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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 × 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 × 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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