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Improved stem cell therapy of spinal cord injury using GDNF-overexpressed bone marrow stem cells in a rat model

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ABSTRACT

The use of stem cell base therapy as an effective strategy for the treatment of spinal cord injury (SCI) is very promising. Although some strategy has been made to generate neural-like cells using bone marrow mesenchymal stem cells (BMSCs), the differentiation strategies are still inefficiently. For this purpose, we improved the therapeutic outcome with utilize both of N-neurotrophic factor derived Gelial cells (GDNF) gene and differentiation medium that induce the BMSCs into the neural-like cells. The differentiated GDNF overexpressed BMSCs (BMSCs-GDNF) were injected on the third day of post-SCI. BBB score test was performed for four weeks. Two weeks before the end of BBB, biotin dextranamin was injected intracrebrally and at the end of the fourth week, the tissue was stained. BBB scores were significantly different in BMSCs-GDNF injected and control animals. Significant difference in axon counting was observed in BMSCs-GDNF treated animals compared to the control group. According to the results, differentiated BMSCs-GDNF showed better results in comparison to the BMSCs without genetic modification. This study provides a new strategy to investigate the role of simultaneous in stem cell and gene therapy for future neural-like cells transplantation base therapies for SCI.

1. Introduction

The Spinal Cord Injury (SCI) defined as damage to any part of the spinal cord or nerves at the end of the spinal canal. About 2.2 to 2.6 million people are suffering from SCI worldwide and 130,000 new cases are reported annually. Difficulties due to paraplegia or quadriplegia have significant effect on quality of life, life expectancy, and also high costs for society [1]. In spite of many attempts to improve the SCI patient's life quality, the SCI is still the most important reason for paralysis and death in worldwide [2]. The SCI treatment strategies are prescribing growth factor (axon and neuron survived intermediate), synaptic plasticity and neurotransmission(3). It seems that, the neurotrophic factor rose from glial cells, GDNF, has a protective effect on neurons. In addition, the GDNF stimulates growing of motor and sensory axons, and the remyelination of neurons [2]. Furthermore, GDNF protects neuronal apoptosis after SCI, along with improvement neural plasticity and neural synaptic formation with integration and neural adherent molecule interaction [2,4,5]. Cell therapy has been proposed as a novel approach for the treatment of many diseases in recent years(6). The main purpose of cell therapy is revived of damaged organs using stem cells. Mesenchymal Stem Cells (MSCs) are more considerable stem cells for their differentiation ability into the different cell lines and makes no immune response after cellular

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injection [7–9].

On the other hand, it has been shown that BMSCs has a very important therapeutic role on the site of the SCI [10–12]. The BMSCs are a kind of mature stem cells that arises from bone marrow and can turn to mesenchymal and non-mesenchymal cell lines. Also, *in vivo* and *in vitro* studies have reported that these cells can differentiate into some neuron-like cells, containing neuron, astrocyte, oligodendrocyte and schwan cell [5,6,9]. This is one of the likely mechanisms for the SCI therapy with BMSCs [13,14]. Considerable neurotrophic factors secretion makes BMSCs a valuable source for repairing of the central nervous system [14–17]. Considering past studies using cell and gene therapy simultaneously, it seems that, the simultaneous use of BMSCs therapeutic effect and GDNF could improve the therapeutic effect.

In this study, we aim to use gene therapy and cell therapy with local injection of the transducted BMSCs with GDNF gene for the first time and make improve the recovery process in a rat model of SCI.

2. Material and methods

The healthy Sprague-Dawley rat of a clean grade, male, aged 8–10 weeks and weighing approximately 210–250 g, were used in this study. All rates were provided by the Karaj Razi Institute. They were classified into 4 groups of 6 randomly. All animal experiments were performed according to guidelines of the Medical Ethics Committee, Shahid Beheshti University of Medical Sciences and Health services (Tehran, Iran). Group one included rats receiving normal saline, group two received BMSCs, group three received BMSCs transducted with lentivirus without the GDNF gene, and group four received BMSCs transducted with lentivirus encoding the GDNF gene.

2.1. SCI model

Rats were individually anesthetized with ketamine (20 mg/kg) and xylotene (2 mg/kg) by intraperitoneal injection and inhaled a mixture of 20% v/v isoflurane and propylene glycol. The dorsal trunk hair was shaved (Fig. 1a). After disinfected with alcohol and Betadine, the surgery was done under the microscope with skin incision and pushing the vertebrate muscle and then was laminectomized at T10 vertebrae. The SCI was induced with Drop Weight method [18] (Fig. 1a and b).

2.2. Stem cell isolation and characterization

Extraction and culture of BMSCs were done by repeated aspiration of the mice femur and frequent change of the medium protocol [19]. The cells were cultured in DMEM medium with 10% of FBS (Invitrogen) in 37 °C, 95% humidity and 5% CO_2. The cell analysis was evaluated after 7–10 passage. Expression of CD90, CD73 and CD105 (as an indicator for BMSCs) and lack of CD45, CD34 (as an indicator for non-BMSCs) were evaluated. After culturing and during time, the cells were detached with trypsin (Invitrogen) and incubated with antibodies and analyzed by flow cytometry cytometry (Attune™ Acoustic Focusing Cytometer). For each expressed antibody were used of negative control with its own isotope, too. Finally, cells were analyzed using FlowJo® software. The mesenchymal stem cells can differentiate to adipocyte and osteoblast in appropriate circumstances. For osteoblastic differentiation, the cultured cells were settled on osteogenic differentiation medium including DMEM high glucose supplemented with FBS 10%, 10 μM of dexamethasone, 50 μg/ml of ascorbic acid and 10 μM of β-glycerophosphate (all from Invitrogen) for 14 days. At the end, the cells were stained with Alizarin red method and observed with the phase contrast microscope. For adipogenic differentiation, the cells were exposed to adipogenc differentiation media including DMEM high glucose supplemented with FBS 10%, differentiation factors, 1 μM of dexamethasone, 200 μM of indomethacin, 1.7 μM of isobutanol methyl xanthine (all from Invitrogen) for 14 days, then evaluated with Oil red staining and phase contrast microscope observing.

2.3. Lentiviral production (plenty-ш-GDNF-GFP)

The lentivirus producing was done in calcium-phosphate protocol with simultaneous transfection of 21 μg of psPAX2 vector (as a covering plasmid), 10.5 mg of PMD2-G vector (as packaging plasmid) and 21 μg of plenty-ш-GDNF-GFP vector transfer (GDNF cloned gene carrier in HEK-293T cell line). All 3 plasmids were purchased from the Stem Cell Technology Research center (Tehran, Iran). After 48 h, the HEK-293T cells were observed under the fluorescent microscope (Nicon, Gapan), to ensure the transfection and the viral and GFP protein expression. The supernatant was removed after 48–72 h after transfection. The ultracentrifuge was done (40000 g for 2 h) to concentration viruses. The serial dilution was prepared from the concentrated viruses to HEK-293T cells. For this purpose, HEK 293T cells were seeded with 4×10^4 cell density per well on 4 wells plate in 250 ml medium/well. 24 h later amounts of 1, 4 and 16 μl of concentrated virus added to wells 2, 3 and 4 respectively. Well number 1 was as negative control. After 72 h, the GFP gene expressed cells were evaluated by flow cytometry for obtaining the unit of virus titration in number of transducing unit (TU) per milliliter [20].

2.4. Virus titration and MOI determination

The gene transferring was done with GDNF carrier lentivirus BMSCs transducing. In consideration of virus titration obtained in before phase, the best MOI (Multiplicity of Infection) was obtained from different concentration of viruses added to cells.

2.5. The evaluation of secreting GDNF

Quantity amount of the secreted GDNF protein from BMSCs was evaluated by ELISA and Promega kit (Cat No: G7620), that was measured the GDNF protein concentration of the media (BMSCs, transducted BMSCs with lack of GDNF gene lentivirus vector and transducted BMSCs with GDNF gene carrier lentivirus) surface layer.

2.6. The differentiation of the GDNF gene expressed BMSC into neural-like cells

In this phase, for further differentiation, the GDNF gene expressed BMSCs were cultured into the differential media including DMEM high glucose with bFGF (Royan, Iran) and N2 (Invitrogen) 1× for 14 days. This medium was changed every two days.

2.7. Immunocytochemistry

A week after differentiation, the differentiated cells were fixed by paraformaldehyde 4% (sigma) for 30 min at room temperature in 4 °C. Then washed with PBS twice and penetrable with triton X-100, they were placed in BSA 5% solution, then incubated with the primary antibody mouse monoclonal anti-Nestin (1:200, Abcam) overnight at 4 °C. In the next phase, the cells were washed and incubated with the secondary antibody PE-goat anti mouse (1:200, Abcam) for 1 h at room temperature, after washing the nucleus was