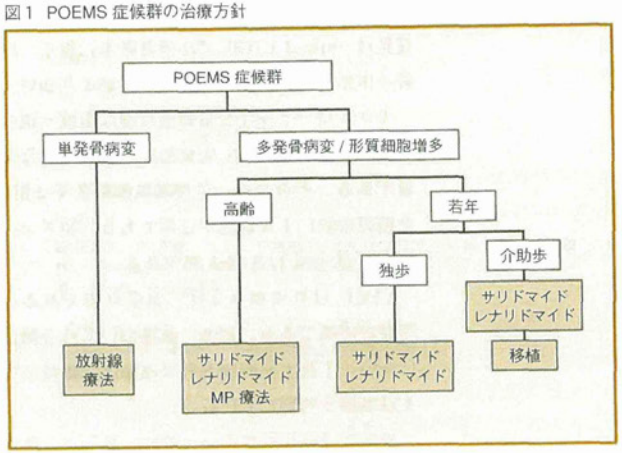
図1 POEMS 症候群の治療方針



胞増多が証明される場合は，化学療法が選択される[8][12]．多くは後者である．しかし，新規治療として国際的に注目されている治療法のほとんどは，保険適応外であるという大きな問題点がある．

2．末梢血幹細胞移植

移植療法の目的は高用量のアルキル化薬投与による形質細胞腫の高度の抑制である．末梢血幹細胞採取は，顆粒球コロニー刺激因子（G-CSF）単独またはシクロホスファミド併用により行う．その後，高用量のメルファランによる前処置を行ったうえで幹細胞移植を行う．通常，VEGF 値は約1～3ヵ月で速やかに低下し，引き続き臨床症状全般の改善が生じる[14]．

適応となるのは原則的には 65 歳以下の若年者である．しかし，関連死のリスクがあること，再発の可能性も少なからずあることから，軽症例への適応は慎重にすべきである．移植療法の最大の利点の1つは，神経障害の比較的速やかな回復である．歩行に介助を要するような中等度以上の神経障害を有する症例では，移植により社会復帰できる可能性があり，おそらく良い適応である．

より安全に移植を行うことを目的に，幹細胞採取前もしくは移植前に下記の化学療法を行うこともオプションと成りうる．その際は，MP 療法［メルファラン，プレドニゾロン］は末梢血幹細胞の採取効率を低下させるため，推奨されない[15]．また，サリドマイド・レナリドミド・ボルテゾミブも長期にわたる使用は幹細胞採取効率に影響する可能性があるため，病勢の安定後は速やかに移植への移行を検討する．

移植後の再発に関する報告も増えつつある．移植後の無増悪生存率は1年で 98％，5年で 75％ とする報告がある[16]．再発後の治療の選択肢としては，再移植・その他の化学療法などが考えられる．移植後再発の予防・再発後の治療選択肢などが，今後の検討すべき課題である．

3．MP 療法，サリドマイド，レナリドミド，ボルテゾミブ

若年軽症例・高齢者における治療選択肢と成りうるのは，MP 療法・サリドマイド・レナリドミド・ボルテゾミブである．用法・用量は骨髄腫と同様である．サリドマイド・レナリドミド・ボルテゾミブはデキサメタゾンと併用されることが多い．また，いずれの治療も VEGF 値の正常化が得られ疾患が安定していると考えられる場合は，減量・休薬を適宜検討すべきである．それぞれの治療の特徴を以下にまとめる．動向としては，主要な治療選択肢は，MP 療法から新規薬であるサリドマイド・レナリドミドへ移行しつつある．しかし，現時点でいずれの薬剤も POEMS 症候群に対する保険適応はない．

MP 療法は骨髄抑制もマイルドで神経毒性もなく，比較的安全に施行できるため POEMS 症候群においても一般的に行われ一定の予後改善が見られた[17]．しかし，メルファランの総投与量が 1,100 mg を超えると骨髄異形成症候群や2次的発がんのリスクが増えるため，一定期間を超えての治療はしにくい．

サリドマイドの本症候群における効果は全身状態不良例を含む9例における治療成績が報告されている．投与開始1年後の寛解率 56％・2年後の生存率 100％ と良好であり，副作用として懸念される末梢神経障害の発現も認めていない[18]．しかし，前述のごとくサリドマイドは本症候群に保険適応がなく，過去の薬害の歴史から管理体制が厳重なため，適応外使用を行うのは困難である．サリドマイドの本症候群への適応拡大を目指した医師主導治験（プラセボ対照二重盲検群間比較試験）が 2012 年現在進行中である（http://www.m.chiba-u.ac.jp/class/neurol/kenkyu/ishisyudou/index.html）．

レナリドミドはサリドマイドの誘導体である．いわゆる第2世代の

薬剤であり，サリドマイドより有効性はおそらく高く，末梢神経障害のリスクは低く骨髄抑制は強い．腎機能・血小板数に応じた用量調整が必要である．レナリドミドの POEMS 症候群における有効性に関する報告も増えつつある[19]．レナリドミドもサリドマイド骨格を持つことから，サリドマイドに準じた厳格な管理体制が必要であり，神経内科医単独の判断に基づく適応外処方は現時点では困難である．

ボルテゾミブはプロテアソーム阻害薬であり，骨髄腫での高い効果が認められている．しかし，最大の問題点は，末梢神経毒性が上記の薬剤のいずれよりも確実に高いことである．POEMS 症候群に対する有効性の症例報告もされ[20]，原疾患の改善による末梢神経障害の改善が神経毒性を上回った可能性などが考察されているが，第1選択としては使用しにくい．骨髄腫領域では神経障害の回避の目的で，投与間隔の延長・皮下注などが試みられつつある．

4．放射線療法

上述のごとく，骨病変が単独かつ骨髄像が正常の場合は，放射線療法による治療も有効である[8]．また，複数の骨病変に大きな骨病変が混じる場合には，効率的に腫瘍量を減少させる観点から，上記の化学療法に加えた局所の放射線照射も選択肢になる．

5．補助療法

POEMS 症候群の一部は経過中に非常に急速な進行・悪化を呈することがある．特に，VEGF による高度の血管透過性亢進に基づくと推定される third space への水分貯留・血管内脱水は，腎機能低下を始めとした多臓器不全につながる．上記の化学療法・放射線療法は効果発現には，数ヵ月単位の期間を要する．メチルプレドニゾロン・デキサメタゾンによるステロイドパルス療法やプレドニゾロン内服は，VEGF の産生を抑制し比較的即効性の得られる治療であり，急性増悪時の治療オプションと成りうる．しかし，ステロイド単独では長期の病勢のコントロールは困難である[8]．

6．再 発

疾患の基盤である形質細胞腫瘍の性格から考えると，いずれの治療を行っても治癒はおそらく困難であり，疾患活動性がいったん低下してもいずれ再発する可能性が高い．そのため，注意深い経過観察が不可欠である．再発の判定には，VEGF 値・Mタンパクが有用である．PET による骨病変のフォローが有用であるとする報告もある[16]．自験例においては，再発例の多くはMタンパクの陽転化・VEGF の上昇に続いて，浮腫・神経症状の悪化などの臨床的な再発を認めた．

今後の展望

骨髄腫治療の進歩により，POEMS 症候群の治療の選択肢は劇的に変わりつつある．予後は確実に改善し，今後はより長期の疾患コントロールの観点から，移植適応・寛解基準・化学療法の減量・休薬基準などの検討が必要である．また，移植関連死・移植後再発の回避を目的とした治療戦略の検討も急務である．

一方，本邦の現状では保険適応の問題などにより，治療が非常にしにくく，治療内容が医療機関により異なりうる．さらに，稀少疾患であるため，認知度も低く，診断が遅れる症例もまれではない．現在進行中の医師主導治験によりサリドマイドが承認されれば，本邦における POEMS 症候群の治療の標準化・認知が進む可能性がある．骨髄腫の治療の発展により，サリドマイドの本症候群における位置付けは早晩変化すると予想される．しかし，科学的な根拠に基づいてプロスペクティブに実施された臨床試験は，疾患の自然歴・活動性マーカーを明らかにして，治療のさらなる発展の土台となる．

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ORIGINAL ARTICLE

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

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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

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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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Neuroprotective Therapy Using Granulocyte Colony–Stimulating Factor for Patients With Worsening Symptoms of Thoracic Myelopathy

A Multicenter Prospective Controlled Trial

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Study Design. An open-labeled multicenter prospective controlled clinical trial.

Objective. To confirm the feasibility of granulocyte colony–stimulating factor (G-CSF) administration for patients with thoracic myelopathy.

Summary of Background Data. Although G-CSF is best known as an important cytokine commonly used to treat neutropenia, it also has nonhematopoietic functions. Previous experimental studies have shown that G-CSF can enhance tissue regeneration of several organs, such as the heart and the brain. We previously reported that G-CSF promotes functional recovery after spinal cord injury in rodents. On the basis of those findings, we started a clinical trial of neuroprotective therapy, using G-CSF for patients with worsening symptoms of thoracic myelopathy.

Methods. Patients whose Japanese Orthopaedic Association (JOA) score for thoracic myelopathy had decreased 2 points or more during a recent 1-month period were eligible for entry. After giving informed consent, patients were assigned to G-CSF and control groups. The G-CSF group (n = 10) received G-CSF 10 μg/kg per day intravenously for 5 consecutive days. The control group (n = 14) received similar treatments as the G-CSF group except for G-CSF administration. The primary outcome was JOA recovery rate at 1 month after G-CSF administration or initial treatment.

Results. There was greater improvement in neurological functioning between baseline and 1-month follow-up in the G-CSF group (JOA recovery rate: 29.1 ± 20.5%) than in the control group (JOA recovery rate: 1.1 ± 4.2%) ($P < 0.01$). No serious adverse events occurred during or after the G-CSF administration.

Conclusion. The results provide evidence that G-CSF administration caused neurological recovery in patients with worsening symptoms of thoracic compression myelopathy.

Key words: neuroprotective therapy, granulocyte colony–stimulating factor, thoracic myelopathy, clinical trial. **Spine 2012;37:1475–1478**

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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 commonly used to treat neutropenia and to mobilize peripheral blood-derived hematopoietic stem cells for transplantation.[1] Several experimental studies have indicated that G-CSF also has nonhematopoietic functions and can enhance the tissue regeneration of several organs such as the heart and the brain.[2,3] We previously reported that G-CSF promotes functional recovery after spinal cord injury in rodents.[4–6]

On the basis of the experimental results described earlier, we hypothesized that administration of G-CSF can effect neurological recovery in patients with progressive compression

myelopathy and started a phase I/IIa clinical trial of G-CSF neuroprotective therapy.[7] In this study, we conducted a multicenter prospective controlled clinical trial (phase IIb) to assess the feasibility of the G-CSF therapy for patients with worsening symptoms of thoracic compression myelopathy.

MATERIALS AND METHODS

This clinical trial was designed as an open-labeled multicenter prospective controlled study and was performed with the approval of the institutional review board of each participating institute. Since April 2010, we recruited patients 20 to 85 years of age, in whom the Japanese Orthopaedic Association (JOA) score (full score = 11 points) decreased 2 points or more during a recent 1-month period.[7]

We assigned patients to a G-CSF group and a control group. Patients in the G-CSF group were given G-CSF 10 μg/kg per day intravenously for 5 consecutive days. Patients in the control group were enrolled in similar treatments as the G-CSF group except for the G-CSF administration. To evaluate neurological improvement resulting from neuroprotective therapy with G-CSF, we planned to follow patients in both groups without surgical treatment for 1 month after G-CSF administration or initial treatment and to provide them with equivalent conservative treatment, such as bed rest. 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.[7] The G-CSF therapy was performed only in the institute to which the corresponding author (MY) belonged. At the other institutes, patients were treated without G-CSF administration.

The primary outcome was the JOA recovery rate at 1 month after G-CSF administration or initial treatment. We evaluated the patients' severity of myelopathy using the JOA score.[7] Then, we evaluated their motor and sensory functions by determining scores for muscle power and pain sensation according to the American Spinal Injury Association score.[7] In this study, 2 orthopedic spine surgeons specializing in thoracic spine surgery evaluated patients' neurological status independently after G-CSF administration and then mean data were calculated. In addition, we analyzed hematological data from the treated patients.

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

RESULTS

Patient Data

Between April 2010 and October 2010, 24 patients (10 patients in the G-CSF group and 14 patients in the control group) were enrolled and examined for 1 month. Patient data for both groups are summarized in Table 1. In the control group, many patients had the most stenotic level at the lower thoracic spine (T9–T12), although no statistical difference was observed in the distribution of the most stenotic level between the G-CSF and control groups. No statistical difference was observed between groups regarding the spinal canal occupation ratio by heterotopic ossification or vertebral spurs at the most stenotic level.

TABLE 1. G-CSF and Control Group Patient Data

	G-CSF	Control
No. of patients	10	14
Sex		
Male	9	11
Female	1	3
Age, *M* ± SD (range), yr	49.7 ± 8.9 (32–74)	53.1 ± 10.6 (22–72)
Diagnosis		
Thoracic OPLL	5	4
Thoracic OLF	2	6
Thoracic spondylotic myelopathy	3	4
Most stenotic level		
Upper thoracic (T1–T4)	4	4
Middle thoracic (T5–T8)	4	2
Lower thoracic (T9–T12)	2	8
Surgical procedure		
Posterior decompression	5	10
Posterior decompression with instrumented fusion	5	4

G-CSF indicates granulocyte colony–stimulating factor; OPLL, ossification of posterior longitudinal ligament; OLF, ossification of ligamentum flavum.

Neurological Recovery

The JOA score immediately before G-CSF administration or initial treatment was 3.8 ± 1.3 in the G-CSF group and 4.1 ± 1.4 in the control group, showing no statistical difference between groups (Table 2). There was greater improvement in neurological functioning between baseline and 1-month follow-up in the G-CSF group (JOA recovery rate: 29.1 ± 20.5%) than in the control group (JOA recovery rate: 1.1 ± 4.2%) ($P < 0.01$) (Table 2).

Regarding the muscle power score, greater improvement between baseline and 1-month follow-up was observed in the G-CSF group (improvement of muscle power score: 2.8 ± 2.8) than in the control group (improvement of muscle power score: 1.6 ± 5.3) ($P < 0.05$) (Table 2).

There was also greater improvement in the pain sensation score between baseline and 1-month follow-up in the G-CSF

TABLE 2. Neurological Recovery

	G-CSF $M \pm SD$ (range)	Control $M \pm SD$ (range)	P
JOA score			
Immediately before treatment	3.8 ± 1.3 (1–5.5)	4.1 ± 1.4 (1.5–6.0)	0.501
One month after treatment	5.7 ± 2.4 (1.0–9.0)	4.3 ± 1.3 (2.5–6.0)	0.061
Recovery rate	29.1 ± 20.5 (0.0–63.6)	1.1 ± 4.2 (0.0–15.8)	<0.01
Muscle power score			
Immediately before treatment	41.9 ± 7.8 (22–50)	37.0 ± 15.5 (0–50)	0.884
One month after treatment	44.7 ± 7.6 (25–50)	38.6 ± 12.6 (20–50)	0.241
Increase of muscle power score	2.8 ± 2.8 (0–9)	1.6 ± 5.3 (0–20)	<0.05
Pain sensation score			
Immediately before treatment	68.3 ± 9. 7 (59–78)	74.1 ± 9.8 (60–92)	0.364
One month after treatment	74. 7 ± 10.4 (62–88)	74.9 ± 8.9 (64–92)	0.578
Increase of pain sensation score	6.4 ± 5.5 (1–17)	1.0 ± 3.2 (0–12)	<0.01

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

Muscle power score (motor: 0–50 points) and pain sensation (pin prick: 0–98 points) score were defined according to the American Spinal Injury Association score.

G-CSF indicates granulocyte colony–stimulating factor; JOA score, Japan Orthopaedic Association score (thoracic myelopathy: 0–11 points).

group (improvement of the pain sensation score: 6.4 ± 5.5) than in the control group (improvement of the pain sensation score: 1.0 ± 3.2) ($P < 0.01$) (Table 2).

Blood Data and Adverse Events

In the G-CSF group, white blood cell count immediately before G-CSF administration was 7.3 ± 1.6 (× 10^3/mm^3). During the administration, it increased up to 36.7 ± 9.4 (× 10^3/mm^3), ranging from 19.2 to 50.3 (× 10^3/mm^3) (Table 3). G-CSF mobilized cells of the neutrophil lineage, but lymphocytes were not affected (Table 3). G-CSF also caused an increase of monocytes. There was no significant change in inflammation during G-CSF administration, as indicated by C-reactive protein levels (Table 3).

In this series, there was no patient who showed bone pain or hepatic dysfunction after the G-CSF administration. No other severe adverse event occurred during or after the administration.

DISCUSSION

To date, 3 clinical trials of G-CSF administration for neurological disorders have been reported; 2 for amyotrophic lateral sclerosis[8,9] and 1 for cerebral infarction.[10] Zhang *et al*[8] reported that the progression of amyotrophic lateral sclerosis symptoms was inhibited by G-CSF administration, although they did not use controls. Neffussy *et al*[9] performed a controlled study, but they showed no significant difference in the progression of amyotrophic lateral sclerosis symptoms between their G-CSF-treated group and controls. A single clinical trial with G-CSF administration for cerebral infarction has been reported by Shyu *et al*.[10] They reported that neurological symptoms were significantly improved by G-CSF administration.

In this study, we conducted the first clinical trial using G-CSF for patients with worsening symptoms of thoracic

TABLE 3. Hematological Data Before and After G-CSF Administration

	Baseline $M \pm SD$ (range)	Peak Value After G-CSF Administration $M \pm SD$ (range)*	P
WBC, ×10^3/mm^3	7.3 ± 1.6 (5.0–10.3)	36.7 ± 9.4 (19.2–50.3)	<0.01
Neutrophils, ×10^3/mm^3	4.6 ± 1.4 (2.1–6.9)	30.6 ± 6.7 (16.6–40.5)	<0.01
Lymphocytes, ×10^3/mm^3	2.1 ± 0.4 (1.5–2.5)	2.4 ± 0.7 (1.5–3.2)	0.29
Monocytes, ×10^3/mm^3	0.4 ± 0.2 (0.2–0.8)	1.9 ± 0.9 (0.7–2.8)	<0.01
CRP, mg/dL	0.1 ± 0.1 (0.0–0.3)	0.3 ± 0.2 (0.1–0.6)	0.08

**Highest level between the first and seventh day after G-CSF administration.*

G-CSF indicates granulocyte colony–stimulating factor; WBC, white blood cell; CRP, C-reactive protein.

compression myelopathy. One month after G-CSF administration, mean recovery rate of JOA score was 29.1%. In contrast, it was 1.1% in the control group at 1 month after initial treatment. In addition, we observed that both motor power and pain sensation scores significantly increased in the G-CSF group compared with the control group at 1 month after treatment. No surgical treatment was performed in patients of either group during the month after G-CSF administration or initial treatment, and they were equally provided conservative treatment such as bed rest. Thus, the present results strongly suggest that G-CSF administration exhibited a neuroprotective effect for the injured spinal cord in patients with worsening symptoms of thoracic myelopathy and improved the myelopathy.

To the best of our knowledge, there has been no other medical treatment that has provided reliable evidence for improvement of thoracic myelopathy. This study provides evidence that G-CSF neuroprotective therapy may be useful as a medical treatment of patients with worsening symptoms of thoracic compression myelopathy. The G-CSF therapy may be especially useful for patients in whom the treatment of complications other than myelopathy needs to be given priority and thus requires a long waiting period before surgery.

In our present trial, no severe side effects occurred. Thus, we suggest that the dose (10 μg/kg per d), duration (5 consecutive days), and route (intravenous administration) of G-CSF administration used in this study are principally safe for the treatment of patients with thoracic myelopathy.

The biggest limitation of this study was that the trial was performed as an open-labeled study and the selection of patients to the G-CSF group and the control group was not randomized. We cannot deny the possibility that a placebo effect of injection may participate in the improvement of neurological symptoms. To increase the level of evidence, in the next stage the study design should be a randomized, double-blind placebo-controlled study. By conducting a phase IIb clinical trial in a large number of patients with the study design described earlier, we will be able to reach a better conclusion regarding the effectiveness of G-CSF neuroprotective therapy for patients with worsening symptoms of thoracic compression myelopathy.

➢ Key Points

- ❑ A multicenter prospective controlled clinical trial was performed to confirm the feasibility of G-CSF administration for patients with worsening symptoms of thoracic myelopathy.
- ❑ For 10 patients with progressive myelopathy, G-CSF (10 μg/kg per day) was intravenously administered for 5 consecutive days.
- ❑ The administration of G-CSF caused neurological recovery in the patients.

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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

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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

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

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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.