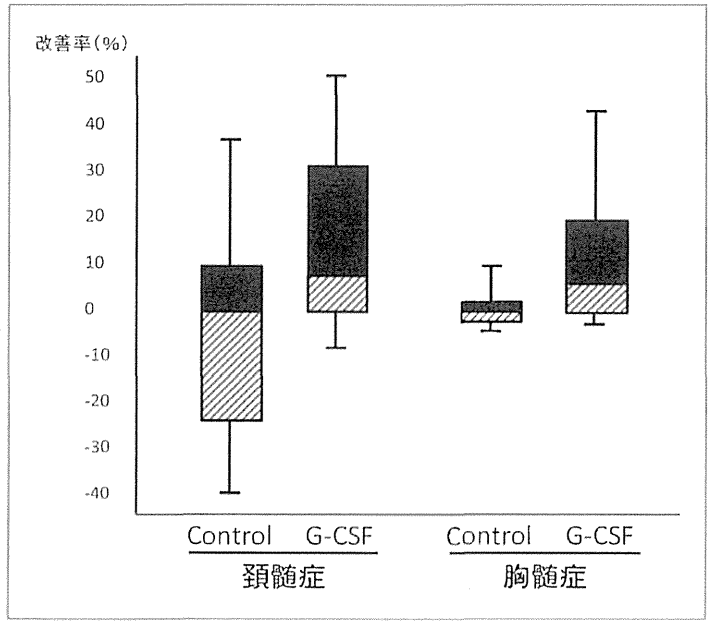
図 1.
圧迫性脊髄症急性増悪患者を対象とするG-CSF神経保護療法のオープンラベル多施設前向き比較対照試験の結果．頚髄症・胸髄症症例ぞれぞれのG-CSF投与後2週時JOAスコア改善率をコントロール症例と比較したところ，有意な改善が得られていた．箱ひげ図は中央値・25および75パーセンタイルと最少・最大値をそれぞれ示した．



て手術を行った．コントロール群では安静など，G-CSF投与以外の治療はG-CSF群と同様に行い，同じく1か月以上の待機期間の後手術を施行した．両群のJOA score獲得点数・改善率を投与後2週の時点で比較した．この試験の症例のうち頚部脊髄症症例では，投与後2週時のJOA score改善率はG-CSF群で13.54±4.0%，コントロール群で−4.03±6.5%と，G-CSF群で有意な改善を認めた(p=0.029，図1)．胸髄症症例に関しても，投与後2週時のJOA score改善率はG-CSF群で11.5±3.5%，コントロール群で1.3±4.5%と，G-CSF群で有意な改善を認めた(p=0.045，図1)．この結果は，G-CSFの圧迫性脊髄症急性増悪例に対する治療効果を示唆するものであった[15]．

この圧迫性脊髄症急性増悪患者を対象とするG-CSF神経保護療法のオープンラベル多施設前向き比較対照試験において，G-CSF治療効果の予後規定因子を検討した．G-CSF投与直前と手術直前でJOAスコアを評価し，改善例を効果あり，不変例と悪化例を効果無しと判定した．年齢，性別，BMI，罹病期間，直近1か月で減少したJOAスコアについて多重ロジスティック回帰分析を用いてG-CSF療法の予後因子を検討した．JOAスコアは平均で8.0点から9.7点に有意に改善した．改善32例，不変9例，悪化2例であった．G-CSF療法の予後規定因子は罹病期間(OR，0.988；95%CI，1.00-1.03)と直近1か月のJOAスコアの減少(OR，2.04；95%CI，0.191-0.962)であった．頚部脊髄症急性増悪患者に対するG-CSF療法は，罹病期間が短く，急性増悪の程度が大きいほど効果が期待できるという結果が得られた．

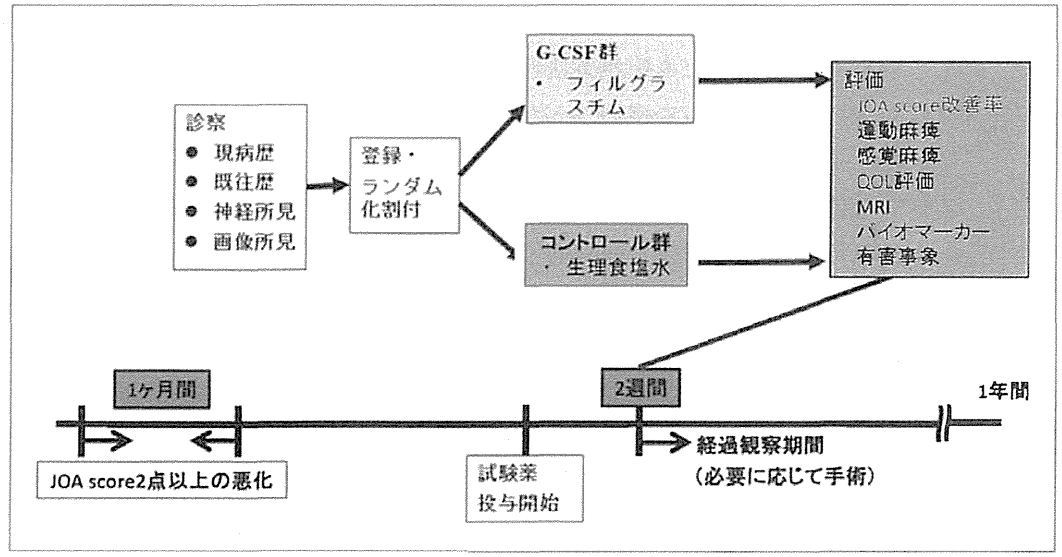


図 2.
試験のフローチャート
現在準備中の圧迫性頚部脊髄症急性増悪患者を対象とするG-CSF神経保護療法のプラセボ対照ランダム化単盲検並行群間比較試験の試験概要をフローチャートに示す．

臨床試験：今後の展望

現在までに施行した臨床試験によりG-CSFの圧迫性脊髄症急性増悪患者に対する治療効果が示唆された．しかし，これまでの試験が非ランダム化・非盲検化のstudy designで行われたため，各種バイアスが結果に影響した可能性を否定できない．そこで，G-CSFの治療効果を証明するための次の段階として，プラセボ対照ランダム化単盲検並行群間比較試験を計画した(**図2**)．対象は，直近の1か月にJOAスコア2点以上の悪化をきたした20～85歳の頚部脊髄症患者さんとした．除外項目としては主として安全性の面から白血病など造血系悪性疾患の既往，過去5年以内の悪性疾患の既往，治療中の心筋梗塞・狭心症，血栓・塞栓症の既往またはその傾向，脾腫，妊婦または妊娠の可能性，その他を設定した．また，試験前にG-CSFまたはコハク酸メチルプレドニゾロンナトリウムエステルの投与を受けた者についても評価が困難になる可能性を考慮し除外とした．対象患者さんをランダムに2群に分け，G-CSF群には10 μg/kg/日の5日間経静脈点滴静注投与．コントロール群には生理食塩水を同量・同様に投与する．両群とも，頚部脊髄症に対する保存療法として，頚椎カラー固定または牽引を行い厳密な頚部の安静を保つ．この試験の主要評価項目は投与後2週の時点でのJOA score改善率(平林法)である．副次的評価項目として，JOA score獲得点数・改善率(平林法)を経時的に観察．American Spinal Injury Association(ASIA)運動scoreにて運動麻痺の程度を，ASIA痛覚scoreにて感覚麻痺の程度を評価する．Euro-QOL 5 dimension(EQ-5D)効用値にて投与前，投与後3か月時・1年時のQOL評価を行う．また，脊髄MRI画像・血中バイオマーカーや有害事象(副作用)に関しても両群を比較する．

上述した圧迫性脊髄症急性増悪患者を対象とするG-CSF神経保護療法のオープンラベル多施設前向き比較対照試験において，全く予想外の作用であったが，神経因性疼痛と思われるビリビリとした自発痛やparesthesiaが軽減する例が少なからず観察された．この鎮痛効果はG-CSF投与中より出現し，約2～3か月間持続した[16]．現在，脊髄損傷モデルの神経因性疼痛に対するG-CSFの鎮痛作用およびその作用機序を明らかにすべく動物実験を開始している．神経因性疼痛は難治性の疼痛で，圧迫性脊髄症においても麻痺と並んで患者さんを悩ませている症状であり有効な治療法が切望されている．こちらも並行して臨床試験の準備を進めている．

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改定：2012年9月

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Clinical Study

Phosphorylated neurofilament subunit NF-H becomes elevated in the cerebrospinal fluid of patients with acutely worsening symptoms of compression myelopathy

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ABSTRACT

It is known that the severity of compression myelopathy sometimes worsens rapidly and results in poor functional recovery because of limited axonal regeneration. Levels of phosphorylated neurofilament subunit NF-H (pNF-H), which indicate axonal degeneration, are elevated in other neurological disorders. To our knowledge, there has been no examination of pNF-H levels in compression myelopathy. Therefore, we conducted a pilot cross-sectional study to evaluate pNF-H levels in the cerebrospinal fluid (CSF) of patients with worsening symptoms of cervical compression myelopathy. From January 2011 to March 2013, 51 samples of CSF were collected from patients at the time of myelography before spinal surgery. The indications for surgery were acutely worsening compression myelopathy (AM) in eight, chronic compression myelopathy (CM) in six, and lumbar canal stenosis (LCS) in 37 patients. The pNF-H levels were measured using a standard enzyme-linked immunosorbent assay. The mean ± standard deviation pNF-H value was 2127.1 ± 556.8 pg/ml in AM patients, 175.8 ± 67.38 pg/ml in CM patients and 518.7 ± 665.7 pg/ml in LCS patients. A significant increase in pNF-H levels was detected in the CSF of patients with AM compared with those with either CM or LCS. The clinical outcome of surgical treatment for patients with cervical myelopathy was satisfactory in both AM and CM patients. Despite the limitations of small sample size and lack of healthy CSF control data due to ethical considerations, our results suggest that pNF-H in CSF can act as a biomarker that reflects the severity of AM.

1. Introduction

Cervical compression myelopathy is one of the most common spinal cord disorders affecting the elderly. It is well known that the mechanism of compression myelopathy is chronic compression of the spinal cord by osteophytes, degenerated discs, thickened ligamenta flava, and ossification of the posterior longitudinal ligament [1]. Usually, a slow and stepwise decline in function is observed after compression myelopathy. However, a rapid progression of motor paralysis and paresthesia with mild or no trauma is occasionally observed. The severity of compression myelopathy has been reported to worsen rapidly in almost 5% of patients [2]. Rapid worsening of compression myelopathy results in severe neurological deficits with poor functional recovery because of limited axonal regeneration [1,3]. To date, the only effective therapy for compression myelopathy is early surgical treatment [4]. Generally, the recovery rate of neurological function after surgical treatment is about 50–70% [5]. However, in some patients, sufficient improvement of neurological function is not achieved. At present we cannot accurately predict the recovery rate before surgical treatment. Moreover, the only indicators to assess the severity of neurological status are subjective, including the Japanese Orthopaedic Association (JOA) score [6]. Therefore, biomarkers that reflect the degree of damage to the spinal cord and the severity of neurological symptoms would be useful.

Phosphorylated neurofilament subunit NF-H (pNF-H) is a structural protein of axon fibers and is not detected in the cerebrospinal

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fluid (CSF) or blood of healthy subjects. However, axonal breakdown increases the level of pNF-H in plasma and CSF [7]. A recent report has indicated that the level of pNF-H in the plasma and CSF is elevated in various neurological disorders such as subarachnoid hemorrhage, traumatic brain injury, amyotrophic lateral sclerosis, and acute spinal cord injury [8–12]. Therefore, pNF-H may be useful to evaluate the severity of progression and the effect of treatment in such disorders.

However, there are no studies examining the level of pNF-H in the CSF or plasma of patients with compression myelopathy to our knowledge. Therefore, we conducted a pilot cross-sectional study to determine the level of pNF-H in the CSF of patients with compression myelopathy.

2. Methods

2.1. Patients and samples

This study was given approval by our University Human Ethics Committee. From January 2011 to March 2013, 51 CSF samples were obtained from patients at the time of myelography before spinal surgery at the Toho University Sakura Medical Center. Informed consent was obtained from all patients. The indications for surgery were cervical compression myelopathy in 15 patients and lumbar canal stenosis (LCS), which was used as a control disorder, in 37 patients. Furthermore, we divided compression myelopathy samples into patients with acutely worsening symptoms (AM) and patients with chronic symptoms (CM). We defined acutely worsening compression myelopathy as that in which the JOA score of patients with cervical myelopathy decreased by 2 points or more during a recent 1 month period [13]. Ultimately, eight patients were allocated to the AM group and six patients to the CM group. Patients who were diagnosed as having cervical spondylotic radiculopathy and cervical spondylotic amyotrophy were excluded from this study. Patients with double lesions (cervical compression myelopathy and LCS) were also excluded.

2.2. Clinical outcome of patients with compression myelopathy

In all patients with compression myelopathy (AM and CM groups), neurological evaluation using a JOA score for cervical myelopathy (scores range from 0 to 17) was performed [6]. The scores were evaluated at the time of myelography before surgery and 6 months after surgery by two orthopedic spine surgeons.

2.3. pNF-H assay

The pNF-H assay was performed using a commercially available enzyme-linked immunosorbent assay kit (ELISA; BioVendor, Brno, Czech Republic). Frozen CSF samples were allowed to thaw, and diluted 1/2 in a buffer. The samples were then loaded onto an ELISA plate. The assay was performed according to the manufacturer's protocol. To standardize the pNF-H value, all samples were tested in duplicate, and the average value for each sample was calculated.

2.4. Statistical analyses

Results are presented as mean ± standard deviation. A one factor analysis of variance with a *post hoc* Tukey–Kramer test was used to evaluate the difference in the pNF-H levels between AM, CM, and LCS patients. Spearman's correlation coefficient by rank test was used to evaluate the correlation between pNF-H and JOA score. $p < 0.05$ was considered statistically significant.

Table 1
Patient characteristics in each group

	AM	CM	LCS
Patients, n	8	6	37
Sex			
Male	4	5	14
Female	4	1	23
Age, years*			
	64.9 ± 10.2 (45–79)	65.0 ± 13.2 (39–75)	70.3 ± 7.9 (55–86)
Preop JOA*	9.25 ± 2.43 (6–14)	10.6 ± 0.80 (10–12)	
Surgical procedure			
Laminoplasty	4	2	
Laminoplasty with posterior fusion	1	2	
Anterior corpectomy and fusion	3	2	

AM = acutely worsening compression myelopathy, CM = chronic compression myelopathy, JOA = Japanese Orthopaedic Association, LCS = lumbar canal stenosis, preop = preoperative.
* Data presented as mean ± standard deviation (range).

3. Results

3.1. Patient characteristics

Table 1 shows the characteristics of each group of patients. The mean age was 64.9 ± 10.2 (range 45–79 years) in the AM group, 65.0 ± 13.2 (range 39–75 years) in the CM group, and 70.3 ± 7.9 (range 55–86 years) in the LCS group. The mean JOA score at the time of CSF sampling in the AM group was 9.5 ± 2.51 (range 6–14), and 10.6 ± 0.80 (range 10–12) in the CM group. The surgical procedure in the AM group was laminoplasty in four patients, laminoplasty with posterior fusion in one patient, and anterior corpectomy and fusion in three patients. The surgical procedure in the CM group was laminoplasty in two patients, laminoplasty with posterior fusion in two patients, and anterior corpectomy and fusion in two patients.

3.2. Levels of pNF-H

Figure 1 shows the level of pNF-H in the CSF of patients from each group. The level of pNF-H was 2127.1 ± 556.8 pg/ml in the AM group, 175.8 ± 27.5 pg/ml in the CM group, and 518.7 ± 665.7 pg/ml in the LCS group. Our findings show that a significant increase in the level of pNF-H was detected in patients in the AM group compared with that in the CM and LCS group ($p < 0.01$). A slightly increased level of pNF-H was detected in CSF from patients in the LCS group compared with levels in the CM group. However, there was no significant difference in the levels between these two groups.

3.3. Evaluation of clinical outcome

Table 2 shows the change of JOA scores after surgery. JOA scores at the time of CSF collection were 9.5 ± 2.51 (range 6–14) in the AM group and 10.6 ± 0.80 (range 10–12) in the CM group. After surgery, neurological improvement was seen in all patients. JOA scores 6 months after surgery were 14.3 ± 1.82 (range 13.5–16.5) in the AM group and 13.9 ± 0.58 (range 13.5–15) in the CM group. The recovery rate of JOA score was 66.0 ± 16.9 (range 46.2–86.7) in the AM group and 51.2 ± 12.5 (range 30–66.7) in the CM group. Although a slightly higher recovery rate of JOA score was seen in the AM group, no statistical difference in recovery rate of JOA score was observed between patients in the AM and CM groups ($p = 0.096$).

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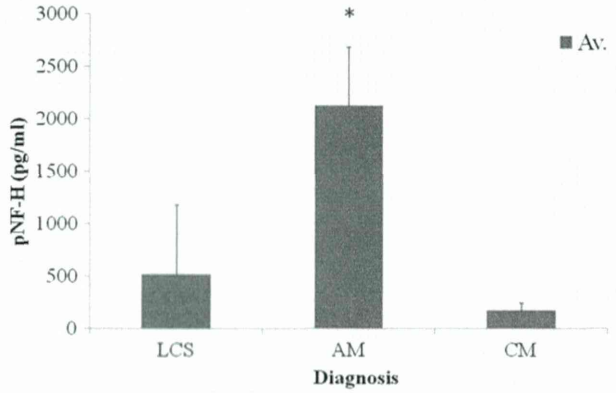

Fig. 1. Levels of phosphorylated neurofilament subunit NF-H. AM = acutely worsening compression myelopathy, Av. = average, CM = chronic compression myelopathy, LCS = lumbar canal stenosis. *$p < 0.01$ compared to lumbar canal stenosis and chronic compression myelopathy group.

Table 2
Recovery of Japanese Orthopaedic Association score

	AM	CM	*p* value
JOA score at the time of CSF collection	9.5 ± 2.51 (6–14)	10.6 ± 0.80 (10–12)	0.146
JOA score at 6 months after surgery	14.3 ± 1.82 (13.5–16.5)	13.9 ± 0.58 (13.5–15)	0.377
Recovery rate of JOA score	66.0 ± 16.9 (46.2–86.7)	51.2 ± 12.5 (30–66.7)	0.096

AM = acutely worsening compression myelopathy, CM = chronic compression myelopathy, CSF = cerebrospinal fluid, JOA = Japanese Orthopaedic Association.

No statistical correlation was found between the level of pNF-H and the recovery rate of JOA score ($p = 0.128$).

4. Discussion

To our knowledge, the present cross-sectional study is the first to determine the level of pNF-H in CSF samples from patients with cervical compression myelopathy and LCS. Our results showed a significant increase in the level of pNF-H of up to 2000 pg/ml in patients with AM. Elevated levels of pNF-H have suggested axonal breakdown in studies of other neurological disorders [7–9,11]. Furthermore, plasma pNF-H was found to be elevated proportional to the severity of acute spinal cord injury (SCI) and to reflect a greater extent of axonal damage because of the secondary damage to the injured spinal cord [10,12]. Increased levels of plasma pNF-H were seen in patients with complete SCI, but not in patients suffering incomplete paralysis [10]. In the present study, we hypothesized that increased levels of plasma pNF-H are not seen in compression myelopathy because of minor injury to the spinal cord compared with SCI. Therefore, we determined the levels of pNF-H in CSF rather than plasma. Although the pathogenesis and prognosis of compression myelopathy remain unclear, inflammation, hypoxia, and excitotoxicity are likely to cause secondary damage in SCI. An increase in the concentration of interleukin-6 has been detected in the CSF of patients with cervical compression myelopathy [14]. An increase in the concentration of interleukin-8 has been detected in the CSF of patients with cervical spondylotic myelopathy [15]. The increased level of pNF-H in the present study suggests that pNF-H reflects the severity of AM, and the pathogenesis in AM may be acute axonal damage followed by secondary damage, as seen in SCI.

In the present study, although a slightly higher recovery rate of JOA score was seen in the AM group, no statistical difference was observed between AM and CM patients. The surgical outcome was satisfactory in patients from both the AM and CM groups. There was no correlation between the level of pNF-H and the recovery rate of JOA score. Although surgical procedures for compression myelopathy are not standardized, our study suggests that early surgical treatment of AM results in sufficient neurological improvement, even in patients with CM.

The present study has several limitations. First, because CSF samples were only collected from patients at the time of myelography before surgery, the sample size was small and there is bias toward more severe disease. We found no statistical correlation between pNF-H and JOA recovery rate. However, a slightly higher JOA recovery rate was seen in AM patients. Further investigation with long-term follow-up after surgery and standardization of both the severity of the myelopathy and the surgical procedure performed are required to support our findings. Second, the collection method for CSF precludes the collection of CSF samples from healthy control subjects because of ethical issues. A slightly increased level of pNF-H was found in the CSF from LCS patients. A rodent study indicated that the level of pNF-is up-regulated in rat dorsal root ganglions [16]. In humans, increased interleukin-6 levels were detected in the CSF of patients with lumbar radiculopathy [14]. The present finding of slightly increased pNF-H levels in the CSF of patients with LCS may reflect axonal damage to the nerve roots or the cauda equina. The average level of pNF-H in the CSF of patients with LCS was about 500 pg/ml. The present findings suggest that pNF-H may be useful in the differential diagnosis of double lesions (cervical myelopathy and LCS). Further investigation using comparison samples from healthy control subjects is required. Third, the detailed pathogenesis of increased pNF-H levels in the CSF of patients with AM or CM remains unclear. Further research using animal models of compression myelopathy may clarify the pathogenesis.

In conclusion, despite the limitations indicated above, a significantly increased level of pNF-H was detected in the CSF of patients with AM. Clinical outcome after surgical treatment for cervical myelopathy was satisfactory in patients with both AM and CM. The present results suggest that pNF-H in CSF may be a biomarker that reflects the severity of AM.

Conflicts of Interest/Disclosures

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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ORIGINAL ARTICLE

Neuroprotective therapy with granulocyte colony-stimulating factor in acute spinal cord injury: a comparison with high-dose methylprednisolone as a historical control

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Abstract
Purpose We performed a phase I/IIa clinical trial and confirmed the safety and feasibility of granulocyte colony-stimulating factor (G-CSF) as neuroprotective therapy in patients with acute spinal cord injury (SCI). In this study, we retrospectively analyzed the clinical outcome in SCI patients treated with G-CSF and compared these results to a historical cohort of SCI patients treated with high-dose methylprednisolone sodium succinate (MPSS).
Methods In the G-CSF group ($n = 28$), patients were treated from August 2009 to July 2012 within 48 h of the injury, and G-CSF (10 μg/kg/day) was administered intravenously for five consecutive days. In the MPSS group ($n = 34$), patients underwent high-dose MPSS therapy from August 2003 to July 2005 following the NASCIS II protocol. We evaluated the ASIA motor score and the AIS grade elevation between the time of treatment and 3-month follow-up and adverse events.
Results The ΔASIA motor score was significantly higher in the G-CSF group than in the MPSS group ($p < 0.01$). When we compared AIS grade elevation in patients with AIS grades B/C incomplete paralysis, 17.9 % of patients in the G-CSF group had an AIS grade elevation of two steps compared to 0 % of patients in the MPSS group ($p < 0.05$), and the incidence of pneumonia was significantly higher in the MPSS group (42.9 %) compared to the G-CSF group (8.3 %) ($p < 0.05$).
Conclusions These results suggest that G-CSF administration is safe and effective, but a prospective randomized controlled clinical trial is needed to compare the efficacy of MPSS versus G-CSF treatment in patients with SCI.

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

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Introduction

Acute spinal cord injury (SCI) is characterized by two pathological phases known as primary and secondary injury [1]. Primary injury occurs when the tissue is destroyed by direct mechanical trauma. Secondary injury occurs when the spinal cord reacts to the primary injury. Neurons and glial cells that were left intact by the initial trauma undergo apoptosis during the secondary phase of injury. Multiple factors exacerbate the secondary phase of injury, including vascular changes, increased concentrations of free radicals and free fatty acids, ionic mechanisms of axonal injury, glutamate excitotoxicity and immune and inflammatory reactions [2]. Secondary injury is, therefore, a rich target for drug therapy [3]. According to the NASCIS II protocol, high-dose methylprednisolone sodium succinate (MPSS) is the standard treatment for attenuation of

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secondary injury after acute SCI [4]. In recent years, MPSS therapy for acute SCI became controversial. Cochran review shows the efficacy of MPSS therapy for SCI [5]. In contrast, the updated guidelines for the management of acute cervical spine and spinal cord injury released by American Association of Neurological Surgeons and Congress of Neurological Surgeons Guidelines Committee described MPSS therapy for SCI as "not recommend" [6]. Hence, new drug therapies for the treatment of secondary injury after acute SCI are needed.

Granulocyte colony-stimulating factor (G-CSF) is a clinically important cytokine that is commonly used to treat neutropenia [7]. Granulocyte colony-stimulating factor also has non-hematopoietic functions and has been suggested as a treatment for neuronal injury [8]. We have previously reported that G-CSF promotes functional recovery in a rodent model of SCI [9–12]. Based on these results, we performed a preliminary phase I/IIa clinical trial and confirmed the safety and feasibility of G-CSF as neuroprotective therapy in patients with acute SCI [13]. The next step is to verify the efficacy of G-CSF compared to standard high-dose MPSS therapy. Toward this end, we retrospectively analyzed the clinical outcome and the incidence of drug-related adverse events in SCI patients treated with G-CSF and compared these results to a historical cohort of SCI patients treated with MPSS.

Methods

Study design

The study was designed as a retrospective comparative analysis using an historical cohort control.

Patient population

Between August 2009 and July 2012, all patients with complete or incomplete C3-C7 cervical SCI who presented to Chiba University Hospital within 48 h of injury were recruited into the study. Exclusion criteria included the following: (1) age younger than 16 years or greater than 85 years, (2) treatment with high-dose MPSS therapy after the SCI event, (3) splenomegaly or altered mental status, (4) history of leukemia, thrombosis or embolism, (5) current treatment of myocardial infarction or angina, and (6) evidence of malignant disease within the last 5 years. Pregnant patients were also excluded. Written informed consent was obtained from all patients prior to G-CSF treatment (G-CSF group).

Patients with acute cervical SCI who received high-dose MPSS therapy following the NASCIS II protocol between August 2003 and July 2005 served as an historical control (MPSS group). Patients were selected based on the same exclusion criteria outlined above. A larger number of patients with complete paralysis (American Spinal Injury Association impairment scale: AIS grade A) were observed in the MPSS group compared to the G-CSF group. No other significant differences in patient background were observed between the two groups, including patient age, sex, injury level and AIS grade (Chi square test).

Standard protocol approvals, registrations, and patient consents

This study was approved by the Institutional Review Boards of both participating institutions. The study was conducted in compliance with the Declaration of Helsinki and the International Conference on harmonization good clinical practice guidelines.

Treatment

G-CSF group

Patients were treated with i.v. Granulocyte colony-stimulating factor (dissolved in normal saline) at a dose of 10 μg/kg/day (administered over 1 h) for five consecutive days. Granulocyte colony-stimulating factor dose regimen was determined by the previous preliminary clinical trial of G-CSF neuroprotective therapy for acute SCI, of which study design was single armed with dose escalation [13].

MPSS group

Methylprednisolone sodium succinate was administered according to the NASCIS II protocol within 8 h after injury. Methylprednisolone sodium succinate was first administered as a bolus dose of 30 mg/kg MPSS. After a 45-min withdrawal period, 5.4 mg/kg was administered intravenously over the next 23 h.

Patients in each group received similar surgical, rehabilitation and nursing care.

Efficacy assessments

Neurologic function was assessed with the American spinal injury association (ASIA) motor and sensory scores immediately upon study entry and after 3 months of follow-up. The primary outcome was the change in ASIA motor score between the time of treatment and 3 months following treatment. The initial analysis included all patients, including those with AIS grade A paralysis. However, because these patients have complete paralysis and typically demonstrate little significant neurological