Journal of the Neurological Sciences 285 (2009) 67–77

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

# A comparison of autologous and allogenic bone marrow-derived mesenchymal stem cell transplantation in canine spinal cord injury

Dong-In Jung [a], Jeongim Ha [d], Byeong-Teck Kang [b], Ju-Won Kim [b], Fu-Shi Quan [f], Jong-Hwan Lee [c], Eung-Je Woo [e], Hee-Myung Park [b,*]

[a] Department of Veterinary Internal Medicine, College of Veterinary Medicine, Gyeongsang National University, Jinju 660-701, South Korea
[b] Department of Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, Seoul 143-701, South Korea
[c] Department of Veterinary Anatomy, College of Veterinary Medicine, Konkuk University, Seoul 143-701, South Korea
[d] Department of Cell and Developmental Biology, School of Dentistry, DRI and Brain Korea 21 Program, Seoul National University, Seoul 110-749, South Korea
[e] College of Electronics and Information, Kyunghee University, Gyeonggi-do 446-701, South Korea
[f] Department of Anatomy, College of Medicine, The Catholic University of Korea, Seoul 137-701, South Korea

ARTICLE INFO

*Article history:*
Received 27 June 2008
Received in revised form 21 May 2009
Accepted 22 May 2009
Available online 24 June 2009

*Keywords:*
Allogenic
Autologous
Bone marrow
Dog
Mesenchymal stem cells (MSC)
Neurotrophic factor
Spinal cord injury (SCI)

ABSTRACT

The purpose of this study is to compare the therapeutic effects between autologous and allogenic bone-marrow-derived mesenchymal stem cell (MSC) transplantation in experimentally-induced spinal cord injury (SCI) of dogs. Thirty adult Beagle dogs (control group = 10, autologous group = 10, and allogenic group = 10) were used in this study. Prelabeled MSCs were intrathecally transplanted through the lumbar spinal cord into the injured lesion at a density of $1 \times 10^7$ cells 7 days after SCI. Neurological signs of dogs in both autologous and allogenic groups were improved in their pelvic limbs after SCI compared with those in control group. Both autologous and allogenic groups showed significantly higher the Olby scores than control group ($p<0.05$). This finding was consistent with results of MRI and histopathological examination in both groups. Immunofluorescence analysis revealed that prelabeled autologous and allogenic MSCs were detected in the injured lesions both at 1 and 4 weeks after transplantation. However, the distribution ratio of MSCs on the injured lesion in allogenic group was significantly decreased at 4 weeks after transplantation relatively to at 1 week after transplantation. The mRNA expression for neurotrophic factors in both allogenic and autologous groups was significantly higher than that in control groups ($p<0.05$). Even though autologous MSC transplantation showed more beneficial effect than that of allogenic MSC transplantation, transplantation of allogenic MSCs also improved functional recovery following SCI.
This study demonstrates that both autologous and allogenic MSC transplantation could be clinically useful therapeutic approaches for treating SCI.



## 1. Introduction

Severe spinal cord injury (SCI), which leads to complete loss of sensory and motor functions, is one of the most serious neurological problems [11,22,23,25,32]. Although it has been long believed that the damaged central nervous system (CNS) does not regenerate upon injury, there is an emerging hope for regeneration-based therapy of the damaged CNS due to the progress of developmental biology and regenerative medicine including stem cell biology [18,22,25]. Previously, several cell therapy studies in experimental rodent SCI models have been reported [22,23,29,32]. In these models, transplantation of different types of cells, including Schwann cells, microglia cells, oligodendrocyte precursors, macrophages, and stem cells, partially improved the functional abilities of the animals by promoting the survival, regeneration, and remyelination of spinal axons [22,23,31,32].

Several studies have suggested that bone marrow cells are a potential source of neural progenitor cells and are clinically important in applications for neuronal tissue repairing [4,16,19,33,37]. MSCs are nonhematopoietic progenitor cells that are initially present in bone marrow [7,19,29,37]. Bone marrow-derived MSCs are also known as bone marrow stromal cells and are capable of in vitro differentiation into marrow and non-marrow cell types, such as adipocytes, chondrocytes, osteocytes, myocytes, and neurons [13,17,19,33,37]. A recent study has shown that canine bone marrow-derived MSC can form neurosphere-like clumps and differentiate into neuron-like cells expressing neuronal markers [19,37]. These cells have attracted interest concerned with their capacity for self renewal in a number of nonhematopoietic tissues, their multipotentiality for differentiation, and their possible use for both cell and gene therapy. Transplantation of adult MSCs into damaged brain and spinal cord reduces functional deficits [1,4,5,12,15,28].

* Corresponding author. Department of Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, # 1 Hwayang-dong, Kwangjin-gu, Seoul 143-701, South Korea. Tel.: +82 2 450 4140; fax: +82 2 450 3037.
*E-mail address:* parkhee@konkuk.ac.kr (H.-M. Park).

0022-510X/$ – see front matter. 
doi:10.1016/j.jns.2009.05.027

Bone marrow-derived MSCs are attractive for transplantation and spinal cord repair as they can be easily isolated, expanded in culture and delivered [7,19,33,37]. Several studies have evaluated the potential of bone marrow-derived MSC for treatment of SCI [1,5,30].

In stem cell research, both autologous and allogenic stem cell transplantation has exhibited considerable therapeutic potential in SCI [1,5,22,23,31,32]. However, all stem cell experiments in SCI have been separately performed by autologous or allogenic. Autologous stem cell transplantation is difficult to attempt on SCI patients in clinical medicine, because of a cell preparatory period and cell transplantation timing. Therefore, allogenic stem cell transplantation has more practical therapeutic value in clinical medicine. Unfortunately, no studies have ever tried to compare the therapeutic efficacy of autologous and allogenic stem cell transplantation in SCI.

The present study investigated the hypothesis that allogenic MSC transplantation is a clinically useful method for treating SCI compared with autologous MSC transplantation. Numerous attempts have been made by researchers to clarify the side effects of allogenic stem cell transplantation [8,14,27,32]. However, here the present study limits the discussion to therapeutic effect of autologous and allogenic MSC transplantation after SCI.

The purpose of this study is to compare the therapeutic efficacy of autologous and allogenic bone-marrow-derived mesenchymal stem cell transplantation in canine SCI.

## 2. Materials and methods

### 2.1. Animals

Thirty adult Beagle dogs (1 to 4 years old, weighing 5.0 to 12.2 kg, female; 19/male; 11) were divided into 3 groups of 10 dogs each, as follows: 1) Control group; no MSC transplantation after SCI, 2) Autologous group; autologous MSC transplantation after SCI, 3) Allogenic group; allogenic MSC transplantation after SCI. Five dogs in each group were euthanized at 2 weeks after SCI, and the other 5 dogs in each group were euthanized at 5 weeks after SCI. Additional not injured 5 normal dogs, exclusive of 30 dogs described above, were euthanized for comparative analysis of neuroprotective factor expression with 3 study groups. All dogs were treated in accordance with the guidelines approved by the Institutional Animal Care and Use Committee (IACUC) of Konkuk University.

### 2.2. MSC isolation and characterization from canine bone marrow

MSC isolation and cultivation from bone marrow was performed according to reports described previously [16,19,33,37]. Cultured mononucleated cells were characterized by fluorescence-assisted cell sorting (FACS) analysis based on previous reports [13,29,37]. For immunophenotypic analysis, third-passage canine MSCs were stained under ice conditions, according to the manufacturer's recommendations regarding the monoclonal antibodies (anti-CD9, anti-CD34, anti-CD44, and anti-CD45; Serotec, USA). All the antibodies used for FACS were shown by the manufacturer to cross-react with canine cells. Positive cells were detected on a Coulter Epics Elite fluorescence-activated cell sorter by using a 1:100 dilution of the secondary antibody (goat anti-mouse fluorescein isothiocyanate (FITC), Goat anti-rat FITC, Jackson Immunoresearch Laboratory, USA). Subsequently, the specimens were analyzed by flow cytometry (FACS calibur flow cytometer, BD, USA) using the CellQuest software (CellQuest, BD, USA).

### 2.3. SCI model

SCI was experimentally-induced using silicone balloon catheter compression methods, as described previously [11]. The dogs were anesthetized by intravenous administration of propofol (Anefol, Hana Pharm, South Korea) at 6 mg/kg with subcutaneous administration of atropine sulfate (Atropine, Jeil Pharm, South Korea) at 0.05 mg/kg. Anesthesia was maintained by inhalation of 3% isoflurane (Terrell, Minrad Inc., USA). Dogs were placed in ventral recumbency on the operating bed, and the dorsal approach was selected to spare the ligaments and the muscles between the spinous processes (between L2 and L3). When the dorsal intervertebral space between L2 and L3 was identified, the distance between the T13 and L2 spinous processes of the vertebrae was measured, and a 6-French silicone balloon catheter (Yushin Medical, South Korea) was inserted into the vertebral canal through the dorsal intervertebral space between L2 and L3. The silicone balloon catheter was inserted in the cranial direction to a distance corresponding to that measured previously between the T13 and L2 spinous processes. Positioning of the catheter was confirmed using fluoroscopy, and the balloon was then inflated to a volume of 1.5 ml in the spinal extradural space by injection of saline. The soft tissues and skin were closed as per standard methods, and the balloon was removed after 20 min.

### 2.4. Transplantation of MSCs after SCI

Seven days after SCI, autologous and allogenic MSCs were prelabeled with a carboxyfluorescein diacetate-succinimidyl ester (CFDA-SE) cell tracer kit (Molecular Probes, USA) and resuspended in 3 ml PBS at a density of $1\times10^7$ cells, and then transplanted into the injured lesion by an intrathecal injection between the L4 and L5 regions using 22-gauge spinal needle under fluoroscopic monitoring. MSCs were transplanted single time for each animal in autologous and allogenic groups under general anesthesia with xylazine (Rompun, Bayer Korea, South Korea; 1.1 mg/kg, IV) and ketamine (Ketamin, Yuhan Corporation, South Korea; 10 mg/kg, IV). In control group, only 3 ml PBS solution was transplanted at 7 days after SCI using the same methods as those used for autologous and allogenic group.

### 2.5. Behavioral analysis based on the Olby score

Behavioral analysis was performed before operation and at 1, 2, 3, 4, and 5 weeks after SCI in order to assess the functional recovery of the pelvic limbs after SCI. Behavioral recovery was scored according to the Olby scoring system [26], which is composed of 15 different criteria. The Olby scoring system is the modified scoring system for dogs based on pelvic limbs, and confirmed the reliability by several investigators [10,24,25]. The gaits of dogs were recorded using video camera, and two different investigators scored the gait.

### 2.6. Magnetic resonance imaging (MRI)

A 3.0-Tesla MRI system (Magnum3, Medius, Korea) was used to examine the location, extent, and progress of each injury. All dogs in each group were examined at 1, 2, and 5 weeks after SCI (at 1 and 4 weeks after MSC transplantation in autologous and allogenic group). Both T1- (TR/TE = 550.0/12.4) and T2-weighted (TR/TE = 4400.0/96.0) transverse and sagittal images with a scan thickness of 4 mm were obtained under general anesthesia with xylazine (1.1 mg/kg, IV) and ketamine (10 mg/kg, IV). The size of injured spinal cord lesion which showed hypointense or hyperintense signals were calculated from midsagittal view MR images using an image analyzer program (ImageJ, version 1.38; National Institutes of Health, USA). The size of lesion at 1 and 5 weeks after SCI was calculated and analyzed among three groups.

### 2.7. Postmorterm examination, histopathological and immunohistochemical analysis

Postmortem examination, histopathological and immunohistochemical changes were evaluated in the following manner: 1) Five dogs in each group were euthanized at 2 weeks after SCI, and 2) Five