Experimental Neurology 239 (2013) 158–162

Contents lists available at SciVerse ScienceDirect

Experimental Neurology

journal homepage: www.elsevier.com/locate/yexnr

Brief Communication

Intra-arterial infusion of human bone marrow-derived mesenchymal stem cells results in transient localization in the brain after cerebral ischemia in rats

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ARTICLE INFO

Article history:
Received 14 May 2012
Revised 21 September 2012
Accepted 27 September 2012
Available online 8 October 2012

Keywords:
Biodistribution
Cell therapy
Cellular engineering
Cerebral ischemia
Mesenchymal stem cells
SPECT imaging

ABSTRACT

Cell therapies from various sources have been under intense research in stroke. Efficient homing of the cells to the injured brain without complications is necessary to realize the therapeutic potential of cell therapy. Intra-arterial (IA) infusion of cells bypasses the filtering organs and directs the cells to the target area more efficiently. Here we studied the biodistribution of human bone marrow-derived mesenchymal stromal/stem cells (BMMSCs) after a direct infusion into the external carotid artery (ECA) in rats. Cells, which were cultured without animal-derived agents and also treated with a proteolytic enzyme to transiently modify cell surface adhesion proteins, were infused 24 h after transient middle cerebral artery occlusion (MCAO). SPECT imaging was used immediately after cell infusion and 24 h thereafter to track ^{111}In-oxine-labeled BMMSC in sham-operated and MCAO rats. IA infusion of BMMSCs in rats resulted in immediate cell entrapment in the brain, but the majority of the signal disappeared during the next 24 h and relocated to the internal organs. In MCAO rats, radioactivity counts 24 h after infusion were higher in the ischemic hemisphere compared to the contralateral hemisphere. Our results showed that IA infusion through ECA is a safe and efficient administration route for BMMSCs resulting in a transient localization of cells in the rat brain.

Introduction

Stroke remains the main cause of disability in adults in western countries. To restore brain function after stroke, cell-based therapies have emerged as a promising approach (Banerjee et al., 2011; Bliss et al., 2010; Lindvall and Kokaia, 2011). Cell homing to the brain is far from the optimal, however, as suggested by limited behavioral recovery in experimental studies (Hicks et al., 2009; Mäkinen et al., 2006).

We have previously reported that intravenous (IV) injection of cells in rats after cerebral ischemia resulted in high accumulation of cells primarily into lungs followed by relocation to the liver and spleen (Lappalainen et al., 2008; Mäkinen et al., 2006). Intra-arterial (IA) infusion represents a route to circumvent the filtering organs, but is complicated by high mortality due to micro-occlusions (Li et al., 2010; Walczak et al., 2008). Recently a modified IA infusion technique with preserved carotid blood flow was described, which produced efficient homing in the brain without increasing mortality (Chua et al., 2011).

Cell type and cell modifications may equally affect homing of cells after intravascular delivery (Lundberg et al., 2012). Use of bone marrow-derived cells is particularly promising, since they home to the injured tissue and secrete factors that promote brain repair (Borlongan et al., 2011). It has been suggested that cell culture conditions, cellular engineering and cell surface modifications may also modulate migratory behavior and immunomodulatory properties of cells and improve/facilitate therapeutic potential (Hatlapatka et al., 2011; Sarkar et al., 2011).

The aim of the present work was to study the whole body biodistribution of IA administered human bone marrow-derived mesenchymal stromal/stem cells (BMMSC) 24 h following transient middle cerebral artery occlusion (MCAO) in rats. Cells were cultured without animal-derived reagents and also treated with a proteolytic enzyme known to transiently modify certain cell adhesion proteins. The biodistribution of ^{111}In-oxine labeled cells was studied by SPECT imaging immediately after IA infusion and 24 h thereafter.

Materials and methods

Animals

Nineteen male Wistar rats (251–359 g, Laboratory Animal Centre, Kuopio, Finland) were used in the study. Rats were maintained in a temperature controlled environment (20 ± 1 °C) with access to food (2016S, Teklad) and water throughout the experiment. Research and animal care procedures were conducted according to the guidelines

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http://dx.doi.org/10.1016/j.expneurol.2012.09.018

set by the European Community Council Directives 86/609/EEC and all the procedures were approved by the Animal Ethics Committee (Hämeenlinna, Finland).

Transient middle cerebral artery occlusion

Focal cerebral ischemia was induced by occlusion of middle cerebral artery by intraluminal filament technique as previously described (Longa et al., 1989). Under halothane anesthesia, the right common carotid artery (CCA), external carotid artery (ECA) and internal carotid artery (ICA) were exposed. Subsequently, the ECA was cut with microscissors and a heparinized nylon filament of 0.25 mm diameter was inserted into stump of ECA and advanced into ICA until a resistance could be observed (1.8–2.1 cm). After the period of occlusion (90 min), the filament was withdrawn and ECA was carefully closed by electrocoagulation leaving a long ECA stump for cell infusion. Sham-operated rats were treated in a similar manner except that the filament was not inserted into the ICA. Buprenorfin (0.03 mg/kg) was administered to relieve postoperative pain. Limb-placing test was used to evaluate the successful MCAO before cell infusion (Puurunen et al., 2001).

Isolation and culture of BMMSCs

Mesenchymal stem cells (MSC) were obtained from bone marrow aspirates from healthy volunteer donors aged 18–33 years after having signed informed consent according to the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the Helsinki University Central Hospital (Finland). Bone marrow was aspirated (20 ml) under local anesthesia from the posterior iliac crest by an experienced physician and collected in heparinized tubes. Mononuclear cells were isolated by density gradient centrifugation (Ficoll-Paque Premium, GE Healthcare, Uppsala, Sweden) according to the manufacturer's protocol. The isolated cells were plated at 4×10^5 cells/cm^2 and further cultured in heparinized low glucose Dulbecco's modified Eagle's medium (DMEM; Gibco, Life Technologies Ltd., Paisley, UK), supplemented with 10% platelet-rich plasma (Finnish Red Cross Blood Service, Helsinki, Finland), 100 U/ml penicillin and 100 μg/ml streptomycin according to Schallmoser et al. (2007) with minor modifications. After three days of culture, the primary cultures were washed thoroughly with phosphate-buffered saline (PBS) to remove non-adherent cells. The medium was changed twice weekly and the cultures were passaged when subconfluent (70–80% confluency) and subcultured at 1×10^3 cells/cm^2. All cells used in this study were of passage 2 (p2).

The subconfluent p2 cells were detached with either trypsin (TryPLe Express, Life Technologies Ltd., Paisley, UK) or 0.5% pronase (Roche, Mannheim, Germany) in PBS and 0.25 mM EDTA. Cell viability was determined for all the samples by Trypan blue exclusion or Nucleocounter NC-100 (Chemometec, Lillerod, Denmark). The cells were cryopreserved in 180 mg/ml human serum albumin (HSA, Albunorm 200 g/l, Octapharma AG, Lachen, Switzerland) and 10% dimethylsulfoxide (DMSO, Sigma-Aldrich, St. Louis, USA).

Characterization of cells

All established BMMSC lines were studied in p2 for multipotency capacity by osteogenic and adipogenic differentiation assays (Dominici et al., 2006) and karyotype. Additionally, the original stromal cell content was determined by the CFU-F assay (Friedenstein et al., 1974) enabling accurate calculation of population doubling (PD) numbers of the used cells. The PD number was calculated with the formula $PD = \log2(N_H/N_1)$, where N_H is the amount of cells in the end of the culture and N_1 the initial stromal cell number = numbers of CFU-F colonies.

BMMSCs were analyzed with flow cytometry for the cell surface epitope expression after the trypsin and pronase detachment. A panel of antibodies against positive and negative cell surface epitopes previously described for MSCs was used in each analysis (Dominici et al., 2006). In addition, the expression of CD44, CD49d, CD49e, CD146, CD166, and fibronectin (FN) (Abcam, Cambridge, UK) was studied. All antibodies were purchased from BD Biosciences unless stated otherwise and were used according to the manufacturer's instructions. Secondary antibody staining was done for FN with either FITC-conjugated goat anti-mouse IgG (Abcam) or Alexa 488-conjugated goat-anti rabbit IgG (Invitrogen, Life Technologies Ltd.). The labeled cells were run with a FACSAria (BD) flow cytometer and the results were analyzed with the FACSDiva software (BD). Adequate isotype control antibodies were always used.

Cell labeling and transplantation

The cryopreserved cells were thawed in water bath at 37 °C. Then, cells were decanted into the thawing medium containing α-MEM (Gibco) and 10% HSA. Cells were centrifuged and medium was removed. Cells were resuspended in labeling buffer (0.2 M Tris, pH 8.0) and ^{111}In-oxine (19.4–32.1 MBq, Nycomed Amersham, Piscataway, NJ) was added. After incubation at 37 °C for 30 min, free tracer was removed by centrifugation and cells were resuspended in PBS. Cell labeling efficiency was 72% based on the amount of ^{111}In that was internalized by cells. Cell viability after labeling was examined by Trypan blue staining. For cell infusion, rats were reanesthetized and the ECA stump was exposed. Labeled trypsin-detached BMMSCs (1.1×10^6) or modified BMMSCs (i.e., pronase-detached) (0.5×10^6) in 500 μl saline were slowly infused through the stump of the ECA while the blood flow was maintained in the internal carotid artery.

SPECT imaging

SPECT imaging of the brain and abdomen was performed using a small animal SPECT/CT (Gamma Medica Inc., Northridge, CA) (Lappalainen et al., 2008). A static 2D SPECT image (acquisition time 120 s) was collected from head and abdomen area 30 min after cell infusion and 24 h thereafter. CT images were taken from the same coordinates (256 projections, 60 kV). The sensitivity of SPECT imaging was around 1000 cells (Lappalainen et al., 2008). After last scanning, animals were sacrificed and samples from the brain, kidneys, liver, lungs, and spleen were collected for radioactivity measurements (Wallac Gammacounter). Data are represented/expressed as % of injected dose/tissue weight.

Histology

Additional rats were perfused for histological analysis immediately and 24 h after cell infusion to investigate perivascular localization of BMMSCs by using sequential double immunofluorescence staining. Vibratome sections (35 μm) were incubated overnight with an antibody specific for human nuclei (MAB1281, 1:1000, Millipore) followed by incubation with secondary antibody Alexa Fluor 488 goat anti-mouse IgG (1:150, Molecular Probes) for 2 h. The sections were then blocked with Fab-fragments (1:500, Jackson ImmunoResearch Laboratories Inc.) for 60 min. To stain blood vessels, the sections were incubated overnight with RECA-1 (1:1000, AbD Serotec) followed by incubation with Alexa Fluor 594 goat anti-mouse IgG (1:400, Molecular Probes) for 2 h. Finally, the sections were mounted on slides and coverslipped. The images were captured using a Zeiss Axio Imager M2 fluorescence microscope equipped with an AxioCam MRm camera. Negative control sections were incubated without primary antibodies and did not show unspecific staining.

Statistical analysis

The statistical differences were tested by SPSS software (IBM SPSS Statistics 19). Radioactivity counts between groups and hemispheres were compared using the Mann Whitney test and the Wilcoxon test,