The effects of selective brain hypothermia on intracerebral hemorrhage in rats

Matthew Fingas, Darren L. Clark, Frederick Colbourne

Abstract

Prolonged hypothermia effectively treats global cerebral ischemic injury in animal models as well as in cardiac arrest victims. Furthermore, clinical trials, based upon encouraging animal findings, are underway to assess efficacy in ischemic stroke. Intracerebral hemorrhage (ICH) is a more devastating stroke, but one that shares mechanisms of injury with ischemia. Accordingly, ICH may be amenable to hypothermia treatment. In this study we tested whether selective brain hypothermia improves outcome after an ICH in rats created by infusing 100 μL of autologous whole blood into the striatum. Striatal hypothermia (∼32 °C) was induced with a novel method (implanted cooling coil) that does not cause systemic cooling, thereby providing a safer and potentially more effective treatment for stroke than systemic hypothermia. Edema occurred for 4 days after ICH, but it peaked at 3 days (∼5%). At this time it was significantly reduced (to ∼2%) by cooling starting 1 h after ICH (3 day duration). Next, we determined whether 1 and 12 h delayed cooling treatments (4 day duration) would lessen functional impairment and lesion size. Untreated (normothermic) ICH resulted in significant forelimb use asymmetry, as well as deficits in walking and skilled reaching. These deficits were unaffected by hypothermia, as was the volume of tissue lost (∼20 mm³) at 1 month. Thus, attenuated edema did not result in behavioral or histological benefit. In conclusion, while additional research with alternative cooling protocols and ICH models are required, these findings suggest that while hypothermia lessens edema, it will not be directly neuroprotective after ICH.

Keywords: Focal hypothermia; Intracerebral hemorrhage; Neuroprotection; Edema; Recovery of function; Stroke

Introduction

Hypothermia (HYPO) is an established neuroprotectant in ischemic stroke (van der Worp et al., in press). For instance, HYPO persistently improves functional recovery and reduces cell death in rodent models of global (Colbourne and Corbett, 1995) and focal ischemia (Colbourne et al., 2000) even when HYPO is initiated after a delay of a few hours. Prolonged cooling provides greater and more enduring protection than brief cooling, which may only transiently protect (Colbourne et al., 1997; Dietrich et al., 1993). Importantly, prolonged HYPO reduces mortality and improves recovery in cardiac arrest survivors (Bernard et al., 2002; The Hypothermia After Cardiac Arrest Study Group, 2002) and in neonates after hypoxia/ischemia (Shankaran et al., 2005). Accordingly, HYPO might improve outcome following intracerebral hemorrhage (ICH), which shares mechanisms of injury with ischemic stroke (Xi et al., 2006).

Although ischemia and ICH share pathological mechanisms (Xi et al., 2006), there are key differences that necessitate the assessment of treatments in ICH models. In this regard, HYPO has had mixed results in rodent models. Notably, systemic HYPO reduces edema after collagenase-induced ICH (Kawai et al., 2002; Kawanishi, 2003), whole blood infusion (MacLellan et al., 2006b), and thrombin injection (Kawai et al., 2001). Hypothermia also reduces inflammation after whole blood infusion (MacLellan et al., 2006b). Unfortunately, HYPO fails to improve functional recovery or to lessen cell death when given soon after collagenase (MacLellan et al., 2004) or whole blood-induced ICH (MacLellan et al., 2006b). Interestingly, more delayed cooling improved outcome in the collagenase model (MacLellan et al., 2004). The failure of early cooling was ascribed to deleterious side effects (e.g., hypertension) that aggravate bleeding when given soon, but not later, after collagenase infusion. Side effects of systemic HYPO occur in patients (Schubert, 1995), which might be mitigated or avoided by using selective brain HYPO. The aforementioned ICH
HYPO studies used systemic cooling. One study (Wagner et al., 2006) in pigs showed that local brain cooling reduced edema after whole blood infusion. Unfortunately, behavior was not assessed.

Selective brain cooling methods are being widely investigated in humans to optimize treatment of ischemia and traumatic brain injury — TBI (den Hertog et al., 2007; Hemmen and Lyden, 2007; Wagner and Zuccarello, 2005). Focal brain cooling can also be safely induced in rats, without affecting heart rate or arterial blood pressure, via a cooling coil implanted underneath the Temporalis muscle (Clark and Colbourne, 2007). Therefore, this HYPO method may benefit ICH and avoid some of the side effects of systemic HYPO. Importantly, prolonged cooling can be used, which may optimize treatment by countering ongoing pathologies such as erythrocyte lysis, iron deposition, inflammation and continuing edema (Xi et al., 2001).

Presently, we investigated the effects of mild, prolonged, local HYPO on edema, histological injury and behavioral recovery in the well-regarded, whole blood model (Andaluz et al., 2002). First, we hypothesized that our focal cooling method (Clark and Colbourne, 2007) would cool the striatum without causing systemic HYPO. Second, we predicted that HYPO would attenuate the peak rise in ICH-induced edema. Third, we predicted that HYPO would alleviate long-term behavioral impairments and reduce tissue loss.

Methods

Animals

One hundred and twenty-one male, young-adult Sprague–Dawley rats, obtained from the Biosciences breeding colony at the University of Alberta, were used. All procedures were approved by the Biosciences Animal Policy and Welfare Committee at the University of Alberta and were in accordance with the Canadian Council on Animal Care guidelines. Aseptic technique was used. Rats were randomly assigned to treatment groups. Additional, step-by-step, details of standard operating procedures (e.g., ICH surgery) are available at: http://web.psych.ualberta.ca/fcolbour/research.htm.

Experiment 1 measured brain ($T_b$) and core temperatures ($T_c$) during focal cooling ($N=9$). Experiment 2 assessed cerebral edema ($N=70$). Experiment 3 assessed long-term behavioral and histological outcome ($N=42$). Data from these three experiments were analyzed with ANOVAs and Scheffé post hoc tests if needed (SPSS v. 15.0). A $p$ value of $<0.05$ was considered to be statistically significant. Pearson r correlations were also performed. Standard deviation (S.D.) error terms are presented.

Intracerebral hemorrhage

Rats were anesthetized with isoflurane (4% induction, 2% maintenance in 60% $N_2O$ and 40% $O_2$) and placed in a stereotaxic frame. A midline scalp incision was made and the skull was balanced between Bregma and Lambda (Paxinos and Watson, 1982). A hole was drilled 3.5 mm lateral to Bregma. This was on the right side in Experiments 1 and 2 and contralateral to the preferred paw, as determined by the single pellet task (below), in Experiment 3. A needle (26 G) was inserted 6.5 mm below the surface of the skull (Fig. 1B) and 100 $\mu$L of blood, withdrawn from the tail artery was infused into the lateral striatum over 10 min (MacLellan et al., 2006b,c). Following this, the needle was slowly removed over an additional 10 min to prevent blood from moving up the needle tract. A metal screw (model MX-080-2; Small Parts, Miami Lakes, FL) was inserted into the burr hole. A local anesthetic (Marcaine; Sanofi Canada, Markham, Ontario, Canada) was applied to the wound at the end of surgery. Rectal temperature was maintained at $\sim 37 \degree C$ during surgery ($\sim 1$ h) using an electric heating pad.

Temperature measurement and control

At the time of ICH, all animals, except some in Experiment 2, were quickly implanted (<1.5 min) with a cooling coil underneath the Temporalis muscle ipsilateral to the ICH (Clark and Colbourne, 2007). Briefly, the Temporalis muscle was retracted to allow for the placement of 8 mm (diameter) by 2 mm (thick) steel coil that rested against the skull. Screws were inserted into two additional burr holes for the well-regarded, whole blood model (Andaluz et al., 2002). First, we hypothesized that our focal cooling method (Clark and Colbourne, 2007) would cool the striatum without causing systemic HYPO. Second, we predicted that HYPO would attenuate the peak rise in ICH-induced edema. Third, we predicted that HYPO would alleviate long-term behavioral impairments and reduce tissue loss.

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holes. These helped secure the cooling coil and an anchoring device for the water lines. The device was made from a 1 mL syringe barrel (20 mm height) that was centered on the injection site and secured to the screws with dental cement. Immediately following the ICH, the HYPO animals were tethered to an overhead swivel (model 1-375/D/20; Instech Solomon, Plymouth Meeting, PA) that connected to a water source. Gravity fed cold water (∼ 11 °C as it enters the coil) was allowed to pass through the coil starting at either 1 (HYPO-1) or 12 h (HYPO-12) following infusion of autologous blood, and remained on for up to 96 h. This cooling method causes rapid cooling of the brain, and following active cooling, a rapid restoration of normothermia (minutes). Control normothermic animals (NORMO) were attached to a flexible cord that simulated the tethering effect of the overhead swivel.

Experiment 1: selective brain hypothermia

A $T_c$ telemetry probe (model TA10TA-F40; Transoma Medical, St Paul, MN, USA) was implanted into the abdominal cavity 4 days prior to ICH under isoflurane anesthesia (MacLellan et al., 2006b). Immediately following ICH the rats were implanted with a cooling coil as described above. Additionally, they were implanted with a telemetry brain probe (Fig. 1A; model VM-FH-BP, Mini-Mitter Co. Inc, Sun River, OR, USA) as described previously (Clark and Colbourne, 2007). Briefly, the cooling coil was implanted as before but a larger bore head cap (from a 5 mL syringe) was made to encase the brain probe. The brain probe was inserted into the same burr hole through which the blood was infused and measured $T_b$ in the center of the ICH. Following anesthesia $T_b$ was measured every 30 s whereas $T_c$ was sampled once per day (noon). Continuous measurement of $T_b$ and $T_c$ with these telemetry probes was not possible owing to signal interference (Clark and Colbourne, 2007; DeBow and Colbourne, 2003). Rats were randomly assigned to HYPO-1 ($N=4$), HYPO-12 ($N=3$) or NORMO ($N=2$) treatments. Animals were killed at 4 days following ICH. Only the temperature data were used for these animals.

Experiment 2: brain water content

Brain water content was measured by the wet–dry weight method. Briefly, rats were anesthetized (isoflurane) then euthanized via decapitation. The brain was quickly extracted and dissected into several regions of interest including the cerebellum, ipsi- and contralateral cortex, and ipsi- and contralateral striatum (MacLellan et al., 2006b). The cortical and striatal regions were from a 4 mm thick coronal section taken surrounding the injection site. These five brain regions were weighed (wet weight), baked at 100 °C for 24 h, and reweighed (dry weight). Water content was determined by: \[ \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \times 100. \]

The time course of edema was established in 30 rats of which 25 were subjected to an ICH and 5 were naïve controls. None of these animals had telemetry probes or cooling coils implanted. The ICH rats were killed at 1 to 5 days following ICH ($N=5$ each).

Three groups of rats were used to determine the effects of HYPO on cerebral edema at 72 h post-ICH as this corresponds to the peak of cerebral edema determined from the above time course experiment. The groups assessed were: HYPO-1 ($N=15$), NORMO ($N=15$) and a group that received an ICH, but no cooling coil or head cap or tethering to a swivel ($N=15$; 10 new rats + the 5 from the above time course experiment). The HYPO-1 and NORMO groups had cooling coils implanted, but telemetry probes were not used. Both groups were tethered to the swivel and only the HYPO-1 group had water perfused through the coil as done in Experiments 1 and 3.

Experiment 3: behavioral and histological outcome

All procedures were done by experimenters blind to group identity. Rats (NORMO: $N=18$; HYPO-1: $N=12$; HYPO-12: $N=12$) were trained in 3 tasks to assess behavior after ICH. Rats survived until 31 days after ICH at which time they were euthanized for histological assessment.

Single pellet task

The single pellet task (Whishaw, 2000) was chosen as it is sensitive to motor system damage including that caused in the whole blood ICH model (MacLellan et al., 2006c). For this test, rats were placed in a clear plexi-glass box (length = 60 cm; width = 14 cm; height = 35 cm) and trained to reach through a 1 cm wide opening to retrieve a food pellet (product F0021; Bio-Serv, Frenchtown NJ, USA) placed on the ledge in front of the opening. Rats were given 20 trials per day over 5 days per week for the 4 weeks preceding the ICH surgery. Early on in training a paw preference (dominant use of either the right or left forelimb) was established and subsequently the pellet was placed in an off-center position to encourage and further train the use of the dominant paw. A ‘successful’ reach was one in which the rat retrieved a pellet and placed it into its mouth in one reaching motion. A reach was a ‘failure’ if the rat failed to grasp the pellet, knocked it off the ledge, or was unable to withdraw the pellet and place it in its mouth. Baseline reaching success was calculated as the average of the last 4 training trials. An exclusion criterion was set at <40% baseline success. Excluded rats were nonetheless trained in this test and used in the rest of the study. Their data were just not included in the single pellet task analysis. Rats were tested on days 7–10 and 28–31 after ICH. Reaching success was defined as (number of successful retrievals/20) × 100.

Forelimb use asymmetry test

The cylinder test of forelimb use was used as it is sensitive to ICH-induced striatal injury (Hua et al., 2002; MacLellan et al., 2006a). Testing was conducted 4 days prior to ICH (baseline) and at 7 and 28 days after ICH. Briefly, rats were placed in a transparent cylinder (20 cm diameter, 45 cm high) for 10 min and their spontaneous forelimb use during wall exploration was determined from the initial placements of a forelimb on the wall and during subsequent lateral movements (Schallert and Woodlee, 2005). Independent forelimb use was expressed as:

\[ \text{(number of contacts with contralateral forelimb + 1/2 both)} \times 100. \]

\[ \text{(ipsilateral forelimb use + contralateral forelimb use + both)} \]
Horizontal-ladder walking test

The horizontal ladder-walking test (Metz and Whishaw, 2002) assesses walking ability and limb-placement of the 4 limbs as a rat traverses a 1 m long array of horizontal bars interspaced 3–5 cm apart. This test is sensitive to striatal injury (MacLellan et al., 2006a). Rats were videotaped crossing the middle 0.5 m segment and the total number of steps and slips made with each limb was determined for four trials per day during baseline (4 days prior to ICH) and at 7 and 28 days post-ICH.

Histology

Rats were euthanized 31 days following ICH with an overdose of sodium pentobarbital (85 mg/kg, i.p.) and were transcardially perfused with 0.9% saline followed by 10% formalin. Forty-micrometer coronal sections were taken every 400 μm using a cryostat and stained with cresyl violet. Using Scion Image J (v. 4.0) the area of the normal tissue was traced and measured in sections encompassing and extending beyond levels with injured brain. In this model of ICH, and at this survival time, the lesion and surrounding dead tissue is easily distinguishable from normal tissue. Lesion volume was calculated as routinely done (MacLellan et al., 2006b,c):

\[
\text{Volume of tissue lost} = \frac{\text{remaining volume of normal hemisphere}}{C_0} \times \frac{\text{distance between sections}}{C_2} \times \text{number of sections analyzed}
\]

Results

No rats died prior to the scheduled euthanasia time in any of these experiments. Three animals were entirely excluded from Experiment 3 due to a technical error with the cooling system (HYPO-1: N = 1; HYPO-12: N = 2).

Experiment 1

Baseline \(T_b\) and \(T_c\) were normothermic in all groups (data not shown). Likewise, \(T_b\) was normothermic following ICH in the NORMO group and up until cooling commenced in the HYPO-1 and HYPO-12 groups (Fig. 1C). The \(T_b\) averaged over the entire cooling period was 32.2 °C±1.6 (S.D.) and 31.5±1.4 °C in the HYPO-1 and HYPO-12 groups, respectively (ca. 37.2±0.5 °C in the NORMO animals). During this period \(T_c\) remained normothermic in all groups (averages ranged from 37.2 to 38.0 °C). Upon cessation of cooling, \(T_b\) re-warmed to normothermia in approximately 5 min in all animals.

Experiment 2

Brain water content (%) (Fig. 2A) significantly increased in the ipsilateral striatum after ICH (i.e., edema occurred), which was statistically significant (vs. naïve rats) on days 1–4 post-ICH (\(p \leq 0.031\)), but not on day 5 (\(p = 0.764\)). Edema peaked at day 3, although this time was not significantly different than days 1–4 post-ICH (\(p \geq 0.077\)) with Scheffé’s test.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>ICH (no treatment)</th>
<th>NORMO</th>
<th>HYPO-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.45±0.05</td>
<td>7.40±0.05</td>
<td>7.43±0.03</td>
</tr>
<tr>
<td>(\rho)CO₂ (mm Hg)</td>
<td>44.3±3.5</td>
<td>42.4±7.1</td>
<td>41.2±5.4</td>
</tr>
<tr>
<td>(\rho)O₂ (mm Hg)</td>
<td>109.9±15.2</td>
<td>116.6±19.3</td>
<td>123.4±11.6</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>15.0±1.7</td>
<td>14.5±1.2</td>
<td>14.5±1.2</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>9.3±2.4</td>
<td>10.0±3.1</td>
<td>10.2±1.2</td>
</tr>
</tbody>
</table>

All measurements were in the appropriate physiological ranges and did not significantly differ among groups (see Results for statistics).

Table 2

<table>
<thead>
<tr>
<th></th>
<th>NORMO</th>
<th>HYPO-1</th>
<th>HYPO-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.43±0.02</td>
<td>7.44±0.02</td>
<td>7.40±0.07</td>
</tr>
<tr>
<td>(\rho)CO₂ (mm Hg)</td>
<td>39.7±4.1</td>
<td>36.6±3.0</td>
<td>39.6±4.7</td>
</tr>
<tr>
<td>(\rho)O₂ (mm Hg)</td>
<td>127.4±21.8</td>
<td>134.1±16.9</td>
<td>129.4±16.1</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>15.6±0.7</td>
<td>15.1±1.3</td>
<td>15.5±0.7</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>9.6±2.3</td>
<td>9.8±3.1</td>
<td>9.9±2.6</td>
</tr>
</tbody>
</table>

All values were in the appropriate physiological ranges and did not significantly differ among groups (see Results for statistics).
significant with less stringent post-hoc tests (e.g., LSD). There was no significant difference among groups in water content in the cerebellum (group main effect: $p = 0.723$). Little to no edema occurred in other structures (data not shown).

The HYPO-1 group had a significantly ($p \leq 0.006$) less edema in the ipsilateral striatum (72 h; Fig. 2B) than the untreated ICH (no cooling coil) and NORMO (with an implanted cooling coil, but no cooling) groups, which were not significantly different ($p = 0.195$). There was no significant difference among groups in water content in the cerebellum (Group main effect: $p = 0.439$). Physiological variables were sampled during ICH in this experiment (Table 1), and all values were in the normal ranges and similar among groups ($p \geq 0.051$).

**Experiment 3**

Blood gases, hemoglobin, pH, and glucose were in normal ranges and did not differ significantly among groups (Group main effect: $p \geq 0.113$; Table 2).

The data for 6 rats were not included in the single pellet task analysis because they failed to reach criterion performance of an average of 40% success over the last four baseline testing sessions. Data from an additional 7 rats were not analyzed because they stopped reaching with their initially-preferred contralateral forelimb, and switched to using their ipsilateral forelimb for reaching after ICH. Regardless, these rats were trained and tested to avoid biasing the use of their cylinder and horizontal ladder test scores, which were analyzed. This left group sizes of: NORMO: $N=9$, HYPO-1: $N=9$ and HYPO-12: $N=7$ for the single pellet test. Data were blocked (averaged) into three sessions: 1) last 4 training days, 2) days 7–10 post-ICH, and 3) days 28–31 post-ICH (Fig. 3). A mixed ANOVA revealed a significant session effect ($p < 0.001$) as all groups performed significantly worse following ICH at both test times ($p < 0.001$); although there was a partial recovery from the second to the third session ($p < 0.001$). The Group main effect ($p = 0.714$) and Group×Session interaction ($p = 0.596$) were not significant. Thus, while this test is sensitive to ICH-induced injury, there were no beneficial effects of HYPO treatment.

For the cylinder test (Fig. 4), a mixed ANOVA revealed a significant Session effect ($p < 0.001$) as overall asymmetry scores were significantly lower for days 7 and 28 post-ICH ($p \leq 0.006$ vs. days 7–10 and 28–31 days post-ICH. All groups were significantly impaired following ICH, but there were no significant differences among groups (see Results for statistics).
baseline), which were not significantly different ($p=0.113$). The Group main effect ($p=0.505$) and Group \times Session interaction ($p=0.150$) were not significant. Thus, this test was sensitive to ICH-induced injury, but there were no beneficial effects of HYPO treatment.

The 4 limbs were analyzed separately for the horizontal ladder data (mixed ANOVA). The ipsilateral (to lesion) hind limb was not impaired following ICH, whereas the ipsilateral forelimb was transiently impaired (day 7 post-ICH). In both cases there were no Group or Group \times Session effects (data not shown). The contra-lateral limbs (Fig. 5) were markedly affected, and showed significant Session effects ($p<0.001$), but no Group ($p \geq 0.370$) or Group \times Session effects ($p \geq 0.142$). Stepping success scores were significantly lower on days 7 and 28 post-ICH ($p=0.821$). Thus, the horizontal ladder test was sensitive to ICH-induced injury, but it did not detect any benefit from HYPO treatment.

A representative diagram illustrating the lesion is given in Fig. 6A. Injury occurred around the needle tract (center of striatum) as well as to the dorsal and lateral striatum. The volume of tissue lost (Fig. 6B), which would account for neuronal and glial cell death, was not significantly different among groups (main effect: $p=0.948$). Lesion volume significantly correlated with average post-ICH performance in the single pellet ($r=-0.488$, $p=0.013$) and horizontal ladder tests ($r=-0.426$, $p=0.008$). The correlation between the cylinder scores and lesion volume was not significant ($r=-0.220$, $p=0.178$).

**Discussion**

Hypothermia is a proven, and perhaps unsurpassed, neuroprotector in global and focal cerebral ischemia. Even with overlapping mechanisms of injury between ischemic and hemorrhagic insults (Xi et al., 2006), HYPO failed to improve either early or late behavioral recovery and to lessen injury in the autologous blood infusion model of ICH in rats. This occurred regardless of having an improved treatment and study design over earlier work. Notably, we used a novel cooling system that affected only the injured hemisphere, thereby avoiding at least some of the complications (e.g., on systemic blood pressure) associated with systemic cooling (Clark and Colbourne, 2007). Second, rats were cooled for 4 days, which is substantially longer than other ICH studies, in order to overlap with the entire period of cerebral edema and to reduce it. Third, we tested early and late treatment interventions. Fourth, we used a sensitive battery of functional tests that should have detected improvements had there been any. Nonetheless, functional and histological benefits were not obtained.

These findings reinforce and extend those using systemic cooling in the whole blood model (MacLellan et al., 2006b) where 2 days of delayed, mild HYPO reduced edema and inflammation, but did not improve behavioral recovery or lessen brain injury. In that study cooling was delayed for 1 or 4 h after a comparable-sized ICH. An earlier study (MacLellan et al., 2004) using the collagenase model showed that 12-h delayed cooling was protective whereas 1- and 6-h delayed HYPO was not. This unexpected pattern was attributed to side effects of cooling (e.g., elevated blood pressure) that negated benefit through increasing bleeding when given soon, but not late, after ICH. Intraparenchymal bleeding continues over hours in the collagenase model, which does not happen in the whole blood model (MacLellan et al., in press). Therefore, it was hypothesized that early cooling would reduce injury in the whole blood model where continued bleeding is not an issue; however, neuroprotection was not found with a 1-h delayed HYPO treatment (MacLellan et al., 2006b). Our present findings confirm this and show that HYPO-12 treatment also fails to mitigate injury contrary to findings in the collagenase model (MacLellan et al., 2004). There are considerable differences between these rat ICH models in pathophysiology, such as the extent of inflammation (Xue and Del Bigio, 2003), and location and time course of injury (MacLellan et al., in press) that might explain these efficacy differences.

Systemic HYPO has been repeatedly shown to reduce edema in rodent models of ICH (Kawai et al., 2002; Kawanishi, 2003; MacLellan et al., 2006b). These studies have tended to use HYPO durations lasting 24–48 h. In Experiment 2 we assessed the effects of HYPO on the peak of edema, which occurred 3 days after ICH, and therefore we cooled for 3 days in that experiment. However, in Experiment 3 we cooled for 4 days in order to cover the entire period of edema (Experiment 2) and because longer bouts of
HYPO provide greater protection from ischemia (Colbourne et al., 1997) and TBI (Jiang et al., 2006). It should be noted, however, that the impact of varying treatment duration has not been rigorously examined in ICH models. Similarly, ICH studies have not systematically compared different depths of cooling. Instead, rodent studies typically have used only one depth of mild hypothermia in a study, which has ranged from 31 to 35 °C. One study (Wagner et al., 2006) selectively cooled the brain (cortex was 14 °C) to reduce edema, but they did not compare across a temperature range to determine whether this level was optimal. Finally, upon cessation of cooling, the Tc rapidly returned to normothermia. While it might be argued that slower re-warming is more effective, there are no data to determine whether re-warming rates are important after local brain cooling as they appear to be after systemic cooling. Indeed, the avoidance of systemic complications (e.g., heart rate and blood pressure changes) with local cooling (Clark and Colbourne, 2007) would suggest that rapid re-warming would be of little to no concern unlike following systemic cooling where cardiovascular effects are prominent (Schubert, 1995).

Notably, slow re-warming was used in our previous study (MacLellan et al., 2006b) that used systemic hypothermia following ICH, and that study found no histological or functional benefit. Thus, while it is possible that alternative HYPO regimens (depth, duration, cooling and re-warming rates) may have greater effects on edema as well as on cell death and recovery, this was beyond the scope of the present study.

Edema is a widely used endpoint in rodent ICH studies and is of great importance to patient survival. Therefore, it is possible that comparable reductions in edema, as presently found with HYPO, may reduce mortality in patients experiencing life-threatening brain swelling. This possibility cannot be easily assessed with the whole-blood model, as there was no mortality despite marked edema as others report (MacLellan et al., 2006b; Xi et al., 2001). Furthermore, we infused 100 μL of blood, which is considerably more than a hematoma size (~50 μL) that approximates the averaged-sized ICH in patients (Deinsberger et al., 1996). Use of greater injection volumes should increase mortality, at least in part due to greater edema; however, it is difficult to produce larger lesions as infusing additional blood would back up the needle tract. Accordingly, this model seems ill suited to testing this hypothesis.

One must also question whether it is possible to achieve much neuroprotection in the whole blood model leading one to question whether this is indeed the “whole blood rejection” model (p. 158, Xue and Del Bigio, 2003). Interestingly, many efficacy studies do not quantify cell death or lesion size, but rely upon edema measurements and simple tests of neurological impairment (neurological deficit scales — NDS; e.g., Nakamura et al., 2006). Furthermore, some treatments reduce edema and improve scores on these tests but fail to lessen brain injury (Belayev et al., 2007). A similar pattern of results has occurred in the collagenase ICH model where many treatments improve NDS scores but fail to lessen lesion volume (Peeling et al., 2001). One limitation of the present study is that we did not assess cell death (or inflammation, etc.) in the peri-hematoma region, thus making it possible that cooling had some beneficial effects. Regardless, any such effects, such as a hypothermia-induced attenuation of inflammation (MacLellan et al., 2006b), clearly did not translate into a discernable reduction in lesion volume or improved functional recovery.

The widespread lack of long-term assessment is an additional serious concern (Stroke Therapy Academic Industry Roundtable (STAIR), 1999) as treatments do not always provide lasting benefit (e.g., brief postischemic HYPO after global ischemia; Dietrich et al., 1993). Furthermore, a simple NDS is not the most sensitive measure of behavioral recovery after ICH owing to marked recovery (MacLellan et al., in press), and insensitivity to lesion size differences (MacLellan et al., 2006a). More sensitive tests, such as of skilled reaching ability (MacLellan et al., 2006a,c), are often not used in ICH studies. Therefore, those studies that have reported improvements on NDS may not have found benefit on more demanding measures (Peeling et al., 2001) such as the ones presently used. Conversely, benefit might have been found had we used a NDS scale or some other test. We did not use a NDS because some of the rats were treated with HYPO for the first 4 days after ICH thereby confounding early testing. We also opted to use tests that detect persistent deficits.

In summary, prolonged, mild, local brain HYPO significantly reduced edema but failed to improve behavioral outcome. These findings along with that from previous systemic HYPO studies, and experimental findings that mild hyperthermia does not worsen ICH outcome in rats (MacLellan and Colbourne, 2005), suggest that ICH-induced injury is not particularly temperature sensitive. Nonetheless, ICH-induced edema is clearly amenable to HYPO therapy and this warrants further consideration. Furthermore, it is possible that other HYPO regimens may provide protection in this or other ICH models that more closely mimic ICH in humans.

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