Neuroprotection following mild hypothermia after spinal cord ischemia in rats

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Objective: We examined the hypothesis that a 1°C reduction in body temperature would reduce gray and white matter injury induced by spinal cord ischemia in rats. In addition, we evaluated the relationship between reactive astrogliosis and gray or white matter injury after spinal cord ischemia with a 1°C reduction in body temperature or normothermia.

Methods: Rats were randomly divided into hypothermia (1°C decrease in body temperature to 36.3°C), normothermia (37.3°C), and sham surgery groups (n = 6/group). Hypothermia was induced 15 minutes before ischemia and maintained during ischemia. Animals were then rewarmed to normothermia. Spinal cord ischemia was induced by a balloon catheter in the thoracic aorta, and the proximal mean arterial blood pressure was maintained at 40 mm Hg for 14 minutes. Hind limb motor function was assessed at 2, 7, 14, 21, and 28 days after reperfusion. At 28 days after reperfusion, gray matter damage was assessed by counting the number of normal motor neurons and white matter damage by the extent of vacuolation. The glial fibrillary acidic protein (GFAP)-positive area fraction (GFAP%) was determined in white and gray matter structures to measure reactive astrogliosis.

Results: Compared with normothermia, hypothermia significantly improved hind limb function at all assessments (P < .01) and increased numbers of normal gray matter motor neurons (39 ± 20 vs 99 ± 13, respectively; P < .001), decreased the percentage area of white matter vacuolation (9.0% ± 2.7% vs 1.6% ± 1.3%, respectively; P = .001), and decreased the GFAP% in gray (P = .003) and white matter (P = .009).

Conclusions: Prophylactic mild hypothermia (1°C reduction in body temperature) preserved hind limb motor function and reduced neuronal death, white matter vacuolation, and astrogliosis in gray and white matter induced by spinal cord ischemia in rats. Thus, mild hypothermia may be useful for perioperative management of thoracoabdominal aortic surgery. (J Vasc Surg 2013;57:173-81.)

Clinical Relevance: Hypothermia (3°-4°C decrease in temperature) is known to protect the spinal cord from ischemia-reperfusion injury; however, hypothermia can also cause serious secondary complications. In this study, a 1°C reduction in body temperature induced before spinal cord ischemia provided marked and persisting neuroprotection and reduced gliosis, without adverse effects. These data suggest that very mild hypothermia may be applied clinically to avoid systemic complications.

Thoracoabdominal aortic surgery can cause spinal cord ischemia-reperfusion during aortic cross-clamping, resulting in postoperative paraplegia, a devastating complication that can severely affect quality of life.1,2 There is some evidence that systemic hypothermia may be beneficial in reducing postoperative paraplegia caused by spinal cord ischemia-reperfusion.1-3 However, hypothermia can also cause serious complications,3-5 and whether the protective action of hypothermia outweighs these detrimental effects remains controversial.

In several experimental animal models, mild hypothermia of 3° to 4°C (33°-34°C body temperature) exerted a potent protective effect against spinal cord ischemia-reperfusion injury.6,7 For example, left ventricular contraction and cardiac output were significantly reduced in pigs during hypothermia at 34°C to 35°C, whereas lowering body temperature to 32°C increased clotting time, even after rewarming.8 In rabbits, hypothermia at 33°C reduced heart rate,7 and in rats, the toxic effects of potassium on the myocardium were increased at a body temperature of 31°C.9 In the clinical setting, a 3°C to 4°C decrease in body temperature may increase the risk of critical arrhythmia,4,5 bleeding, and vasospasm, and may prolong postoperative recovery.5

Further, because it remains unclear the exact temperature spinal cord at which protection is induced after hypothermia, this strategy is largely ignored during emergencies such as immediate shutting off of the descending aorta due to lack of perfusion.
to aneurysm rupture. Importantly, previous reports showed that a 1°C to 2°C decrease in body temperature could effectively attenuate brain injury after experimental ischemia. However, no studies to date have assessed the neuroprotective effect of a 1°C decrease in body temperature on spinal cord ischemia.

After central nervous system (CNS) ischemia, astrocytes are activated with upregulation of glial fibrillary acidic protein (GFAP), resulting in reactive astrogliosis. Reactive astrocytes are important in regulating neuronal cell death after CNS ischemia, whereas mild to moderate hypothermia can reduce reactive astrogliosis after transient or permanent brain ischemia in rats. However, the influence of hypothermia on reactive astrogliosis after spinal cord ischemia is unknown. Finally, a number of studies have suggested that protective strategies against spinal cord ischemia is unknown. Finally, a number of studies have suggested that protective strategies against spinal cord ischemia should be examined over longer recovery periods, based on the delayed onset paraplegia observed clinically and experimentally. Thus, in the present study, we examined the effects of hypothermia (1°C reduction in body temperature) on hind limb motor function, neuronal degeneration, and reactive astrogliosis at 28 days after recovery from spinal cord ischemia–reperfusion in the rat.

METHODS

All animals received humane care in compliance with the Principles of Laboratory Animal Care (National Society of Medical Research) and the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington DC, revised 1996). The Institutional Animal Care Committee of Niigata University approved the experimental protocol.

Animals. Male Sprague-Dawley rats (weight, 380-420 grams) were housed and maintained on a 12-hour light/dark cycle with free access to food and water.

Surgical model. Spinal cord ischemia–reperfusion was induced as previously reported. To avoid circadian changes in body temperature, surgical procedures in all rat groups were done between 9:00 and 11:00 A.M. Rats were fasted overnight and were anesthetized with mixed gas (1 L/min nitrous oxide, 0.7 L/min oxygen, and 1.5-2% [v/v] isoflurane) delivered by an inhalation mask. Paravertebral muscle (T10-11) and rectal temperature were continuously monitored. The rectal temperature was not monitored during ischemia and the subsequent 20 minutes of reperfusion because the rectal thermoprobe was thought to reflect distal arterial pressure.

A PE-50 cannula was inserted into the tail artery for monitoring the distal arterial blood pressure. To control the proximal arterial blood pressure at approximately 40 mm Hg during aortic occlusion, a PE-60 cannula connected to an external blood reservoir (38.0°C) positioned 54 cm above the body of the rat was inserted into the left carotid artery to reduce arterial blood pressure. The left femoral artery was isolated, and a 2F Fogarty catheter was placed into the descending thoracic aorta so that the tip reached directly below the left subclavian artery (11 cm distal from the site of insertion). At the completion of all cannulation, heparin (200 IU) was injected into the tail artery.

To induce spinal cord ischemia, the balloon catheter was inflated with saline, and blood was allowed to flow into the external reservoir. After 14 minutes of spinal cord ischemia, the balloon was deflated and the blood was reinfused during a 60-second period. Protamine sulfate (4 mg) was administered subcutaneously. Blood gases, pH, glucose, and hemoglobin were measured at 1 minute before and 10 minutes after the ischemia. In the sham group, all catheters were inserted in the same manner; however, the balloon was not inflated.

Experimental groups and temperature management protocol. Rats were randomly divided into hypothermia (1°C decrease in body temperature to 36.3°C), normothermia (37.3°C), and sham operation groups (n = 6/group). Temperature was measured with an N550 thermometer (NKS-YSI, Tokyo, Japan), measurable to two decimal places, at two sites. The protocol for temperature control was as follows: The body temperature of the rats was kept constant by using a heat lamp positioned 20 cm above the chest and a heat pad covered by a black sheet. The distance of the heat lamp to the rat’s body was adjusted when paravertebral muscle temperature decreased by 0.03°C from the target temperature. When paravertebral muscle temperature increased by 0.02°C from the target temperature, the heat lamp and the heat pad were removed. If a further increase in temperature was observed, cooling was conducted by placing a plastic plate to the back of the rat and a water bag to the front of the body for a few seconds.

In the hypothermia group, the body temperature was held at 37.3°C until cannulation of the carotid (15 minutes before ischemia) and then cooled to 36.3°C before ischemia by contact with a plastic plate and water bag. The temperature was maintained at 36.3°C during ischemia (14 minutes) and rewarmed after reperfusion by using the heat lamp.
Table I. Physiologic data

<table>
<thead>
<tr>
<th>Variablea</th>
<th>Sham</th>
<th>Normothermiab</th>
<th>Hypothermiab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length, cm</td>
<td>23 ± 0.2</td>
<td>23 ± 0.1</td>
<td>23 ± 0.1</td>
</tr>
<tr>
<td>1 minute before ischemia</td>
<td></td>
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</tr>
<tr>
<td>pH</td>
<td>7.36 ± 0.04</td>
<td>7.38 ± 0.04</td>
<td>7.41 ± 0.03</td>
</tr>
<tr>
<td>PaCO₂, mm Hg</td>
<td>43 ± 4</td>
<td>45 ± 3</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>PaO₂, mm Hg</td>
<td>121 ± 12</td>
<td>125 ± 15</td>
<td>133 ± 20</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>15.9 ± 0.3</td>
<td>15.9 ± 0.9</td>
<td>15.6 ± 0.6</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>134 ± 29</td>
<td>134 ± 29</td>
<td>134 ± 20</td>
</tr>
<tr>
<td>Distal MAP, mm Hg</td>
<td>88 ± 12</td>
<td>84 ± 17</td>
<td>82 ± 15</td>
</tr>
</tbody>
</table>

During ischemia

| | | |
| Distal MAP, mm Hg | 90 ± 16 | 7 ± 2c | 6 ± 2c |
| 10 minutes after ischemia | | | |
| pH | 7.37 ± 0.05 | 7.18 ± 0.06c | 7.19 ± 0.05c |
| PaCO₂, mm Hg | 44 ± 4 | 51 ± 6 | 56 ± 8 |
| PaO₂, mm Hg | 124 ± 8 | 136 ± 29 | 140 ± 31 |
| Hemoglobin, g/dL | 15.6 ± 0.6 | 16.8 ± 0.54d,e | 15.5 ± 0.5 |
| Glucose, mg/dL | 133 ± 29 | 116 ± 43 | 125 ± 38 |
| Distal MAP, mm Hg | 82 ± 21 | 70 ± 10 | 72 ± 11 |

MAP, Mean arterial pressure; PaCO₂, partial pressure of arterial carbon dioxide; PaO₂, partial pressure of arterial oxygen.

aData are presented as the mean ± standard deviation.

bNormothermia and hypothermia: animals receiving spinal cord ischemia with normothermia and hypothermia (1°C decrease), respectively.

cP < .001 vs sham group.

dP = .015 vs sham group.

eP = .013 vs hypothermia group.

lamp and the heat pad. In the normothermia and sham groups, perioperative body temperatures were maintained at 37.5°C. Ischemia in all rats was applied after confirmation of the target temperature ± 0.05°C held for 5 minutes. The temperature of all animals was precisely controlled to hold the target temperature constant up to 3 hours after reperfusion (Fig 1).

Neurologic evaluation. Hind limb motor function was assessed at 2, 7, 14, 21, and 28 days after reperfusion using the Motor Deficit Index (MDI) score22,23 (quantified by ambulation and placing/stepping reflex) by investigators who were blinded to group information. Hind limb ambulation was graded as follows: 0, normal (symmetric and coordinated ambulation); 1, toes flat under the body when walking but ataxia present; 2, knuckle walking; 3, unable to knuckle walk but some movement of the hind limbs; and 4, no movement or drags lower extremities. The placing/stepping reflex was assessed by the dragging movements and responses of the hind paw dorsum when touching the floor surface. A coordinated lifting and placing response (ie, stepping), which was generally evoked when a hind paw touched the ground, was graded as follows: 0, normal; 1, weak; and 2, no stepping. MDI was calculated for each rat as the sum of these scores at each interval. The maximum MDI score was 6 (score of 4 for ambulation and 2 for the placing/stepping reflex).

Histologic preparation of spinal cord. After evaluation of motor behavior, animals were anesthetized with isoflurane, and pentobarbital (100 mg/kg) was administered intraperitoneally. Animals were then transcardially perfused with heparinized saline (100 mL), followed by 10% (v/v) phosphate-buffered formalin (150 mL). The lumbar spinal cord was removed and fixed with the same fixative for another 2 to 7 days. After fixation, the fourth lumbar spinal segment was dissected and embedded in paraffin, and 5-µm-thick serial transverse sections were prepared. For analysis, three representative sections were taken from segments of the fourth lumbar cord with 100-µm interspaces.

Assessment of gray and white matter injury. Gray matter damage was assessed by counting the number of normal motor neurons in the ventral part of the gray matter (anterior to a transverse line drawn through the central canal) at original magnification <X>400. Cells that contained Nissl substance in the cytoplasm, loose chromatin, and prominent nucleoli were considered normal motor neurons. The number of normal motor neurons in each animal was obtained by averaging counts from three different slides stained with Nissl dye.

White matter damage was assessed by the extent of vacuolation in the ventral and ventrolateral white matter on sections stained with hematoxylin and eosin (H&E). A 0.04-mm² area in the ventral and ventrolateral white matter was used for assessment, as previously reported.21,23 Each target area in the white matter was divided into 64 subareas. The number of subareas that were >75% occupied by vacuoles was counted, and the percentage area of vacuolation was calculated (original magnification ×400). The percentage area of vacuolation in each animal was obtained by averaging two areas of the right and left hemispheres of the three different slides (a total of 12 areas).

Immunohistochemical detection and quantitation of GFAP. For immunohistochemistry, sections were deparaffinized and endogenous peroxidase was inactivated using 3% (v/v) hydrogen peroxide in methanol. Sections were rinsed in phosphate-buffered saline (0.1 mol/L,
Table II. Paravertebral muscle temperature (°C)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>Normothermia</th>
<th>Hypothermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>After induction of anesthesia</td>
<td>37.3 ± 0.3</td>
<td>37.2 ± 0.3</td>
<td>37.2 ± 0.2</td>
</tr>
<tr>
<td>30 minutes before ischemia</td>
<td>37.3 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td>37.3 ± 0.1</td>
</tr>
<tr>
<td>Just before ischemia</td>
<td>37.3 ± 0.1</td>
<td>37.3 ± 0.0</td>
<td>36.3 ± 0.0</td>
</tr>
<tr>
<td>5 minutes after ischemia onset</td>
<td>37.3 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td>36.3 ± 0.1</td>
</tr>
<tr>
<td>Just before reperfusion</td>
<td>37.2 ± 0.1</td>
<td>37.2 ± 0.1</td>
<td>36.2 ± 0.1</td>
</tr>
<tr>
<td>After reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 minutes</td>
<td>37.2 ± 0.1</td>
<td>37.2 ± 0.1</td>
<td>36.4 ± 0.2</td>
</tr>
<tr>
<td>20 minutes</td>
<td>37.2 ± 0.2</td>
<td>37.3 ± 0.1</td>
<td>36.7 ± 0.2</td>
</tr>
<tr>
<td>30 minutes</td>
<td>37.3 ± 0.1</td>
<td>37.3 ± 0.2</td>
<td>37.3 ± 0.1</td>
</tr>
<tr>
<td>60 minutes</td>
<td>37.2 ± 0.2</td>
<td>37.3 ± 0.2</td>
<td>37.3 ± 0.1</td>
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<tr>
<td>120 minutes</td>
<td>37.3 ± 0.1</td>
<td>37.3 ± 0.1</td>
<td>37.2 ± 0.2</td>
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<tr>
<td>180 minutes</td>
<td>37.3 ± 0.2</td>
<td>37.2 ± 0.1</td>
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</tbody>
</table>

aData are presented as the median ± standard deviation.

bNormothermia and hypothermia: animals receiving spinal cord ischemia with normothermia and hypothermia (1°C decrease), respectively.

cP < .001 vs sham and normothermia groups.

Table III. Rectal temperature (°C)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>Normothermia</th>
<th>Hypothermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>After induction of anesthesia</td>
<td>36.8 ± 0.3</td>
<td>36.9 ± 0.3</td>
<td>36.9 ± 0.2</td>
</tr>
<tr>
<td>30 minutes before ischemia</td>
<td>36.9 ± 0.2</td>
<td>37.0 ± 0.2</td>
<td>36.8 ± 0.1</td>
</tr>
<tr>
<td>Just before ischemia</td>
<td>36.9 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>36.3 ± 0.1</td>
</tr>
<tr>
<td>After reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>36.8 ± 0.1</td>
<td>36.9 ± 0.2</td>
<td>36.8 ± 0.2</td>
</tr>
<tr>
<td>60 minutes</td>
<td>36.7 ± 0.3</td>
<td>36.8 ± 0.2</td>
<td>36.8 ± 0.2</td>
</tr>
<tr>
<td>120 minutes</td>
<td>36.8 ± 0.2</td>
<td>36.7 ± 0.2</td>
<td>36.7 ± 0.3</td>
</tr>
<tr>
<td>180 minutes</td>
<td>36.7 ± 0.3</td>
<td>36.7 ± 0.2</td>
<td>36.8 ± 0.1</td>
</tr>
</tbody>
</table>

aData are presented as the mean ± standard deviation.

bNormothermia and hypothermia: animals receiving spinal cord ischemia with normothermia and hypothermia (1°C decrease), respectively.

cP < .001 vs sham and normothermia groups.

RESULTS

Physiologic data. Perioperative physiologic data of the experimental groups are reported in Table I. Hemoglobin values at 10 minutes after ischemia were significantly higher in the normothermia group than in the hypothermia group (P = .013) and sham groups (P = .015). Paravertebral muscle and rectal temperatures of rats are reported in Table II and Table III. Just before ischemia to 10 minutes after reperfusion, the paravertebral muscle temperature in the hypothermia group was controlled 1°C lower than that in normothermia and sham groups (Table II).

Neurologic function. MDI-assessed hind limb function is reported in Table IV. MDI scores at all experimental times were significantly different between the groups (Kruskal-Wallis P < .001 for each). MDI scores were significantly lower in the hypothermia group than in the normothermia group at 2, 7, and 14 days (P = .002) and at 21 and 28 days (Scheffé test P = .007).

Gray matter injury. Representative light photomicrographs of the ventral gray matter of Nissl-stained transverse sections taken from the L4 spinal cord segment are
Fig 2. A, Representative light photomicrographs of Nissl-stained sections in the ventral horn gray matter in the sham, normothermia, and hypothermia groups at 28 days after reperfusion. Scale bar: 100 μm. B, Number of normal motor neurons at 48 hours after reperfusion in the sham, normothermia, and hypothermia groups. The number of normal motor neurons in the ventral part of the gray matter was significantly increased in the hypothermia group compared with the normothermia group (*P < .001) and with the sham group (#P < .001). Data are mean ± standard deviation. Normothermia and hypothermia: animals receiving spinal cord ischemia with normothermia and hypothermia (1°C decrease), respectively.

Fig 3. A, Representative light photomicrographs of hematoxylin and eosin–stained sections in the ventrolateral white matter in the sham, normothermia, and hypothermia groups at 28 days after reperfusion. Scale bar: 50 μm. B, Percentage area of vacuolation in the white matter at 28 days after reperfusion in the sham, normothermia, and hypothermia groups. The percentage area of vacuolation in the hypothermia group was significantly lower than in the normothermia group (*P = .001) and in the sham group (#P = .002). Data are mean ± standard deviation. Normothermia and hypothermia: animals receiving spinal cord ischemia with normothermia and hypothermia (1°C decrease), respectively.
shown in Fig 2, A. In the sham and hypothermia groups, neurons exhibited a normal morphology, and gray matter structure was maintained. In the normothermia group, neurons were only rarely observed and there was marked vacuolation. The number of normal motor neurons was higher in the hypothermia group (99 ± 13) than in the normothermia group (39 ± 20; \( P < .001 \); Fig 2, B). Significantly more normal motor neurons were found in the sham group (108 ± 14) than in the normothermia group (\( P < .001 \)).

**White matter injury.** Representative light photomicrographs of H&E-stained sections in the ventrolateral white matter are shown in Fig 3, A. White matter in the sham and hypothermia groups had a homogeneous structure, whereas vacuolation in the white matter was widespread and prominent in the normothermia group. The percentage area of vacuolation in the hypothermia group (1.6% ± 1.3%) was significantly smaller than in the normothermia group (9.0% ± 2.7%; \( P = .001 \); Fig 3, B). The sham group (1.4% ± 0.9%) had a significantly smaller percentage area of vacuolation than the normothermia group (\( P = .002 \)).

**Immunohistochemical outcome in the gray and white matter.** Representative light photomicrographs of the gray and white matter of GFAP-immunostained transverse sections taken from the L4 spinal cord segment are shown in Fig 4, A and Fig 5, A, respectively. The GFAP% values in the gray and white matter were significantly different between groups (Kruskal-Wallis test \( P < .001 \) and \( P < .003 \), respectively). The GFAP% value in the gray matter of the hypothermia group was significantly smaller than in the normothermia group (Scheffé test \( P = .003 \); Fig 4, B). The GFAP% value in the white matter of the hypothermia group was significantly smaller than in the normothermia group (Scheffé test \( P = .009 \); Fig 5, B).

There was a significant negative correlation between the GFAP% value and the number of normal neurons in sham group (\( r = -0.77; P < .001 \) vs without sham group (\( r = -0.81; P = .002 \); Fig 6, A). There was a significant positive correlation between the GFAP% value and the percentage area of vacuolation in the white matter in the sham group (\( r = 0.65; P = .003 \) vs without sham group (\( r = 0.83; P < .001 \); Fig 6, B).

**DISCUSSION**

In the present study, we provide the first evidence that a decrease in body temperature by 1°C initiated before ischemia–reperfusion can reduce hind limb motor dysfunction and histologic damage and expression of reactive astrocytes in the gray and white matter of the spinal cord in rats at 28 days of recovery. Previous animal studies showed that a 2°C reduction in brain temperature could reduce...
neuronal damage in the hippocampal CA1 region after transient cerebral ischemia. Berntman et al also showed that accumulation of lactate in the cerebral cortex at 20 minutes in an acute ischemia model in rats was significantly reduced by a 1°C decrease in rectal temperature and that adenosine triphosphate content was also restored to control group levels. Overall, these data suggest that a very mild decrease in temperature below normothermia can induce substantial neuroprotection and induce ischemic tolerance against spinal ischemia–reperfusion.

Numerous studies have shown that astrocytes can react in response to an ischemic stimulus, which may influence the degree of neurologic damage after reperfusion. After CNS ischemia, reactive astrocytes release glutamate, adenosine triphosphate, d-serine, nitric oxide synthase, free radicals, and inflammatory cytokines, resulting in nerve cell injury. Although astrocytes can take up excess extracellular glutamate, this action is impaired during ischemia. Expression of reactive astrocytes in the ischemia penumbra was also suggested to be associated with delayed expansion of the infarction in different animal models of cerebral ischemia. Further, the effects of reactive astrocytes on injury after cerebral and spinal cord ischemia are equally important in white and gray matter.

Wakasa et al reported a strong correlation between the degree of astrocyte activation and delayed neuronal death after spinal cord ischemia in rats, with a progressive increase in reactive astrogliosis over time in the gray and white matter associated with increased degradation of motor function and cell damage. After cerebral ischemia in the neonatal rat, Hang et al also demonstrated that acute and chronic astrocyte activation in the ipsilateral periventricular white matter exacerbated cognitive function and prolonged neuronal damage.

In the present study, we found a negative correlation between the GFAP% value and the number of normal neurons in the gray matter, and a positive correlation between the GFAP% value and vacuolation in the white matter. Further, hypothermia induced a significant reduction in GFAP% in gray and white matter, suggesting that part of the protective action of hypothermia may relate to suppression of astrogliosis. Insupport, mild to moderate hypothermia (4°C to 9°C decrease from normothermia) was reported to reduce brain cell damage by suppressing astrocyte activation induced after cerebral ischemia. Other evidence shows that reactive astrocytes can exert a neuroprotective effect through brain-derived neurotrophic factor production after brain ischemia. This effect, however, is thought to occur in the late phase (20 weeks), but not in the acute phase, after an ischemic episode. Thus, because most irreversible spinal damage develops in the acute phase after the onset of spinal
ischemia, it is unlikely that reactive astrocytes exert protective effects on spinal dysfunction.

Interestingly, a recent study demonstrated that the incidence of gray matter damage vs white matter damage was dependent on the rewarming rates after cerebral ischemia–reperfusion with mild hypothermia, suggesting that the neuroprotective actions of hypothermia may be different between gray and white matter structures. The white matter consists of axons, oligodendrocytes, and lipids, and has low levels of antioxidative molecules, suggesting that the white matter may be more vulnerable to ischemia than the gray matter.

Clinically, there is evidence of delayed-onset ischemia–reperfusion injury in the spinal cord. Furthermore, Kurita et al reported that white matter vacuolation and hind limb motor function were more impaired at 14 days than at 1 day after reperfusion in a spinal cord ischemia model in rabbits. Thus, longer observation periods are important to examine accurately the neuroprotective effect of hypothermia on spinal cord ischemic reperfusion injury. In a spinal cord ischemia–reperfusion rat model, a 3°C-reduction in rectal and paravertebral muscle temperature during ischemia reduced the incidence of white matter damage at 28 days after reperfusion. Our study showed similar results, despite a smaller degree of hypothermia, suggesting that very mild hypothermia may equally attenuate delayed neuronal degeneration in the white matter.

Our study has some limitations. First, we did not evaluate the neuroprotective efficacy of other levels of hypothermia; nevertheless, the 1°C reduction in body temperature used in the present study showed a protective effect on the spinal cord, without any systemic effects. Second, we did not exam-

Fig 6. A, A significant negative correlation was found in the glial fibrillary acidic protein fraction (GFAP%) and in the number of normal neurons in normothermia and hypothermia groups compared with sham group ($r = -0.77$, $P < .001$) vs without sham group ($r = -0.81$, $P = .002$). B, A significant positive correlation was found in GFAP% and the percentage area of vacuolation in normothermia and hypothermia groups compared with sham group ($r = 0.65$, $P = .003$) vs without sham group ($r = 0.83$, $P < .001$). Data are mean ± standard deviation. Normothermia and hypothermia: animals receiving spinal cord ischemia with normothermia and hypothermia (1°C decrease), respectively.
ine the efficacy of prophylactic hypothermia with longer period of ischemia or with very long-term recovery times. Further studies are required to evaluate the timing of hypothermia onset, longer ischemic times, and long-term observation periods. Finally, although the temperature control shown in this study may be difficult to achieve in the clinical setting, it nevertheless demonstrated the potential protective actions of very mild hypothermia on spinal cord injury.

CONCLUSIONS

We found that prophylactic mild hypothermia (1°C reduction in body temperature) preserved hind limb motor function and reduced neuronal death and astrogliosis in gray and white matter, including white matter vacuolation, induced by spinal cord ischemia-reperfusion in rats. Thus, very mild (1°C) hypothermia may be a potential strategy for perioperative management of thoracoabdominal aortic surgery.

AUTHOR CONTRIBUTIONS

Study conception and design: TS, SS
Conception and design: TS, SS
Analysis and interpretation: TS, SS, HY, MT
Data collection: HY, MT
Writing the article: TS, SS
Critical revision of the article: TS, SS, HY
Final approval of the article: TS, SS, HY, MT
Statistical analysis: HY, MT
Obtained funding: TS
Overall responsibility: TS

REFERENCES


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