MIGRATION OF MESENCHYMAL STEM CELLS THROUGH CEREBROSPINAL FLUID INTO INJURED SPINAL CORD TISSUE

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Introduction
Severe spinal cord injury (SCI) usually results in long-lasting deficits, involving partial or complete paralysis and loss of sensation below the level of the injury. The development of new strategies to treat such injuries is a major clinical challenge to neuroscience. A variety of experimental strategies including stem cell transplantation are emerging to promote regeneration of the injured spinal cord. Bone marrow derived mesenchymal stem cells (MSCs) constitute an alternative source of pluripotent stem cells. Bone marrow is far more accessible than neural stem cells and has the added advantage of having inherent host compatibility. MSCs can be expanded efficiently and manipulated genetically. Differentiation into neuron-like cells expressing neuronal markers has been reported, suggesting that MSCs may be capable of overcoming germ layer commitment.

 Usually cell transplantation have been performed by direct injection into the injured spinal cord. However, direct injection of cells into the lesion would be unrealistic clinically when the lesion is widely spread or multifocal. Less invasive techniques may be better suited for clinical application. We investigated the potential of transplanted MSCs into injured thoracic spinal cord tissue via cerebrospinal fluid (CSF) following more distal lumbar transdural injection.

Methods
[MSCs culture and transfection] MSCs were collected from femurs of adolescent male Lewis rats (5w, 100-125g). Bilateral femurs were harvested and the marrow was flushed out by using a syringe filled with MEM with alpha modification (MEM) containing 10% FBS and 0.1% penicillin/streptomycin. Bone marrow was plated in 10-cm² dishes. About 48 h after plating, supernatant containing nonadherent cells was removed and fresh medium was added. After the cells had grown to near confluency, they were passaged two times by being detached (0.05% trypsin / 0.03% EDTA for 5 min) and replated at a density of 5,000 cells/cm². An adeno viral construct encoding green fluorescent protein (GFP) was used to infect MSC. At the time of infection, defined as day 1, 2.5ml of virus solution and 2.5ml of complete medium were added to the 10⁶ cells. After 24 h incubation, they were replaced to fresh complete medium. On day 4 or 5, Around 10⁶ MSCs were detached (0.3% collagenase for 1 min and 0.05% trypsin / 0.03% EDTA for 5 min) and suspended in 100ul Hank’s buffered saline solution (HBSS).

[Animal Design] A total of 12 adult male Lewis rats weight 250-350 g were used for the vivo experiment. We designed 2 groups and performed a lumbar subdural injection at 3 d (Group 1, n=6) and at 7 d (Group 2, n=6) after thoracic SCI. In each group, 3 rats (2 injected GFP-MSCs and 1 injected PBS for control) had spinal cord tissue harvested at 7 d after transplantation and the other 3 (2 GFP-MSCs and 1 control) were harvested at 14 d after transplantation.

[Spinal Cord Injury] Laminectomy was performed at T9-10 under ketamine HCl anesthesia. The impact rod of the NYU Impactor was centered above T10 and dropped from a height of 25mm causing a consistent SCI. Urinary bladders were emptied manually three times per day for the first week and twice daily thereafter. Cefazolin 25mg/kg were given to prevent urinary tract infection.

[Histological Evaluation] Animals were anesthetized (ketamine HCl) and perfused intracardially with 4% paraformaldehyde. Spinal cords were dissected, postfixed in the same fixative overnight, transferred to 30% sucrose solution, frozen, and cut in a cryostat at 10-um thickness. Longitudinal sections were collected from 20-mm-long spinal cord segments containing the injury sites and thaw-mounted on gelatin-coated slides. Slides were evaluated by using fluorescence microscopy.

Results
All rats suffered SCI were paralyzed in their hindlimbs for several days; then the motion recovered gradually. Lumbar injection did not worsen the hindlimb motion or bladder function. No significant difference of general condition or motor function was seen between MSCs injected rats and control rats.

GFP-labeling to MSCs in vitro was confirmed by using a fluorescent microscopy. Transfected MSCs were stained with green both in the nucleus and cell body.

In animal study, samples harvested at day 7 after injury from Group 1 (transplantation at day 3 after injury) showed many green flushing debris under microscopy. These flushing debris were around the injury site of the spinal cord. Little was seen away from the lesion area. A small number of GFP-positive cells were found. The distribution of these cells was restricted to the lesion site and these cells were not observed in the surrounding intact tissue. The number of GFP-positive cells was increased in samples harvested at day 14 after injection (Fig. 1). Some of these green cells were integrated into the spine tissue surrounding the lesion site (Fig. 2).

The samples from Group 2 (transplantation at day 7 after injury) demonstrated much less flushing debris as well as GFP-positive cells around the lesion area, compared to samples from Group 1. These flushing debris and GFP-positive cells had similar geographical distribution into the zone of injury as those found in Group 1.

Discussion
In this study, we demonstrated that GFP labeled MSCs could migrate from a distant site into the injured spinal cord through the CSF. Furthermore, MSCs appeared to integrate in the host tissue after migration. It is known that the normal CSF works as a trophic factor for fetal primary culture. The fact that GFP-transgenic cells could survive well within the CSF after transplantation into rats with SCI indicates that CSF of injured spinal cord has no obvious harmful effect on injected MSCs.

Lumbar subdural injection is a popular and well-established technique in various clinical situations. Depending on this migration ability of MSCs into the lesioned area of spinal cord, we might be able to deliver various types of transgenic MSCs (like neurotrophic factor genes) in a less invasive manner than direct injection.

In addition, injection timing was very critical. We did not find much MSC migration in the later injection group after SCI. Some host conditions might be changed during the post-injury course to accept cell migration. Further study is necessary concerning the appropriate timing of the injection.