Effects of epidural hypothermic saline infusion on locomotor outcome and tissue preservation after moderate thoracic spinal cord contusion in rats

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Object. Regionally delivered hypothermia has advantages over systemic hypothermia for clinical application following spinal cord injury (SCI). The effects of local hypothermia on tissue sparing, neuronal preservation, and locomotor outcome were studied in a moderate thoracic spinal cord contusion model.

Methods. Rats were randomized to four treatment groups and data were collected and analyzed in a blinded fashion. Chilled saline was perfused into the epidural space 30 minutes postcontusion to achieve the following epidural temperatures: 24 ± 2.3°C (16 rats), 30 ± 2.4°C (13 rats), and 35 ± 0.9°C (13 rats). Hypothermia was continued for 3 hours when a 45-minute period of rewarming was instituted. In a fourth group a moderate contusion only was induced in 14 animals. Rectal (core) and T9–10 (epidural) temperatures were measured continuously. Locomotor testing, using the Basso-Beattie-Bresnahan (Ba-Be-Br) scale, was performed for 6 weeks, and rats were videotaped for subsequent analysis. The lesion/preserved tissue ratio was calculated throughout the entire lesion cavity and the total lesion, spinal cord, and spared tissue volumes were determined. The rostral and caudal extent of gray matter loss was also measured. At 6 weeks locomotor recovery was similar in all groups (mean Ba-Be-Br Scale scores 14.88 ± 3.71, 14.83 ± 2.81, 14.50 ± 2.24, and 14.07 ± 2.39 [p = 0.77] for all four groups, respectively). No significant differences in spared tissue volumes were found when control and treatment groups were compared, but gray matter preservation was reduced in the infusion-treated groups.

Conclusions. Regional cooling applied 30 minutes after a moderate contusive SCI was not beneficial in terms of tissue sparing, neuronal preservation, or locomotor outcome. This method of cooling may reduce blood flow in the injured spinal cord and exacerbate secondary injury.

KEY WORDS • spinal cord injury • hypothermia • neuroprotection • ischemia • rat

T he role of hypothermia as a neuroprotective strategy to reduce the extent of SCI has been controversial since the first promising results were reported in the 1950s, 1960s, and 1970s. Two principal methods have been used to cool the spinal cord—total body cooling (systemic hypothermia) and local spinal cord cooling. The practice of systemic hypothermia derives from studies in which profound cooling was shown to extend the duration of ischemic tolerance of the brain and other organs, including the spinal cord; however, deep systemic hypothermia is associated with potentially life-threatening complications such as ventricular arrhythmia and impaired blood coagulation. Local cooling came to prominence because of the studies of Albin, et al., who conducted experiments in spinal cord–injured monkeys in which they used profound levels of local cooling (11°C). In the past two decades new evidence has accrued that mild degrees of systemic hypothermia (for example, 32.5°C) can be neuroprotective after brain insults and SCI. Still, there are significant concerns about the safety of subjecting patients with acute SCI even to mild systemic hypothermia. These include increased risks of infection and possible myocardial ischemia during rewarming. Patients who have sustained acute SCI have altered autonomic function, and impaired myocardial function has been demonstrated in experimental studies. Patients receiving large steroid doses are relatively immunosuppressed and are at high risk for infections such as pneumonia. In addition, because of shivering, it may be difficult to maintain moderate systemic hypothermia in conscious patients with SCI. The ability to maintain a normal core temperature and avoid the risks of systemic hypothermia makes local cooling strategies attractive for clinical application following SCI. Several methods of local cooling have been shown to be neuroprotective in spinal cord ischemia models in several species. Furthermore, even mild degrees of local hypothermia (30–34°C) have been shown to be protective against transient spinal cord ischemia. A neu-
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The protective effect of local cooling has also been shown in models of spinal cord compression in rats\(^5\) and in canines\(^3\,\,4\,\,5\) as well as in some SCI studies\(^6\,\,7\); however, in a study by Dimar and colleagues\(^8\) involving regional cooling to achieve a 19°C epidural temperature following a severe contusive injury, the authors found no benefit in terms of histopathological, behavioral, and electrophysiological outcomes. When an ischemic compressive injury was added to the initial contusion, local cooling was beneficial. Thus, several groups have shown that local cooling provides a neuroprotective effect after diverse spinal compression/ischemic injuries in several species. In clinical practice, perfusion cooling of the epidural space has been shown to reduce the risk of paraplegia following aortic aneurysm surgery.\(^9\,\,10\) Thus, local cooling is a practical, achievable, and safe strategy, although its efficacy for contusive SCI is unclear. One theoretical advantage of epidural perfusion cooling over other local cooling methods is that it avoids temperature gradients within the spinal cord because the coolant completely surrounds the spinal cord. Clinical temperature gradients within the spinal cord because the coolant completely surrounds the spinal cord. Clinical application could be as simple as placement of a multilumen central venous catheter into the epidural space, which would avoid the need for cumbersome and complex devices after laminectomy.\(^4\) Because epidural perfusion cooling is effective for spinal cord neuroprotection under conditions of spinal cord ischemia\(^4\) and compression and because it has direct potential for clinical translation, we tested its efficacy following a moderate contusive injury (12.5 g/cm) delivered with the NYU injury device. We selected this magnitude of injury because there is considerable preserved tissue after the initial contusion and secondary injury processes substantially propagate the extent of injury. Because the optimal levels of spinal cord cooling are not known, we assessed the effect of three different epidural temperatures on histopathological outcome and Ba-Be-Br locomotor recovery after SCI. We studied the volume of preserved tissue and the extent of gray matter preservation 6 weeks postinjury. In previous experimental studies investigators have used dissimilar methodologies and outcome measures, making comparisons difficult. The development of highly standardized injury devices (for example, the NYU impactor)\(^11\) and outcome assessment scales (Ba-Be-Br locomotor assessment scale)\(^4\) in the field of experimental SCI have enabled the achievement of meaningful comparison of data across studies;\(^4\) however, it remains important to refine the utility of these techniques and explore their limitations. This is particularly true when assessing moderately severe thoracic contusion injuries. In the range to which such contused animals recover (Ba-Be-Br Scale Scores 10–14), the extent of forelimb–hindlimb coordination is used quantitatively. We have found that there is considerable interobserver subjectivity when making this particular assessment. Therefore, we developed a video-based Ba-Be-Br assessment technique to enhance quantification of forelimb–hindlimb coordination and other details such as the degree of paw rotation.

Materials and Methods

All rodent experiments were conducted after approval of Animal Care and Use Committee protocols in accordance with the National Institutes of Health policy on the humane care and use of laboratory animals. Prior to starting the experiment, we confirmed that our NYU impactor produced lesions similar in magnitude to those reported by other investigators.\(^11\) We performed 12.5-, 25-, and 50-g/cm injuries in three groups of five animals, killed the animals after 24 hours, and sent the frozen spinal cord tissue to the laboratory of Dr. Wise Young for determination of the mean lesion volume.\(^11\)

Surgical Procedures

For the epidural hypothermia experiment 56 Sprague–Dawley female rats (mean weight = SD 267 ± 18 g) were randomly assigned to one of four groups. After induction of anesthesia with halothane for 10 to 15 seconds in a sealed jar, the rats were placed on the surgical table and anesthesia was maintained with 2% halothane, 60% oxygen, and 40% nitrous oxide delivered through a humidified anesthetic circuit. The animal’s back was shaved and prepared with betadine. An incision was made to expose the T9–11 laminé. Under a surgical microscope, a complete T-10 laminectomy was performed. The dura mater was preserved to avoid direct saline infusion into the subarachnoid space. The 14 control animals underwent a T-10 laminectomy, and a 12.5-g/cm contusion was induced. The treated animals (Groups I–III) received similar injuries, but 30 minutes postinjury their epidural space was perfused with ice-chilled saline solution for 3 hours consecutively. Rewarming was achieved during a 45-minute period. The target epidural temperatures were 20, 30, and 37.5°C. Three additional animals underwent invasive monitoring of intramedullary temperature. Prior to the randomized experiment, six other animals were used to study the relationship between the perfusate flow rate and the epidural temperature. These animals underwent carotid artery cannulation and measurement of blood pressure as well as PO\(_2\), PCO\(_2\), and pH at various epidural temperatures.

Injury Model

The NYU impactor\(^12\) was used to induce a moderate contusion injury (12.5 g/cm) in the exposed spinal cord. This device allowed us to record the compression distance, compression velocity, and duration of each contusion. These data were used to determine which animals should be rejected from analysis and to establish that intergroup injury magnitudes were similar. Animals in which there was a compression distance greater than 2 SDs from the mean for their group were excluded from analysis.

Cooling Technique

Thirty minutes postinjury, 0.9% ice-chilled saline solution was infused into the epidural space by using an infusion pump (model 55-2222; Harvard Apparatus, South Natick, MA). The cooling setup is shown in Fig. 1. The temperature of a 50-ml syringe was maintained near 4°C by application of ice. The distal end of an Intramedic polyethylene PE-10 catheter (Becton Dickinson, Parsippany, NJ) was located at the base of the T-12 spinal process. Chilled fluid flowed from T-11 to T-9 where an identical catheter was placed and connected to a suction device. The suction was adjusted so that the dura was constantly immersed in fluid. The epidural temperatures were controlled by varying the infusion rates to 18.7 ± 8.3 (Group I), 6.7 ± 3.8 (Group II), and 1.7 ± 0.6 ml/hour (Group III). The rewarming period was achieved by gradually reducing the infusion rate to zero during a 45-minute period. This setup models an epidural perfusion technique that could be readily applied in patients,\(^13\) conceivably without a need for laminectomy. Subdural placement of catheters was not used because subdural placement of catheters can cause additional mechanical trauma to the spinal cord (unpublished data).

Physiological Data Recording

The animal was kept on a homeothermic blanket during the experimental procedure. The blanket’s control unit (model NP 50-7061-R; Harvard Apparatus) was connected to a rectal temperature probe and set to maintain the core temperature at 37°C. The distal end of a minihypodermic thermocouple 0.2-mm-diameter probe (model HYP-O2; Omega, Stamford, CT) was located between the base of the T-9 spinal process and the posterior surface of the dura to record the local epidural temperature at the injury site (Fig. 1B). Epidural and core temperatures as well as O2 saturation and heart
rate were monitored continuously and recorded every 30 minutes. In three animals a second probe was placed into the T-10 parenchyma to a depth of 1.5 mm to study the relative changes in epidural and intraspinal temperatures while infusion rates were varied. Because intraspinal temperature recording is invasive and causes additional SCI we used only epidural temperature recording in the animals studied for locomotor and histopathological outcomes.

**Behavioral Assessment**

Open-field locomotor function was evaluated once a week for 6 weeks by two trained observers who used the Ba-Be-Br locomotor rating scale, and the mean of their scores was recorded. The animals were pretrained in the open-field environment before surgery was performed. The evaluators were blinded as to which treatment group each coded rat had been assigned. Digital video was recorded during the Ba-Be-Br sessions and an FBFVA performed in 28 of the animals, representative of all the groups in the study. A tripod-mounted Sony DCR-PC100 digital camera recorded video at 30 frames per second. For FBFVA, 30-second videoclips were saved as uncompressed files. Sequential frames were displayed at 720 x 480 pixels by using video editing software. The FBFVA allowed us to determine the sequence of limb movements. Each limb was assigned a letter (A, B, C, or D), and a score sheet was used to record each successive limb movement. Every four steps by the animal were defined as a complete stepping cycle, and this cycle was evaluated for coordination. The fulfillment of two criteria were

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**II. SUMMARY OF CONTUSION PARAMETERS**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Rats</th>
<th>Treatment Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>compression (mm)</td>
<td>1.62 ± 0.16</td>
<td>1.60 ± 0.30</td>
</tr>
<tr>
<td>height (mm)</td>
<td>11.96 ± 0.44</td>
<td>12.26 ± 0.44</td>
</tr>
<tr>
<td>velocity (m/second)</td>
<td>0.46 ± 0.02</td>
<td>0.46 ± 0.02</td>
</tr>
<tr>
<td>time (msec)</td>
<td>46.47 ± 2.86</td>
<td>48.62 ± 5.12</td>
</tr>
</tbody>
</table>

* Values are presented as means ± SDs.

**III. COMPARISON OF RECTAL AND EPIDURAL TEMPERATURES**

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Treatment Group (˚C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>rectal</td>
<td>36.07 ± 0.79</td>
</tr>
<tr>
<td>epidural</td>
<td>24.05 ± 2.26</td>
</tr>
</tbody>
</table>

* Values are presented as means ± SDs.
Epidural hypothermic neuroprotection after SCI in rats

**Histopathological Assessment**

Forty-five days after SCI, rats were deeply anesthetized using a rat cocktail (ketamine 43 mg/ml, xylazine 8.6 mg/ml, and acepromazine 1.4 mg/ml) at 1.5 ml/kg and perfused transcardially with heparinized isotonic saline, and then by 4% paraformaldehyde (0.2 M phosphate buffered, pH 7.4). Each spinal cord was assigned a code that was kept by one of the authors (G.R). None of the other authors knew which experimental group the coded spinal cord tissue belonged to until the analysis was completed. Following perfusion, the spinal cords were removed and postfixed for 48 hours, after which they were placed in PBS (0.1 M) for 2 additional days. The transverse site was localized under a dissecting microscope and two 15-mm-long blocks of tissue were selected rostral and caudal to this point. The blocks were embedded in paraffin and axially sectioned every 10 μm by using a rotating microtome. Sequential sections were placed onto gelatin-coated slides. Sections were stained with H & E and Luxol–fast blue. A Neurolucida image analysis system (version 5.04; MicroBrightField, Inc., Williston, VT) was used to trace the spinal cord perimeter and the cavity contours at 200-μm intervals throughout the 30 mm of tissue to reconstruct spinal cord and cavity volumes (Fig. 2).

**Spared Tissue Volume Calculation.** To avoid the confounding effects of the variable distortion of the injured spinal cord between animals, we decided to use cord/cavity ratios. Three-dimensional serial reconstructions and volumetric calculations were computed in Neurolucida/Neuroexplorer. This program permits summation of spinal cord contour and cavity areas to calculate the total spinal cord (Vol\textsubscript{total}) and lesion volumes (Vol\textsubscript{lesion}) within the injury region. The percentage of STV was calculated using the following formula, \( \text{STV} (\%) = \frac{\text{Vol}_{\text{total}} - \text{Vol}_{\text{lesion}}}{\text{Vol}_{\text{total}}} \times 100 \). The epicenter was considered to be the section with the smallest cord/cavity ratio. Based on the localization of the epicenter the rostral and caudal volumes were also derived and compared.

**Statistical Analysis**

To test the hypothesis that hypothermic saline perfusion resulted in improved locomotor recovery among the four groups, the Ba-Be-Br Scale scores during the 45-day survival period were compared using one-way ANOVA with Tukey post hoc tests. To test the hypothesis that FBFVA improved the accuracy of the Ba-Be-Br assessment tool we hypothesized that FBFVA would result in less variation in the Ba-Be-Br Scale scores. Weekly scores per treatment group were averaged and SDs calculated for both real-time (conventional) Ba-Be-Br and FBFVA Ba-Be-Br. The F statistic was calculated to test for equivalence of the SDs. To test the hypothesis that hypothermic perfusion resulted in tissue sparing, the area of white matter spared in the epicenter, and the mean values of the total, rostral, and caudal spared tissue volumes were also compared using ANOVA. The rostral and caudal extent of loss of gray matter was compared among the four groups by using ANOVA. Linear regression analyses were used to test for a correlation between the spared

<table>
<thead>
<tr>
<th>Score</th>
<th>Ba-Be-Br Scale</th>
<th>FBFVA Ba-Be-Br Scale</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td>occasional weight-supported plantar steps, no FL–HL coordination</td>
<td>occasional weight-supported plantar steps, 0% FL–HL coordination</td>
</tr>
<tr>
<td>11</td>
<td>occasional weight-supported plantar steps; occasional FL–HL coordination</td>
<td>occasional weight-supported plantar steps, 0–25% FL–HL coordination</td>
</tr>
<tr>
<td>12</td>
<td>frequent-to-consistent weight-supported plantar steps &amp; occasional FL–HL coordination</td>
<td>frequent-to-consistent weight-supported plantar steps &amp; 25–50% FL–HL coordination</td>
</tr>
<tr>
<td>13</td>
<td>frequent-to-consistent weight-supported plantar steps &amp; frequent FL–HL coordination</td>
<td>frequent-to-consistent weight-supported plantar steps &amp; 50–75% FL–HL coordination</td>
</tr>
<tr>
<td>14</td>
<td>consistent weight-supported plantar steps; consistent FL–HL coordination; predominant paw position rotated when it makes initial contact w/ the surface &amp; just before it is lifted off at end of stance or consistent FL–HL coordination &amp; occasional dorsal stepping</td>
<td>consistent weight-supported plantar steps; consistent FL–HL coordination; predominant paw position is parallel to the body at initial contact &amp; rotated at liftoff</td>
</tr>
<tr>
<td>15</td>
<td>consistent plantar stepping &amp; consistent FL–HL coordination; &amp; no toe clearance or occasional toe clearance during forward limb advancement; predominant paw position is parallel to the body at initial contact</td>
<td>consistent weight-supported plantar steps; consistent FL–HL coordination, predominant paw position is parallel at initial contact &amp; liftoff</td>
</tr>
<tr>
<td>16</td>
<td>consistent plantar stepping &amp; consistent FL–HL coordination during gait; &amp; toe clearance occurs frequently during forward limb advancement; predominant paw position is parallel at initial contact &amp; rotated at liftoff</td>
<td>consistent weight-supported plantar steps; consistent FL–HL coordination, predominant paw position is parallel at initial contact &amp; liftoff</td>
</tr>
<tr>
<td>17</td>
<td>consistent plantar stepping &amp; consistent FL–HL coordination during gait; &amp; toe clearance occurs frequently during forward limb advancement; predominant paw position is parallel at initial contact &amp; liftoff</td>
<td>consistent weight-supported plantar steps; consistent FL–HL coordination, predominant paw position is parallel at initial contact &amp; liftoff</td>
</tr>
<tr>
<td>18</td>
<td>consistent plantar stepping &amp; consistent FL–HL coordination during gait; &amp; toe clearance occurs consistently during forward limb advancement, predominant paw position is parallel at initial contact &amp; rotated at liftoff</td>
<td>consistent weight-supported plantar steps; coordinat ed gait, predominant paw position is parallel throughout stance, consistent trunk stability, tail consistently up</td>
</tr>
</tbody>
</table>

* FL = forelimb; HL = hindlimb.

\[ * \text{FL} = \text{forelimb}; \text{HL} = \text{hindlimb}. \]
tissue area at the epicenter and the spared tissue volume across the entire region of a detectable lesion cavity. Statistical significance was set at a probability value less than 0.05. Results are presented as the means ± SDs.

Results

Eighty animals were used in the study, of which the first 15 were used to verify the reliability of our NYU impactor device. Six animals were used in the pilot study to determine the infusion rates needed to attain target temperatures and to assess the impact of local cooling on physiological variables. Fifty-six animals underwent randomization and contusive SCI. Of these, two animals in Group III were rejected because their contusion parameters were greater than two SDs from the mean (abnormally high compression distances of 2.29 and 2.18 mm, respectively). Seven animals died prematurely and were not included in the analysis.

Validation of the NYU Device

Our device produced consistent injuries similar to those published by other investigators (Fig. 3).

Contusion Parameters

We compared the intra- and intergroup contusion-related variables to exclude significant differences as confounding variables. As shown in Fig. 1 (section II), there were no significant differences in contusion parameters between the four groups.

Epidural and Rectal Temperatures

Rectal temperature recording was used to monitor the core temperature. The overall mean rectal temperature during the 3-hour cooling period was 36.3 ± 0.8°C. No significant intergroup differences in rectal temperatures were found among injured and control rats. The mean epidural temperatures achieved during the cooling phase were 24 ± 2.3°C in Group I (16 rats), 30 ± 2.4°C in Group II (13 rats), and 35 ± 0.9°C in Group III (13 rats) (Fig. 1 [III]). We were unable to obtain consistent epidural temperatures below 22°C using this technique. There was a nonlinear relationship between the rate of chilled fluid infusion and epidural temperature (Fig. 4 upper). Blood pressure, PO2, PCO2, and pH were not affected by epidural perfusion cooling in the six animals in which it was assessed. Epidural and rectal temperatures were similar in rats that received contusion but not epidural fluid perfusion.

Epidural/Intraspinal Temperature Gradients. As the infusion rate was increased, the difference between the epidural and intraspinal temperatures increased with the spinal cord becoming colder than the epidural space (Fig. 4 lower). No significant hydrostatic pressures were created by the perfusion.

Behavioral Testing

The results of hindlimb locomotor assessment using the standard Ba-Be-Br protocol are shown in Fig. 5 upper.
The scores were similar in all groups (14 ± 4.2 [control], 14 ± 1.7 [Group I], 16 ± 3.3 [Group II], 15 ± 2.5 [Group III]) (p = 0.77). The FBFVA Ba-Be-Br scores were lower than the real-time scores because 100% forelimb–hindlimb coordination was rarely observed using the more detailed method of analysis (Fig. 5 lower). In comparing SDs of real-time Ba-Be-Br and FBFVA Ba-Be-Br scores, we found that in 16 of 22 events the SDs of the real-time scores exceeded those of FBFVA Ba-Be-Br scores. Testing for equivalence of the SDs using the F statistic demonstrated that six of 16 instances had significant differences (p < 0.05). Conversely, Ba-Be-Br real-time SDs were less than those of FBFVA SDs in five of 22 events with no statistically significant F value. In two instances the SDs were equivalent. This means that FBFVA reduces variation in the assigned Ba-Be-Br scores, presumably because of enhanced accuracy.

Histopathological Assessment

Figure 6 shows representative epicenter sections from the experimental groups. In some of the sections, necrotic tissue strands were evident within the cavity and this tissue was counted as part of the lesion. A polymorphonuclear inflammatory cell reaction and macrophages were found adjacent to the cavity in almost all of the epicenter sections, but their quantification was not an end point of this study. Qualitatively, no obvious differences were seen between controls and perfusion-cooled animals. An example of a section with three intact neurons is shown in Fig. 7 to illustrate how we made this determination. The preserved tissue area at the epicenter and the total, rostral, and caudal STVs showed nonsignificant differences when comparing the control and treated groups 45 days postinjury (Fig. 8A–D). The longitudinal extent of gray matter loss (Fig. 8E) was 1400 ± 459 μm (in control), 2033 ± 497 μm (in Group I), 1740 ± 458 μm (in Group II), and 1880 ± 179 μm (in Group III rats). The rostral–caudal extent of neuronal loss indicated that gray matter preservation was reduced in the perfusion-cooled animal groups.
compared with controls (p = 0.05). The mean rostral–caudal loss of gray matter following a 12.5-g/cm lesion at 45 days in this study was 1800 ± 478 μm. Therefore, a 30-mm block is adequate to characterize the entire cavity if centered on the epicenter. In all cases the first neurons to be observed within preserved gray matter, rostral or caudal to the lesion were motor neurons. Sections further rostral or distal contained larger numbers of preserved neurons. The relative preservation of gray matter in the control group did not correlate with an improved Ba-BeBr score. Linear regression analysis showed a high correlation between the spared tissue area at the epicenter and the STV over the entire extent of the lesion cavity (r = 0.83, p < 0.0001).

**Discussion**

*Failure of Epidural Perfusion Cooling to Provide Neuroprotection*

Pathomechanisms sensitive to temperature alterations...
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following ischemic and traumatic injury include glutamate release, stabilization of the blood–brain barrier, O2 radical production, nitric oxide metabolism, protein synthesis, energy metabolism, integrity of membrane function, inflammation, activation of intracellular protein kinases, cytoskeletal breakdown, apoptotic cell death, and gene expression. The neuroprotective effects of hypothermia are linked to the magnitude and duration of the insult, the level of hypothermic temperature, the timing of its application, and rewarming.

Despite evidence that hypothermia can reduce the severity of several pathomechanisms after central nervous system trauma or ischemia, we found no beneficial effect. This result contrasts with a beneficial result reported by other investigators who used a similar injury magnitude but conducted systemic hypothermia. The injury parameters between the two studies are almost identical. Our findings also differ from those of Albin, et al., who used cooler temperatures in primates than we could achieve with epidural perfusion in rats. Our findings are consistent with those of Dimar, et al., who, however, used a more severe injury magnitude and a less rigorous histological analysis.

To understand these discrepant results, the following issues should be considered. 1) Local spinal cord perfusion cooling may cause reduced SCBF exacerbating ischemia, whereas systemic hypothermia may not have this effect. 2) Local and systemic hypothermia may affect different pathomechanisms. One effect that may differ between local and systemic cooling is the impact on inflammation, specifically entry of polymorphonuclear leukocytes into the injured spinal cord. Polymorphonuclear leukocytes propagate secondary injury, and their ingress to the injured spinal cord has been reported to be reduced when systemic hypothermia is delivered after SCI. Because the polymorphonuclear leukocytes are constantly rewarmed as they circulate under conditions of local hypothermia, they may still contribute to secondary injury.

Value of FBFVA of Open-Field Locomotion

Our observed Ba-Be-Br scores after the 12.5-g/cm injury are generally similar to those reported by other investigators; however, analysis of FBFV A Ba-Be-Br score results consistently showed reduction in the assigned Ba-Be-Br score. We think this is due to more accurate assessment of forelimb–hindlimb coordination and also of events such as paw rotation that we find difficult to assess in real time. This analysis is particularly valuable in assessing the 12.5-g/cm injury where recovery to varying extents of forelimb–hindlimb coordination is frequently observed.

Increase in Epidural/Intraspinal Temperature Gradient With Increased Rates of Epidural Perfusion

Because cooling reduces the metabolic demand of neural tissue, it seems reasonable that cooling the spinal cord might improve the survival of injured spinal cord tissue; however, this was not observed. In fact, epidural perfusion cooling reduced gray matter survival. In attempting to understand this finding we observed that the epidural/intraspinal temperature gradient increased as the rate of epidural saline perfusion was increased. This observation has several implications. First, it indicates that we cannot assume that intramedullary temperatures will parallel epidural temperatures. Second, one interpretation of this data is that SCBF is decreased by escalating rates of perfusion epidural cooling. We hypothesize that heat is being lost from the spinal cord to the perfusate and that there is inadequate flow of warm blood to maintain tissue temperature. This is the only paradigm that allows us to understand how the spinal cord could become colder than the epidural fluid. This hypothesis is consistent with the decreased survival of gray matter in the epidurally perfused animals. In other studies of local cooling investigators have shown variations in SCBF, with some reporting a decrease and others reporting an increase or no change. Systemic hypothermia (27–28°C), however, has been reported to increase SCBF in phenobarbital-anesthetized rats. The potential to achieve hypothermic neuroprotection may be compromised if cooling leads to a
reduction in SCBF because SCBF is already reduced after SCI.19,20,33,58 Perhaps direct contact of the cool fluid with radicular vessels crossing the epidural space induces vasocostriction.22,62 This underexplored variable may serve to explain some of the inconsistencies in the literature regarding the effects of local cooling on post-SCI outcome. That reduced gray matter neuroprotection was not correlated with Ba-Be-Br score–based outcome indicates the insensitivity of the Ba-Be-Br scoring technique to study thoracic gray matter preservation. The lack of correlation between lesion volumes and gray matter loss may reflect the fact that we set the criteria at three detectable neurons. This quantity of gray matter would represent a very small fraction of the tissue volume.

Conclusions

Regional epidural perfusion cooling applied 30 minutes after a moderate contusive SCI was ineffective at increasing white matter tissue sparing and caused reduced gray matter neuronal preservation. No significant differences were observed in locomotor recovery over the 6-week time course of the study. Regarding open-field locomotor analysis following a moderate contusive SCI, we showed that FBFVA allows a more detailed examination of complex events, such as forelimb–hindlimb coordination, that are difficult to assess using the conventional Ba-Be-Br Scale. Whereas local perfusion cooling has been reported to effectively improve ischemic tolerance of the spinal cord, it appears to be detrimental under the conditions we assessed. Additional studies are required to test the hypothesis that epidural perfusion cooling reduces SCBF. The findings in this investigation validate the reliability of the experimental methods and establish valuable baseline data for subsequent studies of neuroprotection. The data also reinforce the principle that treatments that effectively ameliorate ischemic SCI cannot be assumed to have similar efficacy after contusive spinal cord trauma. We recommend further studies before epidural perfusion cooling is considered for clinical treatment of SCI.

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Manuscript received April 20, 2004. Accepted in final form October 22, 2004.