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Abstract

We studied total nitric oxide (nitrite + nitrate) (NO) levels in cerebrospinal fluid (CSF) of chronic spinal diseases in nonsmokers (133 patients: 76 men and 57 women; mean age, 63 years; range, 15-92 years) by the Griess method to clarify the role of NO in different spinal diseases. The extent of compression in terms of numbers of disc level at the compressed spinal nerve and neurological evaluation were also assessed according to the Japanese Orthopaedic Association scores. The spinal diseases included cervical myelopathy and radiculopathy (cervical disease group), ossification of yellow ligament (thoracic disease group), and lumbar disc herniation, lumbar canal stenosis and lumbar spondylolisthesis (lumbar disease group). NO levels in the spinal disease groups (4.98+/-2.28 micromol/l: mean +/- SD) were significantly higher than that in the control group (2.53+/-0.94 micromol/l). An inverse correlation was detected between the elevated levels of NO and the grade of clinical symptoms in the cervical disorders. The number of disc level at the compressed spinal nerve was positively correlated with elevated NO levels in CSF in the cervical and lumbar disorder groups. These results indicate that nerve compression may elevate NO levels in CSF, and that NO concentration in the CSF might be a useful marker of damage to nervous system in spinal disorders.

KEYWORDS: Griess method, Japanese Orthopaedic Association Score(JOA score), magnetic resonance imaging(MRI), biochemistry assay

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Original Article

Concentration of Nitric Oxide (NO) in Spinal Fluid of Chronic Spinal Disease

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We studied total nitric oxide (nitrite + nitrate) (NO) levels in cerebrospinal fluid (CSF) of chronic spinal diseases in nonsmokers (133 patients: 76 men and 57 women; mean age, 63 years; range, 15-92 years) by the Griess method to clarify the role of NO in different spinal diseases. The extent of compression in terms of numbers of disc level at the compressed spinal nerve and neurological evaluation were also assessed according to the Japanese Orthopaedic Association scores. The spinal diseases included cervical myelopathy and radiculopathy (cervical disease group), ossification of yellow ligament (thoracic disease group), and lumbar disc herniation, lumbar canal stenosis and lumbar spondylolisthesis (lumbar disease group). NO levels in the spinal disease groups (4.98 ± 2.28 μmol/l: mean $\pm$ SD) were significantly higher than that in the control group (2.53 ± 0.94 μmol/l). An inverse correlation was detected between the elevated levels of NO and the grade of clinical symptoms in the cervical disorders. The number of disc level at the compressed spinal nerve was positively correlated with elevated NO levels in CSF in the cervical and lumbar disorder groups. These results indicate that nerve compression may elevate NO levels in CSF, and that NO concentration in the CSF might be a useful marker of damage to nervous system in spinal disorders.

Key words: Griess method, Japanese Orthopaedic Association score (JOA score), magnetic resonance imaging (MRI), biochemistry assay

Nitric oxide (NO) is thought to play 3 distinct roles in the central nervous system (CNS). The first is a normal physiological role. It has been reported that NO induces long-term potentiation about the memory via Ca^{2+} activity in the cerebellum and the hippocampus [1-3]. NO also triggers apoptosis of neurons after inducing a decrease in the level of nicotinamide adenine dinucleotide (NAD) [4-6]. On the other hand, some studies have suggested that NO may reduce the apoptosis of neurons [7-9]. NO also appears to play a neurotoxic role [10-12]. NO changes the composition of $ONOO^-$, then directly damages DNA and the lipid membrane of neurons, especially in diseases such as Alzheimer's disease [13-18]. Delayed neurotoxicity, such as ischemic damage, is induced by glutamate neurotoxity through *N*-methyl-d-aspartate (NMDA) receptors [19-21]. In its third role, NO may also exert protective effects against neurotoxity. NO appears to ameliorate inflammation through reactive oxygen species [22-25]. The radical form of NO ($NO\cdot$) is thought to exert neurotoxic potency, while the nitrosonium ion form of NO (NO^+) may act neuroprotectively [26].

The above studies have focused mainly on the role of NO in the CNS; however, there have been few reports

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on the effects of NO on the whole spine, and fewer still on NO in chronic spinal disease. Asahara reported elevated NO levels in the cerebrospinal fluid (CSF) of patients with lumbar spondylosis [27]. In addition, NO has been shown to cause hypersensitivity to pain in the spinal cord [28-31], and is thought to be involved in the pathogenesis of secondary injuries after spinal cord trauma [32, 33].

The aim of the current study was to clarify the role of NO in spinal diseases by comparing NO levels in 3 groups of chronic spinal diseases: myelopathy and radiculopathy (cervical disease group), ossification of yellow ligament (thoracic disease group), and lumbar disc herniation, lumbar canal stenosis and spondylolisthesis (lumbar disease group). In the first stage of the comparative analysis, we mesured NO levels in the CSF of patients with spinal disease and volunteers without neurological symptoms. We then evaluated the relation between NO level and neurological symptoms, extent of compression, and white blood cell (WBC), protein and glucose levels in CSF. We hypothesized that the NO concentration would vary among each of the 3 anatomical levels of the spinal based on the compressive pathology.

Materials and Methods

We analyzed NO levels in the CSF of 133 patients with chronic degenerative spinal disease (76 males and 57 females; mean age, 63 years; range, 15-92 years). All of them had been hospitalized for further examination and underwent lumbar myelography. The authors classified them into 3 groups corresponding to anatomical levels of the spine; cervical, thoracic and lumbar group. The cervical group consisted of 35 patients (21 males and 14 females; mean age, 65 years; range, 42-78 years), the thoracic group of 5 patients (4 males and 1 females; mean age, 61 years; range, 20-76 years) and the lumbar group of 93 patients (51 males and 42 females; mean age, 62 years; range, 15-87 years) (Table 1). The authors evaluated clinical status based on the Japanese Orthopaedic Association score (JOA score), and recorded the number of disc level at the compressed spinal nerve in these patients in the sagittal and axial views using of magnetic resonance imaging (MRI).

As a control, we also analyzed NO levels in a group of 39 patients without neurological symptoms (14 males and 25 females; mean age, 50 years; range, 16-89 years). Patients with inflammatory disease, myocardiac ischemia or hemodialysis were excluded from this study due to the possibile influence of inflammation or medica-

Table I Subjects and patient data

	Patients (Male, Female)	Mean age	JOA score	NO (μmol/l)
I Control group	Total 39 (14, 25)	50 years		2.53 ± 0.94
II Spinal disease group				
Cervical group	Total 35 (21, 14)	65	10.7/17[a]	4.50 ± 1.83**
a) Myelopathy	27 (16, 11)	66	10.0	4.78 ± 1.87**
b) Radiculopathy	8 (5, 3)	62	13.2	3.52 ± 1.38*
Thoracic group	Total 5 (4, 1)	61	5.6/13[a]	3.95 ± 1.13**
Lumbar group	Total 93 (51, 42)	62	7.2/15[a]	5.21 ± 3.68**
a) Lumbar disc herniation	30 (22, 8)	48	7.2	3.74 ± 2.36*
b) Lumbar canal stenosis	30 (17, 13)	71	7.0	6.08 ± 3.94**, ++
c) Spondylolisthesis	33 (12, 21)	66	7.4	5.74 ± 4.09**, +
	Total 133 (76, 57)	63		4.98 ± 2.28

[a]: The maximum JOA scores in controls were 17, 13 and 15 points for the cervical, thoracic, and lumbar groups, reskectively. Values (μmol/l)are the means $\pm$ SD. *$P < 0.05$ and **$P < 0.01$ *vs.* control group ; +$P < 0.01$ and ++$P < 0.05$ *vs.* lumbar disc herniation

tion upon NO concentration in the CSF or serum. Since the NO levels of smokers in the lumbar disease group were significantly higher than those of nonsmokers (see results), we excluded smokers with chronic spinal disease from the present study, and based all our results on the data for nonsmokers. Informed consent for this study was obtained from all subjects.

Collection of CSF. Two 1.5 ml CSF samples were obtained by lumbar puncture during lumbar myelography in the spinal disease group or during lumbar anesthesia in the control group. The first sample was used for the biochemical assay and the latter was used for the NO measurement. The CSF sample was collected mainly from the L4/5 level. The latter samples were immediately cryopreserved at −15 °C until examination.

Biochemical assay. The WBC in the CSF was counted using the table of Fuchs-Rosenthal. Protein in the CSF was measured with a spectrophotometer (U1000; Hitachi Science Systems, Tokyo, Japan) after reacting with radioactive solution (micro TP-test WAKO; Wako Junyaku Kogyo, Tokyo, Japan). Glucose in CSF was measured according to the electrode method (Glucoroder-GXR; A&T, Tokyo, Japan). Normal WBC, protein and glucose levels in the present examination were 0–5 /mm^3, 10–40 mg/dl and 50–75 mg/dl, respectively.

Analysis of nitrogen oxides. The CSF samples were thawed at room temperature. Protein was removed from CSF samples by addition of 0.3N NaOH and 5% $ZnSO_4$ and centrifugation at 12,000 rpm for 10 min. Then, the supernatant fluid was filtrated with a glass filter (SJAPL04NS; Millipore, Tokyo, Japan).

Nitrite (NO_2^-) and nitrate (NO_3^-) were measured by the Griess method [34, 35]. Briefly, our system used an autoanalyzer (Model TCI-NOX 1000; Tokyo Kasei Kogyo, Tokyo, Japan) to conduct automated flow injection analysis as described by Habu [36]. The carrier solution (A7500) and the Griess reagent solutions (A7502 and A7503) (Tokyo Kasei Kogyo, Tokyo, Japan) were prepared just before the experiment and kept at room temperature. Sodium nitrate solution (NOx standard solution; Tokyo Kasei Kogyo) was loaded by means of a sample injector (Model SVI-6U7; Tokyo Kasei Kogyo) before measurement. Nitrate (NO_3^-) was changed to nitrite (NO_2^-) using an A7200 Cd-Cu reduction column (Tokyo Kasei Kogyo). The total NO_2^- reacts with the Griess reagent to form a purple azo compound. The absorbance at 540 nm was measured with a spectrophotometer (Model S/3250; Somakogaku, Tokyo, Japan). NO_2^- measurements were performed twice for each sample.

Analysis. The NO concentrations were measured in patients of the control group, and the mean concentration was determined. The spinal disease patients were divided into 3 groups (a cervical, a thoracic and a lumbar group) according to clinical signs and image analysis. The cervical group was divided into 2 subgroups by clinical status and MRI, a radiculopathy group (neurological signs in one root area n = 8; 5 males and 3 females; mean age, 62 years; range, 48–80 years) and a myelopathy group (neurological signs in the upper or lower extremities and trunk; n = 27; 16 males and 11 females; mean age, 66 years; range, 38–92 years). The thoracic group included only patients with ossification of yellow ligament. The lumbar group was also divided into 3 subgroups; a lumbar disc herniation group (n = 30; 22 males and 8 females; mean age, 48 years; range, 15–79 years), a lumbar canal stenosis group (n = 30; 17 males and 13 females; mean age, 71 years; range, 53–86 years), and a spondylolisthesis group (n = 33; 12 males and 21 females; mean age, 66 years; range, 25–87 years). Patients with lumbar disc herniation show a tension sign and hypoesthesia from root commpression. The main clinical sign of lumbar canal stenosis is intermittent claudication due to ischemia of the cauda during walking. Spondylolisthesis causes lower back pain. The scoring system (17-2) for cervical myelopathy (JOA score) consists of neurologic symptoms (Table 2). The JOA score for thoracic disease uses the scoring system (17-2) for cervical myelopathy, but excludes the upper extremity signs. The JOA score for lumbar disease consists of a pain scale (category I), neurologic symptoms (category II) and the urinary bladder function (category III) (Table 3). The relation between each clinical status and the level of NO was analyzed. The extent of compression was defined as the number of disc level at the compressed spinal nerve visible in the sagittal view on MRI. The levels of NO were compared with the number of WBC, the protein level and the glucose level in CSF.

Statistical Analysis. The values are expressed as the mean ± SD. Statistical analyses were made with the Stat View-J 4.11 program (Inc. Berkeley, CA, USA). Simple regression was used at the NO level in the control group and the relation between NO levels and the JOA score was assessed in the cervical, thoracic and lumbar groups. Welch's or Student's *t*- test was used to

Table 2 Scoring system (17-2) for cervical myelopathy (Japanese Orthopaedic Association)

Category

A. MOTOR FUNCTION

I. Fingers

0 = Unable to feed oneself with any tableware including chopsticks, a spoon or fork, and/or unable to fasten buttons of any size

1 = Can manage to feed oneself with a spoon and/or a fork but not with chopsticks

2 = Either chopstick-feeding or writing is possible but not practiccal, and/or large buttons can be fastened

3 = Either chopstick-feeding or writing is clumsy but practical, and/or cuffbuttons can be fastened

4 = Normal

II. Shoulder and Elbow

Evaluated by MMT score of the deltoid or biceps muscles, whichever is weaker

-2 = MMT 2 or below

-1 = MMT 3

-0.5 = MMT 4

0 = MMT 5

III. Lower Extremity

0 = Unable to stand up and walk by any means

0.5 = Able to stand up but unable to walk

1 = Unable to walk without a cane or other support on a level

1.5 = Able to walk without a support but with a clumsy gait

2 = Walks independently on a level but needs support on stairs

2.5 = Walks independently when going upstairs, but needs support when going downstairs

3 = Capable of fast walking but clumsily

4 = Normal

B. SENSORY FUNCTION

I. Upper Extremity

0 = Complete loss of touch pain sensation

0.5 = 50% or below of normal sensation and/or sever pain or numbness

1 = Over 60% of normal sensation and/or moderate pain or numbness

1.5 = Subjective numbness of a slight degree without any objective sensory deficit

2 = Normal

II. Trunk

0 = Complete loss of touch and pain sensation

0.5 = 50% or below of normal sensation and/or sever pain or numbness

1 = Over 60% of normal sensation and/or moderate pain or numbness

1.5 = Subjective numbness of a slight degree without any objective sensory deficit

2 = Normal

III. Lower Extremity

0 = Complete loss of touch and pain sensation

0.5 = 50% or below of normal sensation and/or sever pain or numbness

1 = Over 60% of normal sensation and/or moderate pain or numbness

1.5 = Subjective numbness of a slight degree without any ogjective sensory deficit

2 = Normal

C. Bladder Function

0 = Urinary retention and/or incontinense

1 = Sense of retention and/or dribbling and/or thin stream and/or incomplete continense

2 = Urinary retardation and/or pollakiuria

3 = Normal

compare NO levels of the control group to those of the spinal disease groups and subgroups. Kruskal-Wallis test and Fisher's protected least significant difference (Fisher's PLSD test) was used for the relation between NO levels and the number of disc level at the compressed spinal nerve in a disease group.

Results

The NO detection limit was 0.1 μmol/l (95% confidence limit) and the inter-assay and intra-assay coefficients of variation in our experiment were 2.0%, respectively. The mean level of NO in the control group was 2.53 ± 0.94 (mean $\pm$ SD) μmol/l. The NO level was 2.47 ± 0.93 μmol/l in males and 2.63 ± 0.99 μmol/l in females. There was no significant gender difference in mean NO levels. Simple regression: NO concentration $= 0.016 \times$ age $+ 1.75$; $P < 0.05$, $r = 0.33$ (Fig. 1).

The mean values of NO in the cervical, thoracic and lumbar groups were 4.50 ± 1.83, 3.95 ± 1.13 and 5.21 ± 3.68 μmol/l, respectively. All these values were significantly higher than those of the control group (Student's t-test, $P < 0.01$). However, there were no significant differences among the cervical, thoracic and lumbar groups (Table 1).

Within the cervical group, NO concentration was lower in the radiculopathy subgroup (3.52 ± 1.38 μmol/l) than in the cervical myelopathy subgroup (4.78 ± 1.87

Table 3 Assessment of treatment for lower back pain from the Japanese Orthopaedic Association

I. Subjective Symptoms	(9 points)	II. Clinical Signs	(6 points)
A. Lower Back Pain		A. Straight-Leg-Raising Test (including tight hamstring)	
a. None	3	a. Normal	2
b. Occasional mild pain	2	b. 30–70 degrees	1
c. Frequent mild or occasional sever pain	1	c. Less than 30 degrees	0
d. Frequent or continuous severe pain	0	B. Sensory Disturbance	
B. Leg Pain and/or Tingling		a. None	
a. None	3	b. Slight disturbance (not subjective)	2
b. Occasional slight symptom	2	c. Marked disturbance	1
c. Frequent slight or occasional sever symptom	1	C. Motor Disturbance (MMT)	0
d. Frequent or continuous severe symptom	0	a. Normal (Grade 5)	
C. Gait		b. Slight weakness (Grade 4)	2
a. Normal	3	c. Marked weakness (Grade 3–0)	1
b. able to walk farther than 500 meters although it results in pain, tingling, and/or muscle weakness	2	III. Urinary Bladder Function	
		a. Normal	(-6 points)
c. unable to walk farther than 500 meters owisg to leg pain, tingling, and/or muscle weakness	1	b. Mild dysuria	0
		c. Severe dysuria	-3
d. Unable to walk farther than 100 meters owing to leg pain, tingling and/or muscle weakness	0	*Incontinence	-6
		*Urinary retention	

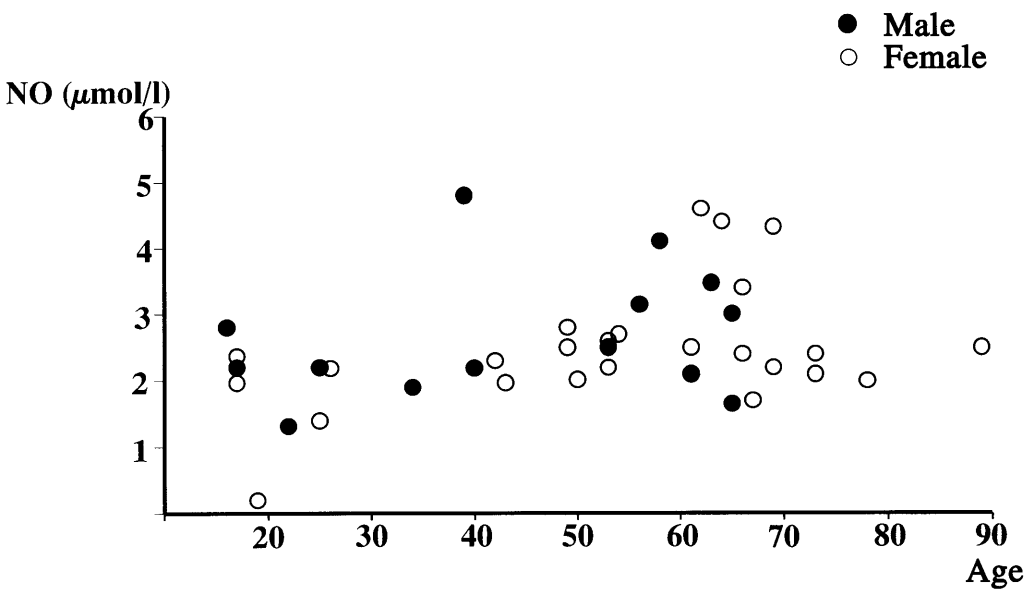

Fig. 1 NO level in the CSF of control group. The mean level of NO in the control group was 2.53 ± 0.94 μmol/l. Simple regression: NO concentration $= 1.75 + 0.016 \times$ age: $P < 0.05$, $r = 0.33$. There was no significant gender difference according to sex.

μmol/l), but not significantly (Table 1). The number of disc level at the compressed spinal nerve correlated positively with the elevated NO concentration. Mean NO concentration was 3.10 ± 1.30 μmol/l in patients with one disc level at the compressed spinal nerve; 3.71 ± 1.39 μmol/l in those with 2 compressing disc levels; 4.75 ± 1.19 μmol/l in those with 3 compressing disc levels; 5.63 ± 0.74 μmol/l in those with 4 compressing disc levels and 7.04 ± 1.46 μmol/l in those with 5 compressing disc level. The NO level in patients with

3, 4, 5 compressing disc levels was significantly higher than that in one compressing disc level, NO level in 4 and 5 compressing disc levels was significantly higher than that in 2 compressing disc levels and NO level in 5 compressing disc levels was significantly higher than in 3 compressing disc levels based on Fisher's PLSD test (Fig. 2). There was an inverse correlation between the level of NO and the JOA score in patients with cervical disease (Fig. 3A). The WBC was 1.9 /mm^3, the protein level was 51.3 mg/dl and the glucose level was 68.2 mg/dl in the cervical group. The density of protein was slightly higher than that of normal. There were no

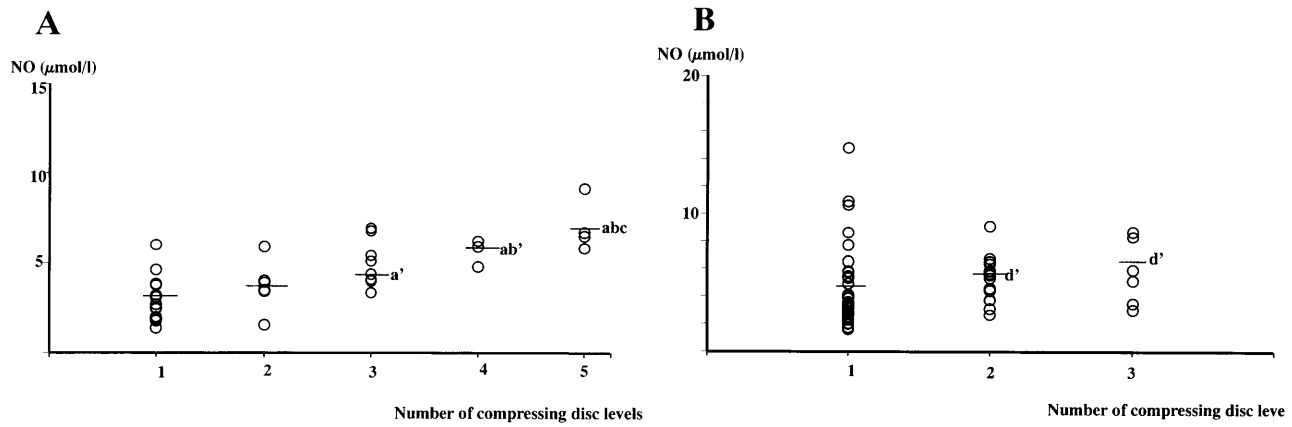

Fig. 2 The relation between NO level in the CSF and the number of disc levels at the compressed spinal nerve in the cervical (**A**) and lumbar (**B**) groups. (**A**) In the cervical group, NO concentration was 3.10 ± 1.30 μmol/l in patients with 1, 3.71 ± 1.39 μmol/l in patients with 2, 4.75 ± 1.19 μmol/l in patients with 3, 5.63 ± 0.74 μmol/l in patients with 4 and 7.04 ± 1.46 μmol/l in patients with 5 compressing disc levels. Fisher's Protected Least Significant Difference, $^{a}P < 0.01$ and $^{a'}P < 0.05$ *vs.* the group with 1 compressing disc level; $^{b}P < 0.01$ and $^{b'}P < 0.05$ *vs.* the group with 2 compressed disc levels; $^{c}P < 0.01$ *vs.* the group with 3 compressing disc levels (Fisher's PLSD test). (**B**) In the lumbar group, NO concentration was 4.41 ± 2.65 μmol/l in patients with 1, 5.75 ± 2.34 μmol/l in patients with 2 and 6.60 ± 3.16 μmol/l in patients with 3 compressing disc levels. Fisher's Protected Least Significant Difference, $^{d'}P < 0.05$ *vs.* 1 compressed disc level.

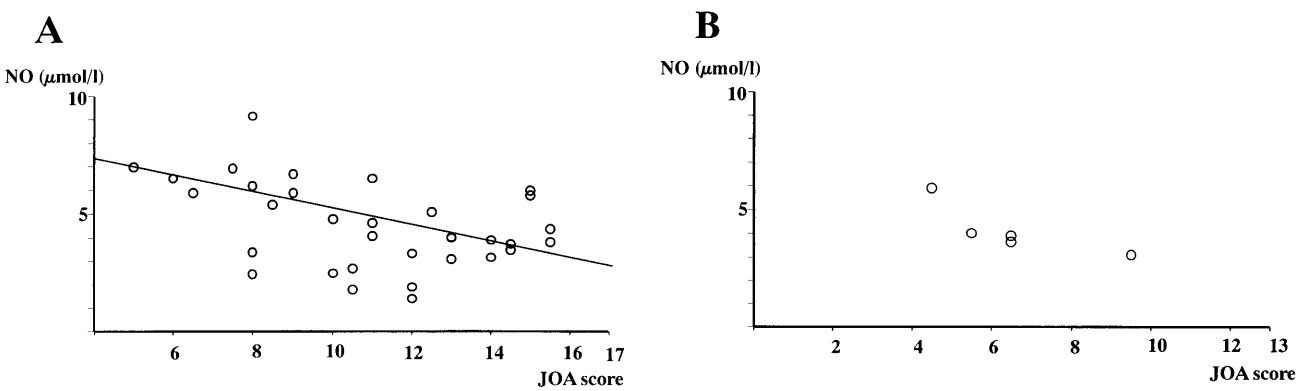

Fig. 3 The relation between NO level in the CSF and JOA score in the cervical (**A**) and thoracic (**B**) groups. (**A**) There was an inverse correlation between the level of NO and the JOA score in the cervical group. NO concentration = 7.30 − 0.25 × JOA score; $P < 0.05$, r = 0.41. (**B**) There was an inverse correlation between NO level and JOA score, but this association did not reach the level of statistical significance. NO concentration = 7.12 − 0.46 × JOA score; $0.05 < P < 0.10$, r = 0.82.

significant differences among the 3 values and NO levels in the cervical group.

In the thoracic group, there was an inverse tendency between the level of NO and the JOA score (Fig. 3B). The WBC was 2.3 /mm^3, the protein level was 81.0 mg/dl and the glucose level was 71 mg/dl. Only protein in the CSF was higher than the normal value. There were no significant differences in the thoracic group in these biochemical indices and NO levels.

In the lumbar group, the NO concentrations of CSF in lumbar canal stenosis (5.63 ± 3.31 μmol/l) and spondylolisthesis (5.43 ± 2.56 μmol/l) were significantly higher than that in lumbar disc herniation (4.07 ± 2.62 μmol/l) (Student's t-test, $P < 0.01$ and $P < 0.05$) (Table 1). The NO concentration was 4.41 ± 2.65 μmol/l in the single disc level at the compressed spinal nerve, 5.75 ± 2.34 μmol/l in the 2 compressing disc levels and 6.60 ± 3.16 μmol/l in the 3 compressing disc levels (Fig. 2). NO levels in patients with 2 or 3 compressing disc levels were also higher than that in patients with one compressing disc level (Fisher's PLSD; $P < 0.05$). The result was that the NO concentration did not correlate with JOA score in patients with lumbar disease. The WBC was 2.4 /mm^3, the protein level 61.0 mg/dl, and the glucose level 67.9 mg/dl in the lumbar group. The protein level was slightly higher than normal. There was no significant correlation between NO level and either WBC, density of protein or desity of glucose in the CSF in the lumbar disease group.

We also compared NO levels in the CSF between smokers and non-smokers of the cervical group (35 nonsmokers and 22 smokers), the thoracic group (5 nonsmokers and 5 smokers) and the lumbar group (93 nonsmokers and 27 smokers). In the cervical group, the mean NO level in the CSF was 4.50 ± 1.83 μmol/l in nonsmokers and 4.90 ± 2.45 μmol/l in smokers. In the thoracic group, the mean NO level in the CSF (3.95 ± 1.13 μmol/l) of the nonsmoker group was lower than that in the smoker group (4.84 ± 0.68 μmol/l). The mean NO level of CSF in the lumbar group was 4.77 ± 2.07 μmol/l in nonsmokers and 6.35 ± 3.56 μmol/l in smokers, and there was a significant difference between the smoker and nonsmoker groups.

Discussion

In 1988, Furchgott first demonstrated NO is an endothelium derived relaxing factor (EDRF) [37-39]. NO is synthesized during the oxidation of L-arginine to L-citrulline by 3 isoforms of NOS: nNOS (neuron-NOS, NOS-1), iNOS (inducible-NOS, NOS-2) and eNOS (endothelium-NOS, NOS-3). eNOS and nNOS are calcium-dependent but iNOS calcium-independent.

The role of iNOS in inflammation has been well defined; the combination of iNOS and inflammation has been shown to cause tissue damage and pain. Activated murine macrophages produce a high level of NO [40]. Because increased levels of NO_2^- and NO_3^- have recently been detected in the serum and airway fluid of patients with rheumatic disease and pneumonia, it is possible that human macrophages can produce NO [41, 42]. Recent reports have also demonstrated increased NO metabolism in the CSF of patients with menigococcal meningitis [43, 44]. Therefore, patients with inflammatory disease were excluded from this study.

NO is known to play physiological, neurotoxic and neuroprotective roles. It is not clear which isoform of NOS is involved in the elevation of NO in the CSF. Recent reports have also demonstrated that iNOS can be induced in motor neurons after spinal root avulsion and that NO may be responsible for the secondary neural injury through perfusion after trauma [45].

There have been few reports about the role of NO in the spinal cord or root. The mechanism of NO production in spinal cord injury is not yet evident. NO is thought to be involved in the pathogenesis of secondary injuries several hours after primary injury in cases of spinal cord trauma. Asahara first reported increased NO metabolism in the CSF in patients with lumbar spondylosis [27]. In this study, we detected elevated NO levels in the CSF of patients with chronic spinal disorders. The mean values of NO in the cervical, thoracic and lumbar groups were significantly larger than that in the control group (Table 1). There were no significant differences in NO levels within the whole spine. Therefore, the mechanism of NO regulation in the spinal cord may be similar to that in the cauda.

The number of disc level at the compressed spinal nerve was positively correlated with the NO concentration. In the cervical group, myelopathy was generally due to spinal cord compression, and radiculopathy was generally due to root compression. Canal stenosis and spondylolisthesis were due to the cauda equina compression, but the root compression was due to disc herniation. The extent of the compression in the nerve system may be one factor of NO production.

At each level of the spine, elevated NO levels were positively correlated with compressive spinal disease in the present study. It has been reported that NO levels in plasma are lower in tobacco smokers [46]. In comparing the resultant ranges of elevated NO and patient histories, an unexpected pattern emerged. Among patients with spinal diseases and especially among patients in the lumbar group, smokers showed greater elevation of NO level than nonsmokers. The findings of this cursory analysis suggest that NO levels in the CSF might be elevated by tobacco smoking, but a detailed analysis of the relevant effects of tobacco use on NO levels in the CSF is well outside the scope of the current study. If gradient levels of NO in the CSF are to be of diagnostic value, then the factors that consistently affect these levels must be identified, and the nature of those effects clarified.

The normal level of NO in human plasma is thought to be 20-30 μmol/l [46, 47]. In the present study, the NO level in serum was higher than that in CSF. This raises the possibility that there may be a barrier similar to the blood brain barrier in the spine. A possible source might be the NO_2^- or NO_3^- in the plasma via permeatation of the tissue membrane.

Several other reports have demonstrated that cNOS is localized in the dorsal horn of spinal cord. This enzyme induces NO production, which acts as a sensory mediator [48, 49]. In the current study, the elevated levels of NO in the cervical and thoracic groups correlated with the grade of neurological deficit; the more severe the symptoms were, the larger the concentration of NO was (Fig. 3). The maximum JOA score in the control group without neurodeficit was 17 points, and the corresponding NO concentration was 3.03 μmol/l. This is the approximate standard value of 2.53 μmol/l of the control group. The compressed CNS cord may regulate cNOS in the posterolateral segments, and might induce elevation of the NO level.

NO plays a role in long-term potentiation by the Ca^{2+} activity in cerebellum and hippocampus, and this potentiation causes hypersensitivity for pain in the spinal cord [27-30]. In the current study, we were unable to detect any significant correlation between NO level and pain scale (category I of JOA score (Table 3)). We could not detect the acerbation of pain status by induced NO. On the other hand, one of the factors related to pain in lumbar disc herniation is said to be inflammation by the chemical factors, IgG, phospholipase A2 or prostaglandin E2 [50-52]. Tamada reported the relation between tension sign and free radicals in the compressed nerve root [53]. In patients with lumbar disc herniation, NO elevation may derive from iNOS production. The pathology of canal stenosis and spondylolisthesis differs from that of disc herniation, and the onset mechanisms differ among 3 pathologies (root compression, cauda compression and spinal instability) in cases of lumbar spine. The symptoms in these disorders are quite different. The authors have concluded that JOA score may not be discriminating enough for clinical assesment of pain for comparative studies of different disorders. On the other hand, in cases of cervical and thoracic spinal disease, we believe that JOA score is a good indicator of neurological signs.

Elevated NO levels in CSF can be used as a reliable marker of extent of neuron damage in clinical applications.

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References

1. Shibuki K and Okada D: Endogenous nitric oxide release required for long-term synaptic depression in the cerebellum. Nature (1991) **349**, 326-328.
2. Ito M and Karachot L: Protein kinases and phosphatase inhibitors mediating long-term desensitization of glutamate receptors in cerebellar Purkinje cells. Neurosci Res (1992) **14**, 27-38.
3. Zorumski CF and Izumi Y: Nitric oxide and hippocampal synaptic plasticity. Biochem Pharmacol (1993) **46**, 777-785.
4. Kruman I, Bruce-Keller AJ, Bredesen D, Waeg G and Mattson MP: Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis. J Neurosci (1997) **17**, 5089-5100.
5. Kiedrowski L, Manev H, Costa E and Wroblewski JT: Inhibition of glutamate-induced cell death by sodium nitroprusside is not mediated by nitric oxide. Neuropharmacology (1991) **30**, 1241-1243.
6. Bonfoco E, Krainc D, Ankarcrona M, Nicotera P and Lipton SA: Apoptosis and necrosis: Two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures. Proc Natl Acad Sci USA (1995) **92**, 7162-7166.
7. Mannick JB, Asano K, Izumi K, Kieff E and Stamler JS: Nitric oxide produced by human B lymphocytes inhibits apoptosis and Epstein-Barr virus reactivation. Cell (1994) **79**, 1137-1146.
8. Melino G, Bernassola F, Knight RA, Corasaniti MT, Nistico G and Finazzi-Agro A: S-nitrosylation regulates apoptosis. Nature (1997) **388**, 432-433.
9. Lei SZ, Pan ZH, Aggarwal SK, Chen HS, Hartman J, Sucher NJ and Lipton SA: Effect of nitric oxide production on the redox modulatory site of the NMDA receptor-channel complex. Neuron (1992) **8**, 1087-1099.
10. Li P, Tong C, Eisenach JC and Figueroa JP: NMDA causes release of nitric oxide from rat spinal cord in vitro. Brain Res (1994) **637**, 287-291.
11. Kitto KF, Haley JE and Wilcox GL: Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. Neurosci Lett (1992)

148, 1-5.

12. Kolhekar R, Meller ST and Gebhart GF: Characterization of role of spinal N-methyl-D-aspartate receptors in thermal nociception in the rat. Neuroscience (1993) **57**, 385-395.
13. Ando Y, Nyhlin N, Suhr O, Holmgren G, Uchida K, el Sahly M, Yamashita T, Terasaki H, Nakamura M, Uchino M and Ando M: Oxidative stress is found in amyloid deposits in systemic amyloidosis. Biochem Biophys Res Commun (1997) **232**, 497-502.
14. Smith MA, Perry G, Richey PL, Sayre LM, Anderson VE, Beal MF and Kowall N: Oxidative damage in Alzheimer's. Nature (1996) **382**, 120-121.
15. Montine KS, Olson SJ, Amarnath V, Whetsell WO Jr, Graham DG and Montine TJ: Immunohistochemical detection of 4-hydroxy-2-nonenal adducts in Alzheimer's disease is associated with inheritance of APOE4. Am J Pathol (1997) **150**, 437-443.
16. Lovell MA, Ehmann WD, Mattson MP and Markesberry WR: Elevated 4-hydroxynonenal in ventricular fluid in Alzheimer's disease. Neurobiol Aging (1997) **18**, 457-461.
17. Calingasan NY, Uchida K and Gibson GE: Protein-bound acrolein: A novel marker of oxidative stress in Alzheimer's disease. J Neurochem (1999) **72**, 751-756.
18. Mattson MP, Fu W, Waeg G and Uchida K: 4-Hydroxynonenal, a product of lipid peroxidation, inhibits dephosphorylation of the microtubule-associated protein tau. Neuroreport (1997) **8**, 2275-2281.
19. Choi DW: Calcium-mediated neurotoxicity: Relationship to specific channel types and role in ischemic damage. Trends Neurosci. (1988) **11**, 465-469.
20. Meldrum B and Garthwaite J: Excitatory amino acid neurotoxicity and neurodegenerative disease. Trends Pharmacol Sci (1990) **11**, 379-387.
21. Hartley DM and Choi DW: Delayed rescue of N-methyl-D-aspartate receptor-mediated neuronal injury in cortical culture. J Pharmacol Exp Ther (1989) **250**, 752-758.
22. Wink DA, Hanbauer I, Krishna MC, DeGraff W, Gamson J and Mitchell JB: Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. Proc Natl Acad Sci USA (1993) **90**, 9813-9817.
23. Nguyen BL, Saitoh M and Ware JA: Interaction of nitric oxide and cGMP with signal transduction in activated platelets. Am J Physiol (1991) **261**, H1043-H1052.
24. Kubes P, Suzuki M and Granger DN: Nitric oxide: An endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci USA (1991) **88**, 4651-4655.
25. Nakamura A, Komoda T, Atsumi M and Shiomi H: A role of the nitric oxide -cyclic GMP pathway in hyperalgesic effects of inflammatory mediators. Jpn J Pharmacol (1994) **64** (Suppl), 93P.
26. Lipton SA, Choi YB, Pan ZH, Lei SZ, Chen HS, Sucher NJ, Loscalzo J, Singel DJ and Stamler JS: A redox-based mechanism for the neuroprotective and neurodestructive effects of nitric oxide and related nitroso-compounds. Nature (1993) **364**, 626-632.
27. Asahara H, Yokoi I, Tamada T, Kabuto H, Ogawa N, Mori A and Inoue H: Increased cerebrospinal fluid nitrite and nitrate levels in patients with lumbar spondylosis. Res Commun Mol Pathol and Pharmacol (1996) **91**, 77-83.
28. Meller ST and Gebhart GF: Nitric Oxide (NO) and nociceptive processing in the spinal cord. Pain (1993) **52**, 127-136.
29. Kitto KF, Haley JE and Wilcox GL: Involvement of nitric oxide in spinally mediated hyperalgesia in the mouse. Neurosci Lett (1992) **148**, 1-5.
30. Malmberg AB and Yaksh TL: Spinal nitric oxide synthesis inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test in rats. Pain (1993) **54**, 291-300.
31. Haley JE, Dickenson AH and Schachter M: Electrophysiological evidence for a role of nitric oxide in prolonged chemical nociception in the rat. Neuropharmacology (1992) **31**, 251-258.
32. Matsuyama Y, Sato K, Kamiya M, Yano J, Iwata H and Isobe K: Nitric oxide: A possible etiologic factor in spinal cord cavitation. J Spinal Disord (1998) **11**, 248-252.
33. Sharma HS, Westman J, Olsson Y and Alm P: Involvement of nitric oxide in acute spinal cord injury: An immunocytochemical study using light and electron microscopy in the rat. Neurosci Res (1996) **24**, 373-384.
34. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS and Tannenbaum SR: Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem (1982) **126**, 131-138.
35. Shintani F, Kanba S, Nakaki T, Sato K, Yagi G, Kato R, and Asai M: Measurement by *in vivo* brain microdialysis of nitric oxide release in the rat cerebellum. J Psychiatry Neurosci (1994) **19**, 217-221.
36. Habu H, Yokoi I, Kabuto H and Mori A: Application of automated flow injection analysis to determine nitrite and nitrate in mouse brain. Neuroreport (1994) **5**, 1571-1573.
37. Furchgott RF and Zawadzki JV: The obligatory role endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature (1980) **288**, 373-376.
38. Furchgott RF: Role of endothelium in responses of vascular smooth muscle. Circ Res (1983) **53**, 557-573.
39. Furchgott RF: Studies on relaxation of rabbit aorta by sodium nitrate: The basis for the proposal that the acid-activatable inhibitory factor from bovine retractor penis is inorganic nitrite and the endothelium-derived relaxing factor is nitric oxide; in Vasodilatation, Vanhoutte PM ed, Raven Press, New York (1988) pp401-414.
40. Sakai N and Milstien S: Availability of tetrahydrobiopterin is not a factor in the inability to detect nitric oxide production by human macrophages. Biochem Biophys Res Commun (1993) **193**, 378-383.
41. Farrell AJ, Blake DR, Palmer RM and Moncada S: Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann Rheum Dis (1992) **51**, 1219-1222.
42. Gaston B, Reilly J, Drazen JM, Fackler J, Ramdev P, Arnelle D, Mullins ME, Sugarbaker DJ, Chee C, Singel DJ, Loscalzo J and Stamler JS: Endogenous nitrogen oxides and bronchodilator S-nitrosothiols in human airways. Proc Natl Acad Sci USA (1993) **90**, 10957-10961.
43. Milstien S, Sakai N, Brew BJ, Krieger C, Viekers JH, Saito K and Heyes MP: Cerebrospinal fluid nitrite/nitrate levels in neurologic diseases. J Neurochem (1994) **63**, 1178-1180.
44. Visser JJ, Scholten RJ and Hoekman K: Nitric oxide synthesis in meningococcal meningitis. Ann Intern Med (1994) **120**, 345-346.
45. Wu W and Li L: Inhibition of nitric oxide synthase reduces motoneuron death due to spinal root avulsion. Neurosci Lett (1993) **153**, 121-124.
46. Node K, Kitakaze M, Yoshikawa H, Kosaka H and Hori M: Reversible reduction in plasma concentration of nitric oxide induced by cigarette smoking in young adults. Am J Cardiol (1997) **79**, 1538-41.
47. Miyasaka N: NO and inflammation: With regard to inflammatory arthritides. Experimental Medicine (1995) **13**, 114-117.
48. Morris R, Southam E, Braid DJ and Garthwaite J: Nitric oxide may act as a messenger between dorsal root ganglion neurones and their satellite cells. Neurosci Lett (1992) **137**, 29-32.
49. Valtschanoff JG, Weinberg RJ, Rustioni A and Schmidt HH: Nitric oxide synthase and GABA colocalize in lamina IIof rat spinal cord. Neurosci Lett (1992) **148**, 6-10.
50. Pennington JB, McCarron RF and Laros GS: Indentification of IgG in

the canine intervertebral disc. Spine (1988) **13**, 909-912.
51. Saal JS, Franson RC, Dobrow R, Saal JA, White AH and Goldthwaite N: High levels of inflammatory phospholipase A2 activity in lumbar disc herniations. Spine (1990) **15**, 674-678.
52. O'Donnell JL and O'Donnell AL: Prostaglandin E2 content in herniated lumbar disc disease. Spine (1996) **21**, 1653-1656.
53. Tamada T, Inoue H and Mori A: Superoxide dismutase activity in cerebrospinal fluid and its relation to compression of the lumbosacral nerve root. Acta Med Okayama (1996) **50**, 197-201.