Significant correlation between cerebrospinal fluid nitric oxide concentrations and neurologic prognosis in incomplete cervical cord injury

Noboru Hosaka · Shinji Kimura · Akiyoshi Yamazaki ·
Xianjun Wang · Hiroshi Denda · Takui Ito ·
Toru Hirano · Naoto Endo

Received: 25 October 2006 / Revised: 24 March 2007 / Accepted: 27 July 2007 / Published online: 22 August 2007
© Springer-Verlag 2007

Abstract In animal models of spinal cord injury (SCI), inducible NO (nitric oxide) synthase is expressed in the spinal cord immediately after sustaining SCI. Excessive NO production has cytotoxic effects and induces neuronal apoptosis, causing neural degeneration and neurodysfunction in the spinal cord. Little is known, however, about the relationship between NO_x (NO metabolites: nitrite and nitrate) levels in the cerebrospinal fluid (CSF) and neurologic severity or recovery in clinical cases. The objective of the present study was to examine the correlation between CSF NO_x levels and neurologic severity or recovery in SCI. Twenty-five patients with incomplete cervical cord injury (CCI) were examined. Eight cases were treated conservatively (non-operated group). Seventeen cases underwent surgical intervention (operated group). NO_x levels in the CSF were measured using the Griess method. The severity of the neurologic impairment was assessed using Frankel's classification and the American Spinal Injury Association motor score (ASIA MS). The degree of neurologic recovery was assessed using Frankel's

classification and the ASIA motor recovery percentage (MRP). There was no significant difference in the NO_x levels between the CCI group (NO_x levels: $5.9 \pm 0.7 \mu\text{M}$) and the 36 control subjects (1 volunteer and 35 patients without neurologic disorders, NO_x levels: $4.9 \pm 0.3 \mu\text{M}$). There was no significant difference in NO_x levels and MRP between the non-operated group and the operated group. The NO_x levels in total SCI group were significantly correlated with the ASIA MS and MRP. There was a significant correlation between CSF NO_x levels and neurologic severity or recovery in incomplete CCI.

Keywords Nitric oxide · Spinal cord injury · Cerebrospinal fluid · Neurologic recovery · Predictor

Introduction

Patients with spinal cord injury (SCI), often suffer permanent paresis, resulting in severe physical, emotional, and economic loss for them and their family. Therefore, immediate treatment and prognostic prediction at the acute stage are required to efficiently rehabilitate the patient to obtain the maximum achievable quality of life.

The prognosis of SCI is usually predicted using the Frankel classification [11, 24] or the findings of magnetic resonance images, such as intramedullary signal changes, the longitudinal length of the haematoma, or edema in the spinal cord at the acute stage [30]. It is not easy to clinically predict the prognosis at the acute stage, however, because the paresis due to spinal cord shock is variable [11]. Currently, there are no quantitative predictors for neurologic improvement at the acute stage.

In SCI, initial mechanical injuries occur, followed by secondary injuries, such as ischemia, edema, and leukocyte

S. Kimura (✉)
Rehabilitation Center, Niigata University,
1-754 Asahimachi-dori, Niigata City,
Niigata 951-8520, Japan
e-mail: skimura@med.niigata-u.ac.jp

N. Hosaka
Department of Orthopedic Surgery,
Niigata Rousai Hospital, Joetsu City, Niigata, Japan

A. Yamazaki
Department of Orthopedic Surgery, Niigata Central Hospital,
Niigata city, Niigata, Japan

X. Wang · H. Denda · T. Ito · T. Hirano · N. Endo
Department of Orthopedic Surgery, Niigata University,
Niigata city, Niigata, Japan

infiltration into the spinal cord parenchyma [31]. The secondary injuries lead to neuronal apoptosis and spinal cord degeneration [4]. Nitric oxide (NO), a gaseous messenger that induces vasodilatation [26] and macrophage cytotoxicity [14], and is involved in neural signaling [17, 18] is a physiologic biofactor involved in secondary injury in SCI.

We previously reported that preoperative concentrations of NO metabolites (NO_x levels) in the cerebrospinal fluid (CSF) are higher in patients with lumbar degenerative diseases than in pain-free controls [19] and are significantly correlated with the degree of pain relief following surgery. Therefore, NO_x levels are possible quantitative predictors of pain relief in lumbar degenerative diseases [20, 21].

In animal experiments of SCI, inducible NO synthase (iNOS) is expressed in the spinal cord immediately after SCI, reaches a maximal value at 12 h, and is maintained at this level of expression for 1 week [13, 28, 33]. Excessive NO production derived from iNOS has cytotoxic effects [5] and induces neuronal apoptosis, causing neural degeneration and neurodysfunction [1, 9]. Compared to wild-type mice, iNOS-gene deficient mice have a significantly better functional outcome after SCI [15]. Administration of a NOS blocker reduces neurologic deterioration [3]. Little is known, however, about the relationship between NO_x levels and neurologic severity or recovery in clinical SCI cases. Therefore, in the present study, we examined whether CSF NO_x levels correlate with the severity and prognosis of neurologic recovery from SCI.

Materials and methods

Subjects

Informed consent was obtained from all participating subjects. Twenty-five patients with incomplete cervical cord injury (CCI group), who were admitted to the hospital between January 1998 and December 2004, were examined. The CCI group consisted of 18 males and 7 females (age range 30–85 years; mean 61 years). The follow-up period ranged from 9 to 45 months (mean 17 months). The CCI group all had initial symptoms such as numbness or paresis immediately after evident trauma (fall 17 cases, traffic accident 6, cervical sprain 1, compression by machine 1). The CCI group was composed of 16 cases with radiologic spondylosis, 2 cases without spondylotic changes, 4 cases with ossification of the posterior longitudinal ligament (OPLL), 1 case with developmental canal stenosis, and 2 cases with cervical fracture and dislocation. The injured levels in the spine were as follows: C1/2 ($n = 1$), C3/4 ($n = 9$), C4/5 ($n = 12$), C5/6 ($n = 2$), C6/7 ($n = 1$).

Eight cases were treated conservatively (non-operated group). Seventeen cases underwent surgical intervention

[operated group; anterior spinal fusion ($n = 7$), cervical laminoplasty ($n = 10$)]. Surgical intervention was indicated in cases of spinal cord compression with OPLL, developmental canal stenosis, or bulging discs; or abnormal spinal instability due to disc injury and/or ligamentous injury of the anterior longitudinal ligament or inter- or supraspinous ligaments. Intravenous methylprednisolone was administered in 21 cases (7 cases in the non-operated group; 14 cases in the operated group) according to the protocol based on the second National Acute Spinal Cord Injury Study (NASCIS II) [3]. Methylprednisolone has an anti-inflammatory effect, and might decrease CSF NO_x levels.

The control group consisted of 36 cases (CON group) with 1 healthy volunteer that had neither pain nor neurologic disorders, and 35 patients with inguinal herniation ($n = 11$), or who were undergoing removal of metal instrumentation following a fracture in the lower extremity ($n = 24$). The CSF in the CON group was obtained just before lumbar anesthesia. The CON group ranged in age from 34 to 76 years (mean 55 years). The CCI and CON groups were matched for age.

Measurement of NO metabolites

In the CON group, CSF (1–2 cc) was collected using a spinal needle (23–25 gauge) at L2/3 or L3/4 under lumbar anesthesia. In a healthy volunteer, CSF was obtained using a spinal needle (25 gauge) at L3/4. In the CCI group, CSF was obtained during conservative treatment or preoperatively. The timing of CSF collection ranged from between 0 and 30 days (mean 5 days; 0–5 days, 18 cases; 6–13 days, 6 cases; 14–30 days, 1 case), usually within 14 days after SCI, although high NO_x levels persist for up to 2 weeks in animal studies. Also, in 7 cases CSF was obtained twice, at the acute phase within 1 day (mean 0.9 days) and at the subacute phase between 5 and 21 days (mean 9.6 days) after the trauma. To elucidate the relationship between changes in NO_x levels and recovery rate, the change in NO_x was calculated using the following equation [20]: changes in NO_x levels = (2nd NO_x levels – 1st NO_x levels)/1st NO_x levels $\times 100$ (%). The specimens were stored at -20°C for later measurement of NO metabolites, NO_2^- plus NO_3^- (NO_x levels). The measurement was performed within 6 months after CSF collection. NO_x in frozen human plasma is stable for at least 1 year [27].

CSF NO_x levels were measured based on the Griess method [12], as reported previously [19]. Methanol (200 μl) was added to deproteinize the CSF (1:1) followed by centrifugation (5,000g, 10 min) to avoid occlusion of the polystyrene polymer column in this system by protein or macromolecules. The assay was performed with an NO

analyzer (ENO-20, Eicom Corp, Kyoto, Japan). In brief, the NO analyzer was first calibrated using a standard solution (100 pM NO_2^- and 100 pM NO_3^-). NO_2^- and NO_3^- were separated on the polystyrene polymer column. NO_3^- was reduced by a cadmium column to form NO_2^- . NO_2^- was mixed with Griess reagent to form a purple azo dye. The absorption at 540 nm was measured by a flow-through spectrophotometer. The concentration of NO_x was measured by assessing the peak area of the absorption using a computer (PowerChrom, Eicom). The minimal detectable level of NO_2^- or NO_3^- was 0.01 μM . The reproducibility and accuracy of this system was previously confirmed [19].

Comparison of NO_x levels between CON and CCI groups

CSF NO_x levels were compared between CON and CCI groups.

Evaluation of neurologic severity and recovery in CCI

The severity of neurologic impairment was assessed using Frankel's classification [11] as well as the American spinal injury association motor score (ASIA MS) [25] at initial admission and the final follow-up. Frankel's classification was categorized as A (complete motor and sensory loss), B (preserved sensory only), C (preserved useless motor activity), D (preserved useful motor activity), and E (complete neurologic recovery). In the present study, Frankel A, B, and C classifications were defined as the Useless group. Frankel D was defined as the Useful group. ASIA MS was based on 10 key muscles associated with the critical neurologic levels of the spinal cord. To investigate the relationship between the NO_x levels and neurologic severity, NO_x levels were compared with ASIA MS.

The degree of neurologic recovery was assessed by Frankel's classification and ASIA motor recovery percentage (MRP) at the final follow-up. The MRP was calculated using the following equation: $\text{MRP} (\%) = \{(\text{motor score at follow-up} - \text{initial motor score}) / (\text{full score} - \text{initial motor score})\} \times 100$ [6, 7, 16]. The MRP was calculated for all 25 cases. The NO_x levels and MRP were compared between non-operated and operated groups.

Data analysis

Data are expressed as the mean $\pm$ SE. The difference in means between two groups was tested using the Mann–Whitney U test. Correlations were performed by Spearman's ranked correlation coefficient. A P value of less than

0.05 was considered statistically significant. No statistical significance is presented as n.s. in the figures.

Results

CSF NO_x levels in CON and CCI groups

There was no significant difference in the NO_x levels between the CON ($4.9 \pm 0.3 \mu\text{M}$) and CCI ($5.9 \pm 0.7 \mu\text{M}$) groups (Fig. 1) although those in the CCI group tended to be higher than those in the CON group and have considerable variability.

Correlation between the NO_x levels and severity of neurologic impairment

The NO_x levels at the initial evaluation in the Useless group ($n = 10$, $7.3 \pm 0.8 \mu\text{M}$) were significantly higher ($P < 0.05$, Fig. 2a) than those in the Useful group ($n = 15$, $5.0 \pm 1.1 \mu\text{M}$). There was a significant correlation between the NO_x levels and ASIA MS ($P < 0.05$, $r = -0.45$, Fig. 2b). These data indicate that NO_x levels in CCI group correlate with the severity of neurologic impairment.

Correlation between the NO_x levels and neurologic recovery

There were no cases classified as Frankel A; 1 Frankel B; 9 Frankel C; 15 Frankel D at the initial evaluation. At the final follow-up, one case classified as Frankel B improved

Fig. 1 Comparison of the NO_x levels in CSF between the CON (control) and CCI (cervical cord injury) groups. *open circle* each datum, *filled square* average in each group, error bars represent standard error of means. There was no significant difference between two groups

Fig. 2 Correlation between the NO_x levels and severity of neurologic impairment. The NO_x levels in the Useless group (Frankel A, B, and C) were significantly higher than those in the Useful group (Frankel D) (a). There is a significant negative correlation between the NO_x levels and the ASIA (American spinal injury association) motor score (b)

to Frankel C. Of 9 cases classified as Frankel C, 7 improved to Frankel D. Of 15 cases classified as Frankel D, 7 improved to Frankel E (Fig. 3a). There was no significant difference in NO_x levels and MRP between the non-operated group ($n = 8$, NO_x levels $7.0 \pm 1.4 \mu\text{M}$, MRP $62 \pm 15\%$) and the operated group ($n = 17$, $5.4 \pm 0.9 \mu\text{M}$, $83 \pm 5\%$). There was no significant correlation between a change in NO_x levels and MRP. There was a significant negative correlation between the NO_x levels and MRP at follow-up in the 25 cases ($P < 0.01$, $r = -0.67$, Fig. 3b), as well as, in the 21 cases ($P < 0.01$, $r = -0.64$) administered methylprednisolone based on the NASCIS II [3], although the two groups with ($n = 21$, MRP $77 \pm 6\%$) and without methylprednisolone ($n = 4$, MRP $75 \pm 25\%$) were not statistically comparable due to the small number patients

Fig. 3 An alteration in Frankel's classification and correlation between the NO_x levels and neurologic recovery. Initial and Follow-up represent Frankel classification at initial evaluation and at the final follow-up, respectively (a). There is a significant negative correlation between the NO_x levels and MRP (motor recovery percentage) (b)

without methylprednisolone. These data indicate that NO_x levels in CCI correlate with neurologic recovery.

Discussion

Increase in NO_x levels in SCI

In animal experiments of SCI, there are several reports indicating a significant increase in iNOS expression or NO around the injured spinal cord. Hamada and colleagues demonstrated that iNOS mRNA at the SCI lesion reaches a maximum 24 h after SCI [13]. Xu and co-workers reported a progressive increase in iNOS expression in the injured spinal cord with maximal expression on day 7, and that the high levels of iNOS expression persist for 2 weeks after SCI [33]. Nakahara reported that NO increases with biphasic changes, such as 1–12 and 24 h to 3 days after injury [28]. These animal studies support the finding that NO metabolites in the CSF increase from 1 day to 2 weeks after SCI, even in human. In the CCI group, however, NO_x levels did not increase significantly in the present study. This might be due to the fact that the CCI group included mostly cases with only mild neurologic deficits (Frankel B, 1; C, 9; D, 15 cases). The NO_x levels were significantly higher in Frankel B, and C groups (Useless group) than in the Frankel D group (Useful) (Fig. 2a). There was a significant correlation between the NO_x levels and ASIA MS (Fig. 2b). Collectively, NO_x levels are indicators of the severity of neurologic impairment.

High NO concentrations induce neural dysfunction

NO, in general, has both cytoprotective and cytotoxic effects, although these effects have not been precisely determined. Physiologic NO levels are not highly toxic. NO reacts with O_2^- to form the powerful oxidant, peroxynitrite (ONOO^-), however, which directly oxidizes lipids, DNA, and proteins [23]. Excessive NO production derived from iNOS has cytotoxic effects [1, 5, 13] and induces neuronal apoptosis [9], causing degeneration and neurodysfunction in the spinal cord. Emery and co-workers [8] confirmed the presence of apoptotic cells in the white matter around the SCI site in clinical cases. These data suggest that high concentrations in NO induce a high rate of neuronal apoptosis and neural degeneration in SCI.

Significant correlation between NO metabolites in CSF and neurologic recovery in CCI

Based on the analysis of 1,461 SCI cases, the Frankel classification at the acute phase is predictive of neurologic

prognosis because 3.0% of cases with Frankel A, 31.3% of cases with Frankel B, and 70.7% of cases with Frankel C recovered to at least a Frankel D at 1-year follow-up [24]. Seldon and co-workers [30] demonstrated that the spreading and lengthening of intramedullary T2 signal intensity in the spinal cord on magnetic resonance images are predictors of neurologic prognosis. In a previous clinical study of SCI, leukotriene C₄ levels, which are a metabolite of arachidonic acid (eicosanoids), in CSF increase at the acute stage of injury and correlate with severity of neurologic impairment [29]. To our knowledge, however, there are no quantitative predictors for neurologic recovery in SCI at the acute stage. The present study indicates that the NO_x levels are significantly correlated with neurologic recovery in SCI based on MRP, although there is a great deal of overlap between the groups; for instance, there were patients with 0% MRP who had the same NO level as patients with 100% recovery (Fig. 3b). Therefore, the NO_x levels are possible quantitative predictors for neurologic recovery in incomplete CCI.

Limitations and problems in the present study

There are several problems regarding the measurement of NO_x levels in CSF. First, subarachnoid hemorrhage might contaminate CSF NO_x levels after SCI because in bovine the NO_x levels in plasma are eight times higher than those in the CSF [2]. In our previous study, however, CSF NO_x levels were not affected by blood contamination [32]. The second is an imbalance in the severity of neurologic impairment in the patient series. The majority of the patients were classified as Frankel C (*n* = 9) and Frankel D (*n* = 15), while the number of severe cases was quite small. This imbalance means that our results might not pertain to severe cases. Therefore, the recovery rate in severe cases should be analyzed further to confirm the relationship between NO_x levels and the neurologic recovery rate. The third is concurrent analysis of the non-operated and operated cases. These two groups might have different outcomes. There are no randomized control studies, however, showing that surgical intervention is superior to conservative treatment [10].

Clinical application for an NOS blocker and related drugs for SCI

Pharmacologic studies indicate that motor function is improved by preventing NO production after SCI in animal experiments. Hamada et al. [13] reported that intravenous injection of an iNOS inhibitor, N^G-nitro-L-arginine methyl ester, reduces motor dysfunction in SCI models. La Rosa

and colleagues [22] also reported that administration of pyroldidine dithiocarbamate (PDTC), which inhibits the activation of NF-κB and thus reduces iNOS expression, significantly improves motor function. These experimental results suggest that a NOS blocker and PDTC are candidate future pharmacologic treatments for SCI in humans. To improve the neurologic prognosis in human SCI, the side effects as well as the clinical effectiveness of these drugs remain to be clarified.

Acknowledgments We thank Yoshiaki Tanaka for his technical assistance. This study was supported in part by a medical research grant on traffic accidents from The General Insurance Association of Japan. This study was approved by the Ethics Committee of Niigata University.

References

1. Bao F, Liu D (2002) Peroxynitrite generated in the rat spinal cord induces neuron death and neurological deficits. *Neuroscience* 115:839–849
2. Blum JW, Morel C, Hammon HM, Bruckmaier RM, Jaggy A, Zurbriggen A, Jungi T (2001) High constitutional nitrate status in young cattle. *Comp Biochem Physiol A Mol Integr Physiol* 130:271–282
3. Bracken MB, Shepard MJ, Collins WF Jr, Holford TR, Baskin DS, Eisenberg HM, Flamm E, Leo-Summers L, Maroon JC, Marshall LF et al (1992) Methylprednisolone or naloxone treatment after acute spinal cord injury: 1-year follow-up data. Results of the second national acute spinal cord injury study. *J Neurosurg* 76:23–31
4. Crowe MJ, Bresnahan JC, Shuman SL, Masters JN, Beattie MS (1997) Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. *Nat Med* 3:73–76
5. Dawson VL, Dawson TM, Bartley DA, Uhl GR, Snyder SH (1993) Mechanisms of nitric oxide-mediated neurotoxicity in primary brain cultures. *J Neurosci* 13:2651–2661
6. El Masry WS, Katoh S, Khan A (1993) Reflections on the neurological significance of bony canal encroachment following traumatic injury of the spine in patients with Frankel C, D, and E presentation. *J Neurotrauma* 10(1 Suppl):S70
7. El Masry WS, Tsubo M, Katoh S, El Miligui YH, Khan A (1996) Validation of the American spinal injury association (ASIA) motor score and the national acute spinal cord injury study (NASCIS) motor score. *Spine* 21:614–619
8. Emery E, Aldana P, Bunge MB, Puckett W, Srinivasan A, Keane RW, Bethea J, Levi AD (1998) Apoptosis after traumatic human spinal cord injury. *J Neurosurg* 89:911–920
9. Estevez AG, Spear N, Manuel SM, Radi R, Henderson CE, Barbeito L, Beckman JS (1998) Nitric oxide and superoxide contribute to motor neuron apoptosis induced by trophic factor deprivation. *J Neurosci* 18:923–931
10. Fehlings MG, Sekhon LH, Tator CH (2001) The role and timing of decompression in acute spinal cord injury. *Spine* 26:S101–S110
11. Frankel HL, Hancock DO, Hyslop G, Melzak J, Michaelis LS, Ungar GH, Vernon JD, Walsh JJ (1969) The value of postural reduction in the initial management of closed injuries of the spine with paraplegia and tetraplegia. Part 1. Paraplegia 7:179–192
12. Griess JP (1964) On a new series of bodies in which nitrogen is substituted for hydrogen. *Philos Trans R Soc Lond* 154:667–731

13. Hamada Y, Ikata T, Katoh S, Tsuchiya K, Niwa M, Tsutsumishita Y, Fukuzawa K (1996) Roles of nitric oxide in compression injury of rat spinal cord. *Free Radic Biol Med* 20:1–9
14. Hibbs JB Jr, Taintor RR, Vavrin Z, Rachlin EM (1988) Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem Biophys Res Commun* 157:87–94
15. Isaksson J, Farooque M, Olsson Y (2005) Improved functional outcome after spinal cord injury in iNOS-deficient mice. *Spinal Cord* 43:167–170
16. Katoh S, El Masry WS, Jaffray D, McCall IW, Eisenstein SM, Pringle RG, Pullicino V, Ikata T (1996) Neurological outcome in conservatively treated patients with incomplete closed traumatic cervical spinal cord injuries. *Spine* 21:2345–2351
17. Kimura S, Uchiyama S, Takahashi HE, Shibuki K (1998) cAMP-dependent long-term potentiation of nitric oxide release from cerebellar parallel fibers in rats. *J Neurosci* 18:8551–8558
18. Kimura S, Yajiri Y, Uchiyama S, Takahashi HE, Shibuki K (1999) Nitric oxide release from substantia gelatinosa of the rat spinal cord in vitro. *Neurosci Lett* 275:199–202
19. Kimura S, Watanabe K, Yajiri Y, Motegi T, Masuya Y, Shibuki K, Uchiyama S, Homma T, Takahashi HE (1999) Cerebrospinal fluid nitric oxide metabolites in painful diseases. *NeuroReport* 10:275–279
20. Kimura S, Watanabe K, Yajiri Y, Uchiyama S, Hasegawa K, Shibuki K, Endo N (2001) Cerebrospinal fluid nitric oxide metabolites are novel predictors of pain relief in degenerative lumbar diseases. *Pain* 92:363–371
21. Kimura S, Ito T, Watanabe K, Hirano T, Yamazaki A, Imura K (2005) Prediction of postoperative pain relief in degenerative lumbar diseases using CSF nitric oxide metabolites: multiple center study. *Spine J* 5:10–11
22. La Rosa G, Cardali S, Genovese T, Conti A, Di Paola R, La Torre D, Cacciola F, Cuzzocrea S (2004) Inhibition of the nuclear factor- κ B activation with pyrrolidine dithiocarbamate attenuating inflammation and oxidative stress after spinal cord trauma in rats. *J Neurosurg Spine* 3:311–321
23. Lipton SA, Choi YB, Pan ZH, Lei SZ, Chen HS, Sucher NJ, Loscalzo J, Singel DJ, Stamler JS (1993) A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. *Nature* 364:626–632
24. Marino RJ, Ditunno JF Jr, Donovan WH, Maynard F Jr (1999) Neurologic recovery after traumatic spinal cord injury. Data from the model spinal cord injury systems. *Arch Phys Med Rehabil* 80:1391–1396
25. Maynard FM Jr, Bracken MB, Creasey G, Ditunno JF Jr, Donovan WH, Ducker TB, Garber SL, Marino RJ, Stover SL, Tator CH, Waters RL, Wilberger JE, Young W (1997) International standards for neurological and functional classification of spinal cord injury. *Spinal Cord* 35:266–274
26. Moncada S, Palmer RMJ, Higgs EA (1991) Nitric oxide; physiology, pathophysiology and pharmacology. *Pharmacol Rev* 43:109–142
27. Moshage H, Kok B, Huizenga JR, Jansen PL (1995) Nitrite and nitrate determinations in plasma: a critical evaluation. *Clin Chem* 41:892–896
28. Nakahara S, Yone K, Setoguchi T, Yamaura I, Arishima Y, Yoshino S (2002) Change in nitric oxide synthase in spinal cord after traumatic injury in rats. *J Neurotrauma* 11:1467–1474
29. Nishisho T, Tonai T, Tamura Y, Ikata T (1996) Experimental and clinical studies of eicosanoids in cerebrospinal fluid after spinal cord injury. *Neurosurg* 39:950–957
30. Selden NR, Quint DJ, Pate N, Hannah S, Papadopoulos SM (1999) Emergency magnetic resonance imaging of cervical spinal cord injuries: clinical correlation and prognosis. *Neurosurgery* 44:785–793
31. Tator CH, Fehlings MG (1991) Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. *J Neurosurg* 75:15–26
32. Wang X, Kimura S, Yazawa T, Endo N (2005) Cerebrospinal fluid sampling by lumbar puncture in rats—repeated measurements of nitric oxide metabolites. *J Neurosci Methods* 145:89–95
33. Xu J, Kim GM, Chen S, Yan P, Ahmed SH, Ku G, Beckman JS, Xu XM, Hsu CY (2001) iNOS and nitrotyrosine expression after spinal cord injury. *J Neurotrauma* 18:523–532