Protective use of *N*-methyl-D-aspartate receptor antagonists as a spinoplegia against excitatory amino acid neurotoxicity

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Objective: Paraplegia remains a serious complication of thoracic and thoracoabdominal aortic operations. To avoid this dreadful complication, *N*-methyl-D-aspartate (NMDA) receptor antagonists have been examined in the ischemic or excitotoxic neuronal injury model. In the present study, we evaluated the protective efficacy of NMDA receptor antagonists that were infused segmentally after aortic clamping, as a spinoplegia, to reduce aspartate neurotoxicity in the spinal cord.

Methods: Infrarenal aortic isolation was performed in New Zealand white rabbits. Group A animals (n = 7) were pretreated with the segmental infusion of MK-801, a noncompetitive NMDA receptor antagonist, followed by segmental aspartate (50 mmol) infusion for 10 minutes. Group B animals (n = 6) received pretreatment with CGS19755, a competitive NMDA receptor antagonist, followed by the same aspartate infusion as group A. Group C animals (n = 7) received vehicle only, followed by aspartate infusion as a control group. In addition, group D animals (n = 6) were pretreated with MK-801 that was administrated intravenously 1 hour before aspartate infusion. Neurologic status was assessed at 12, 24, and 48 hours after operation by using the Tarlov score. The spinal cords were procured at 48 hours for histopathologic analysis to determine the extent of excitotoxic neuronal injury.

Results: Most of the animals in groups A and D revealed full recovery or mild motor disturbance. Group B and C animals exhibited paraplegia or paraparesis with marked neuronal necrosis. In the Tarlov score at 48 hours, group A animals represented better neurologic function than group C ($P < .01$) and similar motor function to group D animals. Severe histopathologic change was not observed in groups A and D. Animals in groups A and D showed a greater number of motor neurons than animals in groups B and C ($P < .01$). The difference could be due to chance between group A and D animals ($P = .08$).

Conclusions: These results showed that the segmental infusion of noncompetitive NMDA receptor antagonist as an intraoperative spinoplegia could have a protective effect on the spinal cord neurons against excitotoxic neuronal injury in vivo. On the other hand, efficacy of the use of competitive antagonist was suggested to be limited in this model, probably because of the insurmountable obstacle of the blood-brain barrier. (J Vasc Surg 2005;42:765-71.)

Clinical Relevance: Paraplegia is a devastating complication during surgical repair of the thoracic and thoracoabdominal aortas. Excitatory amino acids neurotoxicity through the *N*-methyl-D-aspartate (NMDA) receptor is no doubt the pathologic hallmark of ischemic and postischemic spinal cord injury. Systemic administration of either a competitive or noncompetitive NMDA antagonist has been reported to have neuroprotective effect, in terms of preoperative treatment, with dose-related central sympathomimetic and sedative effects. Local administration, particularly of a noncompetitive NMDA antagonist, infused segmentally after aortic clamping could therefore be a potent intraoperative pharmacologic strategy to minimize the effective dose that retains NMDA antagonism without undesirable adverse effects. Our ability to reproduce this model could facilitate pharmacologic prevention or provide a new surgical technique as a spinoplegia for NMDA receptor-mediated neuronal injury.

Paraplegia is a devastating complication of surgical repair of the thoracic and thoracoabdominal aortas. The incidence varies between 1% and 30%, depending on the extent of the aneurysm, dissection, rupture, and aortic clamping time.[1-4] Surgical techniques, such as distal perfusion, hypothermia, spinal fluid drainage, and segmental repair of intercostal arteries, or protective pharmacologic strategies have been developed to reduce the occurrence of this complication by either maintaining adequate spinal cord perfusion or increasing spinal cord ischemia tolerance.[3-5] However, no method yet completely avoids this dreadful complication.

Excitatory amino acids (EAAs), mainly glutamate and aspartate, are major excitatory neurotransmitters in the central nervous system, including the spinal cord. EAAs are also well known to have neurotoxicity under metabolic stress in a manner of activating *N*-methyl-D-aspartate (NMDA) receptor and α-amino-3-hydroxy-5-methylisoazole-4-propionic acid (AMPA) receptor.[6-9] Many clinical and experimental reports have revealed that EAAs contribute to the irreversible and fatal neuronal injury during and after ischemia in the spinal cord; nevertheless, it has been difficult to indicate EAA neurotoxicity in vivo.[10]

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We developed the experimental model that showed detrimental effects of exogenous aspartate on the spinal cord neurons under metabolic stress in vivo.[9] Aspartate is a potent NMDA receptor agonist that is well attenuated by local injection or intravenous administration of NMDA receptor antagonists such as the noncompetitive antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cycloheptan-5,10-imine (MK-801) and the competitive antagonist cis-4-phosphonomethyl-2-piperidine-carboxylic acid (CGS19755).[6,11-17]

In a study using a rabbit model, the intravenous administration of MK-801 (5 mg/kg) starting immediately after spinal cord injury was reported to improve motor recovery and reduce edema formation.[14] Acute therapy with the intravenous administration of CGS19755 (30 to 40 mg/kg) was also evaluated using a rabbit model of spinal cord or focal cerebral ischemia and revealed significant protective effect against ischemic neuronal injury.[15,16] In a recent study, we showed that the preoperative intravenous administration of either MK-801 (6 mg/kg) or CGS19755 (30 mg/kg) had a protective effect on the spinal cord neurons against EAA neurotoxicity.[17]

Evidences were confirmed that treatment of NMDA receptor antagonists ameliorates neuronal injury; however, the effects of segmental infusion have not yet been determined. The purpose of this study was, therefore, to evaluate the protective effect of the segmental infusion of a NMDA receptor antagonist against aspartate neurotoxicity in the spinal cord. In the present study, we administrated two types of NMDA receptor antagonists, MK-801 and CGS19755, immediately after aortic clamping that was designed as a segmental infusion of spinoplegia.

MATERIAL AND METHODS

Subjects. Male New Zealand white rabbits weighing 3.0 to 3.5 kg were used for this study. All experimental animals received humane care and treatment in compliance with the *Guide for the Care and Use of Laboratory Animals*, Institute of Laboratory Animal Resources, Commission of Life Science, National Research Council (Washington: National Academy Press, 1996). This study was approved by the Keio University Institutional Review Board.

Experimental Model. Animals were anesthetized with 1.5% halothane and 98.5% oxygen and were placed in the supine position, maintaining spontaneous breathing. Rectal temperature was kept normal (39°C) with a heating mattress. A median laparotomy was used, and the abdominal aorta was dissected just inferior to the left renal vein and above the bifurcation. Heparin sulfate was used for systemic anticoagulation. A 20-gauge catheter was introduced from the femoral artery to the abdominal aorta, and the tip of the catheter was placed 5 mm above the bifurcation.

The abdominal aorta was clamped inferior to the left renal vein, and the femoral catheter was snared around the bifurcation. The posterior mesenteric artery was also clamped with a vascular clip. Thus the abdominal aorta was isolated with six or seven lumbar arteries. All the rabbits pretreated with segmental infusion received protective agent or vehicle (saline) via the femoral arterial catheter immediately after aortic isolation.

Neuronal injury in the spinal cord was induced by the methods previously described.[9] Briefly, after pretreatment, aspartate solution, which had been incubated at normal body temperature (39°C) and adjusted to the physiologic osmolarity, was infused into the isolated aortic segment via the femoral catheter at a rate of 2 mL/min for 10 minutes. At the end of the infusion, all the clamps were released and the snare around the bifurcation loosened. The abdomen was closed after the femoral arterial catheter was removed.

Pretreatment with NMDA receptor antagonist. MK-801 was obtained from Merck Research Laboratories (Rathway, NJ). CGS19755 was from Novartis Pharmaceuticals Research (Summit, NJ). These compounds were dissolved in saline and incubated at 39°C.

Group A rabbits (n = 7) were pretreated with the local administration of MK-801 (4 mg/kg), which was infused promptly after aortic isolation in a fashion not to exceed the mean arterial pressure via the femoral catheter, and received a segmental infusion of aspartate (50 mmol) at a rate of 2 mL/min for 10 minutes.

Group B rabbits (n = 6) received pretreatment with CGS19755 (30 mg/kg) followed by the same aspartate infusion as group A.

Group C rabbits (n = 7) received only vehicle (saline) followed by aspartate infusion as a control group.

Group D rabbits (n = 6) were pretreated with MK-801 (4 mg/kg) that was administrated intravenously for 3 hours beginning 1 hour before the same segmental infusion of aspartate (50 mmol) via the femoral catheter (MK-801 intravenous).

Five animals were also treated identical to groups A and B but did not undergo aortic clamping (MK-801 and CGS19755 sham).

Neurologic and histopathologic evaluation. Hindlimb function was evaluated at 12, 24, and 48 hours after operation according to the modified Tarlov score (5 = normal hop, 4 = weak hop, 3 = sits alone, 2 = sits with assistance, 1 = slight movement, 0 = no movement). At 48 hours, animals were reanesthetized and sacrificed with an overdose of intravenous pentobarbiturate. The spinal cord was fixed by a perfusion of 10% formaldehyde solution via the femoral arterial catheter in the same manner as the first operation, followed by immersion fixation for 2 weeks.

Cross-sections from the lower thoracic to the sacral cord were stained with hematoxylin and eosin and Luxol-fast blue. For quantitative histopathologic analysis, motor neurons were counted by the methods previously described.[18] Briefly, spinal cords were cut into 10-mm slices from the bifurcation of the third sacral nerve. Motor neurons of normal appearance were counted in each segment of 10 mm, 30 mm, and 50 mm proximal from the third sacral nerve. A normal-appearing motor neuron was defined as >30 μm in size, having no eosinophilic change, and having a clear nucleolus without karyolysis. Histopathologic assessment using a light microscopy was done by a neuropathologist blinded to experimental groups.

Table I. Neurologic outcomes

	12 hours				*24 hours*				*48 hours*			
Talrov score	*Group A**	*Group B*	*Group C*	*Group D**	*Group A*	*Group B*	*Group C*	*Group D*	*Group A*	*Group B*	*Group C*	*Group D*
5	1	0	0	0	2	0	0	1	2	0	0	1
4	2	0	0	1	3	0	0	3	3	0	0	3
3	0	0	0	1	2	0	0	1	2	0	0	1
2	0	1	1	0	0	1	1	1	0	1	0	1
1	0	3	1	0	0	3	1	0	0	3	2	0
0	0	2	5	0	0	2	5	0	0	2	5	0

Group A: MK-801 segmental (n = 7); Group B: CGS19755 (n = 6); Group C: control (n = 7); Group D: MK-801 intravenous (n = 6).
*The motor function of some rabbits could not be evaluated because of the sedative effect of MK-801.

Statistical analysis. Results are expressed as mean ± SD. Statistical significance of hindlimb function was analyzed among the four experimental groups by the Kruskal-Wallis test followed by the Dunn's test for all pair-wise comparisons. We compared numbers of normal-appearing motor neurons using two-way Bonferroni analysis of variance (ANOVA) and physiologic parameters using one-way ANOVA. Data were considered statistically significant at a value of $P < .05$.

RESULTS

In groups A and D, most of the rabbits could not be evaluated because of the sedative effect of MK-801, which lasted for >12 hour after operation. In group A, two of the seven rabbits recovered fully, showing normal hop (Tarlov 5), and the other three exhibited mild disturbance of motor function (Tarlov 4) at 48 hours (Table I). In group D, one of the six recovered fully, and three exhibited mild disturbance of motor function at 48 hours. In group B, pretreated with CGS19755, five of the six rabbits exhibited paraplegia (Tarlov 0) or paraparesis (Tarlov 1) at all the time points (Table I). All group C animals exhibited paraplegia or paraparesis at 48 hours. The median Tarlov score at 48 hours was 4.0 in group A, 5.0 in group B, 0.0 in group C, and 4.0 in group D. Group A animals, pretreated with segmental infusion of MK-801, had significantly better neurologic score compared with group C ($P < .01$) and showed a similar score to group D animals ($P = .28$). The differences could be due to chance between group B and group C animals ($P = .40$).

Most of the rabbits in groups A and D demonstrated normal histology (Fig 1). Some rabbits that exhibited disturbance of motor functions showed minimal neuronal degeneration with shrinkage or eosinophilic change. However, these focal lesions were found in one of several sections of the sacral cord. Histopathology revealed severe neuronal injury in groups B and C. Sections from these groups exhibited severe and extensive gray matter necrosis with prominent vacuolation and numerous degenerated neurons that were predominantly localized in the anterior horns of the lower thoracic and sacral cord. Degenerated neurons were irregularly shrunken or exhibited eosinophilic structureless cytoplasm with their nuclei stained hyperchromatic (Fig 2).

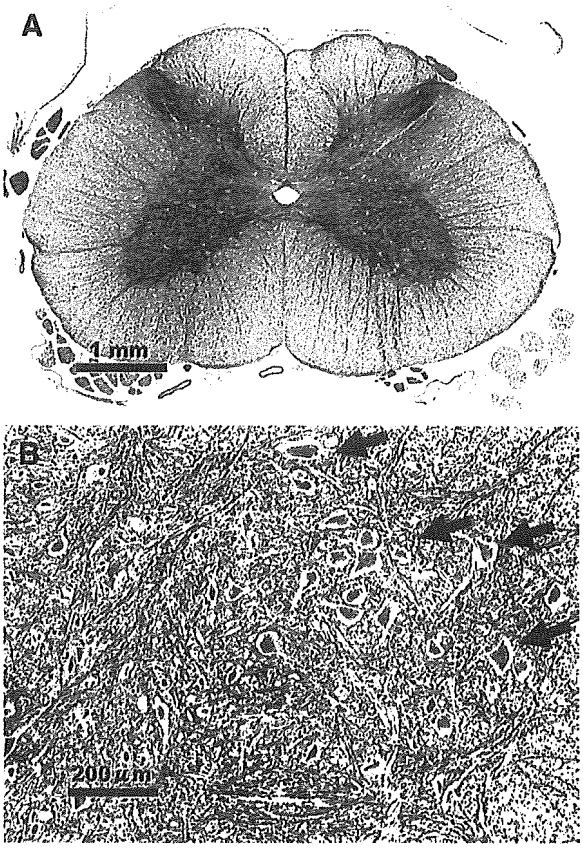

Fig 1. Representative photomicrographs of histologic sections of the spinal cord (stained with hematoxylin and eosin and Luxol-fast blue) from group A showing normal histology. Animals received pretreatment with MK-801 followed by segmental aspartate infusion. Spinal cord neurons were well protected by the segmental infusion of MK-801 *(arrows)*.

The average number of normal-appearing motor neurons among experimental groups in each spinal cord segment is indicated in Fig 3. Group A and D animals had significantly more normal-appearing motor neurons than group B and C animals in all the segments of the spinal cord

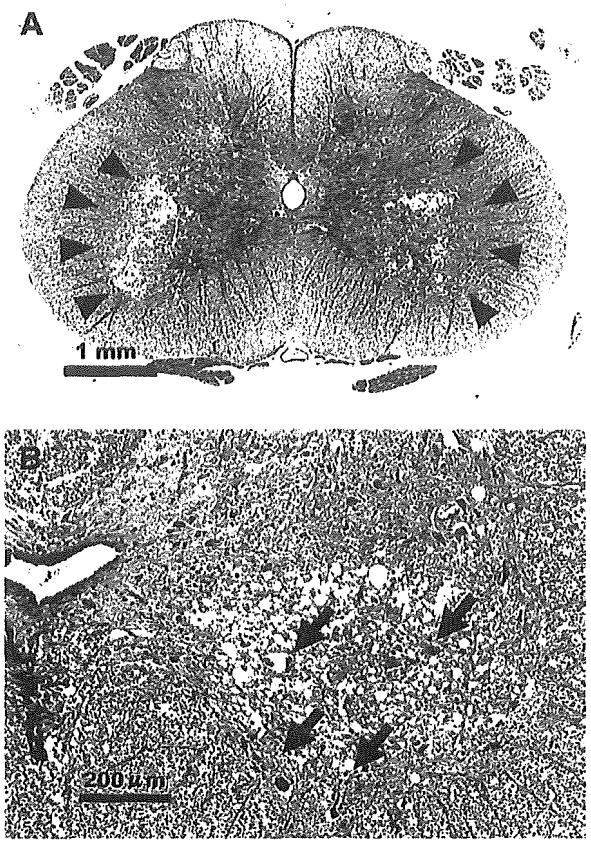

Fig 2. Representative photomicrographs of histologic sections of the spinal cord (stained with hematoxylin and eosin and Luxol-fast blue) from group B and group C demonstrating aspartate neurotoxicity. **A,** Section from group B (pretreated with CGS19755) exhibited severe and extensive gray matter necrosis *(arrow heads)*. **B,** Section from group C received segmental aspartate infusion without pretreatment. Degenerated neurons were irregularly shrunken and exhibited structureless cytoplasm with their nuclei stained hyperchromatic *(arrows)*.

($P < .01$). The difference could be due to chance between group B and C animals ($P = .73$). Animals in group A, pretreated with segmental infusion of MK-801, showed greater number of normal-appearing motor neurons than those in group D, pretreated with intravenous administration (Fig. 3); however, there was no significant difference between the two groups ($P = .08$)

Sham animals in each group exhibited full recovery at 48 hours after operation, although most of the animals receiving MK-801 showed the same sedative effect as those in groups A and D. Sections from those demonstrated normal histology.

Rectal temperatures, before aortic clamping, were similar in groups A, B, C and D. The differences could be due to chance among four groups in mean systemic blood pressure and heart rate during the experiment (Table II).

DISCUSSION

Spinoplegia was first described in the article by Svensson et al,[19] in which segmental infusion of single dose of cold crystalloid with lidocaine was found to be effective in reducing the severity of ischemia during aortic clamping. Particularly, segmental infusion of spinoplegia was reported to be useful for detecting critical arteries that supply the spinal cord.[19,20] Moreover, the local administration of a pharmacologic agent might reduce undesirable adverse effects that could occur when administrated intravenously.

The main finding in the present study was that the segmental infusion of the noncompetitive NMDA receptor antagonist MK-801, which was infused immediately after aortic clamping as a type of spinoplegia, had a protective effect on the spinal cord neurons against EAA-induced neuronal injury; whereas the competitive NMDA receptor antagonist CGS19755 did not reveal neuroprotective effect by segmental infusion. This finding was also of interest because we had previously documented in a rabbit model using NMDA receptor antagonists that the intravenous administration of CGS19755 (30 mg/kg) 1 hour before the insult could have protective effect on the spinal cord neurons against aspartate-induced neurotoxicity.[17]

Competitive NMDA receptor antagonists directly block the glutamate recognition site dose-dependently. These compounds are generally quite polar and show very slow brain uptake in animal studies.[16,21] In the present situation, high concentrations of exogenous agonist (aspartate) at the NMDA receptor would overcome the block produced by a competitive antagonist. On the other hand, noncompetitive NMDA receptor antagonists, which act at the NMDA receptor-operated ion channel as a open channel blocker, are extremely lipophilic and can reach high levels in the brain rapidly after administration.[11,21] Furthermore, in the presence of a noncompetitive antagonist that shows marked agonist dependence, a low physiologic level of NMDA receptor activation could occur, but if this is increased to pathologic levels, excessive activation of the receptor would bring about the noncompetitive block.[12] We estimate that it would be difficult to show the protective effect of competitive NMDA antagonists in acute therapy or preoperative administration just before the neuronal injury.

Aspartate, present ubiquitously in the central nervous system, is a major excitatory neurotransmitter and a potent NMDA receptor agonist. The overactivation of NMDA receptor causes calcium ion influx, and it is followed by activation of several calcium-dependent enzymes that lead to irreversible and fatal neuronal degeneration.[6,9] Since Choi et al[22] demonstrated aspartate neurotoxicity in vitro, it has been difficult to show aspartate neurotoxicity in vivo. The defense mechanism against EAA neurotoxicity is assumed to be due to the activity of rapid and high-affinity reuptake systems that maintain the extracellular aspartate concentration within a nontoxic level.[9,23]

In the present study, the given dose of aspartate in the sham group was cleared by the reuptake systems without toxic effect. Thus, we performed infrarenal aortic clamping

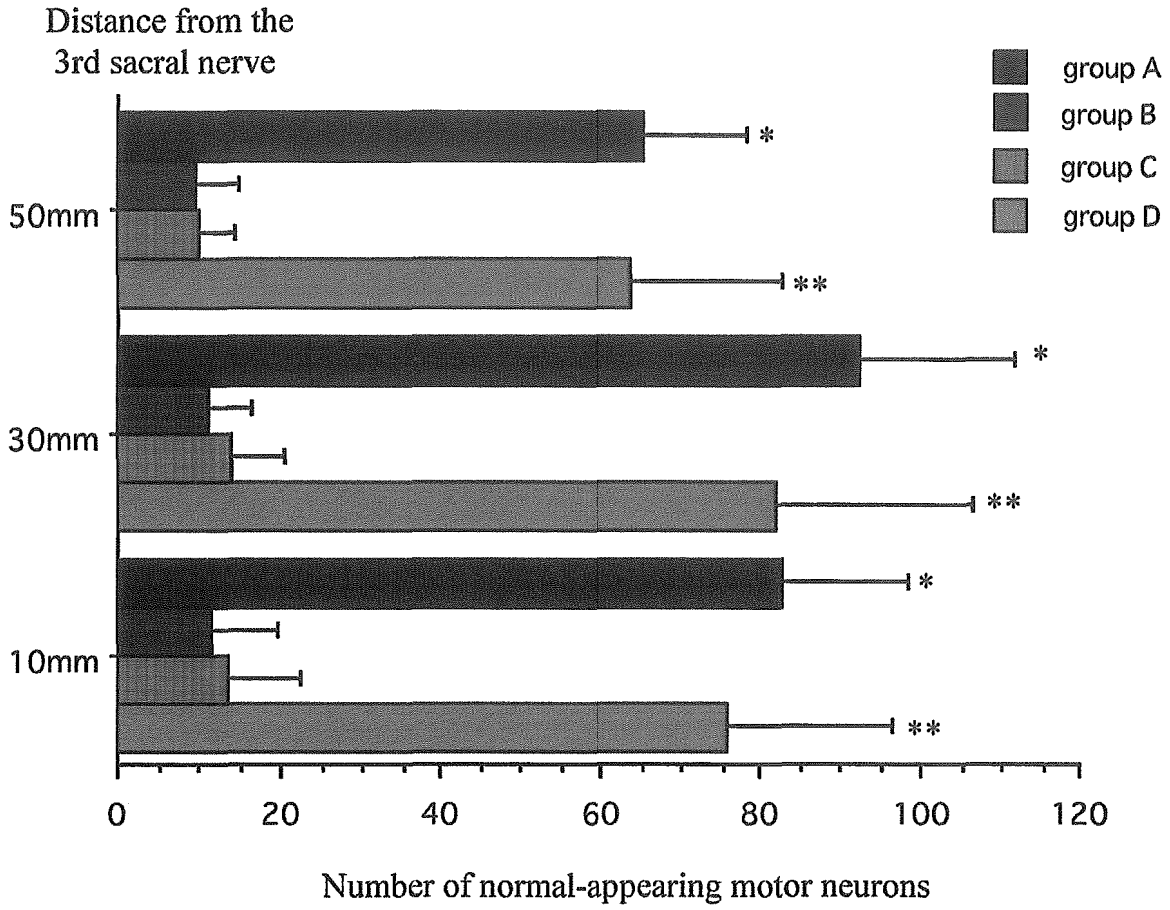

Fig 3. The average number of normal-appearing motor neurons in the level of 10 mm, 30 mm and 50 mm proximal from the third sacral nerve. Group A and D animals showed significantly more normal-appearing motor neurons than group B and C animals in all the segments ($P < .01$). The difference could be due to chance between group B and C animals ($P = .73$). Animals in group A (pretreated with a segmental infusion of MK-801) showed greater number of normal-appearing motor neurons than animals in group D (pretreated with intravenous administration); however, there was no significant difference between two groups ($P = .08$). All values are presented as mean ± SE. *Significant difference between group A and group B; and group A and group C ($P < .01$). **Significant difference between group D and group B; and group D and group C ($P < .01$) (two-way analysis of variance).

Table II. Physiologic parameters

	Group A	*Group B*	*Group C*	*Group D*
Body temperature (°C)	39.4 ± 0.4	39.1 ± 0.6	39.6 ± 0.6	39.1 ± 0.6
Mean arterial blood pressure (mm Hg)	51.7 ± 1.6	52.6 ± 1.6	51.0 ± 4.0	49.8 ± 2.1
Heart rate (beats/min)	322 ± 35	314 ± 22	335 ± 12	327 ± 17

Data are mean ± SD

to reduce the efficiency of the reuptake systems. Furthermore, segmental infusion of aspartate solution (50 mmol) increased the concentration to a level exceeding the physiologic range of aspartate release.

The histopathologic findings in groups B (pretreated with CGS19755) and C (no pretreatment) showed severely injured gray matter, with prominent vacuolation and numerous degenerated neurons that were irregularly shrunken or lightly eosinophilic with their nuclei stained hyperchromatic. In contrast, the white matter was relatively spared, even in the severely injured sections. These pathologic findings were typical in the setting of EAA neurotoxicity.[7,9,24]

Some sections from animals in group A demonstrated minimal neuronal degeneration; however, these sections were proved to have a significantly greater number of motor neurons compared with other groups. Particularly, the average number of normal-appearing motor neurons in group A animals (MK-801 segmental) was similar to those of sham animals that revealed normal histology (data not shown). It was also demonstrated to be greater than in group D animals (MK-801 intravenous), although there was no significant difference between the two groups ($P = .08$). These results suggested that segmental infusion, rather than systemic administration, of noncompetitive NMDA antagonist would be a potent pharmacologic strategy that could retain neuroprotective effect and ameliorate undesirable adverse effects.

During preliminary experiments in this model using various doses of MK-801 (2 mg/kg, 3 mg/kg, 4 mg/kg, and 6 mg/kg) and CGS19755 (10 mg/kg, 20 mg/kg, 30 mg/kg, and 40 mg/kg), we observed that the dose resulting in paraplegia in 50% of animals should lie between 3 mg/kg and 4 mg/kg for MK-801 and between 10 mg/kg and 20 mg/kg for CGS19755. We used segmental infusion of MK-801 (4 mg/kg) and CGS19755 (30 mg/kg) in a relatively high dose, because the initial dose should be high enough to penetrate the blood-brain barrier and to block the vicious cascade that leads to irreversible and fatal neuronal injury.

Most researchers have abandoned NMDA receptor antagonists because human and animal studies have shown that both MK-801 and CGS 19755 have some adverse effects, such as central sympathomimetic and sedative effects. Central sympathomimetic effects observed in animal tests are presumed to be the basis for the few dose-related side effects described in humans. These side effects are restlessness, jitteriness, and agitated confusion.[11,16] In the present study, only animals pretreated with MK-801 revealed sedative effects that were eliminated within 24 hours, and some of these animals showed temporary central sympathomimetic effects such as restlessness and agitated confusion. However, we believe that these adverse effects might be acceptable in trade for protection against irreversible and fatal neuronal injury.

The study regarding spinoplegia showed that segmental infusion of a protective agent was effective in reducing the severity of ischemia during aortic clamping as measured by spinal motor evoked potentials.[19,20] Thus, in the concept of spinoplegia as a means of protecting the spinal cord, it might be necessary to show the data, such as spinal motor evoked potentials and spinal somatosensory evoked potentials, to prove that we actually arrested the spinal cord. However, potential neuroprotective agents that act at the NMDA receptor channel complex are widely accepted to reduce neuronal metabolism in a manner of limiting excitatory neurotransmission.[12,25,26]

CONCLUSION

The segmental infusion of a noncompetitive NMDA receptor antagonist as an intraoperative spinoplegia could have protective effect on the spinal cord neurons against EAA-induced neuronal injury in vivo. On the other hand, the efficacy of the use of competitive antagonist was suggested to be limited in this model, probably because of the blood-brain barrier. Our model may be useful in exploring the mechanism of NMDA receptor-mediated neurotoxicity and to evaluate the ability of protective agents to reduce excitotoxic neuronal injury in the spinal cord.

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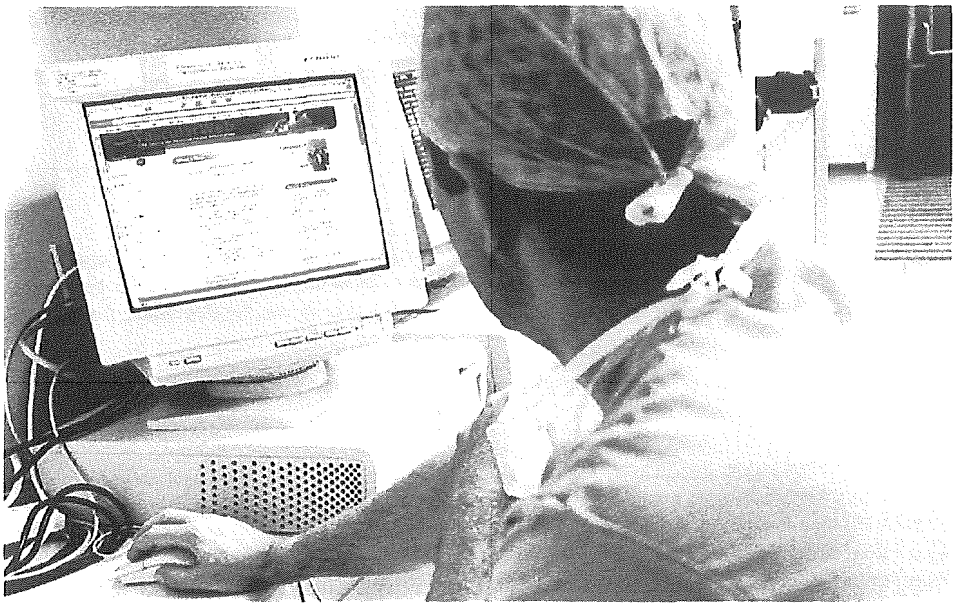

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Serum C-reactive protein elevation predicts poor clinical outcome in patients with distal type acute aortic dissection: association with the occurrence of oxygenation impairment

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Abstract

Background: Acute aortic dissection (AAD) is sometimes complicated by respiratory failure due to severe lung oxygenation impairment. Systemic activation of inflammatory system after aortic injury may play some roles in the development of this complication. The aim of this study was to determine the significance of serum C-reactive protein (CRP) elevation in the development of oxygenation impairment and clinical outcome with distal type AAD.

Methods and results: A total of 61 patients, who were admitted with distal type AAD within 24 h from the onset, were examined. Serum CRP levels, white blood cell (WBC) counts and body temperature were measured serially for at least 4 days. Oxygenation impairment, defined as the lowest PaO_2/FIO_2 ratio ≤200 mmHg, was noted in 31 patients (51%). In patients with oxygenation impairment, peak CRP levels (20.7±7.9 vs. 12.7±3.8 mg/dl, $P<0.001$), peak WBC counts (14,600±3600 vs. 11,800±4300/mm^3, $P=0.008$) and body temperature (38.4±0.5 vs. 38.0±0.6 °C, $P=0.004$) were significantly higher than those without. Peak CRP level was inversely correlated with the lowest PaO_2/FIO_2 ($P<0.001$). Patients who underwent urgent surgical treatment and/or died in the hospital had higher peak CRP levels (25.1±12.3 vs. 16.1±7.4 mg/dl, $P=0.010$) than those who did not. Multivariate analysis revealed that a peak CRP level ≥15 mg/dl (relative risk=12.6, $P<0.001$) was an independent determinant of the development of oxygenation impairment.

Conclusion: The greater serum CRP elevation after distal type AAD was associated with a higher incidence of oxygenation impairment and poor clinical outcome. Systemic activation of the inflammatory system after aortic injury may play an important role in the development of oxygenation impairment.

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Keywords: Acute aortic dissection; C-reactive protein; Inflammation; Oxygenation impairment; Clinical outcome

1. Introduction

In patients with distal type acute aortic dissection (AAD), the prognosis is better compared to proximal type AAD and medical therapy is generally advocated [1–3]. However, multiple organ failure including severe oxygenation impairment requiring mechanical ventilatory support is observed in some cases [4,5]. The mechanisms of the development of such organ failure have not been clarified.

It is known that patients with AAD have systemic activation of the inflammatory system reflected by serum C-reactive protein (CRP) elevation [6,7]. CRP is a clinically useful nonspecific marker of inflammation and is produced in liver by stimulation of proinflammatory cytokines, predominantly interleukin-6 (IL-6). Recently, serum CRP levels have been focused on as a marker of disease progression in patients with vascular diseases, such as coronary artery disease, abdominal aortic aneur-

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ysm (AAA) and peripheral vascular disease, suggesting an important role for vascular inflammation [8–10]. Previous pathological studies showed that inflammatory changes in the aortic wall were observed during the course of AAD [11,12]. It is possible that proinflammatory cytokines secreted from leukocytes, infiltrating into the aortic wall, may cause a systemic inflammatory response after AAD.

The inflammatory response is a necessary consequence for tissue repair and is usually self-limited. However, if the inflammatory system is inappropriately activated, a pathological condition similar to the systemic inflammatory response syndrome (SIRS) may develop. SIRS is known as a condition that is associated with impaired lung oxygenation including acute respiratory distress syndrome (ARDS) and multiple organ failure caused by severe injury or infection [13,14]. Recent evidence supports that CRP is a useful marker for the development of SIRS due to tissue injury or infection [15–17]. We hypothesized that severe organ failure, including oxygenation impairment, in the course of AAD could be predicted by the finding of an initial marked inflammatory response. The aim of this study was to determine the prognostic significance of serum CRP elevation in the development of an adverse clinical outcome with AAD.

2. Methods

2.1. Study population

Between January 1995 and December 2002, 116 patients in our department were diagnosed as the acute phase of distal type AAD. Of them, a total of 75 consecutive patients who were admitted within 24 h after the onset of symptoms were examined. Patients with chronic renal failure, advanced liver disease, heart failure, malignant disease, collagen disease or infectious disease were excluded from this study population. We also excluded patients who died or underwent a surgical procedure before determination of peak CRP levels. A total of 61 patients were eligible for the study.

Table 1
Patient characteristics[a]

	Data
Patient (no.)	61
Age (years)	64±11 (42–88)
Female/Male sex (no.)	9/52
Onset of symptoms to hospital admission (h)	5±6 (0.5–23)
History of hypertension (no.)	37
Systolic BP on arrival (mmHg)	177±36 (108–260)
Diastolic BP on arrival (mmHg)	97±22 (60–150)
DeBakey IIIa/IIIb (no.)	13/48
Open/close type (no.)	30/31
Maximum aortic diameter (cm)	4.2±0.9 (2.7–9.0)

BP=blood pressure.
[a] Data are presented as the mean±S.D. (range) or No. of patients.

Table 2
Clinical findings of the patients with AAD[a]

	No. of patients (%)
Pleural effusion	45 (74)
Involvement of renal or celiac arteries	14 (23)
Persisting pain	7 (11)
Shock	5 (8)
Paraparesis or paraplegia	4 (7)
Hemodialysis	4 (7)
Rupture or impending rupture	3 (5)
Pseudo-coarctation	2 (3)

[a] Data are presented as no. of patients (%).

2.2. Study protocol

Serial 5-mm computed tomography (CT) scans of the chest, the abdomen and the pelvis with nonionic contrast material were performed on admission. A diagnosis of AAD was made on the basis of the detection of two aortic lumens with blood flow or thrombus by contrast-enhanced CT. Blood samples were collected on admission and then every 24 h for at least 4 days. Total white blood cell (WBC) counts were measured by an automated hematology analyzer (Sysmex SE-9000, Toa Medical Electronic, Kobe, Japan). Serum samples were stored at −70 °C until CRP analysis. CRP levels were measured by latex photometric immunoassay (LPIA-CRP, Mitsubishi Chemical, Japan) using an autoanalyzer (Hitachi 7450, Hitachi, Japan) as previously described [18]. Serum cardiac troponin T and/or creatine kinase (CK) were measured in all patients on admission and during hospitalization. Body temperature was measured every 4 h in the armpit. Peak WBC counts and peak body temperature were determined as the highest values within 72 h after admission. Aortic blood samples were obtained on admission and at least every 24 h thereafter for 7 days, and the arterial oxygen tension (PaO_2) was measured using automatic blood gas analyzer system (ABL-520, Radiometer, Copenhagen, Denmark). The PaO_2/fractional inspired oxygen (PaO_2/FIO_2) ratio was calculated to evaluate the severity of oxygenation impairment. During acute phase, arterial blood pressure was monitored and systolic blood pressure was controlled between 100 and 130 mmHg with intravenous administration of antihypertensive agents including β-blockers, sodium nitroprusside and/or calcium antagonists. No patients received non-steroidal anti-inflammatory drugs, corticosteroids, immunosuppressive agents or other medications that would affect inflammatory response. Blood and sputum cultures were performed when blood temperature exceeded 38 °C or respiratory infection was suspected clinically. Urgent surgical treatment was indicated for the patients with distal type AAD complicated by progression with vital organ compromise, rupture, impend-

Table 3
Patient characteristics and peak CRP levels[a]

	Peak CRP level (mg/dl)		*P*-value
	Present	Absent	
Age ≥70 years	18.0±7.5 (17)	16.6±8.6 (44)	0.536
Male sex	17.6±8.8 (52)	13.5±2.9 (9)	0.281
Cigarette smoking	18.0±8.8 (33)	15.7±7.6 (28)	0.283
Hypertension	17.2±9.0 (37)	16.6±7.2 (24)	0.760
Diabetes mellitus	19.6±9.6 (5)	16.7±8.2 (56)	0.460
Hypercholesterolemia	14.9±5.8 (14)	17.6±8.9 (47)	0.288
DeBakey IIIb	18.2±8.2 (48)	12.3±7.3 (13)	0.021
Open type	18.0±8.1 (30)	15.9±8.5 (31)	0.325
Maximum aortic diameter ≥40 mm	18.3±8.6 (34)	15.3±7.8 (27)	0.158
Systolic BP on arrival ≥180 mmHg	16.4±7.2 (31)	17.5±9.4 (30)	0.613
Onset to admission ≤3 h	17.8±8.2 (40)	15.4±8.6 (21)	0.303
β-blockers	15.3±8.3 (36)	19.4±7.8 (25)	0.061
Calcium antagonists	17.2±8.5 (54)	15.2±6.4 (7)	0.548

CRP=C-reactive protein, BP=blood pressure.
[a] Data are presented as the mean±S.D. (no. of patients).

ing rupture or retrograde extension into the ascending aorta.

The following data were obtained: age, sex, risk factors for atherosclerosis including cigarette smoking, hypertension, diabetes mellitus, hypercholesterolemia (total cholesterol level ≥220 mg/dl), elapsed time from the onset of symptoms to hospital admission, blood pressure on arrival and concomitant medications before and after hospitalization. The data of CT scans, including extent of the aortic dissection, open or thrombosed type, maximum diameter of the dissected aorta, were analyzed by two independent radiologists without knowledge of patient's background. The extent of dissection was determined according to DeBakey's classification: the dissecting process was limited to the descending thoracic aorta (DeBakey IIIa) or extended below the diaphragm to involve abdominal aorta (DeBakey IIIb) [19]. Oxygenation impairment was defined as the lowest $PaO_2/FIO_2 \leq 200$ mmHg. The relationship between serum CRP levels and concomitant complications including oxygenation impairment was assessed. The study protocol was in agreement with the guidelines of the ethics committee of our institution.

2.3. Statistical analysis

Continuous data were expressed as the mean±S.D. Comparison between two groups was performed using the Student's *t*-test or the Mann–Whitney *U*-test for continuous variables and Fisher's exact test for categorical variables. Multiple logistic regression analysis was used to assess the effect of various factors on the development of oxygenation impairment. Multivariate analysis included prognostic factors with a *P*-value <0.10 on univariate analysis. To determine the cut-off point of the peak CRP, receiver operating characteristic analysis was performed. A *P*-value <0.05 was considered statistically significant. All statistical analyses were performed using Statview 5.0 software (SAS Institute, Cary, NC).

3. Results

3.1. Patient characteristics

Table 1 shows the patient characteristics. The mean age was 64±11 years (range 42–88); 17 patients (28%) were more than 70 years old. Nine patients (15%) were females and 52 patients (85%) were males. The mean elapsed time from the onset of AAD to admission was 5±6 h; 40 patients (66%) were hospitalized within 3 h. The mean CRP level on admission was 0.6±0.5 mg/dl and peak CRP level was 17.0±8.3 mg/dl. The mean time interval from admission to the CRP peak was 3±1 days. The mean maximum diameter of the dissected aorta was 4.2±0.9 cm. Thirty-seven patients (61%) had hypertension, 20 of whom had been taking antihypertensive agents. The clinical findings at hospitalization are shown in Table 2. No patients showed electrocardiographic signs of myocardial ischemia. Serum cardiac troponin T or CK levels were not significantly elevated in any patients. Table 3 shows the patient characteristics and peak CRP level. The peak CRP level did not differ

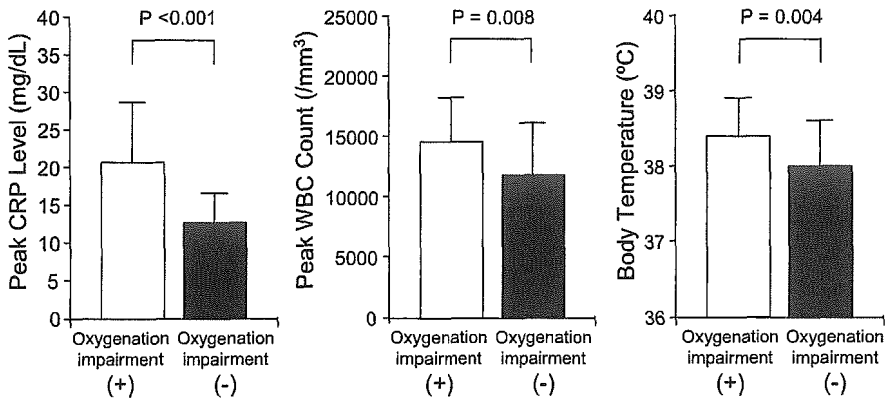

Fig. 1. Patients with acute oxygenation impairment had higher peak CRP levels, peak WBC counts and maximum body temperature compared to those without.

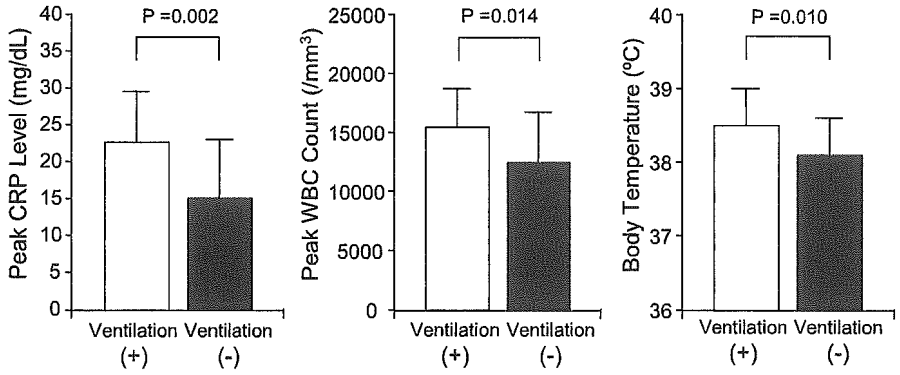

Fig. 2. Patients who required mechanical ventilation had higher peak CRP levels, peak WBC counts and maximum body temperature than those who did not.

significantly due to the presence or absence of hypertension, hypercholesterolemia, diabetes mellitus and smoking history. There was no significant correlation of peak CRP level with type (open or thrombosed) and maximum diameter of dissected aorta. The peak CRP level was significantly higher in patients with DeBakey IIIb than IIIa dissection (18.2±8.2 vs. 12.3±7.3 mg/dl, $P<0.05$) and did not differ between patients with and without β-blockers or calcium antagonists. Sputum and blood cultures revealed no evidence of pneumonia or bacteremia in all patients.

3.2. Incidence of oxygenation impairment in distal type AAD

The mean PaO_2/FIO_2 ratio immediately after admission was 390±70 mmHg. The mean of the lowest PaO_2/FIO_2 ratio was 206±107 mmHg. Thirty-one patients (51%) met the criterion of oxygenation impairment. Swan-Ganz catheter monitoring was performed in 16 patients with oxygenation impairment. The mean pulmonary capillary wedge pressure (PCWP) was 12.1±3.3 mmHg. The PCWP did not exceed 18 mmHg in any patients. The mean cardiac index was 3.9±1.1 l/min/m^2. There was no significant correlation between the PaO_2/FIO_2 ratio and PCWP or cardiac index.

3.3. Relationship between oxygenation impairment and inflammatory response

Patients with oxygenation impairment had higher peak CRP levels than those without (20.7±7.9 vs. 12.7±3.8 mg/dl, $P<0.001$, Fig. 1), though the levels on admission were not significantly different between patients with and without oxygenation impairment (0.6±0.6 vs. 0.5±0.5 mg/dl, P=NS). Peak WBC counts (14,600±3600 vs. 11,800±4300/mm^3, $P=0.008$) and body temperature (38.4±0.5 vs. 38.0±0.6 °C, $P=0.004$) were greater in patients with oxygenation impairment than in those without (Fig. 1). Among them, 15 patients required mechanical ventilatory support. Peak CRP levels (22.7±6.8 vs. 15.1±7.9 mg/dl, $P=0.002$), peak WBC counts (15,500±3200 vs. 12,500±4200/mm^3, $P=0.014$) and peak body temperature (38.5±0.7 vs. 38.1±0.5 °C, $P=0.010$) in patients who required mechanical ventilatory support were also significantly

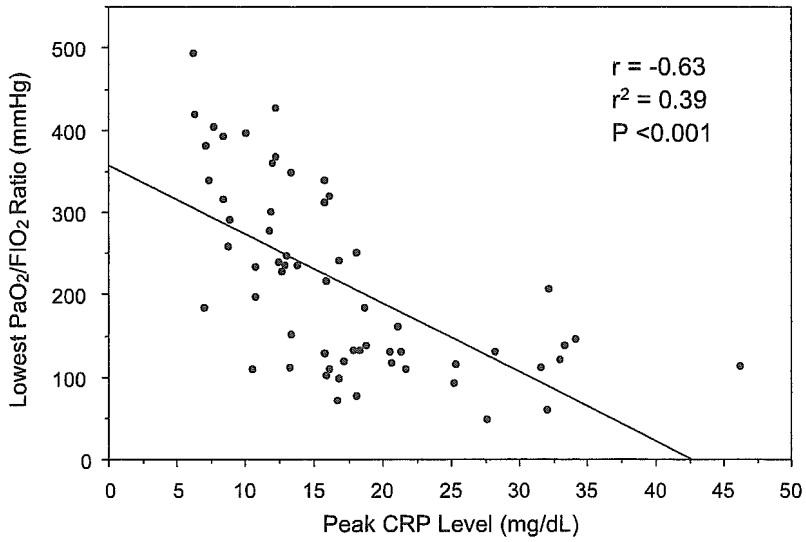

Fig. 3. Correlation between lowest arterial oxygen tension/fractional inspired oxygen (PaO_2/FIO_2) ratio and peak CRP. The lowest PaO_2/FIO_2 ratio showed a significant inverse correlation with peak CRP level.

Table 4
Univariate predictors of oxygenation impairment[a]

	Patients with oxygenation impairment (%)	P-value
Age ≥70 years (*n*=17)	10 (59)	0.570
Male sex (*n*=52)	29 (56)	0.081
Hypertension (*n*=37)	18 (49)	0.795
DeBakey IIIb (*n*=48)	28 (58)	0.031
Open type (*n*=30)	19 (63)	0.074
Maximum aortic diameter ≥40 mm (*n*=34)	20 (59)	0.202
Systolic BP on arrival ≥180 mmHg (*n*=31)	19 (61)	0.127
Onset to admission ≤3 h (*n*=40)	22 (55)	0.426
β-blockers (*n*=36)	15 (42)	0.120
Calcium antagonists (*n*=54)	29 (54)	0.255
Peak CRP level ≥15 mg/dl (*n*=28)	24 (86)	<0.001
Peak WBC counts ≥15,000/mm^3 (*n*=13)	10 (77)	0.059

BP=blood pressure, CRP=C-reactive protein, WBC=white blood cell.
[a] Data are presented as no. of patients (%).

higher than in those who did not (Fig. 2). The lowest PaO_2/FIO_2 ratio was observed at 4±2 days after the onset of AAD. The lowest PaO_2/FIO_2 ratio showed a significant inverse correlation with peak CRP level (r=−0.63, r^2=0.39, P<0.001, Fig. 3).

3.4. Determinants of oxygenation impairment in distal type AAD

To determine the cut-off point of peak CRP level as a predictor of oxygenation impairment with AAD, we performed receiver operating characteristic analysis. The cut-off point of 15 mg/dl was selected as a predictor because of its high sensitivity and specificity to predict this complication (84% and 77%, respectively). Univariate analysis revealed that prognostic factors for oxygenation impairment with a P-value <0.10 were male sex, DeBakey IIIb, open type, peak CRP level ≥15 mg/dl and peak WBC count ≥15,000/mm^3 (Table 4). Multiple logistic regression analysis showed that a peak CRP level ≥15 mg/dl was the strongest predictor of oxygenation impairment (relative risk=12.6, P<0.001), compared with other variables (Table 5).

Table 5
Multiple logistic regression analysis for oxygenation impairment

	Relative risk	95% CI	P-value
Male sex	3.6	0.5–28.4	0.219
DeBakey IIIb	1.3	0.2–8.6	0.767
Open type	1.3	0.3–5.0	0.735
Peak CRP level ≥15 mg/dl	12.6	3.1–52.0	<0.001
Peak WBC counts ≥15,000/mm^3	1.9	0.4–9.7	0.461

CI=confidence interval, CRP=C-reactive protein, WBC=white blood cell.

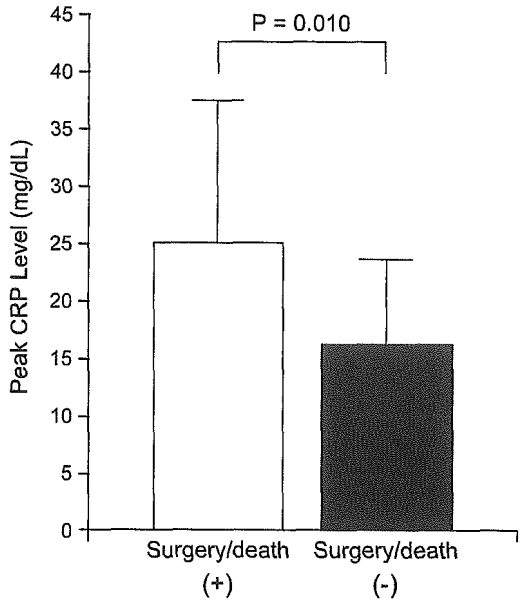

Fig. 4. Patients who underwent urgent surgical treatment and/or died in the hospital had higher peak CRP levels than those who did not.

3.5. In-hospital adverse clinical outcome

Six patients underwent urgent surgical treatment and three patients, consisting of two patients with multiple organ failure including severe respiratory failure and one patient with aortic rupture, died in the hospital after determination of peak CRP. The patients who underwent urgent surgical treatment and/or died had higher peak CRP levels than those who did not (25.1±12.3 vs. 16.1±7.4 mg/dl, P=0.010, Fig. 4).

4. Discussion

A marked elevation of serum CRP levels after distal type AAD was associated with the occurrence of oxygenation impairment and poor clinical outcome. Multivariate analysis showed that a peak CRP level ≥15 mg/dl was an independent determinant of oxygenation impairment, suggesting that systemic inflammation caused by aortic injury may play an important role in the development of oxygenation impairment.

4.1. Inflammatory response and oxygenation impairment in AAD

In the present study, approximately half the patients with AAD demonstrated a lowest PaO_2/FIO_2 ratio less than 200 mmHg. The evidence in Swan-Ganz data showing no significant increase in PCWP suggested non-cardiogenic respiratory failure. An association between AAD and oxygenation impairment has been rarely reported previously [5]. However, there have been several lines of evidence

suggesting that rapid pulmonary damage indicating remote organ injury would follow AAA rupture or vascular surgery [20–22]. Pathological studies of AAD showed the infiltration of macrophages and leucocytes, and higher expression levels of several genes associated with inflammation processes, such as IL-6 and IL-8, in dissected aorta [11,12]. These findings suggest that local aortic insult accompanied by AAD can be the source of humoral factors, which pass through the pulmonary vasculature and activate both resident leucocytes and the pulmonary vascular endothelium. Since the pulmonary vascular bed is known to be an important reservoir of neutrophils, storing a large amount of the total circulating neutrophil pool, the lungs tend to be a major site of tissue damage [23,24]. Activated alveolar macrophage might also play a crucial role in the development of this lung inflammatory response [25]. Thus, it is possible that the powerful assault of intimal rupture and propagation of the dissection into the media may provoke activation of the cellular and humoral inflammatory systems, including leukocytes, macrophages, and various cytokines, and subsequently lead to insufficiency of pulmonary gas exchange.

The present study also showed that high WBC counts and body temperature, in addition to CRP levels, were markers of inflammation in patients with oxygenation impairment during the acute phase of AAD. Elevated WBC counts and body temperature are both manifestations of SIRS [26]. SIRS, which may develop into multiple organ failure, occurs due to a persistent and inappropriate inflammatory response to severe injury. In this regard, oxygenation impairment in patients suffering from AAD could be partly a clinical sign of SIRS.

4.2. Significance of CRP in AAD

CRP is a sensitive and clinically useful marker of inflammation that is produced in liver on stimulation by various cytokines [27]. CRP levels in aortic diseases, including aortic dissection and AAA, have been described previously. A higher CRP level was associated with AAA size [28] and symptoms in patients suffering from AAA [10], suggesting that inflammatory processes play a role in the degradation of the elastic media in aortic diseases. The significance of CRP elevation in the acute phase of aortic diseases is somewhat different because it does not merely occur due to vascular inflammation, but also reflects a systemic inflammatory response after an aortic event [29].

Recently, Schillinger et al. [30] reported that elevated CRP levels on admission were independently associated with higher mortality in patients with acute aortic diseases. Their study included patients who were admitted more than 24 h from the onset of symptoms. In our study, CRP levels on admission were not significantly different between patients with and without oxygenation impairment. This is probably because we included only the patients who were admitted within 24 h and, moreover, 66% of the patients were hospitalized within 3 h after the onset of symptoms. CRP is highly dependent on the elapsed time from the onset. The value remains at low level within 24 h from the onset, but increases to its highest level after a few days, as the peak CRP level in the present study was observed at 3 ± 1 days. Therefore, the peak CRP level is likely to reflect the magnitude of systemic inflammation more precisely than the CRP level on admission.

CRP itself has been reported to have some direct proinflammatory effects on various tissues. CRP induces adhesion molecule expression in cultured endothelial cells and activates the complement system in atherosclerotic vessels or ischemic myocardium [31–33], supporting the notion that CRP may play a direct role in promoting inflammation and damaging the tissue. Within inflamed lungs, CRP is reported to contribute to the pathogenesis of the pulmonary dysfunction by activating alveolar macrophages or interfering with surfactant function [34,35]. Therefore, CRP secreted in response to aortic injury of AAD, although not an initiating insult in pulmonary damage, might enhance and continue lung permeability via an exacerbated inflammatory process.

4.3. Clinical implication

In patients with distal type of AAD, peak CRP levels higher than 15 mg/dl suggest a high risk for oxygenation impairment and poor clinical outcome. CRP is an easily measurable acute-phase protein and may be one of the valuable parameters for risk prediction in these patients. Patients with excessive elevation of CRP levels should be treated carefully and attentively, taking into consideration the possibility of deleterious clinical complications and events.

5. Conclusion

The greater serum CRP elevation after distal type AAD was associated with a higher incidence of oxygenation impairment. Systemic activation of the inflammatory system after aortic injury may play an important role in the development of oxygenation impairment.

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循環器の話題

TOPIC

人工赤血球（人工酸素運搬体）の臨床応用

慶應義塾大学医学部呼吸器外科
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同心臓血管外科
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POINT

● 使用に際し，血液型の判定（クロスマッチ）が必要なく，ウイルスなどの感染の危惧のない，室温で保存可能な人工酸素運搬体が開発されつつある．1つは赤血球よりヘモグロビンのみを回収しこれを脂質二重膜の中に包埋したヘモグロビン小胞体であり，もう1つは合成したヘムをリコンビナントアルブミンに包接させた完全合成系のアルブミンーヘムである．これらは出血性ショックの際の使用ばかりでなく，人工心肺の補塡液，臓器保存液，血液希釈，狭窄した血管の末梢への酸素運搬，固形腫瘍の放射線療法や化学療法に対する増感作用などの効果が期待される．

はじめに

血液は赤血球，白血球および血小板の血球分画と，アルブミン，凝固因子などを含む血漿成分から構成され，それぞれ順に，酸素運搬，殺菌作用，止血，栄養の運搬，血液凝固など実に多彩な機能を持っている．これらすべての役割を代替する人工物を実現することは不可能に近いが，酸素運搬のみに焦点を絞った人工赤血球（人工酸素運搬体）の研究は，近年になってわが国でかなりの成果が得られ，臨床応用を目指した取り組みが具体化している．

人工酸素運搬体としての条件は，赤血球と同等の酸素運搬機能を有するのはもちろん，血液型がなく，ウイルスなどによる感染が起こる危惧もなく，また，長期間の保存が可能なことなどである．従って，緊急時にいつでもどこでも必要に応じて使用でき，また，血液型不一致の異型輸血事故も回避できる．さらに肝炎やエイズ（後天性免疫不全症候群）をはじめ，輸血を介して伝播する「新感染症」の危険性もなくなる．少子高齢化で献血量の減少が危惧される中，人工赤血球の実現に対する期待がこれまで以上に高まってきている．

1．人工酸素運搬体の開発（図1）

血液の中で，水（血漿分画）の酸素溶解度はごくわずかである．必要な酸素は赤血球中に高濃度に存在するヘモグロビン（Hb）に結合された形で肺から末梢組織に運搬される．この役割を代替する人工酸素運搬体としては，これまでに以下の2つが主に開発されてきた．1つは，水よりも高い酸素溶解能を持つ溶液，パーフルオロケミカルズ（perfluorochemicals；PFCs）[1]で，もう

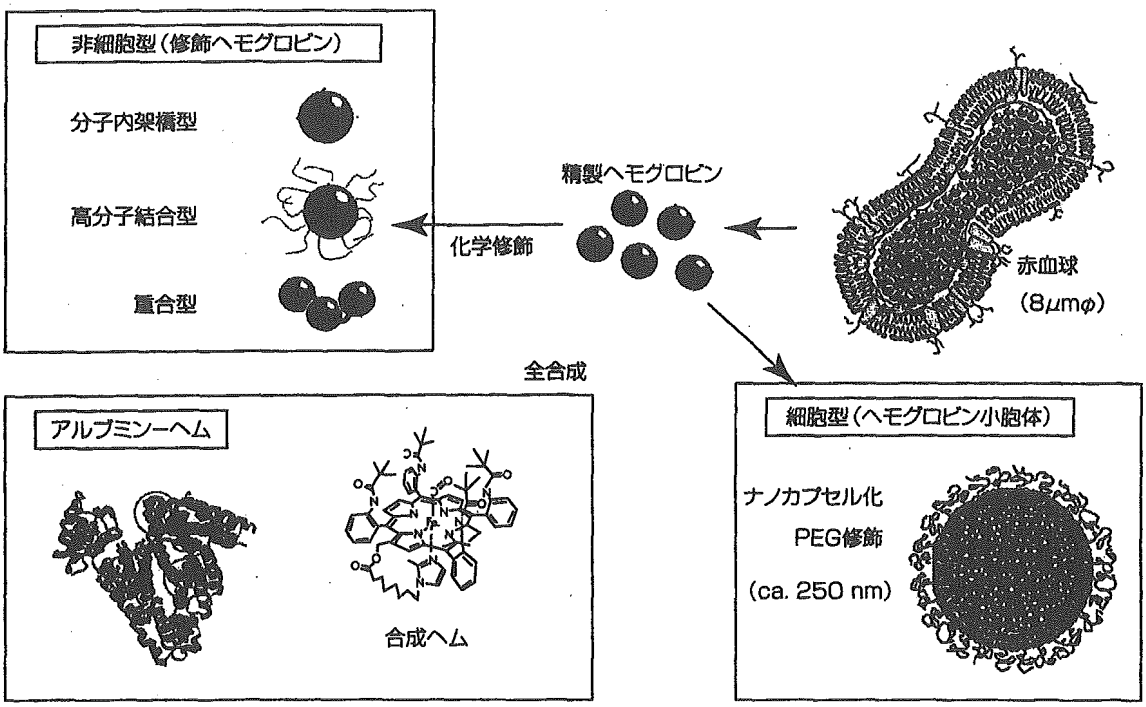

図1　人工酸素運搬体の開発

血液の多様な機能のうち，酸素運搬のみに焦点を絞った人工酸素運搬体の開発は主に1960年代から行われ，現在に至るまでさまざまな種類が開発された．欧米では，高純度ヘモグロビンに化学修飾を加えた非細胞型の人工酸素運搬体の開発が行われているが，副作用も指摘されている．他方，ヘモグロビンをリン脂質の二分子薄膜で包み込んだヘモグロビン小胞体は，赤血球と類似の細胞型構造が特徴である．合成ヘムを組み換えヒト血清アルブミンに包接させたアルブミンーヘムは，大量生産のできる完全合成系酸素輸液である（p.6にカラー写真掲載）

1つは，ヒトの赤血球から精製単離したヘモグロビンを利用したものである．

PFCsは有機フッ素化合物で，酸素を水の数十倍も溶解できる透明な溶液である．これに脂質を加え水相中で乳化して得られる分散液（PFC乳剤）は，わが国でも人工酸素運搬体として開発が進められ，臨床使用されたこともあったが，酸素運搬能の不足（乳化する必要がありその濃度が薄まるため使用に当たって酸素吸入が必要）や体内蓄積などのために最終的には製造が中止されている．その後，酸素溶解量の増大や製造技術の向上に伴い，それまでの欠点を補う次世代のPFCも開発されつつある．

赤血球から精製単離したヘモグロビンを使用する場合，そのままでは，酸素親和度が高いため組織で酸素を放出しないことや，ヘモグロビンの基本構造である4つのサブユニット（四量体）の状態から，血中では二量体に解離し，速やかに血中から消失してしまうなどの課題が明らかになった．そこで，ヘモグロビンに直接加工を加え分子量を増大したり，酸素親和度を適度に調節するなどの試みが主に欧米で行われ，これ

らは修飾ヘモグロビンと呼称されている．臨床試験の最終段階にまで到達したものもあるが，臨床試験の過程で粒子が小さいために血管壁に近接し，血管内皮から放出される一酸化窒素（NO）を吸着するため，血管平滑筋の収縮を来し血圧が上昇するなどの副作用に直面し，いまだ臨床応用に至ったものはない[2, 3]．他方，ヘモグロビン小胞体は，高純度，高濃度ヘモグロビンを脂質二分子膜で被覆し，平均粒径250nmの小胞体とすることにより，赤血球と類似の細胞構造としたものである．また，合成ヘムを組み換えヒト血清アルブミンに包接させたアルブミン－ヘムは，生理条件下で，可逆的に酸素を結合できる完全合成型の人工酸素運搬体であり，その酸素運搬能は天然のヘモグロビンに匹敵する．ヘモグロビン小胞体とアルブミン－ヘムは，わが国独自の技術として早稲田大学が確立し，体内投与試験による酸素運搬機能と安全性の評価研究が，慶應義塾大学医学部と共同で進められ，げっ歯類を用いた基本的な動物投与試験はほぼ終了し，臨床試験の開始が期待されている[4, 5]．

2．人工酸素運搬体の意義

赤血球の寿命の120日と比較し，人工酸素運搬体の半減期は数日程度と短いので，定期的に赤血球輸血を必要とするような慢性的な貧血に対する臨床応用は現時点では困難と考えられる．さらに，優れた特徴（血液型なし，感染源なし，長期保存可能，小粒径）を活用することにより，赤血球では対応が不可能な症例にも使用することが期待できる．まず，緊急医療に必要不可欠となる輸血代替としての酸素輸液（赤血球代替物）は，次世代医療の最も重要な課題に位置付けられており，災害などの緊急時の出血に際し，長期備蓄に耐える安全で血液型のない酸素輸液がいつでもどこでも迅速に供給できるようになれば，危機管理対策の大きな進展になる．加えて最近は，「自己血輸血の支援」としての使用法が注目されている．術前に患者の自己血液を採取して保存し，代わりに人工酸素運搬体を投与して血液希釈することにより，術中出血による損失を少なくすることも可能となる．術中出血に対しては人工酸素運搬体を投与し，術後に採血しておいた患者の自己血液を戻すものである．このように術前血液希釈液として利用すれば手術中に失われる自分自身の血液量を少なくすることが可能になり，結果的に通常の赤血球輸血が回避できる．

さらに，近年になって人工心肺の充填液として人工酸素運搬体を利用する新しい考え方も生まれた．新生児・乳児の開心術では一般的に人工心肺回路の充填液として輸血が用いられているのが現状で，これは低体重の患者で無輸血充填とした場合，人工心肺の運転中に激しい血液希釈状態となり，臓器とりわけ脳の適正な酸素供給が保証されない．ここで人工酸素運搬体を人工心肺回路の充填液に利用すれば，運転中の血液希釈状態においても適正な酸素供給が可能になると考え，山崎らは現在，動物を用いた体外循環モデルを確立し，その可能性について研究を進めている[6]．

さらに，人工酸素運搬体は赤血球よりも小粒径で粘度も低いことから，赤血球が通り抜けられないような狭い部位でも通過でき，体組織や臓器の迅速な酸素化に有効と考えられる．これは赤血球機能の代替という位置付けではなく，人工酸素運搬体の性質を生かした新たな応用として注目されている．心筋梗塞や脳梗塞では血

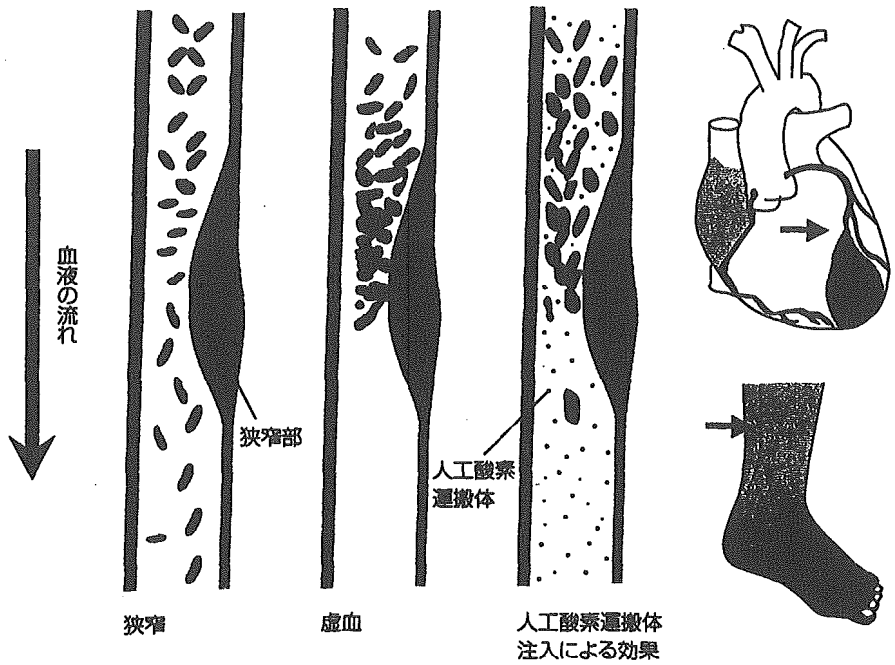

図2　人工酸素運搬体による狭窄血管の末梢への酸素供給

栓で詰まった部位から先には赤血球は到達できず，従って酸素を供給できず虚血性領域となり，その組織は最終的には死に至るが，人工酸素運搬体は狭窄血管あるいは側副経路を経由して，通過できる可能性があり，傷害部位の組織を低酸素から保護し，障害の予防に有用と期待されている[7,8]（図2）．また癌治療薬としての利用も検討されている．一般に腫瘍は低酸素状態であり，放射線照射や抗癌剤治療に対して抵抗性を示す．人工酸素運搬体は赤血球では到達不可能な，血管から離れた腫瘍組織内の低酸素部位にまで酸素を供給することが可能なので，放射線治療や抗癌剤治療への感受性を高めるための検討が行われている[9]．

そのほか臓器移植の際に，摘出臓器の灌流液として使用すれば，その臓器の保存性が向上するとも期待できる．他方では，膜抗原がないことから自己免疫性溶血性貧血への応用，組織再生のための人工臓器細胞培養液としての利用など，その適応範囲はますます拡張している．人工酸素運搬体の完成は，わが国の血液事業の進歩と革新に貢献するばかりでなく，輸血・献血システムの整備が遅れている地域への国際貢献としても極めて重要な意義を持っているのである．

おわりに

人工酸素運搬体の研究は，学際・業際・医工薬の分野を超えた連携の下に強力に推進されて

おり，次世代医療に貢献できる最重要分野に先鞭を付けるべく，世界規模で活発な展開が進められている．わが国でも1997年度より，厚生労働科学研究高度先端医療研究事業「人工血液開発分野」として，「臨床応用可能な人工赤血球の創製に関する研究」[10]に引き続き，現在は，「人工赤血球の安全性向上に関する研究」および「救急災害医療に利用可能な人工赤血球に関する研究」として推進されている．また，人工赤血球，人工血小板，人工抗体の3部門に分かれた活発な研究展開が進められている．今後，さらなるプロジェクト推進により医療に新しい変革をもたらすことが期待されている．

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心臓外科

Knack & Pitfalls

弁膜症外科の要点と盲点

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