Multipotent mesenchymal stromal cells of various origins reduce reactive gliosis in the hippocampal CA1 area during acute ischemia-reperfusion of the rat brain
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Abstract

Neuroprotective therapy for acute cerebrovascular accident is directly aimed at preserving the neurons of the penumbra, but the regeneration of glia in the affected area is also important. Glial cells have fast reactivity and are very sensitive to ischemic cerebral injury. New experimental studies have demonstrated the successful use of multipotent mesenchymal stromal cells (MMSCs) in stroke to modulate microglial activation.

The purpose of the study was to investigate the effect of MMSCs of different origin, MMSC lysate and citicoline on glial components in the brain ischemia-reperfusion model in rats.

Methods. The experiments were conducted on 190 4-month-old male Wistar rats weighing 160-190 g. After modeling ischemia-reperfusion of the brain by bilateral 20-minute occlusion of the internal carotid arteries, the animals were intravenously injected with human umbilical cord Wharton’s jelly-derived MMSCs, human and rat adipose tissue-derived MMSCs at a dose of 1×10⁶ cells/animal. Other groups were injected with fetal rat fibroblasts 1×10⁶ cells/animal in 0.2 mL 0.9 % saline or lysate from umbilical cord Wharton’s jelly-derived MMSCs (0.2 mL/animal). Control animals were injected i.v. with 0.9 % saline. The last group of rats received a single dose of the reference drug citicoline at a dose of 250 mg/kg. On the 7th and 14th days, the area and fluorescence intensity of cells expressing markers of astrocytes (GFAP), microglia (Iba1), and oligodendrocytes (Rip) were quantitatively assessed on sections of the hippocampal CA1 area using immunohistochemical examination with confocal microscopy.

Results. On the 7th and 14th days after ischemia-reperfusion in rats, the fluorescence intensity of GFAP-positive astrocytes and Iba1-positive microglial cells increased, which indicated pronounced reactive astrogliosis and activation of microglia in the CA1 area of the hippocampus. At the same time, ischemia-reperfusion did not significantly affect the content of Rip-positive oligodendrocytes in brain slices. The use of all treatment options (transplantation of MMSCs of different origin, their lysate, or the reference drug citicoline) had a cytoprotective effect and reduced reactive astro- and microgliosis, both on the 7th and 14th days after the injury. The best result was demonstrated by the treatment with human umbilical cord Wharton’s jelly-
derived MMSCs.

**Conclusion.** Brain ischemia-reperfusion induces reactive gliosis by activating GFAP-positive and Iba1-positive glial cells in all layers of the hippocampus. The use of human umbilical cord Wharton’s jelly-derived MMSCs and fetal rat fibroblasts significantly reduces its intensity both on the 7th and on the 14th days after modeling the ischemic brain injury.

**Key words:** ischemic brain injury; hippocampus; glia; neuroprotection; multipotent mesenchymal stromal cells; citicoline

Neuroprotective therapy in acute cerebral blood circulation disorders is directly aimed at preserving the neurons of the penumbra zone, where minimal blood flow remains due to collateral blood flow [1]. However, in addition to neurons, ischemia provokes significant structural and functional disorders of all components of glia – astrocytes, oligodendrocytes, and microglial cells [2].

Astrocytes are key components of the central nervous system that synthesize factors to support the proliferation, differentiation, and migration of neural stem cells. Astrocytes contribute to the development of microglia [3], and their regional density correlates with its density [4]. A change in the structure of astrocytes (reactive astrogliosis) is a pathological sign of an ischemic stroke.

Microglia is the main immune cells of the brain and the first line of defense against a cascade of pathological factors caused by impaired blood circulation in the brain. There is more and more evidence in favor of the important role of microglia in the dynamic interaction with neurons, astrocytes and other neighboring cells of the brain both in normal condition and in stroke [5].

The degree and quality of reactive gliosis depend on the volume of the damage, ischemic environment, disruption of the blood-brain barrier, inflammatory reaction, as well as metabolic, excitotoxic, and oxidative (during reperfusion) stresses. In addition, astrocytes, which rapidly proliferate and secrete pro-inflammatory cytokines, chemokines, and metalloproteinases, accelerate the inflammatory response in coordination with activated microglia [7].

Mature oligodendrocytes are also quite vulnerable to cerebral ischemia, which stop myelin production causing axon dysfunction. Recovery of nervous tissue after ischemia-reperfusion requires the regeneration of the oligodendrocytes pool for the formation of myelin sheaths of new axons [8].

Currently, there are no effective clinical methods of treating ischemic stroke, except for thrombolysis, but up to 60 % of patients do not reach functional independence after the recanalization procedure [9]. A promising therapeutic approach, which consists in stimulating
endogenous regenerative mechanisms with the use of intravenously injected multipotent mesenchymal stromal cells (MMSCs), is of considerable scientific interest. In a number of studies, MMSCs have demonstrated therapeutic efficacy, improving cell migration, angiogenesis, immunomodulation, neuroprotection, and reconstruction of neural circuits [10-12]. In case of impaired cerebral circulation, MMSCs also exhibit a paracrine effect due to neurotrophic effects and functional restoration [13-15]. New studies have demonstrated the successful use of MMSCs in stroke to modulate microglial activation [16]. However, the authors analyzed only the action of cells from one source [17-20], while we previously compared the action of both xenogeneic and allogeneic cells from two sources [23, 24]. The dose of cells used by us coincides with that proposed by other researchers. In the study, we administered therapeutic agents once intravenously immediately after ischemia-reperfusion, because earlier transplantation of MMSCs led to greater neurological recovery and reduced infarct volume, and also required a smaller number of donor cells to achieve a positive effect [17, 18, 26, 27]. Citicoline was chosen as a reference drug due to its ability to enhance neuroregenerative processes. Citicoline stimulates the synthesis of structural phospholipids of neuron membranes and improves the functioning of such membrane mechanisms as ion pumps and receptors, which ensures the normal conduction of nerve impulses. Citicoline was chosen among all available drugs due to its ability to enhance neuroregenerative processes in an experiment on rats and improve cognitive and memory functions in patients with cerebral ischemia [28-31]. Therefore, citicoline is included in the protocol for the treatment of acute disorders of cerebral blood circulation (Order of the Ministry of Health of Ukraine No. 602 dated August 3, 2012) [21].

Thus, glial cells play a significant role in maintaining the physiological function of the CNS and have a very rapid and sensitive response to cerebral damage caused by ischemic stroke [22]. Considering the above, the purpose of the work was to conduct an experimental study on the model of ischemia-reperfusion (IR) of the brain to study the effect of MMSCs of various origins, MMSC lysate or citicoline on the components of neuroglia.

**Materials and methods**

The experiments were carried out using 190 4-month-old male Wistar rats weighing 160-190 g. Animals housed in the vivarium of the National Pirogov Memorial Medical University, Vinnytsia were kept in standard conditions with free access to water and food. MMSCs were isolated from the tissue of human umbilical cords, obtained with informed consent from healthy women after physiological childbirth at 39-40 weeks. Methods of
isolation and characteristics of cells from animals and humans, which were used in the study, were described by us earlier [23]. The research was performed in accordance with the National "General Ethical Principles of Animal Experiments" approved by the First National Congress on Bioethics (Kyiv, Ukraine, 2001) and the Law of Ukraine "On the Protection of Animals from Cruelty" dated February 26, 2006.

The experimental model of ischemia-reperfusion was reproduced by temporarily applying ligatures to the internal carotid arteries on both sides (ischemia) under anesthesia with Propofol-novo (Novofarm-Biosyntez LLC, Ukraine) 60 mg/kg. After 20 minutes, the ligatures were removed (reperfusion). The chosen model reflects the clinical picture of brain infarction and is adequate for the experimental study of potential neuroprotective substances [25]. Experimental animals were divided into 8 groups (Table 1).

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Number of animals</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>sham-operated animals + 0.9 % saline at a dose of 2 mL/kg</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>IR + 0.9 % saline at a dose of 2 mL/kg</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>IR + human umbilical cord Wharton’s jelly-derived MMSCs at a dose of $1 \times 10^6$ cells/animal</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>IR + rat fetal fibroblasts at a dose of $1 \times 10^6$ cells/animal</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>IR + human adipose-derived MMSCs at a dose of $1 \times 10^6$ cells/animal</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>IR + rat adipose-derived MMSCs at a dose of $1 \times 10^6$ cells/animal</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>IR + lysate of Wharton’s jelly-derived MMSCs at a dose of 0.2 mL/animal</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>IR + citicoline 250 mg/kg</td>
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</table>

In the 1st group of sham-operated rats under anesthesia, a skin incision and vessel preparation were performed, excluding ligation of the internal carotid artery, which reproduced the effect of the traumatic conditions of the experiment. The 2nd group is a control group with pathology, in which cerebral ischemia-reperfusion was modeled and 0.9 % saline at the dose of 2 mL/kg was injected once into the femoral vein. A similar dose was administered to rats of the 1st group. The 3rd group of animals immediately after IR were transplanted with human umbilical cord Wharton’s jelly-derived MMSCs at a dose of $1 \times 10^6$ cells/animal once intravenously. The 4th group of animals underwent a single intravenous transplantation of fetal rat fibroblasts at a dose of $1 \times 10^6$ cells/animal immediately after IR. The 5th group of animals with IR received IV $1 \times 10^6$ cells/animal of human adipose-derived MMSCs. The 6th group of animals were administered with rat adipose-derived MMSCs at a dose of $1 \times 10^6$ cells/animal immediately after IR. The 7th group of animals were injected
intravenously with 0.2 mL/animal of lysate from human Wharton's jelly-derived MMSCs immediately after IR. The 8th group of rats were treated with the reference drug citicoline "Neuroxon" (Arterium Corporation, Ukraine) at a dose of 250 mg/kg immediately after IR.

The analysis of the effect of the studied agents on the dynamics of destructive changes in the CA1 area of the hippocampus was performed on the 7th (subacute period of ischemia) and 14th day (recovery period) after IR [32-35]. Animals were euthanized by decapitation after anesthesia with pentobarbital (Penbital, Bivota a.s., Czech Republic) at a dose of 100 mg/kg.

**Immunohistochemical analysis.** Brains were quickly removed from the animals and fixed with a 4 % formaldehyde for 24 hours. After fixation, brains were washed in water, passed through alcohols of increasing concentration and xylene, and after standard histological processing, embedded in Paraplast Plus© (McCormick, USA). Sections 5 μm thick were made on a rotary microtome. Before the immunohistochemical analysis, paraffin sections were deparaffinized in xylene, followed by washing of slides with sections in 0.1 M phosphate buffer. From brains fixed in formaldehyde, which were not embedded in paraffin, 40-μm-thick frontal brain sections were made using a VT1000A vibratome (Leica, Germany). Paraffin or vibratome sections after washing in 0.1 M PBS were blocked in 0.1 M phosphate buffer (pH = 7.4) supplemented with 0.5 % bovine serum albumin and 0.3 % Triton X-100. Incubation with primary antibodies against Glial Fibrillary Acidic Protein (GFAP), Ionized Calcium-Binding Adapter molecule-1 (Iba1) and Receptor Interacting Protein (Rip) was performed for 48 hours at 4 °C (Table 2). Primary antibodies were visualized using secondary antibodies conjugated with Alexa Fluor®488, Alexa Fluor®594, or Alexa Fluor®647 in dilution 1:1000 (Invitrogen, USA). Stained sections were covered with Immu-MOUNT medium (Thermo Scientific, USA).

**Table 2. Antibodies used for immunohistochemical analysis.**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Manufacturer, cat. No.</th>
</tr>
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<tbody>
<tr>
<td>Rabbit anti-GFAP (Glial Fibrillary Acidic Protein)</td>
<td>DakoCytomation, Denmark, Cat. # Z0334</td>
</tr>
<tr>
<td>Rabbit anti-Iba1 (Ionized calcium-Binding Adapter molecule-1)</td>
<td>FUJIFILM Wako Pure Chemical Corporation, USA, Cat. # 019-19741</td>
</tr>
<tr>
<td>Mouse anti-Rip (Receptor Interacting Protein)</td>
<td>Abcam, UK, Cat. # ab72139</td>
</tr>
<tr>
<td>Donkey anti-mouse Ig (H+L) Alexa Fluor®594</td>
<td>Invitrogen, USA, Cat. # A21203</td>
</tr>
<tr>
<td>Donkey anti-mouse Ig (H+L) Alexa Fluor®488</td>
<td>Invitrogen, USA, Cat. # A21202</td>
</tr>
<tr>
<td>Donkey anti-rabbit Ig (H+L) Alexa Fluor®594</td>
<td>Invitrogen, USA, Cat. # A21207</td>
</tr>
<tr>
<td>Donkey anti-rabbit Ig (H+L) Alexa Fluor®647</td>
<td>Invitrogen, USA, Cat. # A31573</td>
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Immunohistochemically stained brain sections were examined using an FV1000-BX61WI confocal scanning microscope (Olympus, Japan) using green helium-neon (HeNeG laser 543 nm), red helium-neon (HeNeR laser 633 nm) and multiline argon (Multi Ar laser 458, 488, 515 nm) lasers. For each examined marker, the same magnification, exposure time, and other laser settings were used. The resulting images were used to quantify the fluorescence intensity of each marker. A total of 5 sections per animal were examined. Color images were converted to black and white to calculate the intensity and area of the fluorescent marker by automatically calculating the mean gray value within the measurement threshold in the ImageJ software (Wayne Rasband, USA). The intensity and area of the fluorescent marker were measured by automatically calculating the average gray value within the measurement threshold. The results are presented as the integral fluorescence density in conventional units (CU), which is equal to the fluorescence intensity multiplied by the fluorescence area (excluding the integral background fluorescence density).

Statistical analysis. To compare the research results of the experimental groups, the analysis of variance (ANOVA) was applied using the Statistica v.6 software (Statsoft Inc., USA). The difference was considered significant at p ≤ 0.05. Integral fluorescence density in a frontal brain slice is shown as the mean value for 5 slices ± standard deviation (M ± SD).

Results and discussion

Immunohistochemical study of frontal brain slices for astrocyte functional activity marker GFAP did not reveal a change in the sham-operated group of rats, which was used as a comparison control. The CA1 area of the hippocampus was the most sensitive to ischemic damage, where GFAP-positive astrocytes had thin, well-branched processes and were located in all layers of the hippocampus. In the stratum oriens layer, astrocyte processes were mostly horizontally oriented, parallel to the stratum pyramidale. In the stratum radiatum layer, they were oriented vertically, parallel to the apical dendrites of pyramidal neurons (Fig. 1A).

In the experimental group of rats with modeled brain injury, it was established that on the 7th and 14th days after ischemia-reperfusion, an increase in the staining intensity of GFAP-positive astrocytes was observed in comparison with the control group of sham-operated animals (Fig. 1B).
Fig. 1. Confocal micrographs of GFAP-positive astrocytes in the CA1 area of the rat hippocampus. A – sham-operated animal; B – an animal on the 7th day after of ischemia-reperfusion. Scale bar – 50 µm.

GFAP-positive astrocytes acquired a hypertrophied form, processes became shorter and thicker, a cluster of astrocytes was observed in the CA1 pyramidal layer of the hippocampus, at the site of neuronal death. That is, pronounced reactive astrogliosis was observed in the hippocampal CA1 area.

A similar reaction to IR was observed on the part of microglial cells. Immunohistochemical analysis for the marker of microglial cells Iba1 showed that, in comparison with sham-operated animals, the intensity of the Iba1-positive signal significantly increased in the CA1 area of the hippocampus, in particular in the pyramidal layer (Fig. 2).

Fig. 2. Confocal micrographs of Iba1-positive microglial cells in the CA1 area of the rat hippocampus. A – sham-operated animal; B – animal on the 7th day after ischemia-reperfusion. Layers are marked: st. or. – stratum oriens, st. pyr. – stratum pyramidale, st. rad. - stratum radiatum. Scale bar – 100 µm.
After IR, Iba1-positive microglial cells changed their resting phenotype, which is normally characterized by well-branched thin processes and a small soma (Fig. 2A), to amoeboid or rod-shaped (Fig. 2B). Iba1-positive microglia with an amoeboid phenotype were observed mainly in the pyramidal layer of the hippocampus, and rod-like microglia was located parallel to the apical dendrites of pyramidal neurons. In general, this indicated reactive gliosis in the hippocampal CA1 area.

IR did not significantly affect Rip-immunoreactivity of brain slices, the difference in fluorescence intensity of Rip-positive oligodendrocytes compared to the control group of sham-operated animals was not statistically significant. Therefore, this marker was not taken into consideration during further comparison of experimental groups.

The next stage was the study of the influence of transplantation of stem cells of different genesis, their lysate or citicoline on the state of glia after IR of the brain. Immunohistochemical analysis of frontal brain slices for the presence of the astrocyte marker GFAP demonstrated that on the 7th and 14th days after IR, all treatment options (the 3rd- the 8th groups) contributed to the reduction of reactive astrogliosis compared to the 2nd group of animals with ischemia only, but did not reach the control value fluorescence in sham-operated rats of the control group 1 (Fig. 3).

**Fig. 3.** Confocal photomicrographs of frontal sections of the hippocampal CA1 area in rats of the studied groups (1-8) stained with the astrocyte marker GFAP. The layers in the CA1 area are marked with letters: a – stratum oriens, b – stratum pyramidale, c – stratum radiatum, d – stratum lacunosum-moleculare. The upper panel of photomicrographs is the 7th day after ischemia-reperfusion, the lower panel is the 14th day. Scale bar – 100 μm.

To quantify the obtained results, the integral fluorescence density index was calculated. The evaluation of the integral fluorescence density of the GFAP-positive signal showed that IR led to the increase in GFAP-immunofluorescence and was 82.4 ± 6.52×10^6 CU on the 7th day,
and 76.2 ± 5.7×10⁶ CU on the 14th day compared to the control group of sham-operated animals (32.6 ± 3.4×10⁶ CU and 30.5 ± 2.9×10⁶ CU, respectively) (Fig. 4).

**Fig. 4. Integral density of fluorescence of GFAP-positive astrocytes on frontal sections of the hippocampal CA1 area of the studied rat groups (1-8).**

Notes: * – p < 0.05 compared to the control; # – p < 0.05 compared to ischemic animals; ** – p < 0.05 compared to the citicoline group; CU – conventional units.

The intensity of GFAP fluorescence was significantly lower in all treatment options (the 3rd-8th groups) compared to the 2nd group of animals with IR only. The best result was demonstrated by the 3rd group with human umbilical cord Wharton’s jelly-derived MMSC transplantation, for which the integral fluorescence density of GFAP-positive astrocytes was 54.3 ± 2.8×10⁶ CU (the 7th day) and 50.8 ± 2.7×10⁶ CU (the 14th day), but did not reach the values of the control group of sham-operated rats. Also, the integral fluorescence density of GFAP-positive astrocytes in this group was statistically significantly lower compared to the reference drug citicoline both on the 7th and 14th days of the study (Fig. 4).

Immunohistochemical analysis of frontal brain slices for the presence of the microglial cell marker Iba1 showed that on the 7th and 14th days after IR, a decrease in reactive gliosis was observed in the 3rd-8th groups of experimental animals compared to the group of animals with only ischemia, but the indicators did not reach the control values of Iba1-immunofluorescence in sham-operated rats of the control group (Fig. 5).
Fig. 5. Confocal micrographs of frontal sections of the hippocampal CA1 area in rats of the studied groups (1-8), stained with the microglial cell marker Iba1. The layers in the CA1 area are marked with letters: a – stratum oriens, b – stratum pyramidale, c – stratum radiatum, d – stratum lacunosum-moleculare. The upper panel of photomicrographs is the 7th day after ischemia-reperfusion, the lower panel is the 14th day. Scale bar – 100 μm.

Quantitative analysis of fluorescence intensity showed that in the group 2 with IR the integral fluorescence density of Iba1-positive microglial cells significantly increased, which was 35.4 ± 2.3×10^6 CU on the 7th day, and 32.6 ± 1.8×10^6 CU on the 14th day compared to the control group of sham-operated animals (9.5 ± 1.4×10^6 CU and 8.4 ± 0.9×10^6 CU, respectively) (Fig. 6).

Fig. 6. Integral density of fluorescence of Iba1-positive microglial cells on frontal sections of the hippocampal CA1 area of the studied rat groups (1-8).
Notes: * – p < 0.05 compared to the control; # – p < 0.05 compared to ischemic animals; ** – p < 0.05 compared to the citicoline group; CU – conventional units.
The intensity of Iba1-immunofluorescence significantly decreased in all treatment options (the 3rd-8th groups) compared to the group of experimental animals with only IR. The best result was demonstrated by the 3rd group with the human umbilical cord Wharton’s jelly-derived MMSC transplantation, for which the integral fluorescence density of Iba1-positive microglial cells was $17.6 \pm 1.8 \times 10^6$ CU (on the 7th day) and $16.4 \pm 1.0 \times 10^6$ CU (on the 14th day), but still did not reach the values of the control group of sham-operated rats. Also, the integral fluorescence density of Iba1-positive microglial cells in this group was statistically significantly lower compared to the reference drug citicoline, both on the 7th and 14th days of the study (Fig. 6).

Thus, immunohistochemical analysis using antibodies against three types of glial cells demonstrated that IR induced reactive gliosis, manifested by increased fluorescence intensity and hypertrophy of GFAP-positive and Iba1-positive glial cells in all layers of the hippocampus. Hypertrophy of GFAP – the main component of the intermediate filaments of astrocytes – is considered a sign of reactive astrocytes in primates and rodents [36, 37]. After an ischemic stroke, reactive astrocytes in the penumbra zone form a glial scar that separates the ischemic core from healthy tissue [37, 38]. Reactive astrogliosis can limit neuronal growth and repair of nerve tissue after ischemia. In many studies, reactive astrocytes have been shown to suppress a wide range of molecules that inhibit axonal regeneration, such as chondroitin sulfate proteoglycans, thereby reducing neuroplasticity of neural tissue after ischemia [39, 40].

In our study, the use of all treatment options with the transplantation of MMSCs of various origin, their lysate, or the reference drug citicoline had a neuroprotective effect and reduced reactive astro- and microgliosis, although statistically significant differences between the investigated parameters on the 7th and 14th days in the groups were not observed. This is likely due to the fact that MMSCs secrete a wide array of trophic and immunomodulatory cytokines, commonly referred to as the MMSC secretome, which has significant potential to treat ischemic brain injury through the induction of endogenous neuroprotection, neurogenesis, and angiogenesis [41, 42]. We suppose that the introduction of the studied agents in our experiment directly after ischemia-reperfusion contributed to neuroprotection both by activating the proliferation of neural progenitors and by preventing their damage and death. Similar conclusions were made with intravenous administration of bone marrow-derived MMSCs in the rat model with middle cerebral artery occlusion, where the transplanted cells showed an immunomodulatory effect by inactivating microglia in the peri-infarct zone [43].
In our study, the best result was shown by the treatment with human umbilical cord Wharton's jelly-derived MMSCs, which also had a statistically significantly better effect compared to the reference drug citicoline on both the 7th and 14th days of the study. Human Wharton's jelly-derived MMSCs induce neurogenesis through a paracrine mechanism, as they express more genes involved in angiogenesis and neurogenesis, especially secretory factors [44]. In general, the revealed neuroprotective properties of stem cell transplantation may indicate the prospect of their use in cell therapy of acute cerebral ischemia.

**Conclusions**

1. According to the immunohistochemical analysis of three types of glial cells, brain ischemia-reperfusion in rats caused reactive gliosis in the form of increased fluorescence intensity of GFAP-positive and Iba1-positive glial cells in all layers of the hippocampus.
2. The use of human umbilical cord Wharton's jelly-derived MMSCs and rat fetal fibroblasts significantly reduced the intensity of reactive gliosis on both the 7th and the 14th day after modeling the ischemic brain injury.
References


22. Lyden J, Grant S, Ma T. Altered metabolism for neuroprotection provided by mesenchymal stem cells. Brain Circ. 2019; 5:140-144. https://doi.org/10.4103/bc.bc_36_19


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