We performed preoperative 3D CTA in all 100 patients to analyze any extra- and intraosseous VA anomalies at the CVJ, described previously.[5] Three-dimensional reconstruction images of the VA were obtained using a volume rendering method. Using these images, we analyzed the extraosseous course of the VA at the CVJ and any asymmetry of bilateral VAs. Using a multiplanar reconstruction method, we reconstructed planes that corresponded to the trajectories of the C1–C2 TAS and the C2 PS. With this method, we analyzed the intraosseous position of the VA at the C2 isthmus.

Statistical analyses were performed using Fisher exact probability test; $P < 0.05$ was considered statistically significant.

RESULTS

In the 100 cases analyzed, there were no complications associated with the performance of 3D CTA. No neurovascular injury occurred during surgery.

Anomaly of Extraosseous VA

In 10 (10%) of the 100 cases, there were abnormalities in the extraosseous course of VA at the CVJ (Tables 3 and 4). In 2 of these 10 cases, fenestration of a unilateral VA was detected. In the remaining 8, a persistent first intersegmental artery (without persistence of the primary VA) was detected at the unilateral or bilateral VAs (Figure 1). Occ-C/T fusion surgery was performed for each of these 10 cases (Table 4). Intraoperatively, we determined the course of the abnormal branch of the VA using Doppler ultrasonography, and carefully exposed the operative site (Figure 1). Interestingly, all 10 cases belonged to the AAS-CSA(+) group (Table 3). Of the 27 cases of the AAS-CSA(+) group, 37% had extraosseous VA anomalies (Table 3).

Anomaly of Intraosseous VA

In 31 (31%) of the 100 cases, a high-riding VA was detected (Table 3). Multiplanar reconstruction images of the CTA showed that the VA groove was located too medially, too posteriorly, and/or too cranially at the C2 isthmus (Figures 1 and 2). In 27 cases of the AAS-CSA(+) group, 14 (52%) had a high-riding VA (Table 3).

Side-To-Side Asymmetry of Bilateral VAs

In 26 (26%) of the 100 cases, the lumen diameter of the VA on one side was more than twice that of the other side (Figure 3 and Table 3). In the 27 cases of the AAS-CSA(+) group, 7 (26%) had side-to-side asymmetry of bilateral VAs (Table 3). There was no difference regarding the incidence of the side-to-side asymmetry among the groups.

Schedule for C1–C2 Transarticular Screw Placement

Preoperatively, the placement of a C1–C2 TAS was scheduled for 42 patients (84 sides) (Table 5). However, based on 3D CTA findings, we considered that C1–C2 TAS placement carried a high risk of VA injury in 24 sides. Therefore, during surgery, the screws were actually inserted in only 60 sides (71%) (Table 5). Many cases in the AAS-CSA(+) group showed potential risk of VA injury and, although screw insertion was scheduled for 38 sides, they were actually inserted in only 22 (58%) (Table 5).

TABLE 3. Vertebral Artery Anomaly According to the Presence of Congenital Skeletal Anomaly

	AAS Group (n = 59)		MLCSL Group (n = 41)	
	CSA(−) (n = 32)	CSA(+) (n = 27)	CSA(−) (n = 40)	CSA(+) (n = 1)
Extraosseous VA anomaly	0 (0%)	10 (37%)*	0 (%)	0 (%)
First segmental artery	0	8	0	0
Fenestration	0	2	0	0
High-riding VA	6 (19%)	14 (52%)†,‡	10 (25%)	1 (100%)
Right	2	4	6	0
Left	1	4	1	1
Right and left	3	6	3	0
Side to side asymmetry	4 (13%)	7 (26%)	14 (35%)	1 (100%)
Right > left	1	4	8	0
Right < left	3	3	6	1

The values are expressed as the number of cases, with the percentage to the total cases of each subgroup in parentheses.

**P < 0.01 vs. CSA(−) cases of AAS group and CSA(−) cases of MLCSL group.*

† P < 0.01 vs. CSA(−) cases in AAS group.

‡P < 0.05 vs. CSA(−) cases in MLCSL group.

AAS indicates atlantoaxial subluxation; MLCSL, middle-to-lower cervical spine lesion; CSA, congenital skeletal anomaly; VA, vertebral artery.

First segmental artery, persistence of the first intersegmental artery.

In the CSA(−) group, C1–C2 TAS insertion was scheduled for both sides of the C2 in 23 patients and actually inserted in only 17 (74%) (Table 6). In the CSA(+) group, TAS insertion was scheduled for 19 patients, but actually inserted in only 9 (47%) (Table 6). In 6 cases of the CSA(−) group and 10 cases of the CSA(+) group, we altered the operative procedure. The details are shown in Table 6. High-riding VA was detected in 2 of the 6 cases in the CSA(−) group and 9 of the 10 cases in the CSA(+) group (Table 6).

Schedule for C2 Pedicle Screw Placement

Preoperatively, the placement of a C2 PS was scheduled in 58 patients (116 sides) (Table 5). However, based on 3D CTA findings, we concluded that the C2 PS placement carried a high risk of VA injury in 27 sides. Therefore, screws were actually inserted in only 89 sides (77%) at surgery (Table 5). Especially in the AAS-CSA(+) group, there were many difficult cases in which the risk of VA injury by the screw insertion was high; thus although it was scheduled for 16 sides, they were inserted in only 5 (31%) (Table 5).

TABLE 4. Summary of Data for 10 Patients With Anomalous Course of Vertebral Artery at the Extraosseous Region of Craniovertebral Junction

Case No.	Age (yr) /Sex	Diagnosis	Type of Congenital Skeletal Anomaly	Anomaly of Extraosseous VA	High-Riding VA	Asymmetry of BL VAs	Method of Surgery
1	5/M	AAS, Down syndrome	Os odontoideum, bifid C1 posteror arch	Fenestration (R)	–	–	Occ-C3 fusion, C1 laminectomy
2	52/F	AAS	Os odontoideum	First intersegmental artery (R)	+ (R)	+ (R>L)	Occ-C5 fusion, C1 laminectomy
3	56/F	AAS	Os odontoideum	First intersegmental artery (L)	+ (R)	+ (R>L)	Occ-C6 fusion, C1 laminectomy
4	16/F	AAS, Down syndrome	Os odontoideum	Fenestration (R)	–	–	Occ-C2 fusion
5	35/F	AAS	Os odontoideum, bifid C1 posteror arch	First intersegmental artery (R, L)	–	–	Occ-C2 fusion, C1 laminectomy
6	35/F	AAS	Os odontoideum, C1 occpitalization	First intersegmental artery (R, L)	–	+ (R<L)	Occ-C2 fusion, FM decompression
7	26/M	AAS, Down syndrome	Os odontoideum	First intersegmental artery (R)	–	–	Occ-C3 fusion, C1 laminectomy
8	72/F	AAS	Os odontoideum	First intersegmental artery (R, L)	+ (R, L)	–	Occ-T1 fusion
9	67/M	AAS	Os odontoideum	First intersegmental artery (L)	+ (L)	–	Occ-C3 fusion, C1 laminectomy
10	53/M	AAS	C1 occipitalization, Klippel-Feil	First intersegmental artery (R, L)	+ (R, L)	–	Occ-C5 fusion, FM decompression

VA indicates vertebral artery; BL, bilateral; AAS, atlantoaxial subluxation; L, left; R, right; Occ-C, occipitocervical; FM, foramen magnum.

First intersegmental artery; persistent first intersegmental artery.

In the CSA(–) group, C2 PS insertion was scheduled for both sides of the C2 in 49 patients, and inserted in only 37 (76%) (Table 6). In CSA(+) group, it was scheduled in 9 patients, and inserted in only 2 (22%) (Table 6). In 12 cases in the CSA(–) group and 7 in the CSA(+) group, we altered the operative procedure. The details of the alterations are shown in Table 6. A high-riding VA was detected in 11 of the 12 cases in the CSA(–) group and each of the 7 cases in the CSA(+) group (Table 6).

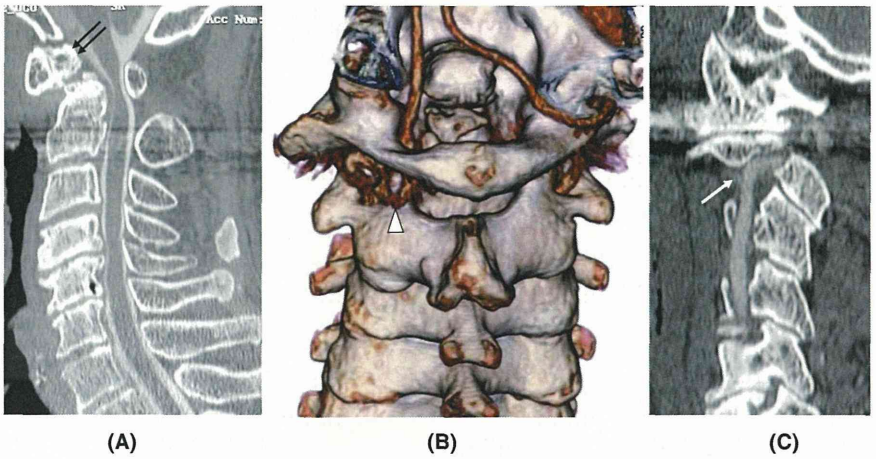
Case Presentations

Case 1 (AAS-CSA[+] Group)

A 67-year-old man presented with myelopathy in association with AAS and os odontoideum (Figure 2A). The 3D reconstruction images of the CTA showed that the left VA was a persistent first intersegmental artery; it turned posteromedially after exiting the C2 transverse foramen and entered the spinal canal between C1 and C2, not passing through the C1 transverse foramen (Figure 2B). We planned a C1 laminectomy and Occ-C fusion using a rod-screw system, with bilateral C2 PS as anchors. However, the multiplanar reconstruction images of the CTA at the trajectories of the screw placement revealed a high-riding left VA (Figure 2C). Therefore, we altered the operative procedure. As anchors, we inserted the PS bilaterally at C3, instead of C2. Intraoperatively, the abnormal course of the left VA was identified by Doppler ultrasonography, and the surgical approach and bone excision were undertaken carefully so as to avoid injury to the left VA (Figure 2D). The surgery was successfully performed (Figure 2E), and neurological disturbances of the patient gradually improved after surgery.

Case 2 (AAS-CSA[+] Group)

A 58-year-old woman presented with myelopathy in association with AAS and os odontoideum (Figure 3A). Instability at C1–C2 was evident, and midsagittal T2-weighted MRI showed an intramedullary high-intensity area at the level of C1 (Figure 3B). Parasagittal reconstruction images of the CTA revealed a high-riding left VA (Figure 3C). We planned a C1–C2 posterior fusion with a rod-screw-atlas claw system, using bilateral C1–C2 TAS as anchors. However, the multiplanar reconstruction images of the CTA at the trajectories of the screw placement showed that the left C1–C2 TAS insertion produced a risk of VA injury. Thus, we altered the operative procedure. Intraoperatively, we used hooks as the anchor to the left side of the C2 (Figure 3D, E). On the right side, we inserted C1–C2 TAS as scheduled (Figure 3F). The C1–C2 posterior fusion was successfully performed. Postoperatively, her neurological disturbances gradually improved.



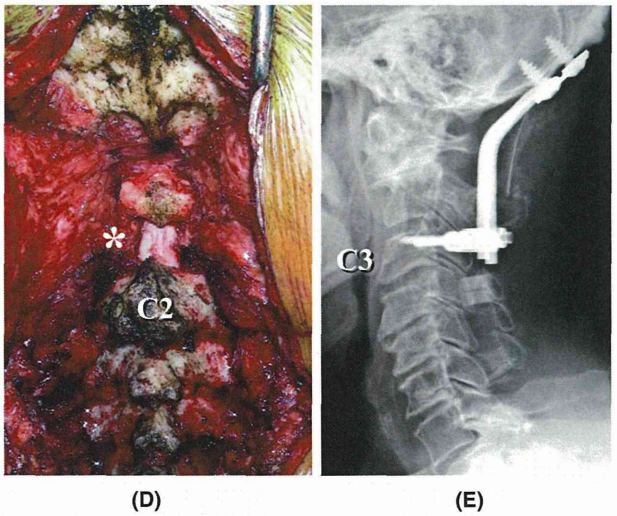


Figure 2. A 67-year-old man presented with myelopathy (AAS-CSA[+] group). Midsagittal reconstruction of a CT myelogram (**A**) showing AAS and os odontoideum (double arrows). Posterior view of 3D CTA (**B**) showed that the left VA was a persistent first intersegmental artery (arrowhead). Left parasagittal reconstruction image of the CTA (**C**) revealing a high-riding left VA (arrow). We chose a C1 laminectomy and occipitocervical posterior fusion using bilateral C3 PSs as anchors for surgery. Intraoperative photograph of the operative field (**D**) showing the abnormal course of the left VA (asterisk). Postoperative cervical lateral radiograph (**E**) showing that occipito–C3 fusion was successfully performed using a screw-rod system. CT indicates computed tomography; AAS, atlantoaxial subluxation; CTA, computed tomographic angiography; VA, vertebral artery; PS, pedicle screw.

Case 3 (MLCSL-CSA[–] Group)

An 81-year-old man presented with traumatic incomplete spinal cord injury. Midsagittal reconstruction CT showed a large ossified anterior longitudinal ligament mass at C3–C7 and segmental OPLL at C3 and C4 (Figure 4A). However, in the ossified mass, there was a gap between C3 and C4, causing mobility at this level (Figure 4A). The 3D reconstruction images of the CTA showed that the lumen diameter of the left VA was less than half that of the right VA, indicating a side-to-side asymmetry (Figure 4B, C). We planned a C2–C7 posterior fusion with a rod-screw system, using bilateral C2 PS as anchors. The surgery was successfully performed as scheduled (Figure 4D).

DISCUSSION

Anomalous Vertebral Artery at the Extraosseous Region

Since 1970, many studies analyzing catheter angiograms have revealed an anomalous VA course at the CVJ.[7–11] Despite these findings, the clinical importance of such VA anomalies had not necessarily been emphasized in the field of spine surgery because most angiographic examinations were performed for patients with cerebrovascular disorders, but not for spinal disorders. In 1983, Rogers reported one fatal case in which a subdural hematoma developed after a lateral C1–C2 puncture and a subsequent postmortem study revealed an anomalous VA within the subarachnoid space at the C2 level.[12] This report alerted spine surgeons to be vigilant for the presence of VA anomalies at the CVJ that might cause serious complications. Recently, instrumentation surgery at the CVJ has developed rapidly. When we insert screws at C1 or C2, or both, wider surgical exposure is required when compared with conventional decompression surgery such as laminectomy of the C1 posterior arch. Thus, the importance of preoperative evaluation of the VA course at the extraosseous region of the CVJ has been emphasized.

Previous studies that analyzed the course of the VA at the CVJ in conventional catheter angiograms for patients free from disease at the CVJ showed that the incidence of persistent first intersegmental artery was 0.60% to 0.67%, and that of fenestration was 0.24% to 1.0%.[13,14] Previous 3D CTA analyses that investigated the course of the VA in patients who did not have osseous anomalies at the cervical spine showed that the incidence of persistent first intersegmental artery and fenestration was 4.7% and 0.6%, respectively.[15] In the present 3D CTA

TABLE 5. C2 Screws Scheduled and Actually Inserted

	AAS Group				MLCSL Group				Total	
	CSA(–)		CSA(+)		CSA(–)		CSA(+)			
	Scheduled	Inserted	Scheduled	Inserted	Scheduled	Inserted	Scheduled	Inserted	Scheduled	Inserted
C1–2TAS	42	34 (81%)	38	22 (58%)*	4	4 (100%)	0	0 (0%)	84	60 (71%)
C2 PS	22	14 (64%)	16	5 (31%)†,‡	76	69 (91%)	2	1 (50%)	116	89 (77%)
Total	64	48 (75%)	54	27 (50%)§	80	73 (91%)	2	1 (50%)	200	149 (75%)

The values are expressed as the number of screws.

The proportion of inserted screws to scheduled screws in each subgroup is expressed in parentheses.

**P < 0.05 vs. CSA(–) cases in AAS group.*

†P < 0.05 vs. CSA(–) cases in AAS group.

‡P < 0.01 vs. CSA(–) cases in MLCSL group.

§P < 0.01 vs. CSA(–) cases in AAS group and CSA(–) cases in MLCSL group.

AAS indicates atlantoaxial subluxation; MLCSL, middle-to-lower cervical spine lesion; CSA, congenital skeletal anomaly; TAS, transarticular screw; PS, pedicle screw.

studies, an anomalous extraosseous VA course was detected in 10 (10%) of the 100 cases. The incidence of a persistent first intersegmental artery was 8% and that of fenestration was 2%. The results indicate a higher incidence of anomalous VA in the extraosseous region than previously reported.[13–15] We propose that the major reason for such a difference is the target group. In the present study, we examined patients who underwent instrumentation surgery at CVJ. In contrast, other studies

TABLE 6. C2 Anchor According to the Presence and Absence of Congenital Skeletal Anomaly

	CSA(–) (n = 72)		CSA(+) (n = 28)	
	Scheduled	Performed	Scheduled	Performed
C1–C2 TAS (BL)	23	17	19	9 (1)
C1–C2 TAS/none		2		2 (2)
C1–C2 TAS/PS		2 (1)		
C1–C2 TAS/hook				1 (1)
C1–C2 TAS/C2–C3 TAS				1 (1)
PS/PS		1		1
C2–C3 TAS/none				1 (1)
SLW/SLW		1 (1)		3 (1)
None/none				1
PS (BL)	49	37 (3)	9	2 (1)
PS/none		3 (2)		1 (1)
PS/SLW				1 (1)
PS/ILS		6 (6)		
SLW/SLW		2 (2)		5 (5)
None/none		1 (1)		

The values are expressed as the number of cases.

The number of cases with high-riding VA is expressed in parentheses.

CSA indicates congenital skeletal anomaly; TAS, transarticular screw; BL, bilateral; PS, pedicle screw; SLW, sublaminar wiring; ILS, intralaminar screw.

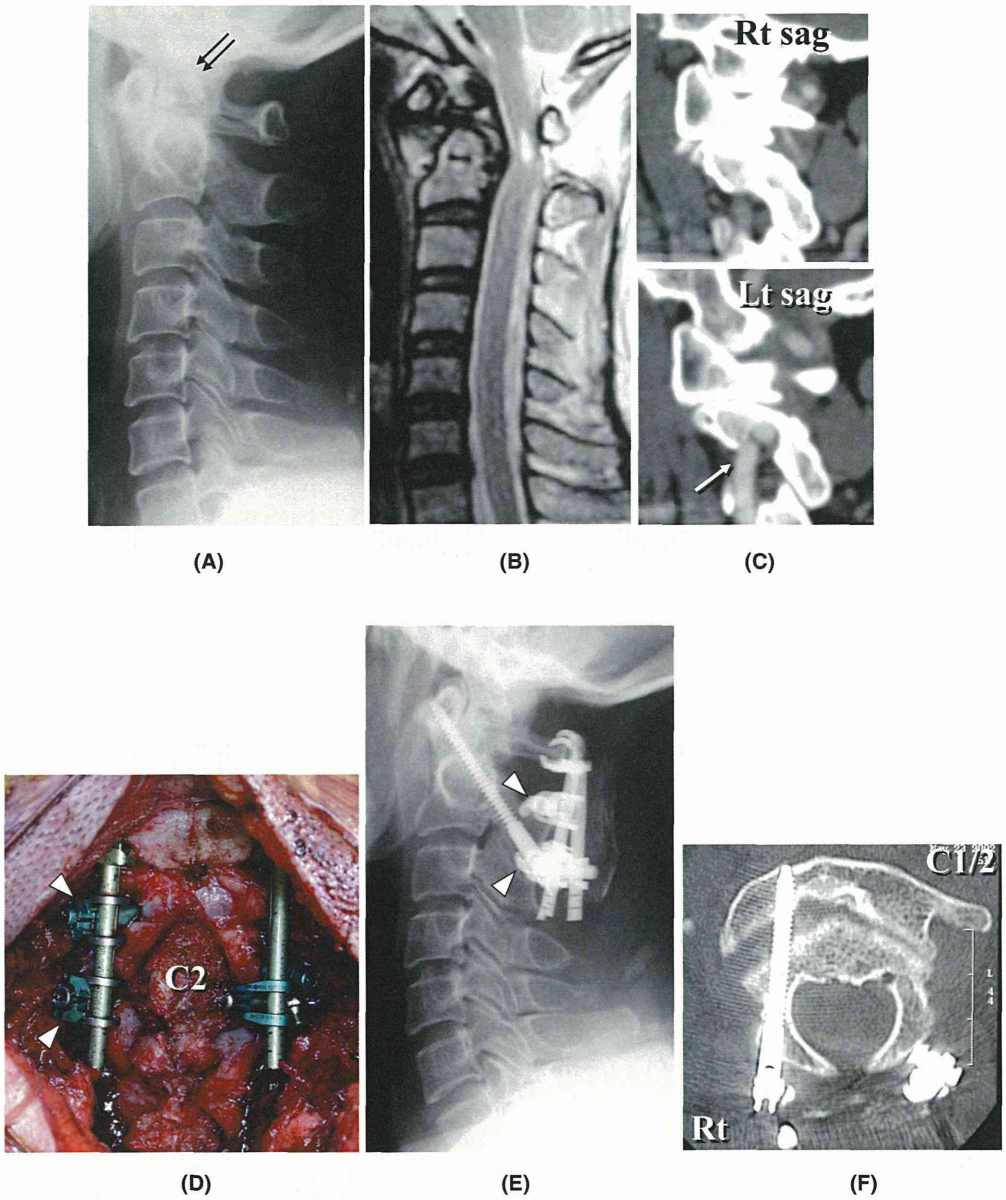


Figure 3. A 58-year-old woman presented with myelopathy (AAS-CSA[+] group). Cervical lateral radiograph (**A**) showing os odontoideum (double arrows). Midsagittal T2-weighted MR image (**B**) showing an intramedullary high intensity area at the C1 level. Parasagittal reconstruction images of the CTA passing bilateral VA grooves (**C**) revealing a high-riding left VA (arrow). We chose C1–C2 posterior fusion surgery using a rod-screw-atlas claw system with right C1–C2 TAS and left C2 hooks. Intraoperative photograph of the operative field (**D**), postoperative cervical lateral radiograph (**E**), postoperative CT reconstruction image through the trajectory of the right C1–C2 TAS (**F**) showing that the C1–C2 fusion was successfully performed using hooks at the left side of C2 (arrowheads). CT indicates computed tomography; AAS, atlantoaxial subluxation; CTA, computed tomographic angiography; CSA, congenital skeletal anomaly; VA, vertebral artery; PS, pedicle screw; TAS, transarticular screw.

examined patients who had no symptoms from cervical spine lesions, and were mainly imaged to screen for brain lesions.[13–15]

There have been several case reports of patients who had an anomalous VA at the CVJ, simultaneously with congenital osseous anomalies at the CVJ such as Klippel-Feil syndrome and C1 occipitalization.[7,9]

Tokuda *et al*[13] analyzed VA catheter angiograms in 21 patients who had osseous anomalies at the cervical spine and revealed a 19.0% incidence of a persistent first intersegmental artery. Furthermore, they reported that the occurrence of this VA anomaly was associated with occipitalization of the atlas and Klippel-Feil syndrome, suggesting that failure of resegmentation of the embryonic sclerotome preferentially contributed to the development of an anomalous course for the VA at the CVJ.[13] Sagittal rearrangement of the intersegmental artery occurs at the cervical region during an embryonic stage and conventional VA formation progresses.[13] Resegmentation of the embryonic sclerotome also occurs at almost similar embryonic stages when such vascular rearrangement progresses.[16] Thus, we observed that the development of an anomalous VA and that of CSA such as os odontoideum or occipitalization of C1 overlap. In the present study, an anomalous extraosseous

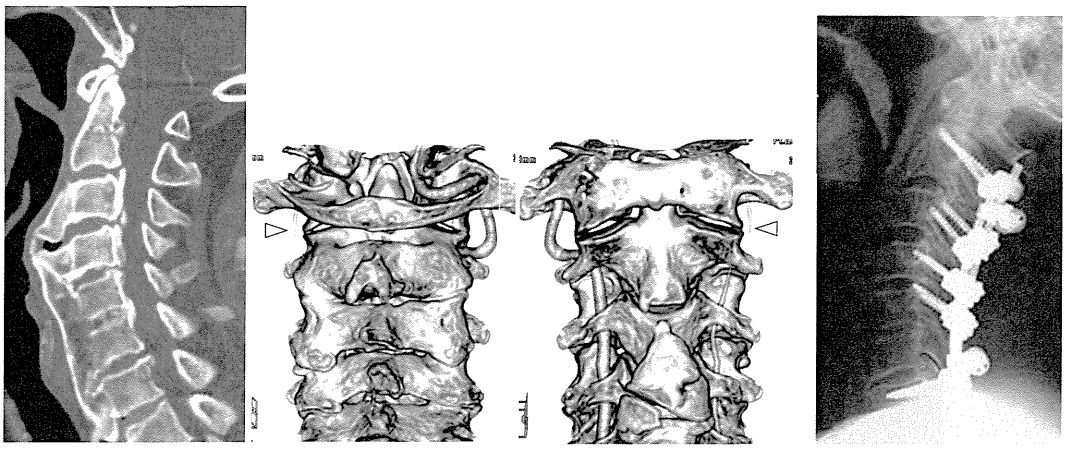


Figure 4. An 81-year-old man with traumatic incomplete spinal cord injury (MLCSL-CSA[–] group). Midsagittal CT reconstruction (**A**) showing a large ossified anterior longitudinal ligament mass at C3–C7, with an osseous gap between C3 and C4. Posterior (**B**) and anterior view (**C**) of 3D CTA showing that the lumen diameter of the left VA was less than half of that of the right VA, indicating a side-to-side asymmetry (arrowheads). We chose C2–C7 posterior fusion surgery using a rod-and-screw system, with bilateral C2 PSs as anchors. Postoperative cervical lateral radiograph showing that the surgery was performed as scheduled (**D**). CT indicates computed tomography; CTA, computed tomographic angiography; VA, vertebral artery; PS, pedicle screw.

VA course was detected in 10 (37%) of the 27 cases in the AAS-CSA(+) group; a proportion higher than that reported previously. This finding supports our hypothesis for the simultaneous development of an anomalous VA and CSA.

Neo *et al*[17] retrospectively analyzed a case in which massive arterial bleeding occurred at the insertion of a C1 lateral mass screw. They concluded that a persistent first intersegmental artery was overlooked before surgery and therefore intraoperative bleeding occurred because of unintentional injury to the anomalous VA. Therefore, the extraosseous course of VAs should be carefully evaluated for anomalies before performing instrumentation surgery at the CVJ. We are convinced that 3D CTA is a useful tool for this evaluation, because it can depict the reciprocal anatomy of complicated VA courses and the surrounding osseous tissue.

In the present study 26 (26%) of the 100 cases had side-to-side asymmetry of bilateral VAs. Even within the 27 cases of the AAS-CSA(+) group, the incidence of the asymmetry was still at 26%. Thus, there seems little correlation between CSA at CVJ and the development of side-to-side asymmetry of VAs.

Anomalous Vertebral Artery at the Intraosseous Region

Anomalous VAs at the intraosseous region of C2 have been reported previously,[18] but such reports have not necessarily attracted the serious attention of many spine surgeons. In 1986, Magerl *et al*[1] reported a new technique of C1–C2 TAS fixation. This procedure greatly altered the strategy for CVJ reconstructive surgery. By using C1–C2 TAS, we were able to perform rigid internal fixation principally without using postoperative halo-vest fixation, and many spine surgeons have introduced this method into their procedures. However, VA injury at the intraosseous region of C2 was reported as a complication of C1–C2 TAS insertion.[2] Thus, the importance of preoperative evaluation of the VA course at the C2 intraosseous region is emphasized, and it has become an important principle for spine surgeons.

A high-riding VA is one that is unusually shifted medially, posteriorly, or cranially at the isthmus of C2. In such condition, the risk of VA injury during the placement of C1–C2 TAS becomes high, because the screws must be inserted through a narrowed isthmus.[19] In previous studies using dry specimens and cadavers, the incidence of a high-riding VA was found to be 10.0% to 22.5%.[20–22] In a clinical study using CT reconstruction, Neo *et al*[19] analyzed 27 consecutive patients who were scheduled for C1–C2 TAS fixation, and reported that 26% of them had a high-riding VA.

Injury to the VA during the placement of a C1–C2 TAS may have lethal complications, especially when it occurs in the dominant side.[2] There are several reports of VA injury as a complication of the C1–C2 TAS fixation—including arteriovenous plexus fistula, occlusion, narrowing, or dissection of the VA—all of which could lead to transient ischemic attacks, stroke, or death.[2,23,24] A large survey of 2492 screws in 1318 patients showed that the risk of VA injury during C1–C2 TAS placement was 4.1% per patient; the risk of neurological deficit from VA injury was 0.2% per patient; and the mortality rate was 0.1% per patient (1.9% per case of VA injury).[2]

Using CT reconstruction images, Yoshida *et al*[25] compared the risk of the placement of C1–C2 TAS and that of C2 PS in patients with high-riding VA. They reported that the insertion of C2 PS posed a similar risk in patients in whom the placement of C1–C2 TAS had a risk of VA injury. Indeed, Jian *et al*[26] reported a case of occipitocervical posterior fusion using bilateral C2 PSs as anchors, in which a unilateral C2 PS was inserted inside of the VA groove. In this case, thrombosis developed at the injured VA and a brain stem infarction occurred. The patient died 6 hours after surgery.

In the present study, 31 (31%) of the 100 cases had a high-riding VA. Of the 27 cases in the AAS-CSA(+) group, 14 (52%) had a high-riding VA. In such cases, there was a considerable risk of VA injury at the insertion of either C1–C2 TAS or C2 PS. Based on our preoperative 3D CTA findings, we altered our surgical procedure and did not use C1–C2 TAS or C2 Ps for such high risk cases. During surgery for cases in the AAS-CSA(+) group, C1–C2 TAS and C2 PS were actually inserted in only 58% and 31% of the planned insertions, respectively. Consequently, no VA injury occurred in the present series.

In conclusion, the present study demonstrates that at instrumentation surgery at the CVJ, the incidence of extra- and intraosseous anomaly of VA is high when patients had AAS and CSA. In such cases, during exposure of the operative field and the placement of C1–C2 TAS or C2 PS, there is a considerable risk of VA injury. Using preoperative 3D CTA, anomalous VAs predicting a risk of VA injury can be precisely identified before surgery, reducing the risk of intraoperative VA injury.

➢ Key Points

- ❑ The frequency of extra- and intraosseous anomalies of the vertebral artery is increased in patients having atlantoaxial subluxation and congenital osseous anomalies at the craniovertebral junction.
- ❑ The increased risk of vertebral artery injury during instrumentation surgery of the craniovertebral junction in patients having osseous anomalies should be considered.
- ❑ Using preoperative 3-dimensional computed tomographic angiography, we can precisely identify anomalous vertebral arteries, and thereby reduce the risk of their intraoperative injury.

Acknowledgment

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ORIGINAL ARTICLE

Neuroprotective therapy using granulocyte colony-stimulating factor for acute spinal cord injury: a phase I/IIa clinical trial

Hiroshi Takahashi · Masashi Yamazaki · Akihiko Okawa · Tsuyoshi Sakuma · Kei Kato · Mitsuhiro Hashimoto · Koichi Hayashi · Takeo Furuya · Takayuki Fujiyoshi · Junko Kawabe · Tomonori Yamauchi · Chikato Mannoji · Tomohiro Miyashita · Ryo Kadota · Masayuki Hashimoto · Yasuo Ito · Kazuhisa Takahashi · Masao Koda

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Abstract
Objective Granulocyte colony-stimulating factor (G-CSF) is a cytokine that is clinically used to treat neutropenia. G-CSF also has non-hematopoietic functions and could potentially be used to treat neuronal injury. To confirm the safety and feasibility of G-CSF administration for acute spinal cord injury (SCI), we have initiated a phase I/IIa clinical trial of neuroprotective therapy using G-CSF.
Methods The trial included a total of 16 SCI patients within 48 h of onset. In the first step, G-CSF (5 μg/kg/day) was intravenously administered for 5 consecutive days to 5 patients. In the second step, G-CSF (10 μg/kg/day) was similarly administered to 11 patients. We evaluated motor and sensory functions of patients using the American Spinal Cord Injury Association (ASIA) score and ASIA impairment scale (AIS) grade.
Results In all 16 patients, neurological improvement was obtained after G-CSF administration. AIS grade increased by one step in 9 of 16 patients. A significant increase in ASIA motor scores was detected 1 day after injection ($P < 0.01$), and both light touch and pin prick scores improved 2 days after injection ($P < 0.05$) in the 10 μg group. No severe adverse effects were observed after G-CSF injection.
Conclusion These results indicate that intravenous administration of G-CSF (10 μg/kg/day) for 5 days is essentially safe, and suggest that some neurological recovery may occur in most patients. We suggest that G-CSF administration could be therapeutic for patients with acute SCI.

Keywords Spinal cord injury · Neuroprotective therapy · G-CSF · Clinical trial

H. Takahashi · M. Yamazaki (✉) · A. Okawa · T. Sakuma · K. Kato · M. Hashimoto · K. Hayashi · T. Furuya · T. Fujiyoshi · J. Kawabe · T. Yamauchi · C. Mannoji · T. Miyashita · R. Kadota · M. Hashimoto · K. Takahashi · M. Koda
Spine Section, Department of Orthopaedic Surgery,
Chiba University Graduate School of Medicine,
1-8-1 Inohana, Chuo-ku, Chiba 260-8677, Japan
e-mail: masashiy@faculty.chiba-u.jp

Y. Ito
Department of Orthopaedic Surgery,
Kobe Red Cross Hospital, Kobe, Japan

Introduction

When spinal cord injury (SCI) occurs, the primary injury is mechanical stress to the spinal cord. After that, the secondary injury occurs, i.e., an inflammatory reaction dependent upon the release of pro-inflammatory cytokines [25]. It is conceivable that methylprednisolone sodium succinate (MPSS) relieves secondary injury to the spinal cord [5, 6]. Based on the Second National Acute Spinal Cord Injury Study (NASCIS-2), administration of high-dose MPSS has been established as a standard treatment for patients with acute SCI. However, several studies have indicated that, after high-dose MPSS therapy, side effects in the respiratory system and digestive organs frequently occur and are often critical for patients [13, 19]. Due to these reports, development of new therapeutic drugs for SCI has been expected.

Granulocyte colony-stimulating factor (G-CSF) is a 19.6-kDa glycoprotein. It is best known as a growth factor for hematopoietic progenitor cells, and is clinically used to treat neutropenia and to mobilize peripheral blood-derived hematopoietic stem cells for transplantation [23, 28]. Recent experimental studies have indicated that G-CSF also has non-hematopoietic functions and can potentially be

used for the treatment of neuronal injury, including stroke and neurodegenerative diseases [10, 16, 18, 30, 31]. Thus, we hypothesized that administration of G-CSF has neuroprotective effects for acute SCI, and examined this hypothesis using SCI models in rodents. We have previously reported that G-CSF promotes functional recovery after compression-induced SCI and contusive SCI in mice and rats [15, 17, 24]. In animal models, G-CSF enhances recovery after SCI through the following mechanisms. In the acute phase, G-CSF mobilizes bone marrow-derived cells to the injured spinal cord, where it directly suppresses neuronal apoptosis, suppresses the death of oligodendrocytes, protects myelin, and suppresses the expression of inflammatory cytokines such as TNF-α and IL-1β [17, 24]. In the subacute phase, G-CSF exerts neuroprotective effects via angiogenesis after SCI [15].

Based on these findings, we initiated a phase I/IIa clinical trial to assess the safety and feasibility of neuroprotective therapy using G-CSF for patients with acute SCI.

Materials and methods

Study design and population

In January 2008, this clinical trial was submitted to the Institutional Review Board of our institute. The application was accepted in March 2008, and the clinical trial was initiated in April 2008. The study was designed as an open-label increasing dosage study. SCI patients were recruited within 48 h after onset. Patients in the following categories were excluded: (1) those <16 years or >75 years of age, (2) those receiving high-dose MPSS therapy after onset, (3) those with intracranial pathologies (e.g., tumors, infection, or ischemia), (4) those having a history of major bleeding requiring blood transfusion or a history of leukopenia, thrombocytopenia, or hepatic or renal dysfunction, severe heart failure, or splenomegaly, and (5) those with evidence of malignant disease within the last 5 years. Patients who were pregnant or nursing were also excluded. Eligible patients gave informed consent for participation in the trial.

Between April 2008 and March 2010, the trial enrolled 16 SCI patients within 48 h of onset. After informed consent was obtained from all patients, they received G-CSF (Gran®, Kyowa Hakko Kirin, Tokyo). In the first step, G-CSF (5 μg/kg/day) was intravenously administered for 5 consecutive days (the 5 μg group) to 5 patients. In the second step, G-CSF (10 μg/kg/day) was similarly administered (10 μg group) to 11 patients (Table 1). All 16 patients were followed-up until 3 months after G-CSF administration. No patients were given MPSS during the follow-up period.

Table 1 Patient data

	G-CSF 5 μg	G-CSF 10 μg	MPSS
Number of cases	5	11	28
Gender			
Male	4	9	23
Female	1	2	5
Age (years)	52.4 ± 11.5 (40–63)	56.0 ± 10.2 (38–68)	56.3 ± 12.7 (18–75)
Cause of injury			
Fall	4	6	17
Road trauma	1	4	10
Sports	0	1	1
Level of injury			
Cervical	4	11	28
Thoracic	1	0	0
ASIA impairment scale (AIS) grade			
A	0	1	7
B	1	0	3
C	4	3	8
D	0	7	10
Time of G-CSF administration after injury (hours)	6.4 ± 2.3 (4–10)	28.5 ± 16.9 (6–48)	NA

NA not administered

Evaluation of safety and feasibility

Adverse events related to G-CSF therapy were evaluated. Patients were asked about common G-CSF therapy side effects. Body temperature was measured twice daily, in the morning and evening, from onset to 7 days after G-CSF administration. If the patients became feverish (>38.5°C) or felt pain, non-steroidal anti-inflammatory drugs (NSAIDs) such as loxoprofen sodium hydrate or diclofenac sodium were administered. Routine biochemical blood tests were performed daily for 7 days after study entry, and thereafter at 1 and 3 months after G-CSF administration, according to protocols provided by the manufacturer.

We also evaluated motor and sensory functions of patients using the American Spinal Cord Injury Association (ASIA) score (motor scores range from 0 to 100, light touch and pin prick scores range from 0 to 112) [20] and ASIA impairment scale (AIS; scores range from A to E). The ASIA score was determined on a daily basis for 7 days after study entry and thereafter at 1 and 3 months after administration. AIS grades were evaluated upon entry and at 3 months after administration.

High-dose MPSS therapy historical control

From August 2003 to July 2005, all patients with cervical SCI were treated with high-dose MPSS within 8 h of their

injuries based on the NASCIS-2 protocol in our institute. From this database, we selected patients who did not have any of the exclusion criteria of the present G-CSF trial, and analyzed them as a historical control. During this period, a total of 38 patients with cervical SCI underwent high-dose MPSS therapy. Among them, 28 patients were selected as the control (the MPSS group) (Table 1).

Statistical analysis

Statistical analysis was performed using a Mann–Whitney *U* test and a Fisher's exact probability test. A *P* value less than 0.05 was considered statistically significant. Results are presented as mean ± standard deviation of the mean.

Results

Patient data

The characteristics of the studied population are shown in Table 1. The mean age at injury was 52.4 years in the 5 μg group and 56.0 years in the 10 μg group. Of the 16 patients, 13 were male and 3 were female. Injuries were caused by falls in 10 patients, road trauma in 5 patients, and sports in 1 patient. The level of injury was cervical in 15 patients and thoracic in 1 patient. In the 5 μg group, the time to initial G-CSF administration after injury was 6.4 ± 2.3 h; 4 patients received G-CSF within 8 h and 1 patient received G-CSF between 8 and 48 h after injury. In the 10 μg group, time to initial G-CSF was 28.5 ± 16.9 h; 2 patients received G-CSF within 8 h and 9 patients received G-CSF between 8 and 48 h (Table 1).

ASIA impairment scale (AIS)

In all 16 patients, neurological improvement was obtained after G-CSF administration. The change of AIS grade between the first examination and 3 months after onset is shown in Table 2. In the analysis of all cases, AIS grade improved by one step in 4 of 5 (80.0%) patients in the 5 μg group, 5 of 11 (45.5%) patients in the 10 μg group, and 9 of 28 (32.1%) patients in the MPSS group. In cases of incomplete paralysis (AIS grade B-D at first examination), AIS grade improved by one step in 4 of 5 (80.0%) patients in the 5 μg group, 5 of 10 (50.0%) patients in the 10 μg group, and 8 of 21 (38.1%) patients in the MPSS group. No statistical differences were observed between groups regarding improvement of AIS grade.

ASIA motor and sensory score

In the analysis of all cases, the ASIA motor score at the first examination was 58.6 ± 10.8 in the 5 μg group, 66.5 ± 25.8 in the 10 μg group, and 50.4 ± 33.3 in the MPSS group (Table 3). Scores were improved at 3-month follow-up in the 5 μg group (points increased 17.2 ± 20.0), the 10 μg group (points increased 19.3 ± 16.6), and the MPSS group (points increased 13.6 ± 11.3) (Table 3).

Table 2 ASIA impairment scale (AIS)

G-CSF 5 μg ($n = 5$)						G-CSF 10 μg ($n = 11$)						MPSS ($n = 28$)					
3 months after onset						3 months after onset						3 months after onset					
1st exam	A	B	C	D	E	1st exam	A	B	C	D	E	1st exam	A	B	C	D	E
A						A	1					A	6	1			
B			1			B						B		2	1		
C			1	3		C				3		C			2	6	
D						D				5	2	D				9	1

AIS grade: A, complete paralysis; *B*, sensory incomplete paralysis, motor complete paralysis; *C*, motor incomplete paralysis (muscle grading < 3/5); *D* motor incomplete paralysis (muscle grading ≥ 3/5); *E*, normal

1st exam AIS grade at first examination

Table 3 ASIA motor score (total cases)

	G-CSF μg ($n = 5$)	G-CSF 1 μg ($n = 11$)	MPSS ($n = 28$)	P^a
At onset	58.6 ± 10.8 (50–77)	66.5 ± 25.8 (27–98)	50.4 ± 33.3 (0–90)	0.195
3 months after injury	75.8 ± 11.9 (65–94)	85.7 ± 18.5 (36–100)	65.8 ± 35.7 (0–100)	0.075
Increased motor score	17.2 ± 20.0 (–12–40)	19.3 ± 16.6 (1–48)	13.6 ± 11.3 (0–48)	0.434

[a] G-CSF 10 μg versus MPSS

Table 4 ASIA motor score (incomplete paralysis cases)

	G-CSF 5 μg ($n = 5$)	G-CSF 10 μg ($n = 10$)	MPSS ($n = 21$)	P^a
At onset	58.6 ± 10.8 (50–77)	70.4 ± 23.4 (32–98)	64.2 ± 25.4 (8–90)	0.597
3 months after injury	75.8 ± 11.9 (65–94)	90.8 ± 8.22 (80–100)	80.3 ± 23.6 (12–100)	0.237
Increased motor score	17.2 ± 20.0 (–12–40)	20.4 ± 17.0 (1–48)	16.1 ± 11.5 (4–48)	0.897

[a] G-CSF 10 μg versus MPSS

In cases of incomplete paralysis (AIS grade of B–D at first examination), the ASIA motor score at the first examination was 58.6 ± 10.8 in the 5 μg group, 70.4 ± 23.4 in the 10 μg group, and 64.2 ± 25.4 in the MPSS group (Table 4). Scores were improved at 3-month follow-up in the 5 μg group (points increased 17.2 ± 20.0), the 10 μg group (points increased 20.4 ± 17.0), and the MPSS group (points increased 16.1 ± 11.5) (Table 4).

The improvements in ASIA score after G-CSF administration are shown in Table 5. The ASIA motor score rose from 58.6 ± 10.8 at onset to 65.6 ± 12.7 1 day after administration in the 5 μg group, and from 66.5 ± 25.8 to 72.2 ± 25.3 in the 10 μg group. A significant increase in ASIA motor score was detected 1 day after G-CSF administration in the 10 μg group ($P < 0.01$). Significant increases in both light touch and pin prick scores were obtained 2 days after administration ($P < 0.05$) in the 10 μg group.

Body temperature and blood data

In both the 5 and the 10 μg groups, no significant increase in body temperature was detected after G-CSF administration.

The changes of blood data are shown in Table 6. White blood cell (WBC) counts before G-CSF administration were 11.3 ± 2.1 ($\times 10^3$ mm^{-3}) in the 5 μg group and 10.4 ± 2.8 ($\times 10^3$ mm^{-3}) in the 10 μg group; these were both higher than normal WBC counts (4.0–9.0 $\times$ 10^3 mm^{-3}). The WBC counts further rose to 28.6 ± 3.2 ($\times 10^3$ mm^{-3}) in the 5 μg group and 26.3 ± 6.3 ($\times 10^3$ mm^{-3}) in the 10 μg group 1 day after the start of G-CSF therapy. During therapy, WBC counts remained elevated compared to those before G-CSF administration ($P < 0.01$). In one patient in the 10 μg group, the WBC increased by more than 50,000 cells/mm^3 during G-CSF administration. One day after the end of G-CSF administration, WBC counts returned to pre-administration levels. No difference in elevation of WBC counts between the 5 and 10 μg groups was observed. In the 10 μg group, a significant elevation of C-reactive protein (CRP) was seen 1 day after administration ($P < 0.05$), but this did not remain elevated. No other blood data changed during or after administration.

Adverse events

No adverse events occurred in the 5 μg group during or after G-CSF administration (Table 7). In the 10 μg group, two patients developed urinary tract infection that was resolved following administration of antibiotics. No relationship was found between the infection and G-CSF administration. In one patient, mild hepatic dysfunction was observed during G-CSF administration, but it resolved spontaneously. No other severe adverse events occurred during or after G-CSF administration. Of the 28 patients in the MPSS group, urinary tract infection developed in 12 (42.9%) patients, pneumonia in 10 (35.7%) patients, gastric ulcer in 4 (14.3%) patients, and hepatopathy in 1 (3.6%) patient. The incidence of pneumonia in the MPSS group was significantly higher than that in the 10 μg group.

Discussion

Non-hematopoietic effects of G-CSF

In experimental studies for acute myocardial infarction (AMI), stem cell mobilization by G-CSF protected the myocardium [14]. In animal models of cerebral infarction, G-CSF suppressed neuronal apoptosis as well as expression of inflammatory cytokines [10, 16, 18, 30, 31]. We made similar observations in animal models for acute SCI [15, 17, 24]. In ALS animal models, stem cell mobilization by G-CSF caused an improvement in ALS-related animal behavior [11, 26]. Based on these results, many clinical trials have been initiated in these diseases, and most of them have reported the safety of G-CSF administration [7, 12, 22, 27, 32–36]. To our knowledge, we are the first group to conduct a clinical trial of G-CSF administration for acute SCI.

In all clinical trials of G-CSF injection for AMI and cerebral infarction, the route of administration was subcutaneous injection. However, a previous report has shown that subcutaneous injection of G-CSF increases WBC counts to higher levels than does intravenous injection [2]. Thus, we elected to use the intravenous route. In many of those clinical trials, the dose and duration of G-CSF

Table 5 Improvement of ASIA score after G-CSF administration

ASIA	Group (μg)	Baseline	Time after initiating G-CSF administration								
			1 day	2 days	3 days	4 days	5 days	6 days	7 days	1 month	3 months
Motor	5	58.6 ± 10.8	65.6 ± 12.7	65.0 ± 12.8	64.6 ± 13.0	65.6 ± 12.6	69.4 ± 12.3	69.4 ± 12.3	71.4 ± 14.0	70.0 ± 16.3	75.8 ± 11.9
	10	66.5 ± 25.8	72.2* ± 25.3	73.5* ± 24.4	75.4* ± 24.2	75.1* ± 25.5	75.4* ± 25.4	75.9* ± 26.0	76.5* ± 25.4	77.5* ± 25.4	85.7* ± 18.5
Light touch	5	68.4 ± 16.3	80.8 ± 24.7	78.0 ± 25.6	80.8 ± 24.7	83.2 ± 23.9	85.2 ± 24.3	85.2 ± 24.4	85.6 ± 24.7	89.8 ± 21.9	92.2 ± 23.6
	10	75.6 ± 30.2	80.9 ± 30.1	83.1** ± 33.4	85.4** ± 33.0	85.8** ± 32.6	85.8** ± 32.5	85.8** ± 32.5	86.6** ± 33.1	84.1* ± 31.8	90.6* ± 26.7
Pin prick	5	61.2 ± 10.1	64.2 ± 10.1	63.0 ± 11.9	67.0 ± 11.6	69.6 ± 12.1	72.8 ± 14.0	71.6 ± 12.5	70.0 ± 11.7	80.2 ± 15.6	81.0 ± 22.4
	10	72.1 ± 32.1	74.6 ± 29.4	74.9** ± 30.1	78.5** ± 30.4	79.5** ± 30.5	79.4** ± 30.5	79.6** ± 30.7	79.6** ± 30.7	79.8** ± 30.9	84.4** ± 26.2

* $P < 0.01$ compared to baseline level

** $P < 0.05$ compared to baseline level

Table 6 Blood data before and after G-CSF administration

Group	Before G-CSF administration	Time after initiating G-CSF administration								
		1 day	2 days	3 days	4 days	5 days	6 days	7 days	1 month	3 months
5 μg										
WBC ($\times 10^3$ mm^{-3})	11.3 ± 2.1	28.6* ± 3.2	27.5* ± 3.9	28.7* ± 4.0	27.7* ± 4.5	24.2* ± 4.9	12.7 ± 3.1	9.1 ± 1.9	6.6 ± 1.5	6.5 ± 0.8
CRP (mg/dL)	0.26 ± 0.4	1.18 ± 0.4	1.98 ± 1.1	2.06 ± 1.7	1.73 ± 1.8	1.05 ± 0.9	1.13 ± 0.9	1.53 ± 1.56	0.75 ± 0.2	0.23 ± 0.25
10 μg										
WBC ($\times 10^3$ mm^{-3})	10.4 ± 2.8	26.3* ± 6.3	28.7* ± 7.4	31.7* ± 7.2	26.9* ± 7.0	26.7* ± 10.9	14.0 ± 4.2	11.0 ± 3.4	7.4 ± 1.8	6.6 ± 1.6
CRP (mg/dL)	1.77 ± 2.0	2.70** ± 2.4	3.08 ± 3.4	2.31 ± 2.0	1.86 ± 1.6	1.26 ± 0.9	1.48 ± 1.76	0.55 ± 0.36	0.89 ± 1.5	0.41 ± 1.5

WBC white blood cells (normal level 4.0–9.0 $\times$ 10^3 mm^{-3}), *CRP* C-reactive protein (normal level <0.5 mg/dL)

* $P < 0.01$ compared to baseline level

** $P < 0.05$ compared to baseline level

Table 7 Side effects

Group	G-CSF 5 μg ($n = 5$)	G-CSF 10 μg ($n = 11$)	MPSS ($n = 28$)	P^a
Urinary tract infection	0 (0%)	2 (18.2%)	12 (42.9%)	0.141
Pneumonia	0 (0%)	0 (0%)	10 (35.7%)	0.021
Gastric ulcer	0 (0%)	0 (0%)	4 (14.3%)	0.249
Hepatopathy	0 (0%)	1 (9.1%)	1 (3.6%)	0.490

[a] G-CSF 10 μg versus MPSS

administration was 5–10 μg/kg/day for 4–6 days. For cerebral infarction patients, Shyu et al. [32] administered G-CSF at 15 μg/kg/day for 5 days. In the present study, to minimize the risks of excessive WBC counts and rupture of the spleen, we utilized lower (5 μg/kg/day) to moderate (10 μg/kg/day) doses of G-CSF.

Side effects of G-CSF

Previous reports have described the side effects of G-CSF administration. Mild symptoms include low back and pelvic pain, fever, listeriosis, headache, nausea, and vomiting [1, 4, 21]. According to these reports, symptoms were transient, and disappeared 2–3 days after cessation of the drug. In the present trial, no significant elevation of body temperature was observed after G-CSF administration. Although two patients developed urinary tract infection, it was resolved following administration of antibiotics. One patient experienced mild hepatic dysfunction that spontaneously resolved.

In contrast, other reports have noted severe symptoms associated with G-CSF therapy, including cerebral infarction, AMI, and rupture of the spleen [3, 8]. When high doses of G-CSF (20 μg/kg/day) were administered, the risks of such events increased. According to reports, if WBC counts remain over 50,000 cells/mm^3, the risk of splenic rupture increases [3]. In the present study, G-CSF at a dose of 10 μg/kg/day increased WBC counts to 50,000 cells/mm^3 in one patient. Thus, it is possible that G-CSF therapy at a dose of 15 μg/kg/day has the potential to cause severe side effects. We suggest that the dose (10 μg/kg/day), duration (5 consecutive days), and route (intravenous administration) of G-CSF administration employed in the present study are generally safe for the treatment for acute SCI. At the beginning of the present clinical trial, we had planned a third step with G-CSF administration of 15 μg/kg/day for 5 days. However, based on the data of the 10 μg group, we canceled the third step.

Neuroprotective therapy with G-CSF for acute SCI

To date, MPSS has been clinically used for the treatment of patients with acute SCI to relieve secondary injury to the spinal cord [4, 5]. In the present study, small (5 μg/kg/day) and moderate (10 μg/kg/day) doses of G-CSF were administered to patients with SCI. Neurologically significant increases in ASIA motor and sensory scores were observed in the 10 μg group. Regarding the improvement of ASIA motor score, patients in the 10 μg group had higher scores than those in the MPSS group, although no statistical differences were detected between groups. This suggests that intravenous administration of 10 μg/kg/day G-CSF for 5 consecutive days has a neuroprotective effect in patients with acute SCI, which is at least as effective as that caused by MPSS treatment based on the NASCIS-2 protocol.

In the present phase I/IIa trial, we administered G-CSF to 11 patients with acute SCI, and confirmed the safety of administering up to 10 μg/kg/day G-CSF. Along with the present study, we have performed another clinical trial of G-CSF neuroprotective therapy for worsening symptoms of compression myelopathy [29]. In that phase I/IIa clinical trial, we administered G-CSF (5 or 10 μg/kg/day) intravenously for 5 consecutive days to 15 patients; the results also indicated that G-CSF administration up to 10 μg/kg/day is safe. Taken together with the present findings, we chose 10 μg/kg/day for 5 days as the final dose and duration for the next phase IIb clinical trial of G-CSF administration for acute SCI.

Regarding the initiation of G-CSF neuroprotective therapy for SCI patients, appropriate timing of the first G-CSF administration has not yet been fully established. In clinical trials of G-CSF administration for AMI, mean time from onset to G-CSF administration varied depending on the study, ranging from 1.4 to 120 h [7, 12, 27, 33, 34, 36]. In the clinical trial for cerebral infarction, Shyu et al. [32] started G-CSF administration within 7 days after onset, and the mean time of initial administration after onset was 48 h. It is known that secondary injury after SCI continues approximately 1 week after injury [9]. When we planned the present phase I/IIa clinical trial, we supposed that if we started the first G-CSF administration within 48 h after injury, the final G-CSF administration (i.e., the fifth administration) would be finished within 7 days after injury, and could be effective for relieving the secondary injury. Thus, we decided that the first G-CSF administration should be performed within 48 h after injury in the present study. As a result, mean time from injury to G-CSF administration was 6.4 h in the 5 μg group and 28.5 h in the 10 μg group. Although the start of G-CSF administration was delayed in the 10 μg group compared to the 5 μg group, considerable neurological recovery was obtained in the 10 μg group. Thus, we suggest that initiation of G-CSF administration within 48 h after injury is not too late to have a neuroprotective effect.

Future investigation

It is known that some neurological improvement is obtained spontaneously in acute SCI. Thus, it is difficult to evaluate the true effects of G-CSF. In the present study, we confirmed the safety of G-CSF treatment. Our next step will be to advance to a phase IIb clinical trial to accurately assess the efficacy of G-CSF therapy. Based of the present results, we will use G-CSF at a dose of 10 μg/kg/day for 5 days. The study design will be a multicenter prospective controlled clinical trial, and a control group without G-CSF administration will be incorporated. By conducting this phase IIb clinical trial, we wish to establish the efficacy of G-CSF neuroprotective therapy for patients with acute SCI.

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Conflict of interest None.

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ORIGINAL ARTICLE

Neuroprotective therapy using granulocyte colony-stimulating factor for patients with worsening symptoms of compression myelopathy, part 1: a phase I and IIa clinical trial

Tsuyoshi Sakuma · Masashi Yamazaki · Akihiko Okawa · Hiroshi Takahashi · Kei Kato · Mitsuhiro Hashimoto · Koichi Hayashi · Takeo Furuya · Takayuki Fujiyoshi · Junko Kawabe · Chikato Mannoji · Ryo Kadota · Masayuki Hashimoto · Kazuhisa Takahashi · Masao Koda

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Abstract
Objective Based on the neuroprotective effects of granulocyte colony-stimulating factor (G-CSF) on experimental spinal cord injury, we initiated a clinical trial that evaluated the safety and efficacy of neuroprotective therapy using G-CSF for patients with worsening symptoms of compression myelopathy.
Methods We obtained informed consent from 15 patients, in whom the Japanese Orthopaedic Association (JOA) score for cervical myelopathy decreased two points or more during a recent 1-month period. G-CSF (5 or 10 μg/kg/day) was intravenously administered for five consecutive days. We evaluated motor and sensory functions of the patients and the presence of adverse events related to G-CSF therapy.
Results G-CSF administration suppressed the progression of myelopathy in all 15 patients. Neurological improvements in motor and sensory functions were obtained in all patients after the administration, although the degree of improvement differed among the patients. Nine patients in the 10-μg group ($n = 10$) underwent surgical treatment at 1 month or later after G-CSF administration. In the 10-μg group, the mean JOA recovery rates 1 and 6 months after administration were 49.9 ± 15.1 and $59.1 \pm 16.3\%$, respectively. On the day following the start of G-CSF therapy, the white blood cell count increased to more than 22,700 cells/mm^3. It varied from 12,000 to 50,000 and returned to preadministration levels 3 days after completing G-CSF treatment. No serious adverse events occurred during or after treatment.
Conclusion The results indicate that G-CSF administration at 10 μg/kg/day is safe for patients with worsening symptoms of compression myelopathy and may be effective for their neurological improvement.

Keywords Neuroprotective therapy · Granulocyte colony-stimulating factor · Compression myelopathy · Clinical trial

T. Sakuma · M. Yamazaki (✉) · A. Okawa · H. Takahashi · K. Kato · M. Hashimoto · K. Hayashi · T. Furuya · T. Fujiyoshi · J. Kawabe · C. Mannoji · R. Kadota · M. Hashimoto · K. Takahashi · M. Koda
Spine Section, Department of Orthopaedic Surgery, Chiba University Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8677, Japan
e-mail: masashiy@faculty.chiba-u.jp

Introduction

Chronic compression of the spinal cord by osteophytes and ossification of the posterior longitudinal ligament (OPLL) causes compression myelopathy [1, 6]. Such myelopathy usually progresses with a slow, stepwise decline in function. In some patients, however, motor paresis and paresthesia rapidly progress with mild or no trauma. According to a previous study, the severity of compression myelopathy rapidly worsened in almost 5% of patients [19]. Rapidly worsening compressive myelopathy results in severe neurological deficits with poor functional recovery because of limited axonal regeneration [1, 3, 6, 24]. To date, early surgical treatment has been the only effective therapy [17, 25].

Granulocyte colony-stimulating factor (G-CSF) is a 19.6 kDa glycoprotein. This cytokine promotes survival, proliferation, and differentiation of cells in the neutrophil lineage [13, 16]. Furthermore, G-CSF can mobilize both immature and mature bone marrow cells into the peripheral blood. As a result, it is used clinically for patients with leukocytopenia and for donors of peripheral blood-derived hematopoietic stem cells for transplantation. Several recent

reports have indicated that G-CSF also has nonhematopoietic functions and can potentially be used for the treatment of neuronal injury, including stroke and neurodegenerative diseases [4, 7, 9, 18, 20]. We previously demonstrated that G-CSF promoted the restoration of damaged spinal cord tissue and the recovery of neural function in experimental spinal cord injury in both mice and rats [8, 14]. In addition, we showed that G-CSF promoted the migration of bone marrow-derived cells into the damaged spinal cord, suppressed apoptosis of neuronal cells and oligodendrocytes, protected myelin, decreased inflammation, and promoted angiogenesis [8, 14]. Based on these results, we have suggested that G-CSF is a candidate for neuroprotective therapy for worsening symptoms of compression myelopathy.

Recently, we began a phase I and IIa clinical trial for the purpose of evaluating the safety and efficacy of neuroprotective therapy using G-CSF for patients with worsening symptoms of compression myelopathy. In the present study, we evaluated the results of this trial.

Methods

This clinical trial was performed with the approval of the Institutional Review Board of our university. We recruited patients 20–75 years of age, in whom the Japanese Orthopaedic Association (JOA) score for cervical myelopathy decreased two points or more during a recent 1-month period. We excluded patients in the following categories: (1) those with intracranial pathologies (e.g., tumors, infection, or ischemia); (2) those having a history of major bleeding requiring blood transfusion or a history of leukopenia, thrombocytopenia, or hepatic or renal dysfunction, severe heart failure, or splenomegaly; (3) those with evidence of malignant disease within the past 5 years. We also excluded patients who were pregnant or nursing. Eligible patients gave informed consent for participation in the trial.

In the first stage of this trial, G-CSF (5 μg/kg/day) was intravenously administered for five consecutive days (the 5-μg group). We conducted an open-label study, and a control group was not used. We evaluated common criteria for adverse event reporting, version 3.0. We also evaluated the patients' severity of myelopathy, using JOA scores (cervical myelopathy scores range from 0 to 17, thoracic myelopathy scores range from 0 to 11) [10]. We then evaluated their motor and sensory functions by calculating scores of muscle power, touch sensation, and pain sensation according to the American Spinal Injury Association (ASIA) score (motor scores range from 0 to 100, light touch and pin prick scores range from 0 to 112) [11]. In the present study, two orthopedic spine surgeons specializing in cervical and thoracic spine surgery evaluated patients' neurological status independently every month until 6 months after G-CSF administration, and calculated the mean data. In addition, we analyzed hematological data from the treated patients. During the first stage (the 5-μg group), we did not restrict the time of surgery of patients and performed surgical treatment according to the patients' directives.

At the second stage, G-CSF (10 μg/kg/day) was similarly administered for five consecutive days (the 10-μg group). We evaluated adverse events, JOA score, scores of muscle power, touch sensation and pain sensation, and hematological data, as done with the 5-μg group. A major difference of the study design between the 5-μg group and the 10-μg group was a restriction of the time of surgery after G-CSF administration. In the 10-μg group, to evaluate neurological improvement resulting from neuroprotective therapy with G-CSF, we planned to follow patients without surgical treatment for 1 month after G-CSF administration. When patients were given informed consent documents, we explained our plans regarding the time of surgery, and we administered G-CSF only to those patients who agreed with the protocol. One month after G-CSF administration, we performed surgical treatment according to the patients' wishes. But when myelopathy progressed and patients wanted to initiate surgery, we abandoned the original schedule and performed surgery according to the patients' requests regardless of the timing relative to G-CSF administration.

Statistical analysis was performed using a Mann–Whitney U test. A p value <0.05 was considered statistically significant. Results are presented as mean values ± standard deviation of the mean.

Results

The 5-μg group

Between June 2008 and May 2009, a total of five patients were enrolled in the first stage of this trial, and all the patients had cervical and/or thoracic myelopathy due to ossification of the spinal ligament, such as OPLL and ossification of the ligamentum flavum (OLF) (Table 1). In all five of the patients, the JOA score decreased two points or more over a recent 1-month period (Table 2). Neurological improvements in both motor and sensory functions were observed in all five patients by the seventh day following the start of G-CSF administration, though the degree of the improvement differed depending on the patient (Table 4). The five patients underwent surgical treatment after G-CSF administration; one patient underwent posterior decompression and four patients posterior decompression with instrumented fusion. The time between the first day of G-CSF administration and surgery ranged from 9 to 115 days.

Table 1 Patients who underwent G-CSF therapy

Case no.	Dose of G-CSF (μg/kg/day)	Age (years)/ gender	Diagnosis	Most stenotic level	Surgical procedure	Time of surgery after G-CSF administration (days)	Follow-up period after G-CSF administration (months)
1	5	61/M	T-OLF	T10–11	PD (T2–3, T9–11)	49	6
2	5	68/M	T-OPLL	T4–5	PDF (T1–T7)	10	6
3	5	51/M	T-OPLL	T1–2	PDF (C7–T5)	10	6
4	5	37/M	T-OPLL	T3–4	PDF (T1–T10)	9	6
5	5	35/M	C- and T-OPLL	C6–7	PDF (C2–T4)	115	6
6	10	46/M	T-OPLL	T7–8	PDF (T4–T11)	59	6
7	10	67/M	C-OPLL	C5–6	NS	NS	6
8	10	75/M	C-OPLL	C3–4	PDF (C2–T1)	49	6
9	10	64/M	C-OPLL	C3–4	PDF (C2–T1)	41	6
10	10	32/M	T-OPLL	T7–8	PDF (T4–T12)	29	6
11	10	67/M	T-OLF	T11–12	PD (T10–12)	33	6
12	10	46/M	CSM	C5–6	PD (C3–7)	94	6
13	10	66/M	CSM	C4–5	PD (C3–7)	73	6
14	10	67/M	CSM	C4–5	PDF (C2–T1)	67	6
15	10	74/M	CSM	C7–T1	PD (C7–T1)	30	6

M male, *T* thoracic, *OLF* ossification of ligamentum flavum, *PD* posterior decompression, *OPLL* ossification of the posterior longitudinal ligament, *PDF* posterior decompression with instrumented fusion, *C* cervical, *NS* no surgery

Table 2 JOA score before and after G-CSF administration (5 μg group)

Case no.	JOA score			Recovery rate
	1 month before administration	Immediately before administration	6 months after administration	6 months after administration
1	6/11	1/11	4/11	30.0
2	5.5/11	3/11	8/11	62.5
3	7/11	3.5/11	11/11	100.0
4	6/11	2/11	6.5/11	50.0
5	4.5/17	2.5/17	6.5/17	27.6
Mean ± SD				54.0 ± 26.4

Recovery rate = (postoperative score − preoperative score/full score − preoperative score) × 100 (%)

JOA score Japanese Orthopaedic Association score (cervical myelopathy: 0–17 points, thoracic myelopathy: 0–11 points)

One day after the start of G-CSF therapy, the white blood cell (WBC) count increased to more than 15,200 cells/mm^3 (Table 5). It remained elevated (from 15,200 to 43,200) during the administration, and returned to preadministration levels within 3 days of the final G-CSF treatment. G-CSF selectively mobilized cells of the neutrophil lineage, while neither monocytes nor lymphocytes were affected (Table 5). There was no change in inflammation during G-CSF administration, as indicated by C-reactive protein levels, except for an instance of surgical site infection (Table 5). One patient (case 4) developed a surgical site infection 14 days after G-CSF administration (5 days after surgery). The infection was relieved by debridement of the infection site and administration of antibiotics. No relation was found between the infection and the G-CSF administration. No other adverse event occurred during or after the administration.

The 10-μg group

Between July 2009 and February 2010, a total of ten patients were enrolled in the second stage of this trial: six patients had cervical and thoracic myelopathy because of ossification of the spinal ligament, such as OPLL and OLF, and four patients had cervical spondylotic myelopathy (CSM) (Table 1). In all ten of the patients, the JOA score had decreased two points or more over a recent 1-month period (Table 3). One month after administration, the mean

Table 3 JOA score before and after G-CSF administration (10 μg group)

Case no.	JOA score				Recovery rate	
	1 month before administration	Immediately before administration	1 month after administration	6 months after administration	1 month after administration	6 months after administration
6	7.5/11	5.5/11	9/11	9/11	63.6	63.6
7	16.5/17	11.5/17	14/17	14/17	45.5	45.5
8	16/17	8.5/17	14.5/17	14.5/17	70.6	70.6
9	14/17	9.5/17	14.5/17	15/17	66.7	73.3
10	6/11	4/11	6/11	6/11	28.6	28.6
11	6/11	4/11	6.5/11	6.5/11	35.7	35.7
12	14/17	11.5/17	14/17	16/17	45.5	81.8
13	12/17	7.5/17	13/17	14/17	57.9	68.4
14	6/17	0/17	4.5/17	11/17	26.5	64.7
15	7.5/11	5/11	8.5/11	8.5/11	58.3	58.3
Mean ± SD					49.9 ± 15.1	59.1 ± 16.3

Recovery rate = (postoperative score − preoperative score/full score − preoperative score) × 100 (%)

JOA score Japan Orthopaedic Association score (cervical myelopathy: 0–17 points, thoracic myelopathy: 0–11 points)

Table 4 Scores of muscle power, touch sensation, and pain sensation before and after G-CSF administration

Group	Before	Time after initiating G-CSF administration		
		7 d	1 m	6 m
Muscle power				
5 μg	81.3 ± 12.1	89.3 ± 9.9		95.5 ± 5.7
10 μg	91.5 ± 6.7		98.2** ± 3.0	99.5** ± 0.9
Touch sensation				
5 μg	78.5 ± 7.4	77.0 ± 8.4		99.5 ± 16.0
10 μg	92.5 ± 14.3		98.3 ± 15.4	106.6* ± 5.9
Pain sensation				
5 μg	78.5 ± 7.4	79.5 ± 12.4		98.0 ± 15.4
10 μg	89.0 ± 14.5		100.5* ± 11.3	106.0* ± 6.1

Scores of muscle power, touch sensation and pain sensation was defined according to the American Spinal Injury Association score (motor: 0–100, light touch and pin prick: 0–112). Before: immediately before G-CSF administration

7 d 7 days after G-CSF administration, *1 m* 1 month after G-CSF administration, *6 m* 6 months after G-CSF administration

* $p < 0.05$ compared with that before G-CSF administration

** $p < 0.01$ compared with that before G-CSF administration

JOA recovery rate was 49.9 ± 15.1% (Table 3), and the muscle power score was significantly improved compared with that before G-CSF administration (Table 4). Nine patients underwent surgical treatment at 1 month or later after G-CSF administration. Six months after administration, the mean JOA recovery rate was 59.1 ± 16.3% (Table 2), and scores of muscle power, touch sensation, and pain sensation were significantly improved compared with those before G-CSF administration (Table 4). One day after the start of G-CSF therapy, the WBC count increased to more than 22,700 (Table 5). It remained elevated (up 12,500 to 50,000) during the administration, and returned to preadministration levels within 3 days of the final G-CSF treatment. G-CSF successfully mobilized cells of the neutrophil lineage, but neither monocytes nor lymphocytes were affected (Table 5). There was no significant change in inflammation during G-CSF administration, as indicated by C-reactive protein levels (Table 5). No adverse event occurred during or after the administration.

Case presentation

Case 7

A 67-year-old man was admitted to our hospital with a complaint of progression of myelopathy. Over the preceding 2 weeks, a loss of muscle power in his upper and lower

Table 5 Blood data before and after G-CSF administration

Group	Baseline	After G-CSF administration								
		1 day	2 days	3 days	4 days	5 days	6 days	7 days	14 days	1 month	6 months
5 μg											
WBC ($\times 10^3$/mm^3)	7.2 ± 1.6	26.7* ± 10.7	25.0* ± 5.5	24.9* ± 6.6	23.3* ± 9.3	20.8* ± 9.6	10.4 ± 3.2	8.2 ± 2.4	8.2 ± 2.4	7.3 ± 2.8	7.2 ± 0.4
Neutrophils ($\times 10^3$/mm^3)	4.5 ± 1.5	22.1* ± 9.2	20.9* ± 5.8	20.6* ± 6.1	19.0* ± 7.7	151.9* ± 7.7	6.8 ± 2.8	5.1 ± 2.0	5.9 ± 2.4	4.7 ± 2.3	4.1 ± 0.1
CRP (mg/dl)	0.7 ± 1.2	0.8 ± 1.3	0.8 ± 1.3	0.8 ± 1.1	0.8 ± 1.0	0.7 ± 1.0	0.7 ± 1.0	0.8 ± 0.9	4.6*,[a] ± 6.9	2.9*,[a] ± 6.1	0.2 ± 0.2
10 μg											
WBC ($\times 10^3$/mm^3)	6.1 ± 1.6	29.3* ± 4.8	31.5* ± 5.6	35.2* ± 7.2	27.8* ± 9.3	25.1* ± 8.0	10.5 ± 2.8	6.7 ± 1.6	4.8 ± 1.9	6.0 ± 1.9	6.8 ± 2.1
Neutrophils ($\times 10^3$/mm^3)	3.5 ± 1.1	25.4* ± 4.2	25.1* ± 8.8	29.8* ± 6.2	22.4* ± 7.7	20.0* ± 6.5	6.6 ± 2.2	3.9 ± 1.2	2.8 ± 1.4	3.4 ± 1.2	4.0 ± 1.6
CRP (mg/dl)	0.3 ± 0.8	0.6 ± 1.3	1.1 ± 2.6	1.6 ± 3.4	1.4 ± 2.4	1.8 ± 2.9	2.0 ± 4.3	1.7 ± 3.3	0.7 ± 1.2	0.4 ± 0.5	0.1 ± 0.1

* $p < 0.05$ compared with the baseline level

[a] Increase due to the surgical site infection of case 4

extremities had rapidly progressed, and gait disturbance developed. Previously, he had undergone surgical treatment for cervical myelopathy because of OPLL: C3–C7 laminoplasty at 64 years of age. After that operation, he could run and slight numbness was present at his finger; his JOA scale score was 16.5 points at 1 month before administration.

On admission, he showed severe loss of sensation below the C6–T1 dermatome level, and muscle strength of his upper extremities decreased to 2–4/5 and lower extremities decreased to 4/5 in manual muscle testing. He could not walk without a cane for assistance. Deep tendon reflexes were hyperactive in bilateral triceps tendons and lower extremities, and Babinski's sign was positive bilaterally. His bladder function was normal, and his JOA score was 11.5 points. Examination with computed tomography (CT) and magnetic resonance (MR) imaging showed anterior compression of the spinal cord by segmental type OPLL at C3–C7 (Fig. 1). Especially at C5–C6, an ossified mass caused severe anterior compression to the spinal cord.

He underwent G-CSF administration (10 μg/kg/day) for 5 days. On the fourth day of G-CSF administration, he felt improved muscle strength in both arms and legs. The G-CSF-induced improvement of motor and sensory functions reached a peak level 2 weeks after G-CSF administration; he could walk without a cane, and no deterioration occurred during the following 6 months. He felt no difficulties in daily life, and he returned to his work 3 months after G-CSF administration.

Discussion

In June 2008, we started a phase I and IIa clinical trial that evaluated the safety and efficacy of neuroprotective therapy using G-CSF for patients with worsening symptoms of compression myelopathy. During the first stage of this trial, G-CSF (5 μg/kg/day) was intravenously administered for five consecutive days. The results indicated that neurological improvements in both motor and sensory functions were obtained in all patients, although the degree of improvement differed depending on the patient. No serious adverse events occurred during or after the administration. Previous studies of G-CSF therapy for acute myocardial infarction, acute cerebral infarction, and amyotrophic lateral sclerosis [2, 5, 12, 15, 21–23, 26, 27] have used a dose of 10 μg/kg/day G-CSF for five consecutive days (Table 6). Therefore, we administered 10 μg G-CSF/kg/day intravenously for five consecutive days for the second stage of this trial. No adverse events occurred, and all patients have shown neurological improvements. This suggests that G-CSF therapy at a dose of 10 μg/kg/day for 5 days is safe for patients with worsening symptoms of compression myelopathy.