Bone Marrow Stromal Cells Can Promote The Neurogenesis in Subventricular Zone in The Rat With Focal Cerebral Ischemia

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ABSTRACT

Introduction: Stroke is one of the most common diseases caused by occlusion or rupture of blood vessels in brain. It brings heavily loads for families and societies. Although some new strategies including treatment of tissue plasminogen activator have been applied in the clinic, these methods do not have perfect effect. Accordingly, more effective therapeutic strategies need to be developed. This study was conducted to investigate the action of bone marrow stromal cells (BMSC) on the neural stem cells in the subventricular zone of the rat after focal cerebral ischemia.

Methods: The rats were induced to permanent focal cerebral ischemia models with middle cerebral artery occlusion (MCA0). Test groups consisted of three groups: MCAO alone, intravenous infusion of 1 ml PBS at 24 hours after MCAO, and intravenous infusion of 2×106 BMSCs 24 hours after MCAO. Then, the groups were divided to investigate at 7 and 14 days after MACO. Neurological functions were detected to use Zausinger evaluation; meanwhile, 5-bromodeoxyuridine was injected to label the proliferating cells in the subventricular zone, and double-immunofluoroscent technologies were used to identify the cell type.

Results: Neurological functional scores of BMSCs-treated group were higher than other two groups ($p<0.05$) at 7 and 14 days after MACO. BrdU-positive cells in SVZ of ipsilateral ischemia of BMSCs-treated group were more than two controls ($p<0.05$) at 14 days after MCAO; double-immunofluorescence label demonstrated that BrdU-positive cells co-located with markers of neuron and astrocyte.

Discussion: BMSCs can promote the neurological function of the rats with permanent focal cerebral ischemia, which may associate with the neurogenesis in the subventricular zone.

Key Words:
Subventricular Zone,
Bone Marrow Stromal Cells,
Cerebral Ischemia,
Rat

1. Introduction

Stroke is one of the most common diseases caused by occlusion or rupture of blood vessels in brain. Although some new strategies including intravenous tissue plasminogen activator treatment (1) have been applied in the clinic, these methods don't have perfect effect. Recently, some studies reported that transplantation of bone marrow stromal cells (BMSCs) may solve this difficulty (2-3). Meanwhile, experimental evidence has shown that BMSCs can secrete some neurotrophic factors (4-6), which may promote the proliferation of neural stem cells. If BMSCs have this effect, it may contribute to recovery of the neuronal function of rat with stroke.

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Therefore, the purpose of this study was to investigate the action of BMSCs on the neural stem cells in the subventricular zone of the rat after focal cerebral ischemia.

2. Methods

2.1. Isolation of BMSCs and cell Culture

Bone marrow was obtained from the femurs and tibias of the rat (Sprague-Dawley, 4-6 weeks old). Then, the cells were suspended in L-DMEM with 15% FBS and incubated at 37 °C in 5% CO2 in flasks for 3 days. Non-adherent cells were removed by replacing the medium and the culture medium was replaced three times a week. After the culture reached confluency, the cells were rinsed with 0.25% trypsin and 1 mM EDTA for 3 min at 37° and cultured until the third passage for transplant.

2.2. Focal Cerebral Ischemia Model

Focal cerebral ischemia model was induced using a previously similar method of the intraluminal vascular occlusion (7). Briefly, adult Sprague-Dawley rats (250-300g) were used in this study. All the care and use of the animals conformed to guidelines of Experimental Animals Committee of Liaoning Medical University. Rats were initially anesthetized with 10% chloral hydrate. The right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were exposed. A monofilament nylon suture (diameter: 0.165 mm) was inserted from the ECA into the ICA until it blocked the origin of the middle cerebral artery (MCA). All the procedures were under the sterile condition. Then, all the models were divided randomly into three groups: MCAO alone, intravenous infusion of 1ml PBS at 24 hours after MCAO, and intravenous infusion of 2×106 BMSCs 24 hours after MCAO.

2.3. Bromodeoxyuridine Labeling

All animals were injected with bromodeoxyuridine (Brdu) (50 mg/kg) 3 times a day in 6 and 13 days after ischemia.

2.4. Behavioral Testing

Behavioral tests were performed with Zausinger'evaluation (8) before ischemia and at 1, 4, 7 and 14 days after ischemia.

2.5. Immunohistochemistry

Brains were removed after perfusion with saline and 4% paraformaldehyde in PBS at 7 and 14 days after ischemia and cut into coronal blocks (corresponding to bregma +1 mm to -1 mm) containing subventricular zone (SVZ). The brain blocks were post-fixed in 4% paraformaldehyde overnight and embedded paraffin. Then, a series of 5-μm -thick sections were cut on microtome (Leica).

2.6. Single Staining of BrdU

After being deparaffinized, the sections were pretreated with 4 N HCl at room temperature for 30 minutes to denature DNA and then incubated in 0.1M boric acid at room temperature for 10 minutes to neutralize residual acid. After antigen retrieved (trypsin at 37 °C for 10 minutes), the sections were incubated in 3% H2O2 at room temperature for 15 minutes, subsequently, in 5% BSA at room temperature for 30 minutes and in mouse monoclonal anti-Brdu antibody (Neomarker; 1:200) at 4 °C overnight. Sections were washed in PBS, and further incubated with biotinylated goat anti-mouse secondary antibody (Boster) for 30 minutes at 37 °C, washed with PBS, and placed in avidin-peroxidase conjugate solution for 30 minutes. The DAB was used to detect the horseradish peroxidase reaction. Finally, sections were dehydrated, cleard and coverslipped.

2.7. Double Staining of Proliferating Cells

To identify cell types, the double-immunofluorescence technique was used. Combination of the primary antibodies included: mouse monoclonal anti-Brdu antibody (Neomarker; 1:200) and rabbit polyclonal anti-NSE antibody (Boster; 1:100), mouse monoclonal anti-Brdu antibody (Neomarker; 1:200) and rabbit polyclonal anti-GFAP antibody (Boster; 1:100).

First, the pretreated procedure of section was the same as the single staining of Brdu. Then, the sections were incubated in the combination of primary antibodies, washed with PBS, incubated in FITC-labeled goat anti-mouse IgG and Rhodamine-labled goat anti-rabbit IgG as the secondary antibodies at 37 °C for 40 minutes. Finally, the sections were washed, coverslipped and detected with Zeiss microscope.

2.8. Data and Statistical Analysis

All values were given as mean ± S.E.M. Statistical analysis was carried out using student's paired t-test and one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Statistical P value less than 0.05 was considered significant.

Table 1. Comparison of BrdU-positive cells in SVZ of three groups

Groups	7 th Day After Ischemia		14 th Day After Ischemia	
	Ipsilateral SVZ	Contralateral SVZ	Ipsilateral SVZ	Contralateral SVZ
MCAO alone	43.93 ± 13.91 *	20.45 ± 6.94	31.82 ± 9.31	25.41 ± 12.21
PBS- Treated	44.85 ± 14.01 *	29.02 ± 16.44	33.75 ± 13.63	28.26 ± 10.65
BMSCs- Treated	49.87 ± 9.75 *	30.61 ± 10.38	66.81 ±15.33 *†	34.67 ± 9.10

* P<0.05 compared with contralateral of ischemia
† P<0.05 compared with sham-treated and PBS-treated group

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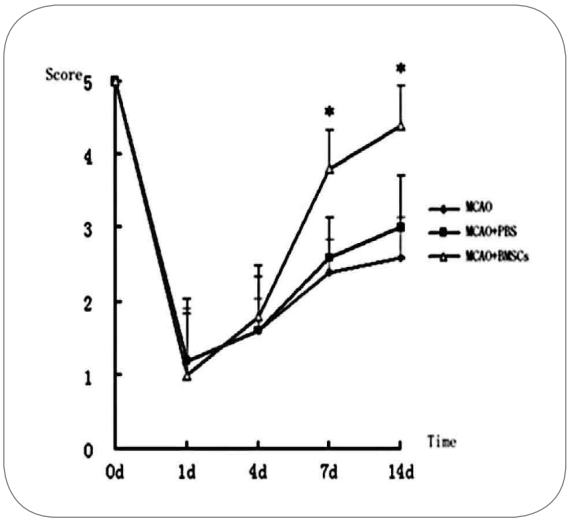

Figure 1. Neurological functional tests

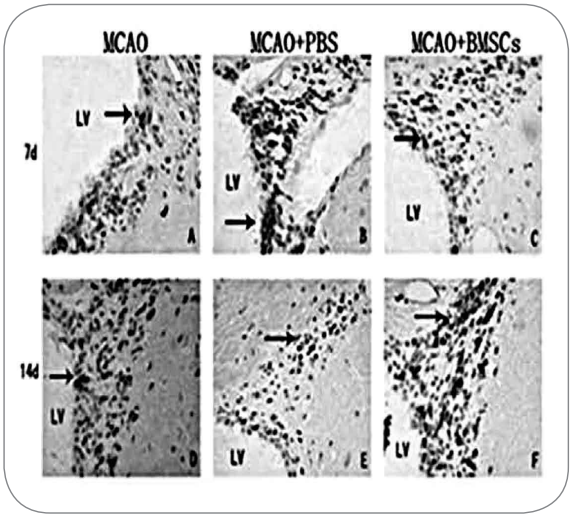

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Figure 2. New generated cells in SVZ ipsilateral ischemia. Sections through SVZ were stained for BrdUrd and visualized with DAB. (A-C) Brdu-positive cells located in the SVZ in three groups at 1 week after ischemia; (D-F)C) Brdu-positive cells located in the SVZ in three groups at 2 weeks after ischemia. (x200) (positive cells arre shown by arrow)

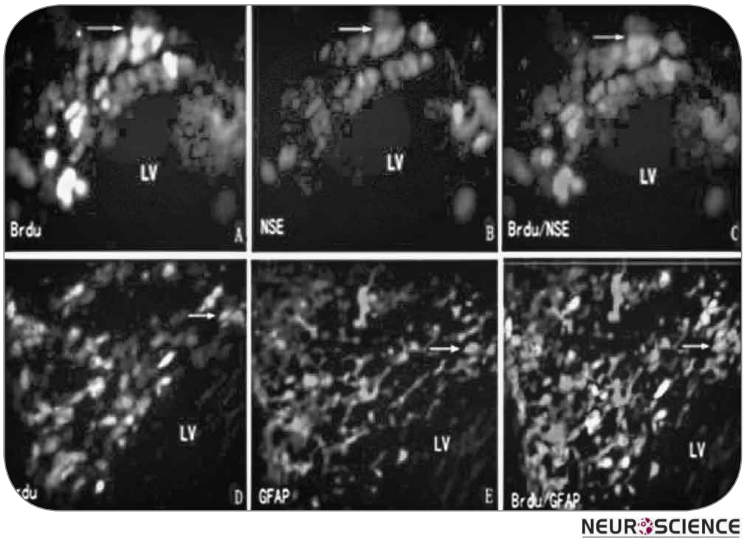

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Figure 3. Implantation of BMSCs promoted neurogensis. Neurogenesis in subventricular zones in the art with focal cerebral ischemia (C) New generated Brdu-positive cells were immunoreactive for the neural markers NSE; (F) New generated Brdu-positive cells were immunoreactive for GFAP, Which is the marker of astrocyte. The neural markers, (A,D) Brdu-positive cells (green); (B) NSE- positive cells (red); (E) GFAP-positive cells (red). (x400) (positive cells are shown by arrow)

3. Results

Body weight and serum glucose level were measured before and at 4th week after the experimcaused a significant reduction in the latter parameter in comparison with untreated-diabetic ones ($P<0.05$). On the other hand, although the weight and serum glucose level of NS-treated control rats was 8.2% and 5.2% lower than untreated-control animals, but the existing difference was not significant.

4. Discussion

In the past decades, many researches have confirmed that neurogenesis occurs in the adult brain and neural stem cells reside in the adult central nervous system (9). However, it is not very effective to restore the function of injured central nervous system only depending on neural stem cells. Recently, some evidence indicated that transplantation of BMSCs could enhance neurological recovery from neurologic disorders, such as stroke, trauma and paraplegia (10-12). Our present observation also demonstrates that this procedure can promote functional recovery in animals with focal cerebral ischemia. These results suggest that cell transplantation may serve as a future restorative therapy for stroke and other neurological disorders.

Many previous researches had focused mainly on the functional outcome of animals and the fates of donor cells (13-14). We showed here the action of transplanted BMSCs on the neurogenesis in the subventricular zone of the rat after focal cerebral ischemia. In the present study, the proliferating cells in the SVZ were labled using the analogue of thymidine bromodeoxyuridine, which will be taken up into the DNA of cycling cells (15). The results indicated that proliferating cells mainly located in the subventricular zone, where the neural stem cells located, which has been recognized. The proliferating cells also can be found in the rostral migratory stream. The distribution of the proliferating cells seems to be like the route of the migrating neural stem cells in the subventricular zone. Meanwhile, double-immunofluorescence technique supports that the proliferating cells were consisted of neuron and astrocyte, which may repair the injured brain and contribute to the recovery of neurological function.

We believe that the most significant factor contributing to the neurogenesis of the cells in the subventricular zone is some neurotrophic factors. At first, several growth factors that can increase proliferation will be stimulated to increase when the brain is subject to ischemic insult. For example, cerebral hypoxia and ischemia may stimulate neurogenesis through stem cells factor (16). Vascular endothelial growth factor (VEGF) stimulates the proliferation of neuronal precursors in murine cerebral cortical cultures and in adult rat brain in vivo (17). Furthermore, evidence in vitro and vivo demonstrated that transplantation of bone marrow stromal cells can also increase some neurotrophic factors to promote the proliferation of the cells within neurogenic regions (18). Munoz et al reported that implanted human bone marrow stem cells expressed ciliary neurotrophic factor (CNTF), nerve growth factor (NGF), vascular endothelial growth factor (VEGF) and neurotrophin-4/5 (NT-4/5), which probably accounted for the neurogenesis (4).

However, at present, it is still unknown whether recovery of neurological function after BMSCs transplantation into the brain of rat with stroke is tissue replacement or the effect of the neurathophic factors. To gain insight into the mechanism of action of BMSCs on the ischemia-injured animal, we believe that the following studies will be focused on biological characteristics of BMSCs in vitro and in vivo.

Our present findings indicated that transplantation of BMSCs can promote neurogenesis in the subventricular zone. This effect of BMSCs which may attribute to the neurofunctional recovery of the rat with ischemia is associated with the expression of some neurotrophic factors.

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