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A simple and effective semi-invasive method for inducing local hypothermia in rat spinal cord

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Abstract— **Hypothermia has been shown to be an effective treatment for spinal cord injury. Local hypothermia is advantageous because it avoids inducing systemic side effects of general hypothermia while providing the opportunity for greater temperature reduction at the site of injury, which may contribute to increased neuroprotection. We report a new semi-invasive method for inducing local hypothermia in rats' spinal cords. Our method does not require laminectomy or penetration of the dura and is more effective at cooling the cord than transcutaneous approaches. We show that we were successfully able to cool the spinal cord to 30.2±0.3°C for 2 hours with rectal temperature maintained at 37.3±0.3°C after a spinal cord contusion injury. We also validated our method in control rats that received only a laminectomy. Furthermore, this method was able to reliably cool and rewarm the cord at a steady rate (Δ5.5°C in 30 min, or 0.2°C/min). Future work will include validating long-term functional improvements of injured rats after treatment and to apply local cooling to other spinal cord injury models, such as compression injuries.**

I. Introduction

Hypothermia treatment is gaining popularity for acute treatment of a number of neurological traumas, such as cardiac arrest [1], stroke [2], and spinal cord injury [3]. There have been several reports of the benefits of acute hypothermia for treatment of spinal cord injury (SCI) [4]. Our lab recently showed that acute 2-hour general hypothermia (32°C) leads to a long-term improvement in the conduction of sensory pathways of the spinal cord, measured by somatosensory evoked potentials, and improved motor behavior [3]. General hypothermia (33°C) for 7.5 hours in rats that underwent compression SCI induced even larger improvements in motor behavior, including the ability to regain weight-supported locomotion, and significantly increased the volume of healthy tissue in the cord [5]. Mild hypothermia also induced neuroprotection in both grey and white matter after ischemic SCI [6].

Although the detailed mechanisms of the role of hypothermia are still unknown, it is accepted that 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 and inflammation. Decreased metabolism brings about reduced consumption of oxygen and glucose and reduced excitotoxic neurotransmitter release; consequently, there is a reduced risk for 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 (reviewed in [7]).

Recently, it was shown that as little as a 1°C reduction in body temperature was enough to reduce grey and white matter damage during spinal cord ischemia [8]. Therefore, more extensive temperature reduction could provide even greater neuroprotection. However, there are a number of complications associated with sustained whole-body cooling, or general hypothermia. These include vasoconstriction, which my cause kidney dysfunction and lead to problems with fluid management; bradycardia possibly leading to cardiac fibrillation; immunosuppression; mild coagulopathy and platelet dysfunction causing risk of heart attach and stroke; and thermoregulatory defense triggering shivering. Cooling could also lead to insulin resistance and hyperglycemia, while rewarming may lead to insulin over-sensitivity and hypoglycemia. [7]

Therefore, it is desirable to explore alternatives using local hypothermia in order to gain the neuroprotective and anti-inflammatory benefits of cooling while avoiding the systemic side effects of general hypothermia. Current methods for inducing local hypothermia include invasive approaches by which epidural catheters are used to perfuse cold water through the epidural space, or transcutaneous approach by applying cooling pads to the back. Here we describe a new semi-invasive approach that does not require removal of lamina or penetration of the dura while still achieving a consistent spinal cord cooling.

II. Methods

A. Spinal cord injury

For all surgical procedures, rats were anesthetized with an i.p. injection of 30.4 mg/kg ketamine, 4.3 mg/kg xylazine, and 0.9mg/kg acepromazine maleate. Nine rats were randomly assigned to receive either a laminectomy surgery (control) or a laminectomy followed by a contusion spinal cord injury. Our contusion SCI model has been previously

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described [3, 9-14]. Briefly, laminectomy was performed by carefully removing the lamina at T8 without damaging the dura. The rat's spinal cord was placed under the vertical shaft of a MASCIS Impactor device (Rutgers University, NJ, USA) and the cylindrical rod was allowed to fall from a height of 12.5 mm to contuse the cord. After surgery, the muscle and skin incisions were sutured closed before inducing local hypothermia.

B. Copper heat exchanger

The heat exchanger was constructed from copper metal tubing with inner and outer diameters of 1mm and 2mm, respectively. The tubing was bent into 4 equal-length rows 11.2cm length and 2 cm in width such that it made an "M" shape (**Fig. 1**). A skin incision was made in the lower back so that the paravertebral muscles were not damaged. Forceps were used to gently separate the connective tissue under the skin to create a pocket between the skin and the paravertebral muscle over the spine. The heat exchanger was tunneled forward under the skin from approximately the T10 to T6 spinal segments. Although the exchanger rested snuggly under the skin, two sutures were inserted to guarantee the device did not shift during the experiment, and the skin incision over the copper tubing was closed. **Fig. 2** outlines the insertion and final position of the heat exchanger.

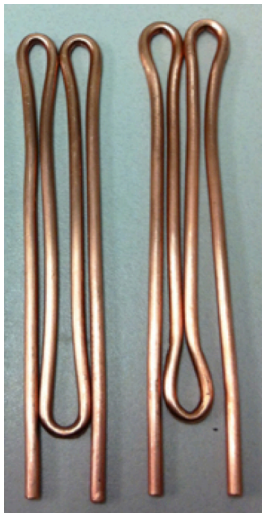

Fig. 1 Photograph of two M-shaped copper heat exchangers used for local spinal cord cooling.

C. Local hypothermia

All rats underwent local hypothermia following laminectomy or contusion injury. The core body temperature was maintained at 37±0.5°C using an electric heating pad for 1 hour after the time of the contusion or laminectomy. Next, local hypothermia was induced using our custom-made copper heat exchanger. To induce spinal cord hypothermia, 17±2.0°C cold water was perfused through the heat exchanger at a rate of 129 ml/min using a peristaltic pump. The temperature of the T8 region of the spinal cord was maintained at 30±0.5°C for 2hrs while the core body was maintained at 37±0.5°C. Following the 2 hours of spinal cord hypothermia, the heat exchanger was removed from the paravertebral muscle and the skin incision was closed. **Fig. 3** shows the inserted heat exchanger in one rat.

D. Temperature acquisition

Following laminectomy and spinal cord contusion (if applicable), a microprobe thermocouple (IT-24P, Physitemp, Clifton, NJ) was placed at the T8 region of the spinal cord without penetrating the dura or cord. The paravertebral muscle incision and the skin incision were closed to ensure an accurate local temperature reading at the dorsal side of spinal cord. The microtemperature probe is thermally insulated such that only the probe tip supplies readings (resolution of 0.01°C). A rat rectal probe was inserted to

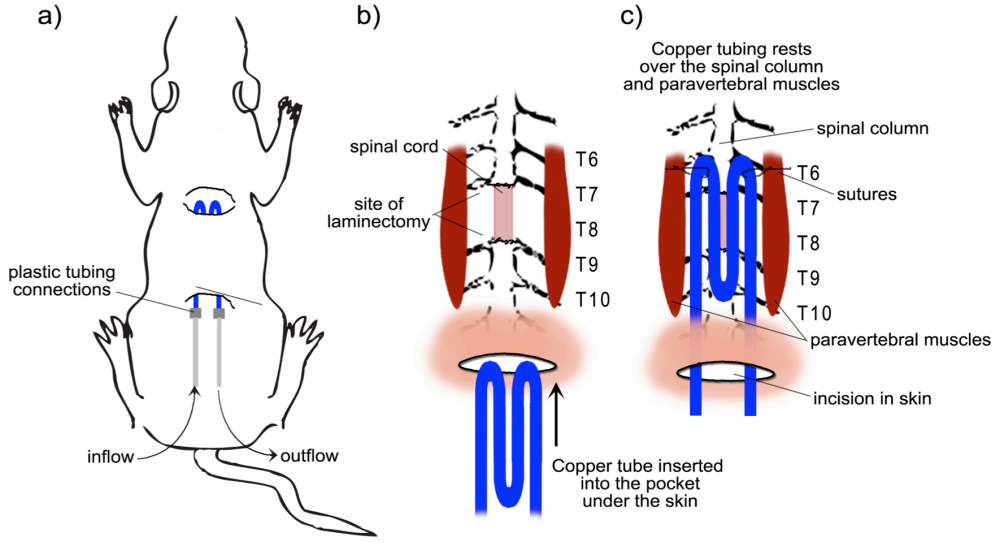

Fig. 2 Diagram of the experimental setup for inducing local hypothermia of the spinal cord. (a) A pocket was opened under the skin on the dorsal side of the rat and the M-shaped copper tubing unit was slipped inside. Plastic tubing connections to allow for inflow and outflow of circulating cold water were attached. (b-c) Laminectomy was performed at T8 to expose the spinal cord. The copper tubing rested over the spinal column and paravertebral muscles; two sutures ensured the tubing does shift during cooling process.

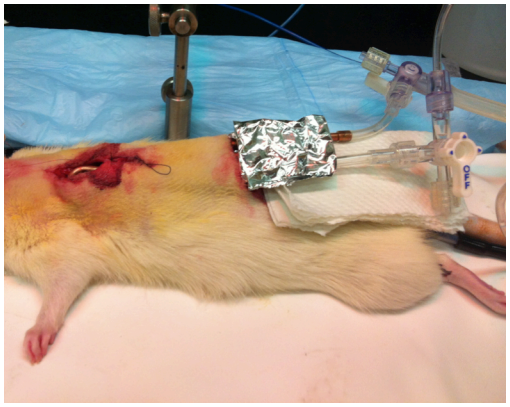

Fig. 3 Photographs of the experimental setup. in place: the copper tubing inserted under the skin, above the spinal column, and the inflow and outflows for circulating the cold water are shown.

monitor core body temperature (resolution 0.1°C). Both probes were interfaced with an electronic thermometer (BAT-10, Physitemp, Clifton, NJ) and temperature readings were manually recorded every 2 min. Temperature was recorded for 1hr pre-hypothermia to acquire the baseline temperature of the spinal cord prior to inducing local hypothermia, and 1hr post-hypothermia to ensure animals regain intrinsic core temperature control. Each recording session lasted 5 hours.

III. Results

To validate our reported method for inducing 2-hr local hypothermia to the spinal cord, we measured the local spinal temperature in n=4 rats with SCI and n=5 rats with laminectomy. In addition, we continuously monitored rectal temperature to verify the cooling method was truly local and that body temperature was maintained at 37±0.5 °C while only the local region of the spinal cord was cooled. **Fig. 4** shows the results of our preliminary study. The dashed lines indicate the target temperature profiles for the rectum and spinal cord. Spinal cord target profile was derived according to 1-hr pre-hypothermia, 30-minute induction to reduce temperature to 30°C (~0.2°C/min), 2-hr hypothermia maintenance, 30-minute rewarming return spinal cord to 37°C (~0.2°C/min), and 1-hour post-hypothermia monitoring. The target rectal temperature was 37°C throughout the experiment.

During the one-hour pre-hypothermia period, we determined the normal temperature of the spinal cord after laminectomy only to be 34.5±0.3°C. Thus, we used this as a target temperature for pre- and post- hypothermia periods in the injured state. During the 2-hour cooling, the spinal cord temperatures for the laminectomy and contusion injury groups were 30.2±0.2°C and 30.2±0.3°C, respectively ($p>0.05$). During the 1-hour post-hypothermia period, the temperatures of for the laminectomy and contusion injury groups were 34.7±0.3°C and 36.1±0.7°C, respectively.

In addition to local spinal cord temperature, we simultaneously recorded rectal temperature. **Fig. 4** shows that the rectal temperatures remained constant throughout the hypothermia induction period. The mean rectal temperatures for the laminectomy and spinal cord injured

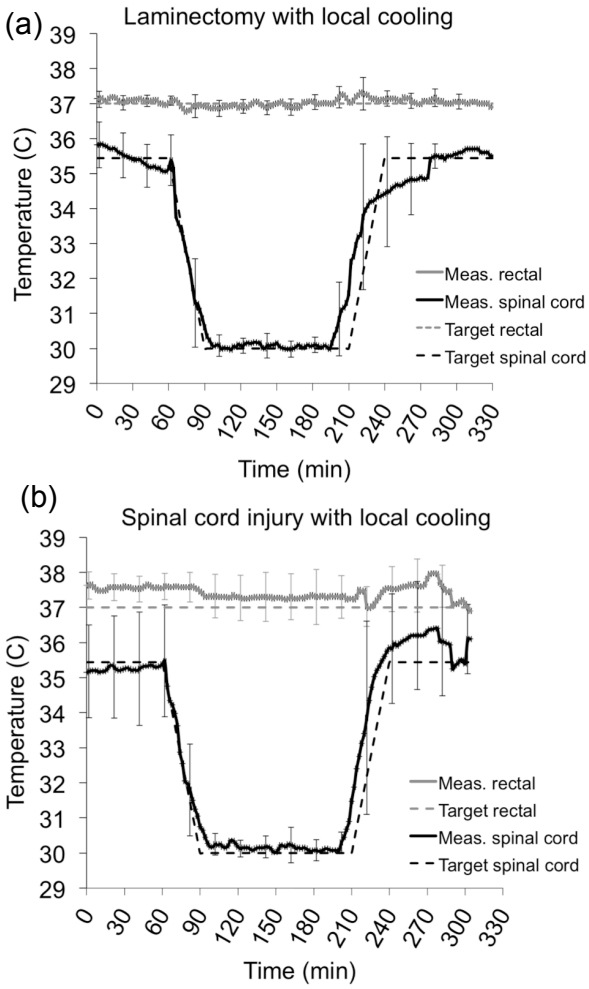

Fig. 4 Results of application of local hypothermia to the spinal cord for (a) rats receiving a laminectomy (n=5) and (b) rats receiving a contusion spinal cord injury at T8 (n=4). Data shows that a constant maintenance of therapeutic hypothermia applied locally to the spinal cord was feasible for both un-injured and injured states. Steady rates of temperature decrease and increase were also achieved. Thick lines represent the average measured temperature; dotted lines represent the target temperature. Error bars are standard deviation.

groups were 37.0±0.1°C and 37.4±0.3°C, respectively ($p>0.05$). The rectal temperature of the spinal cord-injured group was slightly above 37°C, which can be attributed to the body's acute response to the combat the trauma, including inflammation. Nevertheless, the local spinal temperature was successfully maintained within 30±0.5°C.

IV. Discussion

Here we present a new semi-invasive method for inducing local hypothermia at the spinal cord of rats. Our approach differs from previously reported methods of local hypothermia in that 1) the paravertebral muscles, vertebra, and dura mater are not damaged in the process, as only an incision in the skin is required, and 2) no elaborate device is required; common laboratory equipment is sufficient to induce consistent and reliable local hypothermia. Note that although we performed a laminectomy to induce spinal

contusion, a laminectomy is not required. This method could be used in other injury states, such as during tumor compression or spinal cord ischemia, in which a laminectomy is not performed. Furthermore, we verified that during the insertion of the heat exchanger and cooling period, no additional injury is sustained to the animal.

Epidural hypothermia (EH) is one common method for directly cooling the spinal cord, which involves circulating cold water through the epidural space using an epidural catheter or perfusion pump. EH was shown to provide similar benefits to general hypothermia [15, 16], although it is highly invasive. However, shorter duration of EH (30 min) did not provide improved outcomes [17]. Another group designed a novel local cooling device using Peltier modules that forms an arched plate that lays over the lamina [18]. Such as design is advantageous for inducing long-term (48 hour) local hypothermia and the long-term cooling provided functional improvements but requires the custom spinal device.

Less invasive local spinal cord cooling has also been reported, such as transcutaneous cooling in which a cooling pad is placed on the back to exchange heat with the skin and underlying muscles. However, no significant improvement in motor behavior was observed after 1 hour hypothermia even with paravertebral temperature maintained at 28.5± 0.3°C [19]. Another study determined that for moderate cooling of the back (skin temperature 13.66±1.28°C), the spinal cord was only reduced to 24.12±5.7°C [20]. Such a large temperature reduction at the skin is not desirable, and some clinicians have speculated that surface cooling induces increased shivering in patients [7].

We report an approach for cooling that is semi-invasive, without requiring penetration of the dura, but precise enough to directly cool the spinal cord to yield neuroprotective benefits. A previous study reported a subcutaneous spinal cooling technique and correlated paravertebral muscle temperature with intrathecal temperature at the neck, but lacks a report of spinal cord temperature at the site of injury [21]. Our approach was shown to reliably cool the spinal cord to a target of 30°C while maintaining body temperature at 37±0.5°C and thus offers a good trade-off between invasivity and selective cooling. Future work will seek to verify long-term motor behavior improvements and tissue preservation after applying this semi-invasive cooling method to rats with spinal cord injury.

Additional applications of such a cooling device, in addition to traumatic SCI, include treatment of spinal ischemia, tumor compression injures, or inflammation of the central nervous system.

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