Neuroprotective Effects of *Brain-Derived Neurotrophic Factor* and *Noggin*-Modified Bone Mesenchymal Stem Cells in Focal Cerebral Ischemia in Rats

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Background: Administration of bone marrow stromal cells (BMSCs) has been reported to ameliorate functional deficits in rat ischemia models. In the present study, we tried to reveal the underlying mechanism of the improvement of neurological function after stroke by BMSCs transfected with *brain-derived neurotrophic factor (BDNF)* and/or *Noggin*. *Methods:* BMSCs were transfected with *BDNF* or/and *Noggin* using the adenovirus method. Middle cerebral artery occlusion (MCAO) rat models were treated with different types of transfected BMSCs. The treatment effect was assessed by measuring the modified Neurological Severity Score and the expression levels of different stroke-related molecules using Western blot, immunohistochemistry assay (IHC), and enzyme-linked immunosorbent assay (ELISA). *Results:* The injection of *BDNF* or/and *Noggin*-modified BMSCs could significantly improve the neurological function of MCAO animals. Western blot and IHC staining showed that the expression levels of vascular endothelial growth factor, BCL-2, p-GSK3β, and p-Akt were significantly upregulated, while the expressions of Bax, TLR4, and MyD88 were significantly downregulated. Moreover, ELISA assay revealed that the level of matrix metallopeptidase 9 (MMP-9) and reactive oxygen species were also significantly decreased. These results suggested that the treatment of *BDNF* or/and *Noggin*-modified BMSCs may suppress the ischemia-induced apoptosis and inflammation in the model animals, which might be through the Akt/GSK3β and TLR4/MyD88 pathways. *Conclusion: BDNF* or/and *Noggin*-modified BMSCs may exert neuroprotective effects through the Akt/GSK3β and TLR4/MyD88 pathways. Transplantation of *BDNF* or/and *Noggin*-modified BMSCs might be a potential therapeutic method for ischemic stroke in clinics. **Key Words:** Brain-derived neurotrophic factor—Noggin—bone marrow stromal cells—focal cerebral ischemia—neuroprotection—Akt/GSK3β—TLR4/MyD88.

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Introduction

Ischemic stroke is a major health problem leading to high mortality and severe long-term disability worldwide.[1] The disease is characterized by the rapid loss of brain function caused by a lack of brain blood supply and accounts for over 80% of all strokes. Ischemia in the brain will cause local and/or global neuronal death upon oxygen deprivation, causing permanent loss or impairment of body function.[2] Although extensive studies focusing on the ischemic stroke biology have been conducted, few options that may be effective in the treatment of the disease are available due to insufficient effect, unexpected adverse side effects, or time limitation.[1,3] Currently, treatments for ischemic stroke after the acute phase based on understanding the process leading to brain damage are being developed,[3] among which cell therapies with various types of progenitor/stem cells have all been tested in variable experimental stroke models and have shown potential to increase the functional improvement of neural cells.[4-6]

Bone marrow stromal cells (BMSCs) are a class of multipotent adult stem cells with the capacity to differentiate into several cell types of tissues such as osteoblasts, adipocytes, chondrocytes, and neural cells,[7-9] the regenerative potential of which has been assessed in myocardial, limb, and brain ischemia studies.[10,11] When administered intravenously, BMSCs can selectively migrate to the ischemic area and promote recovery after the ischemic stroke.[11] However, only about 1% of grafted BMSCs expressed neuronal markers in rats with cerebral infarction.[12] To solve this problem, variable interventions have been performed to expand the therapeutic effects of the treatment of BMSCs, such as being structured to produce brain-derived neurotrophic factor (BDNF)[13] and Noggin.[14] The former factor has a critical role in the growth, differentiation, and migration of neuron cells,[15] and the latter is an extracellular bone morphogenetic protein (BMP) antagonist, leading to the differentiation of stem cells into neurons.[16]

In the present study, bone mesenchymal stem cells transfected with BDNF and/or Noggin using the adenovirus method were constructed and used as the potential treatment for middle cerebral artery occlusion (MCAO) rat models. To underlie the mechanism of the treatment, possible factors or pathways involved in the recovery processes, including vascular endothelial growth factor (VEGF), BCL-2/Bax, GSK3β/p-GSK3β, Akt/p-Akt, TLR4/MyD88, matrix metallopeptidase 9 (MMP-9), and reactive oxygen species (ROS)[6,17-19] were determined using Western blot, immunohistochemical staining, and enzyme-linked immunosorbent assay (ELISA). We expected that the present study could reveal the mechanism of the *BDNF* and/or *Noggin*-transfected BMSCs in improving the neural function after ischemic stroke and provide some novel therapeutic targets for ischemic stroke in clinics.

Materials and Methods

BMSC Preparation

Four-week-old Sprague Dawley rats were provided by People's Liberation Army Academy of Military Medical Sciences, Beijing, China. All the animal experiments were conducted in accordance with the Institutional Animal Ethics Committee and Animal Care Guidelines of the People's Liberation Army Academy of Military Medical Sciences, China. BMSCs were expanded according to the protocol of Pittenger et al[7]: rats were executed by breaking the neck, and femurs and tibias were removed under sterile condition. Cells from the bone marrow were suspended in 10 mL culture solution Dulbecco's Modified Eagle's Medium (low glucose) (L-DMEM [Gibco, Grand Island, NY] supplemented with 10% fetal bovine serum [Gibco], 100 U/mL streptomycin [Gibco], and 100 U/mL penicillin [Gibco]) and cultured at 37°C with 5% CO_2]. Hematopoietic and nonadherent cells were removed and the culture medium was changed every 3 days. The cells were passaged 3-5 times before being used for experiments.

Adenovirus Infection

High-capacity adenoviral vectors expressing green fluorescent protein (*Ad-GFP*), BDNF (*Ad-GFP-BDNF*), or Noggin (*Ad-GFP-Noggins*) were purchased from Beijing Biosea Biotechnology Co., Ltd. (Beijing, China), with a titer of 10^{11} plaque-forming units/mL. BMSCs were infected at 100 multiplicities of infection, where *Ad-GFP*, *Ad-GFP-BDNF*, and *Ad-GFP-Noggins* were diluted using L-DMEM, low-glucose GlutaMAX, Pyruvate (Cat No. 10567-014; Gibco, China). The detailed process and the optimal multiplicities of infection were conducted according to previous studies.[20]

Animal Grouping, MCAO Model Establishment and Administration of Molecular Modified BMCSs

Forty-eight rats were evenly divided into 6 groups, eight for each group: sham group, surgical nylon suture 5 mm into the external carotid artery during the model establishment; vehicle group, MCAO animals administered with 1 mL .1 M phosphate-buffered saline; BMSC group, MCAO animals administered with *Ad-GFP* transfected BMSCs; BDNF group, MCAO animals administered with *Ad-GFP-BDNF* transfected BMSCs; Noggin group, MCAO animals administered with *Ad-GFP-Noggin*-transfected BMSCs; cotransfected (co-trans) group, MCAO animals administered with *Ad-GFP-BDNF* and *Ad-GFP-Noggin* cotransfected BMSCs.

Rats were anesthetized with 10% chloralhydrate and the transient MCAO model was induced by advancing a 4-0 surgical nylon suture (17.5-18.5 mm determined by body weight) with an expanded (heated) tip from the