Therapeutic effects with magnetic targeting of bone marrow stromal cells in a rat spinal cord injury model

+\textsuperscript{1}Sasaki, H; +\textsuperscript{1}Tanaka, N; +\textsuperscript{1}Nakanishi, K; +\textsuperscript{1}Nishida, K; +\textsuperscript{1}Hamasaki, T; +\textsuperscript{1}Yamada, K; +\textsuperscript{1}Yamamoto, R; +\textsuperscript{1}Nakamae, T; +\textsuperscript{1}Izumi, B; Ochi, M  
+\textsuperscript{1}Hiroshima University, Hiroshima, Japan; +\textsuperscript{1}Hiroshima Prefectural Hospital, Hiroshima, Japan  
hirofumisasaki35@hotmail.com

\textbf{INTRODUCTION}
Subarachnoid injection has been reported as a minimally invasive method of transplantation of bone marrow stromal cells (BMSCs) for spinal cord injury. However, it may be less effective than direct injection into the spinal cord. A larger number of BMSCs may be required to attain the same outcome as direct injection. We previously demonstrated magnetic targeting systems with magnetic liposome or labeled cells. We reported a new magnetic targeting system in which greater numbers of magnetically labeled BMSCs aggregated on the surface of the spinal cord owing to the magnetic force. The purpose of this study is to investigate the therapeutic effects with magnetic targeting of BMSCs in a rat spinal cord injury model.

\textbf{METHODS}

\textbf{Spinal cord injury}
Adult Sprague-Dawley rats (weighing 230-250g) were anesthetized with pentobarbital sodium (50mg/kg, intraperitoneally), and a laminectomy was performed microsurgically at the T7 level of the spinal cord. A 25g rod was placed on the spinal cord for 90 seconds to induce a contusion lesion followed previous report. A neodymium magnet (380mT, 5mm in diameter, 3mm in height) was placed in the para-vertebral muscles at the T7 level of rats in the magnet group, whereas a non-magnetic metal (same material, same size) was placed in a similar manner in the non-magnet group.

Labeling of BMSCs with Feridx
BMSCs were labeled for 24h with 25\mu g Fe/ml Feridx (11.2mg Fe/ml; Takeda, Osaka, Japan) and 375ng/ml PLL (Sigma). Briefly, Feridx and PLL were added to a culture medium and incubated at room temperature for 60 min. Then, this medium was added to the BMSCs culture. After trypsinization, 1 \times 10^7 cells were suspended in 50\mu l of PBS as the injection solution for each rat.

\textbf{Transplantation}
1 day after injury, rats were anesthetized with pentobarbital sodium, and the dura was exposed with partial removal of the L5 spinous process and L4-5 ligamentum flavum. 50\mu l of PBS solution containing 1 \times 10^5 BMSCs were injected into the subarachnoid space with a 29-G needle. We injected 50\mu l of PBS alone in the control group. Each layer of muscle and skin was sutured tightly. After transplantation, rats were kept on a 30 degree slope in the head-down position for 30 minutes.

\textbf{Hind-limb motor function}
Motor function was scored with the BBB scale on days 1-7 and then every week up to the sixth week. Statistical analysis was performed with the Mann-whitney U-test. Significance was set at p<0.05.

\textbf{RESULTS}
At 1 day after transplantation, microscopic findings in sagittal sections demonstrated the aggregations of the GFP-positive cells on the surface of the injured spinal cord in the magnet group, while there were few GFP-positive cells in the non-magnet group. These GFP-positive cells were stained with Prussian blue stain (Fig.1). From these findings, we confirmed that magnetic targeting of BMSCs were successful in a rat spinal cord injury model.

Fig.1. Aggregations of the transplanted BMSCs in the magnet group. There were GFP-positive cells (left) on the surface of the injured spinal cord (sagittal section). GFP-positive cells were stained with Prussian blue (right).

At 6 weeks after injury, the hind-limb motor function scored with the BBB scale improved in both groups. There was no significant difference in the BBB score between the two groups up through 4 weeks after the injury. After 4 weeks, the BBB score of the magnet group demonstrated significant improvement compared to that of the non-magnet group at every week up to the sixth week. At 6 weeks after the injury, the BBB score of the magnet group was 20.0 \pm 1.0 and that of the non-magnet group was 16.5 \pm 2.1, showing a significant difference (p<0.05) (Fig.2).

\textbf{DISCUSSION}
In this study, we demonstrated that magnetic targeting of BMSCs accelerated functional recovery in a rat spinal cord injury model. Transplantation through the cerebrospinal fluid (CSF) has been evaluated as a minimally invasive method in animal spinal cord injury models. However, subarachnoid injection may be less effective than direct injection because of its poor transplant efficiency. Here we showed a new magnetic targeting system in which magnetically labeled BMSCs migrate through the CSF to the desired site in the injured spinal cord. This cell delivery system may be a very attractive method for future clinical application in the treatment of spinal cord injury.