The Effects of Local and General Hypothermia on Temperature Profiles of the Central Nervous System Following Spinal Cord Injury in Rats

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Local and general hypothermia are used to treat spinal cord injury (SCI), as well as other neurological traumas. While hypothermia is known to provide significant therapeutic benefits due to its neuroprotective nature, it is unclear how the treatment may affect healthy tissues or whether it may cause undesired temperature changes in areas of the body that are not the targets of treatment. We performed 2-hour moderate general hypothermia (32°C core) or local hypothermia (30°C spinal cord) on rats that had received either a moderate contusive SCI or laminectomy (control) while monitoring temperatures at three sites: the core, spinal cord, and cortex. First, we identified that injured rats that received general hypothermia exhibited larger temperature drops at the spinal cord (−3.65°C, 95% confidence intervals [CIs] −3.72, −3.58) and cortex (−3.64°C, CIs −3.73, −3.55) than uninjured rats (spinal cord: −3.17°C, CIs −3.24, −3.10; cortex: −3.26°C, CIs −3.34, −3.17). This was found due to elevated baseline temperatures in the injured group, which could be due to inflammation. Second, both general hypothermia and local hypothermia caused a significant reduction in the cortical temperature (−3.64°C and −1.18°C, respectively), although local hypothermia caused a significantly lower drop in cortical temperature than general hypothermia ($p < 0.001$). Lastly, the rates of rewarming of the cord were not significantly different among the methods or injury groups that were tested; the mean rate of rewarming was $0.13 \pm 0.1^\circ\text{C}/\text{min}$. In conclusion, local hypothermia may be more suitable for longer durations of hypothermia treatment for SCI to reduce temperature changes in healthy tissues, including the cortex.

Introduction

HYPOTHERMIA FOR TREATMENT of patients with spinal cord injury (SCI) shows great promise as an acute neuroprotective therapy (Dietrich *et al.*, 2009, 2011; Dietrich, 2012; Maybhatte *et al.*, 2012; Silasi *et al.*, 2012). In addition to SCI, hypothermia has been shown to lead to functional improvements in many other injury types, including cardiac arrest (Arrich, 2007; Madhok *et al.*, 2010; Beddingfield and Clark, 2012), stroke (Groysman *et al.*, 2011; Chang and Marshall, 2012; Lakhan and Pamplona, 2012), ischemia-reperfusion injury (Kawamura *et al.*, 2006), traumatic brain injury (Tomura *et al.*, 2012), and coma (Bouwes *et al.*, 2012). Hypothermia works principally by lowering the overall metabolism of the body. For every 1°C reduction in body temperature, there is 6% reduction in metabolism as well as reduced inflammation (Reuler, 1978). Decreased metabolism

brings about lower consumption of oxygen and glucose and reduced excitotoxic neurotransmitter release. These physiological changes lessen the risk of energy failure. On a cellular level, maintaining stores of energy helps to prevent the failure of sodium pumps and calcium influx, and the end result is prevention of cell death (Choi *et al.*, 2012). For example, it has been shown that as little as a 1°C reduction in body temperature is enough to reduce gray and white matter damage during spinal cord ischemia (Saito *et al.*, 2012).

In the clinic, core temperature is reduced, using either cold-water-circulating pads or intravascular cooling (Levi *et al.*, 2010), inducing temperature changes ranging from 31°C to 34°C $\pm$ 1°C (88°F to 93°F $\pm$ 2°F) throughout the body. In the largest study to-date of cervical SCI patients, a striking 43% of patients who were treated with moderate hypothermia (33°C) showed improvement by at least one grade at follow-up, according to the International Standards

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for Neurological Classification of Spinal Cord Injury (ISNCSCI), which includes both sensory and motor evaluations (Dididze *et al.*, 2012). Therefore, it is highly desirable to undertake studies that further reduce the temperature of the spinal cord after injury (to $<33^{\circ}\text{C}$) in order to achieve the maximum therapeutic benefit. However, in doing so, the therapy must be administered safely to avoid complications.

We previously showed that 2-hour general hypothermia in rats with moderate contusive SCI leads not only to improved motor behavior and increased somatosensory evoked potential (SSEP) amplitude, but also to increased N1-latency of SSEP, for up to 4 weeks after injury (Maybath *et al.*, 2012). Because SSEPs are the response at the primary somatosensory cortex to stimulus of the periphery, a key question is how cortical temperature is affected during the period of hypothermia administration. Importantly, no study has been performed to measure the temperature differences of the cortex under local or general hypothermia. Nor has the effect that injury itself has on core and cortical temperature during hypothermia treatment been reported. Indeed, as patient shock is a primary response of injury, the SCI alone could affect how the body's temperature is regulated. Likewise, during application of general hypothermia, aberrant cooling of the brain, including the hypothalamus, which regulates temperature, may be undesired.

Thus, local hypothermia is attractive for its potential to selectively cool precise regions of the spinal cord while avoiding drastic temperature changes at the brain. In this work, we compare two methods of hypothermia, local and general, for the treatment of moderate contusive SCI. We sought to look at how three sites (core, spinal cord, and cortex) are cooled by comparing the two methods of hypothermia. Such information is critical for evaluating the safety and efficacy of hypothermia treatment, especially if it is to be administered for a longer period of time.

The mean temperature differences achieved at the core, spinal cord, and cortex were calculated for each group. We sought to answer three questions to better understand how modulation of central nervous system (CNS) temperatures differs depending on the injury state and treatment method.

- (1) Do cortical and spinal cord temperatures during hypothermia significantly differ between rats that have undergone only laminectomy (control) and rats subjected to moderate contusion SCI?
- (2) Do local and general hypothermia evoke significantly different temperatures at the spinal cord and cortex during hypothermia treatment?
- (3) Does the rate of rewarming of the spinal cord differ based on the method of hypothermia induction or injury state?

These questions allowed us to determine whether there are changes in cortical and/or spinal cord temperatures that depend on the injury and to identify how each treatment may affect the temperature, not only at site of SCI, but also on the cortex. This is the first study to report the temperature profiles of the core, spinal cord, and cortex during 2-hour general or local hypothermia in rats following SCI.

Methods

All procedures were performed in accordance with protocols approved by the Institutional Animal Care and Use

Committee of the Johns Hopkins University. A total of 20 adult female Lewis rats (200–230 g, Charles River Laboratories, Germantown, MD) were used in this study. Five rats were randomly assigned into one of following four groups: (1) contusion injury followed by general hypothermia, (2) laminectomy followed by general hypothermia, (3) contusion injury followed by local hypothermia, and (4) laminectomy followed by local hypothermia. Rats were housed in individual cages and provided with free access to food and water for the experimental period.

Anesthesia

Anesthesia used for all surgical procedures included a mixture of 30.4 mg/kg ketamine, 4.3 mg/kg xylazine, and 0.9 mg/kg acepromazine maleate administered through intraperitoneal injection (0.12 mL). An adequate level of anesthesia was determined by monitoring the corneal reflex and limb withdrawal to pinch stimuli.

Laminectomy and contusion injury

Our SCI model has been previously described (Agrawal *et al.*, 2008, 2009, 2010a, 2010b; Al-Nashash *et al.*, 2009; All *et al.*, 2009, 2010, 2012; Iyer *et al.*, 2010; Kerr *et al.*, 2010; Bazley *et al.*, 2011, 2012a, 2012b). A laminectomy was performed by removing the T8 thoracic vertebra to expose the dorsal surface of the spinal cord without opening the dura mater. For the injury groups, the vertebrae at T6 and T10 were secured in stabilization clamps to reduce motion of the spinal column during impact of injury. An MASCIS impactor was used to induce contusion injury. The height of the impactor rod (weight = 10 g) was precalibrated to 12.5 mm. The probe was then released to hit the dorsal exposed surface of the T8 spinal cord and induce moderate contusion injury. The impact trajectory, distance, velocity, and time were recorded to make sure consistency among all rodents. Animals for which the error in any of these parameters exceeded 5% were excluded from the study. This is a well-established model for contusion SCI and damage to the rat CNS caused at this height is comparable to moderate injury in patients.

Temperature probe implantation

A rectal probe of 5-cm long and 2 mm in diameter (RET-3; PhysiTemp, Clifton, NJ) was used to monitor core temperature. The accuracy with which rectal temperature parallels core temperature measured from thermistor probes implanted chronically into the chest cavity has been previously established (Poole and Stephenson, 1977). Two microprobe thermocouples (IT-24P Flexible Microprobes; PhysiTemp) were inserted, one in the T8 region of the spinal cord and another at the somatosensory area of the cortex.

To insert the probe at the cortex, under general anesthesia the head region was shaved and local anesthetic of 2% lidocaine HCl was injected under the skin. Next, a midline incision was made and the cranium bone was cleaned by removing the tissue underneath the skin. A dental drill was used to drill one small hole into the cranium. This hole was located on the somatosensory cortex corresponding to the left hindlimb region, which is located 2.5 mm posterior of the bregma and 2.8 mm laterally from the bregma. The temperature probe was inserted through the dura mater without causing compression

to the brain tissue and dental cement was used to cover the small opening in skull. The second probe was securely placed on the spinal cord at the epicenter of injury in the T8 region. The paravertebral muscle and skin were then sutured closed.

Induction of general hypothermia

A group of randomly assigned rats underwent general hypothermia following the contusion injury or laminectomy. Figure 1 outlines the five phases and the target temperatures

associated with each treatment group. Phase 1: *Prehypothermia* refers to the 1-hour period prior to cooling. Phase 2: *Induction* refers to the 30-minute hypothermia cooling phase. Phase 3: *Maintenance* refers to the following 2 hours during which hypothermia was maintained at the target temperature at either the spinal cord (local) or rectum (general). Phase 4: *Rewarming* refers to the 30-minute period during which the cooled body is rewarmed to the prehypothermia temperature. Phase 5: *Posthypothermia* refers to the 1-hour observation period following rewarming.

The phases of general hypothermia are shown in Figure 1A. The core temperature was maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ with the help of a heating pad for 1 hour after the time of contusion or laminectomy and prior to inducing hypothermia. After 1 hour, general hypothermia was induced by spraying the rat with alcohol mist (70% ethanol) and using an electric fan until the core temperature dropped to $32^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, as previously described (Shin *et al.*, 2006; Jia *et al.*, 2008; Maybhate *et al.*, 2012). The induction period lasted 25 to 30 minutes. The temperature was then maintained at $32^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 2 hours. The temperature 32°C was used for general hypothermia because it is within the acceptable range of clinical applications. After 2-hour hypothermia, rats were rewarmed to $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ using the heat pad. The rewarming period lasted 25 to 30 minutes. Rewarming was followed by a 1-hour observation period.

Induction of local hypothermia

A group of randomly assigned rats underwent local hypothermia following moderate contusion injury or laminectomy. For local hypothermia, a heat exchanger was constructed from copper tubing, bent into four equal-length layers, 4.4 inches length and 0.8 inches in width. The heat exchanger was inserted over the paravertebral muscles in the dorsal region of the animal by a subcutaneous tunnel extending from T6 to T10 spinal segments. The small skin incision over the copper tubing was closed. Figure 2 outlines the placement of temperature probes and schematic of the cooling method.

The phases of local hypothermia are shown in Figure 1B. The core temperature was maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ with the help of a heating pad for 1 hour after the time of the contusion or laminectomy and prior to inducing the hypothermia. After 1 hour, local hypothermia was begun. To induce spinal cord hypothermia, cold water at $17^{\circ}\text{C} \pm 2.0^{\circ}\text{C}$ was perfused through the heat exchanger at a rate of 129 mL/min using a peristaltic pump. The cooling period lasted 25 to 30 minutes. The temperature of the T8 region of the spinal cord was maintained at $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ for 2 hours while the core temperature was maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Following the 2 hours of spinal cord hypothermia, the heat exchanger was removed from the paravertebral muscle and the skin incision was closed permanently. The rewarming period lasted 25 to 30 minutes. Rewarming was followed by a 1-hour observation period. The temperature of the T8 region of the spinal cord and the cortex was recorded once every two minutes throughout the procedure.

Statistical analysis

The baseline period was defined as the average of Phases 1 and 5 because the core temperature and spinal cord temperatures were controlled during these periods. Paired *t*-tests

FIG. 1. The five phases of hypothermia treatment and the corresponding target temperatures for general (A) and local (B) hypothermia. During general hypothermia, the rectal temperature was fixed at 32°C while the cortex and spinal cord temperatures were unconstrained. During local hypothermia, both the spinal cord and rectal temperatures were fixed at 30°C and 37°C , respectively, while the cortical temperature was unconstrained.

FIG. 2. Schematic of the experimental setup using the copper heat exchanger for local hypothermia. (a) The M-copper tubing unit was inserted under the skin over the paravertebral muscle extending from T6 to T10 spinal segments. (b) Inflow and outflow allowed for circulation of cold water. Temperature probes were inserted in the rectum, at the epicenter of injury at the spinal cord, and at the somatosensory cortex.

revealed no statistical significance between Phases 1 and 5 for all experimental groups (data not shown). Therefore, these phases were combined and considered the baseline period for determining the temperature change at Phase 3.

Differences in means among groups for each phase were analyzed using a mixed-effect model analysis. This type of analysis takes into account both the fixed effects that result from differences attributed to the various treatments as well as any random effects that occur which are animal dependent. The fixed effects include differences due to the injury state (contusion or laminectomy) and the treatment status (global or local hypothermia). This particular method was used due to the multiple factors involved and the panel data measurements provided for each animal across each phase. Estimates of the difference in means among the temperature measurements were calculated with 95% confidence intervals (CIs).

All analyses were performed using STATA v 11.2 (2011; STATA Corporation, College Station, TX) and MATLAB 2011b (MathWorks, Inc., Natick, MA).

Results

Here we report the changes in temperature profiles of the core, spinal cord, and cortex following local and general hypothermia in rats with and without SCI. The raw data from these experiments showing the measured temperatures of the three sites for each group are shown in Supplementary Figures S1 and S2 (Supplementary Data are available online at www.liebertpub.com/ther). In addition, Supplementary Table S1 reports the mean temperatures and standard deviations at each phase of treatment. During both general and local hypothermia, the spinal cord during Phase 3 was decreased to approximately the same temperature, $30.3^{\circ}\text{C} \pm 0.8^{\circ}\text{C}$ and $30.2^{\circ}\text{C} \pm 0.3^{\circ}\text{C}$, respectively. This illustrates the reliability of both methods for inducing precise cooling of

spinal cord. During general hypothermia, the core temperature of rats was successfully reduced to $32.1^{\circ}\text{C} \pm 0.4^{\circ}\text{C}$. During local hypothermia, core temperature was successfully maintained at $37.3^{\circ}\text{C} \pm 0.7^{\circ}\text{C}$. Importantly, these data show that by using these methods, the core and spinal cord temperatures accurately recapitulated the target temperatures that were set within the experimental design.

General hypothermia resulted in larger temperature changes in injured versus uninjured group

Our first question sought to answer whether the cortical and spinal cord temperatures that change during hypothermia differ between rats receiving a laminectomy (uninjured) and rats receiving a moderate contusion SCI (injury). We considered the cases of general hypothermia and local hypothermia separately for this analysis. First, the general hypothermia injured group was compared with laminectomy (control) to determine whether the effect of general hypothermia differs on injured versus uninjured subjects. During general hypothermia, core temperature was reduced to $32^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$, which induced a constant spinal cord temperature of $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Figure 3A shows that there were significant differences in the Phase 3 temperature decrease between injured and laminectomy groups. This difference was observed in both the spinal cord and cortex ($p < 0.001$). At the spinal cord, the temperature of the injured group decreased by -3.65°C compared with the laminectomy group, which decreased by -3.17°C ($p < 0.001$). Therefore, the spinal cord was cooled by an additional 0.48°C for injured group compared with the laminectomy group. Similarly, cortical temperature decreased by -3.64°C in the injured group but only -3.26°C in the laminectomy group ($p < 0.001$). Therefore, the cortex was cooled by an average of 0.38°C more for the injured group compared with the laminectomy group.

FIG. 3. Comparison of the changes in temperature between injury (light gray) and laminectomy (dark gray) groups. **(A)** During general hypothermia, both spinal cord and cortex have a larger temperature drop compared with laminectomy. **(B)** During local hypothermia, spinal cord temperature change is constant between groups while the laminectomy group exhibited a larger change at the cortex. The bars indicate observed mean temperatures and the error bars represent the 95% confidence intervals (CIs). * $p < 0.001$ for an unpaired t -test between injury and laminectomy groups.

Local hypothermia resulted in smaller reduction in cortical temperature in injured versus uninjured group

Next, we analyzed the temperature reductions during local hypothermia (Phase 3) in the injured group compared with laminectomy (control). During local hypothermia, core temperature was maintained at $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ while the spinal cord was maintained at $30^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$. Figure 3B shows that the reduction in temperature at the spinal cord during local hypothermia was not significantly different between the injured group and laminectomy group ($p = 0.09$). However, at the cortex, there was a measured significant difference between the injured and laminectomy groups. Cortical temperature was reduced by -1.18°C in the injured group and -2.02°C in the laminectomy group ($p < 0.001$). Therefore, the cortical temperature in the injured group is less affected by local spinal cooling, resulting in the reduced temperature difference shown in Figure 3B. This effect is not observed during the general hypothermia condition most likely because the entire body is cooled, including the animal's head.

Local hypothermia induced a smaller change in cortical temperature compared with general hypothermia

The next question we sought to answer was whether local and general hypothermia induce significantly different temperatures at the spinal cord and cortex during Phase 3. Figure 4A shows the change in temperatures at the spinal cord and cortex, comparing general and local hypothermia in the injured group. During general hypothermia, the cortical temperature of the injured group was reduced by -3.64°C , whereas during local hypothermia the cortical temperature is only reduced by -1.18°C . Therefore, the cortex was cooled an average of 2.46°C more during general hypothermia compared with local hypothermia ($p < 0.001$). Such a large reduction in cortical temperature that was exhibited during general hypothermia could have undesired effects on recovery postinjury. Figure 4B shows that the same result was found comparing local and general hypothermia in the groups receiving a laminectomy ($p < 0.001$).

General hypothermia induces equal temperature changes in the cortex and spinal cord

Finally, we sought to evaluate the difference in temperatures between the cortex and spinal cord *within* the same

FIG. 4. Comparison of the changes in temperature between general (light gray) and local (dark gray) hypothermia treatments. Results are shown for injury **(A)** and laminectomy **(B)** groups. Local hypothermia induced a larger temperature drop at the spinal cord, while general hypothermia induced a larger temperature drop at the cortex. The bars indicate observed mean temperatures and the error bars represent the 95% CIs. * $p < 0.001$ for an unpaired t -test between general and local hypothermia.

group. Considering injured groups, Figure 5A shows that the spinal cord and cortex are cooled by the same degree during general hypothermia, whereas there is a large difference in the extent of cooling between the spinal cord and cortex during local hypothermia. During general hypothermia, the spinal cord and cortex are cooled by nearly equivalent amounts (-3.65°C and -3.64°C , respectively, not significant). In contrast, during local hypothermia, the spinal cord is cooled by -5.34°C while the cortex is only cooled by -1.18°C ($p < 0.001$). Considering laminectomy groups, Figure 5B shows that the comparison of cooling at the spinal cord and cortex is similar. In local hypothermia, the cortex was cooled to a much lesser extent.

Rates of rewarming do not differ among injury status or treatment method

The final question we sought to address was whether the rate of rewarming of the spinal cord differs based on the method of hypothermia induction or injury state. A two-way ANOVA revealed no difference among the four groups

FIG. 5. Comparison of the changes between the spinal cord and cortex. The spinal cord and cortex were reduced by the same amount during general hypothermia, but the spinal cord was reduced significantly more than the cortex during local hypothermia. Results are shown for injury (A) and laminectomy (B) groups. The bars indicate observed mean temperatures and the error bars represent the 95% CIs. $*p < 0.001$ for an unpaired t -test between spinal cord and cortical temperatures.

FIG. 6. Rates of rewarming of the spinal cord. Rates between general and local hypothermia, as well as between injury and laminectomy groups, were compared. No comparisons were found to be significantly different. Error bars represent standard error.

($F = 0.15$). The rewarming rates for each group are shown in Figure 6. No statistically significant differences in rates of rewarming among groups were detected, showing that the method of hypothermia treatment nor injury affects the rewarming ability of the rat.

Discussion

Although the complete mechanism of neuroprotective action of hypothermia is unknown, it is accepted that functional benefits of therapeutic hypothermia following SCI are due to a combination of neuroprotection, reduced inflammation, and a resulting decrease in secondary injury response. SCI induces many pathophysiological changes, and therefore the same hypothermia treatment may have a different effect on injured and healthy subjects. It is important to understand these differences prior to assessing the efficacy of the treatment.

Comparing injured with uninjured rats during general hypothermia, we first found that injured animals exhibited larger temperature drops at the spinal cord and cortex than uninjured rats, due to elevated baseline temperatures. This finding could be due to a number of mechanisms. Contusion SCI results in a cascade of secondary injury mechanisms within the CNS, including excitotoxicity, a breakdown of the blood–spinal cord barrier (BSCB), free radical production, and systemic inflammation (Hausmann, 2003). Secondary injury is not limited to the site of spinal injury; for example, the BSCB breakdown extends far away from the insult along the axis of the spinal cord (Schnell *et al.*, 1999; Bartanusz *et al.*, 2011). In fact, we found rats that received local hypothermia after SCI exhibited an elevated core temperature throughout all phases compared with those receiving a laminectomy. This elevated core temperature could be due to inflammation and increased blood flow as a result of the insult.

Next, we compared the cortical temperature of injured and uninjured groups during local hypothermia, and we found that cortical temperature was also higher in the injured group. This was opposite the case for general hypothermia in which injured animals had a lower cortical temperature than uninjured. This phenomenon may illustrate a greater suppression of the inflammatory response when treated with general

hypothermia. As the cortex was minimally affected by local hypothermia, inflammatory processes due to injury may have allowed the cortex to remain elevated over uninjured controls. Few studies have directly compared local and general hypothermia and their effects on inflammation. A recent study by Ok *et al.* (2012) compared epidural hypothermia (EH) with systemic hypothermia in spinal-cord-injured rats and reported neuroprotective effects of both, but reported greater neuroprotective effects of general hypothermia based on measurements of inflammatory-related cells, including terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-positive cells and activated microglia (Ok *et al.*, 2012). Although this study did not investigate inflammatory responses in the cortex, based on findings here, it would be interesting to determine whether there is an optimal cooling of the cortex, which could reduce inflammation without affecting neurological output.

Many studies have illustrated the benefits of hypothermia for neuroprotection after brain trauma. Moderate-to-mild general hypothermia comprises core temperatures in the range of 30–35°C, which can be induced in the clinic by whole-body cooling pads or immersion in cold water (Howes *et al.*, 2010). A study conducted by Polderman *et al.* (2002) tested the effect of hypothermia treatment on 64 patients with severe head injury. Their study showed a marked decrease in intracranial pressure in 62 out of 64 patients within 4 hours of induction of hypothermia (Polderman *et al.*, 2002). Another study by Clark *et al.* (2009) conducted 12 and 48 hours of general hypothermia (33°C) in adult rats 1 hour after permanent middle cerebral artery occlusion, a permanent stroke model. A significant reduction in lesion volume, behavioral, and functional impairment, such as that involving skilled reaching ability as well as enduring histological improvement, was observed in the hypothermia treatment groups (Clark *et al.*, 2009). Numerous factors, such as the duration of hypothermia as well as the method of hypothermia employed, can determine the outcomes. Even the logistics of the hypothermia equipment are crucial for the assessment of safety and efficacy of a hypothermia procedure (Poli *et al.*, 2013). Nevertheless, the effect of hypothermia on an otherwise healthy cortex if the hypothermia treatment is targeting the spinal cord remains to be elucidated.

Although general hypothermia may better suppress the inflammatory response, and has shown benefits for treatment of brain traumas and SCI, the associated hypothermic effect in other healthy tissues may cause complications (Swain, 1988; Hildebrand *et al.*, 2004). These include vasoconstriction, which may cause kidney dysfunction and lead to problems with fluid management; bradycardia possibly leading reduction in cardiac output and even cardiac fibrillation; immunosuppression; mild coagulopathy and platelet dysfunction causing risk of heart attack and stroke; and thermoregulatory defense triggering shivering (Choi *et al.*, 2012). General hypothermia can also induce the phenomenon of cutaneous vasodilation, known as the “hunting effect,” in which reduced contractility of arterioles at temperatures below 8°C causes a reversal of the vasoconstrictive response (Daanen *et al.*, 1997). Such hematological changes can impair many other organs. Neural effects include loss of consciousness, a reduction in cerebral blood flow of about 6–7% per °C, and a loss of cerebrovascular autoregulation at about 25°C (Mallet, 2002). An additional complication that often

occurs in trauma patients and the elderly, known as accidental hypothermia, is characterized by an unintentional fall in core temperature to <35°C (Mallet, 2002). The crucial core temperature for trauma patients is 34°C, below which mortality significantly increases (Jurkovich *et al.*, 1987). At <32°C hypothermia in trauma patients, mortality is near 100%. This is highly applicable to SCI patients, as many such patients are likely to be trauma victims, especially due to motorcar accidents. In such cases, general hypothermia may not be a viable option, opening the door for local hypothermia treatment.

Local hypothermia, which focally cools the spinal cord, circumvents these major systemic side effects by maintaining the core temperature within an acceptable range while reducing spinal temperature to a therapeutic level. Therefore, we have developed a clinically relevant local hypothermia method that provides similar or even greater therapeutic benefits while preventing undesired cooling of the cortex or further damage to the injury or the surrounding vasculatures, peripheral nerves, muscles, and dura mater. Our semi-invasive method to induce local hypothermia of the spinal cord (Bazley *et al.*, 2013) is similar to other local cooling methods like EH. EH is highly effective for locally cooling the spinal cord and involves circulating cold water through the epidural space using an epidural catheter and perfusion pump. Studies have shown that EH provides benefits similar to general hypothermia (Ha and Kim, 2008; Ok *et al.*, 2012). Our semi-invasive method offers an alternative to EH whereby the dura is left intact and thus it offers a good trade-off between invasiveness and selective cooling.

Our previously published study (Maybhathe *et al.*, 2012) showed that treatment with 2-hour general hypothermia following acute moderate contusive SCI in rats led to improvements in SSEP amplitudes postinjury as well as functional recovery of motor behavior. The SSEP amplitude can be used to evaluate the number of intact, functional pathways between the periphery and cortex following an SCI. These increases in SSEP amplitudes were associated with beneficial outcomes, including an increase in motor behavior, measured by BBB score, and an increase in tissue sparing in the spinal cord. However, these same rats also exhibited increased N1-latency of SSEPs, or the time between sensory stimulus and the peak of the SSEP response. Other studies have also reported increased latencies in uninjured rats due to hypothermia treatment (Li *et al.*, 2012; Madhok *et al.*, 2012). One possibility is that the decrease in cortical temperature that was caused during the administration of general hypothermia induced long-term physiological changes in CNS conductivity. To the best of our knowledge, the temperature effect of hypothermia on the cortex, when used to treat SCI, has not been reported. In this study, we found that under general hypothermia, the cortex is cooled by 4°C. Because the cortex originates at a temperature of 34.3°C, it ultimately reaches a temperature of 30.3°C during treatment, even while the core temperature is maintained at 32°C. Thus, one cannot assume that the core temperature accurately reflects the temperature of the cortex. More importantly, such a low cortical temperature, which is intended to preserve spinal cord function, could result in adverse effects at the cortex. Poli *et al.* reported the effects on cortical temperature due to a head-neck cooling device in 11 patients for treatment of severe ischemic or hemorrhagic stroke. Although the treatment

only minimally affected cortical temperature (-0.36°C after 49 minutes), patient side-effects included severe hypertension, intracranial pressure crisis ($>20\text{ mmHg}$), and decreased cerebral perfusion pressure (Poli *et al.*, 2013). It is therefore imperative that the effects of general hypothermia on the cortex are well understood, even when used to treat SCI, as the treatment will inevitably induce temperature changes in other regions of the CNS.

Local hypothermia also induced a significant change in cortical temperature during treatment, although it was far reduced compared with general hypothermia. This comparison is critical because both treatment methods could be applied in the clinical setting for patients with traumatic brain injury or SCI, but their complications and side effects on the body in general, especially the cortex, have not been agreed upon universally by researchers as they are not well studied. The temperature at the cortex during local hypothermia was only reduced to 34.4°C , representing a drop of about 1°C from baseline. Although the effects on the cortex were limited, the temperature drop during local hypothermia was still significant, even while core temperature was maintained at 37°C . A possible explanation of this effect could be due to cooling of circulating cerebrospinal fluid (CSF). The CSF provides a medium of continuity between the brain and the spinal cord. Cooling of the spinal cord leads to the cooling of the CSF, parenchyma, and dura mater surrounding the spinal cord, which could in turn contribute to the decrease in cortical temperature. Therefore, given the significant differences in cortical temperature that are induced by the two methods of hypothermia, a complete study of electrophysiological outcomes comparing general and local hypothermia warrants future investigation.

Lastly, the rate of rewarming is now understood by clinicians to be a critical factor in the application of hypothermia treatment in patients, as recovery is sensitive to a steady and slow rate of rewarming. We found that there were no differences in the rate of rewarming of the spinal cord between the two methods of hypothermia treatment. These results suggest that the methods utilized for rewarming are highly efficient and that the body's thermoregulatory system to these methods is not affected by the cooling techniques employed. It is important for future studies to carefully consider the rate of rewarming, especially for longer durations of hypothermia, and to verify the rates are consistent with this report and do not cause impairment of thermoregulation post-hypothermia.

Conclusions

In this study, we provide the temperature profiles of the core, spinal cord, and cortex during five phases of hypothermia treatment. First, we compared the temperature differences between injured and un-injured rats and found that injured animals were associated with elevated spinal cord and cortical baseline temperatures compared with laminectomy groups. Second, we found that the temperature of the cortex is significantly affected by both general and local hypothermia, but that local hypothermia significantly reduces the amount of cortical cooling compared with general hypothermia. Core and spinal cord temperatures do not necessarily reflect cortical temperature during therapeutic hypothermia. Finally, local hypothermia induced smaller

changes in temperature of the cortex compared with general hypothermia while core temperature was maintained at 37°C throughout. In conclusion, these results strongly suggest that effects on cortical temperature should be carefully considered during administration of any method of hypothermia, even local hypothermia, and further studies are necessary to assess the efficacy of the treatment or whether there are any adverse effects to the cortex due to treatment. Nevertheless, for future studies that seek to increase the duration of hypothermia treatment, local hypothermia may provide the best option for reducing temperature effects throughout the body and minimizing side effects.

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Author Disclosure Statement

The authors declare no conflicts of interest.

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