Naloxone lowers cerebrospinal fluid levels of excitatory amino acids after thoracoabdominal aortic surgery

Takashi Kunihara, MD, PhD,[a] Kenji Matsuzaki, MD,[a] Norihiko Shiiya, MD, PhD,[a] Yasuaki Saijo, MD, PhD,[b] and Keishu Yasuda, MD, PhD,[a] *Sapporo, Japan*

Objective: **Although naloxone has been used to prevent ischemic spinal cord injury (SCI), its effect on excitatory amino acids (EAAs) has not been understood. We investigated the clinical significance of naloxone by measuring EAAs in the cerebrospinal fluid (CSF) in patients undergoing thoracoabdominal aortic surgery.**

Methods and subjects: **Twenty-seven patients (15 men and 12 women; mean age, 66 ± 12 years) undergoing prosthetic replacement of the thoracoabdominal aorta (n = 19) or the descending thoracic aorta (n = 8) from April 1997 to June 2003 under distal perfusion and mild hypothermia were enrolled in this cohort study with historical controls. Their etiology was 7 dissections and 20 nondissections. In 16 patients (naloxone group), intravenous infusion of naloxone (1 μg/kg/h) was continued until the patients became alert. In the remaining 11 patients (control group) naloxone was not given. CSF drainage was used in all patients. CSF levels of EAAs, glutamate, aspartate, and glycine were measured at 6 points in time until 72 hours postoperatively, using a high-performance liquid chromatography method.**

Results: **In 5 patients with SCI (2 patients in control group, 3 in naloxone group), CSF levels of glutamate and glycine continued to increase even at 72 hours postoperatively, and were significantly more elevated than those in patients without SCI ($P < .0001$, glutamate; $P = .0006$, glycine). Postoperative maximum levels of CSF glutamate and glycine were also significantly higher in patients with postoperative SCI than in patients without SCI (glutamate: 215.3% ± 158.6% vs 32.9% ± 37.3% increase from baseline, $P < .0001$; glycine: 309.1% ± 218.2% vs 89.2% ± 103.1% increase from baseline, $P = .0036$). CSF levels of glutamate and aspartate in naloxone group were significantly lower than those in control group ($P = .0161$, glutamate; $P < .0001$, aspartate). Postoperative maximum level of CSF aspartate was also significantly lower in the naloxone group than in the control group (8.3% ± 75.5% vs 119.7% ± 120.6% increase from baseline, $P = .0077$). In multivariate logistic regression analysis, postoperative maximum CSF glutamate >100% from baseline ($P < .001$) and postoperative maximum level of CSF glycine ($P = .005$) were identified as the independent risk factors for SCI. Both SCI ($P < .001$) and postoperative maximum level of CSF glycine ($P = .005$) were the independent predictors for postoperative maximum level of CSF glutamate >100% from baseline.**

Conclusions: **CSF levels of EAAs are elevated in patients with SCI. CSF glutamate is the strongest independent predictor of SCI. Naloxone is effective in lowering CSF levels of EAAs. (J Vasc Surg 2004;40:681-90.)**

Although a large number of experimental works and technical advances have concentrated on improving outcome in thoracoabdominal aortic surgery, spinal cord injury (SCI) still remains the most formidable morbidity to be resolved. Cerebrospinal fluid (CSF) drainage may be one of the most promising procedures to prevent SCI. Acher and associates[1] have reported that combined use of CSF drainage (CSFD) and naloxone hydrochloride (naloxone), an opioid receptor antagonist, reduces the risk of SCI in patients undergoing thoracoabdominal aortic surgery. Recently, CSFD itself proved to be beneficial in reducing the risk of SCI after thoracoabdominal aortic surgery in a prospective randomized clinical trial.[2] However, it has not been investigated whether naloxone alone can, like CSFD, attenuate the risk of SCI clinically.

The mechanisms underlying neurologic injury subsequent to ischemia appear to be multifactorial. The discovery of increased cerebral extracellular concentrations of glutamate and aspartate related to ischemia has triggered enormous interest in this phenomenon.[3] Generally, excessive synaptic accumulation of glutamate during ischemia is associated with neuronal cell damage, which is mediated through overactivation of glutamate receptor followed by excessive influx of calcium. This is the current excitotoxic concept of ischemia-induced neuronal cell death.[4] Neurotransmitter amino acids including glutamate, aspartate, glycine, gamma-aminobutyric acid, and taurine are known as excitatory amino acids (EAAs). Much evidence has suggested that the most changes in EAAs occur selectively in the extracellular space where neuroactive drugs are available.[4] Therefore, EAA-mediated excitotoxicity provides important therapeutic implications for neuroprotective approach in patients suffering from ischemia. Recently, EAA activities in the CSF have been investigated in patients with ischemic insult because it is easier and simpler to obtain CSF than extracellular fluid (ECF) in clinical cases.[5-7] Ele-

From the Department of Cardiovascular Surgery, Hokkaido University Hospital,[a] and the Department of Public Health, Hokkaido University Graduate School of Medicine.[b]

Competition of interest: none.

Additional material for this article may be found online at www.mosby.com/jvs.

Reprint requests: Takashi Kunihara, MD, PhD, Department of Cardiovascular Surgery, Hokkaido University Hospital, N14, W5, Kita-ku, Sapporo, Japan 060-8648 (e-mail: kunihara@med.hokudai.ac.jp).

0741-5214/$30.00

doi:10.1016/j.jvs.2004.07.005

Table I. Patient characteristics

Characteristics	*Group*		P
	Naloxone (n = 16)	*Control (n = 11)*	
Age (y)	69.9 ± 6.7	60.8 ± 16.4	.0556
Gender (male)	10 (62%)	5 (45%)	.3811
Etiology (dissection)	4 (25%)	3 (27%)	.8947
Emergent/urgent operation	0	2 (18%)	.0763
Previous aortic surgery	4 (25%)	3 (27%)	.8947
Extent (thoracoabdominal aorta)	10 (63%)	9 (82%)	.2801
Crawford classification (I/II/III)	5/1/4	2/3/4	.3264
Involved intercostal/lumbar arteries	6.8 ± 3.4	8.0 ± 3.8	.4051
Reattached intercostal/lumbar arteries	2.6 ± 1.5	3.4 ± 1.6	.1857
Total amount of CSFD (mL)	269 ± 251	201 ± 200	.4756
Daily amount of CSFD (mL/d)	88 ± 57	64 ± 60	.3214
Duration of operation (min)	447 ± 228	554 ± 238	.2559
Duration of CPB (min)	119 ± 56	133 ± 75	.5805
Duration of AXC (min)	119 ± 56	113 ± 64	.8313
The lowest rectal temperature (°C)	33.7 ± 1.0	34.1 ± 1.0	.3700
Hospital death	1 (6%)	0	.3981
Spinal cord injury	3 (18%)	2 (18%)	.9702

vated levels of EAAs in the CSF have been reported to be associated with ischemic neurologic insults.[5-7] In the field of thoracoabdominal aortic surgery, it has also been reported that patients with postoperative SCI manifested elevated levels of EAAs in the CSF.[8] Therefore, it is reasonable to speculate that in Acher et al's study,[1] both CSFD and naloxone contributed to a reduction in the risk of SCI by attenuating EAAs in the CSF in addition to reducing CSF pressure.

Although it has been shown that high concentration of naloxone attenuates N-methyl-D-aspartate (NMDA)–mediated neurotoxicity in an animal model,[9] there has been no report to demonstrate that naloxone reduces CSF EAA levels in patients suffering spinal cord ischemia or reperfusion caused by temporal aortic cross-clamping (AXC). Furthermore, little is known about the alterations of CSF EAA levels up to 72 hours after operation, which may interest investigators since neurotoxicity mediated by EAAs is supposed to be a late-onset process.[10-12]

The purpose of this study is to elucidate the alterations of EAA levels in the CSF up to 72 postoperative hours and to test the hypothesis that naloxone given intravenously attenuates EAA levels in the CSF in patients undergoing descending thoracic or thoracoabdominal aortic surgery.

PATIENTS AND METHODS

Twenty-seven patients who underwent prosthetic replacement of the descending thoracic or the thoracoabdominal aorta and CSFD at Hokkaido University Hospital between April 1997 and June 2003 were enrolled in this study. CSFD was approved by the medical ethics committee of Hokkaido University, and a written informed consent was given by all patients. There were 15 male and 12 female subjects, and their ages ranged from 26 to 79 (mean, 66 ± 12). The etiology of disease was dissection for 7 (including 3 Marfan syndrome) and nondissection degenerative disease for 20 patients. Twenty-five patients underwent elective operation, while 2 patients underwent nonelective operation for contained or impending rupture (the former developed paraplegia). Eight patients had aortic diseases in the descending thoracic aorta and 19 patients in the thoracoabdominal aorta (Crawford type I for 7 patients, type II for 4, and type III for 8). Seven patients had undergone previous aortic surgery—from the aortic root to the aortic arch in 2 patients, the distal aortic arch in 2, the abdominal aorta in 2, and the abdominal aorta followed by the aortic arch in 1. As a method to protect the spinal cord, continuous intravenous infusion of naloxone at a rate of 1μg/kg/h was used in the last 16 patients after April 2000. For the analysis of EAAs, the patients were divided into 2 groups according to naloxone administration: (1) the control group consisted of the patients who received no naloxone treatment (n = 11), and (2) the naloxone group consisted of patients who were given naloxone as described previously (n = 16). Characteristics of the patients in each group are demonstrated in Table I. In the naloxone group, mean age was older than in the control group, but there was no significant difference between the 2 groups in patient characteristics except the use of naloxone.

Technique of operation. Except for the use of naloxone, all patients received the same protocol of anesthesia with neuromuscular blockade and intermittent intravenous injection of fentanyl citrate. Separate bronchial ventilation was employed and the left lung was collapsed during the operative procedure. Operations were performed through a left posterolateral thoracotomy, with or without a thoracoabdominal incision. All patients underwent prosthetic replacement of the diseased aorta; 7.3 ± 3.6 (range, 2 to 15) pairs of the intercostal or lumbar arteries were involved in the diseased aortic segments, and 2.9 ± 1.5 (range, 0 to 6) pairs of them were reattached by using evoked spinal cord potential guidance. Partial femorofemoral (F-F) cardiopulmonary bypass (CPB) with mild hypothermia (lowest rectal temperature, 33.9 ± 1.0°C) was employed in all patients.

Because of a report demonstrating that hypothermia decreases the release of EAAs, patients subjected to deep hypothermic operation were excluded.[13,14] Initial flow of F-F bypass was set at 1.8 L/min/body surface area (m^2), and sequential clamp technique was applied as far as possible. When reconstruction of visceral branches was required, each branch was cannulated with a balloon catheter and perfused at a rate of 150mL/min until it was reattached to the graft.

The equipment of CPB was completely heparin-coated (Hepaface; Terumo, Tokyo, Japan) and consisted of a centrifugal pump (Medtronic Bio-Medicus Inc, Eden Prairie, Minn) and a membrane oxygenator (Capiox SX-18; Terumo). This system was primed with lactated Ringer's solution and 5 mg/kg of betamethasone. The same dose of betamethasone was also given intravenously before initiation of CPB. In patients undergoing F-F bypass with cardiotomy sucker (n = 22), 2mg/kg of heparin was used, while 1mg/kg of heparin was given to patients undergoing F-F bypass that was constructed by a closed system without a cardiotomy reservoir (n = 5). A centrifugal cell-saving device (Cell Saver 5; Haemonetics, Braintree, Mass) was used in all patients during the operation. Postoperatively shed mediastinal blood was discarded.

Cerebrospinal fluid drainage and measurement of EAAs. Lumbar CSFD was performed through a 16-gauge in-dwelling catheter, which was inserted into the L4/5 intervertebral space after induction of the anesthesia. CSF was allowed to freely drain if CSF pressure exceeded 13-cm H_2O. CSFD was started just after the induction of anesthesia and was continued until 72 hours after surgery unless no CSF was drained. Samples of CSF were collected just before operation and 0, 12, 24, 48, and 72 hours after operation. The CSF samples were centrifuged and the supernatant was immediately frozen at −80°C for the later measurements. CSF levels of 3 EAAs—glutamate, aspartate, and glycine—were measured by high-performance liquid chromatography (JLC-500V; Nihon Denshi, Ltd, Tokyo, Japan).

Statistical analysis. All values are expressed as mean ± standard deviation. Statistical analysis was performed with the StatView 5.0 program (SAS Institute Inc, Cary, NC) except for logistic regression analysis, which was performed with SPSS (SPSS, Inc, Chicago, Ill). Repeated-measures analysis of variance was used for comparisons of EAA levels between the groups. The Student *t* test was used for comparison of the continuous variables and the χ^2 test was used for comparison of frequencies between the groups. A *P* value <.05 was considered statistically significant.

RESULTS

There was 1 hospital death in the control group due to small intestinal necrosis caused by dissection of the superior mesenteric artery 166 days after the operation for nondissecting thoracoabdominal aortic aneurysm. This event had no effect on the results of this study, since the early postoperative course of this patient was uneventful and dissection occurred 37 days after the operation.

Postoperative SCI occurred in 5 patients (1 with paraplegia and 2 with paraparesis in the naloxone group, 1 with paraplegia and 1 with paraparesis in the control group). One of the paraplegic patients in the control group also had multiple cerebral infarctions in the left hemisphere that resulted in right hemiparesis. This patient underwent an emergent operation for contained rupture of the descending thoracic aortic aneurysm. Spinal cord functions of the 2 patients with paraparesis in the control group recovered almost completely within 2 months. Of these patients, 2 had nondissection degenerative aneurysms on the descending thoracic aorta and 2 on the thoracoabdominal aorta (type I and III); the remaining patient with chronic dissection underwent replacement of the descending thoracic aorta. Neither hospital mortality nor incidence of postoperative SCI was significantly different between the 2 groups. No other neurologic morbidity was experienced.

There were no complications related to CSFD. Mean duration of postoperative CSFD and total volume of CSFD in all patients were 2.8 ± 1.1 days and 238 ± 227 mL (77 ± 58 mL/d). Both the total and daily volume of CSFD was similar between the 2 groups (Table I). Mean duration of operation, CPB, and AXC in all patients were 492 ± 234, 125 ± 64, and 117 ± 58 min, respectively. There was also no significant difference in these intraoperative data between the 2 groups (Table I).

In 5 patients with postoperative SCI, glutamate and glycine levels in the CSF kept increasing even at 72 hours after the operation and were significantly higher than those in patients without SCI ($P < .0001$, glutamate; $P = .0006$, glycine; Figs 1 and 2). CSF aspartate in patients with postoperative SCI peaked at 12 hours postoperatively and decreased gradually up to 72 hours after the operation. The level seemed higher than those in patients without SCI, although this difference was not significant ($P = .3894$; Fig 3). Postoperative maximum levels of CSF glutamate and glycine were also significantly higher in patients who suffered from postoperative SCI than in those without SCI (glutamate, 215.3% ±158.6% vs 32.9% ± 37.3% increase from baseline, $P < .0001$; glycine, 309.1% ± 218.2% vs 89.2% ± 103.1% increase from baseline, $P = .0036$; Fig 4). Postoperative maximum level of CSF aspartate was also higher in patients with postoperative SCI than the rest (84.8% ± 97.4% vs 43.1% ± 111.3% increase from baseline), although it did not have statistical power (Fig 4).

In patients with naloxone treatment, CSF glutamate and aspartate peaked at 48 and 24 hours after the operation, respectively, followed by a gradual decrease. CSF aspartate returned to baseline level at 72 hours after surgery. These 2 EAA levels in the CSF were significantly higher in the control group than in the naloxone group ($P = .0161$ for glutamate and $P < .0001$ for aspartate; Figs 5 and 6). CSF glycine level in both groups kept increasing even at 72 hours after the operation, although there was no significant difference between the groups ($P = .3629$, Fig 7). Postoperative maximum level of CSF aspartate was also significantly lower in patients who underwent naloxone treatment than in those given no naloxone (8.3% ±

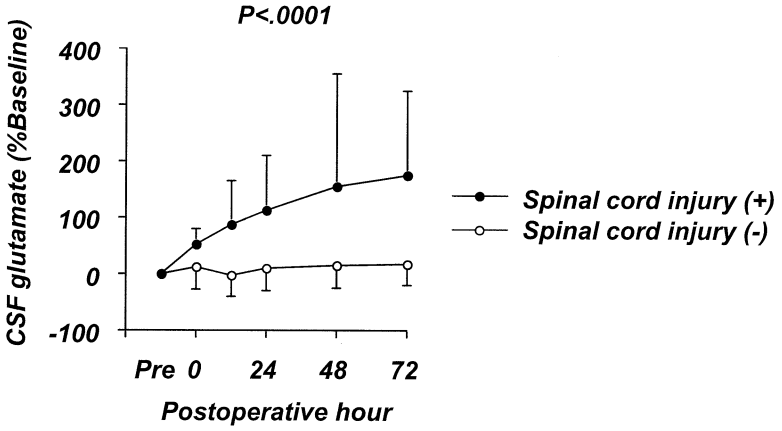

Fig 1. Time course of glutamate levels in the cerebrospinal fluid (*CSF*) in patients with or without postoperative spinal cord injury. All figure error bars represent standard deviation. All data are presented by percent change from baseline.

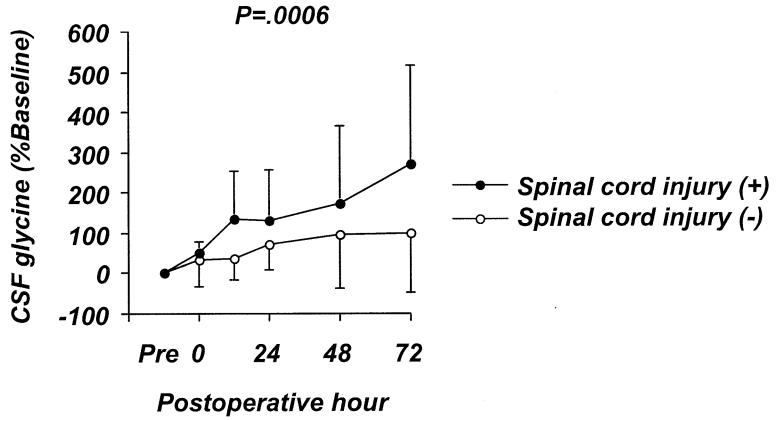

Fig 2. Time course of aspartate levels in the cerebrospinal fluid (*CSF*) in patients with or without postoperative spinal cord injury.

75.5% vs 119.7% ± 120.6% increase from baseline, P = .0077; Fig. 8). The same trend was found in glutamate and glycine, but they did not reach statistical difference (glutamate, 50.1% ± 43.8% vs 80.1% ± 142.5% increase from baseline, P = .4495; glycine, 119.4% ± 118.0% vs 131.9% ± 189.1% increase from baseline, P = .8398; Fig 8). Table II includes original data for CSF EAAs in patients with or without postoperative SCI or naloxone treatment.

To clarify the independent risk factors for SCI or elevation of CSF EAAs, logistic regression analysis was performed. A P value <.20 in univariate analysis was defined for selecting variables for entry into the multivariate model. (See Appendix, online only, for variables used in analysis). In multivariate analysis, postoperative maximum levels of CSF glutamate >100% from baseline (more than twice as large as preoperative values) and postoperative maximum level of CSF glycine were identified as the significant independent risk factors for SCI (Table III). When postoperative maximum levels of CSF EAAs >100% from baseline were defined as the outcome event, no significant independent risk factors were found for aspartate and glycine. However, both SCI and postoperative maximum level of CSF glycine were significant predictors for glutamate. Especially since postoperative maximum levels of CSF glutamate for all patients with SCI were larger than 100% from baseline, SCI and maximum levels of CSF glutamate >100% proved to be the strongest predictor of each other.

DISCUSSION

In the current study, we confirmed that elevated levels of EAAs in the CSF were associated with postoperative SCI for at least 72 postoperative hours. This result not only confirmed a previous report by Brock and colleagues[8] but also disclosed alterations of CSF EAA levels until 72 hours after thoracoabdominal aortic surgery. Furthermore, we were able to clearly demonstrate that naloxone dramatically reduced CSF EAA levels in patients who underwent spinal

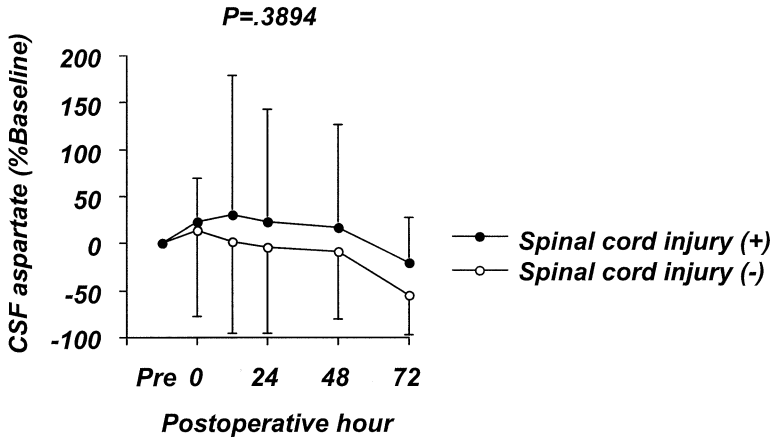

Fig 3. Time course of glycine levels in the cerebrospinal fluid (*CSF*) in patients with or without postoperative spinal cord injury.

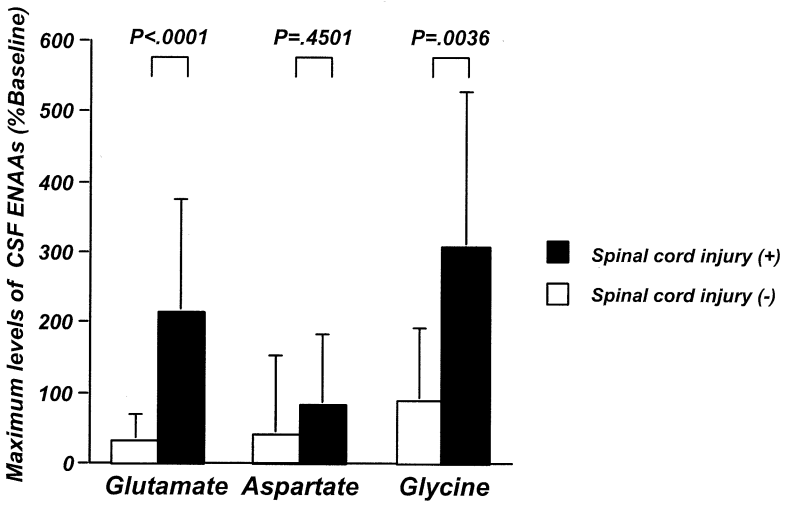

Fig 4. Postoperative maximum levels of *CSF* EAAs for patients with and without spinal cord injury. All data are presented by percent change from baseline.

cord ischemia and reperfusion. To our knowledge, this is the first report to demonstrate that intravenous naloxone reduces CSF EAA levels in patients undergoing thoracoabdominal aortic surgery.

The alteration of EAAs in the CSF of patients with brain injury such as ischemic stroke,[5] traumatic injury,[6] and subarachnoid hemorrhage,[7] or degenerative diseases such as amyotrophic lateral sclerosis[15] and Alzheimer's disease[16] are well documented. However, little is known about the production of EAAs in the CSF during the process of spinal cord ischemia/reperfusion due to thoracoabdominal aortic surgery.[8] Sustained release of glutamate has been observed for several days following traumatic brain injury[17] or massive cerebral infarction.[18] In our findings, glutamate and glycine levels in the CSF in patients with postoperative SCI kept on increasing even at 72 hours after operation. Recently, excitotoxicity has been presumed to be a delayed process.[10,11] It has been reported that glutamate receptor antagonist administered after the ischemic insult could provide neuronal protection.[10,12] Our results suggest that patients subjected to thoracoabdominal surgery are always faced with the risk of delayed onset SCI mediated by excitotoxicity.

After first report by Benveniste and colleagues[3] in 1984 of increased extracellular concentrations of glutamate and aspartate during ischemia, much evidence has supported the idea that several mechanisms and multiple subcellular origins are involved in this phenomenon.[4] Ischemia-induced increase in ECF glutamate can mainly originate from Ca^{2+}-dependent release[19] (ie, neuronal origin) and Ca^{2+}-independent release[20] (ie, metabolic origin). Recent studies have supported the fact that both magnitude and

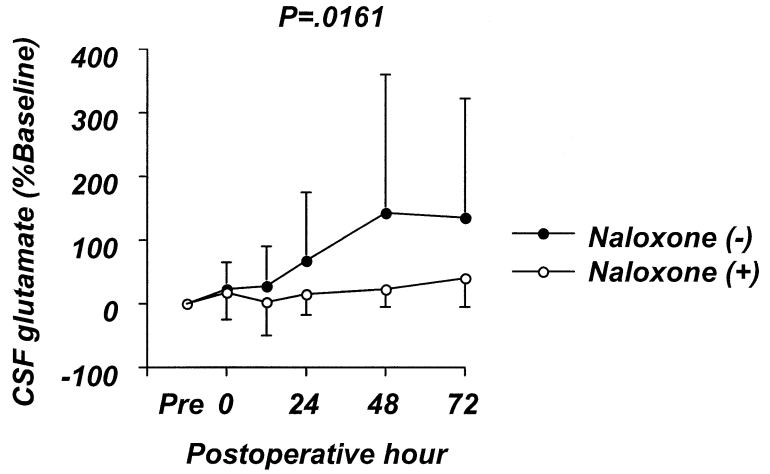

Fig 5. Time course of glutamate levels in the cerebrospinal fluid (*CSF*) in patients given naloxone or not.

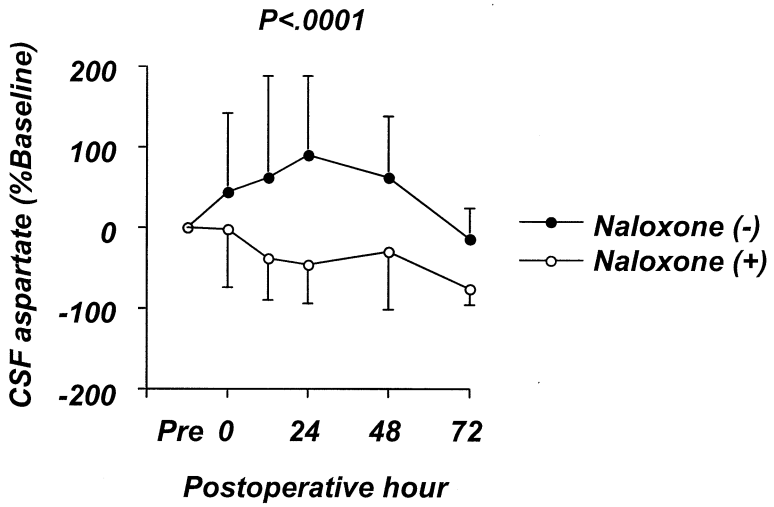

Fig 6. Time course of aspartate levels in the cerebrospinal fluid (*CSF*) in patients given naloxone or not.

duration of Ca^{2+}-dependent glutamate increase related to ischemia and hypoxia seem minor compared with the total elevation of ECF glutamate. It has been well established that accumulated ECF glutamate during ischemia is rapidly cleared during reperfusion.[3,4] Therefore, we speculated that sustained release of EAAs observed in our patients either was mainly of metabolic origin or was caused by sustained ischemia or both. The fact that glutamate receptor antagonist given after the ischemic insult could attenuate neuronal injury[10,12] supports this speculation.

Since Baskin's group reported reversal of ischemic neurologic deficits using naloxone both in animals[21] and humans[22] in the early 1980s, multifactorial mechanisms underlying this beneficial effect of naloxone have been discussed. Several studies have demonstrated that naloxone enhances blood pressure and spinal cord blood flow.[23,24] In patients undergoing thoracoabdominal aortic surgery, systemic blood pressure seems to be maintained steadily. Therefore, this beneficial effect of naloxone may be inferred by improved spinal cord microcirculation rather than by increased blood pressure solely. It is not known whether EAA leak is a direct or indirect effect or cause of SCI. Possible indirect effects of naloxone include the reduction of the degree of ischemic insult and avoidance of sustained ischemia after reperfusion by enhancing spinal cord microcirculation, thereby limiting the leak of EAAs into the CSF. Improved spinal cord microcirculation may also help attenuate EAA release of metabolic origin. The previous findings that ECF glutamate in the cerebral cortex is dependent on cerebral blood flow in a threshold manner may partially support this hypothesis.[25] Another possible mechanism of naloxone in lowering EAAs is mediation by antioxidant action[26] that can control calcium flux[27] and effect cyclic adenosine monophosphate.[28]

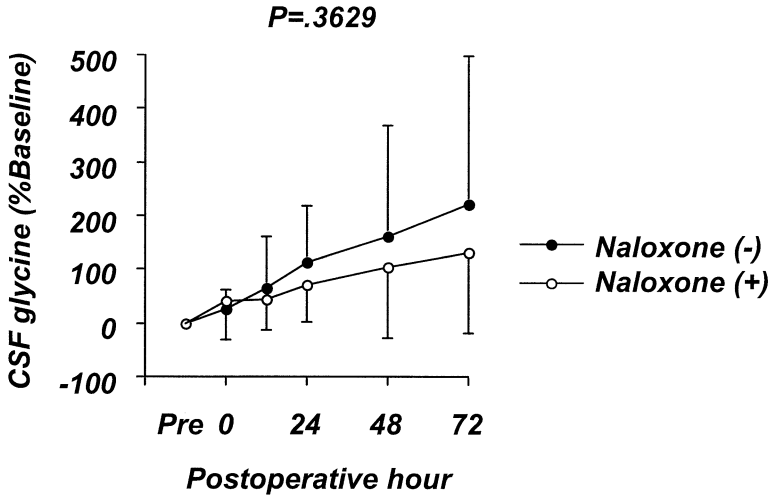

Fig 7. Time course of glycine levels in the cerebrospinal fluid (*CSF*) in patients given naloxone or not.

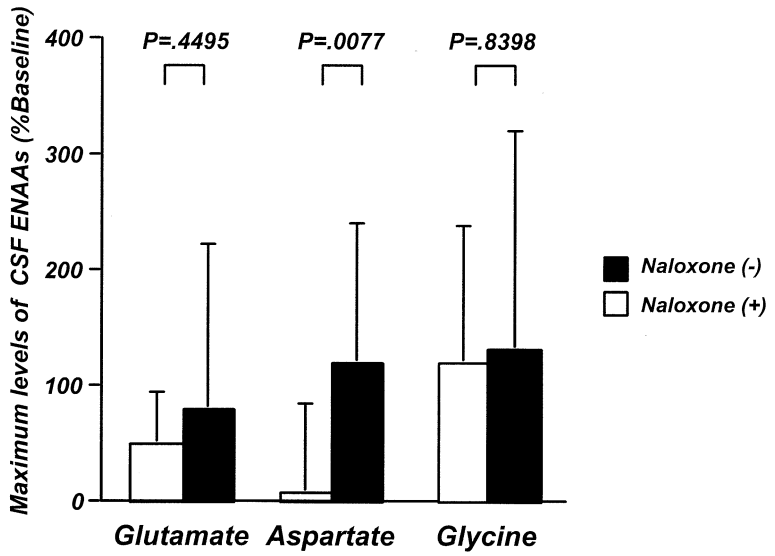

Fig 8. Postoperative maximum levels of *CSF* ENAAs compared between patients underwent naloxone-treatment or not. All data are presented by percent change from baseline.

Glutamate is the most abundant neurotransmitter in the mammalian central nervous system. Excessive synaptic accumulation of glutamate related to ischemia may injure neuronal cells and is mediated through overactivation of glutamate receptors and excessive influx of calcium. This process of neuronal cell death is known as excitotoxicity.[29] Glutamate receptor subtypes are divided into ionotoropic and metabotropic, and the former is further classified into NMDA and non-NMDA types. The mechanism by which naloxone attenuates CSF levels of EAAs remains unclear because naloxone has been believed to be a nonselective opioid receptor antagonist. Kim et al[9] have demonstrated that high concentrations of naloxone could partially antagonize NMDA receptor-mediated neurotoxicity in an animal model. However, it has been unknown whether a very low dose of naloxone, which has been used during thoracoabdominal aortic replacement,[1] can ameliorate NMDA-mediated excitotoxicity or not. A possible explanation is that the sigma opioid receptor may be related to the NMDA receptor channel, where naloxone has weak binding affinity.[30,31] Further experimental studies will be required to clarify the mechanism underlying the effects of naloxone on CSF glutamate.

There is increasing evidence that nonglutamatergic neurotransmitters also play a key role in the pathogenesis of excitotoxicity. Glycine is supposed to be both a coagonist of glutamate on the NMDA receptor that can potentiate the inflow of glutamate-dependent calcium into the cell

Table II. Time course of excitatory amino acid levels (nmol/L) in the cerebrospinal fluid in patients with and without postoperative spinal cord injury or naxolone treatment.

		Spinal cord injury		*Naloxone treatment*	
		(−)	*(+)*	*(−)*	*(+)*
Glutamate	baseline	3.46 ± 2.04	2.40 ± .99	3.54 ± 2.29	3.12 ± 1.73
	0 h	3.42 ± 1.56	3.33 ± 1.67	3.56 ± 1.70	3.31 ± 1.49
	12 h	2.92 ± 1.13	4.92 ± 2.88	3.90 ± 1.81	2.69 ± 1.34
	24 h	3.42 ± 1.28	3.60 ± 2.19	3.85 ± 1.67	3.24 ± 1.25
	48 h	4.46 ± 1.86	7.25 ± 7.93	8.35 ± 7.01	3.91 ± 2.18
	72 h	4.73 ± 1.65	7.40 ± 6.55	8.25 ± 5.81	4.24 ± 1.87
Aspartate	baseline	.29 ± .35	1.55 ± 1.50	.86 ± 1.03	.25 ± .44
	0 h	.30 ± .31	.78 ± .78	.62 ± .27	.23 ± .45
	12 h	.37 ± .48	1.70 ± 1.16	1.01 ± .86	.23 ± .54
	24 h	.79 ± 2.21	.80 ± 1.04	2.08 ± 3.16	.09 ± .10
	48 h	.29 ± .32	2.17 ± 2.71	2.33 ± 2.56	.21 ± .31
	72 h	.09 ± .07	1.87 ± 2.28	1.32 ± 2.13	.15 ± .18
Glycine	baseline	8.94 ± 6.79	10.12 ± 5.70	12.98 ± 8.40	6.56 ± 3.19
	0 h	10.23 ± 6.91	8.88 ± 3.58	13.31 ± 8.85	7.97 ± 3.34
	12 h	12.26 ± 9.36	23.35 ± 14.19	18.73 ± 11.40	10.03 ± 8.82
	24 h	16.40 ± 13.88	12.83 ± 5.65	22.27 ± 13.91	12.23 ± 11.14
	48 h	19.36 ± 20.04	34.12 ± 41.92	41.80 ± 38.27	15.53 ± 18.62
	72 h	24.41 ± 26.04	44.88 ± 55.16	54.62 ± 52.97	18.84 ± 20.76

Table III. Logistic regression analysis to identify independent predictors for spinal cord injury or postoperative maximum levels of CSF EAAs >100% from baseline.

Outcome	*Variable*	*Uni*	*Multi*	*OR*	*95% CI*
Spinal cord injury	Extent (thoracoabdominal aorta)	.119	.285	NA	
	Max. glutamate >100%	<.001	<.001	NA (∞)*	
	Max. glycine	.042	.005	NA	
Max aspartate >100%	Naloxone	.103	.370	2.496	.338–18.427
	Duration of operation	.148	.133	1.004	.999–1.009
	CSFD amount/day	.197	.137	.982	.959–1.006
Max glutamate >100%	Spinal cord injury	<.001	<.001	NA (∞)*	
	Max. glycine	.042	.005	NA	
Max glycine >100%	Spinal cord injury	.150	.722	2.038	.040–103.119
	Max. glutamate	.116	.176	1.022	.990–1.056
	Duration of ventilation	.180	.167	1.645	.812–3.333

CSF, Cerebrospinal fluid; *EAA,* excitatory amino acid; *uni,* univariate; *multi,* multivariate; *OR,* odds ratio; *CI,* confidential interval; *Max,* maximum; *NA,* not applicable.

*Since postoperative maximum levels of CSF glutamate of all patients with spinal cord injury were larger than 100% from baseline, OR and 95% CI could not be calculated.

and the most abundant inhibitory neurotransmitter at a separate receptor in the spinal cord.[8,32,33] Glycine has the highest concentration among all amino acids in the ventral gray matter that is the most vulnerable area to hypoxia/ischemia in the spinal cord.[34] In a swine model of ischemia and reperfusion of the spinal cord, the alteration of ECF glycine level paralleled that of glutamate, which was reversed by a noncompetitive NMDA receptor antagonist.[32] The findings in the current study that postoperative maximum level of CSF glycine was a significant predictor for postoperative maximum level of CSF glutamate >100% from baseline are consistent with this observation. It has also been demonstrated that glycine antagonists attenuate neurotoxicity mediated by glutamate receptors.[35] Clinically, CSF glycine level has been reported to be the most predictive independent factor of severity of stroke at 48 hours after the onset.[5] Our results that CSF glycine level in patients with SCI showed the most pronounced elevation among the 3 investigated EAAs are consistent with this report.

Although the role of aspartate in excitotoxicity is not well established, the high concentration of aspartate has been revealed in animal models[36] and in humans with ischemic neurologic insults.[7] Our patients with SCI also suffered from a higher CSF aspartate level than those without SCI, although it had no statistical power. These findings are also in agreement with Brock et al's study.[8] Furthermore, CSF aspartate level peaked at 24 hours after surgery in patients without naloxone treatment, and both its time course and its maximum level were significantly ameliorated by naloxone. These data are consistent with transient increase of aspartate in animal

models with ischemia and reperfusion of the spinal cord.[14,32,36] Further research will be necessary to explain these results.

The interpretation of our results must be carefully evaluated. First, we performed CSFD continuously until 72 hours after operation. CSFD itself may reduce CSF EAA levels, and may therefore have influenced the results. To clarify the effects of CSFD itself on the alteration of EAAs in the CSF, it is necessary to have a control group in which samples of CSF were collected without continuous CSFD, although it seems unethical. Second, this study is not a randomized study and has a patient selection bias. For example, 2 patients who underwent nonelective operation for contained or impending rupture were included in the current study. However, logistic regression analysis revealed that nonelective operation did not affect incidence of SCI nor CSF levels of EAAs at all. Third, we could not find the risk of postoperative SCI reduced by naloxone itself in our small cohort. Replication in larger prospective randomized clinical trials is needed. In addition, the mechanism of SCI after thoracoabdominal aortic surgery is multifactorial. Although there was no significant difference in involved or reattached intercostals and lumbar arteries or intraoperative variables between groups, the operative technique (ie, duration of spinal cord ischemia) or result of reconstruction (ie, failure or patency of reattached intercostals and lumbar arteries that are critical to the spinal cord) is not uniform between the groups. Furthermore, even postoperative factors that are not directly related to the operative technique, such as perioperative hypotension (postischemic poor perfusion) or thrombosis and embolism of critical intercostals and lumbar arteries, can be associated with SCI. Therefore, our results warrant further investigation in animal models under uniform conditions.

In conclusion, patients with postoperative SCI are exposed to high concentrations of EAAs in the CSF for at least 72 hours after thoracoabdominal aortic surgery. These results may provide partial support for the rationale that excitotoxicity mediated by EAAs is responsible for ischemia/reperfusion injury of the spinal cord that is related to AXC. Sustained elevation of EAAs was reduced by intravenous naloxone, suggesting important therapeutic implications of this compound for patients undergoing thoracoabdominal aortic surgery.

REFERENCES

1. Acher CW, Wynn MM, Hoch JR, Popic P, Archibald J, Turnipseed WD. Combined use of cerebral spinal fluid drainage and naloxone reduces the risk of paraplegia in thoracoabdominal aneurysm repair. J Vasc Surg 1994;19:236-48.
2. Coselli JS, Lemaire SA, Koksoy C, Schmittling ZC, Curling PE. Cerebrospinal fluid drainage reduces paraplegia after thoracoabdominal aortic aneurysm repair: results of a randomized clinical trial. J Vasc Surg 2002;35:631-9.
3. Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem 1984;43:1369-74.
4. Obrenovitch TP, Richards DA. Extracellular neurotransmitter changes in cerebral ischaemia. Cerebrovasc Brain Metab Rev 1995;7:1-54.
5. Castillo J, Davalos A, Naveiro, J, Noya, M. Neuroexcitatory amino acids and their relation to infarct size and neurological deficit in ischemic stroke. Stroke 1996;27:1060-5.
6. Brown JI, Baker AJ, Konasiewicz SJ, Moulton RJ. Clinical significance of CSF glutamate concentrations following severe traumatic brain injury in humans. J Neurotrauma 1998;15:253-63.
7. Kashiwagi S, Fujisawa H, Yamashita T, Ito H, Maekawa T, Kuroda Y, et al. Excitotoxic amino acid neurotransmitters are increased in human cerebrospinal fluid after subarachnoid haemorrhage. J Neurol Neurosurg Psychiatry 1994;57:1442-3.
8. Brock MV, Redmond JM, Ishiwa S, Johnston MV, Baumgartner WA, Laschinger JC, et al. Clinical markers in CSF for determining neurologic deficits after thoracoabdominal aortic aneurysm repairs. Ann Thorac Surg 1997;64:999-1003.
9. Kim JP, Goldberg MP, Choi DW. High concentrations of naloxone attenuate N-methyl-D-aspartate receptor- mediated neurotoxicity. Eur J Pharmacol 1987;138:133-6.
10. Redmond JM, Zehr KJ, Blue ME, Lange MS, Gillinov AM, Troncoso JC, et al. AMPA glutamate receptor antagonism reduces neurologic injury after hypothermic circulatory arrest. Ann Thorac Surg 1995;59: 579-84.
11. Nakamura R, Kamakura K, Kwak S. Late-onset selective neuronal damage in the rat spinal cord induced by continuous intrathecal administration of AMPA. Brain Res 1994;654:279-85.
12. Xue D, Huang Z, Barnes K, Lesiuk HJ, Smith KE, Buchan AM. Delayed treatment with AMPA, but not NMDA, antagonists reduces neocortical infarction. J Cereb Blood Flow Metab 1994;14:251-60.
13. Baker CJ, Fiore AJ, Frazzini VI, Choudhri TF, Zubay GP, Solomon RA. Intraischemic hypothermia decreases the release of glutamate in the cores of permanent focal cerebral infarcts. Neurosurgery 1995;36:994-1001.
14. Rokkas CK, Cronin CS, Nitta T, Helfrich LR Jr, Lobner DC, Choi DW, et al. Profound systemic hypothermia inhibits the release of neurotransmitter amino acids in spinal cord ischemia. J Thorac Cardiovasc Surg 1995;110:27-35.
15. Blin O, Samuel D, Nieoullon A, Serratice G. Changes in CSF amino acid concentrations during the evolution of amyotrophic lateral sclerosis. J Neurol Neurosurg Psychiatry 1994;57:119-20.
16. Csernansky JG, Bardgett ME, Sheline YI, Morris JC, Olney JW. CSF excitatory amino acids and severity of illness in Alzheimer's disease. Neurology 1996;46:1715-20.
17. Baker AJ, Moulton RJ, MacMillan VH, Shedden PM. Excitatory amino acids in cerebrospinal fluid following traumatic brain injury in humans. J Neurosurg 1993;79:369-72.
18. Bullock R, Zauner A, Woodward J, Young HF. Massive persistent release of excitatory amino acids following human occlusive stroke. Stroke 1995;26:2187-9.
19. Wahl F, Obrenovitch TP, Hardy AM, Plotkine M, Boulu R, Symon L. Extracellular glutamate during focal cerebral ischaemia in rats: time course and calcium dependency. J Neurochem 1994;63:1003-11.
20. Ueda Y, Obrenovitch TP, Lok S-Y, Sarna GS, Symon L. Efflux of glutamate produced by short ischemia of varied severity in rat striatum. Stroke 1992;23:253-9.
21. Hosobuchi Y, Baskin DS, Woo SK. Reversal of induced ischemic neurologic deficit in gerbils by the opiate antagonist naloxone. Science 1982;215:69-71.
22. Baskin DS, Hosobuchi Y. Naloxone reversal of ischaemic neurological deficits in man. Lancet 1981;2:272-5.
23. Faden AL, Jacobs TP, Mougey E, Holaday JW. Endorphins in experimental spinal injury: therapeutic effect of naloxone. Ann Neurol 1981; 10:326-32.
24. Faden AL, Hallenbeck JM, Brown CQ. Treatment of experimental stroke: comparison of naloxone and thyrotropin releasing hormone. Neurology 1982;32:1083-7.
25. Shimada N, Graf R, Rosner G, Wakayama A, George CP, Heiss WD. Ischemic flow threshold for extracellular glutamate increase in cat cortex. J Cereb Blood Flow Metab 1989;9:603-6.
26. Marzullo G, Hine B. Opiate receptor function may be modulated through an oxidation-reduction mechanism. Science 1981;208: 1171-3.

27. Guerrero-Munoz F, Guerrero ML, Way EL, Li CH. Effect of β-endorphin on calcium uptake in the brain. Science 1979;206:89-91.
28. Collier HOJ, Roy AC. Morphine-like drugs inhibit stimulation by E-prostaglandins of cyclic-AMP formation by rat-brain homogenate. Nature (London) 1974;248:24-7.
29. Olney JW. Brain lesions, obesity and other disturbances in mice treated with monosodium glutamate. Science 1969;164:719-21.
30. Honey CR, Miljkovic Z, MacDonald JF. Ketamine and phencyclidine cause a voltage-dependent block of responses to L-aspartic acid. Neurosci Lett 1985;61:135-9.
31. Mendelsohn LG, Kalra V, Johnson BG, Kerchner GA. Sigma opioid receptor: characterization and co-identity with the phencyclidine receptor. J Pharmacol Exp Ther 1985;233:597-602.
32. Rokkas CK, Helfrich LR, Lobner DC, Choi DW, Kouchoukos NT. Dextrorphan inhibits the release of excitatory amino acids during spinal cord ischemia. Ann Thorac Surg 1994;58:312-20.
33. Johnson JW, Ascher P. Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 1987;325:529-31.
34. Graham LT Jr, Shank RP, Werman R, Aprison MH. Distribution of some synaptic transmitter suspects in cat spinal cord: glutamic acid, aspartic acid, gamma-aminobutyric acid, glycine and glutamine. J Neurochem 1967;14:465-72.
35. Petel J, Zinkand WC, Thomson C, Keith R, Salama A. Role of glycine in N-methyl-D-aspartate-mediatae neuronal cytotoxicity. J Neurochem 1990;54:849-54.
36. Matsumoto K, Graf R, Rosner G, Taguchi J, Heiss WD. Elevation of neuroactive substances in the cortex of cats during prolonged focal ischemia. J Cereb Blood Flow Metab 1993;13:586-94.

Submitted Apr 12, 2004; accepted Jul 6, 2004.