Unilateral Brain Hypothermia as a Method to Examine Efficacy and Mechanisms of Neuroprotection Against Global Ischemia

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Hypothermia, especially applied during ischemia, is the gold-standard neuroprotectant. When delayed, cooling must often be maintained for a day or more to achieve robust, permanent protection. Most animal and clinical studies use whole-body cooling—an arduous technique that can cause systemic complications. Brain-selective cooling may avoid such problems. Thus, in this rat study, we used a method that cools one hemisphere without affecting the contralateral side or the body. Localized brain hypothermia was achieved by flushing cold water through a metal tube attached to the rats’ skull. First, in anesthetized rats we measured temperature in the cooled and contralateral hemisphere to demonstrate selective unilateral cooling. Subsequent telemetry recordings in awake rats confirmed that brain cooling did not cause systemic hypothermia during prolonged treatment. Additionally, we subjected rats to transient global ischemia and after recovering from anesthesia they remained at normothermia or had their right hemisphere cooled for 2 days (̴32°C–33°C). Hypothermia significantly lessened CA1 injury and microglia activation on the right side at 1 and 4 week survival times. Near-complete injury and a strong microglia response occurred in the left (normothermic) hippocampus as occurred in both hippocampi of the untreated group. Thus, this focal cooling method is suitable for evaluating the efficacy and mechanisms of hypothermic neuroprotection in global ischemia models. This method also has advantages over many current systemic cooling protocols in rodents, namely: (1) lower cost, (2) simplicity, (3) safety and suitability for long-term cooling, and (4) an internal control—the normothermic hemisphere.

Introduction

Rodent models of global ischemia are commonly used to study the mechanisms of ischemic damage and to evaluate neuroprotective treatments. These models can closely reproduce some aspects of the brain damage seen in patients resuscitated from cardiac arrest (Traystman, 2003). For instance, after brief ischemia there is a relatively selective depletion of hippocampal CA1 pyramidal neurons with associated memory impairments. One successful treatment developed in part from using rodent models of global ischemia is prolonged hypothermia, now used to treat some cardiac arrest patients (Bernard et al., 2002; The Hypothermia After Cardiac Arrest Study Group, 2002). Importantly, earlier work in rodents showed that mild hypothermia was neuroprotective not only when applied during ischemia (Busto et al., 1987), but also when initiated after ischemia, provided that the duration of cooling was prolonged (Colbourne and Corbett, 1994).

Many studies have examined mechanisms by which hypothermia provides benefit because it is the gold standard of neuroprotection (Zhao et al., 2007; Yenari et al., 2008; Polderman, 2009). From this work it is hoped that more selective and effective therapies will emerge, and that such treatments will cause fewer side effects than hypothermia. Currently, cardiac arrest patients are cooled systemically (surface blankets, endovascular cooling, etc.), which can cause several serious side effects such as heart arrhythmias and increasing the risk of pneumonia (Polderman, 2009). Similarly, clinical efforts in ischemic and hemorrhagic stroke patients typically use systemic cooling, which increases risk of such side effects. One potential solution, which is more suited for stroke than for cardiac arrest, is to use localized brain hypothermia to avoid systemic complications and potentially to accelerate the rate of cooling. While this is easily achieved in rodents under anesthesia (Nurse and Corbett, 1994; Taniguchi et al., 2005), clinical efforts with devices such as cooling helmets (Mellergard, 1992; Wang et al., 2004) have yet to achieve only a modest level of cooling owing to several factors such as cranial thickness and a robust vascular supply to the scalp. As progress is being made on these and other methods (Konstas et al., 2007; King et al., 2010), it is appropriate to evaluate the efficacy and mechanisms of local brain hypothermia in rodent

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stroke mechanisms. Unfortunately, most studies examining mechanisms of action use either intraischemic or brief post-ischemic hypothermia as these are easily induced in rodents. However, when treatment is delayed, a clinical inevitability, prolonged cooling (e.g., 24–48 hours) is often required for substantial and permanent protection in models of global and focal ischemia (MacLellan et al., 2009). Further, the mechanisms by which cooling mitigates injury likely vary with time since stroke (e.g., metabolic perturbations vs. delayed inflammation). Thus, it makes sense to use prolonged postischemic cooling to study mechanisms of injury as some have done (D’Cruz et al., 2002; Colbourne et al., 2003; Florian et al., 2008).

A number of methods have been used to induce and maintain prolonged hypothermia in rodents, including cold rooms (Yanamoto et al., 1999), fans and water spray (Colbourne et al., 1996), and various drugs (Florian et al., 2008). Each of these methods has limitations and they all induce systemic cooling. Thus, Clark and Colbourne (2007) developed a simple system for inducing focal brain cooling in fully awake rats, which avoids systemic side effects such as changes in blood pressure and the need for anesthesia while allowing for prolonged cooling. The method involves placing a metal coil beneath the Temporalis muscle and adjacent to the side of skull overlying the ischemic region. The coil is cooled by flushing it with cold water while the rat is awake and mobile. Using this technique, we showed that several days of local (hemispheric) hypothermia reduces injury in a rat model of permanent middle cerebral artery occlusion (Clark et al., 2009). Some benefit was also obtained in a rat model of intracerebral hemorrhage (Fingas et al., 2007).

The ability of prolonged focal brain cooling to persistently reduce hippocampal CA1 injury after global ischemia has not been evaluated. Given that this model is commonly used to study mechanisms of cell death relevant to cardiac arrest and ischemic stroke, as well as for evaluating other neuroprotectants, it would be instructive to determine the effects of focal brain cooling on this form of ischemic injury. Thus, the method of Clark and Colbourne, originally developed for focal ischemia and intracerebral hemorrhage models that damage the cortex and striatum, was adapted to focally cool the hippocampus and overlying cortex in one hemisphere while the contralateral hemisphere remained normothermic. We then evaluated whether focal cooling would reduce CA1 sector injury when applied for 2 days starting 1 hour after global ischemia. We hypothesized that focal cooling would result in permanent unilateral protection, which was observed. Thus, this simple, inexpensive method is applicable for studying hypothermic neuroprotection after global ischemia. Importantly, this approach has the advantage of providing an internal control, the contralateral, normothermic hemisphere, which makes this method highly suited to studying mechanisms of action of prolonged cooling after global ischemia.

Methods

Subjects and experimental design

Experiments were performed on 34 male Sprague-Dawley rats (Biosciences breeding colony, University of Alberta) weighing ~300 g at the time of surgery. Water and food were available ad libitum, except during fasting before ischemia. Surgical procedures were performed aseptically. All procedures were in accordance with the Canadian Council on Animal Care and were approved by the Biosciences Animal Care and Use Committee at the University of Alberta.

Three experiments were done. First, we determined brain temperature during focal cooling in both anesthetized and awake rats (n = 5). A second experiment compared the extent of histological damage at a 1-week survival after global ischemia in normothermic rats (NORMO, n = 7) versus those with focal hypothermia (HYPO, n = 7). Third, we extended this experiment to include both histological and behavioral endpoints at a 1-month survival time (NORMO: n = 7; HYPO: n = 8). The use of a long survival time is critically important because certain hypothermia treatments only postpone injury (Dietrich et al., 1993). In the last two experiments, postischemic cooling was initiated after a 1 hour delay and was maintained for 48 hours followed by gradual re-warming over 6 hours.

Experiment 1: brain temperature measurements

A published method (Clark and Colbourne, 2007) for focal brain cooling was modified for use in the current experiments. Instead of using the cooling coil developed in that study, we flattened a stainless steel tube (18 G) to a thickness of 1 mm and a width of 2 mm. A 6 mm length was used as it would overlie the hippocampal formation. Silastic tubing (VWR Scientific; Product # 7-5224) was then attached to each end of the strip in a tight manner not allowing for water leakage (Fig. 1A). The dorsal (vs. lateral) placement of the cooling device is simpler and quicker to implant than the device used in our original study.

To measure brain temperature at multiple points simultaneously, rats were anesthetized with isoflurane anesthesia (4% induction, 2% maintenance in 60% O2, balance N2O) and prepared for surgery by injecting 0.2 mL of Marcaine (Sanofi Canada) subcutaneously on the skull, and shaving the top of the head. After placement in a stereotaxic frame, a midline scalp incision was made and the skull was cleared of connective tissue. Two anchor screws were threaded into the parietal bone approximately 2 mm lateral from the midline. The cooling strip was then positioned on the skull surface (Fig. 1B) adjacent to the right temporal ridge. Dental cement was applied over the anchor screws, the metal strip and a small initial length of tubing to secure the device in place. Additional burr holes were made to allow needle-type thermocouple probes (model HYPO-33-1-T-G-60-SMG-M; Omega) to be stereotaxically placed into the anterior (−3.8 A-P; 1.5 M-L) and posterior (−5.3 A-P; 2.5 M-L) hippocampus of the right hemisphere as well as the contralateral hippocampus (−4.5 A-P; −1.5 M-L). Rectal temperature was maintained at 37°C through a servo regulated warm water blanket. Following baseline temperature recordings from all 3 brain probes focal brain cooling was initiated by pumping chilled water through the cooling strip. The flow rate (115 mL/h) matched the flow rate that would be used in subsequent experiments.

An additional two rats were used to measure hippocampal and body temperature during hypothermia in awake animals. First, a core temperature telemetry probe (model TA10TAF40, Transoma Medical) was surgically implanted into the abdominal cavity as previously described (DeBow and Colbourne, 2003). Four days later, the rats were prepared for a
protect the probe’s electronics, it was encased in a plastic cylinder, which was secured to the skull with dental cement and three anchor screws. The silastic tubes connected to the cooling strip were passed through a flexible spring sheath (model CIH95; 30 cm long; Instech Laboratories) to protect them from damage by the rat. Additional dental cement was applied to the head assembly to secure an initial segment of the metal sheath to the plastic cylinder containing the thermocouple. Once the dental cement had dried, anesthesia was discontinued and the rat was returned to its home cage. The metal sheath and the silastic tubes were connected to an overhead swivel (model 375/D/22; Instech Laboratories) attached to a counterbalance arm (CM375BS; Instech Laboratories) mounted on top of the rat’s cage, as previously described (Clark and Colbourne, 2007). This allowed rats to move about their cage. Brain temperature was measured continually by placing the home cage on a receiver (RPC-1; Transoma Medical) connected to a computer (ART software, v. 2.1; Transoma Medical). Signal interference from the core and brain probes prevented us from measuring both temperatures simultaneously (DeBow and Colbourne, 2003); therefore, the core probes were only activated for short durations before, during and after focal cooling whereas brain temperature was recorded at all other times.

We did not measure brain temperature in this experiment to (1) avoid complicating and prolonging the surgical procedures, and (2) to avoid the potential loss of our brain telemetry probes, which are no longer commercially manufactured.

Three days after surgical implantation of the cooling device the rats were food deprived for ~18 hours to lower blood glucose levels into a consistent range (~6–10 mM) before ischemia surgery. Ischemia was induced for 10 minutes using the 2-vessel occlusion (2VO) model, which involved bilateral carotid artery occlusion combined with systemic hypotension to 35 mmHg (Smith et al., 1984). The later was achieved through exsanguination via the jugular vein. During surgery, core temperature was maintained at 37°C through a rectal temperature probe connected to a warm water blanket. A 100-μl arterial blood sample was collected for blood gas analysis (Radiometer ABL 810; Radiometer), which was within the normal range (PO2: 125–135 mmHg; PCO2: 35.0–45.0 mmHg, pH: 7.350–7.450; data now shown). Skull temperature, measured with a subcutaneous thermocouple probe, was also maintained at normothermia (~37°C) via an overhead infrared lamp (150 W). Immediately after surgery, rats were returned to their home cage and were randomly assigned to either the NORMO or 2 day HYPO treatment starting 1 hour after ischemia. Hypothermia was induced by passing ice cold water, via the overhead swivel, through the cooling device at a point that the water was ~10°C. Rats were free to move about their cage during cooling.

At 7 days postischemia, the rats were transcardially perfused with phosphate buffered saline followed by 10% formalin. Extracted brains were postfixed in formalin for ~24 hours, embedded in paraffin, and sectioned at 10 μm. Sections, stained with hematoxylin and eosin (H&E), were used to quantify the number of remaining neurons in the CA1 field of the hippocampus. Briefly, the number of viable-looking neurons in the medial, middle, and lateral sectors of CA1 (each 0.2 mm long) was counted on a light microscope at

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**Experiment 2: global ischemia with 1-week survival**

second surgery to implant the focal brain cooling strip and a brain temperature telemetry probe. The initial steps of this surgery were identical to above; however, after the placement of the cooling strip on the skull, a single burr hole was drilled over the anterior hippocampus (~3.8 A-P; 2.5 M-L) to accommodate a 5.0 mm cannula (20 G) through which the shaft (8.0 mm long) of a telemetry thermocouple probe (XM-FH-BP; Mini-Mitter Co., Inc.) was lowered 3.0 mm into the brain. To protect the probe’s electronics, it was encased in a plastic cylinder, which was secured to the skull with dental cement and three anchor screws. The silastic tubes connected to the cooling strip were passed through a flexible spring sheath (model CIH95; 30 cm long; Instech Laboratories) to protect them from damage by the rat. Additional dental cement was applied to the head assembly to secure an initial segment of the metal sheath to the plastic cylinder containing the thermocouple. Once the dental cement had dried, anesthesia was discontinued and the rat was returned to its home cage. The metal sheath and the silastic tubes were connected to an overhead swivel (model 375/D/22; Instech Laboratories) attached to a counterbalance arm (CM375BS; Instech Laboratories) mounted on top of the rat’s cage, as previously described (Clark and Colbourne, 2007). This allowed rats to move about their cage. Brain temperature was measured continually by placing the home cage on a receiver (RPC-1; Transoma Medical) connected to a computer (ART software, v. 2.1; Transoma Medical). Signal interference from the core and brain probes prevented us from measuring both temperatures simultaneously (DeBow and Colbourne, 2003); therefore, the core probes were only activated for short durations before, during and after focal cooling whereas brain temperature was recorded at all other times.

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**FIG. 1.** (A) Illustration (modified from Zilles, 1985) showing placement of cooling strip on the skull surface overlying the right hemisphere. Thermocouples were placed as indicated for temperature recordings shown in (C). (B) Photo of the cooling strip with attached tubing and protective sheath. (C) Brain temperature recordings during focal brain cooling in anesthetized rats (HPC = hippocampus). Body temperature was normothermic throughout (data not shown). (D) Brain and body temperature recordings (via telemetry) of an awake rat demonstrate that hypothermia persistently decreases hippocampal temperature during the cooling period, while leaving body temperature unaltered. Due to signal interference between the body and brain probes, body temperature was sampled intermittently. Otherwise, brain temperature was sampled every 30 seconds and averaged for this illustration.
3.7 mm posterior of Bregma (Paxinos and Watson, 1998), as previously done and illustrated (Colbourne and Corbett, 1995). Cell numbers were summed across each region within a hemisphere. Neighboring sections were used for immunolabeling with a Rabbit anti Iba-1 primary antibody (1:1000, product: 019-19741; Wako), and Donkey anti-Rabbit 594 secondary antibody (Jackson Laboratories). Before incubation with the primary antibody, antigen retrieval was performed by boiling the sections in 0.1 M citrate buffer (pH 6.3) for 15 minutes in a microwave (Tang et al., 2007). All incubations were at room temperature, and DAPI (1:500; Sigma) was added during incubation with the secondary antibody to observe cell nuclei. In separate sections, Fluoro-Jade B labeling (Schmued and Hopkins, 2000) was carried out to quantify the number of degenerating neurons within the CA1 region, which was expected to provide similar data to the H&E staining. Fluoro-Jade B and Iba-1-positive cells were quantified within the CA1 pyramidal cell layer in the same regions as H&E cells counts (medial, middle, and lateral sectors).

Experiment 3: 2VO ischemia with 1-month survival

Procedures were identical to experiment 2 except that rats survived for 1 month to determine whether hypothermic neuroprotection was permanent. As well rats were tested on a modified version of the water maze task (Driscoll et al., 2006) from 19 to 30 days postischemia. For the first 2 days the platform remained in the same position within the pool. For the next 2 days it was moved to a new location and so on for a total of 12 days of testing. Each rat was given 8 swim trials per day with each trial starting from one of the 4 cardinal compass points along the edge of the pool. A trial lasted a maximum of 90 seconds and the rats were allowed to stay on the platform for an additional 10 seconds. In cases when a rat did not find the platform it was physically placed on the platform by the experimenter. Performance (including latency, swim speed, and distance traveled) was tracked and analyzed through an overhead camera connected to a computerized tracking system.

Statistical analyses

For histological measures, group differences were initially assessed using two-way between-subject ANOVAs (SPSS v18 Mac). Treatment (HYPO vs. NORMO) and hemispheres (left vs. right) were the factors. While hemisphere may be considered a repeated measures factor, we used the more conservative approach. Due to significant interactions in these two-way analyses, we further examined the data with one-way ANOVAs for treatment and hemisphere comparisons. Behavioral data were analyzed using repeated-measures ANOVA. A p-value of less than 0.05 was considered statistically significant.

Results

There were no exclusions or mortality in these experiments.

Experiment 1: brain temperature measurement

Upon initiation of cooling in anesthetized rats, the ipsilateral hippocampal temperature was lowered to ~32°C, whereas the contralateral side remained normothermic (Fig. 1C). Temperature then quickly stabilized around 31.6°C±0.04°C (mean±SEM). This pattern was replicated in awake animals, as initiation of cooling quickly lowered hippocampal temperature to ~33.2°C±0.05°C (mean±SEM), which was measured with a telemetry probe, and remained in that range for the entire cooling period (48 hour; Fig. 1D). Body (core) temperature, which was sampled intermittently via telemetry before, during and after hemispheric cooling remained unchanged and normothermic. Importantly, we did not observe any adverse behavioral effects of the cooling, as animals often remained engaged in the same behaviors (e.g., grooming, eating) during cooling initiation as just before it.

Experiment 2: 1-week outcome

The two-way ANOVAs for H&E, Fluoro Jade B, and Iba cell quantification data at a 1-week survival all revealed significant interactions (p<0.043), and thus we compared treatments with one-way ANOVAs. Untreated ischemia caused severe CA1 injury (Figs. 2A and 3A), and, as expected, there was no difference between hemispheres within the NORMO group (p>0.299). The HYPO treatment (vs. NORMO) significantly reduced cell loss as measured with H&E (p<0.001, Fig. 2A) and Fluoro Jade B (p<0.001, Fig. 3A) staining in the right hemisphere. This effect was also seen in comparing the cooled (right) hemisphere with the normothermic (left) hemisphere in the HYPO group, which were significantly different (p=0.014). As expected, there was no difference (p>0.269) between groups for the left hemisphere because these were normothermic in both groups. Microglial proliferation was also mitigated in the right hemisphere in the HYPO group (p<0.001, Fig. 4A) relative to NORMO. Surprisingly, there was also a significant decrease of microglial labeling in the left hemisphere in the HYPO group; however, the decrease was significantly greater in the right hemisphere (p=0.001).

Experiment 3: 1-month outcome

Similar to our findings at 1 week, focal cooling increased the number of surviving H&E-stained CA1 neurons in the cooled hemisphere (p=0.026 vs. normothermic side), whereas the hemispheres in the NORMO group were not significantly different (p=0.345, Fig. 2B) and showed marked CA1 injury. Further, only the cooled hemisphere had significantly more remaining neurons in the HYPO group (p<0.001), whereas the group comparison for the normothermic hemisphere was not significant (p>0.252). Fluoro Jade labeling of CA1 neurons was reduced in the cooled hemisphere of the HYPO (p<0.044), whereas there was no difference between hemispheres in the NORMO group (p>0.844, Fig. 3B). A comparison of the hemispheres across groups showed that only the cooled one had a decrease in the number of Fluoro Jade–labeled CA1 cells (p=0.001). In contrast to our results at 1 week, the effects of HYPO on microglia proliferation were restricted to the cooled hemisphere, as HYPO caused a significant decrease in the number of microglia in only the cooled hemisphere (p<0.001, Fig. 4B). The difference between the left and right hemispheres was only significant in the HYPO group (p<0.041).

Both groups showed significant learning in the water maze task (Fig. 5), as the latency to find the platform decreased over testing days (p<0.001); however, the group×day interaction was not significant (p=0.718), indicating that the groups were not different. Additional measures of performance (distance
traveled and swim speed) also indicated no significant group differences ($p \geq 0.673$).

**Discussion**

Our findings demonstrate that focal brain hypothermia can persistently attenuate CA1 sector injury after global ischemia in rat. Protection was observed within the cooled hemisphere, but not in the normothermic side. Selectively cooling one hemisphere would likely have significant advantages over systemic and perhaps even whole-brain hypothermia for ischemic and intracerebral hemorrhagic stroke. Although unilateral treatment is not an ideal strategy for global ischemic insults because brain injury occurs bilaterally, patients may, nonetheless, benefit from brain-targeted cooling when combined with milder systemic cooling. Regardless, we are not advocating that this particular approach or this method be used clinically. Instead, there are meaningful advantages to this line of investigation in animal studies. First, the technique is inexpensive and does not require costly equipment for measuring and regulating temperature (e.g., telemetry probes). Second, the technique is simple, requiring only a quick stereotaxic surgery to implant the cooling device, which is easily made. Third, with this method one can safely cool for prolonged periods, which is required for robust and enduring protection (MacLellan *et al.*, 2009) and similar to current clinical protocols (Polderman, 2008, 2009). Further, focal cooling does not require sedatives, anesthetics, or other drugs that may confound results especially in rodents where one is hampered by their small size and a limited ability to sample.
physiological variables after ischemia (e.g., blood gases). Finally, cooling one hemisphere provides for an internal control—the normothermic hemisphere. Unilateral treatment is a proven strategy to test specificity (e.g., unilateral septo-hippocampal deafferentation) (Buchan and Pulsinelli, 1990).

As noted, one might argue that protection limited to one hemisphere is an important disadvantage of this method for global ischemia research. Certainly, with respect to evaluating behavioral outcome, the fact that only half of the hippocampus is salvaged, at best, means that it may be difficult to observe behavioral protection. In this study, we did not see any significant group effect on water maze scores. Nonetheless, it certainly remains possible (likely) that a more demanding water maze protocol or alternative tests would detect a partial neuroprotective effect (Langdon et al., 2010). Alternatively, one may conduct in vivo or in vitro electrophysiological studies to demonstrate functional preservation as previously done with systemic cooling after global ischemia (Dong et al., 2001). Such an approach would also benefit from having an internal control.

One limitation of this study is that we did not formally evaluate behavior during focal brain cooling (due to tethering to cooling lines), which is also not practical with systemic cooling methods. We did observe that rats appeared normal during cooling without overt signs of distress, which is consistent with physiological measurements taken with telemetry that show normal heart rate and blood pressure during focal cooling (Clark and Colbourne, 2007). This is certainly not the case during systemic cooling (MacLellan et al., 2004). Finally, continued treatment with analgesics, beyond that required to manage postsurgical pain (e.g., local infiltration of Marcaine during surgery), does not seem necessary with this method.

FIG. 4. Number of Iba-positive cells (microglia) at 1 week (A) and 1 month (B) postischemia. Hypothermia treatment significantly reduced this as illustrated in (D), which depicts the cooled hemisphere (Pyr = pyramidal cell layer). By contrast, the normothermic side has extensive microglial infiltration (C). *$p < 0.05$ versus the right side of the NORMO group, whereas $^*p < 0.05$ versus the right side of the HYPO group. Color images available online at www.liebertonline.com/thera

FIG. 5. Average latencies to find the hidden platform in the water maze task for novel (A) and familiar days (B). Groups improved over time in both cases, but were not significantly different from each other. Thus, this test was not able to detect the unilateral neuroprotective effects of focal cooling.

A Novel Days

Mean ± SEM Latency (sec)

B Familiar Days

Mean ± SEM Latency (sec)
Another consideration, based upon previous findings (Clark and Colbourne, 2007), is the occurrence of temperature gradients underneath the cooling device with cortical regions being cooled more than subcortical structures. Thus, it can be very difficult to directly compare focal and systemic hypothermia to determine the more efficacious treatment. Nonetheless, the present findings in global ischemia and our work in focal ischemia (Clark et al., 2009) and hemorrhage (Fingas et al., 2007) show that both strategies are beneficial. As well, it is important to note that the systemic complications of whole-body cooling, such as elevating blood pressure by cooling with fans and water spray (MacLellan et al., 2004), do not entirely account for treatment efficacy in ischemia models. Finally, it should be noted that by varying the water temperature or the flow rate to the cooling device one could manipulate the level of hypothermia achieved (Clark and Colbourne, 2007) as well as changing cooling and re-warming rates. Such research is essential to determine the optimal treatment parameters and the most effective cooling method to bring forth into clinical trials.

Our first experiment in this study and the previous methods article shows that unilateral cooling is achieved with little to no effect on the contralateral side. The lack of histological protection on the left (normothermic) side is the HYPO group further supports this claim. However, at 1 week we did find a moderate but statistically significant reduction in ischemia-induced microglia activation contralateral to cooling. Our experiments do not explain this effect, which may be due to a very modest cooling in the contralateral side. This pattern of results fits a previous study, where a hypothermia protocol insufficient for reducing neuronal death still mitigated microglial proliferation in CA1 (Drabek et al., 2009). Our study with a longer survival time did not find any effects of hypothermia in the normothermic hemisphere, indicating that the reduction in microglia is transient.

We observed slightly more CA1 neurons remaining in the hypothermia-treated group at the longer survival time (4 weeks in experiment 3 vs. 1 week in experiment 2). Given that these are separate experiments, this difference is likely due to chance. However, it is also possible that our “normal-looking” criterion for counting CA1 neurons may slightly underestimate cell counts at early survival times if some of the abnormal-looking cells eventually recover. The increased number of CA1 neurons at the longer survival time is not likely due to proliferation because we recently found that CA1 neurogenesis does not occur after untreated ischemia or in systemically cooled rats in this model (Silasi and Colbourne, 2011).

Conclusion

Prolonged focal cooling persistently reduces CA1 sector injury after 2VO ischemia roughly similar to that observed with systemic treatments in this and other models (van der Worp et al., 2007; MacLellan et al., 2009). These results support the development and use of effective brain-selective cooling technologies for patients suffering stroke and related insults. Finally, our method to unilaterally cool the brain in global ischemia models to provide protection on only one side offers several methodological advantages that make this approach highly suited to further efficacy and mechanistic studies.

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Author Disclosure Statement

No competing financial interests exist.

References


Colbourne F, Grooms SY, Zukin RS, Buchan AM, Bennett MV. Hypothermia rescues hippocampal CA1 neurons and attenuates down-regulation of the AMPA receptor GluR2 subunit after forebrain ischemia. Proc Natl Acad Sci U S A 2003;100:2906–2910.


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Traystman RJ. Animal models of focal and global cerebral ischemia. ILAR J 2003;44:85–95.


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