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| Original Article |

Treatment of Spinal Cord Injury with Bone Marrow-Derived, Cultured Autologous Mesenchymal Stem Cells

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Abstract : A number of animal studies have assessed the neurological benefits of stem cell therapy in spinal cord injury, showing that injected adult stem cells create an environment conducive for axonal regeneration. These studies have also demonstrated the safety of adult stem cell injection. Despite these generally positive findings, few human trials have been performed. Here, we initiated a clinical pilot study to analyze the effectiveness of autologous mesenchymal stem cell therapy for spinal cord injury in humans using changes in electrophysiological studies and magnetic resonance imaging (MRI) to quantitatively evaluate the results. A total of 8×10^6 autologous mesenchymal stem cells (MSCs) were injected into the intramedullary space of ten cervical cord injured patients, and 4×10^7 cells were distributed into the intradural space. During the follow-up period (4 and 8 weeks after the first operation), 5×10^7 MSCs were injected by lumbar tapping. Results were evaluated preoperatively and during the third and sixth postoperative month by assessing Frankel/ASIA motor grade, measuring electrophysiological parameters (electromyography, nerve conduction velocity, somatosensory evoked potential, motor evoked potential), and enhanced MRI. There were no serious complications or side effects in any of the ten patients treated using the indicated protocol. One patient suffered moderate paresthesia during the first 10 months that subsided spontaneously. Six patients exhibited motor power changes; in three of these, performance of daily life tasks was improved. Electrophysiological and MRI changes were observed in five and six patients, respectively. Direct autologous MSC injection into the spinal cord was safe and caused no additional neurological damage. Furthermore, autologous MSC therapy resulted in neurological improvement, electrophysiological changes, and/or MRI changes in some cervical cord injured patients. Additional studies on the use of MSCs for SCI in larger patient populations are warranted to confirm these results.

Key words: *adult stem cell, electrophysiological change, evoked potential, mesenchymal stem cell, spinal cord injury*

1. Introduction

A number of animal studies have assessed the efficacy of stem cell therapy in spinal cord injury (SCI) and assessed the mechanisms of neurological improvement. These studies have shown that injected adult stem cells create an environment conducive for axonal regeneration,[1] and have demonstrated the safety of adult stem cell injection. However, human trials of adult stem cells have rarely been performed in either the spinal cord or the brain.[2-4] To address this understudied question, we initiated a clinical pilot study to analyze the effect of autologous mesenchymal stem cell (MSC) therapy for SCI in humans, using changes in electrophysiological parameters and magnetic resonance imaging (MRI) to evaluate the results.

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2. Materials and Methods

Ten patients with complete motor deficits, paraplegia or quadriplegia due to traumatic cervical SCI (lasting longer than one month after injury), without muscle atrophy or psychiatric problems, and in otherwise in good general condition, were enrolled in this study. Autologous MSCs were harvested from each patient's iliac bone and expanded by culturing for 4 weeks.

All manufacturing and product testing procedures for the generation of clinical-grade autologous MSCs were performed

under good manufacturing conditions (FCB-Pharmicell Co. Ltd., Korea). Mononuclear cells were separated from the bone marrow (BM) by density gradient centrifugation (Histopaque-1077; Sigma-Aldrich, St. Louis, MO, USA) and washed with phosphate-buffered saline (PBS). Cells were resuspended in low-glucose Dulbecco's modified Eagle medium (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco, Australia) and 100 U/mL penicillin/100 μg/mL streptomycin (Gibco), and plated at a density of 2-3×10^5 cells/cm^2 in 75 cm^2 flasks. Cultures were maintained at 37°C in a humidified 5% CO_2 atmosphere. After 5-7 days, nonadherent cells were removed by replacing the medium, and the adherent cells were cultured for an additional 2-3 days. When the cultures approached confluence (70%-80%), the adherent cells were detached by treating with a Trypsin/EDTA solution (Gibco) and replated at a density of 4-5×10^3 cells/cm^2 in 175 cm^2 flasks. Cells for infusion were serially subcultured up to passage five. During culture, some passage one or two cells were harvested and cryopreserved in 10% dimethyl sulfoxide (Sigma-Aldrich) and 90% FBS for the second and third infusions. On the day of injection, MSCs were harvested using Trypsin/EDTA, washed twice with PBS and once with saline solution, and resuspended at a final concentration of 0.8×10^7 cells/mL in saline solution. Criteria for release of MSCs for clinical use included viability greater than 90%, absence of microbial contamination (bacteria, fungus, virus, or mycoplasma) when tested 3-4 days before administration, expression of CD73 and CD105 by more than 90% of cells, and absence of CD14, CD34, and CD45 by less than 3% of cells, as assessed by flow cytometry.

After laminectomy and dura incision, a total of 8×10^6 autologous mesenchymal stem cells (MSCs) were injected into the intramedullary space of study subjects, and 4 x 10^7 cells were distributed into the intradural space. During the follow-up period, 5×10^7 MSCs were injected by lumbar tapping 4 and 8 weeks after the first operation. Results were evaluated preoperatively and during the third and sixth postoperative month by assessment of Frankel/ASIA motor grade; measurement of electrophysiological parameters, including electromyography (EMG), nerve conduction velocity (NCV), somatosensory evoked potential (SEP), and motor evoked potential (MEP); and enhanced MRI.

3. Results

Ten patients with the demographic features shown in Table 1 were treated with above protocol. Four patients were ASIA A grade and the remaining six were grade B. The post-injury duration was minimally 1 month and maximally 108 months. Although there was no change in ASIA grade, there were motor power improvements of the upper extremities in six patients (Patients 3, 4, 6, 8, 9, and 10). In three of these patients (Patients 4, 6, and 9), performance of daily motor tasks was improved. After

Table 1. Patient Profile

	Age(years)	Sex	Duration between injury and surgery	ASIA Grade
Patient 1	56	M	5 mo	A
Patient 2	61	M	52 mo	A
Patient 3	47	M	1 mo	B
Patient 4*	42	F	8 mo	B
Patient 5	35	M	73 mo	B
Patient 6*	44	M	38 mo	B
Patient 7	50	M	108 mo	A
Patient 8	34	F	17 mo	B
Patient 9*	42	M	96 mo	B
Patient 10	49	M	4 mo	A

Table 2. Summary of results

	Motor Change	EMG/NCV/EP change	MRI change	Permanent side effect	F/u duration (months)
Patient 1	-	-	+	-	6
Patient 2	-	-	+	-	6
Patient 3	+	-	-	-	6
Patient 4*	+	+	+	-	11
Patient 5	-	-	+	-	6
Patient 6*	+	+	+	-	6
Patient 7	-	+	+	-	10
Patient 8	+	+	-	-	6
Patient 9*	+	+	+	-	6
Patient 10	+	+	-	-	6

*Improvement in daily living

Table 3. Changes of motor power during follow-up period

	Case 1		Case 2		Case 3		Case 4		Case 5		Case 6		Case 7		Case 8		Case 9		Case 10	
	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
Post OP. (month)	0	3 6	0	3 6	0	3 6	0	3 6	0	3 6	0	3 6	0	3 6	0	3 6	0	3 6	0	3 6
C5 (EF)	0	0 0	0	0 0	3	4 4	2	4 4	5	5 5	4	5 5	1	1 1	4	4 4	5	5 5	5	5 5
C6 (EE)	0	0 0	0	0 0	1	1 1	2	2 2	4	4 4	4	5 5	0	0 0	1	2 2	4	4 4	4	3 4
C7 (WE)	0	0 0	0	0 0	1	1 1	2	4 4	2	2 2	3	3 4	0	0 0	0	0 0	4	4 4	2	1 3
C8 (FF)	0	0 0	0	0 0	1	1 1	0	2 2	0	0 0	0	0 0	0	0 0	0	0 1	3	3 4	0	0 0
T1 (Fab)	0	0 0	0	0 0	1	1 1	0	2 2	0	0 0	0	0 0	0	0 0	0	0 0	2	2 2	0	0 0
Score	0	0 0	0	0 0	7	8 8	6	11 44	11 111	11 111	11 134	11 144	1	1 1	5	7 7	11 88	11 95	11 12	9 9
Score change	0		0		2		16		0		6		0		3		5		6	

treatment, Patient 4 was able to prepare meals by herself with the aid of an elbow brace; Patient 6 could rise to a sitting position from supine position without assistance; and Patient 9 could grasp a water glass unassisted (Table 2, 3). Electrophysiological improvements were observed in five patients. Initially, none of the patients had a measurable MEP, but SEPs initially were detectable in three patients (Patients 6, 9, and 10). After treatment, five patients (Patients 6, 7, 8, 9, and 10) showed improved EP values (Table 2). MRI changes were observed in seven patients by postoperative months six to ten, as shown in Figures.

In Patient 1, who showed no clinical or electrophysiological changes, there was a disappearance of low signal intensity in contusion site and appearance of high signal intensity at the distal part of contusion site. In Patient 2, although there were no

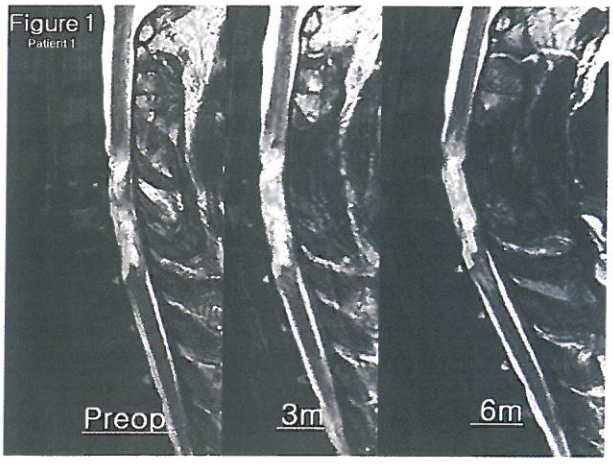

Figure 1. In Patient 1, T2 weighted image of the follow -up MRI showed disappearance of low signal intensity in contusion site and appearance of high signal intensity at the distal part of contusion site.

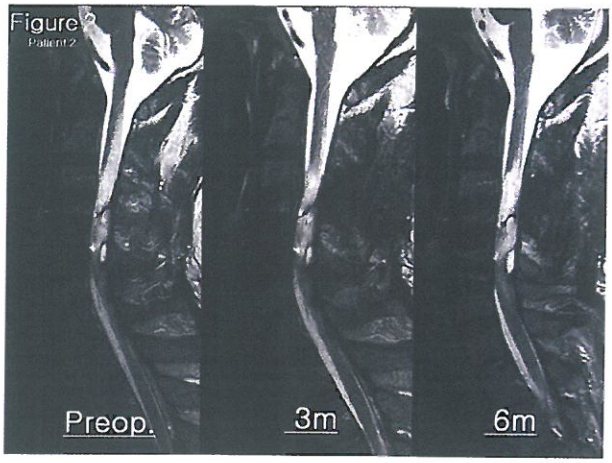

Figure 2. In Patient 2, T2 weighted image of the follow -up MRI showed decrease of high signal intensity in C2 level of spinal cord.

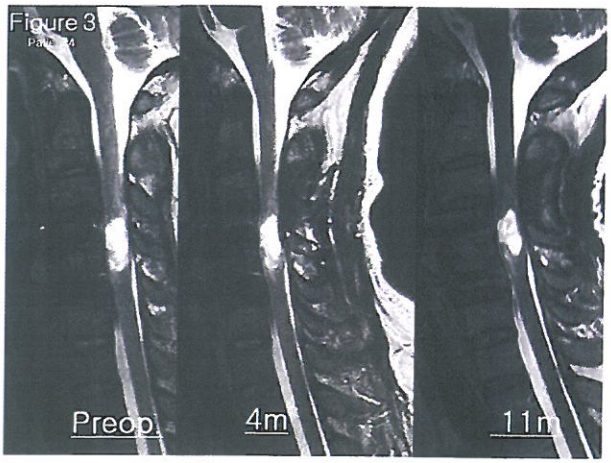

Figure 3. In Patient 4, decrease of cavity size and change of cavity shape was shown in T2 weighted image of the follow -up MRI.

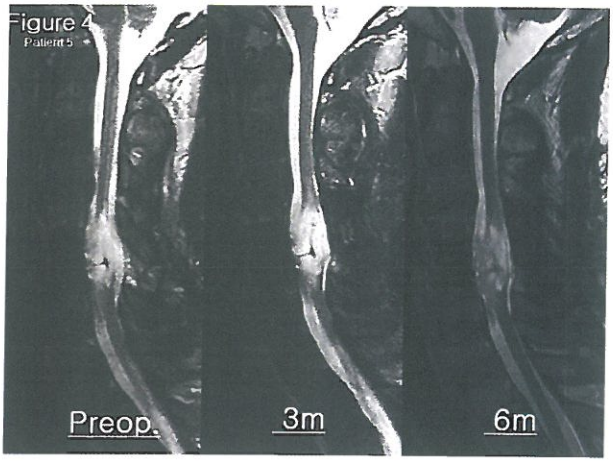

Figure 4. In Patient 5, there was a thickening of the proximal cord above the level of the injury in T2 weighted image of the follow - up MRI.

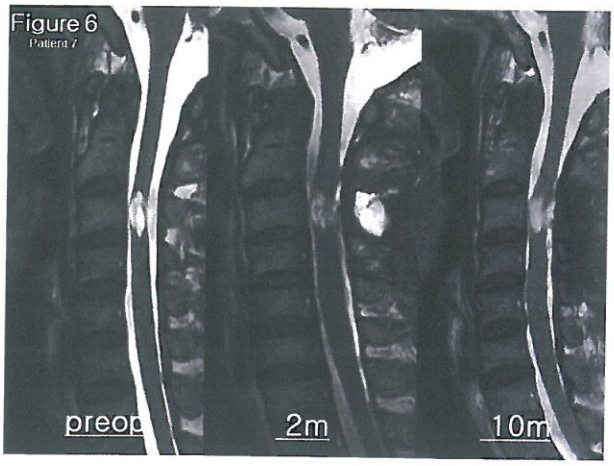

Figure 6. In Patient 7, cavity size was decreased and dorsal side of spinal cord around the cavity was thickened in T2 weighted image of the follow -up MRI.

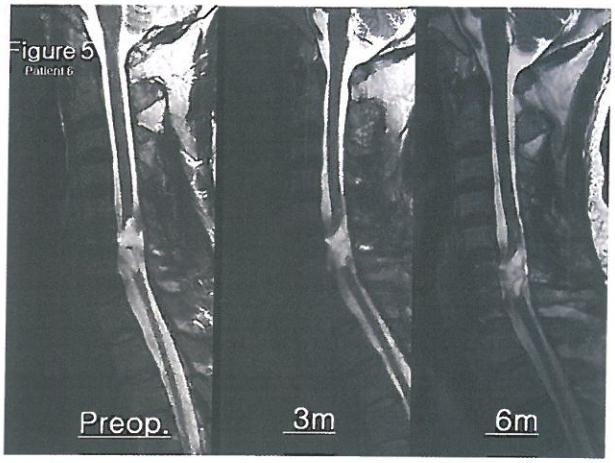

Figure 5. In Patient 6, high signal spot in proximal portion to contusion site of spinal cord was appeared and enlarged in T2 weighted image of the follow -up MRI.

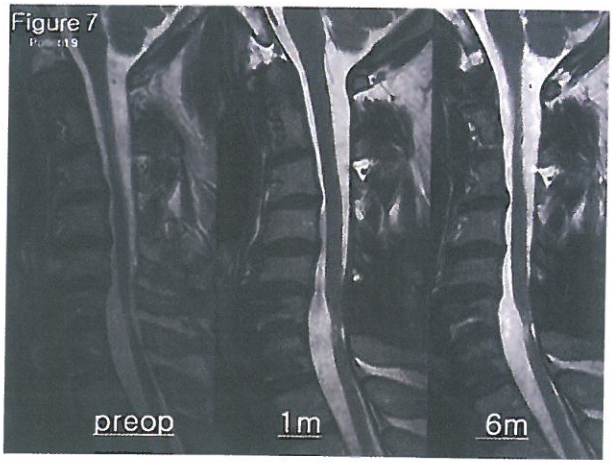

Figure 7. In Patient 9, thickening of spinal cord at proximal and distal portion to injured site was shown in T2 weighted image of the follow -up MRI.

neurological or electrophysiological changes, the high-signal-intensity territory was decreased in T2-weighted MR images. In Patient 3, despite clinical motor improvement, there were no electrophysiological or MRI changes compared to the preoperative state. In Patient 4, upper extremity power was so improved that the patient could eat by herself. In addition, there was slight improvement of compound muscle action potential in upper and lower extremities compared with the preoperative values and MR images showed a slight decrease in high signal volume, indicating decreased cavity size. In Patient 5, although there were no clinical or electrophysiological changes, there was a thickening of the proximal cord above the level of the injury in T2-weighted MR image. In Patient 6, upper extremity motor power, especially elbow extension and flexion, improved, as did MEP and SEP. High signal spot in proximal portion to contusion site of spinal cord was appeared and enlarged on follow–up MRI. In Patient 7, MEP and SEP were improved, although there was no clinical improvement. Follow-up MR image showed decreased cavity size and dorsal side of spinal cord around the cavity was thickened. In Patient 8, wrist extension (both wrists) improved and SEP/MEP also improved, but there were no MRI changes. In Patient 9, bilateral upper extremity motor power and MEP improved, and the patient could grasp a water glass without assistance. MR

image showed a thickening of spinal cord at proximal and distal portion to the injured site. In this patient, trunk sensory responses improved to such an extent that local anesthetics were required for lumbar tapping. Patient 10 exhibited clinical motor and SEP improvements, but MEP and MR images were unchanged. With the exception of one patient (Patient 4), who suffered paresthesia in the trunk and lower extremities for 10 months, no patients exhibited serious complications or side effects of autologous MSC transplantation, including infection, neurological aggravation. And also, in the follow-up MRI images of all cases, there was no syrinx formation or cellular hyperplasia in the spinal cord.

4. Discussion

The brain and spinal cord have traditionally been considered the most difficult organs to regenerate. However, several lines of clinical research have investigated regeneration of the central nervous system in the context of neurological disorders, such as Parkinson's disease, cerebral ischemia, and SCI.[3,5-7]

Treatments of SCI have included biological, surgical, and pharmacological approaches. Treatment with megadoses of steroids has been shown to activate an endogenous response to acute primary SCI and reduce secondary tissue damage, resulting in better functional recovery. However, the effectiveness of steroid treatment is still a topic of debate. Biological approaches to SCI include the use of factors that stimulate neuronal regeneration using several types of stem cells.[8] Transplantation studies have shown that these cells can differentiate and become integrated into the recipient's neural tissue.[3, 5, 9]

In our study, we used autologous MSCs, thus avoiding problems associated with immunologic rejection and graft-versus-host reactions, which frequently arise using allografts. The use of autologous MSCs from BM has several other advantages over other cell sources. First, they are relatively easy to be obtained from BM under local anesthesia, grow well in tissue culture, and can readily be injected into injured tissue. Second, this type of cell therapy is not associated with carcinogenesis, which sometimes occurs with embryogenic stem cell therapy. Third, MSCs can differentiate into a variety of tissue and cell types, including bone, cartilage, and neuronal progenitor cells. Finally, MSCs are eminently suitable for human trials because they can be readily obtained from the patient's own BM.[2,4,10] In the present study, none of our patients showed evidence of immune reactions, carcinogenesis, harvesting problems, or morbidity related to the use of general anesthesia.

Several hypotheses have been proposed to explain the role of BM-derived MSCs in SCI models. First, they improve neurologic deficits by generating either neural cells or myelin-producing cells. Second, transplanted stem cells act to guide axonal regeneration by producing extracellular matrix rather than by differentiating into neurons. Third, transplanted BM-derived cells promote compensatory mechanisms that reorganize the neural network and activate endogenous stem cells. Finally, they facilitate angiogenesis and reduce apoptosis and necrosis at the injection site.[8, 10]

Chronic spinal cord injury doesn't seem to have homing effect which attracts injected stem cells to pathology site by systemic injection. Therefore, we performed direct injection of stem cells into injured spinal cord site. Direct intramedullary injection has been reported to be safe and well tolerated in human trials.[2, 11, 12] Also, stem cell injection through lumbar puncture was reported to be feasible and safe in human trials[3, 13] as well as animal studies.[14, 15] Therefore, to enhance the stem cell therapeutic effect, we performed the second and third injections by lumbar tapping at the first and second months after intramedullary injection. To decide the amount of cells to inject, we adopt the most common amount, because the numbers of stem cells reported in the literatures were variable.

This study is not a randomized controlled trial. However, we can compare our results with those of the relevant previous literature which analyzed spontaneous recovery in spinal cord injury (SCI) patients. In the prospective study[16], the fastest spontaneous motor improvement of upper extremity in cervical spinal cord injury patients occurs over the first three months of injury and becomes steady state at 12 months after injury. When compared to our data, the difference is definite. Six patients (Patients 2,5,6,7,8,and 9) were treated at the time of more than 12 months after injury, and three patients (Patients 6,8,and 9) showed motor improvement (50%) during the follow-up period. In addition, the three patients who showed improved motor performance in daily tasks were treated at the time of chronic status (8, 38, and 96 months for Patients 4, 6 and 9, respectively). This suggests that MSCs played an important role in motor recovery of chronic SCI.

In the EP improvement, it was reported that there was no spontaneous improvement of EP during the follow-up period in ASIA A or B patient.[17] But in our study, six patients (Patients 4,6,7,8,9,and 10) among ten patients of the ASIA A or B (60%) showed electrophysiological improvement after stem cell treatment. In addition, another literature reported that absent initial SEP did not show any recordable SEP throughout the first year of injury except neuropraxy.[18] However, our data showed that SEP recovered from being absent to being present after the treatment in two cases (Patients 7 and 8). These results

after the stem cell treatment showed differences compared to natural course of electrophysiological change.

According to published reports, the presence of initially detectable SEP is associated with a favorable functional and neurological outcome.[18] In our study, three patients (Patients 6, 9, and 10) had initially recordable SEPs; all of these patients showed improved motor power and gradual postoperative improvement of EP. However, EP improvement did not always correlate with motor power improvement (Patients 3, 4, and 7). Motor power improvement was also not strictly correlated with MRI changes. Of the seven patients who showed MRI change, three (Patients 4, 6, and 9) showed motor improvement, and all showed improvement in the performance of daily tasks. This phenomenon suggests that coincidental improvement of motor power and MRI translates into improved recovery. In all MRI studies, gadolinium enhancement was performed. The thickened portions around the injured site were not enhanced in all the cases (Patients 5, 6, 7 and 9) . Therefore, the thickening of spinal cord after stem cell injection was not associated with tumorous condition or inflammatory lesion. If it was ectopic calcification, T1 and T2 images would show low signal intensity, but there was no such low signal intensity lesion. Although we can not explain exactly now, the findings on the thickening of the spinal cord after stem cell injection is supposed to be related with axonal regeneration.

In the prospective analysis of motor recovery from SCI, the rate of recovery is rapid during the first three months and motor improvement is almost completed by nine months after cell therapy.[16] Therefore, we think six to 11 months of follow-up duration is not short period to observe, whether or not motor improvement and MRI changes are developed. In addition, clinical improvement of motor power after stem cell injection was not related with paracrine effect such as in the stem cell treatment of Parkinson's disease, but related with axonal regeneration. Therefore, the motor improvement after stem cell injection would not return to the previous state.

About hydrocephalus, there were no clinical symptoms associated with hydrocephalus such as headache, nausea or vomiting. In addition, when lumbar puncture was performed to inject the stem cells, cerebrospinal fluid pressure was within normal range in all cases. Furthermore, there have been no previous reports about hydrocephalus after cell injection by lumbar puncture.[19, 20, 21] Therefore, we did not consider further evaluation to detect hydrocephalus.

5. Conclusion

Ten patients underwent clinical trial of direct injection of MSC for SCI. Although we were unable to establish the statistical significance of this treatment, some patients showed clinical, radiological, and electrophysiological evidence of improvement. Importantly, there were no significant complications of autologous MSC transplantation. Collectively, our results show that BM-derived stem cell therapy is a promising and safe treatment for chronic SCI. Additional studies on the use of MSCs for SCI in larger patient populations are warranted to confirm the results of this pilot study.

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