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Original Article

Effect of HEPO genetically modified neural stem cell subarachnoid transplantation on rats with cerebral infarction

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Abstract: Objective: To investigate effect of HEPO genetically modified neural stem cell subarachnoid transplantation on functional recovery in rats with nerve injury. Methods: rats' neural stem cells were cultured in vitro, while eukaryotic expression plasmid pcDNA3.1hEPO was established. They were transfected into neural stem cells and divided into three groups: cerebral infarction group, NSC s group, h EPO-NSC s group. Western blot was used to analysis h EPO protein expression in neural stem cells before and after transfection. 65 SD rats were selected. The middle cerebral artery occlusion (MCAQ) model was established. After successfully modeled, they were randomly divided into cerebral infarction groups, NSC s group, and h EPO-NSC s group with 20 in each group. After vein graft, fluorescence microscopy was used to observe the survival and distribution of CM-Di l labeled NSC. RT-PCR and Western Blot detection were used to detect AQP4 and AQP9 genetic changes and protein expression level in rats model with brain infarction; Before modeling, one day, three days, one week, two weeks, three weeks, four weeks after modeling, the modified (m NSS) neurological behavior scoring method was used to evaluate neurological function; the percentage of area use the to calculate infarct area; The rats were sacrificed under anesthesia condition, pathological changes of brain tissue were observed by HE staining; TUNEL assay was used to detect apoptosis. Results: Western blot results showed that human h EPO gene transfected neural stem cells were capable of expressing h EPO; RT-PCR and Western Blot test results showed that AQP4 and AQP9 gene and protein expression level in rats model with brain infarction in h EPO-NSC s group was significantly less than that in the NSC s group and the CI group with statistical significant difference ($P < 0.05$); m NSS neurobehavioral score showed in h EPO-NSC s group it was significantly lower than that in the NSC s group and cerebral infarction group with statistical significant difference ($P < 0.05$); Two weeks after transplantation, compared with and NSC s group and cerebral infarction group, cerebral infarction area and the edema in h EPO-NSC s group were reduced ($P < 0.05$); HE staining results showed that the cerebral tissue surrounded infiltration of inflammatory cells and edema had been significantly alleviated ($P < 0.05$); TUNEL assay showed that in EPO-NSC s group, the number of apoptotic cells was significantly less than the NSC s group and cerebral infarction group ($P < 0.05$). Conclusion: HEPO gene modified neural stem cells can reduce the expression of AQP4 and AQP9 gene and protein around the infarct zone, reduce the impact of neurological damage after cerebral infarction, reduce neuronal apoptosis, and promote the recovery of neurological function.

Keywords: Rat, neural stem cells, transplantation, infarction, HEPO, AQP4, AQP9

Introduction

Cerebral infarction is also known as cerebral stroke, which is a kind of cerebrovascular disease that seriously threatened to people health. Pathogenesis and treatment of cerebral infarction process are very complex. Therefore how to develop effective programs for early prevention and treatment of cerebral infarction has been the focused by cerebrovascular disease specialists. Now it is also a worldwide problem [1-3]. At home and abroad, a number of studies showed that neural stem cells (neural stem cells NSC s) are a kind of self-proliferation and pluripotent seed stem cells. Under certain conditions, it can be differentiated into various cells of the nervous system. In nerve repair aspect, it has broad application prospects [4, 5]. Studies showed that, H EPO was widely presented in various tissues in the ner-

vous system and it played an important role in development, growth, maintenance, and protection of nervous system [6-8]. The present study was designed to investigate the recovery condition of HEPO genetically modified neural stem cell subarachnoid transplantation on rats with cerebral infarction. The detailed are reported in the below.

Materials and methods

Materials

Animals: 10 SD fetuses of gestational age of 15 d, body weighting 10-14 g, (a 15 d-pregnant SD weighed 322 g); infarction modeling: 65 healthy SD rats, body weighting 220-255 g; all the animals in this experiment were purchased from the animal laboratory of Tianjin Medical University; animal quality certification number: SCXK (Tianjin) 20090068.

Methods

Culture of neural stem cells: Culture of neural stem cells: A 15 d-pregnant SD rat was sacrificed and soaked in 75% ethanol for disinfection. Open the rat ventral to remove fetuses; fetal rat brain were removed and soaked in DMEM/F12 solution, removing the meninges and blood vessels. After the removal of the meninges and blood vessels, the fetal brain was soaked in DMEM/F12 medium, pipetted into suspension with a straw repeatedly, and filtered through a 100-mesh sieve holes; the filtered suspension was seeded in culture flask, incubated with EGF (10 μg/mL, b F GF (10 μg/mL), N2 additive in a 37°C, 5% CO_2 incubator. Culture medium was changed after 72 h.

Identification and labeling of neural stem cells: Cells were identified by immune chemical staining method; according to reference [9], the identified NSC s were digested with 0.25% trypsin and prepared into single cell suspension, washed once with serum-free DMEM/F12 medium, and re-suspended with 0.5 mL dilution C cells at a concentration of 2×10^7/mL; According to CM-Di l staining instructions, immunofluorescence staining was performed. Neural stem cells of the third generation were digested into single cell with complex digestive juice, co-incubated with 5 μmol/L of CM-Dil fluorescent dye in dark at room temperature for 5 min, and then incubated at 4°C for 15 min. The labeled cells were reseeded in complete medium and cultured in dark for 72 h (flasks were wrapped with aluminum foil). Labeled cells were centrifuged prior to transplantation; the cell clumps were collected and gently pipetted by glass pipette. After labeling, 1×10^5 cells were immediately collected and washed with PBS once, fixed with 1% paraformaldehyde, using FCM to detect cell labeling rate. Meanwhile fluorescence microscope (Olympus, Tokyo, Japan) was used to observe the survival and distribution of CM-Dil labeled NSC s.

H EPO transfection and Western blot test: According to reference [10], the 4th generation of well-growing neural stem cells were incubated in DMEM medium containing 10% fetal calf serum in a humidified 5% CO_2, 37°C thermostatic enclosed incubator, passaged every two days; cells in logarithmic growth phase were seeded in 24-well plates at a density of 6×10^4 cells/well; after 3 days, rAAV2-h EPO transfection was performed: culture solution was discarded; cells were washed twice with PBS; draw the residual medium from the bottle, add serum-free L-DMEM culture medium-diluted RAAV2-h EPO with multiple infection (MOI) = 10^5 to make it completely cover the cultured area, incubate at 37°C for 2 h, and then add the right amount of fetal bovine serum and L-DMEM medium respectively, continue to culture for 1 week as conventional adherent cells before the following experiments. In 3 d before the test, do not replace the cell culture medium. No-load virus transfection was performed under the same conditions; normal cells under same culture conditions were taken as the CI group. Each cell suspension was centrifuged (radius of 16 cm, 800 r/min, 5 min) and the target cells were collected; after the cell culture medium was discarded, 400 μL protein lysate was added to extract the total protein; protein concentration was determined by Bradford method. After 5% stacking gel 40 V constant for 1 hour, 10% separating gel 60 V constant for 3.5 hours, wet transfer 14 V constant for 14 hours, and closed at 37°C in the shaker for two hours, and 10 min membrane-washing (three times), protein was incubated with h EPO polyclonal rabbit anti-human antibody 1: 800 (Santa Cruz, USA) for 2 h in 37°C shaker; After washing the membrane by TBST for 5 min*4 times, the protein was incubated with goat anti-rabbit antibody 1:700 (US BD Biosciences) for 1.5 h, shaking at 37°C; The membrane was washed by TBST for 5 min*4 times. DAB coloring was performed; the test was repeated three times before Quantity one image analysis; pro-

tein expression levels were evaluated by the optical density ratio of the target band and β-actin band.

Establishment of animal model and grouping: Modified Zea-Longa method was used to make (MCAO) model. 64 healthy Wistar rats were anesthesia with 10% 0.4 ml/100 g chloral hydrate, and spontaneous breathing were preserved. Rats were kept on the operating table with a fixed supine. Regular neck skin preparation and disinfection of shop towels were well prepared. Do an opening in the middle of the neck, and carefully separate the right of free common carotid, internal carotid and carotid artery outside layer by layer, and avoid stimulating the vagus nerve. In the common carotid, external carotid, the internal carotid artery, a 3-0 silk was placed. Ligate the silk on the carotid artery, and ligate carotid artery at 5mm of external carotid and internal carotid bifurcation. Ligate the external carotid artery roots, and put the Nylon thread with a round of baking by ethanol lights into 18 mm of the internal carotid artery, until it reached a little resistance encountered sense then immediately stop. Block the blood supply to the corresponding parts. In this experiment, a total of 65 7-9-day-old Wistar rats were selected. One of BMSC s cultured rat was sacrificed. 64 rats were involved in the modeling. Four rats dead. Finally 60 rats were enrolled in the study. After successful modeling they were randomly divided into infarction group, NSC s group, h EPO-NSC s group with 20 in each group. Cerebral infarction group: Cells were injected into the intrathecal at 1 μl/min with 20 μL DMEM/F12 medium from the foramen magnum; NSC s Group: 20 μL (2×10^6 L-1) NSC s cell suspension were injected into subarachnoid slowly; h EPO-NSC s Group: 20 μL (2×10^6 L-1) h EPO-NSC s cell suspension were injected into subarachnoid slowly (Slowly injected 5 min, grafting once).

m NSS

The experiments performed m NSS scoring respectively before treatment, at 3 d, 1 w, 2 w and 3 w after treatment; improved neurological deficit scores (m NSS) Content and Standards: contents include movement, sensation, balance and reflection 4 parts; total score was 18 points; normal was 0 point; the lower the score, the better the neurobehavioral function in rats; the higher the score, the more severe the neurological dysfunction. In this study, m NSS scoring was performed before treatment, at 3 d, 1 w, 2 w and 3 w after treatment respectively.

TUNEL assay to detect apoptosis

In 7 days after transplantation, five rats in each group were anesthetized by chloral hydrate; infarct brain tissue was collected; using modified TUNEL method [11], 2.0 cm infarcted tissue in the infarct loci was collected and perfusion-fixed in 4% paraformaldehyde; paraffin sections were dewaxed, soaked in 70% alcohol for three minutes, washed with PBS (5 minutes*3 times). After incubation with proteinase K solution (20 ug/ml) at room temperature for 10 min, it was rinsed 3 times with PBS, 5 minutes once. Then it was closed for 15 minutes at 37°C in BSA wet box, balanced for 10 minutes with equilibration buffer, incubated in 37°C T d T enzyme reaction in the closed state for 1 hour, and reacted with horseradish peroxidase solution at room temperature for 20 minutes, and washed with PBS (3 times*5 minutes); After DAB coloring, it was rinsed with water, mounted with gum resin, dried at room temperature and observed under an inverted fluorescence microscope. There were green particles in the nucleus of TUNEL-positive cells; apoptotic cells in 10 photos were counted.

RT-PCR to detect AQP9 and AQP4 gene expression in the brain tissues of rats with cerebral infraction

In 7 days after transplantation, in each group 50 mg cerebral tissue of five rats were randomly collected, respectively, and homogenates were prepared; Trizol method [12] was used to extract total RNA of brain tissue; RNA content was measured by UV spectrophotometer; according to the instructions of MMLV kit, RNA was reverse transcribed into c DNA, and then c DNA were detected by PCR; according to Genebank data, primer design software Primer 5.0 (http://pga.mgh.harvard.edu/primerbank/) was used to determine the optimal primer; then through Blast match, primer was synthesized by Shanghai Sangon Biological Co. AQP4 (305 bp) upstream primer: 5'-CCA GCT GTG ATT CCA AAA CGG AC-3', downstream primer: 5'-TCT AGT CAT ACT GAA GAC AAT ACC TC-3'; AQP9 (305 bp) upstream primer: 5'-CCAGCTGTGATTCCAAAACGGAC-3', downstream primer: 5'-TCTAGTCATACTGAAGACAATACCTC-3'; β-actin (175 bp) upstream primer: 5'-CCATCATGAAGTGTGACGTTG-

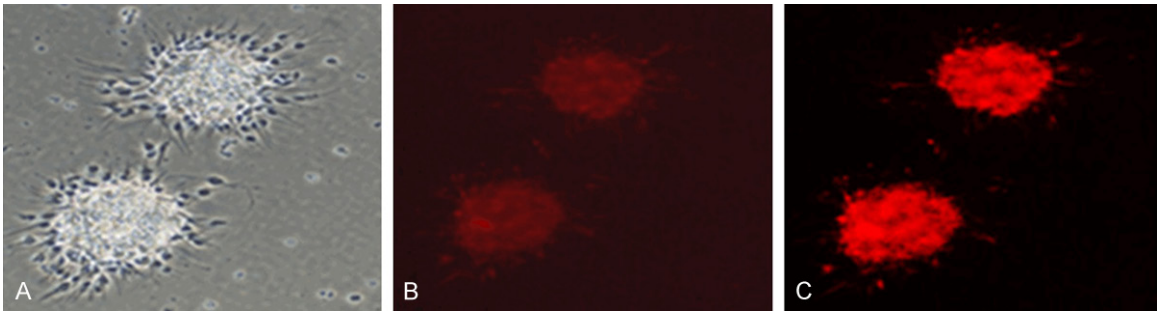


Figure 1. A: The morphology of primary neural stem cells under the Inverted phase contrast microscope (× 40); B: positive Nestin immunofluorescence staining in neural stem cells (× 40); C: CM-Di l-labeled NSC s (× 100).

3', downstream primer: 5'-ACAGAGTACTTGCGCTCAGGA-3'. PCR amplification product was subjected to electrophoresis; gel image analysis system was used for the optical density analysis of electrophoresis maps; the ratio of the integral optical density of AQP4/AQP9 product with β-actin product was calculated as AQP4/AQP9-mRNA expression.

Western Blot to detect AQP9 and AQP4 protein expression in the brain tissues of rats with cerebral infraction

In 7 days after transplantation, the above RT-PCR extract was centrifuged for 30 min; the supernatant was bold protein; protein concentration was determined by Bradford method. After 5% stacking gel 40 V constant for 1 hour, 10% separating gel 60 V constant for 3.5 hours, wet transfer 14 V constant for 14 hours, closed at 37°C in the shaker for two hours, and 10 min membrane-washing (three times), AQP4/AQP9 monoclonal antibody was diluted with TBST (1:200) and protein was incubated at room temperature for 60 min; After washing the membrane by TBST for 10 min*3 times, the protein was incubated with horseradish peroxidase-labeled rabbit anti-mouse antibody (1: 500) at room temperature for 60 min; the membrane was washed by TBST for 10 min*3 times. After washing the membrane for 10 min by TBS, DAB coloring was performed; Bio-Rad gel imaging system was used to scan absorbance values, which were analyzed by Quantity one software; AQP4/AQP9 protein expression levels were evaluated by the absorbance area ratio of the target band and β-actin band.

HE staining

At 4 weeks after transplantation, rats were anesthetized with 2.5% ketamine (20 mg/kg) by intraperitoneal injection; after decapitation, brain tissue was quickly removed, soaked in paraformaldehyde solution for 24 hours, dehydrated, transparent, Immersed in wax, embedded, and serially sectioned in coronal plane (3-4 um), five pieces every 100 um. After conventional dewaxing, sections were washed with distilled water for 2 min, stained with eosin for 1-2 min, stained with HE, and mounted with neutral gum. Under a light microscope, pathological and morphological changes in brain tissue were observed to confirm the degree of recovery. Meanwhile the tissue sections were observed by fluorescence microscopy. 10 horizons were randomly selected for each slice under high magnification (× 200); CM-DIL-positive cells per horizon were counted; the mean was the number of CM-DIL positive cells in each group.

Infarct area calculation

After final scoring, infarct area was calculated using area percent: in each group five rats were randomly selected and decapitated to remove the brain; after removal of the cerebellum, the brain was divided into five sections at an average thickness in the frontal plane, placed in TTC solution and incubated at 37°C for 15 minutes; Infarcted brain tissue sections were not stained; normal brain tissue was stained red; after saline washing and photography, a transparent coordinate paper was used to measure the infarct size in the photo.

Statistical analysis

Measurement data were expressed as mean ± SD; experimental data were analyzed by SPSS 17.0 software (SPSS, Chicago, IL, USA); averages of multiple groups were compared using

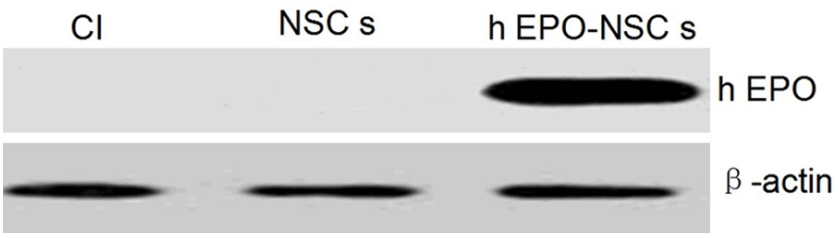


Figure 2. In 48 h after transfection, h EPO protein expression in each group. EPO protein expression was detected in h EPO-NSC s group, while there was no h EPO protein expression in CI group and NSC s group.

Table 1. Neurological deficit scores of rats in three groups

Groups	NSS				
	Before treatment	3 days after treatment	1 week after injury	2 weeks after injury	3 weeks after injury
CI group	15.3±0.62	11.1±0.34	9.9±0.94	8.64±0.75	7.15±0.52
NSC s group	14.26±0.54	10.05±0.45	9.5±0.62	7.92±0.91	5.42±0.66
H EPO-NSC s	10.21±0.43	10.06±0.22	8.6±0.75	4.79±0.87	2.29±0.92

ANOVA and LSD method; averages of two groups were compared using t test; $P < 0.05$ was considered statistically significant.

Results

NSC s vitro culture, identification and labeling

After incubation for 1 d, NSC s increased, small, irregular shaped; a small fraction of cells adherent; after culture for 5 d, NSC s increased, larger, spherical, shown in **Figure 1A**. Immunofluorescence staining showed that, NSC s showed strongly-positive expression of Nestin. Identification of neural stem cells was shown in **Figure 1B**: positive Nestin immunofluorescence staining in neural stem cells (× 40). Labeling of neural stem cells, CM-Di l labeled NSC s showed red fluorescence under a fluorescence microscope, shown in **Figure 1C**. Study confirmed that after CM-Di l-labeling, intracellular fluorescence was stable; positive labeling rate was above 97%; the morphology of labeled cells was good; we can effectively observe the induction and differentiation of cells in vitro, shown in **Figure 1C** CM-Di l-labeled NSC s.

Western blot

At 48 h after the recombinant adeno-associated virus vector-mediated human erythropoietin gene transfection, EPO protein expression was detected in h EPO-NSC s group, while there was no h EPO protein expression in CI group and NSC s group, indicating that the h EPO gene had been stably integrated into the NSC s in h EPO-NSC s group, and the NSC s could stably express the target protein, as shown in **Figure 2**.

Neurological deficit score

Neurological deficit scores of rats in three groups showed that in the first three days after transplantation, there were no statistically significant differences between h EPO-NSC s group and NSC s group, Cerebral infarction group; in two weeks after transplantation, there were statistically significant differences between h EPO-NSC s group and cerebral infarction group ($P < 0.01$), between h EPO-NSC s group and NSC s group ($P < 0.05$); between NSC s group and cerebral infarction group, the difference was statistically significant ($P < 0.05$) (**Table 1**).

TUNEL assay to detect apoptosis

In nucleus of apoptotic neuronal cells, specific green fluorescent particles were observed; under light microscope, apoptotic cells scattered throughout the cerebral infarction zone; apoptotic positive cells also existed in the edge of infarct zone. TUNEL assay showed that in NSC s group, apoptotic cells with green immunofluorescent particles (21.74±4.78) was significantly fewer than the Cerebral infarction group (34.64±6.84) ($P < 0.05$); apoptotic cells in h EPO-NSC s group were the fewest (8.54±2.20), shown in **Figure 3**.

RT-PCR

7 days later, the expression of AQP9 and AQP4 mRNA in surrounding tissues in cerebral infarction group was higher than that in NSC s group ($P < 0.05$); the difference was statistically significant. The expression of AQP9 and AQP4 mRNA in NSC s group was higher than h EPO-NSC s group ($P < 0.05$), and the difference was

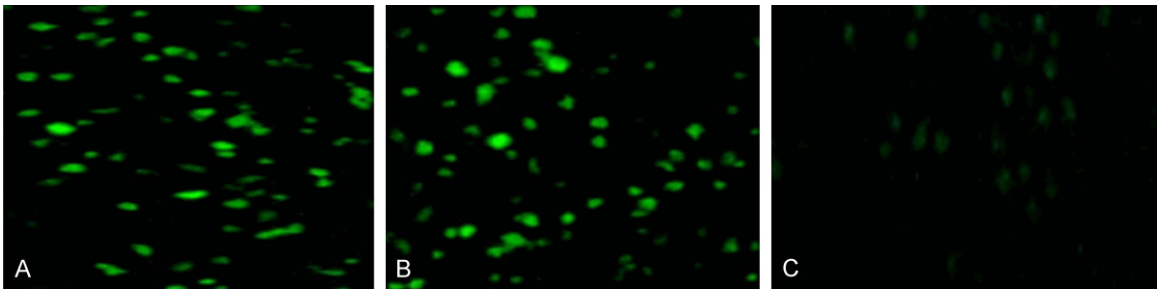
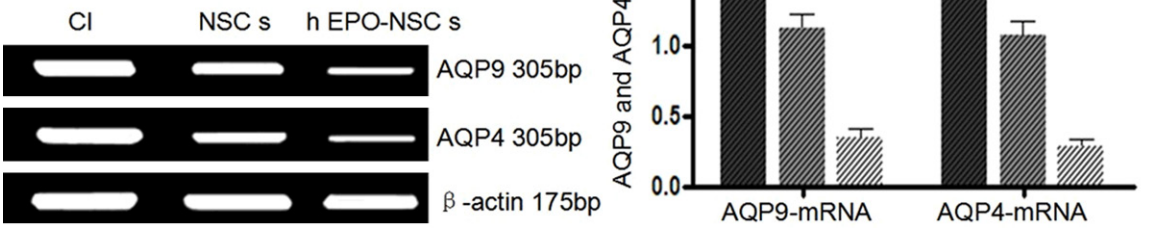


Figure 3. A: Apoptotic cells in CI group (34.64±6.84); B: Apoptotic cells in NSC s group (21.74±4.78); C: Apoptotic cells were the fewest in h EPO-NSC s group (8.54±2.20).

Figure 4. A: Electrophoresis of AQP9 and AQP4-m RNA; B: AQP9 and AQP4-m RNA expression.



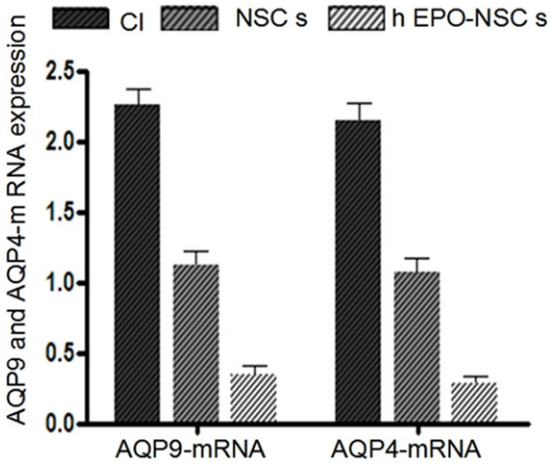
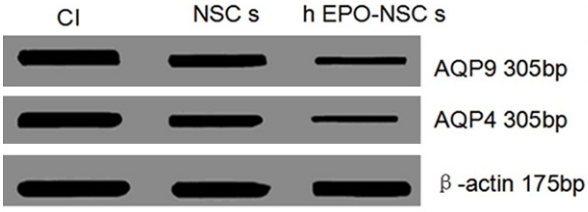


Figure 5. A: Electrophoresis of AQP9 and AQP4-m RNA; B: AQP9 and AQP4-m RNA expression.



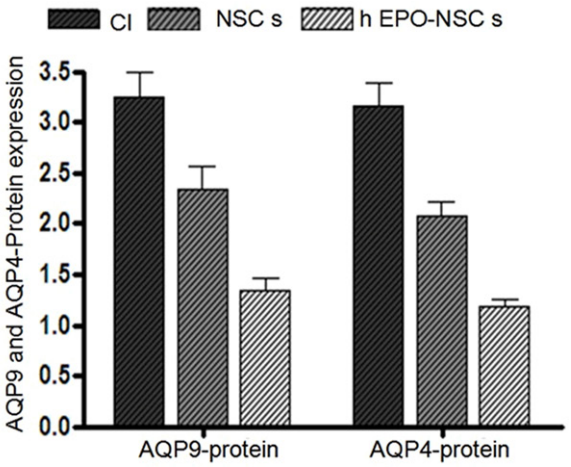


Table 2. Comparison of infarct volume in each group (x±s, %)

Group	Rats (n)	The infarct volume (%)	BWC
CI	5	53.45±6.28	81.63±3.32
NSC s	5	41.39±4.42	72.21±2.35
H EPO-NSC s	5	29.54±3.42[a]	59.05±1.68[a]

Note: Compared with NSC s group and CI group, [a]P < 0.05.

statistically significant, shown in **Figure 4A** and **4B**.

Western blot

7 days later, the expression of AQP9 and AQP4 protein in surrounding tissues in cerebral infarction group was higher than

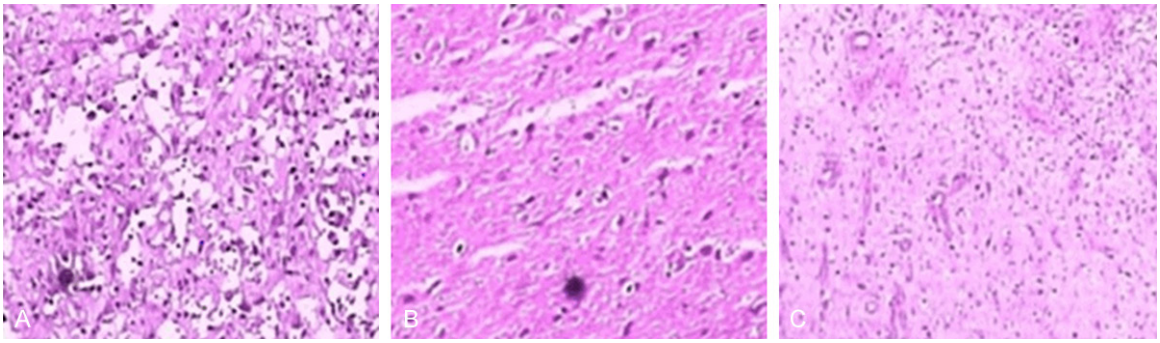


Figure 6. A: In cerebral infarction group, the infract brain tissue was filled with glial cells and glial fiber, forming glial scar and local cerebral atrophy. Malacia foci formed (× 40); B: In NSC s group, typical neuron-like morphological changes were observed; the graft scar and malacia loci were smaller than cerebral infarction group but larger than h EPO-NSC s group (× 40); C: H EPO-NSC s group showed typical neuron-like morphologic changes, disappeared malacia, and a small amount of scar.

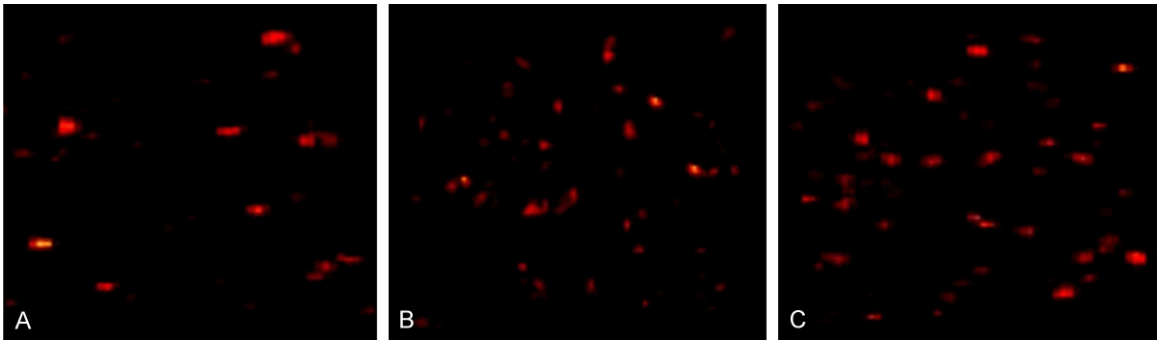


Figure 7. A: No CM-Di I labeled positive cells were observed under a fluorescence microscope from the frozen sections in cerebral infarction group (× 200); B: CM-Di I labeled positive cells observed under a fluorescence microscope from the frozen sections in NSC s group were fewer than h EPO-NSC s group but more than CI group (× 200); C: Under a fluorescence microscope, a large number of CM-Di I labeled positive cells were scattered in the frozen sections in h EPO-NSC s group (× 200).

that in NSC s group ($P < 0.05$); the difference was statistically significant. That in NSC s group was higher than h EPO-NSC s group ($P < 0.05$), and the difference was statistically significant, shown in **Figure 5A** and **5B**.

Comparison of infarct size and brain edema in each group

In 2 weeks after transplantation, compared with the NSC s group and cerebral infarction group, infarct area was reduced and the brain water content decreased in h EPO-NSC s group ($P < 0.05$). The infarct size and brain water content in each group were shown in **Table 2**.

HE staining and fluorescence microscopic observation

In two weeks after transplantation, HE staining showed that: In cerebral infarction group, the infract brain tissue was filled with glial cells and glial fiber, forming glial scar and local cerebral atrophy. Malacia foci formed (**Figure 6A**). In NSC s group, typical neuron-like morphological changes were observed; the graft scar and malacia loci were smaller than cerebral infarction group but larger than h EPO-NSC s group (**Figure 6B**). H EPO-NSC s group showed typical neuron-like morphologic changes, disappeared malacia, and a small amount of scar (**Figure 6C**). Fluorescence microscopy showed that, CM-Di I positive cells in infarct rat brain tissue: cerebral infarction group (**Figure 7A**): (0±0.00)/high power field; NSC s group (**Figure 7B**): (32.94±8.46)/high power field; h EPO-NSC s group (**Figure 7C**): (21.64±4.57)/high power field; after analysis of variance, differences between two groups were compared using Dunnett t test. Differences between the groups were significant ($P < 0.01$).

Discussion

Cerebral infarction is a common neurological disease with high mortality and high morbidity. Its early detection and early treatment is important [13, 14]. In recent years, along with research and development of stem cells, scientists study the application of neural stem cells (NSC s) in the cerebral function recovery from different aspects and different degrees; the results show that the neural stem cells, with strong proliferation and low immunogenicity, are conducive to cell transplantation, which can be used as an effective method to repair nerve dysfunction [15, 16]. Studies have shown that [17, 18], after transplanted into damaged brain tissue, neural stem cells can produce new nerve cells and promote regeneration of the brain. But after transplantation, the differentiating efficiency of NSC s into mature neurons is low, which may be related with the changes in the local microenvironment, severely restricting its application in the clinic.

Some studies have shown that [19], human erythropoietin (h EPO) is a glycoprotein to regulate bone marrow function; it not only regulates the development of central nervous system, but also be neurotrophic and neuroprotective. H EPO shows high expressions in human and animal central and peripheral nervous system; in addition, when the brain tissue is hypoxic, H EPO is also highly expressed, and exogenous H EPO can increase the neuroprotective effect, often in an autocrine or paracrine way [20-22]. Another study showed that [23-25], after cerebral infarction, H EPO play a brain-protective role by anti-inflammation, anti-oxidation, inhibiting free radical chain reaction, anti-apoptosis, promoting angiogenesis, improving intracellular Ca overload, reducing NO, glutamic acid and a variety of endogenous injury factors in brain cell damage and other injuries. In this study, H EPO modified rat neural stem cell was transplanted into rats with cerebral infarction through subarachnoid to observe its effect on functional recovery after nerve injury.

The Western blot results show that h EPO gene-transfected neural stem cells can express h EPO in vitro, suggesting that h EPO has been successfully transfected into NSC s. RT-PCR and Western Blot test results showed that the expression of AQP9 and AQP4 gene and protein in brain tissue of rats with cerebral infarction: in h EPO-NSC s group was significantly lower than NSC s group and the cerebral infarction group, with statistically significant difference, showing that h EPO gene can exhibit stable expression in the m RNA and protein levels. At different time points before and after treatment, m NSS neurological scores in each group showed that, m NSS neurological score in h EPO-NSC s group was significantly lower than that in the NSC s group and cerebral infarction group, indicating that after transplanted into infarction model, h EPO-transfected NSC s can better promote the recovery of neurological function. At 2 weeks after transplantation, compared with NSC s group and cerebral infarction group, infarct area in h EPO-NSC s group was reduced, suggesting that after transplanted into rats with cerebral infarction through the subarachnoid space, H EPO modified rat neural stem cells can reduce infarct size and promote the recovery of cerebral infarction; the findings were consistent with those of Lv Shuhua et al. [26]. HE staining showed that inflammatory cell infiltration and edema of surrounding tissues cerebral had been significantly alleviated compared with infarction group and NSC s group, indicating that H EPO modified rat neural stem cells had exact effect on rat cerebral infarction. It had been observed in TUNEL assay that in h EPO-NSC s group, the number of apoptotic cells was significantly less than the NSC s group and cerebral infarction group, indicating that after transplanted into infarct model, h EPO-modified NSC s can also inhibit cell apoptosis.

In summary, the h EPO successfully transfected into neural stem cells can enhance the proliferation of neural stem cells and promote neural stem cells to play a role better. I believe that with the development of molecular biotechnology, as well as further research on the h EPO neuroprotective mechanism in gene level, h EPO-tranfected neural stem cell transplantation will provide better material for the repairing, regeneration and restoration of nerve function after infarction, and have a profound significance also for the treatment of neurological diseases.

Disclosure of conflict of interest

None.

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