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BMSCs TRANSPLANTATION IMPROVES COGNITIVE IMPAIRMENT VIA UP-REGULATION OF HIPPOCAMPAL GABAergic SYSTEM IN A RAT MODEL OF CHRONIC CEREBRAL HYPOPERFUSION

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Abstract—Bone marrow mesenchymal stem cells (BMSCs) transplantation can ameliorate cognitive impairment in chronic ischemic brain injury, but the underlying mechanism is poorly understood. It is considered that the hippocampus holds the capabilities of memory consolidation and spatial navigation, and the gamma amino butyric acid (GABA)ergic system plays an important role in the control of learning and memory processes. Herein, we investigated whether transplantation of BMSCs could improve cognitive impairment via regulating the hippocampal GABAergic system in a rat model of chronic cerebral hypoperfusion. Animals treated with permanent bilateral occlusion of the common carotid arteries (two-vessel occlusion, 2VO) (a rat model of chronic cerebral hypoperfusion) received intravenous injections of BMSCs or saline as experimental group and control group I, the sham-operated rats received intravenous injections of BMSCs or saline as the sham group and control group II. Four weeks later, the Morris Water Maze was employed to evaluate the cognitive changes of each group, immunohistochemistry and western blotting was used to investigate the GABAergic system expression including GABA, glutamic acid decarboxylase 67 (GAD67) or $GABA_B$ receptor 1 ($GABA_BR1$) in the hippocampus. Our results showed that the 2VO model presented decreased capacities of learning and memory and down-regulated the expression of GABA, GAD67 or $GABA_BR1$ in the hippocampal CA1 subfield in comparison to the sham group ($P < 0.05$), while administration of BMSCs (experimental group) manifested increased performances of learning sessions and probe tasks, as well as up-regulated expression of GABA, GAD67 or $GABA_BR1$ compared with the control group I ($P < 0.05$). Collectively, these findings suggest that transplantation of BMSCs is capable of improving cognitive impairment via up-regulating the hippocampal GABAergic system in a rat model of chronic cerebral hypoperfusion. Hence, BMSCs transplantation could serve as an important tool for cell therapy in chronic cerebral hypoperfusion disorders.

Key words: bone marrow mesenchymal stem cells, hippocampus, GABAergic system, chronic cerebral hypoperfusion.

INTRODUCTION

Multiple previous studies in animal models have suggested that chronic cerebral hypoperfusion is associated with neural impairments and cognitive deficits in aging, vascular dementia (VaD) and Alzheimer's diseases (AD) (Waldau et al., 2010; Zhao et al., 2014; Wang et al., 2014b). Patients with these neurodegenerative diseases show marked reductions in cerebral blood flow and pathophysiological changes such as cerebral hypoperfusion, diffuse brain lesion or memory disturbances (Farkas et al., 2002; Zhao and Gong, 2015). In consideration of unprecedented scale of the aging problems and limited therapeutic measures for these diseases, most studies have focused on the chronic cerebral hypoperfusion to explore the underlying mechanism in aging, VaD and AD (Esposito et al., 2015; Lu et al., 2015; Ran et al., 2015). Permanent bilateral occlusion of the common carotid arteries (2VO) can cause an abrupt reduction of cerebral blood flow in cortical areas and the hippocampus. It also makes the circle of Willis provide compensatory blood flow from the vertebral arteries, which results in the global cerebral hypoperfusion with a diffuse brain lesion and cognitive dysfunction (Murakami et al., 1997). Thus, the 2VO model has been widely approved of inducing chronic cerebral hypoperfusion to investigate the pathophysiological processes of aging, VaD and AD (Ai et al., 2013; Auchter et al., 2014; Lu et al., 2015). It has been reported that chronic cerebral hypoperfusion can cause a wide range of neuronal damage and cognitive deficits (Zhao and Gong, 2015). For cognitive impairment, the hippocampus is considered the target region of 2VO-induced ischemia as the

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Abbreviations: AD, Alzheimer's diseases; ANOVA, analysis of variance; BMSCs, bone marrow mesenchymal stem cells; BrdU, bromo-2-deoxy uridine; EDTA, ethylenediaminetetraacetic acid; GABA, gamma amino butyric acid; $GABA_BR1$, $GABA_B$ receptor 1; GAD, glutamic acid decarboxylase; HCN, cyclic-nucleotide-gated cation nonselective; NGF, nerve growth factor; PBS, phosphate-buffered saline; two-vessel occlusion, 2VO, permanent bilateral occlusion of the common carotid arteries; VaD, vascular dementia; VEGF, vascular endothelial growth factor.

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pyramidal neurons of the hippocampal CA1 region are essential for spatial learning and memory (Bendel et al., 2005). In particular, gamma amino butyric acid (GABA)ergic system deficit in the hippocampal CA1 subfield has been shown as the specific damage of the 2VO model (Wang et al., 2014a; Lu et al., 2015).

Previous study reported that the excitatory-inhibitory balance is mainly maintained by GABAergic system in the central neural system (Gutierrez et al., 2003), and GABAergic system deficit is also the most consistently reproduced finding in hippocampal dysfunctions such as AD (Ma and McLaurin, 2014) and VaD (Li et al., 2014). GABA, the inhibitory neurotransmitter, is synthesized by glutamic acid decarboxylase 65 (GAD65) and GAD67, but the predominating enzyme GAD67 is specialized to synthesize the GABA supporting tonic and synaptic release of GABA in the central neural system (Gutierrez et al., 2003). In addition, previous study indicated that the GABA level is reduced with GAD67 down-regulation, while it remains unchanged in GAD65 knockout mice (Asada et al., 1997). Apparently, GAD67 expression can directly reflect the variation of GABA level in GABAergic system. Meanwhile, GABA can activate $GABA_A$, $GABA_B$ and $GABA_C$ receptors distributed widely throughout neurons in the brain, especially, decrease of $GABA_B$ receptor subunits has been clarified during cerebral ischemia (Huang et al., 2014), activation of $GABA_B$ receptors can restore the balance of cyclic-nucleotide-gated cation nonselective (HCN) 1/HCN2 surface expression in rat hippocampal CA1 area, while HCN 1 channels constrain learning and memory by regulating dendritic integration of distal synaptic inputs to pyramidal cells (Nolan et al., 2004). Moreover, recent study has reported that $GABA_B$ receptor 1 ($GABA_BR1$) is an effective factor to improve cognitive impairment induced by chronic cerebral hypoperfusion (Lu et al., 2015). Therefore, up-regulation of hippocampal GABAergic system such as GABA, GAD67 or $GABA_BR1$ might ameliorate cognitive impairment in a rat model of chronic cerebral hypoperfusion.

Stem cell-based therapy is a promising approach in the treatment of neurological disorders, which provides a strong therapeutic potential for acute ischemic stroke and chronic progressive neurodegenerative diseases (Abe et al., 2012; Yoo et al., 2015). Bone marrow mesenchymal stem cells (BMSCs) are considered as a leading candidate for neurological regenerative therapy due to their multipotency, minimal immunogenicity, culture expandability and paracrine action (Karussis et al., 2008; Abe et al., 2012). Recent studies have reported that BMSCs transplantation is capable of improving cognitive deficits in ischemic stroke (Prasad et al., 2014), VaD (Wang et al., 2014b) and AD (Salem et al., 2014), but the underlying mechanism has not been well known yet. There is growing evidence to support that therapeutic efficacy of BMSCs in ischemic disorders is due to their ability to selectively target damaged areas and release a wide array of trophic factors that drive the endogenous cell repair (Borlongan et al., 2011; Yang et al., 2015). Moreover, it has been shown that BMSCs can selectively increase hippocampal GABAergic pre-synapses, induce glial-dependent neuronal survival and trigger an augmented GABAergic transmission in hippocampal cultures (Mauri et al., 2012). In addition, our previous data demonstrated that BMSCs are capable of secreting GABA, vascular endothelial growth factor (VEGF) and nerve growth factor (NGF) (Long et al., 2013), and improving hippocampal function and neuronal regeneration in the epileptic model (Long et al., 2012). To sum up, BMSCs might hold potential capacity to treat the neurological disorders characterized by hippocampal dysfunction.

However, there has been no research on whether transplantation of BMSCs can ameliorate cognitive deficits via regulating the GABAergic system in a rat model of chronic cerebral hypoperfusion. In this study, we investigated the cognitive changes and hippocampal GABAergic system (GABA, GAD67 and $GABA_BR1$) alterations in a rat model of chronic cerebral hypop erfusion model after BMSCs transplantation.

EXPERIMENTAL PROCEDURES

Experimental animals

Approximate physical condition of 72 male Sprague–Dawley (SD) rats at ages of 4–6 weeks (weight 175–200 g) purchased from the Experimental Animal Center of Xi'an Jiaotong University and raised in a vivarium with 12:12-h light–dark cycle was monitored. Food and water were provided *ad libitum* throughout the experiment. All procedures performed in this study adhered to the guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Cell preparation

BMSCs were isolated from two donor rats as described in our previous report (Long et al., 2012). Briefly, bone marrow was collected from femurs and tibias of the sacrificed animals, and the mononuclear cells were harvested by density gradient centrifugation (400*g*, 25 min) with Histopaques-1077 (Sigma, CA, USA). Viability of the cells was determined using trypan blue solution (Sigma, CA, USA) and 60-100 viable cells/cm^2 in 75 cm^2 flasks were cultured with complete medium containing α-MEM (HyClone, UT, USA) and 10% fetal bovine serum (FBS) (Gibco BRL, MD, USA) (37 °C, 5% CO_2), the culture medium was changed every 3 days. When the adherent cells reached 70–80% confluence, the cells were passaged by 0.25% Trypsin (contained 0.02% EDTA). For detection of the surface antigens, approximately 2×10^5 adherent cells were incubated with primary antibody for 30 min with rabbit polyclonal anti-CD105, CD73, CD90, CD34 or CD11b (1:100 dilution) (Bioss, Wuhan, CHN), then the cells were washed and incubated with FITC goat anti-rabbit IgG (Invitrogen, #65-6111, USA) and analyzed by flow cytometry. To evaluate the multi-potency of these cells, the cultures were treated with StemPro® Osteogenesis (Gibco, A1007201, MD, USA), Chondrogenesis (Gibco, A1007101, MD, USA) or Adipogenesis (Gibco, A1007001, MD, USA) Differentiation Kits (37 °C, 5% CO_2) according to the instructions, and followed by Toluidine