ischemic injury.[11-14] In the clinical arena, drug information on edaravone indicates the possible occurrence of acute renal failure and hepatic functional impairment when the dosage exceeds the clinical dose.[15]

We hypothesized that a reduced dose of edaravone injected into the clamped segment of the aorta might protect the spinal cord from injury, with a reduced risk of liver or kidney complications. However, edaravone injection into the clamped segment of the aorta raises a concern of toxicity in the neuronal cells due to a high concentration of edaravone in the tissue, although there is no evidence of dose-dependent neuronal damage with edaravone.[16] In this study, we examined (1) whether a small dose of edaravone is effective when injected into the clamped segment of the aorta (effectiveness) and (2) whether edaravone injection into the clamped aorta causes neuronal damage (safety).

MATERIALS AND METHODS

Animal Care

Animal care and all procedures were performed in compliance with *The Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. The experimental and animal care protocols were approved by the Research Facilities for Laboratory Animal Science of the Hiroshima University School of Medicine.

Animal Models

Eighteen Japanese domesticated white rabbits, weighing approximately 2.5-3.0 kg, were divided into three groups: transient ischemia followed by treatment with 3 mg/kg of intra-aortic edaravone (group A, $n = 6$), 1 mg/kg of intra-aortic edaravone (group B, $n = 6$), or saline (group C, $n = 6$). The minimum dose of intravenous edaravone that was effective in previous animal studies was 3 mg/kg.[11-14] Since intra-aortic edaravone eventually enters the systemic circulation, a protective effect on the spinal cord was expected in group A (positive control). An intravenous dose of 1 mg/kg is commonly used in clinical practice in Japan, but this dose is below the minimally effective intravenous dose of 3 mg/kg. Group C served as a negative control. All rabbits were allowed free access to food and water before and after the procedure. All rabbits were randomly assigned to group A, B, or C before surgical preparation. We previously showed that transient spinal cord ischemia by clamping only the abdominal aorta below the left renal artery for 15 min consistently resulted in paraplegia in rabbits.[9] This animal model was utilized in this study.

Anesthesia and Monitoring

Anesthesia was induced with intramuscular administration of ketamine hydrochloride at a dose of 50 mg/kg. A 24-gauge catheter was inserted in the marginal vein of the auricle for continuous intravenous infusion of lactated Ringer solution. Cefazoline (10 mg/kg) was injected through the catheter. After a tracheostomy and endotracheal intubation, the rabbits were ventilated mechanically with 2% isoflurane in oxygen to induce sufficient anesthesia. The arterial blood pressure of the proximal and distal aorta was measured by catheters (JMS cutdown tube; C3, Hiroshima, Japan) placed into the right axillary artery and the right femoral artery. Electrocardiograms were also monitored continuously. The operation was performed at normothermia. The rectal temperature was continuously monitored and kept at 38.0 ± 0.5 °C during the operation using a heating blanket.

Surgical Procedure

In the right lateral decubitus position, a left flank skin incision was made parallel to the spine. The abdominal aorta was isolated and encircled between the levels of the renal artery and bifurcation with a retroperitoneal approach. Heparin sulfate was administrated intravenously (100 U/kg). The abdominal aorta was cross-clamped by vascular clamps below the renal artery and above the bifurcation, inducing spinal cord ischemia for 15 min. Immediately after the abdominal aorta was cross-clamped, a 24-gauge catheter was inserted in the aorta and edaravone or saline was injected into the clamped aortic segment for 1 min at room temperature (Fig. 1). A total fluid volume of 6 mL was administered into the clamped aorta in each group. The catheter was immediately removed after injection of edaravone or saline solution, and the point of catheter entry was closed with an 8-0 polypropylene suture (Ethicon, Somerville, NJ). After 15 min, the aortic clamp was removed. The abdomen was closed and all catheters were withdrawn. The animals were allowed to recover from anesthesia and extubated, and routine postoperative care was carried out.

At 7 days after ischemic reperfusion, the animals were killed and spinal cords were immediately extracted. All samples were fixed by immersion in 10% paraformaldehyde and then stored for 1

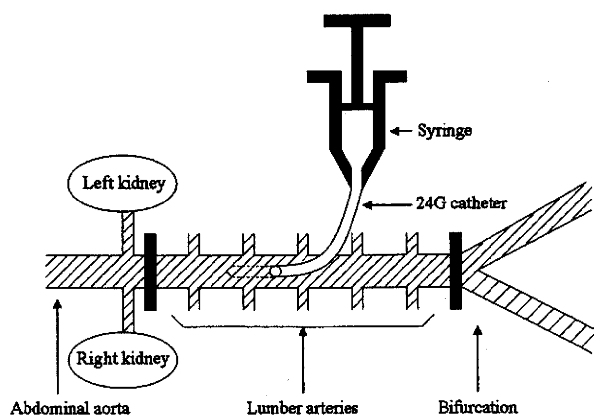

Fig. 1. Diagram of the method of producing spinal cord ischemia and injecting edaravone in this study. The abdominal aorta was cross-clamped by vascular clamps below the renal artery and above the bifurcation. Immediately after the abdominal aorta was cross-clamped, edaravone was injected into the clamped aortic segment through a 24-gauge catheter for 1 min.

week. The specimen at L5 level was embedded in paraffin and sectioned for staining and microscopic examination.

Neurological Assessment

Neurological function was assessed at 8, 24, and 48 hr and 7 days after reperfusion and classified using a five-point scale according to the Tarlov score: 0, spastic paraplegia and no movement of the lower limbs; 1, spastic paraplegia and slight movement of the lower limbs; 2, good movement of the lower limbs but unable to stand; 3, able to stand but unable to walk normally; 4, complete recovery and normal gait/hopping.[17] An observer, blinded to the protocol, graded neurological function.

Histological Assessment

To assess the pathological changes of the spinal cord after ischemia, the sections taken 7 days after reperfusion in each group were stained with hematoxylin-eosin and examined by light microscopy. The numbers of intact large motor neuron cells in the anterior horn of the spinal cord (anterior to a line drawn through the central canal perpendicular to the anterior median fissure) were counted in five sections in each animal. An observer, unaware of animal group and neurological outcome, examined each slide (x200). With hematoxylin-eosin staining, the cells were considered "dead" if the cytoplasm was diffusely eosinophilic and "viable" if the cells demonstrated basophilic stippling (i.e., contained Nissl substance).[18]

TUNEL Staining

Cells with double-strand breaks in DNA, which are suggestive of apoptosis, were examined by means of in situ terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) staining. Paraffin-embedded sections were used for TUNEL staining with Neuro TACS II in situ Apoptosis Detection Kit (Trevigen, Gaithersburg, MD), according to the manufacturer's instructions. In brief, after being deparaffinized, the sections were treated with proteinase and 0.3% H_2O_2. Then, the sections were incubated for 60 min with terminal deoxynucleotidyl transferase at 37 °C. The color was developed with a diaminobenzidine/H_2O_2 solution. The sections were counterstained with hematoxylin. "Apoptosis" was defined when only the nucleus and apoptotic bodies were stained, whereas "necrosis" was defined when the cytoplasm was stained with or without staining of the nucleus. Positive control slides were prepared with HeLa cells treated with ultraviolet (UV) microscope irradiation at 50 J/m^2 by a germicidal lamp with peak emission at 254 nm,[19] and negative control slides were prepared with HeLa cells not treated with UV irradiation.

Statistical Analysis

All results were expressed as the mean ± standard deviation (SD). Statistical significance was assumed at $p < 0.05$. The Mann-Whitney U-test, one-way analysis of variance (ANOVA), and post hoc test with Fisher's protected least significant difference were used to identify the group differences that accounted for the significant p value. We also evaluated the dose-response relationship between the number of intact large motor neurons in the anterior horn of the spinal cord and the applied dose of edaravone using the ANOVA model. The details of the ANOVA model are described in the Appendix.

RESULTS

Physiological Status

No significant differences were noted in the proximal and distal mean arterial pressures and heart rates between the groups during the operation. All 18 animals survived for 7 days, and there were no operative deaths.

Neurological Findings

The results of the neurological assessment are summarized in Table I. There was no interobserver

Table I. Neurological outcome at 8, 24, and 48 hr and 7 days after reperfusion

	Group A (n = 6)				Group B (n = 6)				Group C (n = 6)			
Tarlov score	8hrs	24hrs	48hrs	7days	8hrs	24hrs	48hrs	7days	8hrs	24hrs	48hrs	7days
4	6	6	6	6	6	6	6	6	2	2	2	0
3	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	3	2	1	1
0	0	0	0	0	0	0	0	0	1	2	3	5
Mean	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	1.8	1.7	1.5	0.2

variability in Tarlov score assessment. The Tarlov score was 4.0 ± 0.0 throughout the observation period in groups A and B; motor function in the hindlimbs was well restored in the two edaravone-treated groups. In group C, however, Tarlov scores were 1.8 ± 1.7 at 8 hr, 1.7 ± 1.9 at 24 hr, 1.5 ± 2.0 at 48 hr, and 0.2 ± 0.4 at 7 days after reperfusion. The Tarlov score was significantly higher at all time points in group A than in group C ($p = 0.002$, $p = 0.002$, $p = 0.002$, and $p < 0.001$, respectively). In group B as well, it was significantly higher than in group C ($p = 0.002$, $p = 0.002$, $p = 0.002$, and $p < 0.001$, respectively). There was no significant difference in neurological function between groups A and B (Fig. 2).

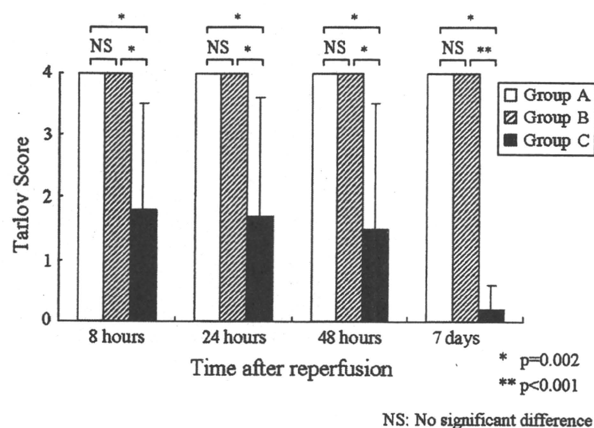

Fig. 2. Changes in Tarlov score at 8, 24, and 48 hr and 7 days after reperfusion. In groups A and B, the Tarlov score was significantly higher at all time points than in group C (*$p = 0.002$, **$p < 0.001$). There was no significant difference in neurological function between groups A and B.

Histological Findings

On the seventh day after reperfusion, a majority of motor neurons remained intact in the anterior horn of the spinal cord in groups A and B (Fig. 3A, B; black arrows), whereas many motor neurons became necrotic (red arrows) in group C. In this group, severe neuronal damage was noted with eosinophilic neuronal degeneration, vacuolization, and frank necrosis and several inflammatory cells were found (Fig. 3C). The number of intact large motor neurons in the anterior horn of the spinal cord was 26.9 ± 4.3 in group A, 24.5 ± 3.3 in group B, and 8.6 ± 3.7 in group C (Fig. 4). Additionally, ANOVA showed that there was a significant difference in the number of intact large motor neurons between groups A and B and between groups B and C ($p = 0.019$ and $p < 0.001$, respectively). There was a dose-response relationship between the number of intact large motor neurons in the anterior horn of the spinal cord and the applied dose of edaravone.

TUNEL Staining

Apoptotic HeLa cells treated with UV irradiation (positive control) showed brownish nuclei with TUNEL staining (Fig. 5A), whereas none of the HeLa cells without UV irradiation (negative control) showed brownish nuclei (Fig. 5B). On the seventh day after reperfusion, there were no brown-stained nuclei in motor neurons in the anterior horn of the spinal cord in groups A and B, indicating the absence of apoptosis (Fig. 5C, D). There was no apparent difference between groups A and B. In group C, several motor neurons in the anterior horn of the spinal cord had cytoplasm that was diffusely stained without brownish nuclei (Fig. 5E). This finding was interpreted as TUNEL-negative, indicating necrosis of spinal cord neurons but not apoptosis.

DISCUSSION

This study demonstrated that (1) edaravone injected into the clamped segment of the aorta prevents both immediate and delayed neuronal injury after spinal cord ischemia, (2) the protective effect is adequate with 1 mg/kg of intra-aortic edaravone, and (3)

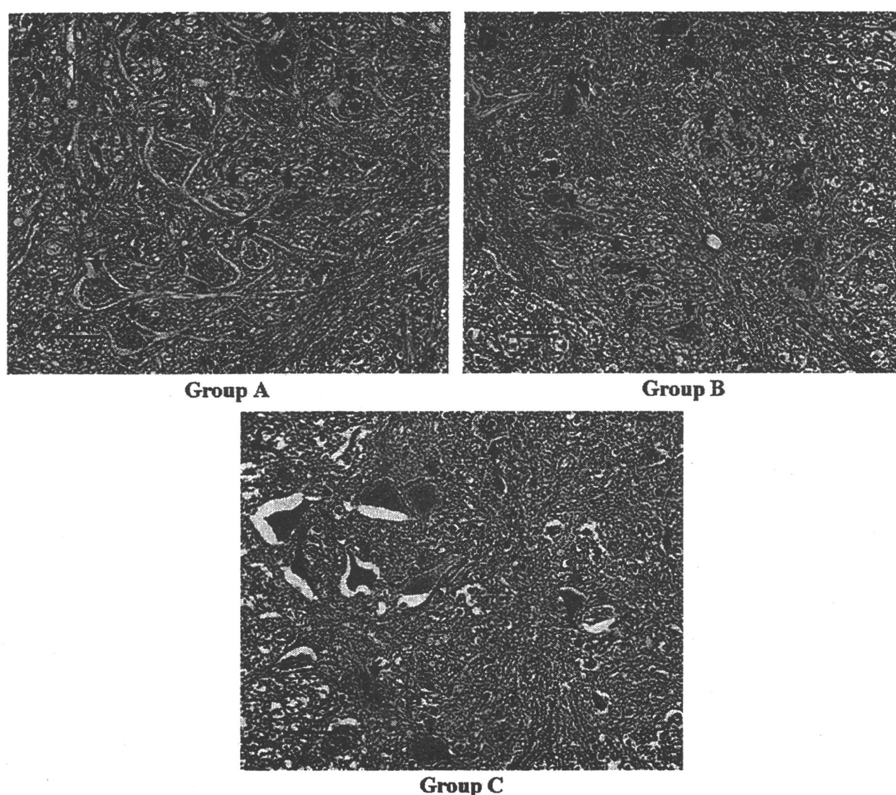

Fig. 3. Histological findings in the anterior horn of the spinal cord at the L5 level stained with hematoxylin-eosin on the seventh day after reperfusion (magnification, ×200). *Black arrows* indicate intact motor neurons; *red arrows* indicate necrotic motor neurons. In group A (**A**) and group B (**B**), a majority of motor neurons remained intact. In group C (**C**), many motor neurons became necrotic; severe neuronal damage was noted with eosinophilic neuronal degeneration, vacuolization, and frank necrosis; and several inflammatory cells were found.

edaravone injection into the clamped aorta does not lead to apparent damage of neuronal cells.

Edaravone injected into the clamped segment of the aorta protected the spinal cord as effectively as that given intravenously. Previous studies reported the mechanisms of the cytoprotective effects of edaravone: scavenging free radicals, reduction of neuronal nitric oxide synthase, increase of endothelial nitric oxide synthase, and reduction of oxidative DNA damage.[11-13] Suzuki et al.[11] reported that edaravone scavenges free radicals that are produced following ischemia-reperfusion injury and that neurological function is restored with prophylactic intravenous injection of edaravone in a rabbit spinal ischemia model.

Based on these results, we sought an effective way of administering edaravone. When given intravenously, 3 mg/kg or more edaravone is effective for protecting the spinal cord but does not provide complete protection since motor function in the hindlimbs sometimes resulted in ataxia (uncoordinated hop)[12,13] or sitting alone.[14] In contrast, 1 mg/kg of edaravone (the clinical dose of intravenous edaravone used to treat cerebral infarction) is effective enough when injected into the clamped segment of the aorta. The drug information alerts to the possible occurrence of acute renal failure or liver damage when the dose exceeds 1 mg/kg, while these adverse effects rarely occur when the dose is within this limit. Edaravone injected into the aorta eventually enters the systemic circulation, and similar adverse effects are likely to occur. Intra-aortic administration of a reduced dose of edaravone may be beneficial for enhancing the protective effect in the spinal cord while minimizing the adverse effects on other organs. Svensson et al.[20] reported that local infusion of cold crystalloid solution with lidocaine added during aortic cross-clamping effectively reduced the attenuation of spinal motor evoked potential amplitude but did not apparently lower the rate of neurological deficit in an animal study. In contrast, a clinically relevant dose of edaravone injected into the clamped segment of the aorta in the present study reduced the neurological deficit rate.

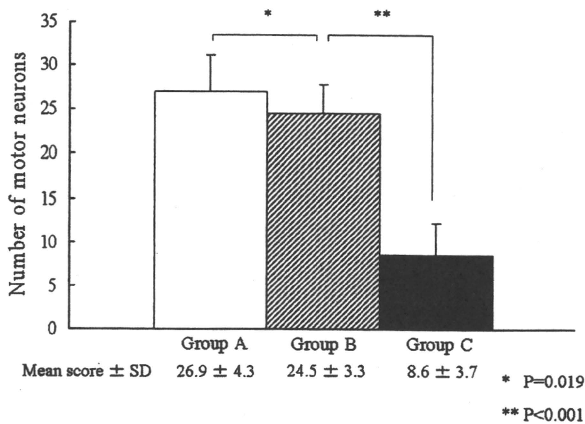

Fig. 4. Number of large motor neurons in the anterior horn of the spinal cord on the seventh day after reperfusion. There was a significant difference in the number of intact large motor neurons between groups A and B and between groups B and C ($*p = 0.019$ and $**p < 0.001$, respectively).

However, edaravone injection into the clamped aorta raises a concern of neuronal cell toxicity due to a high concentration of edaravone in the spinal cord tissue. In the current study, there was no apparent motor dysfunction in the neurological assessment and no histological evidence of neuronal damage. It will be necessary in future studies to determine the minimally effective dose of edaravone that protects against spinal cord injury without causing other organ damage.

There are two patterns in the neuronal injury caused by spinal cord ischemia: immediate and delayed.[21] The former is often caused by severe ischemic insult and rapidly necrotized neuronal cells. The latter is caused either by apoptosis[22-24] or by necrosis.[21,25,26] These previous studies also reported that disruption of infrarenal abdominal aortic blood flow for 15 min caused immediate neuronal injury by 2 days and delayed paraplegia by 7 days.[22-26] In the current study, compared with the negative control group (group C), the edaravone-treated groups (groups A and B) presented with neither immediate neuronal injury nor delayed paraplegia. The histological findings at 7 days in the edaravone-treated groups showed that many intact motor neurons were preserved, with reduced numbers of necrotic motor neurons and no apoptotic motor neurons, whereas in the negative control group the histological findings showed severe neuronal damage with

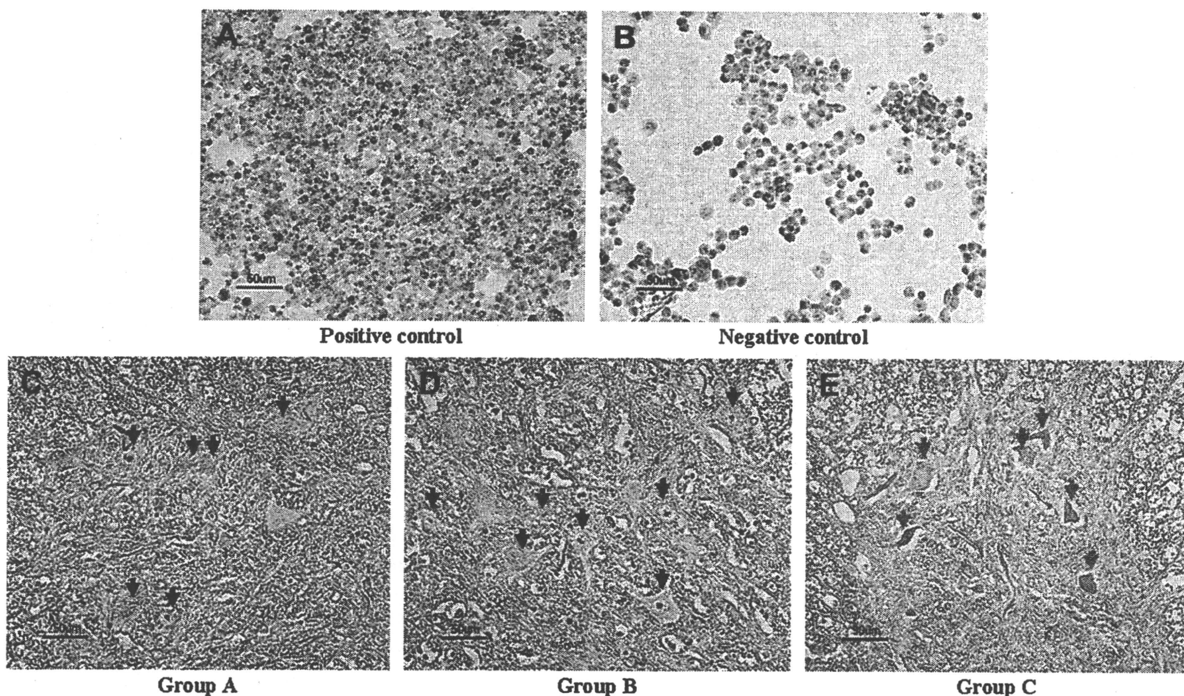

Fig. 5. Typical TUNEL staining of the spinal cord at the L5 level on the seventh day after reperfusion (magnification ×200). In the positive control (**A**), apoptotic HeLa cells treated with UV irradiation showed brownish nuclei with TUNEL staining. In the negative control (**B**), none of the HeLa cells without UV irradiation showed brownish nuclei. In groups A (**C**) and B (**D**), there were no motor neurons with brownish nuclei (*black arrows*). In group C (**E**), several cells had diffusely stained cytoplasm but without brownish nuclei, i.e., TUNEL-negative, indicating necrosis of spinal cord neurons (*red arrows*) but not apoptosis.

a lack of apoptosis and the presence of several inflammatory cells. The fact was that the total neuronal cell death caused by ischemia-reperfusion injury was attenuated with prophylactic intra-aortic injection of edaravone, although the exact mechanism was not certain because it was beyond the scope of this study. Delayed neuronal injury in this model might be an inflammation-mediated event as Papakostas et al. reported,[21] although it is not clearly evidenced in the current study without histological assessments at acute phase.

Histological examination revealed that there were a few necrotic motor neurons in the edaravone-treated groups, indicating that the protective effect of edaravone is not perfect. Further investigation is anticipated for effectiveness and safety. Other administration protocols, such as staged injections or the combined use of edaravone with topical/core cooling, may compensate for this limitation. Although preservation of the feeding artery to the spinal cord is of primary importance, such an artery cannot always be identified preoperatively. The protective effect of edaravone injected into the aorta or the above-mentioned integrated "spinoplegia" protocol may be a potential option in such instances.

In conclusion, edaravone injection into the clamped segment of the aorta is feasible and effective without apparent neuronal damage of the spinal cord in a rabbit model. This method could protect the spinal cord from ischemic injury with a dose of edaravone that is similar to the dose used in clinical practice. This study indicated that this method can potentially be a useful adjunct for spinal cord protection during thoracic and thoracoabdominal aortic operations.

The authors thank Mr. Kazunori Iwase and Ms. Emi Fukuda for their excellent technical assistance.

APPENDIX

We evaluated the dose-response relationship between the number of intact large motor neurons in the anterior horn of the spinal cord (y_i) and the applied dose of edaravone ($x_i^{(A\cup B)}, x_i^{(A)}$) using the following ANOVA model:

$$y_i = \mu_C + \delta_{B|C} x_i^{(A\cup B)} + \delta_{A|B} x_i^{(A)} + \varepsilon_i,\ \varepsilon_i \sim N(0,\sigma^2),\ i = 1,\cdots,90,$$

where $x_i^{(A\cup B)}$ and $x_i^{(A)}$ are group indicator variables defined by

$$x_i^{(A\cup B)} = \begin{cases} 1, & i \in group(A) \cup group(B), \\ 0, & i \in group(C), \end{cases} \quad x_i^{(A)} = \begin{cases} 1, & i \in group(A), \\ 0, & i \in group(B) \cup group(C). \end{cases}$$

The parameters $\delta_{B|C}$ and $\delta_{A|B}$ denote the difference of means between group C and group B and that between group B and group A, respectively.

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Sodium 4-phenylbutyrate protects against spinal cord ischemia by inhibition of endoplasmic reticulum stress

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Objective: Delayed paraplegia after operation on the thoracoabdominal aorta is considered to be related to vulnerability of motor neurons to ischemia. Previous studies have demonstrated the relationship between neuronal vulnerability and endoplasmic reticulum (ER) stress after transient ischemia in the spinal cord. The aim of this study was to investigate whether sodium 4-phenylbutyrate (PBA), a chemical chaperone that reduces the load of mutant or unfolded proteins retained in the ER during cellular stress, can protect against ischemic spinal cord damage.

Methods: Spinal cord ischemia was induced in rabbits by direct aortic cross-clamping (below the renal artery and above the bifurcation) for 15 minutes at normothermia. Group A (n = 6) was a sham operation control group. In group B (n = 6) and group C (n = 6), vehicle or 15 mg/kg/h of sodium 4-PBA was infused intravenously, respectively, from 30 minutes before the induction of ischemia until 30 minutes after reperfusion. Neurologic function was assessed at 8 hours, and 2 and 7 days after reperfusion with a Tarlov score. Histologic changes were studied with hematoxylin-eosin staining. Immunohistochemistry analysis for ER stress-related molecules, including caspase12 and GRP78 were examined.

Results: The mean Tarlov scores were 4.0 in every group at 8 hours, but were 4.0, 2.5, and 3.9 at 2 days; and 4.0, 0.7, and 4.0 at 7 days in groups A, B, and C, respectively. The numbers of intact motor neurons at 7 days after reperfusion were 47.4, 21.5, and 44.9 in groups A, B, and C, respectively. There was no significant difference in terms of viable neurons between groups A and C. Caspase12 and GRP78 immunoreactivities were induced in motor neurons in group B, whereas they were not observed in groups A and C.

Conclusion: Reduction in ER stress-induced spinal cord injury was achieved by the administration of 4-PBA. 4-PBA may be a strong candidate for use as a therapeutic agent in the treatment of ischemic spinal cord injury. (J Vasc Surg 2010; 52:1580-6.)

Clinical Relevance: Spinal cord injury following surgical repair of thoracic or thoracoabdominal aorta is a disastrous complication. Previous studies have demonstrated the relationship between neuronal vulnerability and endoplasmic reticulum (ER) stress after transient ischemia of spinal cord. Sodium 4-phenylbutyrate (4-PBA) is a low molecular weight fatty acid that has been approved for clinical use as an ammonia scavenger in children with urea cycle disorders and for treatment of sickle cell disease and thalassemia. A number of investigators have recently reported utilization of 4-PBA as a chemical chaperone to reverse the mislocalization and/or aggregation of proteins associated with human disease. The 4-PBA of therapeutic dose has low toxicity and uniquely penetrates well into cerebrospinal fluid. In this study, we investigated whether intravenous administration of 4-PBA is beneficial to protecting the spinal cord against ischemic damage in a rabbit model. Spinal cord ischemia was induced by direct aortic cross-clamping while 4-PBA of therapeutic dose was infused intravenously. In this simple and less invasive model, reduction in ER stress-induced spinal cord damage was achieved by intravenous 4-PBA. Our results indicate that 4-PBA of therapeutic dose can be a promising strategy, which is clinically feasible without significant adverse effects. Although further investigations are mandatory before it is applied to the clinical practice, such as optimal timing or route for administration 4-PBA, intravenous administration of 4-PBA may be a new candidate as a therapeutic agent for protecting the spinal card against ischemic damage in thoracoabdominal aortic surgery.

Paraplegia caused by ischemic spinal cord injury is one of the most serious complications of surgery performed on the thoracic and thoracoabdominal aorta, with a reported prevalence of 3% to 23% in different series.[1-3] Various adjunctive procedures for spinal cord protection have been introduced, including cerebrospinal fluid drainage, distal perfusion of the aorta, or use of steroid, barbiturate, or free-radical scavengers, and have successfully reduced the incidence of postoperative paraplegia in recent years. However, postoperative paraplegia remains a devastating complication, and its occurrence is still somewhat unpredictable. Patients undergoing thoracic aneurysm repair who awake without neurologic deficit immediately after the operation may eventually develop paraplegia.[4,5] The exact mechanism of such a delayed vulnerability is not fully understood. In a rabbit spinal cord ischemia model, delayed and selective motor neuron death after transient ischemia has been reported, and it was greatly associated with activated apoptotic signals.[6]

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Competition of interest: none.

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There is evidence to suggest that the activation of apoptosis is initiated by cell-surface receptors or by mitochondrial stress.[7] Recently, another apoptotic-regulatory pathway involved in endoplasmic reticulum (ER) stress has received attention. In the case of mild ER stress, cells develop a self-protective, signal transduction pathway termed the unfolded protein response, which includes induction of molecular chaperones in the ER such as glucose regulated protein 78 (GRP78), translational attenuation, and ER-associated protein degradation.[8,9] However, if the damage is severe, the unfolded protein response ultimately triggers the apoptosis pathway.[10] One of the important molecules relevant to cell death is caspase12 which is regarded to be a representative cell death signal in ER stress.[11] Sodium 4-phenylbutyrate (4-PBA) is a low molecular weight fatty acid that has been approved for clinical use as an ammonia scavenger in children with urea cycle disorders and for treatment of sickle cell disease and thalassemia on the basis of its capacity to activate transcription of β- and γ-globin.[12,13] 4-PBA has been found to prolong life and contribute to therapy for spinal muscular atrophy by altering the pattern of gene expression. In the last few years, a number of studies have described the use of 4-PBA as a chemical chaperone to reverse the mislocalization and/or aggregation of proteins associated with human disease. 4-PBA can act as a chemical chaperone by reducing the load of mutant or mislocated proteins retained in the ER.[14-16] Evidence obtained in recent years has demonstrated that endoplasmic reticulum (ER)-mediated cell death plays an important role in the mechanisms underlying ischemia-reperfusion neuronal damage. Therefore, targeting the ER may provide a therapeutic approach for blocking the pathologic process induced by spinal cord ischemia. However, to our knowledge, no pharmacologic approach for treating spinal cord ischemia-induced ER dysfunction has been reported. In this study, we investigated the protective effects of 4-PBA after transient spinal cord ischemia, apoptotic signaling of caspase12, and induction of GRP78.

MATERIALS AND METHODS

Animal preparation. Animal care and all procedures were performed in compliance with the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. This study protocol was approved by the Research Facilities for Laboratory Animal Science of the Hiroshima University School of Medicine.

Anesthesia and monitoring. We used Japanese female white rabbits (2.5-3.0 kg) in this experiment. General anesthesia was induced with an intramuscular injection of ketamine 50 mg/kg. An ear vein catheter was inserted for the continuous intravenous infusion of lactated Ringer solution. Cefazolin (10 mg/kg) was injected through the catheter. After a tracheostomy and endotracheal intubation, the rabbits were ventilated mechanically with a fraction of inspired oxygen of 1.0 and isoflurane (1.5%-2.0%) to induce sufficient anesthesia. Adequacy of ventilation was monitored by blood gas analysis. The arterial blood pressure was measured by a catheter (JMS cut-down tube; C3, Hiroshima, Japan) placed into the right femoral artery. Electrocardiogram was also monitored continuously. The operation was performed at normothermia. The rectal temperature was continuously monitored and kept at 38.0 ± 0.5°C during the operation using a heating blanket.

Surgical procedures. With animals in the right lateral decubitus position, a flank skin incision parallel to the spine was made on the left side. From a retroperitoneal approach, the abdominal aorta was exposed below the left renal artery and above the iliac bifurcation, and was encircled. After the intravenous administration of heparin (100 U/kg), the abdominal aorta just distal to the left renal artery and just proximal to the iliac bifurcation was cross-clamped and isolated for 15 minutes to produce spinal cord ischemia. To determine the clamping time, we conducted preliminary experiments. Fifteen rabbits were divided into three groups with clamping times of 10, 12, and 15 minutes (n = 5 for each group). Only the abdominal aorta just distal to the left renal artery was clamped. At 7 days after reperfusion, the groups clamped for 15 minutes were all paraplegic.

For the formal experiments, female domesticated white rabbits, weighing 2.5 to 3.0 kg, were divided into three groups: sham operation control group (group A, with only aortic exposure but without transient ischemia), transient ischemia and treatment with vehicle group (group B), and transient ischemia and treatment with 4-PBA group (15 mg/kg/h, group C). Saline solution (vehicle, 1 mL/kg body weight) or 4-PBA (4 mg/mL in saline vehicle) was administered intravenously from 30 minutes before induction of ischemia until 30 minutes after reperfusion. Total operation time ranged from 1.5 hours to 2.5 hours, and the total amount of 4-PBA administration ranged from 46.9 mg to 56.3 mg. After 15 minutes, the aortic clamp was removed. The abdomen was closed, and all catheters were withdrawn. The animals were allowed to recover from anesthesia and extubated, and routine postoperative care was carried out. Immediately after euthanasia, the spinal cord was quickly removed through a laminectomy at L4 and the bone around the spinal cord was chopped off. All samples were frozen in powdered dry ice and stored at −80°C.

Neurologic and histopathologic studies. The neurologic status in groups A, B, and C (n = 6 in each group) was assessed at 8 hours, and 2 and 7 days after reperfusion by an observer blinded to the protocol used for each animal. The motor function of the hind limbs was graded by using the Tarlov score, in which 0 indicates no movement of the hind limbs, 1 indicates perceptible movement of the joints of the hind limbs, 2 indicates good movement of the joints but an inability to stand, 3 indicates the ability to stand and walk, and 4 indicates a complete recovery.[17] The animals (n = 6 in each group) were then euthanized after the completion of the neurologic evaluation at 7 days. Sectioned specimens from the lumbar spinal cord at the L4 level were stained with hematoxylin and eosin and were

studied for ischemic pathology by using standard light microscopy. Neuronal ischemic injury was evaluated at a magnification of ×200 by an observer blinded to the treatment groups. Normal motor neurons in the anterior horn of the spinal cord (anterior to a line drawn through the central canal perpendicular to the anterior median fissure) were counted for each animal and averaged. Ischemic neurons were defined by the following criteria: (1) shrinkage of the cell body, (2) an eosinophilic cytoplasm with a loss of Nissl granules, (3) triangular and pyknotic nuclei, and (4) a homogenizing change of the neuron.

Caspase12 and GRP78 immunohistochemistry. Immunohistochemistry was used to evaluate the changes in expression of caspase12 and GRP78, at 8 hours and 1 day after reperfusion in groups A, B, and C (n = 3 for each group at each time point). After endogenous peroxidase activity was quenched by exposing the slides to 0.3% H_2O_2 and 10% methanol for 30 minutes, the spinal cord sections were blocked in 2% normal horse serum for 2 hours at room temperature. Next, they were incubated with primary antibodies for 20 hours at 4°C. The primary antibodies used were as follows: rat monoclonal anti-caspase12 antibody (SC-12747; Santa Cruz Biotechnology Inc, Santa Cruz, Calif) diluted 1:50 and goat polyclonal anti-GRP78 antibody (SC-1050; Santa Cruz Biotechnology) diluted 1:200. The slides were rinsed in PBS and incubated for 3 hours with biotinylated antirat IgG (PK-6104; Vector Laboratories, Burlingame, Calif) and biotinylated antigoat IgG (PK-6105; Vector Laboratories) at 1:200 dilution in PBS containing 0.018% normal horse serum, respectively. Subsequently, they were incubated with avidin-biotin-horseradish peroxidase complex (PK-6104 and PK-6105). The slices were colonized with DAB/H_2O_2 solution and cytoplasm, and counterstained with hematoxylin. To ascertain specific binding of antibody for protein, a set of sections were stained in a similar way without primary antibody.

Statistical analysis. All results were expressed as means ± SD. Statistical significance was assumed at a *P* value of <.05. The Mann-Whitney *U* test, one-way analysis of variance, and post hoc tests with the Fisher protected least significant difference test were used to adequately identify which group differences accounted for the significant *P* value.

RESULTS

Physiologic status. There were no significant changes in the distal mean arterial pressures and heart rates between the groups during the operation. All 18 animals survived for 7 days without significant intra- or postoperative complications.

Neurologic outcomes. Fig 1 shows the neurologic outcomes of rabbits in each group. The Tarlov scores in groups A, B, and C were 4.0 ± 0.0, 4.0 ± 0.0, and 4.0 ± 0.0 at 8 hours; 4.0 ± 0.0, 2.5 ± 1.2, and 3.9 ± 0.4 at 2 days; and 4.0 ± 0.0, 0.7 ± 0.8, and 4.0 ± 0.0 at 7 days after reperfusion, respectively. While all sham-operated control animals (group A) demonstrated normal neurologic function, the group B rabbits presented with progressive deterioration of neurologic function, as was anticipated from the results of the preliminary experiments. Five out of six rabbits in group B demonstrated severe paraparesis by 7 days. In group C, neurologic function slightly deteriorated at 2 days but recovered by 7 days. There was no significant difference in Tarlov score between groups A and C across all time points. The score was significantly higher in group C than in group B at 2 and 7 days ($P < .0001$).

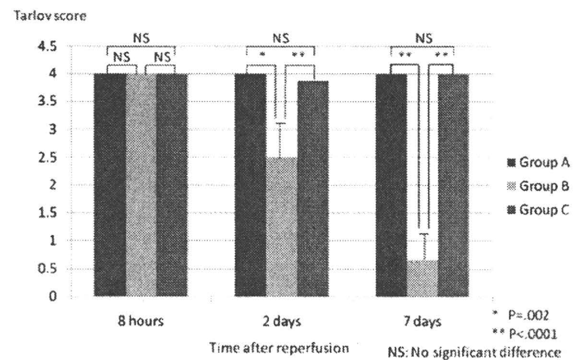

Fig 1. Changes in Tarlov score at 8 hours, and 2 and 7 days after reperfusion. In groups A and C, the Tarlov score was significantly higher at 2 and 7 days than in group B (*$P = .002$, **$P < .0001$). There was no significant difference in neurologic function between groups A and C.

Histopathologic assessment. Fig 2 shows the typical histopathologic findings of the spinal cord from a group B rabbit with paraplegia and a group C rabbit with normal neurologic function in comparison with those in group A. In group B (Fig 2, *B*), the motor neurons in the anterior horn were shrunken or had pale homogenous cytoplasm compared with those in group A, consistent with ischemic change. In group C (Fig 2, *C*), no apparent histologic changes were detected. The number of motor neurons with a normal appearance in the anterior horn of the spinal cord at 7 days after reperfusion was 47.4 ± 5.7, 21.5 ± 6.0, and 44.9 ± 7.9 in groups A, B, and C, respectively (Fig 3). The number of normal neurons was significantly less in group B than in groups A and C ($P < .0001$). There was no significant difference in the number of normal neurons between groups A and C ($P = .019$).

Histochemical study. Immunohistochemistry results for caspase12 and GRP78 are shown in Figs 4 and 5. In the sham-operated animals, there was no evidence of caspase12 and GRP78 expression in any cells of the spinal cord. In group B, caspase12 and GRP78 were present in motor neurons after 8 hours of reperfusion (Fig 4, *A* and Fig 5, *A*) but were apparently reduced at 1 day (Fig 4, *B* and Fig 5, *B*). In group C, expression of caspase12 and GRP78 in motor neurons was not observed at either 8 hours or 1 day (Fig 4, *C* and *D*, and Fig 5, *C* and *D*).

DISCUSSION

This study has demonstrated that the peripheral administration of 4-PBA at therapeutic doses prevents both im-

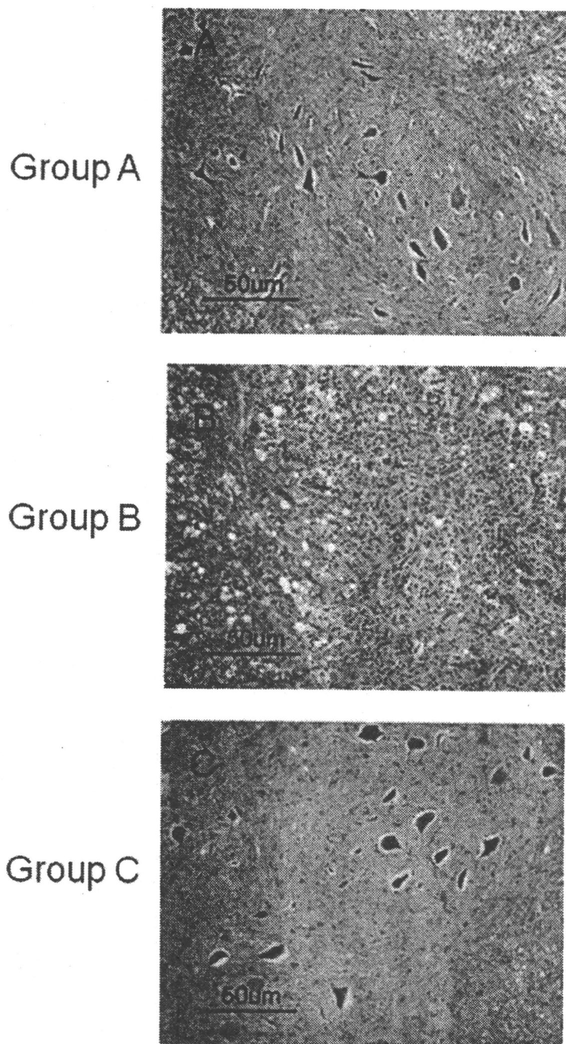

Fig 2. Histologic findings of spinal cord 7 days after 15 minutes of ischemia (hematoxylin and eosin staining). **A,** Sham-operated control spinal cord was intact. **B,** In spinal cord of group B, about 60% of motor neurons were lost. **C,** In group C, no ischemic damage was detected, and there were many normal motor neurons.

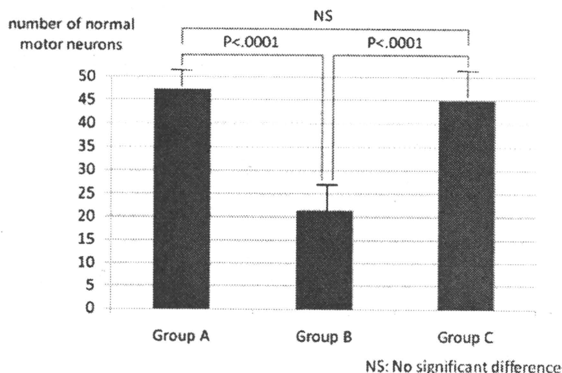

Fig 3. The number of viable neurons in the gray matter of lumbar spinal cord sections stained with hematoxylin and eosin. Group C had significantly more viable neurons than group B ($P < .0001$ compared with group B).

mediate and delayed neuronal damage of the spinal cord following an ischemic insult. Neurologic function was preserved with little histologic change observed in motor neurons, most probably due to suppression of ER stress.

When cells are exposed to stress, ER function is disrupted. Because the ER regulates the folding and quality control systems of membrane and secretory proteins, ER stress leads to an accumulation of unfolded proteins in the ER lumen and ER-mediated cell death can be induced.[18] In ER stress, several signaling pathways from the ER such as the unfolded protein response (UPR) are simultaneously activated. Accumulation of unfolded proteins in the ER is sensed by inositol-requiring protein 1 (IRE1), a serine/threonine kinase with an endoribonuclease domain in the ER membrane, and transcription induction of chaperone genes such as glucose-regulated proteins (GRPs) including GRP78 is promoted to re-fold the unfolded and accumulated proteins.[19,20] Caspase12, the first identified ER-associated member of the caspase family, is regarded as a representative molecule implicated in the cell death-pathway seen in ER stress.[11]

ER stress has been reported to contribute to ischemia-reperfusion injury in the brain and spinal cord by inducing apoptosis of neuronal cells.[8,10,21,22] We speculated that targeting the ER-associated apoptotic pathway might be an effective way to minimize neuronal cell damage, and explored the effect of an ER stress inhibitor, 4-PBA, on minimizing the ischemic injury using a rabbit spinal cord ischemia model.

A low molecular weight fatty acid, 4-PBA, can act as a chemical chaperone, assisting with protein folding and thus relieving the ER stress of the cell.[16,23-25] In the present study, 4-PBA administration was associated with decreased expression of caspase12 and GRP78 as well as the number of apoptotic cells, suggesting it has beneficial effects on attenuating ER stress. It appears to effectively reduce the neuronal damage, which progresses after reperfusion. Treatment with 4-PBA 30 minutes before and after ischemia notably improved the neurologic outcome. We used the 4-PBA dose of 15 mg/kg/h, based on phase 1 and pharmacokinetic studies of 4-PBA by peripheral infusion.[26,27] It has low toxicity and good penetrations into the cerebrospinal fluid.[28] It has been clinically used for treatment of urea cycle disorders in children, sickle cell disease, thalassemia, and cystic fibrosis.[29] Our study has suggested that 4-PBA at therapeutic doses can be a potential agent for preventing spinal cord ischemia. We found that 4-PBA seems to widely suppress ER stress-mediated apoptotic signaling under conditions of ischemia. Considering the findings of the present study, it is reasonable to conclude that the protective effect of 4-PBA on ER stress-mediated injury may involve the activity of chemical chaperone.

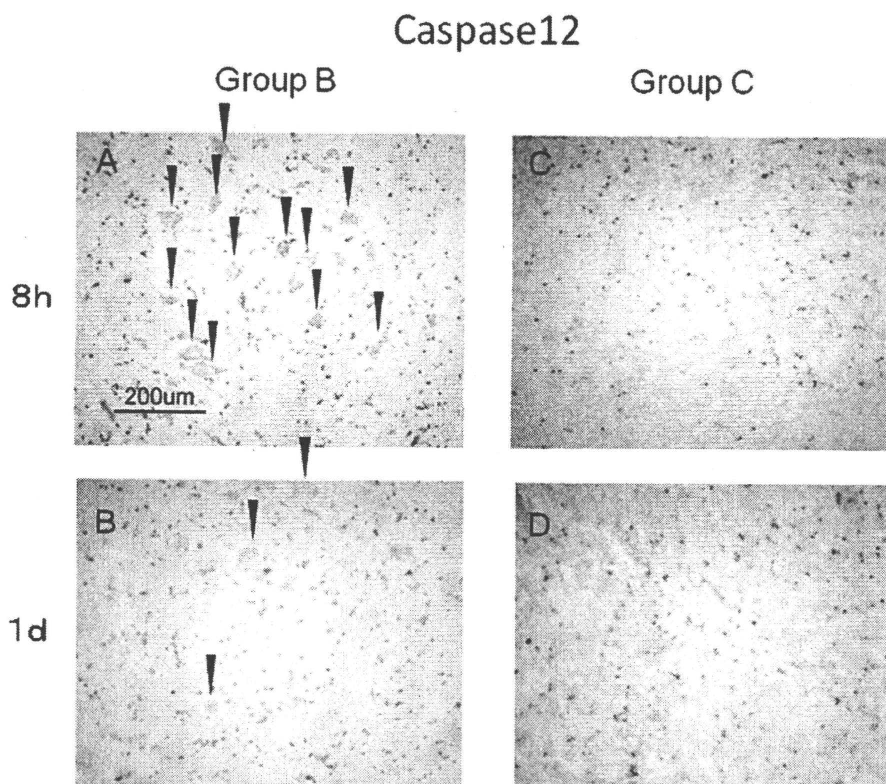

Fig 4. Immunohistochemical staining for caspase12 in spinal cords at 8 hours (**A**) and 1 day (**B**) in group B, and at 8 hours (**C**) and 1 day (**D**) in group C. Density of caspase12 immunoreactivity in group C was reduced relative to that in group B *(black arrows)*.

There are two patterns of neuronal injury due to ischemia: immediate and delayed.[30] The former is often caused by a severe ischemic insult and rapidly leads to necrotic neuronal cells. The latter is caused either by apoptosis[6,31,32] or necrosis.[30,33,34] Disruption of the infrarenal abdominal aortic blood flow for 15 minutes causes immediate neuronal injury by 2 days and delayed paraplegia by 7 days.[6,31-34] In the current study, 4-PBA prevented not only immediate neuronal injury but also delayed paraplegia. In the 4-PBA-treated groups, many intact motor neurons were preserved, whereas in the negative control group, the immunohistochemical findings showed expression of caspase12 in motor neurons. To date, 14 caspases have been discovered. They are supposed to be activated in a multiple cascade fashion, activated by caspase9,[35] an initiator caspase. Under appropriate stress signals, caspase12 is not only activated within the ER but also translocates to the cytosol, where it can activate caspase9 in a noncytochrome-c–dependent manner.[36] Activation of caspase 12 is considered to be one of the potential mechanisms by which the ER contributes to apoptosis. Expression of caspase12 precedes the appearance of neuronal damage and might, therefore, be implicated in the activation of apoptosis.

There are a few points to consider when 4-PBA is applied to clinical situation. First, histologic examination shows that there is some loss of motor neurons in the 4-PBA-treated groups, indicating that the protective effect of 4-PBA is not perfect. One strategy that may enhance the effectiveness of 4-PBA without increasing its adverse effects is topical injection into the clamped segment of the aorta or combined use of 4-PBA with topical/core cooling. Although preservation of the feeding artery to the spinal cord is essential, such an artery cannot always be identified preoperatively. The protective effect of 4-PBA administered intravenously or the above-mentioned integrated "spinoplegia" protocol may be a potential option in such instances.

Second, the spinal cord anatomy and circulation in rabbits differ significantly from humans. The main source of blood in the rabbit spinal cord is a segmental supply from the abdominal aorta.[37] Thus, the lumbar spinal cord of rabbits becomes ischemic by clamping of the infrarenal aorta. Despite the different anatomy, rabbits are often used as the experimental model of spinal cord ischemia because of the simplicity and reproducibility of the model. Some studies have occluded the infrarenal aorta with an intra-aortic balloon.[6,31,32] Although this procedure is easy and less invasive, it is unclear whether aortic occlusion is complete or not. We chose a direct clamping of the aorta through a retroperitoneal approach to precisely determine

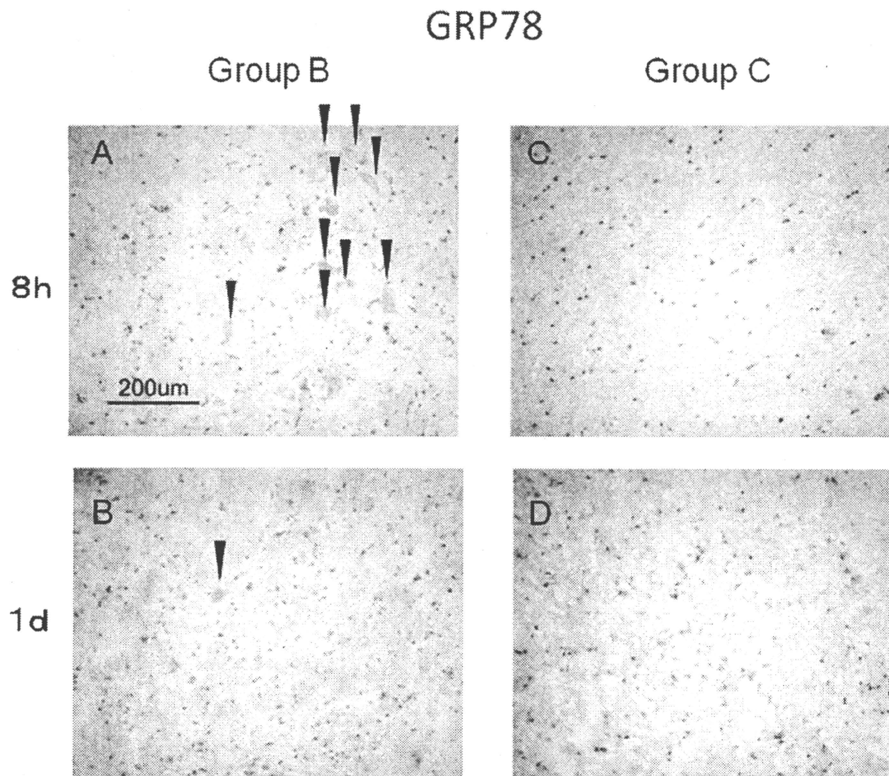

Fig 5. Immunohistochemical staining for GRP78 in spinal cords at 8 hours **(A)** and 1 day **(B)** in group B, and at 8 hours **(C)** and 1 day **(D)** in group C. Density of GRP78 immunoreactivity in group C was reduced relative to that in group B *(black arrows)*.

the ischemic time of the spinal cord for the reproducible development of paraplegia. On this basis, the current study showed the beneficial effect of 4-PBA.

Third, the drug toxicity of intravenous administration of 4-PBA and the peak time of ER stress at the onset of ischemia are other concerns that will likely limit the clinical application of 4-PBA, although reported side effects are not considerable.[26] An additional experiment that examines the timing of injection, injection method and dosage of 4-PBA administration would be required before any clinical applications.

In conclusion, administration of 4-PBA intravenously is feasible and effective without apparent neuronal damage of the spinal cord in a rabbit model. This study indicated that this method can potentially be a useful adjunct for spinal cord protection during thoracic and thoracoabdominal aortic operations.

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AUTHOR CONTRIBUTIONS

Conception and design: TM, KOrihashi, MH
Analysis and interpretation: TM, KOrihashi
Data collection: TM, BH, ST
Writing the article: TM
Critical revision of the article: KOrihashi, KOkada
Final approval of the article: TS
Statistical analysis: TM, KOrihashi
Obtained funding: Not applicable
Overall responsibility: TM

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Cold blood spinoplegia under motor-evoked potential monitoring during thoracic aortic surgery

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Objective: Motor-evoked potential monitoring is used to prevent paraplegia during thoracic aortic surgery. Multidetector computed tomography has been used preoperatively to detect the Adamkiewicz artery, but the hemodynamic significance of the Adamkiewicz artery is controversial. This study aims to evaluate whether the multidetector computed tomography–defined Adamkiewicz artery is hemodynamically essential and needs to be reconstructed with cold blood spinoplegia under motor-evoked potential monitoring.

Methods: From 2005 to 2008, both preoperative multidetector computed tomographic analysis and intraoperative neurogenic motor-evoked potential monitoring with cold blood infusion into the clamped segment of the aorta were done in 15 patients. A motor-evoked potential decrease to less than 50% of the initial value at 3 minutes after cold blood infusion determined the hemodynamic significance of the multidetector computed tomography–defined Adamkiewicz artery. Adamkiewicz arteries determined to be essential were reconstructed, and those determined to be nonessential were sacrificed.

Results: The Adamkiewicz artery was involved in the clamped segment of the aorta in 11 cases. After cold blood infusion, 8 patients experienced no significant motor-evoked potential decrease, and Adamkiewicz artery ligation was undertaken, whereas a moderate motor-evoked potential decrease was noted in 1 patient, prompting reconstruction. None of these 9 patients had permanent neurologic deficits. In 2 patients, the Adamkiewicz artery was reconstructed based on motor-evoked potential findings, with paraparesis occurring in 1 patient. In 4 patients without Adamkiewicz artery involvement in the clamped segment, there was no neurologic deficit.

Conclusions: Cold blood infusion accelerates motor-evoked potential changes and might enable decision making regarding the need for reconstruction of multidetector computed tomography–defined Adamkiewicz arteries. Cold blood–loaded motor-evoked potential is beneficial to minimize Adamkiewicz artery reconstruction time and limit spinal cord ischemia. (J Thorac Cardiovasc Surg 2011;141:755-61)

Paraplegia, paraparesis, or both remain serious complications of thoracic and thoracoabdominal aortic aneurysm repair and occur in 2% to 16% of patients[1-5] despite the implementation of various adjunctive procedures, including distal perfusion, mild hypothermia, cerebrospinal fluid drainage, and protective drugs. Two strategies that are commonly accepted for preserving essential spinal cord perfusion are (1) preoperative identification of the Adamkiewicz artery (AKA) by means of 64-row multidetector computed tomographic angiographic (MDCTA) or magnetic resonance angiographic analysis and (2) intraoperative monitoring of the motor-evoked potential (MEP).

The former modalities enable visualization of the continuity of the intercostal or lumbar arteries, the greater radicular artery, and the anterior spinal artery in more than 95% of patients.[6-8] Despite preoperatively identified AKAs being elaborately reconstructed in many institutions to minimize the risk of spinal cord injury, they are often found to be occluded on postoperative assessment, suggesting that they were not necessarily essential.

MEP monitoring has proved to be a sensitive monitor of spinal cord ischemia and is beneficial to improving surgical outcomes.[9,10] However, it can take longer than 10 minutes to determine ischemic changes of MEP in normothermia. For this reason, we introduced cold blood infusion into the clamped segment of the aorta, aiming to prompt MEP changes by topically cooling the spinal cord.[11] The MEP amplitude significantly decreased within 3 minutes after cold blood infusion, whereas no patient showed delayed onset of MEP decrease. Cold blood–loaded MEP appeared to clearly delineate the presence or absence of MEP changes within 3 minutes.

Recently, Griepp and Griepp[12] postulated the "collateral network theory" and reported excellent results. The intercostal and lumbar arteries between T7 and L1 were sacrificed while the MEP and somatosensory-evoked potential (SSEP) were monitored during thoracic and thoracoabdominal repair.[13] These reports make us reconsider the significance of the AKA.

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Abbreviations and Acronyms

AKA	= Adamkiewicz artery
CSF	= cerebrospinal fluid
CT	= computed tomography
MDCTA	= multidetector computed tomographic angiography
MEP	= motor-evoked potential
SSEP	= somatosensory-evoked potential
TEE	= transesophageal echocardiography
TEVAR	= thoracic endovascular aneurysm repair

Thus we hypothesize that (1) a prompt MEP decrease indicates hemodynamic significance of the AKA and need for reconstruction and (2) hemodynamic insignificance is responsible for an occlusion of the reconstructed AKA. The purpose of this article is to report the preliminary results of this study.

MATERIALS AND METHODS

Subjects

From 2005 to 2008, 29 patients underwent aortic repair for descending thoracic or thoracoabdominal aortic aneurysms in our institute, excluding emergency cases. In 15 of these patients, preoperative assessment of the AKA was done with MDCTA, and MEP was monitored intraoperatively. In the remaining 14 patients, MEP monitoring was not used because the aneurysm was located proximal to the T6 level or far distal to the AKA visualized with MDCTA, or cold blood infusion was not performed because of aortic dissection with a large false lumen, and these patients were excluded from the study. Demographics of the patients are shown in Table 1. There were 11 male and 4 female patients with ages ranging from 59 to 86 years (mean age, 72.7 ± 7.6 years). All patients had several comorbidities, including hypertension. Seven patients had descending thoracic aortic aneurysms, and 7 patients had Crawford type I and 1 patient had Crawford type IV thoracoabdominal aortic aneurysms.

Preoperative MDCTA Study

Helical computed tomographic (CT) angiographic analysis was performed with a 64-row multidetector CT scanner (Light Speed VCT; GE Healthcare, Waukesha, Wis). After 100 mL of contrast medium was administered intravenously, scanning was started when the CT value in the lumen of the thoracic aorta (T7) reached 200 Hounsfield units. Three-dimensional image reconstruction was performed with analysis software (Virtual Place Raijin; AZE, Ltd, Tokyo, Japan). The AKA was defined as a continuous vessel from the aorta through the intercostal or lumbar artery and the radiculomedullary artery to the anterior spinal cord artery to eliminate venous signatures from being mistaken as arterial.

MEP Monitoring

Neurogenic MEP monitoring was used in every case to obtain discrete waveforms during adequate muscle relaxation. On the day before the operation, a pair of bipolar, platinum electrode catheters (UKG-100–5PM; Unique Medical, Tokyo, Japan) were placed in the dorsal epidural space for intraoperative evoked spinal cord potential monitoring: a stimulating electrode was placed at the C5-C6 level, and a recording electrode was placed at the lumbar level. A cerebrospinal fluid (CSF) drainage catheter was inserted at a low lumbar location (L3–L4).

After induction of anesthesia with fentanyl/remifentanyl and propofol with vecuronium bromide, a pair of bipolar, screw-type platinum electrodes was placed on the bilateral anterior parietal region for transcranial electrical stimulation of the cerebral motor cortices. A pair of bipolar surface electrodes was placed on the bilateral peroneal nerves for the SSEP. The MEPs and SSEPs were recorded at the lumbar electrodes. Stimulation and recordings were performed with the EpochXp system (Nihon Koden, Tokyo, Japan; Figure 1). The stimuli (MEP: intensity, 100 mA; duration, 0.2–1.0 ms, single pulse; SSEP: intensity, 25–50 mA; duration, 0.2 ms, single pulse) were delivered at a rate of 3.3 to 7.3 Hz. Each evoked potential was filtered between 20 and 1500 Hz and was amplified by averaging a total of 20 to 50 responses. All data were stored in the hard disc of this system.

Surgical Procedures

The aorta was exposed through a left thoracotomy with or without a paramedian retroperitoneal approach. Femorofemoral partial cardiopulmonary bypass was established with a pump flow of 2.2 to 2.6 L · min^{-1} · m^{-2} after intravenous injection of steroids and the oxygen radical scavenger edaravone. The rectal temperature was maintained at 34°C. CSF drainage was continued for 2 days to maintain the CSF pressure at less than 10 mm Hg. The distal perfusion pressure was maintained at a level higher than 60 mm Hg. The intercostal arteries, including the preoperatively identified AKA, were exposed.

After aortic crossclamping, cold blood (4°C) was infused into the clamped segment of the aorta through a 14-gauge needle at a flow rate of 100 to 200 mL/min for 3 minutes (Figure 2) while MEP amplitudes were monitored. The puncture site was carefully determined based on meticulous assessment by means of transesophageal echocardiographic (TEE) analysis, and cold blood inflow was visualized with TEE analysis to avoid unintended detachment of a mural thrombus. The amount of infused cold blood depended on the size of the aneurysm and ranged from 300 to 600 mL.

Decision Making Based on MEP Monitoring

The AKA in the clamped segment was diagnosed as essential when the MEP amplitude decreased to a level less than 50% of the original amplitude after cold blood infusion within 3 minutes (Figure 3). In patients with a critical decrease in MEP, the AKA was immediately reconstructed after aortotomy with a vascular prosthesis (10 mm in diameter) or a saphenous vein and was perfused with warm blood at a flow rate of 40 mL/min and a perfusion pressure of up to 150 mm Hg. After the aorta was repaired, this reconstructed intercostal graft was anastomosed to the main graft. When there was no decrease of MEP amplitude after cold blood infusion, the AKA identified by means of preoperative MDCTA study was determined to be not essential and was ligated extraluminally before opening the aneurysm. If the other intercostal arteries were exposed, they were also ligated extraluminally before opening the aneurysm; otherwise, they were ligated intraluminally.

RESULTS

A total of 17 AKAs were identified in 15 patients undergoing preoperative MDCTA analysis: a single AKA in 13 patients and 2 AKAs in 2 patients. The AKA entered the spinal canal at T7 (2 AKAs), T9 (3 AKAs), T10 (5 AKAs), T11 (3 AKAs), T12 (2 AKAs), or L1 (2 AKAs). Fifteen AKAs were on the left side, and 2 AKAs were on the right side. No complications related to the placement of epidural electrodes for MEP monitoring were encountered in any case.

Duration of the operation, duration of extracorporeal circulation, and aortic clamp time were 418 ± 182, 92 ± 40,

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TABLE 1. Patients' characteristics

No. of patients	15
Age, y (range)	72.7 ± 7.6 (59 - 86)
Sex (male/female)	11/4
Preoperative variables	
Hypertension	13 (87%)
Hyperlipidemia	4 (27%)
Diabetes mellitus	3 (20%)
COPD	1 (7%)
Coronary artery disease	4 (27%)
Renal insufficiency	2 (13%)
Cerebral infarction	1 (7%)
Smoking	4 (27%)
Marfan syndrome	0 (0%)
Coactation of aorta	0 (0%)
Previous chest pain	3 (20%)
Aortic dissection	4 (27%)
Traumatic	0 (0%)
Aortic infection	0 (0%)
Aortic rupture	0 (0%)

COPD, Chronic obstructive pulmonary disease.

and 77 ± 31 minutes, respectively. There was no paraplegia, but 1 patient experienced paraparesis caused by cerebral infarction, probably secondary to multiple thromboembolism during the proximal aortic clamping. There were no operative or hospital deaths.

Table 2 shows the level of the AKAs, involvement of the AKA in the aneurysm, changes in MEP amplitudes, and surgical outcomes. Although the AKA was not involved in the clamped segment of the aorta in 4 patients (patients 1–4), the origin of the AKA was within the clamped segment in 11 patients (patients 5–15). In the former 4 patients, there were no MEP changes after cold blood infusion. No neurologic deficit was encountered in any of these patients. Among the latter 11 patients, there was no significant MEP decrease within 3 or 20 minutes in 7 patients (patients 5–11). Although every intercostal artery in the clamped segment, including the supposed AKA, was ligated, none of them presented with neurologic deficit. The MEP amplitude decreased but remained at a level greater than 50% of the initial value in 2 patients (patients 12 and 13). Delayed MEP decrease within 20 minutes was not appreciated in either of these patients. The AKA was ligated in both patients, but it was finally reconstructed in 1 patient because of the reason described below. The MEP amplitude decreased to a level less than 50% of the initial value in only 2 patients (patients 14 and 15), both of whom underwent reconstruction of the AKAs.

Although patient 12 presented with a gradual decrease of MEP to 70% of the initial value during the operation, motor function of the lower limb was temporarily restricted after the operation and was recovered during the hospital stay. Patient 13, who had previous repair of the proximal thoracic aorta for acute aortic dissection, underwent replacement of the distal descending aorta. The MEP amplitude decreased to 70% at 3 minutes of cold blood infusion and gradually recovered to the initial level. However, the MEP amplitude decreased to 70% again during a period of hypotension caused by sustained bleeding and did not recover to the

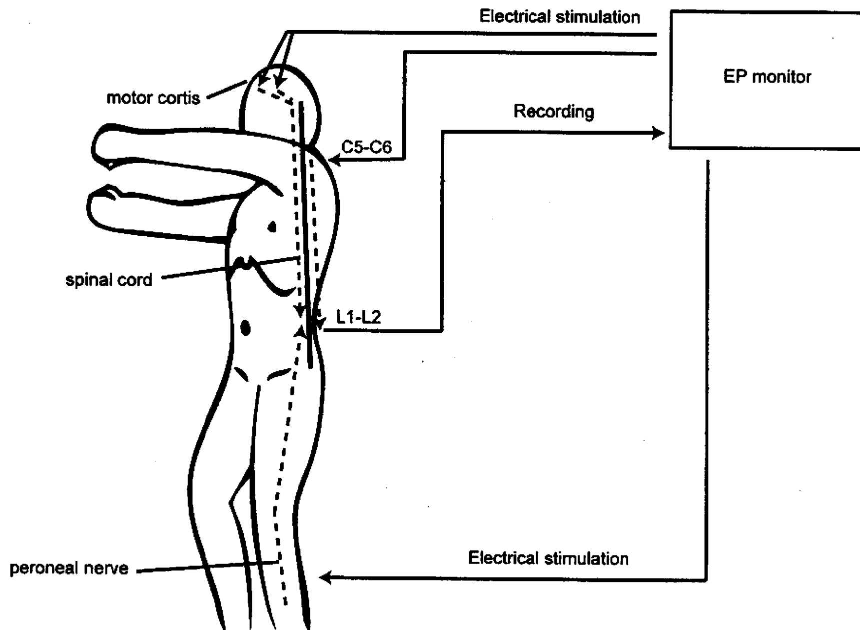

FIGURE 1. Evoked potential *(EP)* monitoring system and conduction pathway of evoked potential *(dotted line)*. Transcranial electrical stimulation passed through motor cortices and the spinal cord was recorded by using the epidural electrode (L1-L2; transcranial motor-evoked potential). Descending evoked spinal cord potentials (C5-C6 to L1-L2) and somatosensory-evoked potentials (peroneal nerve to L1-L2) were also recorded for multilateral observation.

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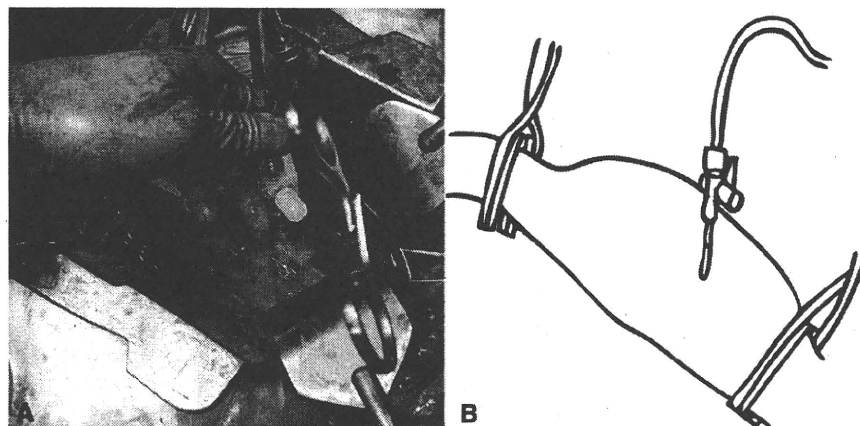

FIGURE 2. Procedure of cold blood infusion. A, A 14-gauge needle was punctured into the aorta. B, Schema of the picture. Cold blood was infused into the clamped segment of the aorta.

initial level. In this case, we initially ligated the AKA but decided to reconstruct the AKA at T10 to ensure adequate blood flow because the spinal cord perfusion appeared to be partially dependent on the AKA. This patient did not have a neurologic deficit. Postoperative CT scanning showed occlusion of the reconstructed intercostal artery.

In patients 14 and 15, the intercostal arteries were reconstructed based on the significant and immediate MEP changes. In patient 14, the MEP recovered after reperfusion, and no neurologic deficit developed. In patient 15, however, MEP disappeared at one point and later recovered, although only to 50% of the initial level. Aortic perfusion pressure was maintained at greater than 100 mm Hg during the operation and during the postoperative period. The patient in this case had paraparesis postoperatively. In this patient, the aorta was diffusely and severely atherosclerotic, and fragile intima was likely to have caused left cerebral infarction from a multiple thromboembolism caused by proximal aortic clamping. Furthermore, selective perfusion of the intercostal artery was not feasible because of atherosclerotic stenosis of the AKA, and ischemic time was prolonged. Postoperatively CSF drainage pressure increased to greater than 15 mm Hg, and CSF drainage was continued. The cranial magnetic resonance image showed multiple left cerebral infarctions. The left upper limb function was normal, but the left lower limb movement was impaired, which was unexplainable based on the cranial magnetic resonance image. Therefore we concluded that the patient had lower limb paraparesis with right hemiplegia caused by both stroke and spinal cord ischemia.

DISCUSSION

Despite the fact that thoracic endovascular aneurysm repair (TEVAR) has achieved a lower incidence of paraplegia and paraparesis than open surgical intervention, neurologic complications have not been completely eliminated, suggesting a possibility of occluding the essential AKA by the endovascular graft.[14] Siegenthaler and associates[15] reported delayed temporary paraparesis that appeared after successful TEVAR, but the deficit was corrected by increasing the blood pressure. These findings indicate that spinal cord perfusion depends not solely on AKAs but partially

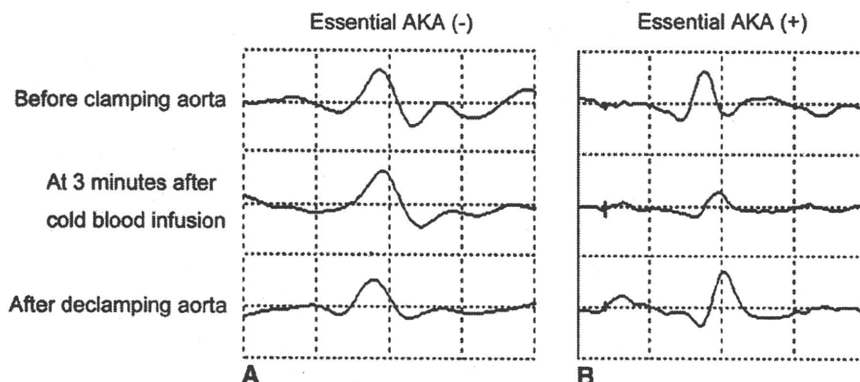

FIGURE 3. Changes in motor-evoked potential (MEP). A, MEP amplitude did not change. B, MEP amplitude decreased after cold blood infusion within 3 minutes and was restored after Adamkiewicz artery *(AKA)* reconstruction and declamping of the aorta.

TABLE 2. MDCTA-defined AKA and results

Patients no.	Type of aneurysm	AKA (MDCTA)	AKA in aneurysm	MEP at 3 min	Amplitude (%)		Reconstruction of branch artery	Neurologic outcome
					Lowest	At end		
1	DTAA	T9L, T12L	N	100%	100%	100%	None	No deficit
2	DTAA	T11L	N	100%	100%	100%	None	No deficit
3	I	T12L	N	100%	100%	100%	None	No deficit
4	IV	T11L	N	100%	100%	100%	None	No deficit
5	DTAA	T7L	Y	100%	100%	100%	None	No deficit
6	DTAA	T7L	Y	100%	100%	100%	None	No deficit
7	I	T9R	Y	100%	100%	100%	None	No deficit
8	DTAA	T10R	Y	100%	100%	100%	None	No deficit
9	I	T11L	Y	100%	100%	100%	None	No deficit
10	I	T9L	Y	100%	80%	100%	None	No deficit
11	DTAA	T10L	Y	100%	70%	100%	None	No deficit
12	I	L1L	Y	80%	70%	70%	None	No deficit
13	I	T10L, L1L	Y	70%	70%	80%	T10L	No deficit
14	DTAA	T10L	Y	45%	45%	90%	T10L	No deficit
15	I	T10L	Y	40%	0%	50%	T10L	Paraparesis

The patients are in order in relation to motor-evoked potential change and not in chronologic order. Reconstructed branch arteries were patent in patients 13 and 14 and occluded in patient 15. *MDCTA*, Multidetector computed tomographic angiography; *AKA*, Adamkiewicz artery; *MEP*, motor-evoked potential; *DTAA*, descending thoracic aortic aneurysm.

on collateral circulation, although the latter can be inadequate under low perfusion pressure.

The role of collateral circulation has been emphasized in the collateral network concept of Griepp and Griepp.[12] Etz and colleague[13] reported that sacrifice of the intercostal and lumbar arteries during thoracic and thoracoabdominal aortic aneurysm repair resulted in low rates of immediate and delayed paraplegia. They described a patient with immediate paraplegia who experienced precipitating intraoperative dissection resulting in 6 hours of lower body ischemia. This case indicates the importance of collateral circulation.

Two questions arise. First, should every AKA be elaborately reconstructed at a risk of prolonged ischemia of the spinal cord? Second, is it the best strategy to create a situation in which spinal cord perfusion depends only on collateral circulation? A possible solution is to reconstruct only the essential AKAs and sacrifice the other nonessential AKAs to minimize the ischemic insult on the spinal cord.

Improved quality of 64-row MDCTA and magnetic resonance angiography has enabled visualization of AKAs in more than 95% of cases.[6-8] Three-dimensional CT images discretely visualize the continuity from the intercostal artery to the anterior spinal cord artery and facilitate identification of the AKA in the operative field. This development has led to a situation that AKAs need to be reconstructed in most cases, and the majority of grafts are found to be occluded postoperatively.[16] It is unclear whether the occluded graft was effective in avoiding spinal cord ischemia in the perioperative period or whether reconstruction was even necessary. A considerably lower incidence of paraplegia after TEVAR, despite occlusion of every branch artery, appears to support the latter. In another respects, however, TEVAR might create a new Achilles heel: persistent susceptibility to hypotension, even if the patient can survive the neurologic deficit in the acute phase. Thus it is desirable to identify and reconstruct only the essential AKAs among the morphologically defined AKAs.

We previously reported that cold blood infusion into the clamped segment of the aorta induces a rapid change in MEP amplitude.[11] A decrease in MEP amplitude might be related to local hypothermia by cold blood.[5] Ueno and coworkers[17] reported that intra-aortic infusion of cold lactated Ringer's solution (3°C) cooled the spinal cord by 6°C to 7°C. Because hypothermia induces a decreased electrophysiologic response in cerebral and peripheral neurologic activity,[18,19] an immediate MEP decrease is likely to be caused by cooling of the spinal cord. Furthermore, because cold blood saturated with oxygen is infused, infusion of cold blood does not induce spinal cord ischemia and just accelerates MEP changes. We hypothesized that cold blood infused into the essential AKA topically cools the spinal cord and decreases MEP amplitude. We did not perform a morphologic assessment but rather a functional assessment.

In this study, as well as a previous study, the MEP was recorded at the intrathecal electrode (neurogenic MEP).[11,20] The advantage of a neurogenic MEP is a simple waveform compared with a compound waveform recorded in a myogenic MEP.[13,21] However, it takes a longer time to detect ischemic changes in a neurogenic MEP than in a myogenic MEP. de Haan and associates[22] reported that the median time to a 50% reduction in epidural and myogenic MEP amplitudes after aortic occlusion was 11.3 minutes and within 2 minutes, respectively. We previously showed that an MEP decrease was caused by cooling of the spinal cord in a dog model.[23] Despite the longer time to detect changes in neurogenic MEP during ischemia, cold blood infusion might be helpful for identifying

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hemodynamically significant AKAs based on an early change in the neurogenic MEP.

We routinely monitor both afferent and efferent conduction to check for damage of the spinal cord or in case the MEP recording is unexpectedly interrupted for some unknown reason. SSEPs were used to distinguish between spinal cord ischemia and systemic factors or technical problems. The monitoring of both MEPs and SSEPs is considered to be a safety system to prevent spinal cord ischemia.

Our current strategy for descending aortic repair is described as follows:

(1) If there is no AKA in the clamped segment, the branched arteries are not preserved.
(2) If the AKA is present in the clamped segment, MEP changes after cold blood infusion into the clamped segment of aorta are monitored.
   (a) Without a significant MEP change, the AKA is not reconstructed.
   (b) When MEP amplitude decreases to a level less than 50% of the initial value, the AKA is determined to be essential and is reconstructed.

The cutoff value of MEP amplitude as 50% of the baseline value is based on the report of van Dongen and coworkes[10] that the relative risks of paraplegia in patients with an MEP amplitude of 50% or less than the baseline value after 5 minutes of reperfusion and during closure of the skin were 21.7 and 30.9 times higher, respectively, compared with those of patients with an MEP of greater than 50% of the baseline value. We evaluated the MEP changes at 3 minutes after cold blood infusion based on the findings in our previous studies.[11,20] The MEP amplitude did not decrease to a level less than 50% of the baseline value after 3 minutes of cold blood infusion in any patient unless there was an apparent cause, such as considerable systemic hypotension. If our hypothesis was wrong, neurologic deficit should have occurred in several patients in whom the AKA in the clamped segment of the aorta was sacrificed (patients 5–12). In this series, MEP amplitude was unchanged after cold blood infusion in 7 of 11 patients, and this might explain the high incidence of graft occlusion of reconstructed AKAs.

It remains uncertain whether the AKA should be revascularized when the decrease in MEP amplitude is mild.[20] Kawanishi and colleagues[24] reported that 8 of 9 patients whose MEP amplitude decreased to less than 75% of baseline value at the end of the procedure had a neurologic deficit postoperatively. Because our study had a small number of patients, it is difficult to make a definitive conclusion, especially when the MEP is between 50% and 99% of the baseline value. In 2 patients, the MEP amplitude decreased to 70% to 80% of the baseline value. Although 1 patient had no paraplegia with the immediate decision to reconstruct the AKA, postoperative CT scanning revealed occlusion of the reconstructed intercostal artery, and this patient often noticed discomfort and slight palsy of the lower limbs during periods of hypotension associated with daily hemodialysis. The other patient had a transient paraparesis, probably as a result of sustained hypotension, which was caused by a large amount of oozing from the broadly dissected surface in this redo case. The events in these patients appear to be comparable with those in the patient undergoing successful TEVAR with delayed paraplegia caused by hypotension. These results suggest that the AKA should be reconstructed if there is any decrease in MEP amplitude. Hypotension during the operation and the postoperative period should be avoided. This will be our new strategy in the future based on the results of the current study.

Neurologic deficit in surgical cases is likely to be caused by (1) occlusion of the essential AKA, (2) intraoperative hypotension that leads to hypoperfusion of the spinal cord for a prolonged period of time, or both. Acher and associates[25] reported desirable outcomes of descending aortic surgery by maintaining the blood pressure at greater than 90 mm Hg and mentioned that the mean arterial pressure during aortic clamping was a significant factor for paralysis. By using a multidisciplinary strategy in aortic surgery, the incidence of neurologic deficit can be decreased to a level comparable with that of TEVAR. Reconstruction of all AKAs detected in the preoperative assessment can adversely prolong the duration of cardiopulmonary bypass and increase the amount of bleeding. It might be beneficial to limit the number of reconstructions by identifying the essential functional AKA by means of MEP monitoring under cold blood infusion. If the essential AKA can be successfully reconstructed without neurologic deficit, it can be beneficial in reducing the future risk against unpredicted hypotension in various situations.

In 4 patients (patients 1–4), the AKA was located close to the aneurysm on the preoperative CT scan, and there was a risk of including this artery in the clamped segment. The level of the aortic clamp might be altered when the anastomosis is difficult. In such a situation, the nearby AKA can be included in the clamped segment. Thus we have used our method in these patients and included it in this study.

The small number of patients in the current series is a limitation of this study. Nevertheless, our preliminary results demonstrate that the majority of MDCTA-defined AKAs can be sacrificed without neurologic sequelae. The need for puncture and cold blood infusion into the aneurysm can be another limitation because it might raise a concern of possible thromboembolism. However, careful TEE assessment for an appropriate puncture site and subsequent observation during infusion has shown that this procedure can be safely performed.

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