Epidural cooling for the prevention of ischemic injury to the spinal cord during aortic occlusion in a rabbit model: Determination of the optimal temperature

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Purpose: This experiment was designed for the determination of the optimal epidural cooling temperature for the allowance of spinal cord protection with minimal side effects during an aortic occlusion-induced spinal cord ischemia model in rabbits.

Methods: Spinal cord ischemia was induced in rabbits with infrarenal aortic occlusion for 40 minutes. Spinal cord cooling was effected with epidural infusion of normal saline solution at the following different temperatures: group 1, 17°C (n = 6); group 2, 24°C (n = 6); group 3, 32°C (n = 6); and group 4, 39°C (n = 3). Sham-operated rabbits without aortic occlusion were used as controls with epidural infusion at healthy body temperature (39°C; n = 3). Motor function was assessed at 48 hours with Tarlov’s criteria, and the animals were killed. The spinal cord was sectioned into multiple segments, and semiquantitative histologic scoring (0 to 5) was used to grade ischemic injury.

Results: Cooling solution and spinal cord temperatures showed linear correlation (r = 0.95). All the rabbits in groups 1 (except one with mild weakness), 2, and 3 were neurologically intact, and all in group 4 had paraplegia develop (P < .001). One rabbit in group 1 died from increased intracranial pressure (ICP). Mean blood pressure, ICP, and body temperature were similar among the groups. Histology correlated with the clinical findings. In groups 1 and 2, minimal histologic changes were noted. Low-grade ischemic changes were present in group 3 in the low-lumbar and mid-lumbar segments. Severe ischemic injury occurred at the same segments in group 4 (P < .05).

Conclusion: These study results suggest that in rabbits satisfactory spinal cord protection during aortic occlusion can be achieved at moderate regional hypothermia (24°C). Large volume infusion for the achievement of profound hypothermia may cause deleterious effects of increased ICP and is not warranted. (J Vasc Surg 2002;35:547–55.)

Spinal cord ischemic injury remains a most compelling challenge of thoracoabdominal aortic aneurysm (TAA) repair.1–6 In one report, the incidence rate of paraplegia or paraparesis was 16% in 1509 patients who underwent TAA operations.8 In our experience, the incidence rate of these complications in 181 patients who underwent operation for TAA was 8%.5 Various measures, such as short aortic cross-clamp time, left atrial-femoral bypass grafting, cardiopulmonary bypass grafting with circulatory arrest, improved anesthesia, and systemic hypothermia, have been used for the prevention of ischemic spinal cord injury. However, none of these procedures has completely prevented this devastating complication.7–10 Although systemic hypothermia is effective against neuronal ischemia, it is not without adverse effects, such as cardiac arrhythmia, coagulopathy, and suppressed cardiopulmonary functions.11–13 Regional hypothermia, such as epidural cooling (EC), is void of the systemic side effects and has been shown to be effective in the reduction of spinal cord ischemic injury in both experimental and clinical settings.14–19 A significant limitation of EC is the rise in cerebrospinal fluid (CSF) pressure, which diminishes spinal cord perfusion pressure and causes intracranial hypertension.20,21 This study was conducted to test the hypothesis that there is an optimal spinal cord temperature at which maximal spinal cord protection can be achieved and that further decrease in the temperature does not add to the neuroprotection but increases the risk of side effects.

MATERIAL AND METHODS

Animal care was in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” (NIH Publication No. 86–23, revised 1985). The experiments were performed in two phases. The first phase was the search for appropriate noninvasive monitoring of the spinal cord temperature. In this phase, we also aimed to determine the greatest degree of spinal cord cooling possible without significant increase in the ICP. In the second phase, we planned to determine the optimal temperature for EC.

New Zealand white rabbits, weighing from 2.8 to 3.3 kg, underwent anesthesia with an intramuscular cocktail of...
Ketamine hydrochloride (68 mg/kg) and xilazine (9 mg/kg). Blood pressure was monitored via the central auricular artery. The infrarenal aorta was exposed through a retroperitoneal approach and looped with a Rummel’s tourniquet (Fig 1). In the rabbit, the aorta right below the most caudal renal artery corresponds to the second lumbar vertebrae (L2) level. The rabbit then was placed in a prone position, and the head was placed in a stereotactic frame. Through a skin incision over the temporoparietal region, the cranium was exposed. A burr hole was drilled, and a pressure transmitter was placed into the subdural place for ICP monitoring. Through a posterior midline skin incision, the spinal process of L6 was resected to expose the L5-L6 intervertebral space. A transverse incision was made on the ligamentum flavum, and a 17-gauge spinal needle was inserted into the epidural space. An 18-gauge epidural catheter then was advanced through the needle into the epidural space for 2 cm. Lack of spasm assured the proper placement of the catheter into the epidural space. The needle then was removed, and the catheter was secured to the prevertebral fascia. To allow egress of coolant, the proximal epidural space was vented at the level of L3 and the L3 spinal process was resected through a separate posterior skin incision (Fig 2). Two 33-gauge temperature probes (Omega Engineering, Stamford, Conn), at L2-L3 and L5-L6, were placed directly into the spinal cord. A 21-gauge temperature probe was inserted into the paraspinal muscle at the level of L5, and another temperature probe was used for the measurement of the coolant temperature at the entry point into the epidural space. Core body temperature was monitored in the rectum. After systemic heparinization (100 U/kg), the EC was initiated. At the same time, the control of infrarenal aorta was obtained and confirmed with the loss of Doppler scan signal over the femoral artery.

The spinal cord was cooled with an extracorporeal perfusion system that consisted of a roller pump (Minipuls 2 Gilson, Inc, Middleton, Wis) and a glass coil (Mayo Clinic Department of Engineering, Rochester, Minn) immersed in a water bath (D1-L Circulator, Thermo Haake, Karlsruhe, Germany). A heating blanket was used for the maintenance of healthy body temperature throughout the procedure. All throughout the cross-clamp time, data were continuously displayed on a data-logger (Omega Engineering) and printed every minute. Once steady state was reached, which took about 8 minutes, the temperature of the spinal cord was changed to the next level with the adjustment of the temperature of the water bath. Nine rabbits were used for this part of the experiment. The lowest spinal cord temperature achievable was determined with the infusion of iced saline solution at 5 mL/min. The animals were killed at the conclusion of the experiment.

Directly measured spinal cord temperature was plotted against cooling fluid temperature. Four temperature points at similar intervals (17°C, 24°C, 32°C, and 39°C) between the lowest achievable spinal cord and healthy body temperature were arbitrarily selected along the linear segment of the correlation curve (Fig 3).

In the second set of experiments, the animals were divided into four groups according to the degree of spinal cord cooling: 17°C (group 1, n = 6), 24°C (group 2, n = 6), 32°C (group 3, n = 6), and 39°C (group 4, n = 3). The rabbits were prepared in a similar manner as described previously, except for the lack of temperature probes in the spinal cord and in the paraspinal muscle. Epidural venting was obtained at the L3 level in the same way as in the first phase of the study. Spinal cord cooling for each group was achieved with the adjustment of the temperature of the saline solution through acting on the water bath tempera-
ture, while the flow rate of the cooling fluid was kept constant at 5 mL/min. Because EC was performed during the 40 minutes of aortic occlusion, the amount of fluid infused was equivalent to 200 mL. At the conclusion of the experiment, the skin layer was closed and the rabbits were allowed to recover. The motor function was assessed at 48 hours with the Tarlov’s criteria (Table I). A control group without aortic occlusion was infused at 39°C for 40 minutes at a rate of 5 mL/min (n = 3).

After 48 hours, the rabbits underwent anesthesia with an intramuscular cocktail of ketamine hydrochloride (68 mg/kg) and xilazine (9 mg/kg). Through a median sternotomy, the pericardium was opened and the left ventricle was cannulated with a 14-gauge catheter. The animal was exsanguinated through the right atrium, and perfusion fixation of the cord was performed with infusion of 250 mL of saline solution at 80 mm Hg for 3 minutes, followed by 800 mL of 10% neutral buffered formalin at 80 mm Hg for 10 minutes.

The spinal cord was harvested for histologic examinations. The spinal cord was sectioned through the dorsal root ganglia from the low thoracic level to the low lumbar level into the following five segments (the numbers in parenthesis refer to the vertebral levels): low thoracic (LT = T9-T11), thoracolumbar (TL = T11-L1), upper lumbar (UL = L1-L3), mid lumbar (ML = L3-L5), and low lumbar (LL = L5-L6) segments. From each segment, two 7-µm sections, 500 µm apart (level 1 and level 2), were cut and stained with hematoxylin and eosin. An examiner (G.M.), blinded for group designation, reviewed all the slides and graded the extent of ischemic changes (Table II).23-25

Table I. Tarlov’s scoring for neurologic assessment

<table>
<thead>
<tr>
<th>Neurologic assessment</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td>No voluntary movement</td>
<td>0</td>
</tr>
<tr>
<td>Perceptible movement of joints</td>
<td>1</td>
</tr>
<tr>
<td>Good movement of joints but inability to stand</td>
<td>2</td>
</tr>
<tr>
<td>Ability to stand and walk</td>
<td>3</td>
</tr>
<tr>
<td>Complete recovery</td>
<td>4</td>
</tr>
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**Statistical analysis.** All the clinical data were expressed as the mean ± the standard error of the mean. Clinical scoring was compared with Friedman test. The results of the histologic grading were analyzed with Kruskal-Wallis analysis of variance test followed by the Scheffé multiple comparison test. Statistical significance was assumed at a P value of less than .05.

**RESULTS**

The mortality rate of the procedure was 4.2%: one rabbit in group 1 died 10 minutes after epidural infusion from intracranial hypertension. Postmortem study results revealed that the epidural space at the L3 site had not been adequately opened.

The paraspinal muscle temperature did not show association with the directly measured spinal cord temperature (data not shown), therefore the paraspinal muscle temperature probes were omitted early on during the experiment. The lowest achievable temperature of the spinal cord was 9.2°C. The cooling fluid temperature, measured at the...
skin level of the animal, correlated the best with the spinal cord temperature measured at L₅-L₆. Linear correlation was shown between the cooling fluid and the spinal cord temperature (r = 0.95) if the cooling fluid temperature was kept in the 10°C to 40°C range (Fig 3). During 40 minutes of aortic cross-clamping, the mean core body temperature, the mean ICP, and the mean arterial blood pressure did not differ between the groups (Table III).

All the control rabbits without aortic occlusion recovered without neurologic deficit at 48 hours. All but one animal in groups 1, 2, and 3 were neurologically intact. One in group 1 had mild weakness. By contrast, all the rabbits in group 4 had paraplegia develop (P < .001; Table IV). Histologic assessment results of the upper two-thirds of the spinal cord (from LT 1 to UL2) showed no or only minimal injury in each group, although minor histologic changes occurred more frequently in group 4 (39°C; Fig 4). The only animal in groups 1 to 3 with mild neurologic deficit (Tarlov’s criteria 3) had minimal changes (histologic score = 1) in LT1 and LT2. In the ML1 and ML2 segments in groups 3 (32°C) and 4 (39°C), partial anterior horn necrosis was noted. Significant differences were found in the ML segments between group 2 (histologic score, ML₁ = 0; ML₂ = 0) and group 4 (histologic score, ML₁ = 3.6; ML₂ = 3.6; P < .05; Fig 5). The LL₁ and LL₂ segments were void of ischemic insults in groups 1 to 3 (histologic score = 0). However, in group 4, the LL segments showed bilateral necrosis of the entire anterior horn (histologic score = 5). At the LL level of the spinal cord, differences between group 4 and any other groups were statistically significant (P < .05; Fig 5).

**DISCUSSION**

Neurologic complications after repair of TAA are dictated by multiple, interdependent variables, such as metabolic demand of the cord, ischemia time, and reperfusion injury. Currently available methods of spinal cord protection can be largely divided into two main approaches: maintenance of local blood flow and neuroprotection. The former is achieved with intercostal reimplantation, left heart bypass grafting, and CSF drainage, and the latter is obtained with hypothermia and various neuroprotective pharmacologic agents.26

There are several mechanisms by which hypothermia protects neurons from ischemic insults. Early studies showed that hypothermia reduces metabolism and oxygen consumption in the central nervous system.27 Oxygen demand of the central nervous system decreases by approximately 5% per every degree (°C) drop in the cord temperature. Hypothermia mediates neuroprotective effects by decreasing loss of adenosine triphosphatase, phosphocreatinine, and reduced nicotinamide adenine dinucleotide
phosphate, by stabilizing cellular membrane, and by reducing release of excitatory neurotransmitters and other mediators of reperfusion injury. CSF production is reduced with hypothermia, which then leads to a reduction in CSF pressure and thereby improves collateral circulation to the spinal cord. Hypothermia may also decrease thrombus formation and prevent microembolism.

Although systemic hypothermia has been used for spinal cord protection since 1945, its adverse effects on bleeding and cardiopulmonary function precluded its widespread use. Thus, various techniques of regional spinal cord cooling have been advanced. These include hypothermic perfusion of the intercostal arteries, subarachnoidal cooling, and EC.

Selective perfusion of excluded segment of the aorta has been shown to be effective in the reduction of neurologic injury in animal models. However, variations in blood supply to the spinal cord from the aorta and interruption of the blood flow during aortic reconstruction limit its use. Subarachnoidal space, filled with CSF, is an ideal space for the cooling of the spinal cord. Subarachnoidal cooling poses a risk of rise in CSF pressure, of CSF dilution, and of intrathecal infection.

EC has been shown to be an effective tool in spinal cord protection in both experimental and clinical settings. Our laboratory has shown the benefit of EC of the spinal cord during thoracoabdominal cross-clamping in a canine model. It was noted that EC with 0°C Ringer’s lactate almost completely prevented ischemic spinal cord injury after 60 minutes of aortic occlusion. Studies with the rabbit model have shown that the lowering of the spinal cord temperature to 15°C with EC prevented any ischemic injury after 60 minutes of aortic occlusion. Similar protection was observed with the

**Table III.** Core body temperature, intracranial pressure, and arterial blood pressure in rabbits during 40 minutes of aortic occlusion and epidural cooling at various temperatures (mean ± standard error of mean)

<table>
<thead>
<tr>
<th>Core body temperature (°C)</th>
<th>ICP (mm Hg)</th>
<th>Arterial blood pressure (mm Hg)</th>
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<tr>
<td>Group 1 (17°C)</td>
<td>36.3 ± 1.9</td>
<td>8.2 ± 3.4</td>
</tr>
<tr>
<td>Group 2 (24°C)</td>
<td>36.6 ± 1.0</td>
<td>8.5 ± 4.8</td>
</tr>
<tr>
<td>Group 3 (32°C)</td>
<td>36.6 ± 0.8</td>
<td>7.3 ± 3.4</td>
</tr>
<tr>
<td>Group 4 (39°C)</td>
<td>37.2 ± 0.3</td>
<td>7.7 ± 0.9</td>
</tr>
<tr>
<td>Control (39°C) (no occlusion)</td>
<td>38.3 ± 0.6</td>
<td>7.1 ± 2.2</td>
</tr>
</tbody>
</table>

Differences remained nonsignificant.

ICP: Intracranial pressure.

**Fig 5.** Mean histologic scores of ischemic injury at different levels of spinal cord after aortic occlusion with epidural cooling at various temperatures (with Kruskal-Wallis analysis of variance, P < 0.05.) *39°C versus 24°C. **39°C versus any other temperature groups. LT, Low thoracic; TL, thoracolumbar; UL, upper lumbar; ML, mid lumbar; LL, low lumbar. Levels 1 and 2 of each.
cooling of the spinal cord to 21°C with an aortic occlusion of 40 minutes.25 However, EC is not without limitation. High EC fluid flow may be necessary for the maintenance of the desired level of hypothermia resulting in increased CSF pressure.20,21 These study results showed that, in a rabbit model of spinal cord ischemia, moderate regional hypothermia at 24°C protected the spinal cord as effectively as profound hypothermia (17°C) and mild hypothermia (32°C) was partially effective. Profound hypothermia provided no additional neuroprotection as compared with moderate hypothermia. In a clinical setting, a 11.5% absolute risk reduction in the incidence rate of neurologic deficit after repair of TAA with EC has been reported.42 However, with moderate epidural hypothermia (CSF temperature 26°C), neurologic complications still occur in 12% of patients with type I and II TAA.42 The cause of paraplegia in these patients is variable and likely includes embolism or thrombosis of the spinal arteries. With perfusion of saline solution in humans, EC may indeed be associated with significant CSF pressure increase. The amount of the perfused cooling solution, therefore, should be kept to a minimum. Our data suggests that deep hypothermia would not add to the spinal cord protection but would only increase the harmful effects of CSF pressure increase. Therefore, attempts to achieve hypothermia lower than 24°C with the perfusion of larger amount of cooling solution should be abandoned.

In summary, moderate regional hypothermia achieved with EC is an effective method of spinal cord protection against ischemic spinal cord injury in an experimental model. Moderate hypothermia (24°C) is as effective as profound hypothermia (17°C) without the attendant side effects of the latter. Because the study was performed in rabbits and was limited to 48 postoperative hours, no implication should be made on the development of delayed onset paraplegia or other neurologic deficit.

REFERENCES
18. Tabayashi K, Niibori K, Konno H, Mohri H. Protection from postis-