Original Paper

Transplanted Dental Pulp Stem Cells Migrate to Injured Area and Express Neural Markers in a Rat Model of Cerebral Ischemia

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Key Words

Focal cerebral ischemia • Dental pulp stem cells • Cell transplantation

Abstract

Background/Aims: Ischemic stroke is a major cause of disability and mortality worldwide, while effective restorative treatments are limited at present. Stem cell transplantation holds therapeutic potential for ischemic vascular diseases and may provide an opportunity for neural regeneration. Dental pulp stem cells (DPSCs) origin from neural crest and have neuro-ectodermal features including proliferation and multilineage differentiation potentials.

Methods: The rat model of middle cerebral artery occlusion (MCAO) was used to evaluate whether intravenous administration of DPSCs can reduce infarct size and to estimate the migration and trans-differentiation into neuron-like cells in focal cerebral ischemia models. Brain tissues were collected at 4 weeks following cell transplantation and analyzed with immunofluorescence, immunohistochemistry and real-time polymerase chain reaction (RT-PCR) methods. **Results:** Intravenously administration of rat-derived DPSCs were found to migrate into the boundary of ischemic areas and expressed neural specific markers, reducing infarct volume and cerebral edema. **Conclusions:** These results suggest that DPSCs treatment may serve as a potential therapy for clinical stroke patients in the future.

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Introduction

Ischemic stroke resulting from abrupt interruption of cerebral blood flow represents one of the major causes of disability and mortality worldwide [1]. The central nervous system has limited neuronal regenerative potential in response to stroke. Exploring reparative

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approaches is a promising field of investigation. Stem cell transplantation provides an alternative strategy for replacing the lost neurons and repairing the damaged tissues. Dental pulp stem cells (DPSCs) are originated from cranial neural crest, have neuro-ectodermal features with stronger proliferation, and exhibit neurogenic, odontogenic, chondrogenic, adipogenic and myogenic differentiation abilities after long term cryopreservation [2-4]. DPSCs have received much attention in the field of regenerative medicine because of their easy isolation, expansion and storage [5]. Since these cells are applicable for autologous tissue regeneration and cell-transplantation therapies without the risk of an alloimmunization reaction, less ethical issues are involved in their clinical application. For potential use in neurological conditions, DPSCs can secrete neurotrophic factors with neuroprotective effect, enhancing neurogenesis and angiogenesis [6-8]. Adult human DPSCs may promote blood-brain barrier permeability via expressing vascular endothelial growth factor, also supporting their use in therapy for neurological disorders [9]. In fact, it is suggested that DPSCs may offer therapeutic potentials for neurodegenerative diseases [10], cranial nerve injury [11, 12], spinal cord injury, cerebral ischemia and so on. In this study, we evaluated whether intravenous administration of DPSCs can reduce infarct size and estimated the migration and trans-differentiation of transplanted DPSCs into neuron-like cells in a rat model of focal cerebral ischemia.

Materials and Methods

Animals

Pathogen free Male Sprague-Dawley (SD) rats weighing 240-250 g were purchased from the animal center of the second affiliated hospital of Harbin Medical University. The experimental procedures and study design were conducted according to institutional guides for animal experiments approved by the Institutional Animal Care and Use Committees of Harbin Medical University.

Experiment design

Rats were randomly allocated into two groups after middle cerebral artery occlusion (MCAO), i.e., saline group received saline injection 24h after MCAO (n=12) and DPSCs group subjected to DPSCs transplantation 24h after MCAO (n=12). A subset of animals (n=4/group) were allowed to survive for 1 week following MCAO to assess the establishment of the ischemic model. Other animals were allowed to survive 4 weeks after MCAO, with halves of animals subjected to histological (n=4/group) and biochemical (i.e., RT-PCR, n=4/group) studies, respectively. The 4 week surviving point was chosen considering that this time would allow the transplanted cells to home and possibly integrate in the brain and exhibit putative protective impact on stroke recovery. In addition, pilot study was carried out to familiarize with and establish the model of MCAO, with animals (n=10) sacrificed 24h after MCAO occlusion and brains stained with triphenyltetrazolium chloride (TTC) to visualize the infarct areas.

Isolation, culture, labeling and transplantation of rat derived DPSCs

Dental pulp was harvested from the incisors of 4-week-old male SD rats and was digested with 0.3% collagenase in phosphate-buffered saline. The cells were placed into 75 cm² plastic flask and then cultivated in 12 ml of alpha modification of the Eagle's medium (α -MEM), including 10 % fetal bovine serum, 1% penicillin (100 U/ml) and streptomycin (100 mg/ml), at 37°C in humidified atmosphere with 5% CO₂. After 72 hours, new culture medium was added and the non-adherent cells were removed. When adherent cells grew to 80% confluency, cells were incubated with 0.25% trypsin and 1 mM ethylenediaminetetraacetic acid at 37°C for 2 minutes. The above prepared passage 3 DPSCs were used for this experiment. To identify and monitor the transplanted cells in the injured regions, noninvasive green fluorescent dye PKH 67 labeling was used. A total of 1×10^6 cells DPSCs in 0.5 ml saline was injected into the MCAO rats via tail vein 24 h after the induction of stroke, i.e., a subacute phase of stroke. Equal amount of saline was injected into control rats. All of these procedures were conducted under aseptic protocols. The following measurements were performed four weeks after the DPSCs transplantation or saline injection.

Induction of focal cerebral ischemia

Focal cerebral ischemia was established through the occlusion of middle cerebral artery (MCAO). Animals were anesthetized by intraperitoneal injection of 10 % chloral hydrate (3.5 ml/100 g). Body temperature was maintained at $(37 \pm 0.5) ^\circ\text{C}$ by using heating lamps during the surgery. A ventral midline incision in the neck was made to expose and isolate the right internal carotid artery (ICA), external carotid artery (ECA) and common carotid artery (CCA) bluntly. The CCA was ligated close to its origin with a 4-0 silk suture, then a 4.0 monofilament nylon suture with a rounded silicone wrapped tip was inserted into the right ICA, and advanced 18-20 mm into the origin of right middle cerebral artery (MCA) until a slight resistance was felt. After 120 min of the occlusion of the right MCA, the 4.0 monofilament nylon suture was removed to allow reperfusion. The sham group received the same procedure except for the occlusion of the right MCA. When the procedures were finished, the incision was sutured and the animals returned to their cages. The induction of brain ischemia via neck dissection and vessel ligation could avoid a major concern for regenerative therapies for CNS diseases caused by intracranial surgery or disruption of the blood brain barrier.

TTC staining

Triphenyltetrazolium chloride (TTC) stain was used to visualize the infarct volume as described previously [13]. At 24h after reperfusion, the brains were rapidly removed on ice, frozen at $-20 ^\circ\text{C}$ for 25min and cut into five consecutive coronal slices, which were incubated in 1% TTC in 0.1 M sodium phosphate buffered saline (PBS, pH 7.4) at $37 ^\circ\text{C}$ for 25 min. In the slices red area represented normal brain tissue and pale area was infarct tissue. All brain slices were photographed with a digital camera, with the total infarct zone ratio for each brain calculated as follows: $(\text{left hemisphere area} - \text{right non-infarcted area}) / (\text{left hemisphere area} \times 2)$ [14].

HE staining

Animals were perfused transcardially with 0.9% saline followed by 4 % paraformaldehyde. The brains were cut into frontal slices and then embedded with paraffin. Paraffin sections were treated with xylene, 100% ethanol for 5 minutes twice, and 95%, 90%, and 80% ethanol each for 3 minutes, stained with hematoxylin for 10-15 minutes, and washed with tap water for 10 minutes. After this, 1% hydrochloric acid alcohol was added for 1 second to visualize the blue color, washed with tap water for 10 minutes and further stained with eosin for 5 minutes. HE stained sections were dehydrated with 80%, 95%, and 100% graded ethanol for 1 minute twice, cleaned with xylene and coverslipped with neutral gum before light microscope examination.

Immunofluorescence

Rats were anaesthetized with intraperitoneal injection of 10% chloral hydrate and then perfused transcardially with 0.9% NaCl at 4°C and followed by 4% paraformaldehyde in PBS (pH 7.4). The brains were removed, kept in 4% paraformaldehyde at 4°C overnight and then immersed in 20% and 30% phosphate-buffered sucrose at 4°C until sinking into the bottom. The brains were cut into 35 micron thick frontal sections in a cryostat. After blocking with PBS containing 5% normal goat serum, the frozen sections were incubated with primary antibodies including rabbit anti- β III tubulin, rabbit anti-doublecortin (DCX), mouse anti-nestin and mouse anti-neurofilament H (NF-H) overnight at 4°C . The sections were washed three times with 0.1 M PBS, 5 min each time and incubated with rhodamine conjugated anti-rabbit/mouse IgG/IgM (1:400) for 1.5 h at room temperature. The nuclei were stained with 4'-6-diamidino-2-phenylindole (DAPI). Co-localization of PKH 67, neural specific markers and DAPI was conducted by laser scanning confocal microscopy at wavelengths of Alexa Fluor 594 nm (red), Alexa Fluor 488 nm (green) and Alexa Fluor 405nm (blue).

Immunohistochemistry

The rats were deeply anaesthetized at four weeks after cell transplantation by an overdose of 10% chloral hydrate. The rat brains were fixed by transcardiac perfusion with saline, followed by perfusion and immersion in 4% paraformaldehyde in 0.1 M PBS solution (pH 7.4). The sections were then rinsed with Tris buffer and treated with 0.3% H_2O_2 to block endogenous peroxidase. Following this, the sections were blocked with 10% normal goat serum and 0.1% Triton X-100 in PBS for 30 minutes at room temperature,

incubated with rabbit anti- β III tubulin, rabbit anti-doublecortin (DCX), mouse anti-Nestin, mouse anti-neurofilament H (NF-H) overnight at 4°C, with goat anti-mouse or anti-rabbit IgG (1:500) for 1 hour and with an avidin-biotin-peroxidase complex (Vectastain ABC kit) for 1 hour at room temperature. After brief washing in PBS, sections were counterstained with hematoxylin, dehydrated, mounted, then observed and analyzed under a bright-field microscope to determine the number of positive cells in the brain tissues.

RT-PCR analysis

RNA was harvested from ischemic brains by using Trizol reagent and reverse transcription was conducted according to conventional protocols. All the primer sequences of neural specific markers were confirmed by established GenBank sequences. The primers were used to amplify the duplicate PCRs, while β -actin was used as a control. Each sample was analyzed in triplicate. Cycling procedures included pre-incubation at 95°C for 15 seconds, annealing and extension at 57°C for 60 seconds, followed by 95°C for 15 seconds and finally cooling at 4°C for 5 minutes.

Statistical analysis

Data were expressed as the mean $\pm$ Standard deviation (SD). $P < 0.05$ was considered statistically significant. All statistical analyses were performed with Graph Pad prism5.0.

Results

Characterization of DPSCs

The DPSCs started to form colonies in 4-5 days and attached to the plate surfaces. DPSCs displayed ramified, triangular, oval and typical fibroblast-like cell morphology and showed high proliferative capacity (Fig. 1). Around 7 days, adherent DPSCs reached full confluency.

Focal cerebral ischemia animal model

TTC and HE staining were used to analyze and verify the focal cerebral ischemia model. White zone in brain tissues represented infarct areas caused by ischemic injury, and red zone represented normal brain tissues (Fig. 2). Brain infarcts of right hemisphere were induced by the occlusion of middle cerebral artery during the acute phase. Rats in sham group displayed red staining, suggesting no ischemic damage. HE staining showed remarkable decrease of neurons in the ischemic brains. Neuronal arrangement was irregular with vacuoles, eosinophilic cytoplasm and increased gap formation between cells. Inflammatory cells infiltration was detected.

DPSCs reduced cerebral infarct volume

To investigate whether intravenously transplanted DPSCs could decrease infarct volume and cerebral edema in cerebral ischemic models, TTC staining was conducted

Fig. 1. Morphology of dental pulp stem cells in culture. A: P0: DPSCs start to form colonies 4-5 days in vitro and attach to the plate surfaces. B~C: P2~P3: DPSCs display ramified, triangular, oval and typical fibroblast-like cell morphology. D: PKH67 labeled DPSCs emit green fluorescence under fluorescence microscope.

to measure infarct volume and brain edema at 1 week after cell transplantation. Data showed that cerebral infarct volume was about 30.20 ± 2.14 % in saline group, whereas the white zone was about 20.85 ± 1.86 % in DPSCs group ($p < 0.05$) (Fig. 3). Therefore, brain edema and neuronal damage was also significantly relieved in DPSCs group compared with saline group.

DPSCs migrate into ischemic areas

The spatial distribution of DPSCs was observed and analyzed following transplantation into the cerebral ischemic models. The grafted green fluorescent dye pre-labeled cells could easily be identified under fluorescent microscope at higher magnification, while the signal could not yield sufficient cellular resolution at low magnification (data not shown). Compared with the saline group, we detected transplanted cells that were colocalized with cell tracker PKH 67, neural specific markers and DAPI under laser scanning confocal microscopy in DPSCs group. PKH 67 have low cytotoxicity and emit green fluorescent signal *in vivo*. PKH 67 positive cells expressed neural specific markers β III tubulin, DCX, Nestin, NF-H, which showed cytoplasm in yellow and DAPI-stained nuclei in blue (Fig. 4). The cells were almost gathered in the boundary of ischemia brain tissues, and were not detectable in the contralateral hemisphere.

Grafted DPSCs express neural specific markers

Immunolabeling for neural specific markers were used to detect the differentiation activity of DPSCs in the ischemic models. β III tubulin, an early neuronal marker, is a constituent of neuronal microtubules and plays an important role in axon growth and in the development

Fig. 2. Histopathological features of brain tissue 24 h after the occlusion of middle cerebral artery. A: TTC staining of brain slices from MCAO models. B: TTC staining of brain slices from the sham group. The red color represents normal tissue and pale areas are infarct regions. C: HE staining of animals in sham group represents normal histology in brain. D: HE staining of MCAO models indicates remarkable decrease of neuronal profiles. The infarct area contains irregular vacuoles, eosinophilic cytoplasm and increased gap formation around the cells.

Fig. 3. Infarct volume was analyzed and brain edema were measured with TTC staining at one week after cell transplantation. Saline group exhibited that cerebral infarct volume was about 30.20 ± 2.14 %, whereas the DPSCs decreased the white zone to 20.85 ± 1.86 % ($p < 0.05$).

of normal brain [15, 16]. DCX, a specific marker of immature neurons [17], is a valuable alternative marker used to measure the levels of neurogenesis [18]. Nestin, an intermediate filament protein, is expressed in dividing cells during the early stages of development in the central nervous system, peripheral nervous system and in myogenic and other tissues [19]. NF-H is a marker of mature neurons. Immunofluorescence data suggested that grafted DPSCs express neural specific markers. Immunohistochemistry data illustrated that DPSCs group had more positive signals for neural specific markers compared with saline group (Fig. 5). Moreover, RT-PCR suggested that the gene expression of neural specific markers was up-regulated in DPSCs group relative to saline group (Fig. 6).

Discussion

In this study, rat incisors derived DPSCs were successfully isolated and cultured. Transplanted DPSCs exhibited neuroprotective effect on brain ischemia rats, by reducing the infarct volume and enhancing the neurological function recovery after cerebral ischemic injury. The results of the present study suggest that transplanted DPSCs can survive, proliferate and migrate into and locate around the ischemic areas in a model of MCAO. DPSCs provide a novel platform for ischemic stroke treatment with the most appealing potentials. DPSCs have advantages of less invasive isolation from autologous dental pulp, superior proliferation and inherent propensity to differentiate into neural cells. Intravenous infusion is the gold standard delivery method in the administration of stem cells with the least damage compared with other administration routes such as

Fig. 4. Green fluorescence represent PKH 67, red fluorescence represent neural specific markers β III tubulin, DCX, Nestin and NF-H, blue fluorescence represent DAPI. PKH 67 positive cells express neural specific markers, which show cytoplasm in yellow and DAPI-stained nuclei in blue. The cells are almost gathered in the boundary of ischemic brain tissues, which are not detectable in the contralateral hemisphere.

Fig. 5. The expression of neural specific markers β III tubulin, DCX, Nestin and NF-H is up-regulated in DPSCs group compared with saline group by immunohistochemistry.

Fig. 6. The gene expression of neural specific markers is up-regulated in DPSCs group compared with saline group by RT-PCR.

intra-arterial, intrastriatal and intraventricular injection. Intravenous administration is safe, optimal and effective in present study and is suitable for clinical application. Transplanted DPSCs are identified with lipophilic green fluorescence dye PKH 67, and at 4 week post-transplant they express neural cell markers β III tubulin, DCX, Nestin & NF-H and are colocalized with DAPI in ischemic brain regions. Gene level analysis of the differentiating DPSCs was verified by Real-time PCR analysis.

A large number of PKH 67 labeled cells mainly gathered in the boundary of brain ischemic areas. This distribution pattern suggests that transplanted DPSCs could traverse the blood-brain barrier without the use of a permeabilizer. DPSCs survived, proliferated and migrated towards the injured regions and did not induce teratomas formation. Noninvasive tracing using green fluorescent dye PKH 67 provides an accurate and practical method to track grafted cells *in vivo*, which can be widely used in the research of cell transplantation therapy.

The mechanism underlying the migration of intravenously transplanted DPSCs to the ischemic lesions remains unclear. Several studies have reported that stem cells mobilization mechanism may involve cytokines, chemokines, and growth factors released at the injured site and hypoxia environment [20]. Stromal cell-derived factor 1 (SDF-1), which is a strong chemoattractant, is widely expressed by a variety of tissues such as the bone marrow, heart, liver, lung, lymph nodes, kidney, and brain [21]. SDF-1 and its receptor CXCR4 play vital roles in the homing of stem cells [22]. Our results illustrated that the groups which received DPSCs had a decreased infarct volume compared with the sham group. Another possible hypothesis is that interaction of DPSCs with the host brain may activate endogenous stem cell to proliferate and self-repair. In addition, DPSCs may attenuate stroke-induced inflammation and improve microenvironment in subcortical regions of the ischemic brains, protecting the

brains against ischemia. There are numerous preclinical studies using DPSCs transplantation needed to be investigated for neurological diseases. This beneficial treatment may become an important regenerative therapeutic strategy for clinical stroke patients in the future.

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Disclosure Statement

The authors have nothing to disclose.

References

- 1 Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, Moran AE, Sacco RL, Anderson L, Truelsen T, O'Donnell M, Venketasubramanian N, Barker-Collo S, Lawes CM, Wang W, Shinohara Y, Witt E, Ezzati M, Naghavi M, Murray C, Global Burden of Diseases I, Risk Factors S, the GBDSEG: Global and regional burden of stroke during 1990-2010: findings from the Global Burden of Disease Study 2010. *Lancet* 2014;383:245-254.
- 2 Ibarretxe G, Crende O, Aurrekoetxea M, Garcia-Murga V, Etxaniz J, Unda F: Neural crest stem cells from dental tissues: a new hope for dental and neural regeneration. *Stem Cells Int* 2012;2012:103503.
- 3 Alge DL, Zhou D, Adams LL, Wyss BK, Shadday MD, Woods EJ, Gabriel Chu TM, Goebel WS: Donor-matched comparison of dental pulp stem cells and bone marrow-derived mesenchymal stem cells in a rat model. *J Tissue Eng Regen Med* 2010;4:73-81.
- 4 Zhang W, Walboomers XF, Shi S, Fan M, Jansen JA: Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. *Tissue Eng* 2006;12:2813-2823.
- 5 Woods EJ, Perry BC, Hockema JJ, Larson L, Zhou D, Goebel WS: Optimized cryopreservation method for human dental pulp-derived stem cells and their tissues of origin for banking and clinical use. *Cryobiology* 2009;59:150-157.
- 6 Mead B, Logan A, Berry M, Leadbeater W, Scheven BA: Paracrine-mediated neuroprotection and neuritogenesis of axotomized retinal ganglion cells by human dental pulp stem cells: comparison with human bone marrow and adipose-derived mesenchymal stem cells. *PLoS One* 2014;9:e109305.
- 7 Nosrat IV, Widenfalk J, Olson L, Nosrat CA: Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons *in vitro*, and rescue motoneurons after spinal cord injury. *Dev Biol* 2001;238:120-132.
- 8 Nosrat IV, Smith CA, Mullally P, Olson L, Nosrat CA: Dental pulp cells provide neurotrophic support for dopaminergic neurons and differentiate into neurons *in vitro*; implications for tissue engineering and repair in the nervous system. *Eur J Neurosci* 2004;19:2388-2398.
- 9 Winderlich JN, Kremer KL, Koblar SA: Adult human dental pulp stem cells promote blood-brain barrier permeability through vascular endothelial growth factor- α expression. *J Cereb Blood Flow Metab* 2016;36:1087-1097.
- 10 Apel C, Forlenza OV, de Paula VJ, Talib LL, Denecke B, Eduardo CP, Gattaz WF: The neuroprotective effect of dental pulp cells in models of Alzheimer's and Parkinson's disease. *J Neural Transm (Vienna)* 2009;116:71-78.
- 11 Mead B, Logan A, Berry M, Leadbeater W, Scheven BA: Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury. *Invest Ophthalmol Vis Sci* 2013;54:7544-7556.
- 12 Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Okano T, Ogiuchi H: Tubulation with dental pulp cells promotes facial nerve regeneration in rats. *Tissue Eng Part A* 2008;14:1141-1147.
- 13 Gao L, Ji X, Song J, Liu P, Yan F, Gong W, Dang S, Luo Y: Puerarin protects against ischemic brain injury in a rat model of transient focal ischemia. *Neurol Res* 2009;31:402-406.

- 14 Ostrowski RP, Colohan AR, Zhang JH: Mechanisms of hyperbaric oxygen-induced neuroprotection in a rat model of subarachnoid hemorrhage. *J Cereb Blood Flow Metab* 2005;25:554-571.
- 15 Katsetos CD, Legido A, Perentes E, Mork SJ: Class III beta-tubulin isotype: a key cytoskeletal protein at the crossroads of developmental neurobiology and tumor neuropathology. *J Child Neurol* 2003;18:851-866; discussion 867.
- 16 Tischfield MA, Engle EC: Distinct alpha- and beta-tubulin isotypes are required for the positioning, differentiation and survival of neurons: new support for the 'multi-tubulin' hypothesis. *Biosci Rep* 2010;30:319-330.
- 17 Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG: Transient expression of doublecortin during adult neurogenesis. *J Comp Neurol* 2003;467:1-10.
- 18 Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N, Bogdahn U, Winkler J, Kuhn HG, Aigner L: Doublecortin expression levels in adult brain reflect neurogenesis. *Eur J Neurosci* 2005;21:1-14.
- 19 Michalczyk K, Ziman M: Nestin structure and predicted function in cellular cytoskeletal organisation. *Histol Histopathol* 2005;20:665-671.
- 20 Naaldijk Y, Johnson AA, Ishak S, Meisel HJ, Hohaus C, Stolzing A: Migrational changes of mesenchymal stem cells in response to cytokines, growth factors, hypoxia, and aging. *Exp Cell Res* 2015;338:97-104.
- 21 Wurth R, Bajetto A, Harrison JK, Barbieri F, Florio T: CXCL12 modulation of CXCR4 and CXCR7 activity in human glioblastoma stem-like cells and regulation of the tumor microenvironment. *Front Cell Neurosci* 2014;8:144.
- 22 Yu Y, Wu RX, Gao LN, Xia Y, Tang HN, Chen FM: Stromal cell-derived factor-1-directed bone marrow mesenchymal stem cell migration in response to inflammatory and/or hypoxic stimuli. *Cell Adh Migr* 2016;10:342-359.