Acta Histochemica 116 (2014) 1075–1084

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Comparison of administration routes for adipose-derived stem cells in the treatment of middle cerebral artery occlusion in rats

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ARTICLE INFO

Article history:
Received 28 March 2014
Received in revised form 11 May 2014
Accepted 12 May 2014

Keywords:
Adipose-derived stem cells
Ischemic stroke
Middle cerebral artery occlusion
Delivery route
Rat

ABSTRACT

Given that adult adipose tissue is an abundant, accessible and safe source of stem cells, the use of adipose-derived stem cells (ADSCs) provides a promising approach in ischemic stroke. The delivery route, however, for transplantation of ADSCs in clinical application remains controversial regarding the time window, cell type, safety issues, 'first pass' effect and therapeutic effect. To determine the optimal administration route in transplantation of ADSCs, we compared the therapeutic effect of the three mainly used administration routes of ADSCs in a middle cerebral artery occlusion (MCAO) rat model. Cells isolated from the adipose tissue of adult rodents were differentiated and characterized in vitro, and further transplanted in vivo by intravenous, intra-arterial or intra-ventricular delivery. The infarct volume, expression of neurotrophic factors and the neurobehavioral improvements were evaluated after the equal dose of BrdU labeled ADSCs transplantation. Our results indicated that the equal dose of ADSCs delivered intravenously were effective in improving the neurological outcome and reducing the infarct volume after ischemic brain injury in long term duration in contrast to intra-arterial and intra-ventricular delivery. At 1–7 days after transplantation, the increased expression levels of BDNF, VEGF, bFGF, Bcl-2, IL-10 and decreased levels of caspase-3 and TNF-α in the intra-ventricular and intra-arterial groups were significant in contrast to the intravenous group. There was no significant difference among the three groups after 7 days. Our findings suggest that compared with the intra-ventricular delivery, intravascular injection allows higher dose injection with fewer invasions and appears to be optimal in application with regard to therapeutic efficacy, safety and feasibility.

Introduction

Stroke is the third leading cause of death worldwide and nearly half the survivors are left with moderate to severe disabilities that seriously affect their daily lives (Roger et al., 2011; Lackland et al., 2014). The lifetime risk of stroke in adults is estimated at 8–10% (Seshadri et al., 2006). Ischemia accounts for 80% of stroke subtypes. Tissue plasminogen activator (tPA) was once considered an effective intervention in the treatment of ischemic stroke; however, fewer than 5% of ischemic patients can receive tPA treatment due to the risk of hemorrhage and a narrow therapeutic window (Donnan and Fisher, 2008; Kufner et al., 2013; Liu et al., 2014). Therefore, an effective treatment for ischemic stroke is urgently needed.

With the emergence and development of stem-cell biology and tissue-engineering technology, grafting stem cells may provide a means of regenerating nerve tissue damaged by ischemic stroke. Zuk et al. (2001) isolated a fibroblast-like population of stem cells with multipotency and self-renewal ability from human adipose tissue. These are now widely known as adipose-derived stem cells (ADSCs) (Zuk et al., 2001). As adult adipose tissue is an abundant, accessible and safe source of stem cells, the use of ADSCs circumvents ethical and immunological concerns and provides a promising application of stem-cell therapy for stroke patients (Mitchell et al., 2006; Kim and de Vellis, 2009; Mahmoudifar and Doran, 2010). However, the choice of delivery route for stem-cell transplantation remains controversial among clinicians, with debate over treatment windows, cell types, safety, the 'first-pass' effect and therapeutic outcomes (Meamar et al., 2013).

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http://dx.doi.org/10.1016/j.acthis.2014.05.002
0065-1281/

Therefore, the aim of this study was to compare the therapeutic effects of three major routes for the administration of ADSCs, using the middle cerebral artery occlusion (MCAO) rat model. The cells were isolated, differentiated and characterized in vitro. Infarct volume, expression of neurotrophic factors and neurobehavioral improvements were evaluated after the transplantation of BrdU-labeled ADSCs by intravenous, intra-arterial and intraventricular delivery routes.

Materials and methods

Animals

Pathogen-free, adult male Sprague-Dawley (SD) rats weighing 260–280 g were obtained from the Experimental Animal Center of the First Teaching Hospital of Xinjiang Medical University. All the animals were housed in polycarbonate cages containing wood shavings in an air-conditioned room (12/12-h light/dark cycle with humidity of 60 ± 10%, 22 ± 4 °C), with unlimited access to food pellets and water. The protocols for the experimental study received the approval of the Ethics Committee of the First Teaching Hospital of Xinjiang Medical University.

Isolation, culturing and characterization of ADSCs

The ADSCs were isolated from the adipose tissue of the rats as previously described, with some modifications (Zuk et al., 2002). In brief, subcutaneous adipose tissue was obtained from the inguinal regions of male SD rats euthanized with 10% chloral hydrate (350 mg/kg, i.p.). The isolated adipose tissue was washed three times with sterile phosphate-buffered saline (PBS) to remove contaminating debris and blood cells. The washed tissue was minced and digested with 0.075% collagenase (Type I, Sigma–Aldrich, St. Louis, MO, USA) at 37 °C in a water-bath shaker with gentle agitation for 40 min. Collagenase activity was neutralized with an equal volume of low-glucose Dulbecco's modified Eagle's medium (L-DMEM, Thermo Scientific, HyClone, Logan, UT, USA) containing 10% fetal bovine serum (FBS, HyClone, USA) and antibiotics (100 U/ml penicillin G, 100 g/ml streptomycin, Sigma–Aldrich, USA). The undigested tissue was removed by filtration through a 200-mesh filter. The filtrate was centrifuged at 1000 × *g* for 5 min. The pellet was resuspended in L-DMEM containing 10% FBS and antibiotics, then cultivated for 24 h at 37 °C in a 5% CO_2 incubator. After the unattached cells and debris had been removed, fresh L-DMEM medium was added to the adherent cells, which were then continuously cultured at 37 °C in a 5% CO_2 incubator. The medium was changed for the first time after 24 h, and thereafter every 3 days. When the cell density of the colonies reached approximately 90% confluence, the cells were digested with 0.25% Trypsin (HyClone, USA) and then passaged at a ratio of 1:2. The third generation of ADSCs was collected for use in subsequent experiments.

Immunohistochemical analysis was carried out to determine the identity of the isolated cells by assessing their surface markers. Briefly, the cultured ADSC cells (passage 3) were harvested and washed three times with PBS solution. Next, they were fixed in cold acetone for 8 min, washed three times with PBS, treated with 3% H_2O_2 for 10 min and again washed three times with PBS. The diluted primary antibodies (CD34 and CD44, Boster Bio-engineering, Wuhan, China) were consecutively added to the sample overnight during incubation in a 4 °C humid-chamber. Polymer enhancer was added sequentially to the sample at room temperature for 10 min. After being washed three times with PBS, the samples were visualized with a fresh preparation of diaminobenzidine (DAB). The nuclei were stained with hematoxylin, and the samples were mounted in a glycerine-gelatin mountant and observed under a phase-contrast microscope. Photographs were taken using a digital camera.

Differentiation of ADSCs in vitro

Adipogenic differentiation and identification of ADSCs: the cultured ADSC cells (passage 3) were harvested and seeded into 6-well plates with sterilized coverslips at a density of 1×10^5 cells per well, and then cultured in an adipogenic-induction medium, namely a DMEM/F12 medium containing 10% fetal bovine serum, 1 μmol/L dexamethasone (Sigma, USA) and 10 μmol/L insulin (Sigma, USA). The culture medium was changed every 3 days. After 14 days of induction, the culture slide was stained with Oil Red O. The sample was mounted with glycerine-gelatin mountant and observed under a phase-contrast microscope. Photographs were taken using a digital camera.

Neuronal differentiation and identification of ADSCs

The cultured cells (passage 3) were harvested and seeded into 6-well plates with sterilized coverslips at a density of 1×10^5 cells per well, and then cultured in a neural stem cell induction medium. After a week, the neural stem cell induction medium was exchanged for a DMEM/F12 medium containing 10% fetal bovine serum for further development. Half of this culture medium was replaced every 2 days. The expression of non-specific esterase (NSE) was identified by immunohistochemical analysis.

Animal grouping and transient middle cerebral artery occlusion

One hundred male adult SD rats were randomly divided into five groups: (1) a sham group; (2) a middle cerebral artery occlusion group; (3) a ventricular-transplantation group; (4) an arterial-transplantation group; and (5) a venous-transplantation group ($n = 20$ per group). The modified Longa method was used to establish a transient focal cerebral ischemia model (Longa et al., 1989). In brief, all of the rats were anesthetized with 10% chloral hydrate (350 mg/kg, i.p.), and a feedback-regulated water-heating system was used to maintain their rectal temperature at 37 °C. The right common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) of each rat were exposed via a midline neck incision. The tip of a 4–0 nylon monofilament was rounded by heating over a flame and coated with silicone. To occlude the origin of the middle cerebral artery (MCA), the monofilament was moved carefully into the ICA lumen via the ECA lumen until a slight resistance was felt. After 1.5 h, the suture was slowly withdrawn to allow reperfusion. The rats in the sham group received all of the surgical procedures in the absence of a suture.

Cell labeling and delivery routes for transplantation

At passage 3, the attached ADSCs were cultured with 20 μmol/L BrdU at 37 °C and 5% CO_2 for 48 h. The ADSCs labeled with BrdU were collected and counted. Cells at concentrations of 5×10^{10} L^{-1} and 1×10^9 L^{-1} were prepared for transplantation.

At 24 h after MCAO modeling, the rats were anesthetized with i.p. injections of 10% chloral hydrate. The rats in the intraventricular-transplantation group were fixed in a stereotaxic apparatus. A 1-mm hole was made in the skull of each rat with a dental drill, and 10 μL of BrdU-labeled ADSCs (5×10^{10} L^{-1}) were injected into the right lateral ventricle at the following coordinates: 0.8 mm posterior, 1.5 mm lateral and 4.0 mm ventral to the bregma. In the intra-arterial transplantation group, the ECA stump was exposed and 0.5 mL of BrdU-labeled ADSCs (1×10^9 L^{-1}) were