the hippocampus and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus on Day 7 after IR, $r = -0.90$, $p < 0.05$), thus, there is a feedback indicating the regenerative potential of the umbilical cord hWJ MSCs after experimental IR, which may be explained not by the substitution of affected cells in the ischaemic zone, but by their release of bioactive substances that facilitate neurogenesis and defend brain tissue from ischaemic damage [19]. Mechanisms underlying favourable outcomes in MSC transplantation include "bystander" effects, paracrine mechanisms, or restorative effects mediated by extracellular vesicles [10].

Thus, the study by A.A. Taei *et al*. [20] found that MSC secretome therapy reduced inflammation, apoptosis, and neuronal loss in the ischaemic brain. On Day 14 day after IR, strong direct correlations were found between the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus and the S% in the hippocampus ($r = 0.90$, $p < 0.05$) and DNA fragmentation level also in the hippocampus ($r = 0.90$, $p < 0.05$). Thus, under the influence of umbilical cord hWJ MSCs in rats on Day 14 after IR, positive correlations were observed in the hippocampus towards endogenous neuroregeneration and switching of necrotic neuronal death to apoptotic. It is known from literary sources that programmed cell death by apoptosis occurs in the late stages of ischaemic stroke progression and in neurons of the ischaemic penumbra. During apoptosis, DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, development of apoptotic bodies, which are subsequently subjected to phagocytosis, occur in the cell. That is why the apoptotic death of neurons has certain advantages over necrosis and is a "lesser evil" for the brain, although the total number of cells will be reduced [21]. The results of the study are consistent with those obtained by H. Cao *et al*. [12]. The authors found that treatment with umbilical cord MSCs can stimulate neurogenesis while reducing the degree of damage and inflammation, and improve neuroprotection after the ischaemic brain lesion.

In Group 4 rats having rat embryonic fibroblasts transplantation just after IR modelling, a direct correlation was found between the G2 + M% in the somatosensory cortex and the damaged nuclei number in the somatosensory cortex (on Day 14 after IR, $r = 0.90$, $p < 0.05$). Analogous correlations were observed in the hippocampus between the G2 + M% and the damaged nuclei number (on Day 14 after IR, $r = 0.97$, $p < 0.01$). Thus, after intravenous transplantation of rat embryonic fibroblasts on Day 14 after IR, positive correlations were found in the brain damage in rats due to paracrine effects on neurogenesis in both the somatosensory cortex and hippocampus.

In rats from the Group 5 (i.e., rats with a model of brain IR followed by human adipose tissue MSCs transplantation), there was no correlation in the somatosensory cortex between the flow cytometry parameters and the damaged neuronal nuclei number in the periods studied after IR. There was a direct correlation between the S% in the hippocampus and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus (on Day 7 after IR, $r = 0.90$, $p < 0.05$), which may indicate the activation of endogenous neurogenesis in the hippocampus by paracrine signalling of human adipose tissue MSCs through trophic factors. The results of the current research are consistent with the data of K. Yatsenko *et al*. [21], according to which the described effects are intermediated by the secretome of human adipose tissue MSCs, since these cells do not have direct contact with the brain of experimental animals when injected intravenously.

In rats with a model of brain IR followed by the therapeutic MSCs transplantation of rat adipose cells, there was no correlation in the hippocampus between flow cytometry parameters and the damaged neuronal nuclei number in the subacute and recovery periods of IR. A strong direct correlation was found between the G0G1% in the somatosensory cortex and the damaged nuclei number (karyopyknosis, karyorrhexis) in the somatosensory cortex (on Day 14 after IR, $r = 0.90$, $p < 0.05$), which reflects the protective potential of MSCs of the rat adipose tissue on nervous cells present in the somatosensory cortex.

A study by C.T. Van Velthoven *et al*. [22] found that MSCs reduce ischaemic damage that occurs during experimental reversible occlusion of the middle cerebral artery in newborn rats, protect white matter in a brain, and make better long-term functional curing after a focal stroke. They found a positive correlation between the white matter integrity (which was affected by MSCs) and functional performance analysed in ischaemic newborn rats. In Group 7 rats with a model cerebral insult followed by the MSCs transplantation of cell lysate from hWJ multiple linear correlations between flow cytometry parameters and the damaged neuronal nuclei number in the period after the insult in both the somatosensory cortex and hippocampus are noteworthy.

The author found multiple negative correlations between the S% in the somatosensory cortex and DNA fragmentation level in the somatosensory cortex with the damaged nuclei number (karyopyknosis, karyorrhexis) in the somatosensory cortex (on Day 14 after IR, $r = -0.90$ in both cases, $p < 0.05$), which may indicate that the MSCs transplantation of cell lysate from hWJ has no effect on neurogenesis and neuroapoptosis in the somatosensory cortex after IR in rats. In addition, the transition from an inverse correlation (on Day 7 after IR) to a direct correlation (on Day 14) between the G0G1% in the somatosensory cortex and the damaged nuclei number (karyopyknosis, karyorrhexis) in the somatosensory cortex (on Day 14 after IR, $r = 0.90$, $p < 0.05$), in authors' opinion, may indicate the neuroprotective effect of cell lysate from hWJ MSCs on the neurons of the somatosensory cortex.

However, MSCs of the cell lysate from hWJ had no protective action on hippocampal neurons against ischaemia-reperfusion injury on Day 7 after IR. This was evidenced by a strong inverse correlation between the G0G1% in the hippocampus on Day 7 and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus (on Day 7 after IR, $r = -0.95$, $p < 0.05$). There were strong direct correlations between the G2 + M% in the hippocampus and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus (on Day 7 after IR, $r = 0.95$, $p < 0.05$) and between DNA fragmentation level in the hippocampus and the damaged nuclei number (karyopyknosis, karyorrhexis) in the hippocampus (on Day 14 after IR, $r = 0.90$, $p < 0.05$), indicating activation of endogenous neurogenesis in the hippocampus and switching of necrotic neuronal death to apoptotic one under the influence of cell lysate from hWJ. The obtained results of the correlation analysis between morphometric and cytometric indicators in the hippocampus and somatosensory cortex of rats with cerebral ischaemia-reperfusion confirm that MSC transplantation effectively protects ischaemic neurons of the penumbra zone.

CONCLUSIONS

The correlation analysis revealed strong direct and inverse line dependencies between the flow cytometry parameters and the damaged neuronal nuclei number on Day 7 and Day 14 after IR modelling in rats, both in the somatosensory cortex and hippocampus. It was confirmed that subtotal reversible brain IR in rats caused early damage to hippocampal neurons by activating neuroapoptosis. MSCs usage having different ancestry and cell lysate from hWJ as a therapy for ischaemia-reperfusion injury of brain structures showed different correlations (both direct and inverse) between the flow cytometry parameters and the damaged neuronal nuclei number during the experimental period. Thus, transplantation of umbilical cord hWJ MSCs after IR modelling caused positive correlations directed to the endogenous neuroregeneration and switching of necrotic neuronal death to apoptotic.

Transplantation of rat embryonic fibroblasts in rats revealed strong direct correlations with neurogenesis in both somatosensory cortex and hippocampus on Day 14 after model IR. Transplantation of human adipose tissue-derived MSCs caused strong direct correlations aimed at activation of neurogenesis in the hippocampus on Day 7 after model IR in rats. In general, MSCs transplantation from rat adipose tissue for IR therapy caused a strong direct correlation aimed at protecting somatosensory cortex neurons from ischaemia-reperfusion injury in rats. MSCs administration of cell lysate from hWJ caused strong direct correlation dependencies aimed at protecting somatosensory cortex neurons from ischaemia-reperfusion injury and activation of endogenous neurogenesis in the hippocampus.

Thus, the results demonstrated that MSCs having different ancestry exerts a distinct neuroprotective effect aimed at restoring neurogenesis in brain structures and suppressing the intensity of neuroapoptosis in post-perfusion injuries. The data obtained during the correlation analysis will be used to determine the most effective MSC class as a neuroprotective agent, in order to further develop an injectable medication for the cure of patients with the ischaemic stroke. The results experimentally substantiate the expediency of clinical trials of an injectable drug based on human Wharton's umbilical cord MSCs for a new purpose, namely, as a cytoprotector in patients with the ischaemic stroke.

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CONFLICT OF INTEREST

None.

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Кореляційні особливості між морфометричними та показниками проточної цитометрії у соматосенсорній корі та гіпокампі щурів із церебральною ішемією-реперфузією та її корекція

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Анотація. Зростання захворюваності на ішемічний інсульт стало однією з головних проблем у світі через високу інвалідність та смертність. Метою роботи стало проведення аналізу кореляцій між ушкодженими нейронами та показниками проточної цитометрії у соматосенсорній корі та гіпокампі щурів в умовах терапії церебральної ішемії-реперфузії. Досліджено вплив мезенхімальних стромальних клітин (отриманих із пуповини людини, жирової тканини щура та людини, а також ембріональних фібробластів щурів та їх лізату) на показники морфометрії та проточної цитометрії в гіпокампі та соматосенсорній корі статевозрілих щурів лінії Вістар (вік – 3-4 міс, маса тіла – 160-190 г, яким проведено модельну ішемію-реперфузію головного мозку). У соматосенсорній корі та CA1 ділянці гіпокампа підраховано загальну кількість ядер нейронів на 1 мм^2, а також визначено співвідношення кількості неушкоджених ядер нейронів та ядер із патологічними змінами (каріорексис та каріопікноз). Для визначення зв'язку між окремими параметрами використано непараметричний кореляційний ранговий аналіз Спірмена. При застосуванні мезенхімальних стромальних клітин різного походження та їх лізату при терапії ішемічно-реперфузійного ураження структур головного мозку виявлено різноспрямовані кореляції (як прямі, так і зворотні) між параметрами проточної цитометрії та кількістю ядер уражених нейронів на 7 та 14 добу після ішемії, як у соматосенсорній корі, так і в гіпокампі. Отже, результати кореляційного аналізу демонструють наявність у мезенхімальних стромальних клітин різного походження виразної нейропротекторної дії спрямованої на відновлення нейрогенезу в структурах головного мозку та пригнічення інтенсивності нейроапоптозу при постреперфузійних пошкодженнях. Отримані дані кореляційного аналізу будуть використані з метою визначення найбільш ефективного класу мезенхімальних стромальних клітин у якості нейропротектора та подальшого створення на його основі ін'єкційного препарату для лікування хворих з ішемічним інсультом

Ключові слова: мезенхімальні стромальні клітини; церебральна ішемія-реперфузія; морфометрія; нейроапоптоз; кореляційний аналіз