Research Report

Delayed localized hypothermia reduces intracranial pressure following collagenase-induced intracerebral hemorrhage in rat

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A B S T R A C T

Brain injury, such as from intracerebral hemorrhage (ICH), causes edema and raises intracranial pressure (ICP) – a potentially life-threatening complication. Clinical studies suggest that therapeutic hypothermia (TH) reduces edema and ICP after ICH. Similarly, animal studies show that TH can sometimes reduce edema, but whether ICP would be attenuated is not known. Here we tested whether 24-h delayed TH reduces edema and ICP in rats with severe striatal ICH (collagenase model). First, we showed that ICH increased epidural ICP (mean of 18 vs. 6.5 mm Hg in controls), measured via telemetry. Second, we confirmed that delayed TH did not affect hematoma size at 7 day (C2465 vs. C2461 mm Li n controls). A cranial cooling device lowered striatal temperature to C24331 C from 24 to 72 h after ICH. Third, we compared normothermic rats to those with TH that were rewarmed immediately or over 6 h. Both TH protocols significantly reduced average and peak ICP by the second treatment day, and benefits persisted after rewarming. However, TH with slow rewarming failed to mitigate edema at 96 h (83.2% vs. 83.6% in controls) whereas rapid rewarming worsened edema (85.7%). Finally, we compared normothermic and TH rats without rewarming and found no impact on edema at 72 h (~81%). In summary, it appears that 24-h delayed local TH lowers ICP by a mechanism other than edema. Rapid rewarming worsens edema after local cooling, but this did not markedly impact ICP. Thus, TH should reduce ICP in patients with severe ICH, but not necessarily through mitigating edema.

1. Introduction

Intracerebral hemorrhage (ICH) is a devastating subtype of stroke resulting from the rupture of an intraparenchymal blood vessel. This event accounts for ~15% of all strokes and it has a 30-day mortality rate of ~40% (Qureshi et al., 1999). Primary injury from the mechanical trauma of blood spilling into the brain, and numerous secondary mechanisms of injury combine to cause considerable neurologic impairment that leaves many disabled (Wang, 2010; Balami and Buchan, 2011). Outcome is worse in those with large hemorrhages, and in part, this is because of pathological intracranial pressure (ICP) levels that result from the hematoma mass coupled with edema growth (Zazulia et al., 1999; Fernandes et al., 2000). Thus, it is thought that reducing edema will
lesser ICP thereby improving survival and recovery, especially in those with severe ICH. It is because of this and the assumed utility of edema to act as a surrogate marker of injury that many use edema as a primary endpoint in animal ICH neuroprotection studies (MacLellan et al., 2012).

Intracerebral hemorrhage is most commonly produced in rat striatum by either inducing autologous whole blood or bacterial collagenase, each having advantages and disadvantages (Andaluz et al., 2002; MacLellan et al., 2012). Notably, they both reproduce key features of ICH including blood-brain barrier damage, edema, inflammation, cell death, etc. However, the models are different with regard to the nature of bleeding, extent of injury, and the level of impairment, even with comparable hematoma volumes (MacLellan et al., 2008). As well, the collagenase model results in more edema and a higher ICP than that found after infusing a roughly comparable volume of blood into the striatum of rat (Hilploylee and Colbourne, 2014). The course of edema is roughly similar between these models with a peak occurring around the 2nd or 3rd day and complete resolution within about a week (Yang et al., 1994; Fingas et al., 2007; Kraft et al., 2014). In patients, edema growth is highest in the first 48 h and peaks up to 2–3 weeks, but there is some controversy on whether edema independently predicts outcome (Zazulia et al., 1999; Arima et al., 2009; Venkatasubramanian et al., 2011) with more recent studies suggesting edema does have an independent negative effect (Appelboom et al., 2013; Murthy et al., 2015; Volbers et al., 2015; Yang et al., 2015). In addition to edema, other factors determine ICP (e.g., cerebrospinal fluid (CSF) production), so a change in edema may not necessarily translate into comparable changes in ICP. Animal studies in focal ischemia and ICH support this contention (Hilploylee and Colbourne, 2014; Murtha et al., 2015).

Numerous animal studies in global and focal ischemia and hypoxia showed that therapeutic hypothermia (TH) is neuroprotective (van der Worp et al., 2007, Tagin et al., 2012), and these data predicted the translation of this treatment to neonatal hypoxia and cardiac arrest in adults (Bernard et al., 2007; Tagin et al., 2012). Currently, 2 ongoing clinical trials are assessing the efficacy of TH in acute ischemic stroke (Hemmen et al., 2012; van der Worp et al., 2014). Induced TH is a multimodal treatment strategy. In ICH, it mitigates perihematoma edema by targeting several underlying mechanisms of injury such as inflammation and blood-brain barrier damage along with other potentially protective effects (MacLellan et al., 2006; Wagner et al., 2006; Kawanishi et al., 2008; Sun et al., 2013). In comparison to historical controls, Kollmar et al., 2010 reported that mild (35 °C) endovascular cooling for up to 10 days prevented the progression of edema and improved outcome in patients with large spontaneous ICH. These findings prompted the current Cooling in Intracerebral Hemorrhage (CINCH) trial, which is assessing the efficacy of TH in reducing edema and ICP after ICH (Kollmar et al., 2012). Animal models also show that TH can often lower edema after an ICH (Kawanishi, 2003; Wei et al., 2013), but it is not known whether ICP rises are mitigated.

Choice of cooling methods and treatment parameters are obviously important to patient comfort, determining the extent of protection, and the risk of side effects. For instance, localized TH should cause fewer complications (e.g., shivering) than whole body cooling. Complications that arise during the induction, maintenance or rewarming phases can detract from the neuroprotective potential of TH. Notably, experimental and clinical data in ischemia and traumatic brain injury show that rewarming is a critical factor because fast rewarming can cause sudden vasodilation, reduced cerebral perfusion pressure, rebound increases in ICP and excitotoxicity (Polderman, 2009). Thus, from these findings it is assumed that fast rewarming would also negatively impact the effects of TH after an ICH. As such, recent clinical trials with TH, including for ICH, use slow rewarming rates such as 0.1 °C/h (Hemmen et al., 2012; van der Worp et al., 2014). However, the optimal course of rewarming is not yet known for ICH or for focal cooling.

In this study, we evaluated the hypothesis that brain-selective cooling reduces edema and ICP in adult rats subjected to a large collagenase-induced ICH. We predicted that fast rewarming would be harmful by further elevating edema and ICP. We used the collagenase model because earlier work in our lab (Hilploylee and Colbourne, 2014) identified an appropriate dose to produce severe edema and high ICP, which reflects our target clinical population. We measured epidural ICP using transmitters implanted in freely moving rats (Silasi et al., 2009; Hilploylee and Colbourne, 2014). Focal TH was used as it is thought to be safer than systemic TH, as discussed, and there is clinical interest in such approaches. We used a 48 h period of mild TH based upon efficacy studies in animal models of global and focal ischemia (Clark et al., 2009) and similar protocols are used in patients (Abdullah and Husin, 2011; Rincon et al., 2014; Su et al., 2015). However, treatment onset was delayed for 24 h because localized TH can aggravate intraparenchymal hemorrhaging even when administered up to 12 h after collagenase infusion, but not after a 24 h delay (John et al., 2014). Finally, we assessed cerebral edema because it is a contributor to ICP and since it is widely used in previous ICH studies evaluating TH (Fingas et al., 2007; Kawanishi et al., 2008; Kollmar et al., 2010).

2. Results

2.1. Mortality and exclusions

Of 107 animals, a total of 23 were excluded because of technical problems (e.g., faulty cooling device) or experimental error. Five animals were excluded from Experiment 1 (3 sham, 2 ICH), 2 animals were excluded from Experiment 2 (1 NORMO, 1 HYPO), and 6 animals were excluded from Experiment 3 (3 HYPO-f, 3 HYPO-s). Nine ICH-treated animals in Experiment 3 (3 NORMO, 3 HYPO-f, 3 HYPO-s) spontaneously died within 18 h post-insult. One additional NORMO animal from Experiment 3 was euthanized early because of feeding difficulties and excess weight loss. Their ICP data suggests that at least some of these resulted from ICP spikes. Indeed, peak ICP (r = 0.555, p < 0.001) predicted mortality better than average ICP (r = 0.231, p = 0.157). As all spontaneous deaths occurred prior to 24 h, which was the scheduled time to start TH, we were unable to determine whether TH influences mortality in this model. As such, mortality does not confound group comparisons.
The large dose of collagenase caused substantial bleeding within the striatum as measured at 7 days. This can be compared to the natural blood volume of ~15 μL in the brain’s vasculature using this method (John et al., 2014). Inducing TH starting 24 h after collagenase infusion did not affect hematoma volume (p=0.481 vs. NORMO, Fig. 2). The volume of blood in cerebellum control samples was similar between groups (p=0.866).

2.3. Experiment 2: 24 h intervention delay does not worsen bleeding

As there were potentially more transient effects that would need to be included in the analysis, average (mean ± SD) was used. Although not formally assessed, a hematoma was seen during tissue processing for all collagenase-treated rats.

The SHAM group had normal watery content in striatum (Fig. 1B) whereas the ICH group had substantially more (p<0.001). The cerebellum control samples were normal and not different between groups (p=0.346). When all rats were included in the analysis, average (r=0.749, p=0.013) and peak ICP (r=0.808, p=0.005) predicted edema. However, average (r=0.144, p=0.817) and peak ICP (r=0.168, p=0.788) did not predict edema within just the ICH group (excluding SHAM animals).

2.4. Experiment 3: TH reduced ICP but not edema

As in experiment 1 (Section 2.2), the ICH rats had above normal ICP values on day 1. As the groups were not treated differently at this time, the average (r=0.527, Fig. 3A) and peak ICP values (r=0.686, Fig. 3B) were similar among groups. Although there was a trend for the HYPO group to have lower average and peak ICP values on day 2, which is the first 24 h period of TH, these effects were not statistically significant (main effect for average ICP: p=0.337; main effect for peak ICP: p=0.257). However, the main effects for average and peak ICP data comparisons were significant on days 3 and 4 (p ≤ 0.042). Tukey post-hoc comparisons were done to identify those comparisons that were significant as illustrated in Fig. 3. For instance, on day 3 (2nd day of cooling), both HYPO groups significantly reduced peak ICP (p ≤ 0.047 vs. NORMO). Similarly, peak ICP remained lower in both HYPO groups upon rewarming (day 4, p ≤ 0.047).

As the HYPO-f and HYPO-s groups were treated identically up to the point of rewarming (end of day 3), we combined these data for a more powerful comparison to the NORMO group (for days 1–3). The average (r=0.819, NORMO: 13.6±4.1 vs. combined HYPO: 13.2±4.4, mean±SD) and peak ICP (r=0.837, 20.5±7.5 vs. 20.0±6.2) comparisons on day 1 were not significantly different, which was expected because all groups were normothermic during the first 24 h. On day 2, the first day of TH, there were non-significant trends for cooling to reduce average (p=0.143, 15.6±5.4 vs. 13.1±4.3) and peak ICP (p=0.127, 22.0±7.8 vs. 18.1±6.4). In contrast, TH significantly lessened average (p=0.009, 13.7±2.6 vs. 10.3±3.9) and peak ICP (p=0.002, 21.5±5.1 vs. 15.6±4.9) on day 3, the 2nd day of cooling. Overall, HYPO lowered average ICP by approximately 3.5 mm Hg on average (e.g., see 2nd day of cooling) whereas the reduction in peak ICP was greater at times (e.g., up to ~10 mm Hg less on the day after re-warming).

The HYPO-f and HYPO-s groups did not significantly differ for any of the above ICP post-hoc comparisons (p ≥ 0.233). As there were potentially more transient effects that would
Fig. 3 – (A) Epidural ICP measurements were recorded every min and averaged every 30 min. Cooling was initiated at 24 h, followed by either fast (i.e. instantaneously, HYPO-f) or slow rewarming over 6 h (HYPO-s) starting after 48 h of cooling. There was no effect of localized TH on mean ICP until days 3 and 4. Asterisk (*) denotes a significant difference from NORMO ($p < 0.05$). Values expressed as mm Hg (mean ± SD). (B) Peak ICP was significantly reduced in treatment groups on day 3 and 4. Asterisk (*) denotes a significant difference from NORMO ($p < 0.05$). (C) This graph depicts the ICP data starting from 6 h prior to rewarming or a comparable time in controls. There were no obvious spikes in ICP, such as at the start of rewarming in either the HYPO-f or HYPO-s groups.

Fig. 4 – (A) Cooling did not reduce day 4 brain water content in either treatment group. In fact, fast rewarming worsened edema (*$p <= 0.016$ vs. NORMO, #*$p <= 0.010$ vs. HYPO-f). Water content in the cerebellum was not statistically different among groups ($p = 0.818$). (B) In a separate experiment, significant edema was found on day 1 in untreated ICH rats (Fig. 4B; $p = 0.001$ for comparison with SHAMs from Experiment 1 – Section 2.2). Edema after normothermic ICH significantly increased from day 1–3 (*$p = 0.001$). Edema after hypothermic ICH was also significantly increased (#$p <= 0.010$ vs. day 1 NORMO). Nonetheless, TH, which started at 24 h post-ICH, did not lessen edema on day 3 ($p = 0.801$ vs. day 3 NORMO). (C) There were no significant linear relationships between day 4 water content (ipsilateral striatum) and average ICP ($r = 0.057$, $p = 0.747$) or peak ICP ($r = 0.078$, $p = 0.654$, all three groups included). Similarly, there were no significant relationships for each group.
be missed in the above analyses, we examined the hourly data starting 6 h prior to rewarming or the equivalent time in the NORMO group until 12 h later (Fig. 3C). There were no obvious spikes in ICP, such as at the start of rewarming in either treated group.

Significant edema occurred in all groups 4 days after ICH (e.g., vs. sham animals in experiment 1 – Section 2.2, Fig. 4A). There were no group differences for the cerebellum, which had normal water content ($p=0.818$). There was a treatment main effect for the striatal edema ($p=0.005$), and post-hoc tests revealed that the HYPO-f group had significantly more edema than the NORMO ($p=0.016$) and HYPO-s ($p=0.010$) groups. The HYPO-s and NORMO groups were not different ($p=0.967$). As found in Experiment 1 when only the ICH group was included, a linear correlation analysis showed no relationship between either peak ICP on day 4 and edema ($r=0.078$, $p=0.654$), or average ICP on day 4 and edema ($r=0.057$, $p=0.747$) (all three ICH groups included, Fig. 4C). Likewise, there were no significant relationships for each individual group ($p \geq 0.092$).

2.5. Experiment 4: TH does not reduce edema 3 days post-ICH

Owing to the worsening of edema in the HYPO-f group and a complete lack of efficacy in the HYPO-s group (Fig. 4A), we examined whether edema was established by day 1, and whether cooling lessened edema when assessed in rats euthanized at 72 h post-ICH. Significant edema was found on day 1 in untreated ICH rats (Fig. 4B; $p=0.001$ for comparison with SHAMS from Experiment 1 – Section 2.2). Edema after normothermic ICH significantly increased from day 1–3 ($p<0.001$). Nonetheless, TH, which started at 24 h post-ICH, did not lessen edema on day 3 ($p=0.801$). These animals were subjected to TH up to a few min prior to euthanasia. Cerebellum control samples were normal in all cases.

3. Discussion

A number of important conclusions arise from our experiments. First, a large infusion of collagenase in rat striatum leads to significantly elevated ICP with values that are ~3 fold higher than normal over the first 3 days. The initial peak rise in ICP predicts mortality within the first day. Thus, the large-dose collagenase model has good face validity in representing clinical cases of severe ICH with ICP elevation. Second, use of mild TH localized to the injured hemisphere significantly attenuates the rise in ICP after severe ICH, and this effect persists beyond treatment. These findings indicate that localized TH will effectively lower ICP elevations in patients suffering a severe ICH. Third, TH reduced ICP without affecting cerebral edema suggesting that other mechanisms, at least in some settings, can completely underlie the beneficial effects of TH on ICP. Fourth, rewarming rate from mild local TH did not influence ICP values. However, rapid rewarming apparently did cause re-bound edema. This suggests a potentially harmful effect that was insufficient to affect ICP, but nonetheless should be avoided.

Our previous study (Hiploylee and Colbourne, 2014) was the first to show that moderate to large collagenase-induced ICHs result in significant and persistent elevations in ICP, a finding we now replicate. The present experiments also support our previous conclusions that ICP values exceeding ~25 mm Hg increase the risk of spontaneous death in rats (Silasi et al., 2009; Hiploylee and Colbourne, 2014). It should be noted, however, that this is an estimate and further study, with significantly more samples (mortality), is needed to more accurately estimate risk of death based upon ICP data. Several animals died within the first 18 h after ICH in this study, and ICP data revealed that these spontaneous deaths were related to transient ICP spikes more so than average ICP. Again, more cases of mortality are needed to have sufficient confidence in concluding that peak ICP is the better predictor. Several studies reporting on the high mortality rate for ICH patients indicate that 20–50% of these deaths occur within the first 2 days (Fogelholm et al., 2005). While there is similarity to our ‘severe’ collagenase model, it is clear that our mortality rate is still considerably lower. This could perhaps be addressed by further increasing lesion size (collagenase dose) to cause even higher ICP values and greater mortality; however, this would lead to significant animal welfare issues from severe disability and unanticipated deaths. Perhaps this might make it even more difficult to unambiguously assess neuroprotectants.

Numerous studies in ischemic stroke and TBI suggest that TH can reduce ICP (Schwab et al., 2001; Steiner et al., 2001; Flynn et al., 2015). In ICH, both animal and clinical data support the use of TH as an anti-edema therapy (e.g., Kawanishi et al., 2008; Kolimar et al., 2010), and presumably a promising candidate for ICP management. Our animal data strongly support previous clinical observations (Kolimar et al., 2012) as discussed, and extends them to show that brain-selective TH can significantly reduce ICP even when initiated 24 h after ICH begins. Interestingly, in our study, TH lowered peak and to a lesser extent average ICP even though there was no reduction in cerebral edema, at least on the days assessed. This suggests that other mechanisms can at least sometimes account for TH’s beneficial effects on ICP (e.g., reduction in CSF production), a conclusion also supported by recent focal ischemia data (Murtha et al., 2015). Mechanisms of action will likely vary among treatment protocols, and thus it may be unwise to only rely upon singular mechanisms, such as edema, to gauge treatment efficacy. This issue coupled with differences among clinical and experimental studies make it difficult to discern optimal TH parameters. Our present model allows us to address some of these issues that are not easily studied clinically. As for other mechanisms, our previous work suggests that blood pressure is not appreciably changed in this ICH model (MacLellan et al., 2004; Hiploylee and Colbourne, 2014), and that our local cooling protocol does not impact blood pressure in normal rats (Clark and Colbourne, 2007; Auriat et al., 2012). Nonetheless, additional studies ought to consider whether cooling impacts blood and cerebral perfusion pressure in ICH rats treated with TH.

While the majority of animal studies cool for <6 h (van der Worp et al., 2007), several ischemia studies suggest that prolonged TH is more effective than brief TH at global
(Colbourne and Corbett, 1994) and focal ischemia (Yanamoto et al., 2001; Clark et al., 2008). We suspect that while brief cooling is highly effective in some situations such as during or early after stroke (Murtha et al., 2015), the use of protracted hypothermia provides superior benefit when greater intervention delays occur and with more severe ischemic insults (Colbourne and Corbett, 1994; Colbourne and Corbett 1995). It is not yet entirely clear how this applies to ICH, but given the protracted nature of edema and raised ICP, it is likely that extended cooling is needed. Furthermore, it is likely that cooling may not be safely applied as quickly as with ischemic stroke owing to the risk of aggravating cerebral bleeding in some ICH patients. In the collagenase model, cerebral bleeding is aggravated when TH is induced up to 12 h after injury (John et al., 2014) likely due to the protracted (several h) nature of bleeding in this model (Rosenberg et al., 1990; MacLellan et al., 2008). Our present findings confirm our earlier work that 24-h delayed cooling does not worsen bleeding (John et al., 2014), and so this does not confound the present experiments. However, it is possible that much better protection could have been achieved had it been safe to cool sooner, which should be possible in the majority of ICH patients that do not have ongoing hematoma growth. If so, studying delayed TH in the collagenase model likely underestimates the true potential for using TH after ICH. The alternative is to use the whole blood model where cooling could be induced sooner, but we found that infusing 100 μL of blood, the standard model for rat, did not cause a significant and persistent rise in ICP (Hiploylee and Colbourne, 2014). We are currently optimizing this model to better mimic severe ICH with persistent ICP elevations.

Besides intervention delay and treatment duration, the rate of rewarming has received considerable attention for ischemic and traumatic brain injury. Although the best rewarming protocol is not known, it is clear that fast rewarming can be detrimental, at least after systemic TH. For example, fast rewarming can lead to sudden vasodilation and rebound ICP increases (Polderman, 2009). In our study, we used brain-selective TH, which theoretically should not cause as many or as severe complication as would occur with rewarming from systemic TH. Nonetheless, we compared rapid and a relatively slow rate of warming. The fast rewarming apparently caused re-bound edema, which surprisingly did not affect ICP. Regardless, elevated edema suggests a harmful effect (not presently studied), which should be avoided by using slower rewarming. Our data suggests that a 6 h period is sufficiently slow, but additional study is needed (after varying depths and durations of cooling, young and old subjects, etc.). Finally, a study design issue should be considered when interpreting these data. Specifically, we used separate groups of rats to compare edema among groups and over days. The wet-dry weight measurements are taken post-mortem and thus we could not determine edema in the same animals prior to, during and after rewarming. Thus, we can only infer that re-bound edema occurred after rapid re-rewarming because our HYPO treatment had no effect on edema in rats that were not re-rewarmed.

There are a number of additional limitations with our study that warrant discussion. First, we were unable to measure brain temperature in our studies owing to the use of ICP sensors placed on the skull, which prevented us from placing temperature sensors there. However, we can infer that mild TH was applied to the striatum based upon our earlier work that directly measured brain temperature (Clark and Colbourne, 2007; Fingas et al., 2007; John et al., 2014). Second, we did not assess histological or behavioral endpoints in the current series of experiments. In these studies we wished to avoid any potential confounds of animal handling and behavioral testing on ICP, and the endpoints chosen (edema and blood volume) were incompatible with histological assessment. Others and we have previously evaluated the impact of cooling on behavioral deficits and cell death after ICH in rodents. Those studies have yielded mixed results (MacLellan et al., 2006; Kawanishi et al., 2008; Fingas et al., 2009), which likely results from model differences (e.g., insult severity), a wide range in treatment parameter (e.g., TH duration), and variable side effects (e.g., aggravated bleeding-MacLellan et al., 2004; John et al., 2014). Further work is needed to address these issues in relevant animal models. Third, we did not address other factors, besides edema, that determine ICP. It has been speculated that in the initial stages after brain trauma, the CSF volume (slowed production, increased drainage) counters maladaptive changes in ICP (Gabriel et al., 2010; Murtha et al., 2015) and it is likely that this applies to ICH as well, but further study is needed. We did find that peak and average ICP predicted edema, but only in the first experiment and then only when SHAMs were included (also see Hiploylee and Colbourne, 2014). When only the ICH animals were assessed (Exp. 1 and 3), we found that edema and ICP were not significantly related, which implies that other factors are either more important or they obfuscate the relationship between edema and ICP. As well, it must be kept in mind that rapid changes in edema and ICP are possible and this may account for the lack of correlation observed. Fourth, out of necessity we had to implant ICP sensors immediately after ICH surgery because the sensors would have made it very difficult to inject collagenase. This means that ICP values are initially underestimated (e.g., for an hour) as the sensor system equilibrates after surgery (e.g., starting out near 0). As well, it means that we were unable to correct for individual differences in resting ICP values, which result from innate differences among animals as well as technical issues (e.g., subtle differences among surgeries). Although this is a limitation, such noise did not prevent us from easily detecting an effect of ICH on ICP (Fig. 1A), which replicates an earlier study from our lab (Hiploylee and Colbourne, 2014). As well, the use of the first day average and peak ICP values were not significantly different among ICH groups (Fig. 3A, and B) suggesting that all groups were similar up to the time of treatment. Regardless, the ability to record ICP prior to stroke would undoubtedly improve our study design and perhaps allow us to detect more subtle differences.

4. Conclusions

In summary, we report that mild brain-selective TH significantly reduces mean and peak ICP after a severe collagenase-induced ICH. This supports the clinical interest in using TH
for ICP control after ICH. We are also the first to demonstrate that fast rewarming after local TH appears to cause rebound edema in ICH rats. Surprisingly, this did not noticeably impact ICP. Similarly, TH significantly reduced ICP but not by affecting edema, which remained unchanged with our 24 h delayed TH protocol. Although TH is a promising strategy, at least for ICP control after ICH, further preclinical work is needed to test whether the present findings with HYPO can be replicated, and if so then to optimize its effectiveness and to better understand mechanisms of action.

5. Experimental procedures

5.1. Subjects

All protocols followed the Canadian Council of Animal Care Guidelines and were approved by the University of Alberta’s Animal Care and Use Committee for Biosciences. A total of 107 male Sprague Dawley rats were obtained from the Biosciences breeding colony at the University of Alberta. Rats (~300-600 g, ~13–24 weeks old) were housed separately in polycarbonate cages in a temperature and humidity controlled room kept on a standard 12 h light/dark cycle with free access to Purina rat chow and water. Immediately after surgery and for several days, the rats were also given Purina rat chow that was softened by mixing it with peanut butter and water. Animal weight and age ranges were consistent within each experiment. All animals were randomized to applicable groups.

5.2. Experiments

5.2.1. Experiment 1: effects of ICH on ICP and Edema

Seven rats were given a striatal ICH (ICH) and 8 had a sham surgery (SHAM). Epidural ICP was recorded for 72 h after this surgery at which time they were euthanized to assess peak brain edema (Fingas et al., 2007).

5.2.2. Experiment 2: effect of TH on Bleeding Volume

A total of 18 rats were subjected to an ICH followed by TH (HYPO-24, n=9) or normothermia (NORMO, n=9). The HYPO-24 rats had their injured hemisphere cooled starting 24 h after surgery and this treatment lasted for 3 days. The NORMO controls were not cooled but were otherwise treated the same. All animals were euthanized 1 week after surgery, and hematoma volume was measured using a hemoglobin spectrophotometric assay (Choudhri et al., 1997; John et al., 2014) to determine whether TH caused additional bleeding.

5.2.3. Experiment 3: comparing rewarming rates

Animals were randomized to normothermic controls (NORMO, n=16), TH with fast rewarming over several min (HYPO-f, n=18), or TH with rewarming over 6 h (HYPO-s, n=17). Cooling began 24 h after ICH and was maintained for 2 days. Epidural ICP was recorded immediately after surgery for 4 days when they were euthanized to determine edema.

5.2.4. Experiment 4: effects of TH on edema

To further assess the influence of TH on edema, we compared 3 groups: NORMO at a 24 (n=5) and 72 h survival (n=9) and HYPO (n=9) at a 72 h survival. The HYPO group was cooled up to a few minutes before being euthanized. It is assumed that some rapid rewarming would occur during the euthanasia process, but this is not expected to have affected brain water content.

5.3. ICH surgery

All animals were anesthetized with isoflurane (4% induction, 2% maintenance in 60% N2O and balance O2) while rectal temperature was maintained at 37 °C. Rats were placed in a stereotaxic frame, a midline scalp incision (~2.5 cm) was made and a burr hole was drilled 3.5 mm to the right and 0.07 mm posterior of Bregma (MacLellan et al., 2008; John et al., 2014). A 26 G Hamilton syringe was lowered 6.5 mm below the skull surface into the striatum and 0.2 U bacterial collagenase in saline (Sigma; Type IV-S) was injected over 5 min. The needle was left in place for an additional 5 min to prevent backflow. A solid metal (Experiments 2 and 4) or hollow plastic (Experiments 1 and 3) screw was inserted into the burr hole (Small Parts Inc., Miami Lakes, FL; model MX-080-2 and MN-0080-02P-25 respectively). A hollow screw was used to act as a cannula guides for a 26 G needle to sit flush with the bottom of the skull. All animals were hydrated with 5 mL S.C. of sterile saline after surgery.

5.4. Cranial apparatus and ICP implant

After striatal injection, the burr hole was sealed with a hollow nylon screw (C212SGN, PlasticsOne, Roanoke, VA) to guide a 23 G needle just into the epidural space. A 2 cm long PE20 tube (Smiths Medical International Ltd., Kent, UK) was attached to the other end of the cannula. Cannulas were filled with sterile saline prior to surgery. All telemetry probes were first sterilized in 2% glutaraldehyde and rinsed several times in sterile saline. The catheter of a PA-C10 probe (Data Sciences, St. Paul, MN) was then slowly secured into the PE20 tube similar to our previous work (Silasi et al., 2009; Hiploydie and Colbourne, 2014).

A 5 mL syringe barrel was cut to ~2.5 cm in length to sit vertically on the skull and encase the telemetry probe. Small holes were drilled near the edge of one end of the barrel into which metal screws were inserted to help anchor the cranial apparatus to the cooling coil and to the skull. The barrel was filled with dental cement to cover the screws. The PA-C10 probe was then lowered into the barrel to sit on top on the dental cement. The barrel was sealed with a rubber plunger to prevent movement of the telemetry probe.

Immediately after surgery, data was recorded from freely moving rats every min, averaged every 30 min, and stored using ART software (DataSciences Int., A.R.T. v 2.3). In all experiments, room pressure recordings were collected at least 2 h prior to surgery to allow for the correction of pressure-offset errors.
5.5. Local brain TH

Immediately after positioning the anchor screws, an 8 mm wide cooling coil was implanted between the skull and temporalis muscle on the right side (Clark and Colbourne, 2007; Fingas et al., 2007). The coil was attached to a metal spring encasing the PE-50 tubing that allowed for cold water to be flushed through the coil. The apparatus was held in place with dental cement. After surgery, the cooling tubing was attached to overhead swivels (model 375/20, Instech, Plymouth Meeting, PA) to allow animals to move freely.

Cooling was produced by perfusing cold water through the coil starting at 24 h after collagenase infusion. This was followed by rapid (water flow turned off) or gradual rewarming (step reductions in flow over 6 h) depending on the experiment. Based on published data, a flow rate of 96–120 mL/h, which we presently used, caused cooling of ~33 °C at the injection site (Clark and Colbourne, 2007; Auriat et al., 2012; John et al., 2014). However, striatal temperature on the cooled side would vary somewhat among animals and over time (e.g., range from 31–34 °C) owing to unavoidable inconsistencies in the production of cooling coils and surgical procedures, as well as from natural circadian rhythms in brain temperature along with changing behaviors (e.g., rest vs. active). Finally, body temperature remains normothermic during cooling (Clark and Colbourne, 2007; Auriat et al., 2012).

5.6. Euthanasia and hemoglobin assay

The spectrophotometric hemoglobin assay, which measured hematoma volume, was adapted from previous work (Choudhri et al., 1997, MacLellan et al., 2004, John et al., 2014). Briefly, animals were anesthetised with isoflurane and decapitated. The brain was removed and divided into left (contralateral) and right (ipsilateral) hemispheres. The cerebellum served as a control. Each region was weighed and transferred to a tissue homogenizer (Kimble Chase, 7 mL, Pestle “B”). Distilled water was added in a 1:4 tissue to water ratio (weight: volume). Tissue homogenates were incubated on ice for 7 min to allow for additional osmotic lysing of red blood cells. Homogenate samples were then transferred to 1.5 mL Eppendorf tubes and centrifuged at 15,800g for 35 min. 100 μL aliquots of hemoglobin-containing supernatant were reacted with 600 μL of diluted Drabkin’s reagent (Sigma) in 1.5 mL cuvettes for 15 min. Absorbance was measured at 540 nm with a spectrophotometer (Model 4001/4; Thermo Fisher Scientific, Waltham, MA), and compared to a previously generated standard curve to determine total blood volume in each brain region. This would include intra- and extravascular blood.

5.7. Water content assessment

Animal were anesthetised with isoflurane and immediately decapitated. Brains were blocked from 2 mm anterior to 4 mm posterior to the injection. Ipsilateral (infusion side) and contralateral (no injury or edema, data not shown) striatum, as well as the cerebellum (control) were separated and weighed before (wet weight) and after (dry weight) being baked for 24 h at 100 °C in an oven. Normal striatal water content is ~78% (Fingas et al., 2007).

5.8. Statistical analysis

All data was analyzed using SPSS (v. 21, SPSS Inc., Chicago, IL). ICP and edema data, which appeared normally distributed and with no significant heterogeneity, were analyzed using ANOVA and when needed additional post-hoc Tukey tests. Mortality was analyzed using a point biserial correlation (Pearson r). Comparisons were considered significant when p ≤ 0.05. Data are presented as mean, and standard deviation bands are plotted where applicable.

Author disclosure statement

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References


