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Original article

EFFECT OF MESENCHYMAL STROMAL CELLS OF DIFFERENT ORIGIN ON DNA FRAGMENTATION IN RAT HIPPOCAMPAL NEURONAL NUCLEI AFTER ISCHEMIA-REPERFUSION

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Background. The treatment of cerebral blood circulation disorders remains a pressing issue due to their prevalence in the elderly. Brain tissue ischemia caused by such disorders leads to necrotic and neuroapoptotic changes. To mitigate neuroapoptosis in the ischemic zone during the subacute period of the process, neuroprotectors are used. In recent years, the neuroprotective properties of mesenchymal stromal cells (MSCs) have been actively studied.

Objective. To compare effect of MSCs of different origin and the cell lysate of human MSCs from Wharton's jelly (hWJ-MSC) on neuroapoptotic changes in the hippocampus of the rat brain after ischemia-reperfusion (IR).

Methods. A 20-minute bilateral transient IR of the internal carotid arteries was performed on 165 four-month-old male Wistar rats. Following IR modeling, MSCs derived from hWJ-MSCs, as well as human and rat adipose tissue, were injected intravenously into the femoral vein of the rats. Other groups of rats received intravenous injections of fetal rat fibroblasts and cell lysate from hWJ-MSCs. Only an intravenous injection of physiological solution was administered to the control group of rats. The level of DNA fragmentation in the nuclei of hippocampal neurons on the 7th day after IR was assessed via flow cytometry.

Results. Experimental IR caused a 4.9-fold increase in the level of fragmented DNA in the operated rats compared to the sham-operated animals. The use of MSCs of various origins and hWJ-MSC lysate reduces the intensity of DNA fragmentation in the nuclei of rat hippocampal neurons, with the most pronounced effects observed in groups treated with rat fetal fibroblasts (by 4.8 times), human adipose tissue MSCs (by 2.5 times), and hWJ-MSC cell lysate (by 2 times).

Conclusions. A persistent focus of necrotic and apoptotic death of neurons in the hippocampus of rats is formed after experimental 20-minute IR of rats' brain, as evidenced by increased levels of fragmented DNA. Intravenous transplantation of MSCs of various origin and cell lysate from hWJ-MSC demonstrated a significant effect in the IR model: neurodestruction and neuroapoptosis at the area of the ischemic brain damage get less intensive. MSCs derived from human adipose tissue demonstrated superior neuroprotective potential compared to rat adipose tissue MSCs in the IR model of the rat brain.

Keywords: ischemia-reperfusion; hippocampus; neuroapoptotic changes; flow cytometry; mesenchymal stromal cells.

Introduction

Acute cerebrovascular accident (ACVA) is one of the causes of stroke. The development of the last pathological process is preceded by ischemia of nervous tissue, which begins with the formation of an acute neuronal energy deficit and further manifests itself in a sequence of reactions of the "ischemic cascade" leading to irreversible damage of the nervous tissue [1]. Nowadays thrombolytic therapy is used for the treatment of the ischemic stroke as a standard [2–4]. But the restoration of blood supply to ischemic tissue, not surprisingly, deepens the disruption of metabolic processes in the brain tissue, which contributes to the occurrence of reperfusion injuries [1]. The desmosomes destruction and remoteness of neurons from each other lead to the spread of free radicals, as well as secondary messengers, resulting in secondary damage to previously intact neurons and an increase in the area of the lesion [5–7]. It is worth noting that in conditions of the ACVA apoptotic death of neurons dominates over their necrotic death, the reason for this is the induction of all those factors that occur during secondary damage to nerve cells. In ischemia-reperfusion (IR) which clinically corresponds to post-perfusion brain damage after thrombolysis the majority of neurons die by apoptosis [8]. A question may arise, why exactly the hippocampus became the object of research in the IR model. It has been proven that in case of acute IR injury of the brain, the first morphological changes will appear in neurons that are particularly vulnerable to ischemia (pyramidal neurons of the CA1 zone of the hippocampus) [9]. In addition, post-ischemic damage to the hippocampus contributes to the development of vascular dementia because in the hippocampus, IR induces neuronal death as a result of oxidative stress and zinc (Zn^{2+}) dyshomeostasis of neurons [10].

That is why cytoprotective therapy should be used to prevent and inhibit apoptosis. In our opinion, in the conditions of model IR, it is expedient to evaluate and compare effect of mesenchymal stromal cells (MSCs) of different origin and cell lysate from human MSCs from Wharton's jelly (hWJ-MSC) on neuroapoptotic changes in the hippocampus of the rat brain after ischemia-reperfusion.

Materials and methods

Rats (165 animals, exclusively white four-month-old sexually mature males of the Wistar line, with a body weight 160 to 190 g), bred in the vivarium of the Vinnytsia National Pirogov Memorial Medical University, were kept in standard conditions (free access to food and water) and were used for IR simulation. During the research, we scrupulously followed both the methodological recommendations of the Ministry of Health of Ukraine and the requirements of the "General ethical principles of animal experiments" approved by the First National Congress of Bioethics (Kyiv, Ukraine, 2001), as well as the provisions of the Law of Ukraine "On the Protection of Animals from Cruel Treatment" dated February 26, 2006.

Experimentally, the IR model was created by imposing ligatures on the common carotid arteries bilaterally (for the 20 minutes). The rats were previously anesthetized (propofol anesthesia was used (Propofol-novo, Novofarm-Biosintez LLC, Ukraine) at the dose 60 mg/kg of body weight). This IR model simulates the clinical manifestation of cerebral infarction and is used to experimentally study the action of neuroprotective substances [11]. MSCs of various origin at a dose 10^6 cells/rat (suspended in 0.2 ml of 0.9% NaCl solution) were injected intravenously into a catheter being in the femoral vein immediately after IR. The MSCs injection into the femoral vein was performed immediately after applying ligatures to the internal carotid arteries with the aim of more effective neurological recovery and reducing the area of the infarcted area. In addition, such the technique allowed using a smaller dose of MSCs (the only 10^6 cells/rat) for a favourable effect [12–15]. MSCs of different origins (both allogeneic and xenogeneic) were used in order to compare the effect on the DNA destruction process in neurons of the hippocampus of the rats' brain.

Methods for isolation of all the cell types used in this study are described in details in previous work [16]. Briefly, umbilical cords were taken into investigation after written consent of women. All the recommendations of the International Society of Cell Therapy (2006) were taken into account (cell adhesion, cell morphology, CD-markers and differentiation capacity) [17]. Cells had spindle-shaped form, were adhesive to plastic. According to FACS-analysis with specific antibodies to CD-markers (CD_{34}, CD_{45}, CD_{73}, CD_{90} and CD_{105}) more than 98% of the cells belonged to MSCs. Differentiation into adipo-, osteo- and chondrocytes in special media confirmed this conclusion (data not shown). Rat fetal fibroblasts were isolated from muscle tissue of 15-day-old rat embryos; the gestation period was determined by the copulation plug. There are no specific markers of fibroblasts, they express common with MSCs ones [18], so cells were identified by spindle-shaped morphology and hematoxylin-eosin staining They were used as heterologous analogue to xenogenic hWJ-MSCs as their characteristics including differentiation capacity are very close [18, 19]. The obtained results are the next part of our studies [20–22].

Research design

All the experimental rats were divided into 7 groups (Table 1).

The 1st group included sham-operated rats, i.e. rats, which successively were subjected to anesthesia, skin incision, vascular preparation (excepting ligation of the internal carotid artery (ICA)), which taken together modelled the impact of traumatic experimental conditions. After such the subsequent interventions the rats were injected with 0.9% NaCl solution into the femoral vein at a dose of 2 ml/kg. Rats of the 2nd group (this group is considered as the control one) were subjected to 20-minute ischemia of the brain (I) by placing a ligature on the ICA. In 20 minutes the ligatures from the ICA were taken off thereby providing reperfusion (R) of the brain tissue with next injection of 0.9% NaCl solution into the femoral vein at the dose of 2 ml/kg. The 3rd group of animals immediately

Table 1: Division of the experimental animals into groups

The group of rats	Rats number	Description of the group
1st group	10	Sham-operated animals + 0.9% NaCl solution (2 ml/kg)
2nd group (control)	40	IR + 0.9% NaCl solution (2 ml/kg)
3rd group	20	IR + hWJ-MSCs (10^6 cells/animal)
4th group	20	IR + rat fetal fibroblasts (10^6 cells/animal)
5th group	25	IR + MSCs from human adipose tissue (10^6 cells/animal)
6th group	25	IR + MSCs from rat adipose tissue (10^6 cells/animal)
7th group	25	IR + cell lysate from hWJ-MSC (0.2 ml/animal)

Note. IR – ischemia-reperfusion, MSCs – mesenchymal stromal cells.

after IR received a transplantation of hWJ-MSCs at the dose of 10^6 cells/animal. The 4th group of rats underwent a single transplantation of fetal rat fibroblasts at the dose of 10^6 cells/animal immediately after IR. The 5th group of animals with IR received MSCs of human adipose tissue at the dose of 10^6 cells/animal (also at once after IR). Rats of the 6th group were injected immediately after IR with stem cells obtained from rat adipose tissue at the dose of 10^6 cells/animal. Immediately after IR the 7th group of animals was given a single dose of cell lysate from hWJ-MSC (in a volume of 0.2 ml/animal). Standardization of hWJ-MSC lysate was based on the equal amounts of intact cells and cells that were lysed. As in all the experimental groups we used 10^6 cells, the same cell amount were subjected to lysis.

It would be correct to indicate the concentration by the amount of proteins at least. This has not been done as there was no control for this measurement – only cell amount. According to this protein content should be the same in both variants.

Out of 165 rats taken at the beginning of our own research, 35 animals made it to flow cytometry (5 rats in each study group).

Next step in the experimental procedure was to analyze the effect of MSCs of different origin and cell lysate from hWJ-MSC on the neuroapoptotic changes in the hippocampus of rats on 7th day after IR (such the time interval was taken because it corresponds to the subacute period of ischemia). It is known the process of neuroapoptosis begins with the destruction of cellular nuclei and deoxyribonucleic acid (DNA) damage, so fragmented DNA presence may be considered as a marker of apoptosis [23]. The method of flow cytometry permits to estimate neuroapoptotic changes in the brain tissue (in particular in the hippocampus of rats). With this purpose 5 rats from each group were taken and decapitated using pentobarbital anesthesia ("Penbital", Bioveta JSC, Czech Republic, 100 mg/kg), their brain was rapidly ablated at once after decapitation [24, 25]. Nuclear suspensions of the hippocampal biopsies were prepared immediately after the material was collected and washed with a cold (+4–8 °C) phosphate-salt buffer with a pH of 7.4 (Sigma). SuStain DNA from Partec Company (Germany) was added to the tissue to obtain a nuclear suspension according to the instructions of the producer. CellTrics 50 μm disposable filters (Partec, Germany) also were used. Flow cytometry was carried out on a flow cytometer "Partec PAC" of the Partec company, Germany. An ultraviolet light was used to excite the fluorescence of the nuclear DNA label – diamidinophenylindole. 20.000 events were analyzed from each sample of nuclear suspension. Flow analysis of DNA fragmentation was made using FloMax software (Partec, Germany) by highlighting Sub-G1 regions on DNA histograms (SUB-G0G1 regions on DNA histograms are RN1 before the G0G1 peak, which indicates cell nuclei with DNA content <2 DNA subunits (i.e. DNA defragmentation). Both necrotic and apoptotic cell death can be assessed by flow cytometry. However, the proposed model of subtotal IR ensures the predominance of apoptotic changes over necrotic ones [24]. It was established that after the restoration of blood flow in the occluded vessel cell death by apoptosis prevails over the necrotic process, since reperfusion can induce all the factors that occur during secondary damage to neurons. At the same time, the death of cells by apoptosis is not accompanied by the development of inflammation, since the integrity of their membranes is not disturbed. During apoptosis, DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, formation of apoptotic bodies, which are subsequently subjected to phagocytosis by phagocytizing cells, occur in the cell [26].

The statistical analysis of the results was carried out using the methods of parametric (descriptive statistics, Student's test) statistics in the case of a normal distribution of the variation series (tested by the Shapiro–Wilk test) and non-parametric (Wilcoxon–Mann–Whitney U-test) statistics.

Results

The statistically significant increase in the level of fragmented DNA (by 4.9 times) was found in the control group of rats on the 7th day of experiment (those who were subjected to IR and 0.9% NaCl solution injection at a dose of 2 ml/kg) in comparison with the group of the sham-operated animals (Figs. 1, 2, Table 2). Such the dynamic shows formation of brain tissue lesions by neuroapoptosis in the rat hippocampus.

The statistically processed flow cytometry data (SUB-G0G1 %) are presented in Table 2.

As can be seen, the use of transplanted MSCs of various origin and cell lysate from hWJ-MSC is quite effective in the treatment of neuron destruction occurring in the hippocampus after experimental IR: thus, the intensity of DNA fragmentation in the nuclei of hippocampal neurons of the rat brain in comparison to the group of control pa-

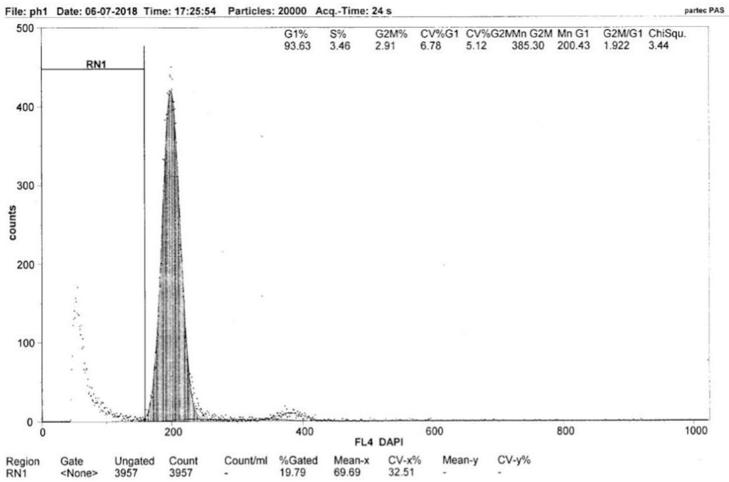

Figure 1: RN1 (Sub-G1) – 17.35 ± 1.21%. DNA fragmentation in the nuclei of hippocampal neurons in the control group of rats (cerebral ischemia-reperfusion). Flow cytometry

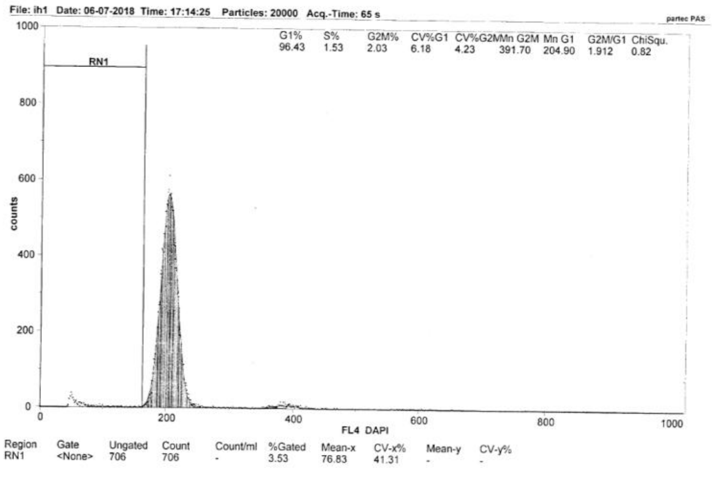

Figure 2: RN1 (Sub-G1) – 3.52 ± 0.18 %. DNA fragmentation in the nuclei of hippocampal neurons of the rat brain in the group of sham-operated rat. Flow cytometry

thology (IR without treatment) probably decreased from 1.4 to 4.8 times (Table 1, Figs. 3–7), excluding the group of rats that were injected the stem cells from rat adipose tissue.

We emphasize that transplantation of MSCs obtained from rat adipose tissue to experimental animals with IR (the 6th group) did not in any way affect the intensity of DNA fragmentation in the nuclei of hippocampal neurons of rats. At the same time transplantation of fetal rat fibroblasts and MSCs obtained from human adipose tissue and cell lysate from hWJ-MSC in rats with IR had a cerebroprotective effect, which was indicated by a probable decrease of DNA fragmentation in the nuclei of hippocampal neurons (relative to the control group) by 79.1%, 60.2%, and 48.9%, respectively.

Thus, inhibition of neuroapoptosis intensity in a hippocampus of the rats' brain under the action of fetal rat fibroblasts, MSCs obtained from human adipose tissue and cell lysate from hWJ-MSC, explains a significant reduction in the ischemic focus due to the preservation of the number of morphologically intact neurons, shown by us earlier [22, 28], and is one of the mechanisms of cerebroprotective action of MSCs in postreperfusion brain damage. Morphological confirmation of this opinion is the results of our research, published previously [22, 28].

Table 2: The effect of intravenous injection of mesenchymal stromal cells (MSCs) of different origin and cell lysate from hWJ-MSC on DNA fragmentation of the nuclei in hippocampal neurons in rats on 7th day after transient bilateral ischemia-reperfusion of the internal carotid arteries (M ± m)

Experimental conditions	Group number (rats' number)	SUB-G0G1 %
Sham-operated animals + 0.9% NaCl solution	1 (*n* = 5)	3.52 ± 0.18
IR + 0.9% NaCl solution (the control group)	2 (*n* = 5)	17.35 ± 1.21*
IR + hWJ-MSCs	3 (*n* = 5)	12.69 ± 2.76*
IR + rat fetal fibroblasts	4 (*n* = 5)	3.63 ± 0.70#$
IR + MSCs from human adipose tissue	5 (*n* = 5)	6.91 ± 1.40#$
IR + MSCs from rat adipose tissue	6 (*n* = 5)	19.09 ± 2.37*
IR + cell lysate from hWJ-MSC	7 (*n* = 5)	8.87 ± 1.28#$

Notes. SUB-G0G1% – areas on DNA histograms – RN1 before the G0G1 peak, which indicates cell nuclei with DNA content <2 spirals; * – $p < 0.05$ relative to the sham-operated animals; # – $p < 0.05$ relative to the control group; $ – $p < 0.05$ relative to animals with ischemia-reperfusion (IR) + stem cells from rat adipose tissue.

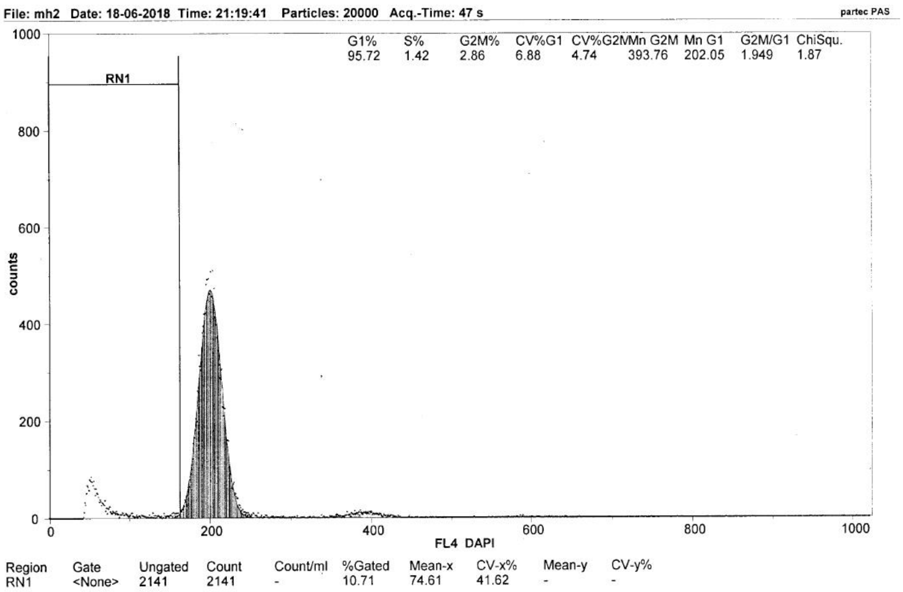

Figure 3: RN1 (Sub-G1) – 12.69 ± 2.76%. DNA fragmentation in the nuclei of neurons in the hippocampus of rats with model cerebral ischemia-reperfusion with transplantation of human mesenchymal stromal cells from Wharton's jelly

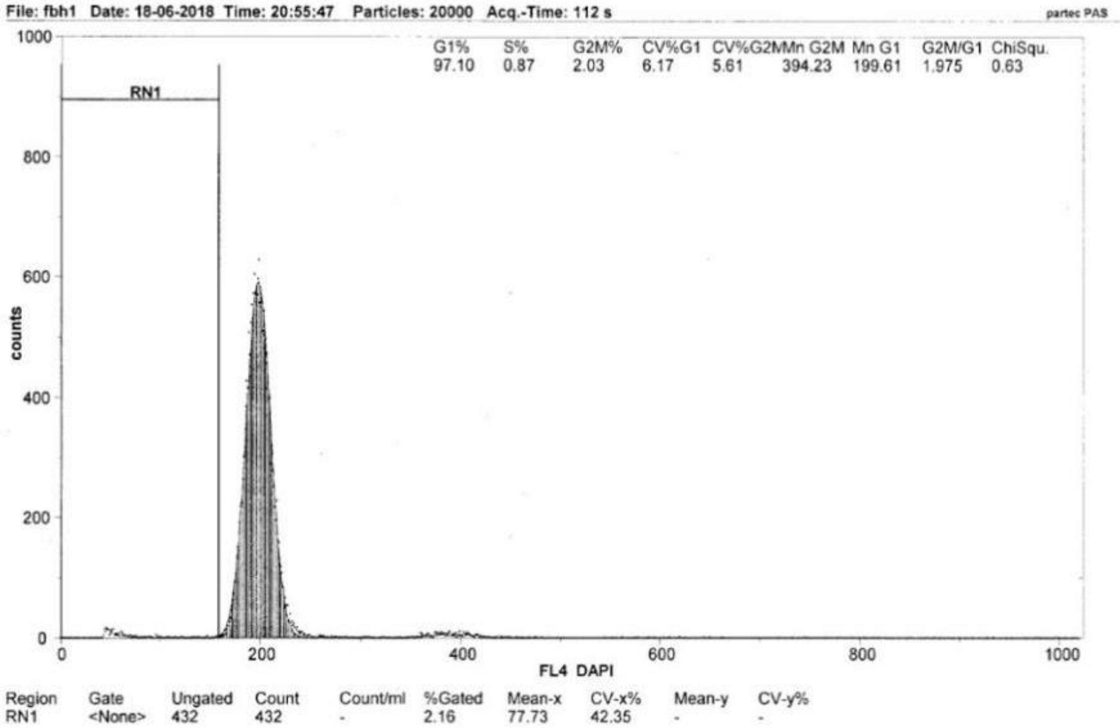

Figure 4: RN1 (Sub-G1) – 3.63 ± 0.70%. DNA fragmentation in the nuclei of neurons in the hippocampus of the rats with model cerebral ischemia-reperfusion with transplantation of rat fetal fibroblasts

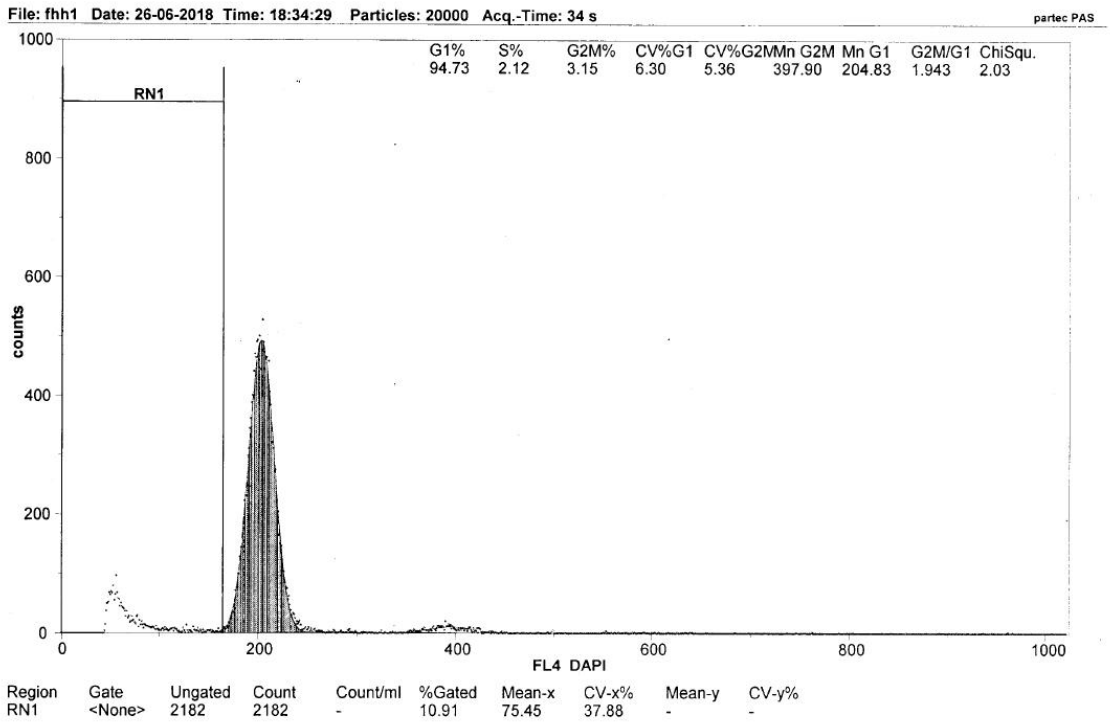

Figure 5: RN1 (Sub-G1) – 6.91 ± 1.40%. DNA fragmentation in the nuclei of hippocampal neurons of the rats with model cerebral ischemia-reperfusion with transplantation of mesenchymal stromal cells from human adipose tissue

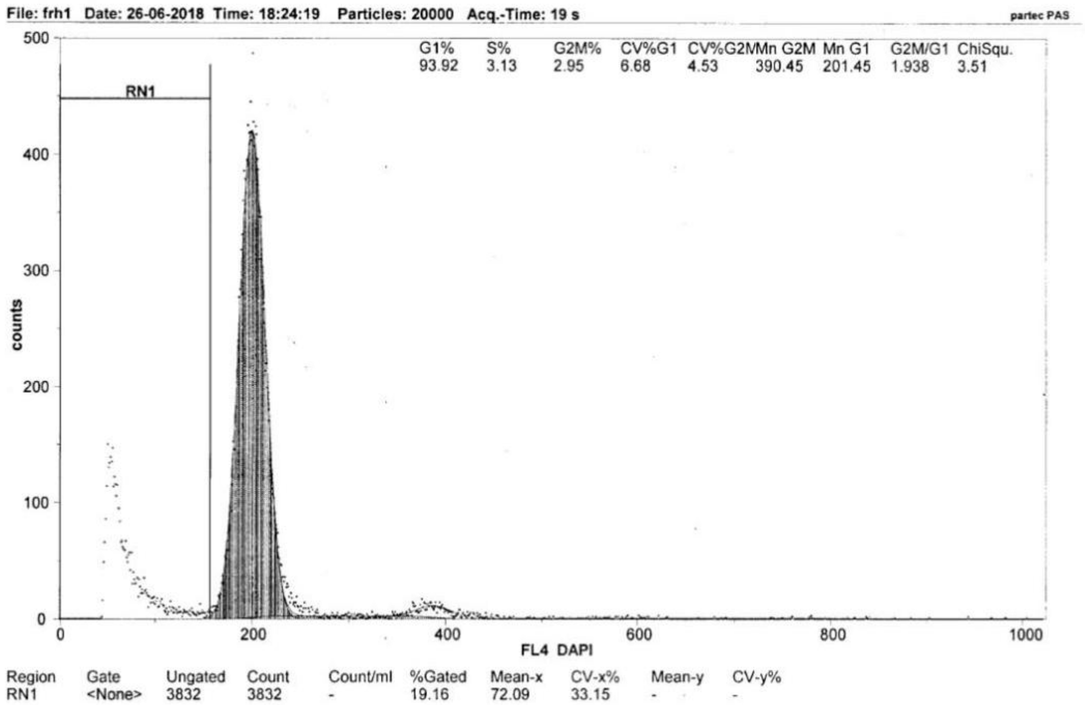

Figure 6: RN1 (Sub-G1) – 19.09 ± 2.37%. DNA fragmentation in the nuclei of neurons in the hippocampus of the rats with model cerebral ischemia-reperfusion with transplantation of mesenchymal stromal cells from rat adipose tissue

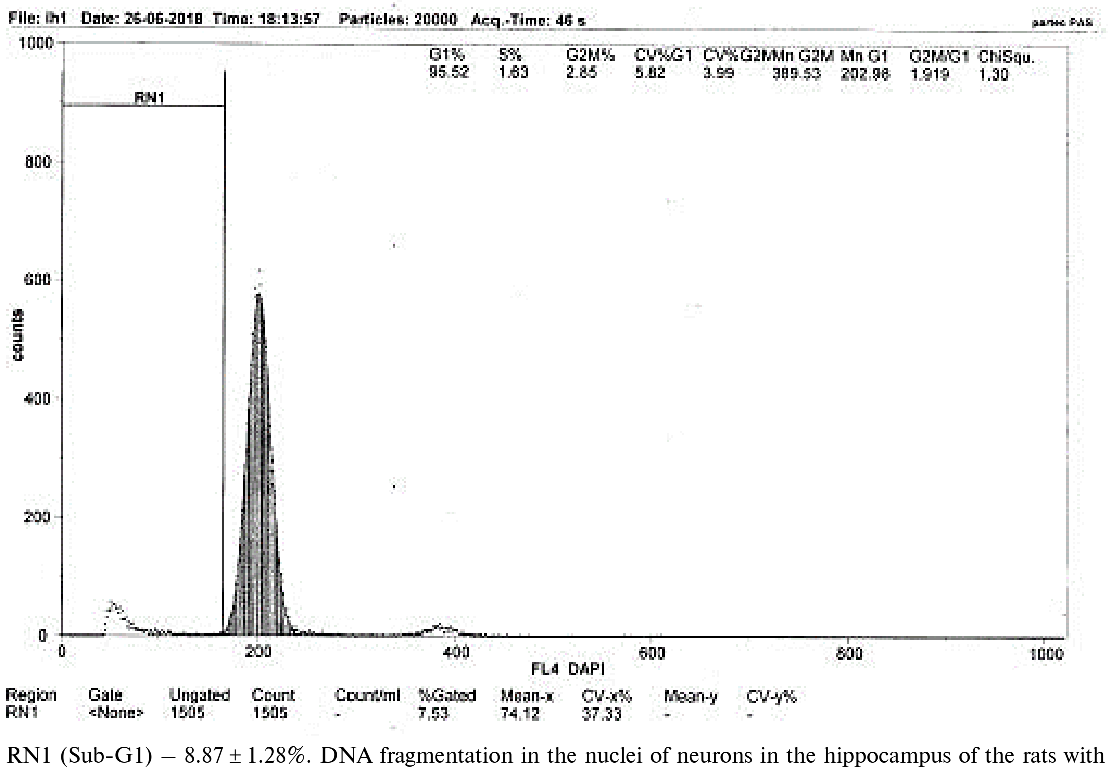

Figure 7: RN1 (Sub-G1) – 8.87 ± 1.28%. DNA fragmentation in the nuclei of neurons in the hippocampus of the rats with model cerebral ischemia-reperfusion with intravenous administration of cell lysate from human mesenchymal stromal cells from Wharton's jelly. Flow cytometry

Discussion

Improvement of cerebral blood circulation after thrombolysis additionally to positive dynamics on the course of the penumbra zone formation also can have at least one negative effect – it can activate the process of neuroapoptosis [29].

One of the neuroapoptosis markers is the level of fragmented nuclear DNA. DNA fragmentation is one of the main biochemical signs of programmed cell death [30].

Taking this into account it was appropriate to characterize the influence of MSCs (of various origins) and cell lysate from hWJ-MSC on neuroapoptotic changes in the rat brain hippocampus by the method of flow cytometry (which is the modern and one of the best methods for assessing of DNA fragmentation in neurons) in the postreperfusion cerebral ischemia conditions on the IR model. The IR model choice was due to the fact that clinically this model corresponds to a post-perfusion injury of the brain after thrombolysis, and the majority of neurons die as a result of apoptosis [31].

Early brain injury after IR induces morphological changes in neurons with cytosolic microvacuolation. Microvacuoles appear within 15 min after reperfusion in particularly vulnerable neurons (hippocampal CA1 pyramidal neurons, cortical pyramidal neurons in the layers 3 and 5) [9].

As a part of the regenerative strategy stem cell transplantation in ischemic stroke became a new impetus [32, 33].

The modern views of many scientists are focused on the ability of stem cells to secrete substances that interfere with many pathogenetic cascades and contribute to the survival, migration, differentiation and functional integration of transplanted cells into the brain during acute ischemia [34].

The impetus for this was the results of experiments on animal models and several clinical trials [35, 36].

MSCs have several advantages over other stem cells due to easier methods of their obtaining, low risk of tumorigenicity and lack of ethical problems [37].

Our study shows that intravenous administration of fetal rat fibroblasts, MSCs from human adipose tissue and cell lysate from hWJ-MSC after the model IR leads to a decrease in the fragmented DNA level, to a decrease of apoptosis in the rat hippocampus 7 days after the IR. Thus suppression of the neuroapoptosis intensity in the rat brain hippocampus under the influence of MSCs, as well as cell lysate from hWJ-MSC, indicates reduction in the focus of the ischemic penumbra due to preservation of the morphologically intact neurons number and is one of the leading mechanisms of the neuroprotective effect of the investigated substances in post-perfusion brain damage.

Xenotransplantation of the MSCs from human adipose tissue has a significantly better neuroprotective effect on the rat hippocampus after the IR. In our opinion, this is due to the fact that transplanted MSCs from human adipose tissue release biologically active substances that can increase the plasticity of the host's brain (in the case of experimental stroke). The obtained results require in-depth research, becuase in the experiment of Gutiérrez-Fernández *et al.* [38] it has been shown that transplantation of human adipose MSCs (xenogeneic transplantation) and rat adipose MSCs (allogeneic one) in permanent middle cerebral artery occlusion (pMCAO) in rats used as a treatment for cerebral infarction demonstrated no difference in the recovery and reduction of ischemic brain damage in rats, had no side effects and did not form tumors. And in the study of Chung *et al.* [39] it has been established that the neuroprotective effect of MSC treatment from human adipose tissue (xenogeneic transplantation) may be associated with the prevention of the blood-brain barrier destruction and endothelial damage and the reduction of neutrophil infiltration, and the use of MSCs from human adipose tissue is highly effective.

Fetal rat fibroblasts were used in this study with the aim to change xenogeneic hWJ-MSCs by heterogenic (rat) cells with close characteristics and compare their effect. As it is known that fetal MSCs and fetal fibroblasts have common signs [18] we think such substitution is legitimate. That’s why, to our opinion, their transplantation in the IR model in rats, caused a better immunomodulatory effect.

Fetal rat fibroblasts belong to embryonic stem cells are poorly differentiated. This may be partially mediated by the induction of angiogenesis and neurotrophic factors and the inhibition of the expression of inflammatory and apoptotic factors [40]. As for MSCs from human or rat adipose tissue, they are adult stem cells, and therefore have many similar properties to other MSCs. Numerous researchers have pointed out that the neuroprotective effects of MSCs on the ischemic brain are not realized directly through their differentiation, but by the way of paracrine signalling through trophic factors, which promotes functional recovery by multiple mechanisms, including immunomodulation, proangiogenic signalling, neurotrophic factor secretion, and neuronal differentiation [41–44]. Thus, in investigation of Taei A *et al.* it was shown that the neuroprotection of the hippocampus in conditions of ischemia is provided by the secretome of MSCs obtained from human embryonic stem cells [40]. In our opinion, cell lysate from hWJ-MSC had a positive effect on DNA fragmentation through remote modulation (as a result of the release of various bioactive molecules).

The results of our study are consistent with results of the recent investigations showing that MSC treatment can stimulate neurogenesis while reducing the extent of damage and inflammation, and improve neuroprotection after IR brain injury in the rat hippocampus [38, 39, 45].

The regenerative potential of MSCs during experimental IR is explained not by replacement of damaged cells in the ischemia zone, but by their release of bioactive substances that contribute to neurogenesis and protection of brain tissues from ischemic damage [40]. Thus, intravenous transplantation of MSCs can increase the therapeutic efficiency of reperfusion therapy in cerebral ischemia, which indicates the prospect of their use in cell therapy of acute cerebral ischemia.

Conclusions

20-minute bilateral transient ischemia-reperfusion of the internal carotid arteries of rats causes a stable focus of necrotic and apoptotic destruction of neurons in hippocampus, which is manifested with an increase in fragmented DNA level (by 4.9 times). Purely morphological confirmation of this conclusion is presented in the materials of our previous studies.

Intravenous injection of MSCs of various origin and cell lysate from hWJ-MSC exerts a significant effect in model IR and leads to decrease in neurodestruction and neuroapoptosis processes in hippocampus of rats.

MSCs obtained from human adipose tissue demonstrate better neuroprotective potential than MSCs from rat adipose tissue in model IR of the rat brain.

Interests disclosure

The authors declare that there are no conflicts of interest.

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ВПЛИВ МЕЗЕНХІМАЛЬНИХ СТРОМАЛЬНИХ КЛІТИН РІЗНОГО ПОХОДЖЕННЯ НА ФРАГМЕНТАЦІЮ ДНК ЯДЕР НЕЙРОНІВ ГІПОКАМПУ ГОЛОВНОГО МОЗКУ ЩУРІВ ПІСЛЯ ІШЕМІЇ-РЕПЕРФУЗІЇ

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Проблематика. Лікування порушень мозкового кровообігу залишається актуальною проблемою через їх поширеність серед літніх людей. Ішемія мозкової тканини, викликана такими порушеннями, призводить до некротичних і нейроапоптичних змін. Для зменшення нейроапоптозу в ішемічній зоні під час підгострого періоду процесу використовуються нейропротектори. Останніми роками активно досліджуються нейропротекторні властивості мезенхімальних стромальних клітин (МСК).
Мета. Порівняти вплив МСК різного походження та клітинного лізату МСК із Вартонових драглів пуповини людини на нейроапоптичні зміни в гіпокампі мозку щурів після модельної ішемії-реперфузії (ІР).
Методика реалізації. На 165 чотиримісячних самцях щурів лінії Вістар виконували 20-хвилинну двобічну транзиторну ІР внутрішніх сонних артерій. Після моделювання ІР МСК із Вартонових драглів пуповини людини, а також МСК із жирової тканини людини та щура вводили внутрішньовенно у стегнову вену щурів. Іншим групам щурів внутрішньовенно вводили фетальні фібробласти щура і клітинний лізат із Вартонових драглів пуповини людини. Контрольній групі щурів вводили лише фізіологічний розчин. Рівень фрагментації ДНК у ядрах нейронів гіпокампу на 7-му добу після ІР оцінювали методом проточної цитометрії.
Результати. Експериментальна ІР викликала 4,9-кратне збільшення рівня фрагментованої ДНК у оперованих щурів порівняно з псевдооперованими тваринами. Використання МСК різного походження та лізату Вартонових драглів пуповини людини знижує інтенсивність фрагментації ДНК у ядрах нейронів гіпокампу щурів, причому найвираженіший ефект спостерігався в групах, яким вводили фетальні фібробласти щурів (у 4,8 разу), МСК з жирової тканини людини (у 2,5 разу) та клітинний лізат МСК із Вартонових драглів пуповини людини (у 2 рази).
Висновки. У гіпокампі щурів після експериментальної 20-хвилинної ІР мозку формується стійкий осередок некротичної й апоптотичної загибелі нейронів, що проявляється збільшенням фрагментованої ДНК. Внутрішньовенна трансплантація МСК різного походження та лізату МСК із Вартонових драглів пуповини людини демонструє значний ефект у моделі ІР: нейродеструкція та нейроапоптоз у зоні ішемічного ураження головного мозку стають менш інтенсивними. МСК, отримані з жирової тканини людини, показали вищий нейропротекторний потенціал порівняно з МСК із жирової тканини щура в моделі ІР мозку щурів.

Ключові слова: ішемія-реперфузія; гіпокамп; нейроапоптичні зміни; проточна цитометрія; мезенхімальні стромальні клітини.