A Phase III Clinical Trial Showing Limited Efficacy of Autologous Mesenchymal Stem Cell Therapy for Spinal Cord Injury

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BACKGROUND: In our previous report, 3 of 10 patients with spinal cord injury who were injected with autologous mesenchymal stem cells (MSCs) showed motor improvement in the upper extremities and in activities of daily living.

OBJECTIVE: To report on the results of a phase III clinical trial of autologous MSCs therapy.

METHODS: Patients were selected based on the following criteria: chronic American Spinal Injury Association B status patients who had more than 12 months of cervical injury, and no neurological changes during the recent 3 months of vigorous rehabilitation. We injected 1.6×10^7 autologous MSCs into the intramedullary area at the injured level and 3.2×10^7 autologous MSCs into the subdural space. Outcome data were collected over 6 months regarding neurological examination, magnetic resonance imaging with diffusion tensor imaging, and electrophysiological analyses.

RESULTS: Among the 16 patients, only 2 showed improvement in neurological status (unilateral right C8 segment from grade 1 to grade 3 in 1 patient and bilateral C6 from grade 3 to grade 4 and unilateral right C8 from grade 0 to grade 1 in 1 patient). Both patients with neurological improvement showed the appearance of continuity in the spinal cord tract by diffusion tensor imaging. There were no adverse effects associated with MSCs injection.

CONCLUSION: Single MSCs application to intramedullary and intradural space is safe, but has a very weak therapeutic effect compared with multiple MSCs injection. Further clinical trials to enhance the effect of MSCs injection are necessary.

KEY WORDS: Clinical trial, Diffusion tensor imaging, Electrophysiological study, Intramedullary injection, Mesenchymal stem cells, Spinal cord injury

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Spinal cord injury (SCI) can result in severe disability including impairment of motor and sensory function and loss of bowel and bladder function. As a result, SCI patients and their families may experience long-term medical care, high burden of disability, and low quality of life.[1,2] Over the past decades, various surgical techniques and intensive rehabilitation therapy options for SCI have been developed, but they remain ineffective in cases of chronic SCI.

The central nervous system, including the brain and spinal cord, is vulnerable to injury and degenerative changes and does not easily regenerate.[3] Therefore, several clinical trials and studies on stem cell therapy have been performed for various diseases such as Parkinson disease, stroke, and other neurodegenerative diseases.[4-6] For SCI, several types of cell therapies using olfactory ensheathing cells, Schwann cells, mesenchymal stem cells (MSCs), and other neural stem cells/progenitor cells have been investigated.[7-11] Among these types, MSCs have certain advantages compared with other cell therapies. MSCs can exert the activities related to neuronal regeneration.[12-16] Moreover, MSC

ABBREVIATIONS: ADL, Activities of Daily Living; **ASIA,** American Spinal Injury Association; **BM,** bone marrow; **DTI,** diffusion tensor imaging; **EP,** electrophysiological studies; **KMFDS,** Korean Ministry of Food and Drug Safety; **MEP,** motor evoked potential; **MSC,** mesenchymal stem cell; **SCI,** spinal cord injury; **SEP,** somatosensory evoked potential

transplantation not only enhances neuronal protection but also reduces the inflammatory response and microglial reactivity by its immunosuppressive feature.[17,18] In addition, MSCs are easy to harvest from the bone marrow (BM) of each patient at the bedside,[15,19-22] and the use of MSCs can overcome ethical problems and rejection complications because MSCs can be extracted from the patients themselves.

We previously published a pilot study on BM-MSC injection therapy for patients with chronic SCI.[15] In that report, 6 of the 10 patients showed neurological improvement of the upper extremities, 3 of whom showed improvement in Activities of Daily Living (ADL). On the basis of those results, we designed a phase III nonrandomized clinical trial on autologous MSC therapy for patients with chronic SCI, changing the protocol from multiple cell injections to a single application according to government regulations that usually prohibit multiple applications as a phase III trial. The effectiveness of this clinical trial was determined based on neurological examination, magnetic resonance imaging (MRI) with diffusion tensor imaging (DTI), and electrophysiological analysis.

METHODS

Patient Selection

The selected number of patients was initially 32: this number, calculated statistically, was the minimum number to achieve positive results. However, during the study, the final results were expected to be unsatisfactory, so we decided to finish the study earlier than we planned. Therefore, 20 patients with chronic American Spinal Injury Association (ASIA) grade B quadriplegia caused by traumatic cervical SCI were selected for enrollment in this study at the Asan Medical Center from August 2008 to December 2012. All participants were given a full explanation of the study and signed the informed consent form. Inclusion criteria were as follows: (1) age 16 to 65 years, (2) traumatic cervical cord injury more than 1 year after the accident, (3) no neurological improvements during the recent 3 months of vigorous rehabilitation, (4) an ASIA impairment scale grade B, and (5) no gross severe muscle atrophy and joint contracture. The exclusion criteria were: (1) an aspartate aminotransferase/alanine transaminase value more than 3 times the normal value, (2) the creatinine level more than 1.5 times the normal value, (3) penetrating injury of the spinal cord, (4) a severe infectious condition (ie, urinary tract infection, pneumonia), (5) positive for penicillin or autologous MSC by skin test, or (6) positive viral markers of hepatitis B virus, hepatitis C virus, human immunodeficiency virus, and venereal disease research laboratory test. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this study.

Preparation of Autologous MSCs

We used the same technique for the harvest and culture of BM cells, which was described in our previous report.[15] BM was aspirated from the posterior superior iliac crest. Then mononuclear cells were separated from BM by density gradient centrifugation (Histopaque-1077, Sigma-Aldrich, St. Louis, Missouri) and was washed using phosphate-buffered saline, mixed with low-glucose Dulbecco modified Eagle's medium (Gibco, Grand Island, New York) containing 10% (vol/vol) fetal bovine serum (Gibco). The culture medium was replenished by 100 U/mL penicillin and 100 mg/mL streptomycin (Gibco). The culture flasks were incubated at 37°C in a humidified 5% (vol/vol) CO_2 atmosphere for 5 to 7 days, and refreshed by replacing the same medium that is described above to remove nonadherent cells. When the adherent cells approached enough confluence (70%-80%), the adherent cells were detached from the medium with a trypsin/ethylenediamine tetraacetic acid solution (Gibco). Cells for infusion were continuously cultured and injected within 4 weeks after BM aspiration. On the day of injection, MSCs were extracted with trypsin/ethylenediamine tetraacetic acid, washed with phosphate-buffered saline, and then moved to saline solution. The final solution contained 4.8×10^7 cells/3 mL and was transferred to the operating room for stem cell injection therapy.

Surgical Technique and Follow-up Treatment

Cell injection was conducted per the technique described in our previous study.[15] After laminectomy and a dural incision at the injury site, 1.6×10^7 autologous MSCs in 1 mL of normal saline were injected into the intramedullary area at 5 points including the normal proximal area and the injured site (Figure 1). The depth of injection was 3 to 4 mm. Each injection was done gently through a 27-gauge needle over 10 seconds, and the needle was withdrawn after 1 minute. Fibrin glue was applied to the injection site during removal of the needle to prevent cell leakage. In addition, 3.2×10^7 autologous MSCs in 2 mL of normal saline were administered to the subdural space around the injection site before dural closure. One week after surgery, the patients were transferred to the department of rehabilitation and underwent a standardized rehabilitation therapy 6 days per week for a total of 4 weeks. The rehabilitation therapy included physical therapy twice a day, lying on the tilting table for 30 minutes, and mat exercises for 30 minutes. The mat exercises included active assistive range of motion for upper and lower extremities as well as functional activities such as rolling and sitting. Functional electrical stimulation was also applied on the wrist extensor and quadriceps. The duration of 4 weeks was decided to observe closely postoperative neurological status as well as complications

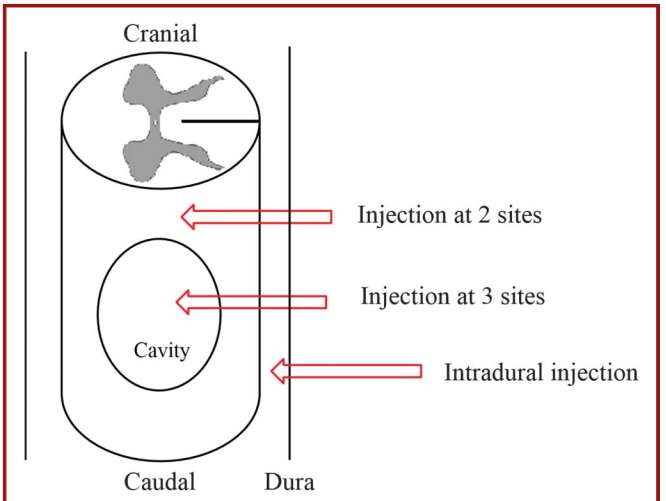

FIGURE 1. *Diagram of stem cell injection. 1.6×10^7 autologous MSCs were injected into the intramedullary space: 2 sites in the proximal normal spinal cord just above the injured area and 3 sites in the injured spinal cord area. 3.2×10^7 autologous MSCs were administered to the subdural space around the injection site before dural closure. MSC, mesenchymal stem cell. Color version available online only.*

associated with stem cell injection and to evaluate first postoperative neurological examination at postoperative 1 month without revisit.

Outcome Measurements

Neurological motor examination was conducted according to the International Standards for Neurological Classification of Spinal Cord Injury[23] by the same rehabilitation physician, and observations were made 1, 3, and 6 months after MSC injection (Figure 2). The positive results of this phase III trial were defined by the Korean Ministry of Food and Drug Safety (KMFDS) and the definition was composed of 2 criteria: (1) an improvement in motor grade in more than 2 joints from grade 2 or below to grade 3 or above, and (2) more than 30% of effective patients in the total patients followed up.

We also measured motor evoked potential (MEP) and somatosensory evoked potential (SEP) before MSC injection and at 3 and 6 months after surgery. MEP was recorded by using the Magstim Rapid^2 (Magstim, Carmarthenshire, United Kingdom) and was measured on the abductor pollicis brevis muscle (median nerve), abductor digiti minimi muscle (ulnar nerve), extensor carpi radialis muscle (radial nerve), and tibialis anterior muscle (deep peroneal nerve). MEP stimulation was conducted by applying transcranial magnetic stimulation using a coil magnetic stimulator, and the potential was recorded with a needle-type electrode placed in each muscle. SEP was performed with the support of Synergy (Natus Medical, Middleton, Wisconsin). For SEP investigation, the median, ulnar, and posterior tibial nerves were stimulated. The median and ulnar nerve were stimulated on the wrist and responses were recorded by using scalp needle electrodes placed at the contralateral C3′/C4′. The posterior tibial nerve was stimulated on the ankle, recorded by the active electrode placed at contralateral Cz′. We defined a clinically significant change in MEP and SEP as follows: (1) new appearance of wave that was not present at the preoperative study, (2) more than 50% increase in the amplitude, or (3) more than 10% decline in the latency.

The spinal MRI was obtained to observe changes in the spinal cord before MSC injection and 6 months later. With the use of an Achieva 3.0-T scanner (Philips Healthcare, Best, the Netherlands), 3-mm-thickness T1-, T2-, and T1-enhanced sagittal and axial images were obtained. To observe the change of spinal cord atrophy on the injured site, we evaluated the spinal cord diameter on the injured site via PACS (Picture Archiving Communication System) Petavision (Asan Medical Center, Seoul, Korea) in the sagittal and axial T1- and T2-weighted MRI. Moreover, DTI was performed to examine continuity of nerve fiber around the injured site. The DTI parameters included 15 diffusion gradient directions, *b*-value 600 s/mm^2, slice thickness of 2 mm, 20 slices of sagittal sections, repetition time/echo time 3380/56 ms, number of averages of 4, sensitivity encoding (SENSE) factor 4 and acquisition time of 3 minutes, 39 seconds. Fiber tractography was generated from the DTI data by using the FiberTrak software program (Philips Healthcare, Best, the Netherlands), and we compared preoperative fiber tractography with a postoperative image to find any change in the white matter tract. We used high SENSE factor to reduce the distortion from metallic artifacts and to compensate for the decrease in signal-to-noise ratio; we increased the number of averages and used low *b*-value. When the continuity between proximal and distal white matter tract appeared in postoperative fiber tractography, we defined it as a "newly generated continuity of the tract."

Statistical analyses involved the Student *t* test for continuous variables (age, duration between SCI and stem cell therapy). The Fisher exact test was performed to analyze the categorical variables (sex, treatment protocol, the association between motor improvement and change of MRI or electrophysiological studies). All tests were considered significant if the *P* values were less than .05. We used SPSS software for Windows (Statistical Product and Service Solutions, version 12.0, SSPS Inc, Chicago, Illinois) to perform statistical analysis.

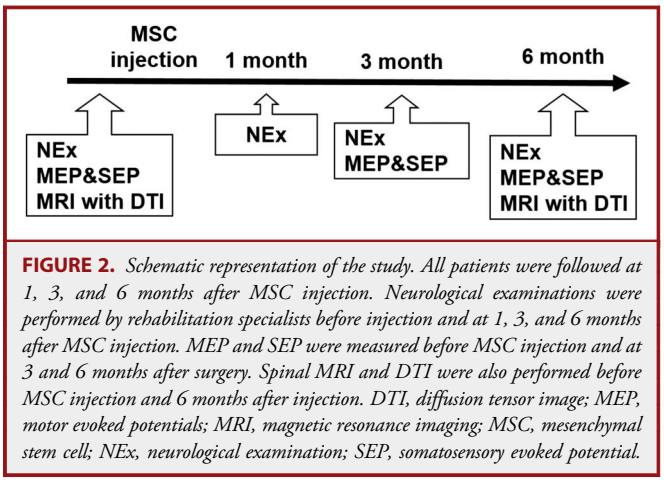

FIGURE 2. *Schematic representation of the study. All patients were followed at 1, 3, and 6 months after MSC injection. Neurological examinations were performed by rehabilitation specialists before injection and at 1, 3, and 6 months after MSC injection. MEP and SEP were measured before MSC injection and at 3 and 6 months after surgery. Spinal MRI and DTI were also performed before MSC injection and 6 months after injection. DTI, diffusion tensor image; MEP, motor evoked potentials; MRI, magnetic resonance imaging; MSC, mesenchymal stem cell; NEx, neurological examination; SEP, somatosensory evoked potential.*

RESULTS

Patient Characteristics

Among the 20 enrolled SCI patients, 4 (20.0%) were excluded: dropout during follow-up in 1 patient, refusal to undergo therapy after BM harvest in 1 patient, and a positive skin test for MSCs in 2 patients. Therefore, 16 (80.0%) patients who were followed up for the entire period were included in the analysis (Table 1). This cohort comprised 15 men (93.8%) and 1 woman (6.2%), with a mean age of 40.9 years (range, 18-65 years). The average duration from the date of SCI to this study was 62.5 months (range, 24-181 months).

Neurological Improvement

Two (12.5%, cases 9 and 13) of the 16 patients showed improvement in motor grade of the upper extremities (Table 2); they did not fulfill the criteria of effectiveness. No changes were noted in the other 14 patients (87.5%) at the 6-month follow-up (Table 1).

MRI Findings

In 5 patients, changes were noted at T2-weighted MRI images. Increases in spinal cord diameter were observed in 5 patients (cases 2, 9, 10, 11, and 13), and disappearance of the cavity was observed in 1 patient (case 2) (Figure 3). Blurring of the cavity margin (cases 9, 11, and 13) and appearance of fiber-like streaks (cases 9, 11, and 13) were observed. Newly generated continuity of the tract in the spinal cord was observed in 2 patients on DTI (cases 9 and 13) (Figures 4 and 5), whereas the others did not show this finding (Figure 6).

MEP and SEP Study

Four patients (cases 7, 8, 12, and 15) showed significant SEP improvement, and 6 patients (cases 1, 2, 4, 6, 9, and 12) showed

TABLE 1. Characteristics of Patients[a]

Case	Age	Sex	Level of Injury	Type of Surgery	AIS at Injured Time	Duration After Injury (mo)	Motor Change	MRI Changes	Appearance of Continuity in DTI	Electrophysiological Improvement	
										MEP	SEP
1	31	M	C5-6 dislocation	ACDF, C5-6	B	84	No	No	No	Yes	No
2	50	M	C5-6 dislocation	ACDF, C5-6	B	24	No	Yes	No	Yes	No
3	18	M	C5 burst fracture	AIF, C4-6	B	25	No	No	No	No	No
4	38	M	C5-6 dislocation	ACDF, C5-6	B	181	No	No	No	Yes	No
5	43	M	C3-4 dislocation	ACDF, C3-4	B	132	No	No	No	No	No
6	64	F	Traumatic HNP and OPLL, C3-4, 4-5	No	B	24	No	No	No	Yes	No
7	26	M	C5 burst fracture	AIF, C4-6	B	36	No	No	No	No	Yes
8	41	M	C5 burst fracture	AIF, C4-6	B	66	No	No	No	No	Yes
9	57	M	C6-7 dislocation	ACDF, C6-7	B	60	Yes	Yes	Yes	Yes	No
10	42	M	Traumatic HNP, C5-6	ACDF, C5-6	B	73	No	Yes	No	No	No
11	43	M	C3-4 dislocation	PSF, C3-4	B	84	No	Yes	No	No	No
12	23	M	C5-6 dislocation	ACDF, C5-6	B	49	No	No	No	Yes	Yes
13	65	M	C4-5, 5-6 dislocation	AIF, C4-6	B	25	Yes	Yes	Yes	No	No
14	32	M	Traumatic HNP, C3-4	ACDF, C3-4	A	44	No	No	No	No	No
15	36	M	C5-6 dislocation	ACDF, C5-6	B	60	No	No	No	No	Yes
16	45	M	C5 burst fracture and C5-6 dislocation	AIF, C4-7 and PSF, C3-7	B	34	No	No	No	No	No

[a]ACDF, anterior cervical discectomy and fusion; AIS, ASIA impairment scale; AIF, anterior interbody fusion; DTI, diffusion tensor image; HNP, herniated of nucleus pulposus; MEP, motor evoked potentials; MRI, magnetic resonance imaging; OPLL, ossification of posterior longitudinal ligament; PSF, posterior screw fixation; SEP, somatosensory evoked potential.

noticeable changes in MEP. None of the 4 patients with SEP improvement showed recovery of motor grade. Among the 6 patients with MEP improvement, only 1 (case 9) patient had an improvement in motor grade (Table 1).

Adverse Effect

No serious adverse effects requiring a revision operation, such as cerebrospinal fluid (CSF) leakage or infection, were seen in any of the patients. However, 8 patients developed mild adverse effects. One patient showed sensory deterioration in the right lower extremity after MSC injection. Two patients showed increased muscle rigidity in the posterior neck, bilateral shoulder, or arm. Five patients reported worsened symptoms of tingling sense requiring pain medication, but these symptoms had resolved in all of these patients within a few months.

TABLE 2. Characteristics of Patients Who Showed Neurological Improvement During Follow-up[a]

	Patient 1 (Case 9)				Patient 2 (Case 13)			
	Baseline		Follow-up		Baseline		Follow-up	
	Right	Left	Right	Left	Right	Left	Right	Left
C5	V	V	V	V	V	V	V	V
C6	V	V	V	V	III	III	IV[b]	IV[b]
C7	V	V	V	V	I	I	I	I
C8	I	I	III[a]	I	0	0	I[b]	0
T1	0	0	0	0	0	0	0	0

[a]Roman numerals, motor grade.
[b]Parameter that showed a change.

Representative Cases

Patient 1 (Case 9)

A 57-year-old male patient experienced a cervical fracture and dislocation at C6-7 due to a car accident. His neurological status was ASIA grade B and did not change for 60 months despite rehabilitation therapy. Six months after MSC injection, the motor grade of his right hand had improved slightly, with grasp motor (C8 segment) improving from grade 1 to grade 3 (Table 2). Final MRI indicated blurring of the cavity margin, thickening of the spinal cord, and appearance of dark fiber-like streaks within the contusion site on T2-weighted images and newly generated continuity in fiber signal on DTI (Figure 4). During the follow-up period, SEP of the median nerve had not improved: the preoperative latency and amplitude were 19.6 milliseconds and 1.8 μV on the right side and 18.9 milliseconds and 3.0 μV on the left side, whereas the postoperative latency and amplitude were not detected. The SEP of the ulnar and posterior tibial nerves was not detected at preoperative and last follow-up measurement. However, the MEP of ulnar nerve and deep peroneal nerve showed significant changes. Preoperatively, MEP wave of the ulnar nerve was not observed in both sides, but slight MEP signals were observed at the last follow-up, in which the latency

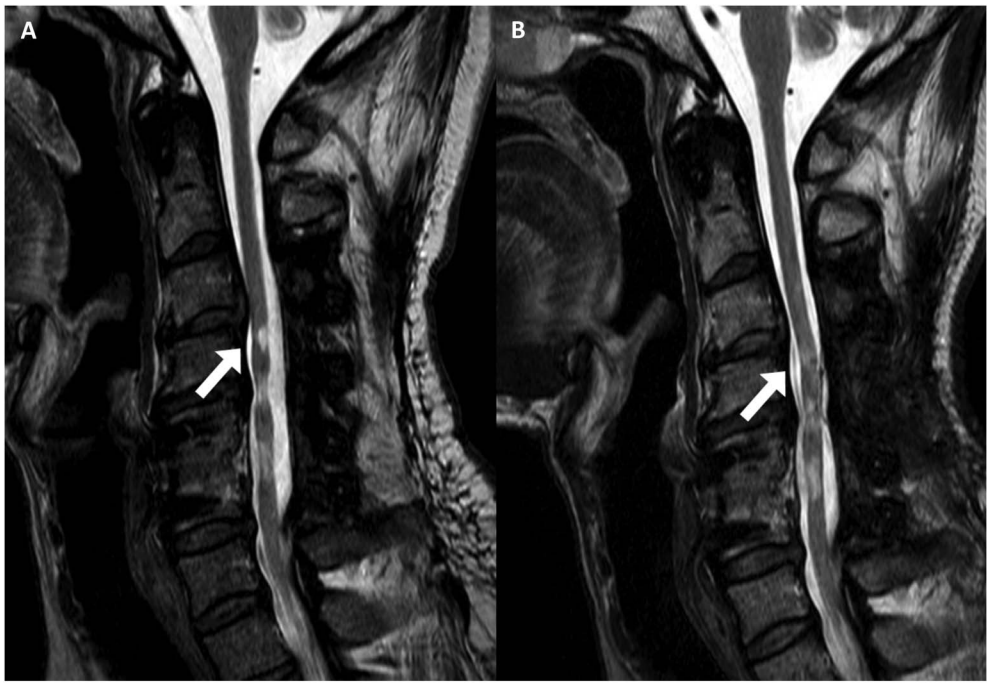

FIGURE 3. *In case 2, preoperative MRI (A) showed a cystic cavity at C4 level (arrow), which was not found on MRI 6 months after MSC injection (B). MRI, magnetic resonance imaging; MSC, mesenchymal stem cell.*

and amplitude were 16.7 milliseconds and 120.0 μV on the right side, and 23.1 milliseconds and no detectable amplitude on the left side. Preoperatively, MEP wave of the deep peroneal nerve was not observed in both sides, but at the last follow-up, the latency and amplitude were 20.3 milliseconds and 995.0 μV on the right side, and 25.7 milliseconds and no detectable amplitude on the left side.

Patient 2 (Case 13)

A 63-year-old male patient became quadriplegic after a blow injury (ASIA grade B). Despite a long period of rehabilitation, the patient showed no neurological improvement. MSCs were administered at 25 months after SCI. During follow-up, the motor grade of his bilateral wrist extension (C6 segment) improved from grade 3 to grade 4 and his right hand grasp motion (C8 segment) improved from grade 0 to grade 1 (Table 2). Six months later, an MRI indicated new changes, similar to those seen in patient 1 (case 9) ie, slight thickening at the injured spinal cord level, blurring of the cavity margin, and the appearance of dark fiber-like streaks (Figure 5). Additionally, the spinal cord at the injected site was enhanced (Figure 7). The appearance of continuity in fiber signal was also detected on DTI (Figure 5). We followed up this patient 9 months after surgery to verify the change in enhancement found on postoperative MRI. The enhancement finding had decreased slightly (Figure 7). The MEP and SEP did not show any significant change between baseline and 6 months later. Preoperative latency and amplitude of median nerve SEP were 20.3 milliseconds and 4.3 μV on the right side and 21.2 milliseconds and 1.6 μV on the left side, and the postoperative latency and amplitude were 20.0 milliseconds and 0.68 μV on the right side and 19.6 milliseconds and 0.83 μV on the left side. Preoperative latency and amplitude of radial nerve MEP were 13.9 milliseconds and 151.7 μV on the right side and 16.5 milliseconds and 401.7 μV on the left side. However, postoperative latency and amplitude of radial nerve MEP were not improved: the latency and amplitude were 15.7 milliseconds and 20.0 μV on the right side and 15.25 milliseconds and 83.3 μV on the left side.

DISCUSSION

Before the development of MSC isolation and culture technologies, some reports described the use of mononuclear cells harvested from BM.[24,25] Currently, there are more studies using cultured MSCs for the treatment of subacute or chronic stage SCI.[15,21,22,26] MSCs are multipotent progenitor cells that have the facility to differentiate into mesodermal lineages and induce trophic activities related to neural cells.[12] Another function of MSCs is to eliminate glial scars in the injured spinal cord.[15] Moreover, implanted MSCs can fill the cavity formed by the contusion in the spinal cord, producing bridge material for axonal regeneration through the cavity,[13,16] and

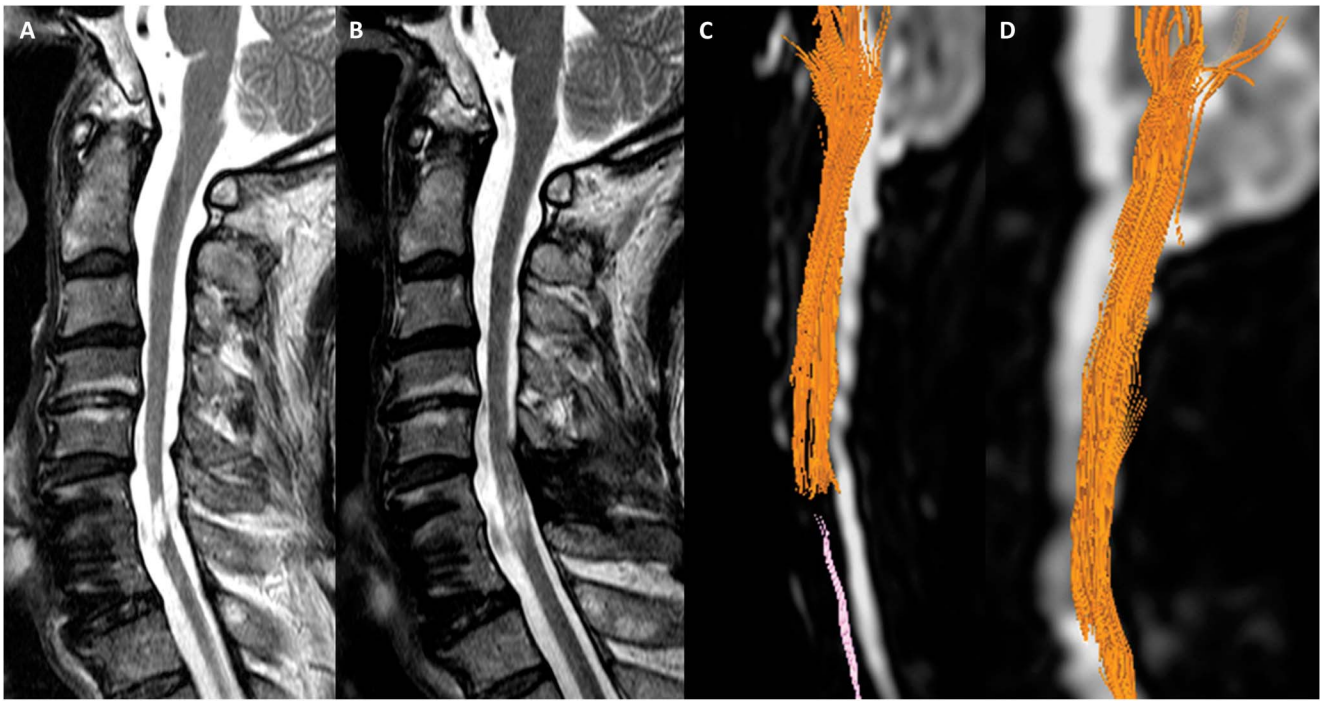

FIGURE 4. *MRI and DTI in patient 1 (case 9). Preoperative sagittal T2-weighted image (A) showed a traumatic cavity at the C6-7 level of the spinal cord. Six months after MSC injection, a sagittal T2-weighted image (B) showed blurring of the cavity margin, thickening of the spinal cord, and the appearance of dark fiber-like streaks within the contusion site. DTI evaluated at 6 months after the operation (D) revealed newly generated continuity in fiber signals compared with preoperative DTI (C). DTI, diffusion tensor image; MRI, magnetic resonance imaging; MSC, mesenchymal stem cell. Color version available online only.*

are known to activate intramedullary endogenous stem cells.[14] Meanwhile, determining the appropriate administration route for MSCs is important for safe and efficient treatment. According to several reports on animals, intrathecal injection is more effective for cell engraftment to the injured site than intravenous injection.[27,28] However, other barriers are associated with intrathecal injection: large stem cell numbers are necessary to reach the appropriate amount of cells in the target site, and subarachnoid or perimedullary adhesion may act as an obstacle for stem cells to reach the target.[8] In addition, the therapeutic cell homing effect is not expected in chronic SCI.[15,29] For these reasons, many studies including our previous report have used intramedullary injection at the injured site; this technique has been reported not to evoke many adverse effects such as CSF leakage, intramedullary hemorrhage, or increased neurological problems.[8,15,19,21] For these reasons, we administered MSCs to both the normal proximal area and injured site in the spinal cord via intramedullary injection (Figure 1). The normal proximal spinal cord above the injured site is the optimal site for the survival of MSCs, but there is a limitation to injecting an adequate amount of MSCs on this site because of high tissue pressure and high risk of normal spinal cord damage. On the contrary, a sufficient amount of MSCs can be injected in the injured site; however, this is not an optimal environment for the survival of MSCs because of lower vascular perfusion. In addition, the site of contused cavity is a good environment for the resolution of glial scars and bridging for axonal regeneration. Therefore, we decided to inject both the normal proximal spinal cord and the injured area to take advantage of both sites, and the injection was conducted through the avascular surface to prevent spinal cord damage. In addition to intramedullary injection, we applied supplementary subdural MSCs to increase the cell numbers with the hypothesis that subdural MSCs would migrate into the spinal cord by the homing effect that would be newly developed from intramedullary injection.

In our previous report, 6 (60%) of a total of 10 patients showed motor grade improvement of the upper extremities, three of whom showed improvement in ADL.[15] ADL improvement means the increase of daily activity tasks such as moving and feeding resulting from significant motor improvement. However, in the current study, we did not evaluate ADL improvement but only measured the change of motor grade. The therapeutic effectiveness in this study was defined as an improvement in motor grade in more than 2 joints from grade 2 or below to grade 3 or above. If the patients in this study fulfilled this condition, they would also show ADL improvement; therefore, the specific evaluation criteria for ADL improvement were not included.

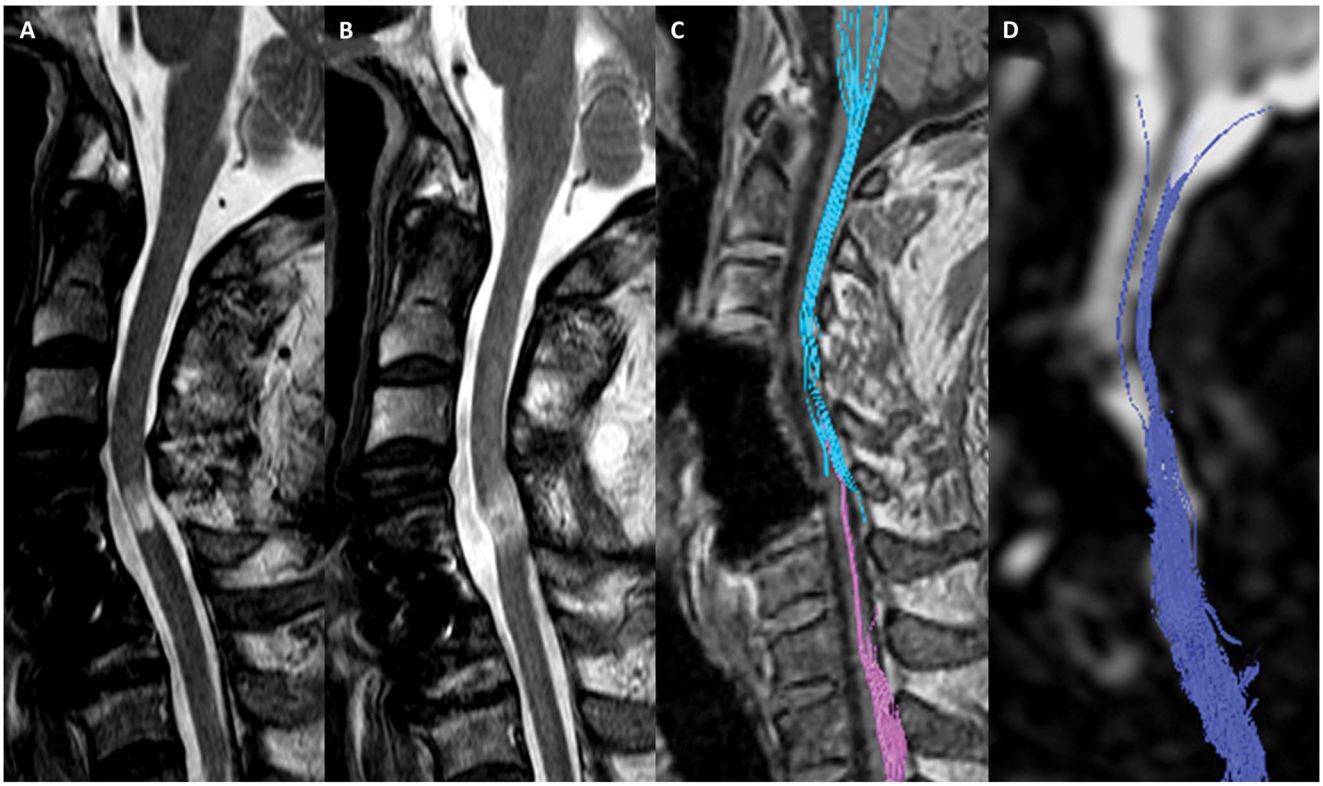

FIGURE 5. *MRI and DTI in patient 2 (case 13). Preoperative sagittal T2-weighted image (A) showed high signal cavity at the C5 level. At the 6-month follow-up, a sagittal T2-weighted image (B) indicated new changes, similar to those observed in patient 1 (case 9): slight thickening at the injured spinal cord level, blurring of the cavity margin, and the appearance of dark fiber-like streaks. Preoperative DTI (C) demonstrated discontinuity of white matter fiber. On follow-up DTI (D), however, downward continuity of fiber signal was newly found at the injury site. DTI, diffusion tensor image; MRI, magnetic resonance imaging. Color version available online only.*

We used a single MSC application to intramedullary and intradural space in this trial without additional intrathecal injection unlike the previous pilot study. Instead, the intramedullary-injected number of MSCs was increased twice compared with the previous study, and standardized rehabilitation exercises over 4 weeks were added. The reason for this modification in the protocol is due to the regulation of KMFDS in the phase III trial. Based on the regulation, protocols using a single application of a new drug are generally approved. The stem cells are classified as a drug by the pharmaceutical safety and efficacy regulation in Korea. Our data showed that only 2 patients (12.5%) showed improvement in motor grade of the upper extremities, which is disappointing in comparison with the results of our previous study. In our previous report, 8×10^6 autologous MSCs were injected into the intramedullary space and 4×10^7 cells were distributed in the intradural space. Afterward, additional intrathecal injections of 5×10^7 MSCs were given 4 weeks and 8 weeks, respectively, after the first injection via lumbar tapping. In contrast, in our current study, 1.6×10^7 autologous MSCs were injected into the intramedullary area, and 3.2×10^7 autologous MSCs were spread into the intradural space only once. As a result, we believe that the increase of the cell number entering into intramedullary space and addition of rehabilitation exercise did not contribute significantly to motor improvement. We compared the patients' characteristics and the results of our 2 studies in Tables 3 and 4. Although the number of cases in our 2 studies is small, we can assume that application number of stem cell may be an important factor in therapeutic effectiveness, because improvement of the motor grade was significantly higher in the previous study that conducted multiple MSCs application including intrathecal lumbar injection.

We also evaluated previous as well as recent electrophysiological studies to identify the correlation with motor recovery in the previous and present study (Table 3). In animal studies, there are many reports that demonstrate close affinity between neurological recovery and electrophysiological studies after cell therapy.[30-33] Similarly, in human studies, electrophysiological studies (EP) were used as a measurement for neurological recovery in clinical trials and reported to have relevance to the neurological outcome.[34-36] However, such association was not found in some studies.[37,38] In our previous study, all patients with ADL improvement showed EP change. Therefore, we speculated that

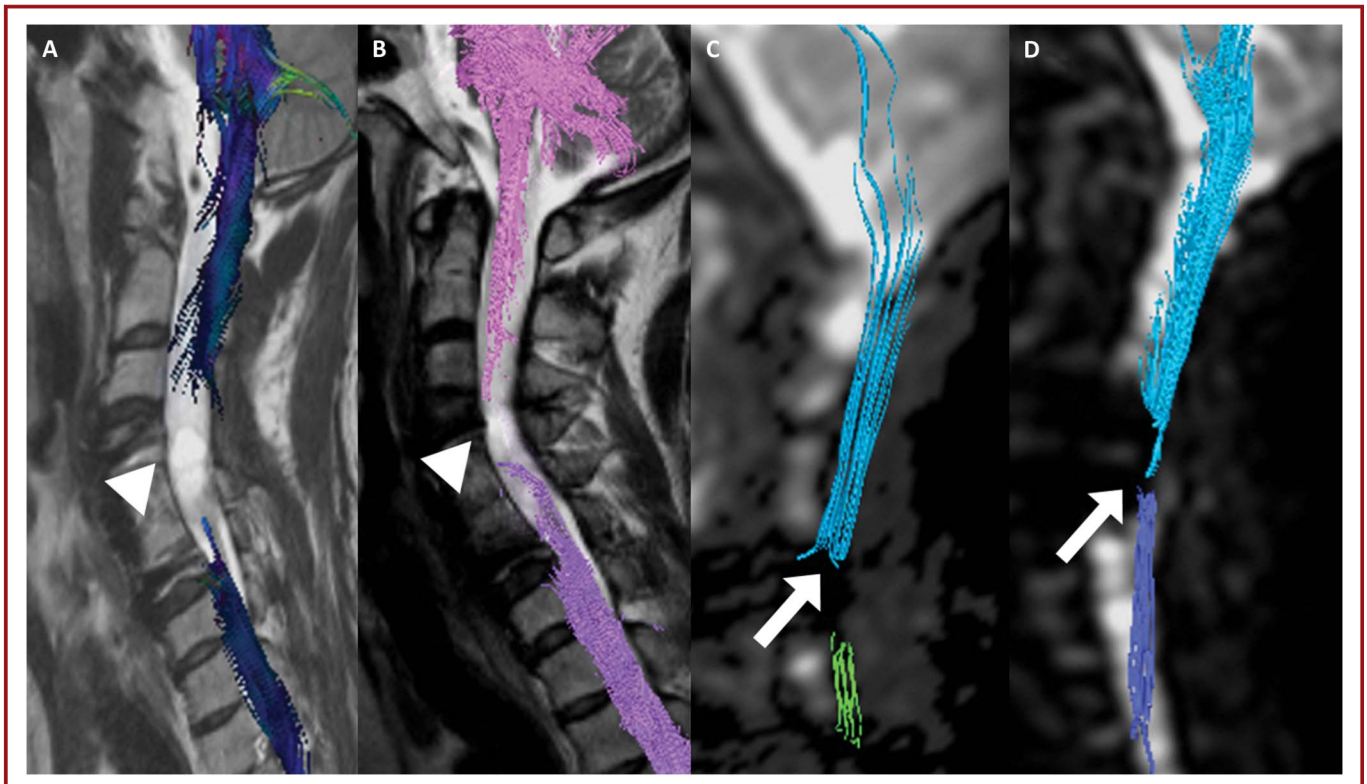

FIGURE 6. *DTI of case 8 (A, B) and 10 (C, D). Discontinuity of white matter fiber on preoperative DTI (arrow and arrowhead) (A, C) remained on DTI 6 months after MSCs injection (arrow and arrowhead) (B, D). DTI, diffusion tensor image; MSC, mesenchymal stem cell. Color version available online only.*

the EP change is a necessary condition for significant motor recovery enough to show ADL improvement. However, these findings were not able to be confirmed in present studies because the number of improved patients was insufficient.

Previous studies have reported various MRI changes after MSC or BM cell application for the treatment of SCI.[15,19] Park et al[15] reported that neurologically improved patients showed increases in cord diameter, disappearance of intramedullary cavity margin, and appearance of longitudinal, fiber-like streaks at the injured spinal cord in the MRI findings. Yoon et al[19] reported that increases in the diameter of the spinal cord on MRI were more frequently seen in the BM mononuclear cell injection group than in the control group, but the difference was not statistically significant. Even though such changes in MRI findings were usually detected in patients with motor function improvement, previous literature suggested that those findings could also occur in patients with no neurological improvement. We also could not find the correlation between motor recovery and the MRI change. Furthermore, the disappearance of the cavity margin and the appearance of fiber-like steaks in the cavity on conventional MRI seem to be nonspecific findings in terms of axonal regeneration. To overcome these false-positive findings of conventional MRI in SCI study, we conducted DTI, which was presented in central nervous system disorders such as stroke, multiple sclerosis, and various demyelinating diseases to discriminate differences between MRI and clinical status.[39,40] DTI is known to be useful for the prediction of neurological recovery as well as accurate visualization and assessment of white matter tracts in SCI patients.[41-45] In our present study based on DTI, 2 patients who had neurological improvement showed the appearance of fiber continuity at the injury site in the cord. Therefore, we speculate that the appearance of fiber continuity on DTI, which was not continuous before stem cell therapy, may be an indicator of axonal regeneration. Nevertheless, because all patients enrolled in this study had fixation surgery with the use of a titanium metal device, we have to remove metal artifact and improve the accuracy of DTI to conclude about this speculation. Although we have adjusted the parameters appropriately, further study is needed to increase the reliability of the image.

We found in our current investigation that a single MSC application therapy is very safe, but only produces a weak therapeutic effect. Therefore, an alternative method is needed to raise the effectiveness of MSC therapy. Multiple MSC injections may be more effective as seen in our previous study. In addition, many studies have reported the combined application of stem cells and trophic factors, which stimulates neuroregeneration.

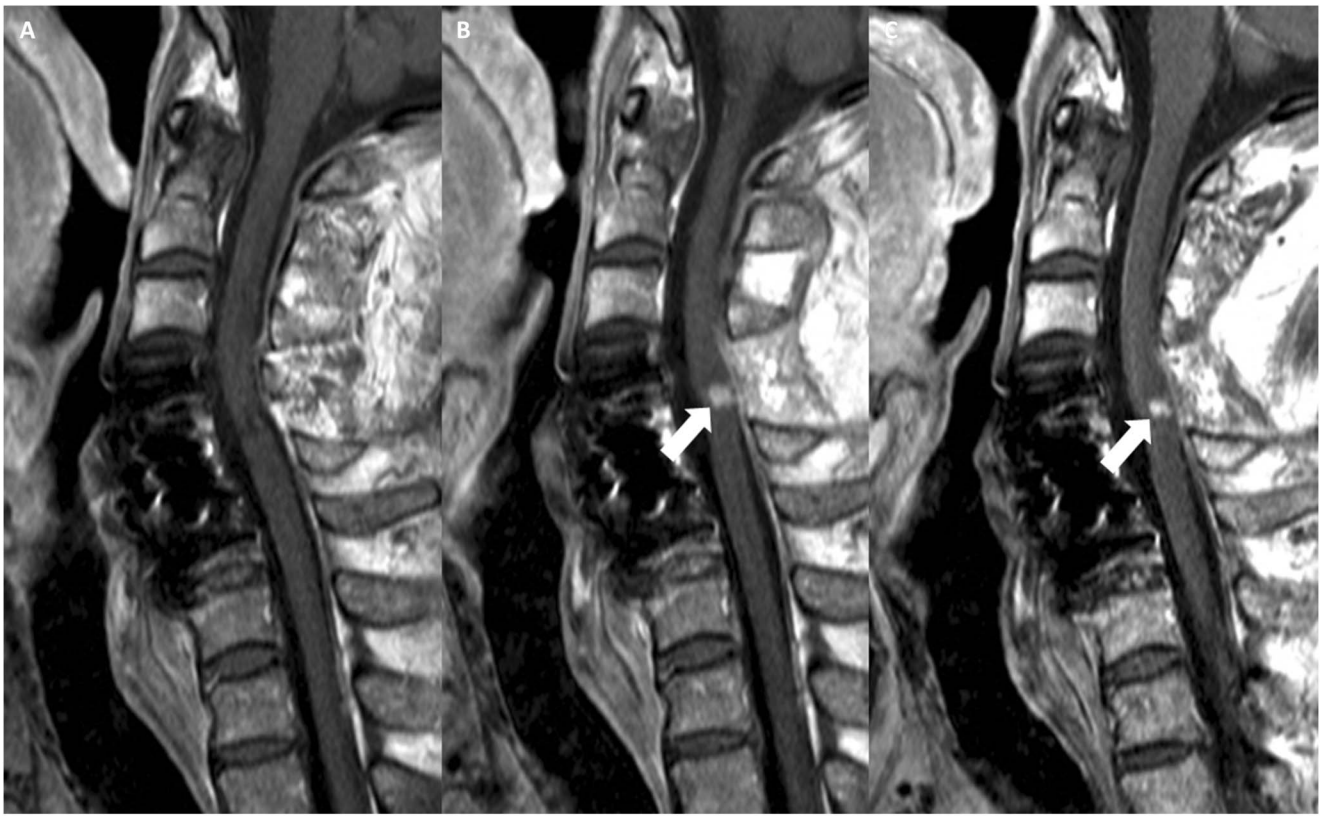

FIGURE 7. *Six months after the operation, the MRI of patient 2 (case 13) showed enhancement of spinal cord at the injected site (arrow) (B), which was not found on preoperative MRI (A). Therefore, additional follow-up MRI was obtained 9 months after the operation to clarify the changes of enhancement, which showed a decrease in enhancement of the lesion (C). MRI, magnetic resonance imaging.*

For example, Yoon et al[19] performed intramedullary injection of autologous BM cells with the administration of granulocyte macrophage-colony stimulating factor. Moreover, in animal studies, using combined factors such as chondroitinase,[46] methylprednisolone,[47] and neurotrophin[48] or gene modification of cells for secreting additive molecules[16,49] has been proposed as next-generation methodology. Therefore, such approaches should be considered for stem cell therapy for SCI on the basis of the safety of MSCs. The appropriate timing of transplantation of stem cells is also an important issue for enhancing the effectiveness of MSC therapy. We speculate that the optimal timing of stem cell transplantation is the subacute phase of the SCI. The acute phase is not appropriate for stem cell application because implanted stem cells in this phase would be exposed to cytotoxic environments owing to excitatory transmitters, reactive oxygen, and inflammatory molecules.[50] In addition, the ischemic situation of the acute stage could also be hostile to implanted stem cells.[51] Regarding the chronic status of SCI, substantial scar tissue acts as a barrier that mechanically interferes with axonal regrowth. There are some studies that report less axonal growth or neurological recovery following cell therapies in the chronic stage compared with acute or subacute stage therapies.[19,52,53] In animal studies, a decrease of neuronal growth ability in chronic SCI is reported to be associated with a decreased production of injury-induced growth-stimulating proteins,[54,55] and the homing effect of the stem cell cannot occur in this stage.[29,56,57] Therefore, the subacute stage as an optimal phase should be considered as another aspect to promote effectiveness of MSC therapy in SCI.

The individual variations in MSC effectiveness can be explained with several reasons. First, there should be a quantitative difference in the number of cells that stayed and survived in the spinal cord even though the cells were applied with the same method. In this study, 1.6×10^7 MSCs were injected into the intramedullary site, but we observed very slight leakage of the cells through the needle tract after injection. Even if we applied fibrin glue to prevent this leakage, a perfect prevention would have been difficult. In addition, there might be an environmental variation among the patients regarding cell survival even if the cells stayed successfully in the spinal cord after injection. Finally, the disparities in the functional activities of MSCs, as we described previously, including activation of endogenous stem cells, formation of

TABLE 3. Summary of Changes in the Motor and Electrophysiological Studies in Previous and Present Study[a]

Previous Study					Present Study				
Case Number	AIS	Motor Change	MRI Changes	EP Change	Case Number	AIS	Motor Change	MRI Changes	EP Change
I	B	Yes[b]	Yes	Yes	1	B	No	No	Yes
II	B	Yes[b]	Yes	Yes	2	B	No	Yes	Yes
III	B	Yes[b]	Yes	Yes	3	B	No	No	No
IV	A	No	Yes	No	4	B	No	No	Yes
V	A	No	Yes	No	5	B	No	No	No
VI	B	Yes	No	No	6	B	No	No	Yes
VII	B	No	Yes	No	7	B	No	No	Yes
VIII	A	No	Yes	Yes	8	B	No	No	Yes
IX	B	Yes	No	Yes	9	B	Yes	Yes	Yes
X	A	Yes	No	Yes	10	B	No	Yes	No
					11	B	No	Yes	No
					12	B	No	No	Yes
					13	B	Yes	Yes	No
					14	B	No	No	No
					15	B	No	No	Yes
					16	B	No	No	No

[a]Roman numerals, previous study case number; Arabic numerals, present study case number; AIS, ASIA impairment scale; EP, electrophysiological studies; MRI, magnetic resonance imaging.
[b]The patients who showed improvement in activities of daily living (ADL).

matrix in the cavity, and reduction of diminishing glial scar, seemed to result from the different effects of MSC application.

Limitations

The first limitation of this study is that this study was designed as a single-arm study rather than a case-control study. In our previous pilot study,[15] we found that ADL improvement was achieved in patients with chronic ASIA B status. Therefore, based on the pilot study, this clinical trial targeted patients with chronic ASIA B status, and the criteria were made and confirmed strictly as we described in the Methods section. However, there was an insufficient number of patients with chronic ASIA B status who satisfied the criteria to enroll both control and treatment groups. In addition, neurological improvement in these patients is very hard to attain, and we considered that the neurological improvement after MSCs treatment in these patients is caused by the treatment. Moreover, we conducted this trial as a single-institution study, in which it is easy to standardize the treatment including surgery, but has a disadvantage in collecting patients. For these reasons, with the approval of the KMFDS, we performed this phase III clinical trial as a single-arm study without a control group. Another limitation is the small number of cases in this study. The enrollment of patients with chronic ASIA B status is not easy, and we need a large number of cases in the future for further comprehensive analysis. The third limitation of this study is the relatively short period of follow-up. In our previous study, the neurological improvement with motor recovery was detected within 6 months in all cases with motor recovery and there has been no significant change since

TABLE 4. Comparison of the Characteristics in Previous and Present Study[a]

Variables	Previous Study (n = 10)	Present Study (n = 16)	*P* Value
Age (average years)	46.0	40.9	.297
Sex (%)	8 men (80.0)	15 men (93.8)	.538
	2 women (20.0)	1 woman (6.2)	
Duration between SCI and cell therapy (average months)	40.2 ± 40.3	76.1 ± 60.5	.111
Effect of treatment, n (%)			
Motor improvement (−)	4 (40)	14 (87.5)	.026[b]
Motor improvement (+)	6 (60)	2 (12.5)	

[a]SCI, spinal cord injury.
[b]Statistically significant.

then.[15] In addition, the patients of this study were enrolled with the following criteria: chronic ASIA B status with a history of 12 months after SCI and no neurological improvement despite vigorous rehabilitation during the past 3 months. In this regard, we decided on the follow-up period as 6 months, but long-term follow-up to increase the preciseness is needed in the future.

CONCLUSION

In this phase III clinical trial, only 2 of the 16 patients with chronic cervical SCI treated with a single MSC application to the intramedullary and intradural space, accompanied by active rehabilitation, showed neurological improvement in the upper extremities, none of whom fulfilled the criteria of effectiveness. We believe that further vigorous studies to determine the appropriate protocol of MSC treatment for SCI are needed on the basis of the safety of MSCs.

Disclosures

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COMMENT

Spinal cord injury (SCI) is a devastating event that results in substantial neurological deficits and that also has important social and economic repercussions for patients, family members, and society.[1] In view of the limited regenerative capacity of the adult human central nervous system (CNS), it was previously thought that neurological recovery from a SCI was an unlikely prospect. However, the recent emergence and rapid development of stem cell biology has given hope that some recovery may be possible.[2] Among the variety of cell types that have been examined for possible transplantation, mesenchymal stem cells (MSCs) have long been investigated because they can be readily harvested from the bone marrow of a SCI patient.[3,4] MSC transplantation for SCI has been shown to have beneficial effects in repairing damaged neural tissue and promoting functional recovery in rodent models.[3,4] However, limited reports have evaluated their safety and efficacy in human SCI,[4-7] making the results from the current clinical trial of particular interest.

The authors previously conducted a pilot study examining the effects of multiple injections of MSCs into a cohort of 10 patients classified as ASIA B after SCI and showed improvements in motor and Activities of Daily Life (ADL) outcomes. The aims of the current study were to demonstrate safety and to evaluate motor improvement as assessed by neurological examination, magnetic resonance imaging (MRI) with diffusion tensor imaging (DTI), and electrophysiological analysis in a small non-randomized clinical trial of 16 patients with SCI. This article reconfirmed the safety of these cells for transplantation and further evaluated the efficacy of intramedullary and intradural injection. In terms of neurological improvement, although only 2 of the 16 patients (12.5%) showed improvement, it was reported that several patients showed apparently positive effects on MRI and electrophysiological analysis. Although the latter outcomes are encouraging, the results also need to be interpreted with caution because the numbers of patients in the study are small and the trial was nonrandomized.

Although these results are promising, further study is needed to make firm conclusions regarding the efficacy of this treatment owing to the small number of patients in the study and the lack of a control group. In addition, the efficacy of the injections might differ depending on whether single or multiple injections of MSCs are administered. Furthermore, the injection methods will need to be optimized with a view to obtaining better neurological recovery. Finally, further work is required to define the mechanism of action of MSCs, which remains poorly defined.

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