Effects of Noggin-Transfected Neural Stem Cells on Neural Functional Recovery and Underlying Mechanism in Rats with Cerebral Ischemia Reperfusion Injury

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Objective: To investigate neuroprotection of noggin-transfected neural stem cells (NSCs) against focal cerebral ischemia reperfusion injury (IRI) in rats. *Methods:* Eighty Wistar rats were randomly divided into the sham, IRI, NSCs, and noggin + NSCs groups. Noggin containing adenoviral vectors was transfected into rat NSCs. Rats were subjected to 2.0 hours middle cerebral artery occlusion and reperfusion 1.0 hour, followed by infusion into the lateral ventricles of NSCs alone, noggin-transfected NSCs, and saline at 3 days in the NSCs, noggin + NSCs, and sham groups, respectively. All rats were sacrificed on 1, 3, 7, and 28 days after transplantation; the colorimetric method was used to detect the levels of superoxide dismutase (SOD) and the malondialdehyde (MDA) content after the behavior capability determined. Western blot was performed for detecting the expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) proteins. The TUNEL-positive and BrdU/nestin double-positive cells were observed under a light microscope and quantitative analysis was performed by morphometric technique. *Results:* Noggin-transfected NSCs significantly decreased the infarct volume and improved the neurological scores. Noggin-transfected NSCs also reduced the percentage of apoptotic neurons and relieved neuronal morphological damage. Noggin-transfected NSC transplantation markedly decreased the MDA levels and increased the SOD activity, and simultaneously downregulated the BMP4 (bone morphogenesis protein), VEGF, and bFGF proteins. *Conclusions:* The present study demonstrates that grafting NSCs modified by noggin gene provides better neuroprotection for cerebrovascular disease. **Key Words:** Noggin—neural stem cell—cerebral ischemia—dentate gyrus—neural functional recovery—neuron.

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Data availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

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Introduction

Cerebrovascular disease is a consequence of impairment of the blood supply to the brain, and ischemic strokes account for 85% of all strokes.[1] Ischemia in the brain will cause local or global neuronal death upon oxygen deprivation, causing permanent loss or impairment of body function.[2] Reperfusion plays an important role in curing cerebral ischemic injury. Restoration of blood flow to the ischemic brain as early as possible is a way to rescue the patients. However, reperfusion itself also has the potential risk to produce additional injuries such as irreversible brain damage and neuronal injury in the ischemic brain.[3] Until now, there is still no effective therapeutic treatment that can improve the reconstruction of damaged cerebral tissue and its functional recovery. Despite decades of intense research, current treatments for acute ischemia stroke are far from optimal. Ischemia stroke triggers a cascade of pathophysiological events, including excitotoxicity, oxidative stress, apoptosis, and inflammation.[4] In addition, neural regeneration, synaptogenesis, astrocyte activation, and angiogenesis are stimulated within the brain post stroke.[5] Although intrinsic progenitor cell proliferation and differentiation occur in response to cerebral ischemia in order to provide functional benefits, this is not sufficient to prevent irreversible brain damage.[6] In recent years, experimental studies and clinical trials have indicated that cell-based therapies offer promise as a stroke treatment.

Neural stem cells (NSCs) have recently aroused a great deal of interest not only because of their importance in basic research of neural development but also for their broad potential for stem cell-based therapy in neurological diseases, such as stroke, Parkinson's disease, and spinal cord injury.[7] NSCs are immature cells with the ability to renew themselves and give rise to neurons, astrocytes, and oligodendrocytes.[8] This strategy has offered hope for recovery through the ability of the NSCs to differentiate and integrate appropriately into host cytoarchitecture. Previous studies demonstrated that intravenously transplanted human NSCs could differentiate into various neural cell types and compensate for the neurological deficits following cerebral ischemia.[9,10] These studies, however, also showed that the differentiating rate of grafted NSCs into mature neurons was very low, and very few of the grafted cells survived in given time. Therefore, improving the surviving rate of the grafted cells and inducing their differentiation into neurons are key points in stroke treatment with NSCs.

Bone morphogenesis proteins (BMPs) are a group of cytokines. These molecules play an important role during development of the nervous system.[11] The previous studies have indicated that expression of BMP4 inhibited oligodendrocyte differentiation.[12] Noggin is a negative well-known regulator of BMP signaling pathway that plays a key role in the regulation of neural tube patterning.[12,13] Binding of noggin to some BMPs inhibits bone formation through blocking of BMP signaling.[14] The increased expression of noggin as antagonist of BMP was accompanied by neural differentiation in neurosphere cultures.[12] Furthermore, in vitro studies have revealed that exposure to noggin could facilitate differentiation of embryonic stem cell into neuronal lineage.[6] Besides, the blockage of BMP signaling pathway using the BMP inhibitor noggin led to differentiate human embryonic stem cells into neuroectodermal fate.[15] Noggin did not affect self-renewal of neurospheres, but promoted the differentiation of both oligodendrocytes and neurons, which was inhibited by BMP4, simultaneously decreasing astrocyte differentiation both in vivo and in vitro.[16] However, the mechanisms underlying and neural functional recovery remain largely unknown.

In the present study, NSCs transfected by noggin gene 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 involved in the recovery processes, including vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), were determined using Western blot, apoptosis, and nestin-positive BrdU (5-bromo-2′-deoxyuridine) cells in the ipsilateral dentate gyrus detected by immunohistochemical staining. We expected that the present study could reveal the mechanism of noggin-transfected NSCs in improving the neural function after ischemic stroke and provide some novel therapeutic targets for ischemic stroke in clinics.

Materials and Methods

Experimental Groups

In total, 80 rats were randomly divided into 4 groups, with 20 animals in each group, which were the sham, ischemia reperfusion injury (IRI), NSCs, and noggin + NSCs groups. The experimental time points for each group were 1 day, 3 days, 7 days, and 28 days after the transplantation. The IRI models were prepared by MCAO for 2.0 hours and reperfusion for 1.0 hour.

Establishment of MCAO Model

We obtained healthy, male Wistar rats, aged 3-4 months, and weighing about 250-280 g, which were purchased from the Experimental Animal Center of the Guizhou Medical University, China (Clean grade, License No. SYXK(qian) 2002-0001). The rats were individually housed at 22°C-24°C with 55%-60% relative humidity and 12-hour dark-light cycle, and they were allowed free access to food and water. Rat models of IRI were developed by occluding rat middle cerebral artery following suture occlusion method, which was established using previously described methods from Longa et al.[17] Briefly, rats were weighed and anesthetized using .3 ml/kg of 10% chloral