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| Original Article |

Immunohistomorphometric Analysis of Transplanted Umbilical Cord Blood-Derived Mesenchymal Stem Cells and The Resulting Anti-Inflammatory Effects on Nerve Regeneration of Injured Canine Spinal Cord

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Abstract : In order to investigate the beneficial mechanism of transplanted canine umbilical cord blood-derived mesenchymal stem cells (UC-MSCs) on spinal cord injury (SCI), we evaluated recovery in a canine SCI model by examining neurological function and nerve regeneration. Recovery was assessed by clinical observation and by examining regenerated nerve cells by immuno-histomorphometric analysis and by assessing anti-inflammation by measuring mRNA expression of inflammation-related cytokines. Extradural compression of the spinal cord in 21 dogs (4.6±0.4 kg, ~2-3 yrs) was performed using a balloon catheter for 12 hours. All of the dogs showed hind limb paralysis after compression. Functional recovery of the hind limbs was evaluated by the Olby score and Revised modified tarlov scale. Experimental dogs were divided into cUCB and control groups. In the cUCB group (n=12), UC-MSCs were infected with a lentivirus-vector labeled GFP gene and injected into the SCI site. In the control group (n=9), only PBS was injected into the SCI site. Seven dogs (control = 3, cUCB = 4) were euthanized and their injured spinal cords were collected at 1, 4 and 8 weeks after transplantation. Nerve regeneration was assessed on longitudinal sections at the epicenter. After transplantation of UC-MSCs, functional improvement up to 5 points of the Olby score and 4 points of Revised modified tarlov scale were observed. Compared to the control group, immuno-histomorphometrical analyses showed that cells labeled with GFAP were significantly reduced and cells labeled with Tuj1 and NF160 were increased in the cUCB group ($p < 0.05$). In western blot analysis, total gliosis in the cUCB group was reduced by 35% compared to controls and surviving nerve cells in the SCI lesion were increased by more than 50 % compared to controls at 8 weeks after transplantation. However, it must be noted that a small number of nerve cells were derived from the transplanted UC-MSCs. In addition, the expression of COX2, IL1, IL6, TNF and TGF-β, in the cUCB group were down regulated. These findings suggest that improvements of neurological function seen after transplantation of UC-MSCs into injured spinal cord might be due primarily to reduced gliosis by anti-inflammation, increased survival of endogenous nerve cells and enhanced function of survived endogenous nerve cells by engrafted cells.

Key words: *anti-inflammation, canine umbilical cord blood, immuno-histomorphometric analysis, spinal cord injury, mesenchymal stem cell*

1. Introduction

After injury, spinal cord regeneration is very limited. Spinal cord injury (SCI) leads to cell death, particularly in neurons, and later culminates in glial scarring.[1-3] Because neuronal phenotypes of ependyma-derived cells are not generated following SCI,[4,5] cell transplantation therapy using adult stem cells has recently been identified as a potential therapeutic modality in SCI.[6,7] Adult mesenchymal stem cells (MSCs) exhibit neuroprotective properties through putative mechanisms including secretion of nerve regeneration-related factors and transdifferentiation in SCI.[8,9] Transplanted umbilical cord blood (UCB) stem cells differentiate into various neural cells and have been shown to improve motor function in cord-injured rodent models.[10] More thorough experiments are needed to evaluate how UCB stem

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cells modulate improvement after SCI and whether these cells possess the potential of tissue plasticity.[11] It is also unclear whether the enhanced functional recovery results from a regeneration of injured neural cells by engrafted cells or by trophic support that spares the surviving neuron that would otherwise degenerate. Although we previously reported that umbilical cord blood-derived MSCs (UC-MSCs) were effective in a canine SCI model, there was a weak histologic evidence of spinal cord tissue regeneration.[12] Thus, the relationship between nerve regeneration and functional recovery after SCI remains unresolved and mechanistic explanations are needed. Therefore, in this study, we evaluate the effect of transplanted UC-MSCs on the recovery of neurological function by nerve regeneration and by anti-inflammatory action in a canine SCI model.

2. Materials and Methods

2.1 Animals

Twenty one healthy adult mixed-breed dogs (4.6±0.4 kg) were used. We certify that applicable institutional and governmental regulations concerning the ethical use of animals were followed during the course of this research. The Institute of Laboratory Animal Resources of Seoul National University approved the study in accordance with the guidelines for the care and use of laboratory animals. SCI was induced by epidural balloon compression. The dogs were randomly assigned to 6 groups based on treatment and the survival duration after transplantation. The control group (n = 9) was treated with only phosphate-buffered saline (PBS) at the lesion site. Three dogs from the control group were harvested at 1, 4 and 8 weeks after injection. The cUCB group (n = 12) received transplantation of canine UC-MSCs at the site of SCI and four dogs were harvested at 1, 4 and 8 weeks after transplantation.

2.2 Induction of SCI

The experimental dogs were placed under general anesthesia and their spinal cords were then compressed so that SCI resulted.[12] Briefly, dogs were medicated and anesthetized with intravenous administration of tramadol (Toranzin; Sam Sung Pharm. Ind, Korea) at a dose of 4 mg/kg, and 6 mg/kg propofol (Anepol; Ha Na Pharm, Korea) with 0.04 mg/kg atropine sulfate (Atropine; Je Il Pharm, Korea) given subcutaneously. Anesthesia was maintained by inhalation of 2% isoflurane (Aerane; Ilisung, Korea). Datex-Ohmeda (Microvitec Display, UK) was used to monitor physiological measures including rectal temperature, oxygen saturation and pulse rate during anesthesia. The dogs were placed in a ventral recumbent position. The hemilaminectomy was performed by a left paramedian approach at the fourth lumbar segment (L4). A 3.5 mm hole was made in the left vertebral arch at L4 using a high-

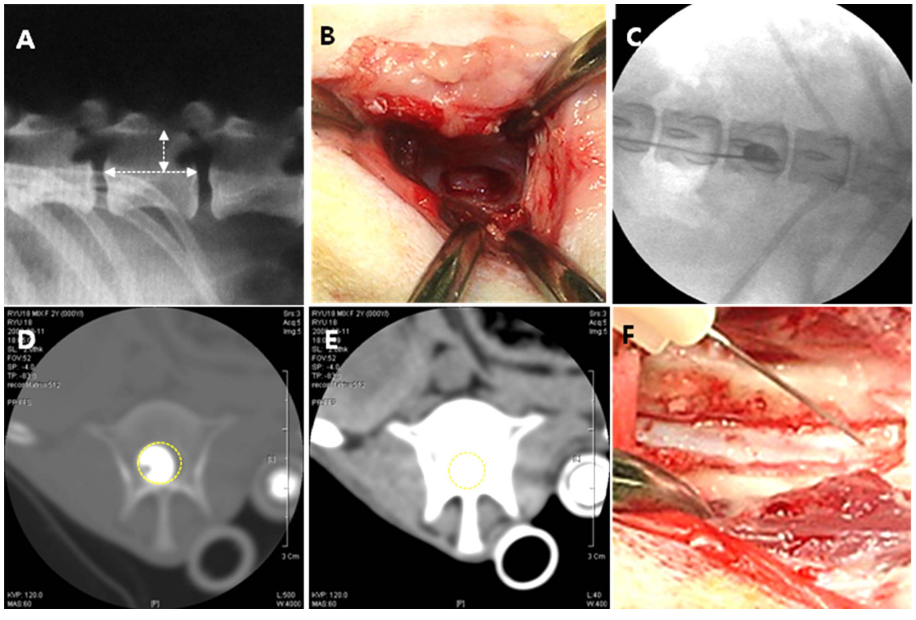

Figure 1. Induction of spinal cord injury (SCI) model. (A) Longitudinal axis is a length of lumbar 1 (L1) spinal canal. Vertical axis is the major axis of L1 spinal canal. (B) and (C) A 3-french embolectomy catheter was inserted into the epidural space through a left hemilaminectomy hole made in L4 vertebral arch. The balloons were inflated with a contrast agent at L1 level. (D) and (E): Transverse CT images of the epicenter of the lesion after balloon compression. The area of spinal canal in vertebra window (D) and the area of balloon inflation in spine window (E) spinal cord occlusion assessed using the formula, spinal cord occlusion ratio is the area of spinal canal in vertebra window / the area of balloon inflation in spine window × 100. (F) Transplantaion of UC-MSCs after dorsal-laminectomy at L1.