Brief Hyperthermia does not Worsen Outcome After Striatal Hemorrhage in Rats

Mark Penner¹, Gergely Silasi², Shannon Wowk¹, Lindsey Warkentin¹ and Frederick Colbourne *¹,²

¹Department of Psychology, University of Alberta, Edmonton, Alberta
²Center for Neuroscience, University of Alberta, Edmonton, Alberta

Abstract: Hyperthermia accelerates and increases ischemic brain damage. Owing to overlapping mechanisms of injury, many assume that hyperthermia also worsens outcome after intracerebral hemorrhage (ICH). However, clinical data do not conclusively prove this, and there is only one animal study examining the impact of hyperthermia. In that study (MacLellan and Colbourne, 2005), several hyperthermia protocols were administered after collagenase-induced ICH in rats; none worsened injury. While the collagenase model is widely used, it differs in important ways from another common model – injecting autologous blood directly into the brain. Thus, we evaluated the impact of immediate hyperthermia (HYP, 39 °C for 3 hr) after a 100-µL infusion of blood into the striatum of rats. This treatment, which markedly increases ischemic damage, was compared to control rats kept normothermic (NOR, 37 °C). Three separate experiments were done to measure: 1) edema at 24 hr, 2) edema at 72 hr, and 3) behavioral impairment and lesion size out to 1 month post-ICH. The HYP treatment did not significantly affect edema at 24 hr, but surprisingly, it modestly reduced edema at 72 hr and partly improved behavioral outcome. However, there were no last effects of HYP on behavior (e.g., skilled reaching) or the volume of tissue lost (NOR: 14.0 mm³ vs. HYP: 14.5 mm³). In summary, our findings do not support the common belief that hyperthermia worsens outcome after ICH. Additional research is needed to determine whether more severe or prolonged heating or fever and its cause (e.g., infection) affect morbidity and mortality after ICH.

Keywords: Stroke, intracerebral hemorrhage, hyperthermia, temperature, striatum, rat.

INTRODUCTION

Intracerebral hemorrhage (ICH) is a devastating stroke resulting in high mortality and considerable disability [1]. Despite a growing understanding of its pathophysiology [2] no clinically-proven neuroprotective treatments exist. Nonetheless, aggressive medical management appears to improve outcome [3], but whether and how specific physiological factors influence outcome remains unclear. Notably, many ICH patients become febrile [4-7], which current guidelines recommend treating to maintain normothermia. This practice is based upon limited clinical data. Indeed, some studies report that fever is detrimental after ICH [8, 9], whereas others find no causative relationship between fever and outcome [5, 6].

In contrast, clinical studies repeatedly show that fever is an independent predictor of mortality and poor outcome after cerebral ischemia [10-13]. While antipyretic treatments have not yet been proven to help [14], there are several randomized controlled trials confirming the benefits of mild hypothermia for cardiac arrest in adults [15, 16] and hypoxic-ischemic encephalopathy in newborns [17]. Clinical studies have not yet determined efficacy after ischemic stroke [18], but there are numerous animal studies showing that hypothermia mitigates ischemic injury whereas hyperthermia worsens it [19-22].

For ICH, clinical trials suggest that hypothermia will be helpful [23], but large randomized controlled trials are needed. Furthermore, animal experiments show that while hypothermia is somewhat beneficial, there is considerably less protection than in ischemia [19]. Thus, despite overlapping mechanisms of injury, ICH appears to be relatively temperature-insensitive compared to various cerebral ischemic insults. Accordingly, it may be premature to assume that hyperthermia / fever will equally affect outcome after hemorrhagic and ischemic stroke. To date, there is only one published study on hyperthermia following experimental ICH [24]. That study used a bacterial collagenase model of ICH to study several hyperthermia conditions. In two groups, hyperthermia (38.5 °C) was maintained for 24 hr and initiated either immediately or at 24 hr post-ICH. In a third group, hyperthermia (40 °C) was initiated 24 hr after ICH and maintained for 3 hr. These groups were compared to a normothermic control group. Each of these hyperthermia protocols had no effect on functional outcome, the number of peri-hematoma macrophages, or lesion volume. In contrast, previous experiments using similar hyperthermia treatments clearly demonstrate that they aggravate and accelerate ischemic and traumatic injury [25-29].

In order to further study the role of hyperthermia following ICH, we currently used the autologous whole-blood model of ICH [30]. There are several key differences between this model and the collagenase model (e.g., bleeding rate, inflammatory response, and extent of injury), and between these models and humans (e.g., white matter involvement) that necessitate using multiple animal models [31, 32]; a practice that is widely recommended in the stroke field [33]. In this study, we completed 3 experiments to
evaluate whether 3 hr of post-ICH hyperthermia (39 °C, HYP) affects outcome compared to normothermic controls (NOR). In experiment 1, edema was measured at 24 hr post-ICH. In experiment 2, behavior was evaluated and edema was determined at 72 hr post-ICH. In the third experiment, groups were evaluated with additional sensitive behavioral tasks over 1 month post-ICH at which time we determined the volume of tissue lost. Use of a long survival time and behavioral assessment is a necessary step in preclinical assessment [33]. We hypothesized, based upon findings in the collagenase model [24], that hyperthermia would have little impact on edema, behavioral dysfunction and final lesion size.

**MATERIALS AND METHODS**

**Subjects**

All procedures were approved by the Animal Care and Use Committee (Biosciences) at the University of Alberta. Procedures also conformed to Canadian Council on Animal Care guidelines. We used 98, male, Sprague Dawley rats weighing between 200 and 350 g at study onset. They were housed in a humidity and temperature-controlled room and maintained on a 12-hr light-dark cycle (on: 07:00 – 19:00). Rats were given free access to standard rodent food and water, except during food restriction occurring 2 days prior to and during single pellet reach training and testing.

Rats were randomly assigned to either the HYP or NOR control group for all experiments. In experiment 1, brain water content (BWC) was measured at 24 hr (n = 21 per group). In experiment 2, we measured BWC at 72 hr (n = 15 per group) in addition to assessing functional outcome using several behavioral tasks. In experiment 3, we assessed long-term (32 days post-ICH) behavioral and histological outcome (n = 13 per group). Experimenters blind to group assignment analyzed all data.

**Surgical Procedures**

All surgical procedures were done in rats under aseptic conditions with isoflurane anesthesia (4% induction; 2.0 - 2.5% maintenance in 60% N2O, balance O2). Each rat had a telemetry probe (model TA10TA-F40; Transoma Medical, St. Paul, MN, USA) implanted into the peritoneal cavity [34]. Briefly, a 2 cm off-midline incision was made through the skin and underlying abdominal muscle. The sterilized telemetry probe was inserted and the abdominal muscle and fascia were sutured closed. Marcaine (Sanofi Canada, Markham, Ontario) was infiltrated underneath the scalp, which was subsequently cut to make two burr holes in the skull at 3.5 mm lateral to Bregma, one on each side. One was used for injecting the blood whereas the other was to hold a guide cannula. A third burr hole was drilled 5 mm posterior to the guide cannula and a metal screw (model MX-080-2; Small Parts, Miami Lakes, FL) was inserted for support. The cannula was held in place by applying dental cement around the cannula and screw. Once the cannula was secure, we lowered a 30 gauge thermocouple probe (HYP1-30-1/2-T-G-60-SMPW-M, Omega, Stanford, Conn.) 5 mm below the surface of the skull in the contralateral-to-ICH striatum.

The tail artery was catheterized to monitor mean arterial blood pressure (MABP), pH, pCO2, pO2, hemoglobin and glucose (model ABL810, Radiometer, Copenhagen) and to obtain blood for the intraparenchymal infusion. This was infused via a 26 G needle into the striatum (AP: 0; ML: 3.5; DV: 6.5 mm). We injected 100 µL of arterial blood over ten minutes. In experiments 1 and 2 this was done in the left hemisphere whereas in experiment 3 we injected on the side contralateral to paw preference as determined in the single-pellet reaching task. The needle remained in place for 10 min before being slowly raised to help prevent blood from going up the needle tract. Afterwards, the holes were sealed with metal screws.

**Temperature Manipulation**

While still anesthetized, the rats were subjected to NOR or HYP treatments. In the latter, the rats’ brain temperature was elevated to 39°C with a heating pad and overhead infrared lamp (250 W) starting immediately after blood infusion. Their temperature was maintained for 3 hr. Hyperthermia was induced under anesthesia because it allowed for precise and easy control while physiological variables were measured. We initiated hyperthermia immediately after ICH because this protocol has been repeatedly shown to aggravate ischemic and traumatic brain injury [25, 28, 29, 36]. Afterwards, the rats were allowed to spontaneously cool over 30 min. The total anesthetic time was less than 5 hr. The NOR group remained at 37 ºC throughout anesthesia (same duration as HYP). We then sutured the tail and head wounds and applied Marcaine. We discontinued anesthesia and placed rats in their home cage to monitor body temperature via telemetry. Rats were weighed each day and given a mixture of rat chow, peanut butter, and honey for the first few days to minimize weight loss.

**Brain Water Content (Experiments 1 and 2)**

We assessed BWC at 24 hr post-ICH in experiment 1 and at 72 hr in experiment 2. Brains were extracted after anesthetization and decapitation. A thick coronal section from 2 mm anterior to 2 mm posterior to the site of injection was taken and separated into cortex and striatum for each side. The whole cerebellum served as a control. We calculated BWC as follows: BWC = ((Wet Weight - Dry Weight) / Wet Weight) × 100 as routinely done [35]. Wet weight was the measurement taken prior to baking at 100 °C for 24 hr. The
Dry weight was the measurement taken after baking to remove all water.

Behavioral Evaluation

Neurological Deficit Scale

We used a modified version of the neurological deficit score (NDS) scale, which is sensitive to striatal ICH [37, 38]. The tasks included beam walking (scored 0 to 3), spontaneous circling (scored 0 to 3), bilateral forepaw grasp (scored 0 to 3), hind limb replacement (scored 0 to 3), forelimb flexion (scored 0 to 2), and vibrissae-elicited forelimb placing. For forelimb placing, rats were given a score of 0 (10/10 correct placements), 1 (6 to 9 correct placements), 2 (1 to 5 correct placements), or 3 (0 correct placements). A score of 17 indicated maximum impairment. A baseline NDS score was collected 2 days prior to ICH. Post-ICH testing was done on days 1, 2 and 3 in experiments 2 and 3 whereas rats in experiment 3 were also evaluated on days 11 and 32.

Cylinder

Limb use asymmetry [37, 39] during wall exploration of a vertical cylinder was evaluated in experiments 2 and 3. Briefly, rats were placed in a clear Plexiglas cylinder (45 cm in height; 20 cm in diameter) for 10 min while their behavior (paw contacts with wall) was videotaped and subsequently analyzed. An asymmetry score was calculated as follows: (contralateral forelimb contact + ½ both) / (ipsilateral forelimb contact + contralateral forelimb contact + both) × 100. Rats normally score around 50% whereas those with striatal ICH show diminished use of the contralateral forelimb. In order to avoid erroneously extreme values, we excluded rats from this analysis, but not from the rest of the study, when they did not display at least 10 independent wall touches in the cylinder. A baseline measure was taken 2 days prior to ICH. They were tested in this apparatus at 3 days post-ICH in experiment 2 whereas testing was also done on days 11 and 32 in experiment 3.

Horizontal Ladder Test

Walking ability was evaluated with the horizontal ladder test [40], which is sensitive to striatal ICH [37]. Briefly, rats traversed a horizontal ladder (1 m) made up of variable spaced steel rungs (3 - 5 cm) oriented perpendicular to the rats’ direction of travel. The number of steps and slips were calculated and averaged over 4 sessions. However, rats that did not cross the ladder at least twice were excluded from this analysis. Baseline performance with the contralateral-to-ICH forelimb was determined 2 days prior to ICH and testing was done at 3 days post-ICH in experiment 2 whereas those in experiment 3 were also evaluated on days 11 and 32.

Corner Turn Test

The corner turn test, which is sensitive to striatal ICH, was used to assess turn preference in experiment 3 [39, 41]. Briefly, rats were placed in front of two Plexiglas walls (41 cm in height; 30.5 cm in length) separated by 1 cm at a 30° angle. The direction turned to exit the corner was recorded with previous findings showing that normal rats usually exhibit no bias whereas rats with striatal injury turn ipsilateral. Two days of baseline testing (10 trials per day) were done before the ICH, and we excluded rats from this analysis that had a baseline turn preference of > 70% in either direction. Rats were tested on days 3, 11 and 32 post-ICH (10 trials per testing session).

Single Pellet Reaching

Skilled reaching ability, affected by striatal ICH [37, 42], was determined in the single pellet task [43]. For training and testing the rats were first food restricted to 90% of free-feeding body weight. Rats were placed in a clear Plexiglas box (60 cm long, 14 cm wide, 35 cm high) and trained to reach through a 1 cm wide vertical slot to grab a food pellet (45 mg; Bio-Serv, Frenchtown, NJ, USA) sitting in a well on a shelf in front of the opening. Paw preference was quickly determined and then the rats were trained to reach over 20 days (5 days a week for 4 weeks; 25 trials per day). A successful reach was achieved when the rat grabbed the pellet and brought it to its mouth in one motion. An unsuccessful reach occurred when the pellet was knocked from the well, or the rat failed to grasp the pellet or failed to bring the pellet to its mouth. The final four days of training were considered their baseline performance. Testing occurred on days 7 – 10 and 28 – 31 post-ICH. We averaged performance for each period. Overall success rate was defined as: (number of successful reaches / trials) × 100. Rats that did not successfully reach on 40% of the trials were excluded from this analysis.

Histology

Rats in experiment 3 were euthanized with an intraperitoneal injection of sodium pentobarbital (100 mg/kg) at 32 days post-ICH. They were transcardially perfused with 0.9% saline followed by 10% formalin. Coronal sections (40 µm every 400 µm) were taken with a cryostat and stained with cresyl violet. Scion Image J 4.0 (Scion Corporation, Frederick, MD, USA) was used to calculate total tissue loss, which includes lesion volume and ventricular dilation, from digitally scanned slides. The volume of each hemisphere was calculated as: area of remaining tissue × distance between sections × number of sections analyzed. The volume of tissue lost was calculated as: remaining volume of normal hemisphere – remaining volume of injured hemisphere as previously done [24, 37]. Lesion volume was used instead of a measure of peri-hematomat cell death because the latter is normally complete much earlier in the whole blood model [44], which unlike the collagenase model of ICH, does not show ongoing striatal tissue loss over weeks [45].

Statistical Analysis

All data are presented as mean ± SEM except for the NDS data, which is reported as medians. Tissue loss and physiological variables (e.g., body temperature) were analyzed with one-way ANOVA (SPSS v. 17.0; SPSS Inc., Chicago, IL, USA). The corner turn, single pellet, horizontal ladder, and forelimb asymmetry test data were analyzed with repeated measures ANOVA. We controlled for multiple comparisons with the Tukey HSD post-hoc test. We analyzed the ordinal NDS data with Mann-Whitney U tests.
RESULTS

Experiment 1

One rat (NOR) died during surgery of unknown cause. There were no other exclusions.

Physiological variables were kept within the normal physiological range, but there were some trivial but statistically significant differences (Table 1). Temperature during surgery was regulated as desired and similar among groups afterwards (Fig. 1). Notably, rats were normothermic after ICH, without any evidence of fever. Furthermore, all rats displayed normal temperature profiles prior to surgery (data not shown).

Table 1. Physiological Variables, Including Blood pH (Temperature Corrected), pCO₂ (mmHg, Temperature Corrected), pO₂ (mmHg), ctHb (g/dL), MABP (mmHg) and Body Weight (g), for Experiment 1, 2 and 3. Data are Presented as Mean ± SEM (* Denotes p ≤ 0.05)

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
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<td>pH</td>
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<tr>
<td>pO₂</td>
<td>130.0 ± 10.0</td>
<td>136.0 ± 13.6</td>
<td>126.3 ± 7.7*</td>
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<td>9.2 ± 1.4</td>
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<td>ctHb</td>
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<td>MABP</td>
<td>86.7 ± 10.7</td>
<td>88.5 ± 17.8</td>
<td>93.9 ± 8.1</td>
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<td>84.3 ± 17.1</td>
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<td>Surgery Weight</td>
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<td>364.5 ± 10.5</td>
<td>413.0 ± 14.9</td>
<td>406.4 ± 17.0</td>
<td>391.7 ± 8.7</td>
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<td>Euthanasia Weight</td>
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<td>389.3 ± 13.3</td>
<td>381.0 ± 14.5</td>
<td>472.3 ± 11.9</td>
<td>460.7 ± 14.2</td>
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Fig. (1). Average rectal and core temperatures (°C) during and after surgery for experiment 1. Rectal temperature was recorded during surgery, whereas core temperature was subsequently measured with a telemetry probe (sampled every 30 seconds). Temperature profiles from experiments 2 and 3 (not shown) were similar to experiment 1. Brain temperature was measured only during surgery (via thermocouple probes, see Methods) and kept at the desired level (NOR: 37°C; HYP: 39°C).

Brain water content was measured at 24 hr post-ICH. Edema (higher BWC vs. contralateral side) was present in cortex \((F_{(1,38)} = 17.060, \ p < 0.001)\) and striatum \((F_{(1,38)} = 31.030, \ p < 0.001)\). However, there were no group differences in the ipsilateral striatum \((F_{(1,38)} = 0.881, \ p = 0.354)\), ipsilateral cortex \((F_{(1,38)} = 0.104, \ p = 0.748)\), contralateral striatum \((F_{(1,38)} = 0.760, \ p = 0.389)\), contralateral cortex \((F_{(1,38)} = 0.008, \ p = 0.354)\) or cerebellum \((F_{(1,38)} = 0.046, \ p = 0.832)\) as shown in Fig. (2A). Thus, the HYP treatment did not significantly affect cerebral edema at 24 hr.

Fig. (2). Brain-water content (BWC) in the striatum, cortex, and cerebellum (mean ± SEM) at 24 (A) and 72 hr post-ICH (B). The BWC was determined by the wet-dry weight method. Hyperthermia (vs. NOR) significantly reduced BWC (edema) in the striatum at 72 hr post-ICH (*p ≤ 0.05); all other group comparisons were not significant, and the control regions (e.g., cerebellum) had the expected and normal BWC values.
Experiment 2

One rat died during surgery (unknown cause) and one was excluded due to processing error, both in the HYP group. Group sizes were reduced further for the horizontal ladder (NOR = 14, HYP = 12) and cylinder tests (NOR = 10, HYP = 8) due to failure of some rats to reach criterion levels of performance.

Physiological variables were similar between groups with one small but statistically significant difference (Table 1). Temperature was controlled as desired and similar to experiment 1 (data not shown).

Edema occurred in the ipsilateral cortex ($F_{(1,26)} = 75.997$, $p < 0.001$) and striatum ($F_{(1,26)} = 44.428$, $p < 0.001$) as shown in Fig. (2B). The HYP treatment surprisingly reduced BWC at 72 hr post-ICH in the ipsilateral striatum ($F_{(1,26)} = 4.430$, $p = 0.045$), but it had no effect on the ipsilateral cortex ($F_{(1,26)} = 3.381$, $p = 0.077$), contralateral cortex ($F_{(1,26)} = 0.007$, $p = 0.935$), contralateral striatum ($F_{(1,26)} = 1.463$, $p = 0.237$) or cerebellum ($F_{(1,26)} = 1.547$, $p = 0.225$).

Unexpectedly, the HYP group had better (lower) NDS scores than the NOR group on days 1 ($p = 0.056$, nonsignificant), 2 ($p = 0.040$) and 3 ($p = 0.006$) as shown in Fig. (3A). There was no difference prior to ICH. A repeated-measures ANOVA was used to analyze the contralateral

Fig. (4). Stepping error rate (mean ± SEM) in the horizontal ladder test for experiments 2 (A) and 3 (B). For this test, rats walk across a series of parallel bars and the slip rate (paw falls below the bars) is measured. Baseline data were collected prior to ICH and, as expected, groups were similar (i.e., normal performance). The ICH caused a significant increase in slips in the NOR group, which HYP treatment significantly reduced on day 3 post-ICH (*$p \leq 0.05$). In Experiment 3, the error rate spontaneously improved over time in the NOR group and there were no significant effects of HYP beyond day 3.

and day 3 data, with the HYP group making significantly less errors on day 3 post-ICH than NOR rats ($F_{(1,24)} = 11.905$, $p = 0.002$). There were no baseline differences between groups ($F_{(1,24)} = 1.846$, $p = 0.187$). There was a main effect of Time ($F_{(1,16)} = 13.472$, $p = 0.002$) in forelimb asymmetry (cylinder) task where each group used their contralateral-to-ICH forelimb less on day 3 post-ICH relative to baseline (data not shown). However, there was no Time × Group interaction ($F_{(1,16)} = 4.267$, $p = 0.055$) or Group main effect ($F_{(1,16)} = 2.189$, $p = 0.158$).

Experiment 3

Three rats were excluded due to unexpected mortality (HYP = 2, NOR = 1) during surgery or in the days following ICH. Additional rats were excluded in the ladder test (1 per
Physiological variables were similar between groups (Table 1). Temperature was controlled as desired and similar to experiment 1 (data not shown).

Neurological deficits (Fig. 3B) were not significantly different between groups at any time (p ≥ 0.121). For the horizontal ladder test (slip error rate), there was a significant Time × Group interaction ($F_{(3,57)} = 3.938, p = 0.013$) as shown in Fig. (4B). Further analysis showed that HYP treatment caused fewer forelimb slips on day 3 post-ICH ($F_{(1,19)} = 4.647, p = 0.044$), but not on the other testing days (p ≥ 0.129). In the corner turn test (Fig. 5A), there was a significant Time main effect ($F_{(3,63)} = 5.814, p = 0.001$). Rats showed an ipsilateral turning preference on day 3 (p = 0.003) and day 11 post-ICH (p = 0.008) but not on day 32 post-ICH (p = 0.230) relative to baseline. There was no Time × Group interaction ($F_{(3,57)} = 0.913, p = 0.440$) or Group main effect ($F_{(1,19)} = 0.012, p = 0.915$). In the single pellet reaching task (Fig. 5B), the Time main effect was significant ($F_{(2,32)} = 15.436, p < 0.001$). Indeed, rats were significantly impaired in skilled reaching during days 7 - 10 (p = 0.002 vs. baseline) and 28 – 31 post-ICH (p = 0.023). In contrast, neither the Time × Group interaction ($F_{(2,32)} = 2.037, p = 0.147$) nor the Group main effect ($F_{(1,10)} = 0.625, p = 0.441$) was significant. The cylinder task analysis showed a significant Time main effect ($F_{(3,57)} = 7.844, p < 0.001$). Rats used their contra-lateral-to-ICH forelimb less on days 3 (p = 0.005 vs. baseline) and 11 post-ICH (p = 0.010), but not on day 32 post-ICH (p = 0.122). However, there was no Time × Group interaction ($F_{(3,57)} = 1.348, p = 0.268$) or Group main effect ($F_{(1,19)} = 0.307, p = 0.586$; data not shown).

The ICH caused injury to the striatum and ipsilateral corpus callosum as previously observed and illustrated in Fig. (6A). However, HYP treatment did not significantly alter lesion volume at a 32 day survival ($F_{(1,21)} = 0.008, p = 0.929$) as shown in Fig. (6B).
DISCUSSION

As reviewed by many (e.g., [20, 46]), hyperthermia significantly accelerates and worsens ischemic and traumatic brain injury. In contrast, we report that 3 hr of hyperthermia (39 °C) induced immediately following ICH does not worsen edema, behavioral recovery or lesion size in the whole blood model. These findings support our previous work using the bacterial collagenase model of striatal ICH [24]. Considered together with studies showing modest to no benefit with therapeutic hypothermia in these ICH models [19], one is led to believe that ICH is a relatively temperature-insensitive insult unlike cerebral ischemia. Accordingly, animal findings suggest that preventing moderate bouts of hyperthermia will have little clinical impact.

There are important limitations with this study, however, that must be considered. First, more substantive episodes of hyperthermia and especially fever may be harmful. For instance, fever from an infection correlates with higher body temperature and worse mortality for ischemic patients [22]. Second, this and our previous study [24] were done in young male rats. Age [47-49] as well as gender and hormones [50, 51] influence outcome after ICH. Accordingly, these factors may worsen the impact of hyperthermia / fever. Finally, there are important differences between rodent models and humans [32], such as the extent of white matter involvement, the location of injury and presence of co-morbidities, that may alter responsiveness to any treatment, including temperature. Another notable difference is that our rats did not spontaneously experience fever following ICH, confirming earlier studies using telemetry measurements in these rat ICH models [24, 35, 52]. Thus, lack of spontaneous fever in rodent experimental ICH models is an important limitation to consider. Unfortunately, while some simple manipulations, such as lipopolysaccharide administration, can induce fever in rodents, it minimally affects temperature in ICH rats (MacLellan and Colbourne, unpublished data) and does not match that found in ICH patients. Thus, the critical questions of whether fever worsens while anti-pyretic treatments improve outcome are not so simply addressed in the autologous blood and collagenase models.

The observations of slightly (statistically significant) improved behavioral scores (NDS, ladder test) in the HYP group soon after ICH were surprising. One possible explanation is that HYP rats had significantly less edema on day 3 and this transiently improved some behavioral scores. The lack of long-term functional benefit was not surprising given that lesion volume was identical in both groups. Our study did not determine how HYP treatment facilitates edema resolution, but it might relate to the well known effect of hyperthermia augmenting immune surveillance [53, 54]; thus, the removal of erythrocytes and dead tissue may have been accelerated after ICH. Indeed, stimulating phagocytosis by macrophages and microglia with drug treatment has been shown to improve outcome in mice subjected to ICH [55]. As well, the early HYP treatment may have affected many other processes (clotting rate, heat shock protein response, etc.) that then mitigated downstream mechanisms of injury. Regardless of mechanism(s), no lasting therapeutic effect was observed.

Animal studies have also shown that cooling significantly reduces edema, inflammation and blood brain barrier disruption, but unfortunately these effects result in modest or no behavioral benefit [35, 52, 56-59]. There is either a complex, perhaps ‘n’-shaped, relationship between temperature and edema (behavior), or the differences among studies relate to other factors such as the timing and duration of temperature manipulation (brief hyperthermia vs. prolonged cooling). Regardless, the reductions in edema found in experimental temperature studies have not resulted in much behavioral benefit. Unfortunately, we do not know whether attenuating edema in rodent models accurately predicts the benefits of reducing edema in humans.

We used a battery of behavioral tasks as no one test perfectly predicts striatal injury after ICH [37, 60]. While each test used was previously shown to detect striatal damage in this model, the pattern of results varied among tests and experiments suggesting that chance variability might be a factor. Thus, we combined data from experiments 2 and 3 to determine whether there were any significant effects on the NDS and ladder tests. When these data were pooled, HYP treatment still significantly reduced stepping error rate in the ladder test while attenuating neurological deficits at day 3 post-ICH, consistent with the lower edema values found at this time in the second experiment. Regardless, no other protective effects were observed, including in the single pellet test, which is arguably one of the most sensitive tests for this model [42]. Thus, we conclude that early HYP treatment modestly and transiently improves some behavioral scores.

In summary, our current findings along with our earlier work in the collagenase model show that moderate bouts of hyperthermia do not notably affect outcome after ICH. Accordingly, they suggest that anti-pyretic drug treatment or use of other methods to enforce normothermia (e.g., cooling blankets) may not benefit ICH patients who experience mild to moderate elevations in body temperature. However, the cause, timing, magnitude and duration of temperature elevation must be considered in future animal and clinical studies in order to identify conditions where temperature control may be helpful. Further, an enhanced ability to select patients that should benefit from temperature control will minimize adverse events associated with temperature control.

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