Long-term Results of Spinal Cord Injury Therapy Using Mesenchymal Stem Cells Derived From Bone Marrow in Humans

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BACKGROUND: Although the transplantation of mesenchymal stem cells (MSCs) after spinal cord injury (SCI) has shown promising results in animals, less is known about the effects of autologous MSCs in human SCI.

OBJECTIVE: To describe the long-term results of 10 patients who underwent intramedullary direct MSCs transplantation into injured spinal cords.

METHODS: Autologous MSCs were harvested from the iliac bone of each patient and expanded by culturing for 4 weeks. MSCs (8×10^6) were directly injected into the spinal cord, and 4×10^7 cells were injected into the intradural space of 10 patients with American Spinal Injury Association class A or B injury caused by traumatic cervical SCI. After 4 and 8 weeks, an additional 5×10^7 MSCs were injected into each patient through lumbar tapping. Outcome assessments included changes in the motor power grade of the extremities, magnetic resonance imaging, and electrophysiological recordings.

RESULTS: Although 6 of the 10 patients showed motor power improvement of the upper extremities at 6-month follow-up, 3 showed gradual improvement in activities of daily living, and changes on magnetic resonance imaging such as decreases in cavity size and the appearance of fiber-like low signal intensity streaks. They also showed electrophysiological improvement. All 10 patients did not experience any permanent complication associated with MSC transplantation.

CONCLUSION: Three of the 10 patients with SCI who were directly injected with autologous MSCs showed improvement in the motor power of the upper extremities and in activities of daily living, as well as significant magnetic resonance imaging and electrophysiological changes during long-term follow-up.

KEY WORDS: Activities of daily living, Axon regeneration, Human, Magnetic resonance imaging, Mesenchymal stem cells, Spinal cord injury

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Cell therapies for human spinal cord injury (SCI) have given us hope of neuronal regeneration of the spinal cord.[1-7] The efficacy and safety of direct injection of olfactory ensheathing cells into the spinal cord has been shown in several studies.[1-5] However, its side effects include syrinx formation, myelomalacia, and perioperative morbidity, suggesting that this procedure does not meet international standards for either safety or efficacy.[8,9] Intramedullary Schwann cell transplantation for human SCI has been shown to be safe, although resulting in unsatisfactory motor and functional improvement.[10] Although embryonic stem cells are also regarded as pluripotent cells that have the capability to differentiate into nearly all cell types, including neuronal and glial cells, there are several concerns regarding the safety of transplantation of human embryonic stem cells in humans, including the controversial formation of teratomas.[11-13] A clinical trial using human embryonic stem cells has been delayed because further studies of human embryonic stem cell–derived neural cells in animal models are needed.[12]

ABBREVIATIONS: ADL, activities of daily living; **ASIA,** American Spinal Injury Association; **BM,** bone marrow; **MEP,** motor evoked potential; **MSC,** mesenchymal stem cell; **P,** passage; **SCI,** spinal cord injury

Although the possibility of malignant transformation also exists,[14-16] mesenchymal stem cell (MSC) transplantation after SCI has shown promising results in animals and humans.[6,7,17-27] MSCs are multipotent cells that can differentiate along several mesenchymal pathways, including those leading to the development of chondrocytes, osteoblasts, and adipocytes.[28] In addition, MSCs have garnered considerable interest as possible donor cells in cell transplantation therapies for SCI.[24,26,29-32] The injection of MSCs in animal models of SCI has resulted in significantly increased tissue preservation, decreased cyst and injury size, and recovery of function,[19,21,29,33-37] but there are no reports of morphological changes in the human spinal cord as a result of this treatment.

MSCs are an attractive source of stem cells in patients with SCI because MSCs can be easily and reproducibly isolated from bone marrow (BM) aspirates and reintroduced into patients as autografts.[17,34,38,39] Although intrathecal injection of MSCs has been reported to be of therapeutic value in such patients,[17,22] to date, there is no report of intramedullary MSC injection to treat human SCI, with the exception of our previous report on short-term (6-month) outcomes in 10 SCI patients who underwent MSC transplantation in the subacute and chronic state.[20] Of these 10 patients, 4 were classified as American Spinal Injury Association (ASIA) grade A and 6 as ASIA grade B. We observed motor improvement in the upper extremities of 6 patients, magnetic resonance imaging (MRI) changes in 7, and electrophysiological changes in 6; no patient experienced permanent complications during follow-up. Of the 6 patients showing motor improvement, 3 showed improvement in activities of daily living (ADL). Here we describe the long-term results in these 3 patients, including clinical outcomes and changes in MRI and electrophysiological patterns, as well as the latest results from the other 7 patients.

MATERIALS AND METHODS

Cell Preparation

Autologous MSCs were harvested from the iliac bone of each patient and expanded by culturing for 4 weeks. All procedures for the generation of clinical-grade autologous MSCs were performed under good manufacturing conditions (FCB-Pharmicell Co, Seoul, South Korea). Mononuclear cells were separated from BM by density gradient centrifugation (Histopaque-1077; Sigma-Aldrich, St. Louis, Missouri), washed with phosphate-buffered saline, resuspended in low-glucose Dulbecco modified Eagle's medium (Gibco, Grand Island, New York) containing 10% (vol/vol) fetal bovine serum (Gibco), and 100 U/mL penicillin/100 μg/mL streptomycin (Gibco), and plated at a density of 2 to 3×10^5 cells/cm^2 in 75-cm^2 flasks. The cultures were maintained at 37°C in a humidified 5% (vol/vol) CO_2 atmosphere for 5 to 7 days, after which nonadherent cells were removed by replacing the medium, and adherent cells were cultured for an additional 2 to 3 days. When the cultures approached confluence (70%-80%), adherent cells were detached by treatment with a trypsin/ethylenediamine tetraacetic acid solution (Gibco) and replated at a density of 4 to 5×10^3 cells/cm^2 in 175-cm^2 flasks. Cells for infusion were serially subcultured up to passage (P) 5. During culture, some cells of P1 or P2 were harvested and cryopreserved in 10% (vol/vol) dimethyl sulfoxide (Sigma-Aldrich) and 90% (vol/vol) fetal bovine serum for use as second and third infusions. P5 cells were used in all the treatment procedures. Directly cultured P5 cells were used for the first injection, but for the second and third injections, P5 cultured cells were used after cryopreserved P1 cells were defrosted. The same differentiation potency of 2 kinds of P5 cells was also identified.

On the day of injection, MSCs were harvested with trypsin/ethylenediamine tetraacetic acid, washed twice with phosphate-buffered saline and once with saline solution, and then resuspended in saline solution at a final concentration of 0.8×10^7 cells/mL. Criteria for the clinical use of MSCs included a viability greater than 90%, an absence of microbial contamination (bacteria, fungus, virus, or mycoplasma) as confirmed 3 to 4 days before administration, the expression of CD73 and CD105 by more than 90% of cells, and an absence of CD14, CD34, and CD45 (expression of each by $<3\%$ of cells), as assessed by flow cytometry.

Operation and Follow-up Injection

After laminectomy and a dura incision are performed, 8×10^6 autologous MSCs in 1 mL of normal saline were injected into the intramedullary space, with 0.2 mL of normal saline being injected into each of 2 sites of the proximal spinal cord just above the cavity and in each of 3 sites in the cavity itself. The depth of injection was determined as the half diameter of the preoperative midline sagittal MRI. Vascular structures were avoided. Each injection took 10 seconds, and a 26.5-gauge needle was kept in place for 30 seconds before removal. After intramedullary injection, fibrin glue was used to cover the injection site to prevent cell leakage. In addition, 4×10^7 cells in 5 mL of normal saline were distributed into the intradural space of each subject during watertight dural closure (Figure 1). An additional 5×10^7 MSCs in 8 mL of normal saline were injected into each patient at 4 and 8 weeks via lumbar tapping.

Patient Selection Criteria

A previous study enrolled 10 patients with complete motor deficits, paraplegia, or quadriplegia caused by traumatic cervical SCI (lasting >1 month after injury), without muscle atrophy or psychiatric problems, and otherwise in good general condition. All patients were followed for 6 months after MSC treatment, and 3 patients who showed ADL improvement during this period were evaluated with long-term follow-up. All patients consented to participate in this study.

Neurological examinations were performed by rehabilitation specialists. No rehabilitation program was scheduled before or after MSC injection.

MRI was performed using a 1.5-T Siemens Magnetom Avanto scanner (Siemens, Erlangen, Germany), and T1-, T2-, and T1-enhanced images were obtained for all patients. The parameters of T2 sagittal imaging used for quantitative assessments included 3-mm slice thickness, 280 field of view, 4360 TR, 107 TE, and 291×448 spatial resolution.

RESULTS

Patient 1

A 47-year-old woman became quadriplegic (ASIA grade B) after a traffic accident. At that time, she underwent cervical spinal surgery consisting of decompression and stabilization. She received active rehabilitation for 3 months after fusion surgery. Eight

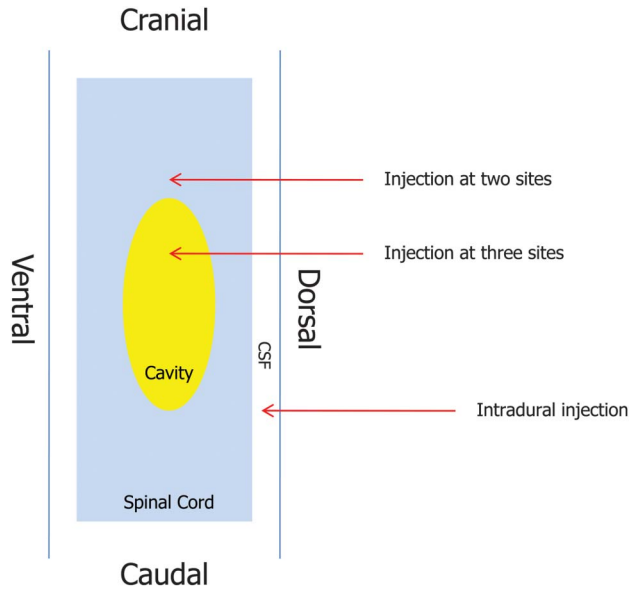

FIGURE 1. *After laminectomy and a dura incision are performed, 8×10^6 autologous mesenchymal stem cells in 1 mL of normal saline were injected into the intramedullary space, with 0.2 mL of normal saline being injected into each of 2 sites of the proximal spinal cord just above the cavity and into each of 3 sites in the cavity itself. In addition, 4×10^7 cells in 5 mL of normal saline were distributed in the intradural space of each patient during watertight dural closure. The depth of injection was determined as half diameter of preoperative midline sagittal magnetic resonance imaging.*

months after surgery, she was referred to our hospital in a stable neurological state (Table 1). She did not undergo rehabilitation before or after MSC injection. T2-weighted MRI scans of her spinal cord showed high signal intensity at the C4-5 level (Figure 2). After stem cell therapy, her motor power grade improved gradually, with her elbow power improving to grade IV (V at right elbow flexion). However, she experienced moderate paresthesia during the first 10 months, which subsided spontaneously. At 36 months after treatment, electrophysiological examination showed slight improvement in the compound motor action potentials of the muscles of both her upper and lower extremities. No somatosensory evoked potential (SSEP) signal was obtained before injection, and no change was noted during follow-up. By contrast, a motor evoked potential (MEP) signal, which was absent before injection, was obtained at the last follow-up. At 36 months, cortical stimulation of the right radial nerve showed a latency of 14 milliseconds and an amplitude of 528.3 μV, whereas stimulation of the left radial nerve showed a latency of 13.4 milliseconds and an amplitude of 596.7 μV. With the aid of an elbow brace, she gained the ability to prepare meals and to type on a keyboard (Table 2). MRI assessment also showed a gradual decrease in cavity size at the injured spinal cord level and at the margin of the cavity; although the cavity wall was visible at 24 months, it disappeared by 32 months (Figure 2). Follow-up MRI showed no significant enhancement that might indicate the appearance of a neoplasm. A computed tomography (CT) scan at the last follow-up visit showed no evidence of any high-density lesion that might indicate ectopic calcification (Figure 3).

Patient 2

A 49-year-old male patient experienced a cervical fracture and dislocation in a car accident. He underwent spinal fusion at the time of the injury. His neurological status was ASIA grade B (Table 1), which had not changed for 7 years despite active exercise. After MSC treatment, he showed gradual improvement in the motor power of his upper extremities, especially in elbow flexion and extension, without any rehabilitation therapy (Table 2), and he gained the ability to rise from a supine to a sitting position without assistance. Electrophysiological examination showed that the amplitudes of compound motor action potentials had increased in his median and posterior tibial nerves. Preoperatively, no MEP of the median nerve was evident after cortical stimulation. Three months after MSC injection, however, weak MEP signals from the right (latency 16, 75 milliseconds) and left (latency, 85 milliseconds) median nerves were observed after cortical stimulation, but with amplitudes so weak that they could not be measured. Although his right median nerve SSEP signal (latency, 18.3 milliseconds) was weak preoperatively and the left median nerve SSEP signal was absent, right (latency, 19.95 milliseconds) and left (latency, 19.4 milliseconds) median nerve SSEP signals were observed 3 months after stem cell injection, although the weak amplitudes could not be measured.

MRI showed the appearance in his spinal cord of a high signal spot proximal to the contusion site, which became enlarged during follow-up, as well as the appearance of fiber-like streaks (Figure 4, arrows). Although the cavity wall was visible until 20 months after injury, it began to disappear after 32 months (Figure 4). No

TABLE 1. Basic Characteristics of Patients With ADL Improvement During 6-Month Follow-up[a]

Patient	Age, y	Sex	Duration Between Injury and Surgery, mo	ASIA Grade	Injury Level	Follow-up Duration, mo
1	42	F	8	B	C4-5	36
2	44	M	38	B	C6-7	39
3	42	M	96	B	C6-7	30

[a]ASIA, American Spinal Injury Association.

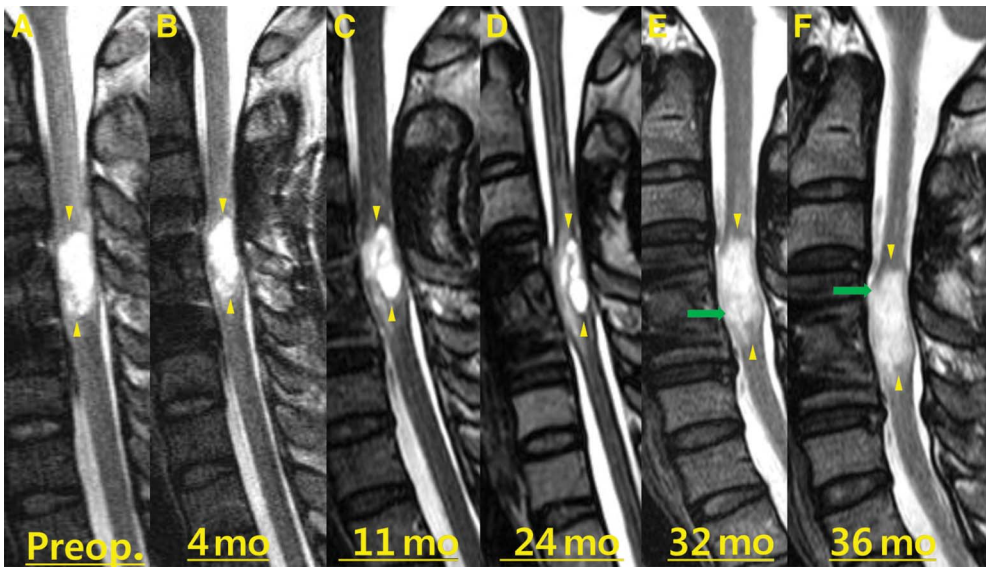

FIGURE 2. *T2-weighted sagittal magnetic resonance imaging of patient 1 showing the site of injury and the cavity at the C4-5 level. Mesenchymal stem cells were injected into 2 sites in the proximal spinal cord just above the cavity and into 3 sites of the cavity itself. Gradual decreases in cavity size were observed at the injured spinal cord level and at the margins of the cavity until 24 months postoperatively. The cavity margin (arrowheads) completely disappeared and fiber-like streaks (arrows) were observed at 32 and 36 months, respectively. The cavity sizes on T2-weighted midline sagittal magnetic resonance imaging at each time point were 8.1 × 22.6 mm (A); 8.1 × 20.86 mm (B); 7.8 × 19.65 mm (C); 6.86 × 18.35 mm (D); no cavity margin (E); and no cavity margin (F).*

significant lesion enhancement was noted, and the last follow-up CT scan showed no evidence of calcification (Figure 3).

Patient 3

A 46-year-old male patient became quadriplegic after a car accident (ASIA grade B) (Table 1), with MRI showing severe spinal cord atrophy. He underwent rehabilitation therapy for about 3 months after injury and fusion surgery, followed by MSC treatment 96 months after SCI. During follow-up, his right hand grasp improved to grade 5 and his left hand grasp improved to grade 3 to 4+, and he was able to hold a water glass unassisted (Table 2). Preoperatively, he showed weak SSEPs of both median nerves and MEP signals from both radial nerves, with weak MEPs of the radial nerve and no MEP signal of the median nerve emanating. His right and left SEP latencies were 19.7 and 19.3 milliseconds, respectively, but the amplitudes could not be measured. Six months after injection, the latency and amplitude of SSEPs were 20.35 milliseconds and 4.4 μV, respectively, on the right side, and 20.15 milliseconds and 2.8 μV, respectively, on the left side. His preoperative right and left radial MEP

TABLE 2. Long-term Changes in Motor Power of the Upper Extremities in 3 Patients With ADL Improvement During 6-Month Follow-up[a]

Upper Extremity Motor Power	Patient 1								Patient 2								Patient 3							
	Right				Left				Right				Left				Right				Left			
Months	0	3	6	40	0	3	6	40	0	3	6	39	0	3	6	39	0	3	6	30	0	3	6	30
C5 (EF)	II	IV	IV	V	II	IV	IV	IV	IV	V	V	V	IV	V	V	V	V	V	V	V	V	V	V	V
C6 (WE)	II	II	II	IV	II	II	II	IV	IV	V	V	V	IV	V	V	V	IV	IV	IV	IV	IV	IV	IV	IV
C7 (EE)	II	IV	IV	IV	II	IV	IV	IV	III	III	IV	VI+	III	IV	IV	V	IV	IV	IV	IV	IV	IV	IV	IV
C8 (FF)	0	II	II	II	0	II	II	II	0	0	0	I	0	0	0	I	III	III	IV	V	I	II	II	III
T1 (Fab)	0	II	II	II	0	II	II	II	0	0	0	0	0	0	0	I	II	II	II	V	I	II	II	IV+

[a]ADL, activities of daily living; 0 months, preoperative state; EF, elbow flexion; WE, wrist extension; EE, elbow extension; FF, finger flexion; Fab, finger abduction; Roman numerals, motor power grade of upper extremities; IV+, the motor power between grade IV and V.

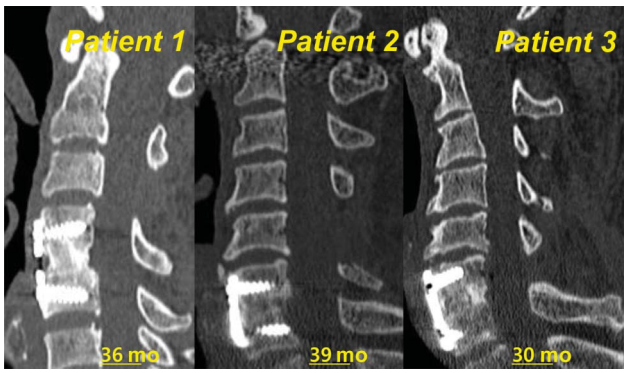

FIGURE 3. *Computed tomography images of all 3 patients at their last follow-up visits, showing no evidence of high-density lesions that might indicate ectopic calcification.*

latencies were 19.65 and 19.1 milliseconds, respectively, but the amplitudes could not be measured. Six months after treatment, however, the latency and amplitude of his radial nerve MEPs were 17.15 milliseconds and 0.7 μV, respectively, on the right side, and 17.05 milliseconds and 0.5 μV, respectively, on the left side. In addition, the median nerve MEP showed a latency of 27.4 milliseconds and an amplitude of 0.2 μV on the right side, and a latency of 26.3 milliseconds and an amplitude of 0.1 μV on the left.

Although the spinal cord atrophy did not change, follow-up T2-weighted images showed thickening of the spinal cord, both proximal and distal to the injured site (Figure 5). MRI assessment showed no significant enhancement, suggesting tumor formation, and the last follow-up CT scan showed no evidence of calcification.

The motor improvements of all 3 patients are described in detail in Table 2. No patient experienced any postoperative permanent complication, such as infection and neurological aggravation, except for transient moderate paresthesia in patient 1. Follow-up MRI showed no syrinx or neoplasm formation, and cervical CT studies at the last follow-up showed no evidence of ectopic calcification (Figure 3).

Patients With No ADL Improvement

The remaining 7 patients showed no ADL improvement during short-term (6-month) follow-up and were not assessed annually. Two patients (patients IV and VII in Tables 3 and 4) underwent their last follow-up examinations in outpatient clinics, and 2 patients (I and II in Tables 3 and 4) were assessed only by telephone interviews. Patients I and II reported no neurological improvement after 6 months, but no aggravation of abnormal sensations, such as allodynia and paresthesia. Patient III showed mild motor improvement until 6 months, but died of pneumonia 3 years after MSC treatment. Patient IV showed a slight improvement in right elbow extension with no aggravation of abnormal sensations, such as allodynia and paresthesia, after 62 months. There were no changes in the electrophysiological results, and no abnormalities (including calcification) were evident in MRI or CT scans after 62 months. Patient V did not experience any neurological changes for the first 6 months after treatment and refused to participate in a follow-up interview. Although patient VI showed motor improvement for the first 6 months, she was admitted to a psychiatric hospital for depression and could not be reached. Patient VII was followed for 55 months after treatment, during which time he showed motor improvement in left finger flexion and abduction and no aggravated abnormal sensations, such as allodynia and paresthesia. However, no changes were evident on the electrophysiological recordings, and there were no abnormalities (including calcification) on MRI or CT scans after 55 months (Tables 3 and 4).

DISCUSSION

We previously reported that several of our 10 patients who underwent autologous MSC transplantation for SCI showed neurological and electrophysiological improvement and MRI changes during short-term follow-up.[20] We describe here the

TABLE 3. Characteristics of the 7 Patients With No ADL Improvement During 6-Month Follow-up[a]

Patient	Age, y	Sex	Duration Between Injury and Surgery, mo	ASIA Grade	Injury Level	Last Follow-up, mo	
I	56	M	5	A	C5-6-7	6	F/U was not done for personal reasons
II	61	M	52	A	C4-5	6	F/U was not done for personal reasons
III	47	M	1	B	C5-6	6	Died of pneumonia
IV	35	M	73	B	C4-5-6	62	
V	50	M	108	A	C3	6	Patient refused contact with us
VI	34	F	17	B	C5-6	6	F/U could not be done because of patient's depression
VII	49	M	4	A	C5-6	55	

[a]ADL, activities of daily living; ASIA, American Spinal Injury Association; F/U, follow-up.

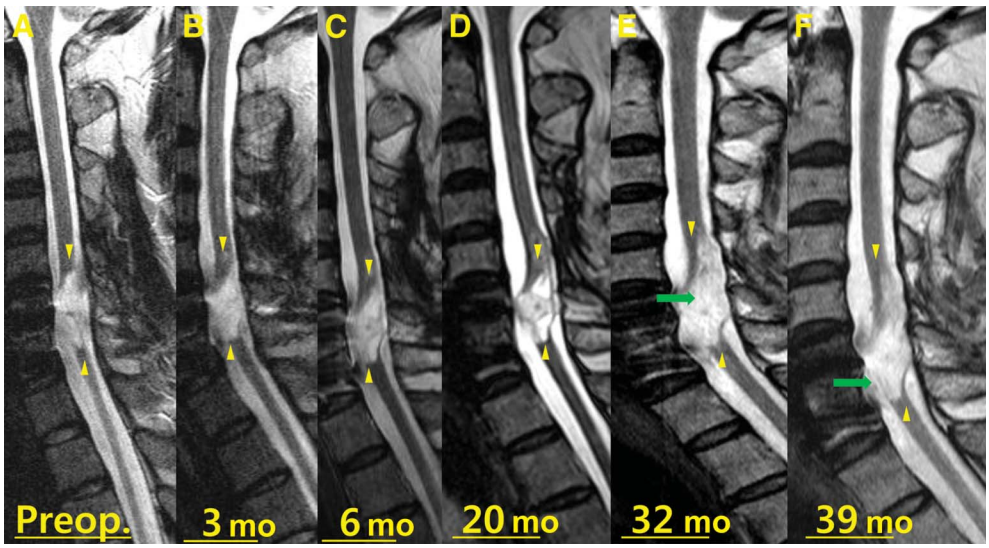

FIGURE 4. *T2-weighted sagittal magnetic resonance imaging of patient 2 showing the site of injury and the cavity at the C6-7 level. Mesenchymal stem cells were injected as described in the legend to Figure 1. A high signal spot was observed proximal to the contusion site of the spinal cord. This spot became enlarged on follow-up and was accompanied by the appearance of fiber-like streaks (arrows). The cavity wall was seen until 20 months postoperatively, but the disappearance of the cavity margin began after 32 months (arrowheads). The cavity sizes on T2-weighted midline sagittal magnetic resonance imaging at each time point were 13.4 × 25.5 mm (**A**); 11.8 × 26.5 mm (**B**); 10.8 × 25.3 mm (**C**); 11.4 × 25.1 mm (**D**); no cavity upper margin (**E**); no cavity upper margin (**F**).*

long-term results of the 3 patients who showed improvement in ADL during short-term follow-up.

Stem cell treatment of patients with irreversibly damaged spinal cords has yielded good results.[17,20,22,27,40] We used autologous MSCs, thus avoiding the problems associated with immunological rejection and graft-vs-host reactions that frequently arise when performing allogeneic transplantation.[41] Autologous MSCs from BM have several advantages over other sources of cells. MSCs are relatively easy to obtain from the BM under local anesthesia; the cells grow well and expand ex vivo and can be readily injected into injured tissue.[42,43] MSCs also secrete bioactive molecules, such as growth factors and cytokines, provide structural support, suppress inflammation, and reduce apoptosis. Finally, MSCs are particularly suitable for use in human trials because they can be readily obtained from the BM of the patient.[17,42,44-46] None of our patients showed evidence of tumorigenesis, any harvesting problem, or morbidity associated with the use of general anesthesia.

The translation of cellular transplantation strategies into clinical procedures requires a safe and efficient means of cell delivery. Less invasive methods for delivery include intravascular delivery[47-49] and delivery into the cerebrospinal fluid, which has been reported to be superior to intravascular delivery in animal models.[36,50-52] However, the most common method of delivery in animal models of SCI is direct injection into the injury site.[29,53,54] Although this allows the delivery of a defined number of cells, there is a risk of further injury to the spinal cord, and this may therefore be inappropriate for humans. In animals, intrathecal delivery via lumbar puncture has been reported to be safer and more effective than direct injection.[18] However, the wound-healing process has already ended and the homing effect, ie, the process by which stem cells can migrate to the pathologic sites, has disappeared in the chronic stage of SCI, unlike the acute stage.[55-57] Therefore, we believe that direct injection of MSCs in the spinal cord is most effective for delivering MSCs to the optimal site, which is a chronic lesion without a homing effect. In addition, we hypothesize that second and third intrathecal injections of MSCs can be delivered to the contusion site of the spinal cord using the homing effect, which occurred by previous injections of MSCs in the spinal cord. Although intrathecal injection of MSCs alone has been used in patients with SCI,[17,22] no study on direct intramedullary injection of MSCs into humans has been reported. We used a 26.5-gauge needle and 5 injection sites, 2 in the proximal spinal cord just above the cavity and 3 in the cavity itself. We hypothesized that the proximal spinal cord just above the cavity would be the optimal target for neuronal regeneration, but injection into this area may result in increased tissue pressure, causing leakage of injected MSCs. We therefore used fibrin glue to seal the injected sites. By contrast, injection into the cavity is not associated with a risk of leakage because of the lower tissue pressure at that site, as well as the resolution of glial scars, but the cavity may be a hostile

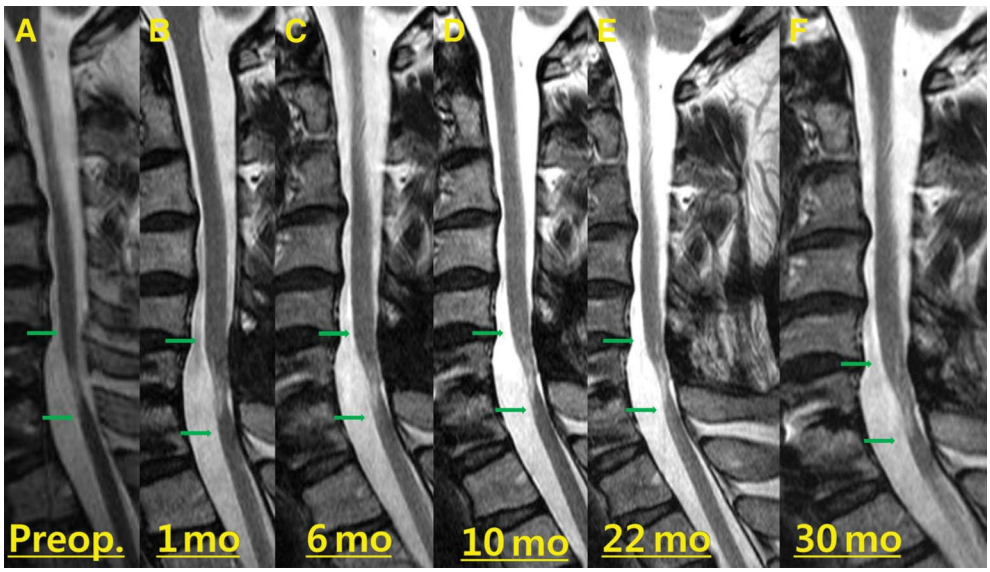

FIGURE 5. *T2-weighted sagittal magnetic resonance imaging of patient 3 showing the site of injury and atrophy at the C6-7 level. Mesenchymal stem cells were injected into 5 sites in the proximal spinal cord just above and around the site of atrophy. Severe spinal cord atrophy was observed before stem cell injection, but follow-up magnetic resonance imaging showed thickening of the spinal cord proximal and distal to the injured site as well as at the injured site. The anteroposterior diameters of the spinal cord at the level of the C5-6 intervertebral disc and C7 mid-body level on T2-weighted midline sagittal images (arrows) were 3.86 and 4.54 mm (**A**); 6.52 and 4.67 mm (**B**); 5.47 and 4.38 mm (**C**); 5.30 and 4.10 mm (**D**); 5.46 and 4.59 mm (**E**); and 5.46 and 4.89 mm (**F**).*

environment for cell survival because of lower vascular perfusion. Using our method of MSC delivery, no patient experienced any surgery-related permanent complications, indicating that direct intramedullary injection of MSCs is safe. Although there have been several researches reporting the formation of tumors induced by MSCs,[14-16,58] we did not see any tumor formation in the 5 patients who were followed long term with MRI. We believe that further studies with more cases are needed.

The 3 patients described here showed functional recovery and changes in MRI results. In the literature,[59] the most rapid spontaneous motor improvement of the upper extremities occurred during the first 3 months after injury, reaching a steady state 12 months after injury. By contrast, 2 of the 3 patients described here showed gradual improvement, even after 12 months, suggesting that motor power improvement of the upper extremities in these patients was associated with MSC transplantation.

The presence of a glial scar has been regarded as a major impediment to axon regeneration.[60] In 2 of our patients (patients 1 and 2), we observed the disappearance of cavity walls on MRI scans 32 months after MSC transplantation. A reduction in the amount of fibrotic tissue in infarcted myocardium has been reported in animals after MSC injection,[61] suggesting that the disappearance of cavity margins on the MRI scans of our patients was caused by a similar mechanism. In other words, BM-derived MSCs are thought to have the function of diminishing glial scars in human spinal cords. In addition, MRI scans of these 2 patients showed the appearance of longitudinal, low-signal, fiber-like streaks in the injured spinal cords, which may provide indirect evidence of axon regeneration in these patients. Alternatively, these streaks may represent the mesenchymal cells themselves, which tend to assume a fibroblastic morphology and elongate in the direction of the long axis of the spinal cord, or they may represent collagen produced by MSCs. The MRI scan of patient 3 showed gradual thickening in the peripheral region of the injured site. Such long-term changes seen on MRI are objective and suggest that the use of MSCs for axon regeneration is promising.

The 3 patients also showed significant electrophysiological improvement in MEPs and SSEPs, especially when compared with the 2 patients (patients IV and VII) who showed minimal motor improvement at the last follow-up. The ability to record early SSEPs has been reported to be related to outcomes in SCI patients.[62] Although we could not collect SSEP data for 2 patients (patients 2 and 3) during their periods of acute injury, their pretreatment SSEPs were recordable, which may be associated with their better clinical outcomes. In the literature, a positive SSEP response after stem cell injection into SCI patients was reported to not correlate with clinical outcomes.[40] However, although we were unable to collect whole data sets for all 10 patients, we observed positive associations between long-term clinical outcome and electrophysiological improvement.

The changes in the motor, MRI, and electrophysiological studies are summarized in Table 5. All 3 patients who showed ADL improvement were classified as ASIA grade B before treatment, whereas 3 of the 7 who did not show ADL improvement

TABLE 4. Last Follow-up Results of Motor Power Changes in the Upper Extremities in the 7 Patients With No ADL Improvement During 6-Month Follow-up[a]

Upper Extremity Motor Power	Patient I								Patient II								Patient III							
	Right				Left				Right				Left				Right				Left			
Months	0	3	6	F/U was not done for personal reasons	0	3	6		0	3	6	F/U was not done for personal reasons	0	3	6		0	3	6	Died of pneumonia	0	3	6	
C5 (EF)	0	0	0		0	0	0		0	0	0		0	0	0		III	IV	IV		III	IV	IV	
C6 (WE)	0	0	0		0	0	0		0	0	0		0	0	0		I	I	I		I	I	I	
C7 (EE)	0	0	0		0	0	0		0	0	0		0	0	0		I	I	I		I	I	I	
C8 (FF)	0	0	0		0	0	0		0	0	0		0	0	0		I	I	I		I	I	I	
T1 (Fab)	0	0	0		0	0	0		0	0	0		0	0	0		I	I	I		I	I	I	

Upper Extremity Motor Power	Patient IV								Patient V										Patient VI					
	Right				Left				Right				Left						Right			Left		
Months	0	3	6	62	0	3	6	62	0	3	6	Patient refused contact with us	0	3	6	0	3	6	F/U could not be done because of patient's depression			0	3	6
C5 (EF)	V	V	V	V	V	V	V	V	I	I	I		I	I	I	IV	IV	IV				IV	IV	IV
C6 (WE)	IV	IV	IV	IV	IV	IV	IV	IV	0	0	0		0	0	0	I	III	III				I	II	II
C7 (EE)	II	II	II	III-	II	II	II	II	0	0	0		0	0	0	0	0	0				0	0	0
C8 (FF)	0	0	0	I	0	0	0	I	0	0	0		0	0	0	0	0	0				0	0	0
T1 (Fab)	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0				0	0	1

Upper Extremity Motor Power	Case VII							
	Right				Left			
Months	0	3	6	55	0	3	6	55
C5 (EF)	V	V	V	V	V	V	V	V
C6 (WE)	IV	IV	IV	IV	III	III	IV	IV
C7 (EE)	II	III	III	III	I	I	III	III
C8 (FF)	0	0	II	II	0	0	0	II
T1 (Fab)	0	0	0	0	0	0	0	II

[a]ADL, activities of daily living; 0 months, preoperative state; EF, elbow flexion; WE, wrist extension; EE, elbow extension; FF, finger flexion; Fab, finger abduction; F/U, follow-up; Roman numerals, motor power grade.

TABLE 5. Summary of Changes in the Motor, MRI, and Electrophysiological Studies[a]

		ASIA Grade	Motor Change	MRI Change	Electrophysiological Change	F/U Duration, mo
ADL improvement	Patient 1	B	Yes	Yes	Yes	36
	Patient 2	B	Yes	Yes	Yes	39
	Patient 3	B	Yes	Yes	Yes	30
No ADL improvement	Patient I	A	No	Yes	No	6
	Patient II	A	No	Yes	No	6
	Patient III	B	Yes	No	No	6
	Patient IV	B	Yes	Yes	No	62
	Patient V	A	No	Yes	Yes	6
	Patient VI	B	Yes	No	Yes	6
	Patient VII	A	Yes	No	Yes	55

[a]MRI, magnetic resonance imaging; ASIA, American Spinal Injury Association; F/U, follow-up; ADL, activities of daily living.

were ASIA grade B and 4 were ASIA grade A. This treatment might be most effective for the patient who has residual neurological function. Therefore, MSC treatment is more likely to enhance the remaining neurological function rather than regeneration. It also indicates that this treatment could be more effective for patients with incomplete injuries rather than complete injuries. However, more studies are needed to know whether the mechanism of MSC treatment enhances residual neurological function or neuronal regeneration. We could detect SSEPs in 2 of 3 patients who had ADL improvement. Only 1 patient showed SSEPs of the 7 patients who had no ADL improvement. However, because few patients have undergone this treatment, we could not measure accurate statistics.

CONCLUSION

Three of 10 patients with SCIs who received direct injections of autologous MSCs showed continuous and gradual motor improvement in the upper extremities and significant MRI and electrophysiological changes during long-term follow-up. Direct intramedullary injection of MSCs into SCI patients did not result in permanent complications, such as infection, tumor formation, syrinx formation, ectopic calcification, and aggravated chronic pain, including allodynia and paresthesia. Further studies are needed to determine the clinical significance of these MRI changes and the effects of transplanted MSCs on axon regeneration.

Disclosures

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COMMENT

The article by Park et al describes the long-term outcome of 3 spinal cord injury (SCI) patients who initially showed positive results at 6 months after direct injection of mesenchymal stem cells (MSCs). Outcome was assessed by magnetic resonance imaging (MRI), electrophysiology, motor power grade, and activities of daily living (ADL). On MRI, these subjects also appeared to exhibit reduced cyst size. None of the patients showed any adverse side effects after transplantation of the cells. Stem cell transplantation is an attractive and active avenue of research for the treatment of SCI (for a review, see Wright et al[1]). Stem cells can be a source for cell replacement, but can also promote neuroprotection and regeneration. Whether MSCs can transdifferentiate into neural cells remains controversial. Evidence suggests that MSCs may bring about improvement in SCI through anti-inflammatory properties. MSCs can also produce trophic factors including brain-derived neurotrophic factor, nerve growth factor, and vascular endothelial growth factor. Moreover, it has been suggested the MSCs may also offer physical support to regenerating axons by acting as cellular bridges. Nonetheless, the literature shows that the interaction between MSCs and the SCI environment is highly complex. To date, worldwide, 2 clinical trials using ex vivo expanded autologous MSCs have been reported by Saito et al[2] and Pal et al.[3] Both studies reported positive results with in regard to the safety of MSC transplant, and no serious complications were observed. Both studies reported only slight motor improvement. Overall, the subject of this article by Park et al contributes to the field of MSC transplantation into the spinal cord by providing additional evidence that no adverse affects arise from MSC transplantation. Moreover, this study shows promising results of reduced cavity size and motor improvement. However, the exact mechanisms behind the observed findings are yet to be defined. The small number of individuals analyzed (n = 3) and the variability within subjects are additional weaknesses of this report. In summary, more rigorous research will add to our understanding of the use of stem cell transplantation for SCI and will be critical to the design of future clinical trials.

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