Brain, Behavior, and Immunity 34 (2013) 56–66

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Bone marrow mononuclear cells exert long-term neuroprotection in a rat model of ischemic stroke by promoting arteriogenesis and angiogenesis

Jianping Wang [a,*], Lie Yu [a], Chao Jiang [a,b], Ming Chen [a], Chunying Ou [a], Jian Wang [b,*]

[a] Department of Neurology, The Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou, 450052 Henan, China

[b] Department of Anesthesiology/Critical Care Medicine, Johns Hopkins University, School of Medicine, Baltimore, MD, USA

ARTICLE INFO

Article history:
Received 11 May 2013
Received in revised form 6 July 2013
Accepted 17 July 2013
Available online 24 July 2013

Keywords:
Angiogenesis
Arteriogenesis
BMMNCs
Cerebral ischemia
Functional recovery
Infarct volume

ABSTRACT

Transplanted bone marrow-derived mononuclear cells (BMMNCs) can promote arteriogenesis and angiogenesis by incorporating into vascular walls and differentiating into smooth muscle cells (SMCs) and endothelial cells (ECs). Here, we explored whether BMMNCs can enhance arteriogenesis and angiogenesis and promote long-term functional recovery in a rat model of permanent middle cerebral artery occlusion (pMCAO). Sprague–Dawley rats were injected with vehicle or 1×10^7 BMMNCs labeled with BrdU via femoral vein 24 h after induction of pMCAO. Functional deficits were assessed weekly through day 42 after pMCAO, and infarct volume was assessed on day 7. We visualized the angioarchitecture by latex perfusion on days 14 and 42. BMMNC transplantation significantly reduced infarct volume and neurologic functional deficits compared with untreated or vehicle-treated ischemic groups. In BMMNC-treated rats, BrdU-positive cells were widely distributed in the infarct boundary zone, were incorporated into vessel walls, and enhanced the growth of leptomeningeal anastomoses, the circle of Willis, and basilar arteries. BMMNCs were shown to differentiate into SMCs and ECs from day 14 after stroke and preserved vascular repair function for at least 6 weeks. Our data indicate that BMMNCs can significantly enhance arteriogenesis and angiogenesis, reduce infarct volume, and promote long-term functional recovery after pMCAO in rats.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

After ischemic stroke, blood supply to the injured brain is decreased, leading to dysfunction of the brain tissue in that area. This type of stroke is usually precipitated by progressive atherosclerosis or an embolus from the heart or neck vessels (Shuaib et al., 2011). The only treatment currently available for ischemic stroke is thrombolytic therapy. However, it would be very beneficial to be able to restore anterograde perfusion of the ischemic territory through arterial revascularization (Bang et al., 2011; Shuaib et al., 2011).

The circle of Willis, leptomeningeal anastomoses, and microvasculature in the peri-infarction zone are important paths of collateral perfusion for the injured brain tissue (Carmeliet and Jain, 2011; Shuaib et al., 2011). Promoting additional collateral circulation may represent a potential treatment for ischemic stroke (Carmeliet and Jain, 2011; Shuaib et al., 2011). Collateral vessels bring bulk flow to ischemic cerebral tissues and may be sufficient to replace the stroke-induced loss of perfusion (Bang et al., 2011; Shuaib et al., 2011). Establishment of collateral circulation and new capillaries can be achieved by neovasculogenesis, which refers to the development of a new vascular network and comprises two different processes: arteriogenesis and angiogenesis (Ruiz-Salmeron et al., 2011; Troidl and Schaper, 2012). Arteriogenesis occurs when preexisting collateral arterioles transform into functional collateral arteries, whereas angiogenesis is the expansion of the vascular network via sprouting in the ischemic border (Carmeliet and Jain, 2011; Sugiyama et al., 2011). Evidence suggests that therapeutic arteriogenesis and angiogenesis can be stimulated by cell transplantation (Hao et al., 2011; Imada et al., 2005; Li et al., 2011; Ruiz-Salmeron et al., 2011; Yoon et al., 2010).

One cell type that might be useful for promoting collateral vessel growth through autologous or allogeneic transplantation is the bone marrow mononuclear cell (BMMNC). The arteriogenic effects of BMMNCs have been confirmed in limb ischemia and myocardial infarction (Imada et al., 2005; Li et al., 2011; Ruiz-Salmeron et al., 2011; Yoon et al., 2010). Transplanted BMMNCs also have been shown to cross the blood–brain barrier and to convert into neuronal and glial cell types in a cerebral ischemic animal model (Iihoshi et al., 2004; Zhang et al., 2011). Additionally, BMMNCs can

* Corresponding authors. Tel.: +86 371 68322417; fax: +86 371 66965783 (J. Wang). Tel.: +1 410 955 3640; fax: +1 410 502 5177 (J. Wang).
E-mail addresses: wjpwfy666@126.com (J. Wang), jwang79@jhmi.edu (J. Wang).

0889-1591/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbi.2013.07.010

promote functional recovery by decreasing neurodegeneration, promoting angiogenesis, reducing the level of proinflammatory cytokines such as tumor necrosis factor-α, and releasing numerous trophic factors such as nerve growth factor (Brenneman et al., 2010; Giraldi-Guimaraes et al., 2009; Hao et al., 2011; Ribeiro-Resende et al., 2009).

Although bone marrow harvest and reinfusion of autologous BMMNCs has been shown to be feasible and safe in patients with acute ischemic stroke (Savitz et al., 2011), no study has examined whether transplanted BMMNCs can promote arteriogenesis (especially for leptomeningeal anastomoses) and whether they can incorporate into the adult vasculature. We hypothesized that BMMNCs would promote neovasculogenesis by differentiating into vessel cells [smooth muscle cells (SMCs) and endothelial cells (ECs)] in a rat model of ischemic stroke. In the current study, we focused on testing the arteriogenic effects of BMMNCs and their direct impact on the prognosis of ischemic stroke. We found that transplantation of BMMNCs induced arteriogenesis and angiogenesis, alleviated ischemic brain damage, and promoted long-term functional recovery. To the best of our knowledge, this is the first report of using BMMNCs to promote cerebral arteriogenesis in a rat model of ischemic stroke.

2. Materials and methods

2.1. Animals and experimental groups

Adult male Sprague–Dawley rats (300–310 g, 3 months old; supplied by the Animal Experimental Center of Zhengzhou University) were randomly divided into four groups: sham-operated group ($n = 26$), untreated ischemic group ($n = 34$), vehicle-treated ischemic group ($n = 36$), and BMMNC-treated ischemic group ($n = 54$). All protocols were approved by the Animal Care and Use Committee of Zhengzhou University. All rats were given free access to food and water throughout the study.

2.2. Permanent middle cerebral artery (MCA) occlusion (pMCAO)

We induced pMCAO by intraluminal vascular occlusion (Wang et al., 2010) and used laser Doppler to assess regional cerebral blood flow (CBF) as described previously (Chen et al., 2009). Briefly, rats were anesthetized with an intraperitoneal injection of 4% (350 mg/kg) chloral hydrate. Rectal temperature was maintained at 37 ± 0.5 °C throughout the surgical procedure by means of a feedback-regulated water-heating system. An incision was made in the scalp to expose the skull. For detection of CBF on the MCAO side, the fiber optic probe (tip diameter, 0.5 mm) of a computer-controlled laser Doppler flowmeter (moorVMS-LDF, Moor Instruments Ltd., UK) was fixed to the surface of the skull on the left side (2 mm posterior, 6 mm lateral to bregma). Then, the left common carotid artery, external carotid artery, and internal carotid artery were isolated through a midline incision. The ipsilateral internal carotid artery was occluded with Heifetz aneurysm clips. To block the origin of the MCA, we inserted a nylon wire (0.22–0.24 mm in diameter, 18.0–19.5 mm in length) with a rounded tip from the left common carotid artery into the lumen of the internal carotid artery through a small incision. Successful MCAO was defined as ⩾80% decrease in CBF and was confirmed by laser-Doppler flowmetry. Sham-operated rats were subjected to the same surgical procedure except that the wire was not inserted. Untreated rats were subjected to pMCAO only. Twenty-four hours after pMCAO, cell-treated rats were anesthetized with 4% (350 mg/kg) chloral hydrate, and blunt dissection was used to isolate the femoral vein. A 26-gauge needle was inserted into the lumen of the vein, and approximately 10 million BMMNCs in 300 μL total fluid volume of phosphate-buffered saline (PBS) were delivered over a 3-min period based on a previous study (Yang et al., 2011). Vehicle-treated rats were injected with an equal volume of PBS. After completion of the injection, the syringe was withdrawn and pressure was applied to the femoral vein for approximately 1 min to stop bleeding. Skin was sutured after thorough disinfection.

2.3. BMMNC preparation

5-Bromo-2′-deoxyuridine (BrdU, B5002, Sigma–Aldrich, St Louis, MO, USA) was used as a tracer for newly formed DNA of bone marrow cells. We injected adult male Sprague–Dawley rats intraperitoneally with BrdU (50 mg/kg) once daily for 14 days before harvesting the bone marrow (Li et al., 2001). BMMNCs were isolated as previously described (Giraldi-Guimaraes et al., 2009; Ribeiro-Resende et al., 2009). The rats were anesthetized by an intraperitoneal injection of 4% chloral hydrate and then sacrificed. Their femurs were aseptically dissected and both ends cut; bone marrow was extruded with serum-free DMEM F12. The extracted bone marrow was subjected to density-gradient centrifugation (260g, 25 min) in 1.083 g/mL Histopaque 1083 (Sigma–Aldrich). The mononuclear cell layer was recovered from the gradient interface and washed with DMEM F12 in three consecutive steps of centrifugation (5 min) and resuspension. After cells were counted and the viability checked, BMMNCs were resuspended in cold, sterile PBS to a final concentration of 3×10^7 cells/mL and used immediately for implantation. This process required approximately 2 h.

2.4. Fluorescence-activated cell sorting (FACS) analysis

To evaluate the purity of the isolated bone marrow cells as putative BMMNCs, we subjected a portion of the cell pellet to FACS analysis to determine the expression of various immunophenotypic markers. Thus, approximately 1×10^7 cells of each rat ($n = 5$) were incubated in 2% fetal bovine serum in PBS at 4 °C for 30 min with 1 μL of monoclonal antibody specific for CD34 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), CD45, CD90 (BD Biosciences, San Jose, CA, USA), and CD117 (Abcam, Cambridge, MA, USA). To confirm the labeling rate, we incubated some cells with 2 M HCl to depurinate the DNA. Cells were washed with PBS containing 0.1% fetal bovine serum albumin and 0.2% Tween-20 and incubated with 1 μL of anti-BrdU-fluorescein isothiocyanate (BD Biosciences) for 30 min (Montes et al., 2011). As a negative control, some cells were incubated in buffer without primary antibodies.

2.5. Tests of neurologic function and body weight

An investigator blinded to treatment group tested the rats on days 1, 7, 14, 21, 28, 35, and 42 after pMCAO or sham surgery with the modified neurological severity score (mNSS; $n = 12$/group) (Narantuya et al., 2010). The mNSS comprises tests of motor function, sensory function, balance, reflex, and general movement on a scale of 0–22 (0 = no deficit, 22 = maximal deficit). In motor function tests, the rats are raised by their tail to evaluate the flexion of limbs and head movements in the vertical axis, or they are placed on a flat surface for gait analysis. In sensory function tests, the rats are examined for visual and tactile functions, and a proprioception test is performed by pushing the paw against the table edge to stimulate limb muscles. Balance is tested by placing the rats on a slim beam and counting the number of limbs that fall from the beam and the time before the rat falls off the beam. Reflexes tested were the pinna reflex, corneal reflex, and startle reflex. The pinna reflex is tested by touching the auditory meatus and observing whether the rat shakes his head. The corneal reflex is tested by lightly stimulating the cornea with cotton and observing whether the rat blinks. The startle reflex is tested by observing

Download English Version:

https://daneshyari.com/en/article/7281823

Download Persian Version:

https://daneshyari.com/article/7281823

Daneshyari.com