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Cold Saline Injection Attenuates Motor-evoked Potential in the Spinal Cord by Cortical Electrical Stimulation in the Dog

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ABSTRACT

Changes in the motor-evoked potential of the spinal cord with transcranial stimulation are monitored for spinal cord function during thoracoabdominal aortic aneurysm surgeries. We examined the effects of changes in motor-evoked potential with cold saline injected into the clamped segment of the aorta, and compared the effects to lidocaine and warm saline injection.

Eighteen dogs were divided into three groups according to the injected agents: Warm saline group (37°C, 20 ml), Cold saline group (4°C, 20 ml), and Lidocaine group (5.0 mg/kg of lidocaine in 20 ml of warm saline), (n=6, each group). Changes in the peak-to-peak MEP amplitude and the indirect wave (I wave) amplitude were measured during aortic cross-clamping.

In the peak-to-peak MEP amplitude, the cold saline and lidocaine groups attenuated to 80% of the control value but were not significantly changed. In the I wave amplitude, the cold saline group showed a significant attenuation 1 min after injection ($p<0.0001$) and the lidocaine group 4 min after injection ($p=0.0230$), when compared with the warm saline group. Attenuation of the I wave amplitude in the cold saline group was significantly larger than that in the lidocaine group ($p=0.0003$).

Changes in the I wave amplitude appeared within 4 min in both the cold saline and lidocaine groups. Cold saline injection into the clamped segment of the aorta is a diagnostic procedure for determining presiding critical arteries in the segment without experiencing the pharmacological side effects observed with lidocaine injection.

Key words: *Spinal cord ischemia, Cold saline injection, Motor-evoked potential, Thoracoabdominal aortic surgery*

Many methods have been contrived to protect against ischemic spinal cord injury during thoracoabdominal aortic aneurysm (TAAA) surgery including: 1) hypothermia[13,19,20], 2) distal perfusion during aortic cross-clamping[18], 3) identification and reconstruction of the Adamkiewicz artery[10,22], and 4) cerebrospinal fluid drainage during and after surgery[5,18]. However, a complete spinal cord protection has not been established. Aortic cross-clamping time is one of the key factors for protecting the spinal cord from ischemic injury. To detect spinal cord ischemia, we used motor-evoked potential (MEP) to determine spinal cord ischemia in TAAA. However, it takes longer than 15 min to detect MEP change after aortic cross-clamping[6,21,24], and the spinal cord may be damaged during this warm ischemic time. Thus, aortic cross-clamping time has to be shortened as much as possible to avoid spinal cord injury. The injection of lidocaine, a popular local anesthetic, was reported to evoke change in the MEP wave[23]. However, it has a toxic effect on the spinal cord[29]. In this study, we injected cold saline into the clamped segment of the aorta instead of lidocaine solution and examined MEP change.

This study aims to examine the acceleration of MEP change following cold saline injection for shortening the aortic cross-clamping time, and to compare with that following lidocaine regarding the expedition and distinctness of change.

MATERIALS AND METHODS

Animal Preparation

Animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the Institute of Laboratory Animal

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Resources and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health. This study protocol was approved by the Guiding Principles on Animal Experimentations in Research Facilities for Laboratory Animal Science, School of Medicine, Hiroshima University.

Surgical Procedures

Anesthesia was induced with intramuscular injections of ketamine hydrochloride (0.3 ml/kg) and atropine sulfate (0.5 mg). Lactated Ringer solution was continuously given through a forelimb vein. After the intravenous administration of thiamylal sodium (15 mg/kg) and pancronium bromide (0.05 mg/kg), endotracheal intubation was performed. General anesthesia was maintained with isoflurane (1.5–2.0%) carried by O_2 (2 liters/min) mixed with nitrous oxide (4 liters/min) under volume-controlled mechanical ventilation. Adequate ventilation was adjusted by blood gas analysis at 37°C. Continuous monitoring of blood pressure was done at the proximal and distal portion of the clamped segment of aorta (PAP and DAP) from the right forelimb artery and the right femoral artery, respectively. A catheter was placed in the left subclavian artery and left common carotid vein, and was connected to a siliconized reservoir, which was placed at 108 cm above the dog to maintain 85 ± 5 mmHg of systolic PAP despite aortic cross-clamping. After systemic heparinization (100 unit/kg, intravenously), blood was withdrawn until the systolic PAP was reduced to 80 mmHg. After 15 min of aortic cross-clamping, the blood was returned using a roller-type pump (50–100 ml/min). During the experiment, body temperatures were kept at around 37°C using a warm blanket.

This model was established in a previous study using 12 beagle dogs. It was designed to reproduce complete paraplegia. Aortic cross-clamping of the descending aorta failed to cause paraplegia due to a rich collateral blood supply to the spinal cord as well as reactive hypertension following aortic cross-clamping. Spinal cord ischemia was induced by aortic cross-clamping at the proximal descending aorta. Substantial ischemia in the critical lumbar region was produced. We estimated both MEP and the hindlimb motor function in six dogs after aortic cross-clamping only for 60 min (Group A) and in six dogs after aortic cross-clamping for 40 min with blood pressure control using a reservoir (Group B). In group A, two dogs showed minimal changes in their MEP amplitudes and four dogs showed a significant amplitude reduction for up to 45 min. In contrast, MEP amplitudes decreased significantly for 30 min in all of the dogs from group B. Moreover, in group A, paraplegia was confirmed in 4 dogs and two had no neurologic deficits 48 hours after ischemia, whereas in group B, all of the animals showed paraplegia with necrotic changes in the gray matter of the lumbar cord. Based on these results, this model was thought to be suitable to reproduce paraplegia in dogs.

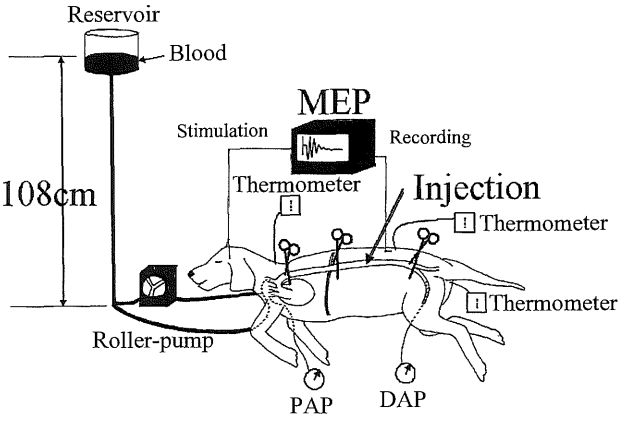

Fig. 1. Schematic illustration demonstrating the animal model for spinal cord ischemia.
Spinal cord ischemia was induced by clamping the descending thoracic aorta just distal to the left subclavian artery, at the aortic hiatus, proximal to the bifurcation, and at every visceral branch of the abdominal aorta. Drugs were administered into the clamped segment of aorta. MEP was monitored for spinal cord function.

In the current study, eighteen adult beagle dogs weighing 9.0 to 11.0 kg were used. Spinal cord ischemia was induced by aortic cross-clamping the descending thoracic aorta just distal to the left subclavian artery, at the aortic hiatus, proximal to the bifurcation to the common iliac arteries, and at every visceral branch of the abdominal aorta (Fig. 1).

Drug Administration

A catheter was inserted into the center of the clamped segment of the abdominal aorta, between the aortic hiatus and proximal to the bifurcation to the common iliac arteries, for injection. Drugs were administered into the clamped segment of the aorta after aortic cross-clamping at 80 ml/min using a syringe pump. The eighteen dogs were divided into three groups according to the injected agents: Warm saline group (37°C, 20 ml), Cold saline group (4°C, 20 ml), and Lidocaine group (5.0 mg/kg of lidocaine in 20 ml of warm saline), (n=6, each group).

Physiological Parameters Measurement

Proximal, distal aortic pressure, and heart rate were continuously monitored during the procedure. Temperatures were also monitored at the rectum, and cervical (C5) and lumbar (L5) spinal cords using a digital thermometer, PTW-100A (Unique Medical Co. Ltd., Tokyo, Japan).

Measurement of MEP

MEP was measured as the spinal cord potential evoked by electrical transcranial stimulation of the motor cortex. The stimulation (intensity, 0.1 A; pulse duration, 0.5 ms; pulse rate, 4.0 Hz, single-pulse, and filter; 20 Hz-1.5 kHz) was applied to the bilateral temporal scalp using 2 needle type electrodes. The recording was done by an electrode which was placed in the lumbar subarachnoid space (L5). The spinal cord potential was amplified and averaged for 50 impulses per recording using a Nicolet Viking Quest system (Nicolet Biomedical, Inc., Madison, WI). The aortic cross-clamping time was fixed at 15 min because MEP did not show any significant ischemic change during the first 15 min in a preliminary study using the same model. The concentration of isoflurane was fixed at 1.5% during the recording. MEP was recorded at 1 min prior to the aortic cross-clamping, and at 1, 4, 7, 10 and 15 min after aortic cross-clamping. MEP at 1 min prior to the aortic cross-clamping was defined as a control value. MEP was evaluated as the percent-change in the peak-to-peak MEP amplitude of the wave, and that in the indirect wave (I wave).

Statistical Analysis

Data were expressed as means ± standard deviation. To adjust for multiple comparisons when ANOVA (analysis of variance) showed a significant difference between groups ($p<0.05$), Fisher's Protected Least Significant Difference post-hoc test was used to identify which group difference accounted for the significant p value.

RESULTS

There was no significant difference in aortic pressures and in heart rate following aortic cross-clamping among the three groups. There was no statistically significant decrease of the peak-to-peak MEP amplitude during aortic cross-clamping in the three groups (Fig. 2).

The I wave amplitude significantly decreased 1 min after cold saline injection when compared with warm saline injection ($p<0.0001$), and I wave amplitude in the case of cold saline injection remained low during aortic cross-clamping. In the lidocaine group, the amplitude did not decrease significantly at 1 min. However, it decreased significantly 4 min after injection ($p=0.0230$), when compared with that in the warm saline group. The I wave amplitude attenuation was more significant in the cold saline group than in the lidocaine group at 1 min after injection ($p=0.0003$) (Fig. 3).

Typical changes in waveform in the three groups are presented in Fig. 4. In the warm saline group, there was no change in waveform at either 1 or 4 min after injection compared with the control waveform. In the cold saline group, the waveform markedly changed, especially the I wave,

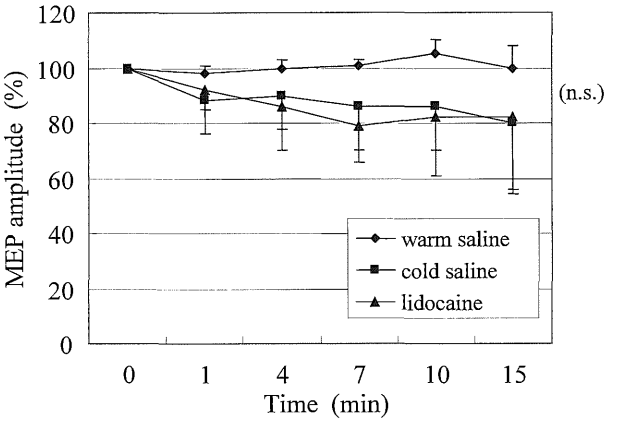

Fig. 2. Changes in the peak-to-peak MEP amplitude. The amplitudes in the cold saline and lidocaine groups attenuated to approximately 80% of the control value, but were not significantly different among the three groups.

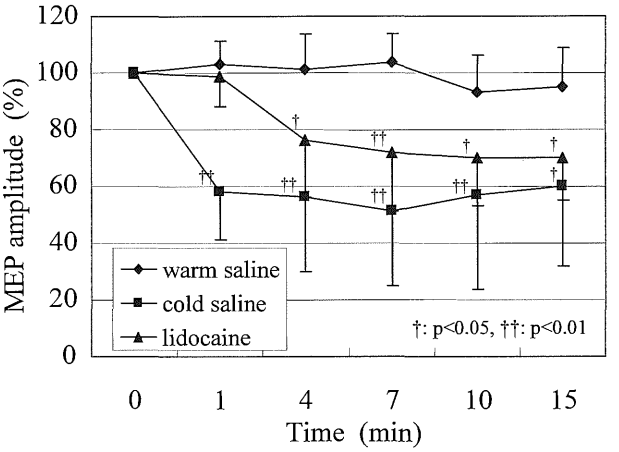

Fig. 3. Changes in the I wave amplitude. At one minute after injection, a significant decrease was recognized only in the cold saline group. The amplitude attenuation was significantly larger in the cold saline group than in the lidocaine group.

which was obviously attenuated 1 min after injection compared with the control. Four minutes after injection, the I wave was more attenuated than at 1 min. On the other hand, there was no obvious change 1 min after injection in the lidocaine group. The I wave amplitude decreased 4 min after injection compared with that of the control value.

After the cold saline injection, the temperature in the lumbar spinal cord dropped within 1 min. The cooling effect was maintained for 15 min with a single cold saline injection until the aorta was declamped (Fig. 5). In contrast, the temperature in the cervical spinal cord and that in the rectum did not change significantly.

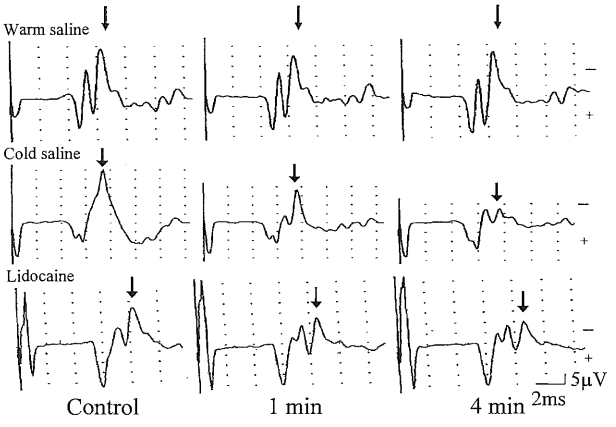

Fig. 4. Changes in the typical waveform of each group.
Warm saline group (upper), Cold saline group (middle), Lidocaine group (lower). Arrows show the change of I wave. The waveform of I wave in the cold saline group was the most remarkable difference among the three groups.

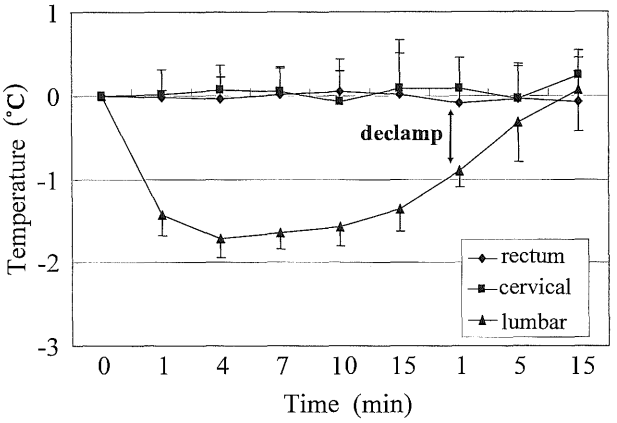

Fig. 5. Changes in the temperature of the cold saline group.
Spinal cord temperature quickly decreased by approximately 2°C and a reduction of about 1.5°C was maintained from 1 min after the injection to the aortic declamping.

DISCUSSION

This study demonstrated that cold saline injection into the clamped segment of the aorta drastically changed MEP amplitudes within a short time and quickly lowered the temperature at the corresponding lumbar spinal cord. This result shows that we can diagnose whether critical arteries are included or not in the clamped segment of the aorta within 1 min. As a result, this method is able to shorten the aortic cross-clamping time and to decrease spinal cord injury.

A beagle dog is ideal for animal experiments in spinal cord protection because electrophysiologic spinal cord monitoring and cerebrospinal fluid drainage can be performed. However, spinal cord blood flow is complex and extremely variable in dogs[8]. Especially, dogs have an extensive collateral blood supply to the spinal cord[28]. Thus, we derived a special means of aortic cross-clamping, as a result of which the critical area of the individual spinal cord can be compromised in a highly reproducible manner. In this model, we established a simple method that reduces the perfusion pressure in caudal-direction collateral vessels originating from the aorta to the critical region of the spinal cord. This decreases the proximal AP at the targeted level, controls the proximal AP constantly and inhibits the initial rise in the proximal AP during aortic cross-clamping. Cross-clamping at both the thoracic aorta and the terminal abdominal aorta reduced regional blood flow in the lumbar cord to 20% of the blood flow before the clamping, and it was restored to up to 70% after initiating distal perfusion[14]. This evidence supports the existence of rostral-direction collateral vessels originating from the iliac system. In the current concept, including proximal AP management by the blood reservoir, the contribution of collateral blood flow in these two directions can be minimized during the reproduction of spinal cord ischemia. This model demonstrated that it reproduced substantial spinal cord injury using a simple technique supplementary to conventional aortic occlusion. We believe that our new method has several advantages over aortic cross-clamping alone or aortic occlusion associated with proximal-to-distal aortic bypass technique.

MEP could estimate spinal cord function under general anesthesia. MEP change was accurate in detecting spinal cord ischemia and predicting the paraplegia[24]. MEP represents the activity of motor pathways and assesses the function of motoneurons, including the corticospinal tract, extrapyramidal tract system and the ventral horn of the spinal cord. MEP consists of an early negative deflection (direct wave: D wave) followed by a series of subsequent low-voltage deflections (indirect wave: I wave). The former results from direct activation of pyramidal units and represents unrelayed pyramidal activity, while the later results from the indirect activation and combined excitation of many different neural elements including the motoneurons and interneuron networks to the spinal cord[1,9,15,27].

Hypothermia is commonly used in TAAA surgery to minimize ischemic injury of the organ. Hypothermia is advantageous for spinal cord protection because it reduces metabolic activity (5~7% per 1°C decrease), decreases potential amplitude and nerve conduction velocity, and depresses synaptic transmission[3,17,26]. Systemic hypothermia using extracorporeal circulation was previously the only method of cooling the spinal cord. However, systemic hypothermia may induce an abnormality of the coagulation cascade and

platelet function, induce cardiac arrhythmias, and increase the risk of infection[11,16]. Topical cooling of the spinal cord using epidural, intrathecal or continuous cold blood perfusion has been reported[4,12]. Other investigators developed core cooling of the spinal cord by infusing cold saline into the clamped segment of aorta like cardioplegia[23,25]. We applied this concept for rapid MEP change with a small dose of saline. In this study, the spinal cord temperature quickly dropped by approximately 2°C from the control temperature with 20 ml of cold saline. All abdominal branched arteries except lumbar arteries were clamped in the clamped segment of the aorta. Thus, cold saline certainly perfused the anterior spinal cord artery and the spinal cord. Cold saline injection appears to have some cooling effect on the spinal cord if the Adamkiewicz artery is included in the clamped segment of the aorta. Rapid change of MEP amplitude indicates that the Adamkiewicz artery is included in the injected segment and that spinal cord activity is suppressed by cooling. In the current study, the peak-to-peak MEP amplitude dropped to only 80~90% of the control value. However, the I wave amplitude dropped to nearly 50% of the control after cold saline injection. The difference here indicates that cold saline acted on the spinal cord, especially the spinal interneurons and motoneurons, and exercised a cooling effect on the spike conduction velocity, synaptic transmission and interneuronal transmission of the spinal cord. It also suggests the inclusion of the Adamkiewicz artery in the injected segment. Thus, if we use this method during an operation, the I wave may be useful as an indicator of the presence of the Adamkiewicz artery.

Several investigators have reported using lidocaine infusions into the aorta or epidural spaces[23,29]. The effects of lidocaine include the stabilization of cell membranes, suppression of synaptic transmissions, the blockage of Na^+ channels, and the inhibition of rapid axonal transport[2,7]. In some studies, 120 ml of cold saline solution was used with lidocaine (100 mg/dl). However, about 50% change of MEP amplitude from the control value demanded over 25 min. In our study, the peak-to-peak MEP amplitudes for both groups decreased to about 80% of the control, and the I wave amplitudes decreased below 70% of the control following an injection of a low concentration of lidocaine in 4 min. These changes suggested that lidocaine injection suppressed spinal cord activities, especially those of the interneurons and spinal ventral horn, selectively. However, it required a long time or showed little change compared with cold saline. It is better not to infuse a higher dose of lidocaine because lidocaine has adverse effects such as bradycardia and/or cardiac arrest. Although a large concentration of glutamate is neurotoxic to the spinal cord, lidocaine administered intrathecally increases the glutamate concentration in cerebrospinal fluid and demonstrates histopathologically severe vacuolations in the dorsal funiculi[29].

Cold saline injection showed some advantages over lidocaine injection in our study. Cold saline injection can be used for suppression of spinal cord metabolism. However, further investigation is mandatory to determine the appropriate temperature, dose and timing of cold saline injection for clinical applications. We confirmed the existence of the critical spinal cord arteries in the clamped segment of the aorta under MEP monitoring. In the future, we will also research how to identify the Adamkiewicz artery safely.

CONCLUSION

Cold saline injection into the clamped segment of aorta induced rapid MEP change, especially in I wave amplitude, and cooled the spinal cord during aortic cross-clamping period. This method can facilitate the diagnosis of MEP change during aortic cross-clamping and can shorten the time for reconstruction of the Adamkiewicz artery.

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