artifacts in the cervical spinal cord.[13–17] Therefore, rFOV DTI is a novel modality that can indicate the degree of spinal cord damage in a tract specific manner.

We sought to quantify spinal cord damage at the tract level in patients with CCM using rFOV DTI.

Materials and Methods

Participants

All subjects provided written, informed consent prior to taking part in the research, which was approved by institutional review board. We enrolled 10 healthy volunteers and 20 patients with CCM in this study. Potential participants with a history of neurologic injury, spinal surgery or neurodegenerative diseases causing any spinal cord disorder were excluded. Healthy volunteers included 7 men and 3 women with a mean age of 42.9 ± 9.2 years. In the group of patients with CCM, there were 11 men and 9 women with a mean age of 67.6 ± 11.3 years. The clinical diagnoses included 11 patients with cervical spondylotic myelopathy, 6 with ossification of the posterior longitudinal ligament, 2 with cervical disc herniation, and 1 with atlantoaxial dislocation because of rheumatoid arthritis. Those who had a history of neurologic injury, spinal surgery or neurodegenerative diseases causing spinal cord disorder were excluded.

Clinical Evaluation

We evaluated clinical symptoms using Japanese Orthopaedic Association (JOA) scores. JOA scores are a widely used physician-based functional assessment scale for cervical spine myelopathy.[18–19] (Table 1) We also recorded JOA subscores for motor dysfunction of the lower extremities (LE) and upper extremities (UE). Total score and each domain constituting the JOA

score were assessed by 3 experienced spinal surgeons. If there was a discrepancy of JOA score between the surgeons, the score was determined by majority consensus.

Conventional MRI

The patients were studied using a 3.0 T MR system (Discovery MR 750, GE Healthcare, Waukesha, WI). An 8-ch head neck spine receiver coil was used. Conventional MRI scans consisted of T1-weighted (T1W) and T2-weighted (T2W) sagittal images, and T2-weighted axial images were also obtained. For sagittal imaging, the acquisition settings were as follows: FOV = 24 × 1.0 cm^2, slice thickness = 3 mm echo time (TE)/time of repetition (TR) = 8.4/500 ms (T1W) and TE/TR = 105/2710 ms (T2W). For axial imaging, the acquisition settings were as follows: FOV = 16 × 1.0 mm^2, slice thickness = 4 mm, TE/TR = 105/4000 ms.

Diffusion Tensor Imaging

For DTI acquisitions, diffusion-weighted spin-echo rFOV single-shot echo-planar imaging was used, with diffusion gradients applied in 6 noncollinear spatial directions and 1 baseline image with b = 700 s/mm^2. The thickness of each slice was 5 mm and the sequence parameters for DWI were: TE = 74.9 ms, TR = 3000 ms, FOV = 14 × 0.3 cm^2, NEX = 16 and the acquisition data matrix = 176 × 44. Transverse images covering the cervical spinal cord from C1 to T1 were acquired, each of which was placed at the center of either a vertebrae or intervertebral disc.

Image Analysis

We quantified diffusion tensor parameters using DTIStudio software (Johns Hopkins Medical Institute, Johns Hopkins University). Regions-of-interest (ROI) for the lateral column (LC) and

posterior column (PC) tracts were determined based on the geometry of the cord on the axial image of the FA map at one slice cephalic to the maximum compression level, and FA values were measured (Fig. 1). We analyzed the correlation between FA values and JOA score (total score and subscore for motor dysfunction of upper and lower extremities). We recorded increased signal intensity (ISI) of the spinal cord based on sagittal T2W images. The morphometry of the spinal cord was analyzed using the anteroposterior diameter at the level of highest compression in axial T2W images.

Reliability of ROI Measurement for DTI Analysis

ROI measurements were made by 2 experienced spinal surgeons 2 times at 2-week intervals. Intraclass correlation coefficients (ICC) were used to determine the interobserver and intraobserver reliabilities. We provide 95% prediction limits for the errors in measurements.

Statistical Analysis

A Mann–Whitney *U* test was used to compare FA values between patients with CCM and healthy controls, and was also used to compare JOA scores between patients with and without ISI. Correlation between anteroposterior diameters and JOA scores, and correlation between FA and JOA scores were determined using Spearman's correlation coefficient. The ICC values were classified as poor (less than 0.40), fair (0.40–0.59), good (0.60–0.74), and excellent (0.75–1.00).[20] $P < 0.05$ was considered significant. All the analyses were conducted using JMP version 10.0.2 (SAS Institute, Cary, NC).

Results

Clinical Symptoms

Seventeen of 20 patients with CCM presented gait disturbance and 15 of 20 patients presented hand clumsiness. The mean total JOA scores and subscores for motor dysfunction of upper and lower extremities in this cohort were 10.7 (range 5.5–17), 2.9 (range 1.0–4.0), and 2.2 (range 1.0–4.0) respectively.

FA Values

Tract-specific analyses revealed that the FA values in patients with CCM were significantly lower than those in healthy volunteers in both LC and PC tracts. The mean FA values in LC tracts in patients with CCM and healthy volunteers were 0.59 (range 0.40–0.76) and 0.71 (range 0.66–0.75) respectively ($P = 0.01$). The mean FA values in PC tracts in patients with CCM and healthy volunteers were 0.58 (range 0.40–0.82) and 0.72 (range 0.68–0.76) respectively ($P < 0.01$; Table. 2).

Correlation between JOA Score and FA Values

In patients with CCM, total JOA score moderately correlated with FA values in LC ($\rho = 0.48$, $P = 0.03$) and PC tracts ($\rho = 0.48$, $P = 0.03$). JOA subscores for motor dysfunction of the UE weakly correlated with FA value in PC tracts ($\rho = 0.23$, $P = 0.32$), whereas JOA subscores for motor dysfunction of the UE did not correlate with FA value in LC tracts ($\rho = 0.16$, $P = 0.50$). JOA subscores for motor dysfunction of the LE showed a strong correlation with FA value in LC ($\rho = 0.76$, $P < 0.01$) and PC tracts ($\rho = 0.74$, $P < 0.01$; Fig. 2).

Reliability of ROI Measurement for DTI Analysis

The ICC value for interobserver reliability of ROI measurements was 0.80 in LC and 0.72 in PC

tracts. The ICC value for intraobserver reliability of ROI measurements was 0.92 in LC and 0.90 in PC tracts. The inter- and intraobserver reliability of tract specific ROI measurements were found to be reliable for analyzing the FA maps of patients with CCM.

Conventional MRI Parameters

The mean anteroposterior diameter of the spinal cord in patients with CCM was 4.4 ± 1.5 mm at the most apparent cord compression level. The spinal cord diameter weakly correlated with total JOA score ($\rho = 0.21$, $P = 0.36$) and subscores for LE motor dysfunction ($\rho = 0.35$ $P = 0.13$), whereas there was no significant correlation between the spinal cord diameter and subscores for UE motor dysfunction ($\rho = -0.05$, $P = 0.82$; Fig. 3).

Fifteen of 20 patients (75%) showed ISI. The mean total JOA scores in patients with and without ISI were 9.8 (range 5.5–17) and 13.4 (range 12–16) respectively ($P = 0.013$). The mean subscores for motor dysfunction of UE in patients with and without ISI were 2.8 (range 1.0–4.0) and 3.0 (range 2.0–4.0) respectively ($P = 0.81$). The mean subscores for motor dysfunction of the LE in patients with and without ISI were 2.0 (range 1.0–4.0) and 2.9 (range 2.0–4.0) respectively ($P = 0.051$; Fig. 4). There was a significant difference between total JOA scores in patients with ISI and without ISI. Although there was a trend toward lower subscores for LE motor dysfunction in patients with ISI than in those without ISI, this difference was not significant.

DISCUSSION

The present study showed that it was feasible to evaluate specific tracts of the cervical spinal cord of patients with CCM using rFOV DTI. FA values in LC and PC tracts measured using rFOV DTI correlated strongly with severity of gait disturbance. In other words, rFOV DTI can

indicate the degree of damage in the long tracts of the spinal cord. To our knowledge, this is the first report showing correlations between FA of specific spinal cord tracts and severity of myelopathy in patients with CCM.

rFOV DTI of the spinal cord enables the acquisition of high resolution images with which to evaluate specific spinal cord tracts and distinguish white matter from gray matter.[16, 21] DTI analyses of the spinal cord have been applied not only to CCM, but also to demyelinating disease. Naismith et al. evaluated patients with multiple sclerosis and neuromyelitis using DTI. They found correlations with spinal cord tract DTI parameters and specific clinical functions carried by those tracts.[22] Spinal cord morphology is preserved in patients with demyelinating disease, therefore it is easier to evaluate spinal cord at the tract level in patients with demyelinating disease than it is in patients with CCM and compression-induced spinal cord deformity. There have been several reports describing DTI analyses in patients with CCM. Recently, DTI was used to evaluate somatosensory tracts in patients with cervical spondylotic myelopathy (CSM).[10] Patients with abnormal somatosensory-evoked potentials had decreased FA in the dorsal column. However, no investigation of the correlation between DTI parameters and motor/sensory function of the patients was reported. In another recent study, Cui et al. used diffusion tensor tractography to evaluate specific columns in the spinal cord of patients with CSM. They reported FA is significantly lower in the LC and PC, while mean diffusivity, axial diffusivity, and radial diffusivity in the LC and PC are higher in patients with CSM compared with healthy subjects.[11] However, they did not find any correlation between DTI parameters and clinical symptoms. By contrast with earlier reports of DTI of patients with CCM, we report the evaluation of DTI parameters relevant to clinical symptoms in the present study.

Compared with conventional MRI findings such as ISI of the spinal cord and diameter of the

spinal canal, DTI measures FA, which indicates the severity of myelopathy. We found that conventional MRI parameters, including ISI of spinal cord on T2-weighted images and diameter of the spinal canal, had only weak correlations with the severity of myelopathy. ISI on T2W images reflects a variety of pathological changes induced by spinal cord compression including edema, ischemia, necrosis, myelomalacia, and cavitation.[23,24] Association between the presence of ISI and clinical symptoms remains controversial. Some investigators reported that the increased severity of neurological deficits are concomitant with ISI, whereas others noted no correlation between ISI and clinical presentation.[23–27] In the present study, patients with ISI presented lower JOA scores than patients without ISI. However, the presence of ISI is only a qualitative assessment and cannot quantify the severity of myelopathy.

It is well known that a discrepancy exists between the degree of spinal cord compression and the clinical symptoms.[28,29] Although in population studies there is correlation between narrow spinal canal diameter and cervical myelopathy, there is a considerable degree of overlap between the frequency histograms for minimum anteroposterior diameter of the asymptomatic population and those with CSM.[30] The present study also showed only weak correlations between anteroposterior diameter and JOA score. Therefore, spinal cord morphology evaluated by conventional MRI does not necessarily reflect pathology of spinal cord.

Consistent with previous DTI studies of patients with CSM, in the present study we found a decrease in FA at the compression site.[4,6–12] DTI has shown theoretical promise for assessing the integrity of white matter tracts, and decrease in FA is considered to reflect demyelination and axon damage in the spinal cord.[31–33] The lateral corticospinal tract, which is located in the LC, is the principal descending motor fiber tract conducting voluntary movement in the human spinal cord. PC mainly conducts deep sensation including position and proprioception, both of which

are important to coordinate voluntary movement.[34] Therefore, damage to LC and PC tracts, which can be detected by DTI as a reduction of FA, leads to gait disturbance. Reduction of FA at compressed levels showed weak correlation with total JOA scores compared with JOA subscores for LE motor dysfunction. In the JOA score of 17 points, 6 points reflect sensory disturbance assessed by a pinprick test and 3 points reflect bladder function. Because LC and PC tracts do not contribute to sensory disturbance of bladder function, it is reasonable that FA of these tracts is less strongly correlated with total JOA scores. Hand myelopathy reflects not only long tract symptoms, but also segmental and/or radicular symptoms at the lower cervical spine in patients with CCM. Considering a decrease in FA reflects dysfunction, particularly in the white matter, it is reasonable that a reduction of FA in the LC and PC tracts did not significantly correlate with finger disability.

By contrast with conventional MRI findings, DTI successfully revealed a neurological deficit in patients with CCM in the present study. The present results suggest that DTI may become an indispensable diagnostic modality for patients with CCM. A study is currently underway to determine the association between FA and functional outcomes after decompression surgery. This might suggest DTI as a predictor of surgical outcomes, to assist decision making for surgical intervention in patients with CCM.

A major limitation of the present study is the significant difference in age between the healthy volunteers and patients with CCM ($P < 0.01$). FA in the cervical spinal cord decreases with age.[35, 36] This may contribute to the significantly higher FA of the spinal cord of healthy volunteers. Therefore, age should be matched in future studies. Another limitation of the present study is the difficulty of defining the ROI in patients with severely compressed spinal cords. Deformity of the spinal cord leads to deviation of specific tracts, possibly reducing the

reproducibility of ROI determination. To reduce this problem, we placed the ROI at one slice cephalic to the level of maximum compression. In patients with CCM having prolonged latency of somatosensory-evoked potentials, FA decreases not only in the compression lesion, but also at the level cephalic to the lesion.[10] We showed high reliability and reproducibility using these strategies.

Conclusions

Using rFOV DTI, it is feasible to evaluate the cervical spinal cord at the tract level. Reduction of FA in LC and PC tracts strongly correlated with the JOA subscore for motor dysfunction of the LE. These findings suggest that FA indicates white matter damage in patients with CCM and is a candidate imaging biomarker for spinal cord impairment.

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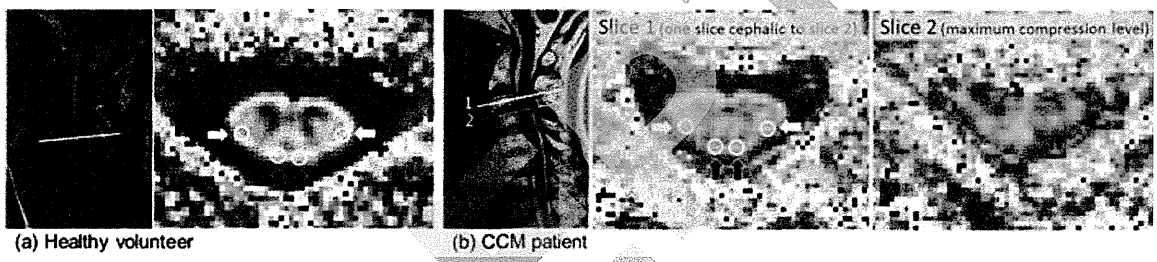

Figure 1. FA maps of (a) a healthy volunteer and (b) a patient with CCM. In the patients with CCM, ROIs were defined on axial images of the FA map at one slice cephalic to the level of maximum compression. ROIs were placed in the lateral columns (the white arrows) and posterior columns (the black arrows).

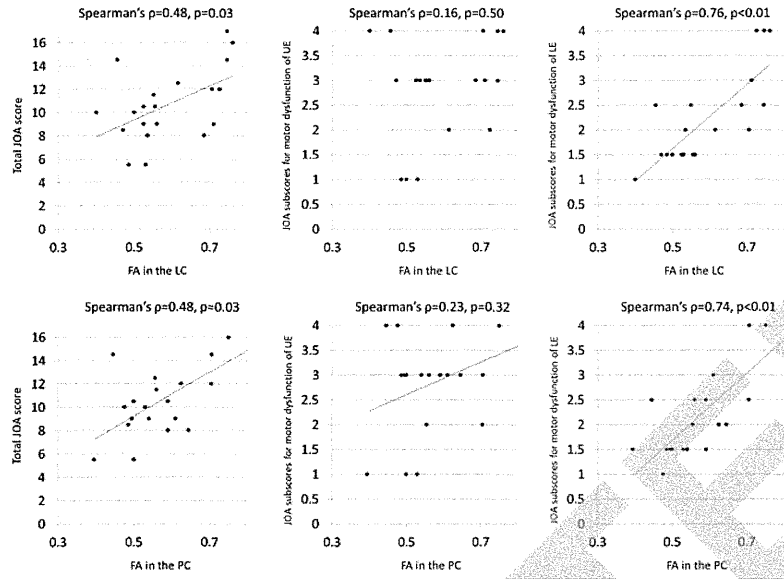

Figure 2. Sum of JOA score (left column), JOA subscore of motor dysfunction of UE (middle column) and JOA subscore of motor dysfunction of LE (right column). FA in the LC is shown in the upper row and FA in the PC is shown in the lower row.

FA: fractional anisotropy, LC: lateral column, PC: posterior column, UE: upper extremity, LE: lower extremity

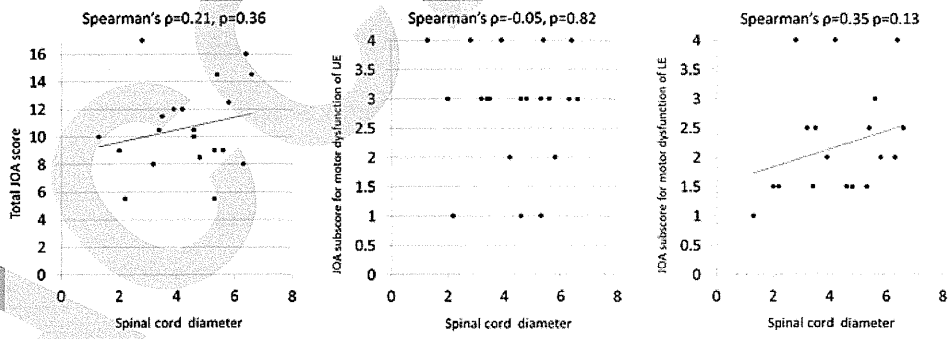

Figure 3. Spearman's correlation between JOA score and spinal cord diameter. The spinal cord diameter correlated only weakly with total JOA score and subscores for motor dysfunction of LE and did not correlate with subscores for motor dysfunction of UE.

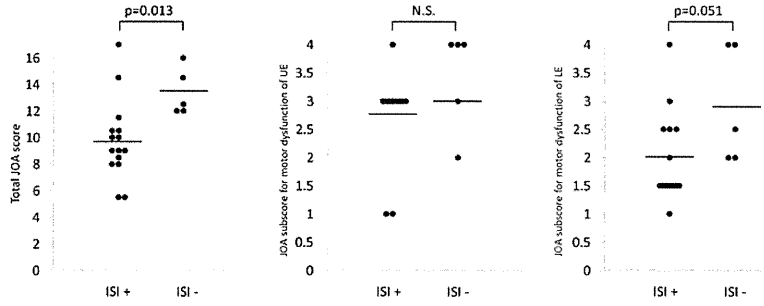

Figure 4. Comparison of JOA scores between patients with and without ISI. There was significant difference in total JOA scores and subscores for motor dysfunction of LE between patients with ISI and without ISI.

ISI: increased signal intensity, N.S.: not significant, UE: upper extremities, LE: lower extremities

Table 1. Japanese Orthopaedic Association Scoring System for Cervical Myelopathy

Function	**Grade**
Motor function	
Fingers	
Unable to feed oneself with any tableware including chopsticks, spoon, or fork, and/or unable to fasten buttons of any size	0
Can manage to feed oneself with a spoon, and/or fork but not with chopsticks	1
Either chopstick-feeding or writing is possible but not practical, and/or large buttons can be fastened	2
Either chopstick-feeding or writing is clumsy but practical, and/or cuff buttons can be fastened	3
Normal	4
Shoulder and elbow (evaluated by MMT score of the deltoid or biceps muscles, whichever is weaker)	
MMT 2 or less	-2
MMT 3	-1
MMT 4	-0.5
MMT 5	0
Lower extremity	
Unable to stand up and walk by any means	0
Able to stand up but unable to walk	0.5
Needs cane or aid on flat ground	1
Able to walk without support but with a clumsy gait	1.5
Walks independently on flat ground but needs support on stairs	2

Able to walk independently when going upstairs, but needs support when going downstairs	2.5
Capable of fast but clumsy walking	3
Normal	4
Sensory function	
Upper extremity	
Complete loss or touch and pain sensation	0
50% or less normal sensation and/or severe pain or numbness	0.5
More than 60% normal sensation and/or moderate pain or numbness	1
Subjective numbness of slight degree without any objective sensory deficit	1.5
Normal	2
Lower extremity	
Complete loss or touch and pain sensation	0
50% or less normal sensation and/or severe pain or numbness	0.5
More than 60% normal sensation and/or moderate pain or numbness	1
Subjective numbness of slight degree without any objective sensory deficit	1.5
Normal	2
Trunk	
Complete loss or touch and pain sensation	0
50% or less normal sensation and/or severe pain or numbness	0.5
More than 60% normal sensation and/or moderate pain or numbness	1
Subjective numbness of slight degree without any objective sensory deficit	1.5
Normal	2
Bladder function	
Complete retention	0
Severe disturbance (sense of retention, dribbling, incomplete continence)	1
Mild disturbance (urinary frequency, urinary hesitency)	2
Normal	3

MMT: Manual muscle test.

Table 2. Mean FA values in CCM patients and healthy volunteer

FA value	Controls	CCM patients	p
Lateral column	0.71 (range 0.66–0.75)	0.59 (range 0.40–0.76)	0.01
Posterior column	0.72 (range 0.68–0.76)	0.58 (range 0.40–0.82)	<0.01

FA values in CCM patients were significantly lower than that in healthy volunteers in both LC and PC

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Transplanted peripheral blood stem cells mobilized by granulocyte colony-stimulating factor promoted hindlimb functional recovery after spinal cord injury in mice

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Running head: G-CSF-mobilized peripheral blood stem cell for SCI

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Abstract

Granulocyte colony-stimulating factor (G-CSF) mobilizes peripheral blood stem cells (PBSCs) derived from bone marrow. We hypothesized that intraspinal transplantation of PBSCs mobilized by G-CSF could promote functional recovery after spinal cord injury.

Spinal cords of adult non-obese diabetes/severe immunodeficiency mice were injured using an Infinite Horizon impactor (60 kdyn). One week after the injury, 3.0 μL of G-CSF-mobilized human mononuclear cell (MNC; 0.5 x 10^5/μL), G-CSF-mobilized human CD34-postive PBSC (CD34; 0.5 x 10^5/μL) or normal saline was injected to the lesion epicenter. We performed immunohistochemistry. Locomotor recovery was assessed by Basso Mouse Scale.

The number of transplanted human cells decreased according to the time course. The CD31-positive area was significantly larger in the MNC and CD34 groups compared with the vehicle group. The number of serotonin-positive fibers was significantly larger in the MNC and CD34 groups than in the vehicle group. Immunohistochemistry revealed that the number of apoptotic oligodendrocyte was significantly smaller in cell transplanted groups and the areas of demyelination in the MNC- and CD34-transplanted mice was smaller than that in the vehicle group, indicating that cell transplantation suppressed oligodendrocyte apoptosis and demyelination. Both the MNC and CD34 groups showed significantly better hind limb functional recovery compared with the vehicle group. There was no significant difference between the 2 types of transplanted cells.

Intraspinal transplantation of G-CSF-mobilized MNC or CD 34-positive cells promoted angiogenesis, serotonergic fiber regeneration/sparing and preservation of myelin, resulting in improved hind-limb function after spinal cord injury in comparison with vehicle-treated control mice.

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Transplantation of G-CSF-mobilized PBSCs has advantages for treatment of spinal cord injury in the ethical and immunological viewpoints, although further exploration is needed to move forward to clinical application.

Keywords: G-CSF, peripheral blood stem cell, cell transplantation, angiogenesis, spinal cord injury

Introduction

The central nervous system, including the spinal cord, is a delicate tissue that cannot tolerate damaging physiological conditions. Thus, poor recovery following injury is generally attributed to the hostile local milieu created at the site of injury. Spinal cord injury (SCI) is characterized by so-called secondary injuries that are triggered by the initial mechanical insult (29). These include hemorrhage, destruction of the blood-spinal cord barrier and infiltration of inflammatory cells. The damage is mediated by a complex cascade of deleterious events that lead to further degenerative damage, including spinal cord ischemia beyond the site of initial injury (8). Those events suggest that angiogenesis-promoting treatments might be valuable for SCI treatment in both the acute and sub-acute phases.

Granulocyte colony-stimulating factor (G-CSF) is a 19.6 kDa glycoprotein that is best known as a growth factor for hematopoietic progenitor cells. It is clinically used to treat neutropenia and to mobilize peripheral blood-derived hematopoietic stem cells for transplantation (20, 24). Furthermore, G-CSF mobilizes peripheral blood stem cells (PBSCs) derived from bone marrow. The mobilized PBSCs reach the injured spinal cord by migrating through the disrupted blood-spinal cord barrier. However, only modest numbers of mobilized PBSCs reach the site of spinal cord injury (17). Thus, we hypothesized that intraspinal transplantation of PBSCs mobilized by G-CSF could promote better functional recovery after contusive SCI.

In the current study, we tested whether intraspinal transplantation of human G-CSF-mobilized mononuclear cells or CD34-positive PBSCs promote angiogenesis and accelerate hind limb functional recovery in mouse contusive SCI.

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Material and Methods

All experimental procedures were performed in accordance with the Chiba University School of Medicine guidelines pertaining to the treatment of experimental animals. The approval of the Animal Committee of Chiba University Graduate School of Medicine was obtained before we started the experiments (the approval number was 25-72).

Spinal cord injury

A total of 48 adult non-obese diabetes/severe immunodeficiency (NOD/SCID) mice (8 to 9 weeks old, average weight 25 g; Charles River Japan, Yokohama, Japan) were used in this study. Animals were anesthetized with inhaled 1.0 - 1.2% halothane (Wako, Osaka, Japan) in 0.5 L/min oxygen. Laminectomy was performed at the thoracic (T) 9 vertebral level, leaving the dura intact. The T8 and T10 spinous processes of the vertebra were clamped to fix the spine. Then, their spinal cords were injured using an Infinite Horizon impactor (60 kdyn, Precision Systems and Instrumentation, Lexington, KY). After that, muscles and skin were sutured layer to layer, and the mice were placed in warm cages overnight. Food and water were given ad libitum. All animals were given saline with antibiotics (Cefmetazon, Daiichi-Sankyo Pharmaceuticals, Tokyo, Japan) by subcutaneous administration to avoid dehydration and infection once a day for 3 days. Manual bladder expression was performed twice a day until recovery of the bladder reflex.

Cell transplantation

One week after the initial injury, the mice were randomly allocated to 3 groups and the spinal cords were re-exposed. In the mononuclear cell (MNC) group (n = 16), 3.0 μL of G-CSF-mobilized human MNC (0.5 x 10^5/μL) were injected into the lesion site with a fine glass pipette attached to a Hamilton syringe (Hamilton Company, Reno, NV). In the CD34+ group (n = 16), 3.0 μL of G-CSF-mobilized human CD34-postive PBSC (0.5 x 10^5/μL) were injected into the lesion site. The cell dosage was determined according to the previous studies (16, 18).

In the vehicle group (n = 16), normal saline (3.0 μL) was injected in the same fashion as the transplanted groups. We purchased human cells mobilized by G-CSF