Investigating the Influence of Therapeutic Hypothermia on Stroke-Induced Intracranial Pressure in a Rat Model of Intracerebral Hemorrhage

by

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A thesis submitted in partial fulfillment of the requirement for the degree of

Master of Science

Centre for Neuroscience
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ABSTRACT

Elevated intracranial pressure (ICP) is a potentially life threatening complication after intracerebral hemorrhagic stroke (ICH). Animal and clinical studies suggest that mild (~33ºC) therapeutic hypothermia (TH) reduces ICP after ischemic stroke and traumatic brain injury (TBI). Since TH has also been shown to reduce edema after several neurological conditions, and edema is widely considered a key contributor to ICP, it is hypothesized that ICP management might be achieved through edema reduction. Thus, the current thesis evaluated the influence of brain-selective TH on ICP in a rat model of ICH. This thesis evaluated whether brain selective-cooling aggravated bleeding in the collagenase-induced ICH model, and tested whether brain cooling reduced ICP after ICH, or the re-warming rate mattered. ICP was measured for 4 days using telemetry pressure transmitters in untethered awake rats subjected to a large-collagenase induced ICH. Delayed cooling 24 hours after ICH did not worsen bleeding, and so TH treatment was delayed for 24 hours in all cooling experiments. Brain-selective hypothermia significantly reduced mean and peak ICP. On the contrary, fast-rewarming worsened edema on day 4, but this did not noticeably affect ICP responses. Lastly, increases in edema did not correlate with increases in ICP. These findings suggest that factors other than edema may better predict ICP, and further pre-clinical work is needed to provide better insight into cerebral pressure management.

PREFACE

This thesis is an original work and received research ethics approval from the University of Alberta Animal Care and Use Committee for Biosciences, Project AUP960.

Chapter 2 of this thesis has been published as John RF, Williamson MR, Dietrich K, and Colbourne F, "Localized hypothermia aggravates bleeding in the collagenase model of intracerebral hemorrhage," Therapeutic Hypothermia and Temperature Management, vol. 5, issue 1, 19-25. John RF was responsible for the concept formation, data collection and analysis as well as the manuscript composition. Williamson MR assisted with the data collection and manuscript composition. Dietrich K assisted with the manuscript composition, and Colbourne F was the supervisory author, and was involved with concept formation, data analysis, and manuscript composition.

Chapter 3 of this thesis has been submitted for publication as John RF and Colbourne F, "Localized Hypothermia Reduces Intracranial Pressure Following Collagenase-Induced Intracerebral Hemorrhage in Rat" to the journal of Experimental Neurology. John RF was responsible for concept formation, data collection, data analysis, and manuscript composition. Colbourne F was the supervisory author, and was involved with concept formation, data analysis, and manuscript composition.

DEDICATION

I wish to dedicate my thesis to my parents, Alfred and Theresa John.

ACKNOWLEDGEMENTS

First, I would like to express my deepest gratitude to my supervisor, Dr. Fred Colbourne, for his excellent guidance, patience, and providing me with a comfortable atmosphere to grow as a researcher. Your continuous encouragement throughout my study is forever appreciated.

I would also like to thank my committee members, Dr. Ken Butcher, Dr. Ian Winship, and Dr. Clayton Dickson, for their valuable feedback.

I would like to thank all my lab mates for their support and input. I would also like to thank Dr. Yonglie Ma for his technical assistance, and former lab mate, Carmen Hiploylee, for helping me to learn key techniques even when preparing for her own thesis defense, and her advice thereafter.

To my mother and father, I am beyond grateful for all the sacrifices you have made on my behalf. Mom, your daily prayers have sustained me thus far. Dad, thank you for the late nights you spent listening to me speak about my work. Thank you both for teaching me to extend beyond life's obstacles and challenges.

Finally, I would like to express my sincere appreciation for Joel Lumbala. Thank you for worrying about my safety during my late time points, and spending countless hours learning with me.

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List of Abbreviations

BBB	Blood brain barrier
BP	Blood pressure
BWC	Brain water content
CBF	Cerebral blood flow
CSF	Cerebral spinal fluid
CPP	Cerebral perfusion pressure
ICH	Intracerebral hemorrhage
ICP	Intracranial pressure
MAP	Mean arterial pressure
PHE	Perihemorrhagic edema
SAH	Subarachnoid hemorrhage
SD	Standard deviation
TBI	Traumatic brain injury

CHAPTER 1

1.1 Introduction

This thesis describes the effectiveness of therapeutic hypothermia to reduce intracranial pressure (ICP) and edema in a rodent model of intracerebral hemorrhage (ICH). An ICH is a devastating subtype of stroke resulting from the rupture of blood vessels within the brain parenchyma. Outcome is presumably worse as the risk of a potentially fatal ICP response to brain injury increases. Mild therapeutic hypothermia (TH, 33-35°C) is a promising ICP management strategy after ICH by perhaps reducing secondary consequences of ICH such as inflammation, oxidative stress, blood brain barrier (BBB) damage, and most notably edema. However, TH does not consistently provide benefit in ICH studies. This might be explained by the lack of a clear established TH protocol (e.g. induction, duration, and rewarming rate) and complications in ICH. Thus, it was hypothesized that brain-selective cooling reduces edema and ICP in adult rats subjected to a large collagenase-induced ICH. The rationale behind the experiments is explained in the following introductory sections. Background on relevant topics such as stroke epidemiology, brain edema, experimental models of stroke, methods of cooling, and ICP-reducing therapies are provided. The influence of onset of treatment and rewarming rates after hypothermia are also considered.

1.2 Stroke

1.2.1 Epidemiology

Stroke is defined as an interruption of blood supply to the brain, causing damage to brain tissue (World Health Organization, 2015). In Canada, stroke is the leading cause of disability, and the third leading cause of death. An estimated 50,000 Canadians suffer a stroke each year -

one in every ten min. According to the Heart and Stroke foundation, the risk of stroke-related deaths increases by 13% after age 60. However, stroke rates amongst young adults (ages 24-64) are steadily increasing, and recent international studies predict a startling doubling of stroke rates in this cohort within the next fifteen years (Heart and Stroke Foundation, 2014).

Death rates for stroke victims have significantly decreased by more than 75% over the past decade due to advanced diagnoses, treatments, and coordinated practices (Heart and Stroke Foundation, 2014). With more stroke survivors yearly, this implies that more people are living with disabilities, as most stroke survivors do not fully recover. Approximately 75% of stroke victims live with disabilities, and moderate to severe impairments such as aphasia, memory loss, neglect, paralysis, anxiety, and depression (World Health Organization, 2013). This increases the burden on family members, caregivers, and the economy. Each year, Canadian stroke victims spend more than 639,000 days in acute care, and 4.5 million days in residential care facilities. This costs the Canadian economy approximately 3.6 billion dollars a year in physician services, hospital costs, lost wages, and decreased productivity (Canadian Stroke Network, 2011). Besides the obvious toll that the disease has on individuals and the family, the economic burden motivates advances in treatments and technology to reduce the cost on society.

1.2.2 Intracerebral Hemorrhage

Approximately 80% of all stroke cases are ischemic in nature – interruption of cerebral blood flow (CBF) due to thrombosis (clot builds at the site of occlusion) or embolism (clot travels through bloodstream and builds at site of occlusion). Generally, hemorrhagic stroke account for approximately 20% of all strokes (Gebel and Broderick, 2000). A hemorrhagic stroke is caused by the rupture of cerebral vessels, resulting in collection of blood in the

subarachnoid space (subarachnoid hemorrhage, SAH) or in brain tissue (intracerebral hemorrhage, ICH). An ICH (9-15% of all strokes) occurs twice as often as a SAH, (Sudlow et al., 1997) and accounts for 10% to 30% of all stroke hospital admissions, and a devastating 30-day mortality rate of 30% to 50%, with up to 50% of deaths occurring within the first 2 days after ICH (Broderick et al., 1993; Broderick et al., 1994; Rincon et al., 2012). Mortality in the acute phase of injury is reportedly associated with several factors including age, hematoma volume, intraventricular extension (bleeding in the ventricular system), infrantentorial origin, and low levels of consciousness. Patient outlook can be scored using the Glasgow Coma Scale (lower scores indicate worse injury) in the acute phase of injury has been correlated with 30-day mortality. Of the surviving patients, only 12-39% have been reported to return to independent functioning, although below pre-stroke capabilities (van Asch et al., 2010). This emphasizes the need for a dedicated treatment for ICH.

1.2.2.1 Clinical Manifestation

Clinical presentation of ICH depends on the location and severity of the insult. Subcortical regions of the brain (e.g. striatum, thalamus) that receive inputs from motor and somatosensory cortex are particularly vulnerable to hypertensive hemorrhages (Xi et al., 2006). Hemorrhages in lobar regions are the second most frequent (one-third of primary ICH), and typically the result of cerebral amyloid angiopathy (Labovitz et al., 2005; Morgenstern et al., 2010). Symptoms such as seizures or visual deficits are particular to the lobe affected (Qureshi et al., 2001). Seizures occur in 4-20% of patients, with an 8% risk of seizures within the first month, and others suffer non-convulsive seizures that are often missed unless continuous EEG monitoring is used (Bladin et al., 2000; Passero et al., 2002). During a seizure, blood flow and

pressure is elevated, which can cause re-bleeding or increased bleeding after an ICH (Meldrum et al., 1976; Passero et al., 2002). This may cause further complications and increase mortality (Balami et al., 2011).

Discerning hemorrhagic strokes from ischemic stroke can be challenging based on neurological examinations alone. Neurological deficits develop over a few minutes to hours, along with nausea, headaches, impaired consciousness, limb weakness, slurred speech, and elevated blood pressure (BP). However, stroke can often be confused with other neurological conditions that share similar symptoms such as seizures, syncope (short loss of consciousness due to decrease in blood flow to the brain), and sepsis (whole-body inflammation caused by an infection) (Nor et al., 2005). In addition, symptoms of syncope, coma, seizures, and nausea are common in ischemic stroke, but typically absent in ICH (Nor et al., 2005).

1.2.2.2 Risk Factors

Risk factors of ICH can be broadly classified into modifiable and non-modifiable risk factors. Modifiable risk factors for ICH include hypertension, anti-coagulant therapies, thrombolytic therapies, and cocaine use (Magistris et al., 2013). Hypertension accounts for 60% of all ICH cases, and results in small aneurysms at the bifurcation of high-volume flow arterioles (Ariesen et al., 2003; Lovelock et al., 2007). Non-modifiable risk factors for hemorrhagic stroke include old age, Negroid and Asian ethnicity, coagulopathies, and hereditary cerebral amyloid angiopathy. Stroke incidence in the Asian population is especially high, but incidence rates have fallen dramatically due mainly to a decrease in the prevalence of hypertension (Sacco et al., 1997; Magistris et al., 2013; Wu et al., 2013). In the elderly, cerebral amyloid angiopathy is the most common cause of lobar intracerebral hemorrhage, and is caused by abnormal amyloid

protein deposits in cerebral blood vessels. Similar to hypertensive hemorrhages, this process weakens blood vessels and causes hemorrhagic lesions. Lobar regions are especially vulnerable to cerebral amyloid angiopathy, while hypertensive hemorrhages occur most frequently in the thalamus-basal ganglia (Thanvi, 2006). However, the greatest non-modifiable risk factor for stroke in general is age because the risk of stroke doubles in each decade after 55 years of age due to continued progression of cerebrovascular diseases (Magistris et al., 2013).

1.2.2.3 Rodent Models of ICH

Stroke can be induced in a number of different animal models to better understand mechanisms of injury and evaluate treatments. Animal models of ICH have been developed using pigs, rabbits, dogs, cats, and rodents. Pig models have a unique advantage as they have comparable white matter content to humans, but are very costly (Andaluz et al., 2002). There is no perfect ICH model but the two most common ICH models are the whole blood model and the collagenase model. Other ICH models include inflatable balloons, infusion of blood components (e.g. thrombin or FeCl2), and spontaneously hypertensive rats. However, these other models do not closely resemble the natural course of injury after ICH (MacLellan et al., 2010; Kirkman et al., 2011; Manaenko et al., 2011). For example, microballoon insertion does not cause blood brain disruption or address blood toxicity (Sinar et al., 1987).

Ropper and Zervas initially performed the whole blood model in 1982 in which allogeneic blood was infused into the basal ganglia of unanesthetized rats. Bullock et al., 1984 later adapted the model by infusion whole blood under arterial BP to be more clinically relevant, but did not find consistency in hematoma size. A study by Deinsberger et al., 1996 successful adapted this model by first allowing a small amount of blood to clot and prevent backflow into

the needle tract. The remaining blood was subsequently infused. Similar to other findings (Rynkowski et al., 2004; Wang et al., 2004), MacLellan et al., 2008 demonstrated that a 100 µl blood injection results in a hematoma volume of 85.34 ± 6.25 (standard error) 12 hours after ICH. Thus, this presents a crucial complication as not all infused blood stays in the brain, and may even travel back along the needle tract or into the epidural or ventricular space. A study by Deinsberger et al., 1996, modified the single blood injection model using a double arterial blood injection by infusing autologous blood and waiting for 10 minutes to allow for clot formation before injecting the remaining blood. Therefore, the double injection model minimizes blood reflux through the needle tract. However, there was an increased potential of clot formation because of the time lag between injections (Ma et al., 2013). Other limitations of the autologous blood injection model include ventricular extension of blood, inconsistent lesion shape and location, quick resolution of hematoma and behavioural deficits, and an extensive inflammatory response (Bullock et al., 1984; Nath et al. 1986, MacLellan et al., 2008).

The collagenase infusion model is popular for experimental spontaneous ICH. Collagenase is a bacterial enzyme that breaks down the basal lamina of cerebral blood vessels to create a spontaneous bleed. This technique was first developed by Rosenberg et al., 1990 in which collagenase was injected into the brain using microinfusion pumps. Bleeding was observed as early as 10 minutes, hematoma expansion 1-4 hours after infusion, and a mature hematoma was developed by 4-24 hours. This model is advantageous because the hematoma size and location can be easily modified and reproduced. On the other hand, bleeding in the collagenase model is diffuse and due to the rupture of small vessels around the injection site in contrast to patients bleeding from an arterial source. In comparison the whole blood model, the

collagenase model is associated with greater functional deficits, edema, inflammatory response, and continued bleeding or re-bleeding (MacLellan et al., 2008; Nguyen et al., 2008).

Although both models are widely used to assess treatment efficacies, many successful preclinical therapies have failed to translate to the clinic (Gladstone et al., 2002; Mergenthaler and Meisel, 2012). These failures might be due to several possibilities. There is likely a disconnect between experimental methodologies and clinical practices. For instance, many studies rely on rodent data but there are likely fundamental differences (e.g. relative amount of white matter) between rodents and humans that prevent translation of findings (Stroke Therapy Academic Industry Roundtable (STAIR), 1999). However, the use of other mammals such as pigs or primates can be very costly. Preclinical studies also often rely on treatment administered immediately or within a few h after injury, whereas treatments may be delayed in the clinic for several reasons (e.g. hospital admission time, stabilizing the patient, diagnoses, etc.). Therefore, it is important to consider treatment windows (Gladstone et al., 2002). Furthermore, several study designs rely on histology to suggest efficacy with little to no long term functional benefit, making it all the more likely to fail in clinical trials . Other factors include inadequate study designs, exaggerated importance of findings or unwillingness to report negative findings, and inappropriate models (MacLellan et al., 2008; Mergenthaler and Meisel, 2012). Major practices mentioned in the STAIR recommendations include reporting sample size calculations, proper randomization, and allocation concealment. However, Philip et al., 2009 reported that only 10% of studies report blinded treatments and assessments. Likewise, a systematic analysis of all studies published in the Journal of Cerebral Blood Flow and Metabolism in 2008 revealed that only 22% of studies reported randomization, 8% allocation concealment, 15% blinded assessment of outcome, and 14% inclusion/exclusion criteria (Vesterinen et al., 2011). When

MacLellan et al., 2012 reviewed the study quality of experimental ICH research; the authors found that only 12% of studies reported no significant treatment effect on any measures which may reflect a publication bias. In addition, may studies failed to report methodological details (e.g. group sizes, exclusions, randomization, etc.). This makes it difficult for investigators to replicate another laboratory's data. Thus, improvements in these measures are encouraged to ensure research validity.

With respect to the two common ICH models discussed earlier, there are some differences in the extent and time course of injury. The collagenase model simulates a spontaneously bleed, and the severity can be easily modified depending on the dose (Terai et al., 2003). However, it is associated with greater secondary injury when compared to the whole blood model (MacLellan et al., 2008; Nguyen et al., 2008). Long-term functional deficits (up to 28 days) are also observed with the collagenase model, whereas functional deficits return to baseline levels by 21 days in the whole blood model with simple tests (MacLellan et al., 2008). Therefore, selection of a particular model is dependent upon study objectives.

1.2.2.4 Pathophysiology

A number of events occur during ICH, which can be broadly classified into primary and secondary injury. The spontaneous rupture of cerebral blood vessels influenced by the aforementioned risk factors (Xi et al., 2006) causes the initial bleed. Primary damage is a result of the mechanical trauma of blood dissecting through the brain parenchyma. Secondary injury involves the mass effect of the growing hematoma, blood brain barrier (BBB) damage, toxicity of blood products, and cerebral edema.

Primary damage after ICH is influenced by the mass effect of hematoma expansion (Zazulia et al., 1999; Venkatasubramanian et al., 2011). Hematoma expansion occurs within hours after the initial onset due to the mechanical trauma of bleeding (Staykov et al., 2011). In a third of patients, up to 73% hematoma growth occurs in patients scanned within 3 hours of symptom onset (Davis et al., 2006). However, the ability to detect expansion and its frequency depends on the time from symptom onset to initial evaluation. This might explain why the frequency of hematoma expansion varies across studies (Brouwers and Greenberg, 2013). In addition, the frequency of hematoma expansion in patients within 6 h of symptom onset is between 13 and 32%, while 48% of patients present 6 hours or more after symptom onset (Brouwers and Greenberg, 2013). Generally, hematoma expansion is defined as a 40% increase in hemorrhagic volume or an increase of 12.5 to 20 cm^3, occurring in about a third of patients within the first 24 hours after ictus (Brott et al., 1997; Rodriguez-Luna et al., 2011). In these patients, the growing hematoma contributes to the midline shift of brain structures and progressive neurological deterioration (Broderick et al., 1990; Zazulia et al., 1999). In addition, perihematomal ischemic regions may develop due to increased volumes within the skull and compression of brain tissue (e.g. brain herniation). Studies have shown that cerebral blood flow (CBF) adjacent to a hematoma decreases below 25 $mL/100g^{-1}/min^{-1}$, but this reduction lasts less than an hour and returns to baseline within 3 to 4 hours (Nath et al., 1987; Yang et al., 1994). However, the CBF threshold for ischemic injury is 15-20 $ml/100g^{-1}/min^{-1}$, suggesting that critical levels of hypoperfusion do not occur after experimental ICH (Mendelow et al., 1984; Bell et al., 1985; Diringer et al., 1998). Similar findings of CBF reduction have been reported where an early and temporary hypoperfusion zone was observed but no markers of ischemia or structural damage was identified (Qureshi et al., 1999; Xi et al., 2001). It remains unclear whether

secondary cerebral ischemia occurs in the brain after ICH or whether milder reductions in CBF influence outcome.

‘Toxic’ blood products such as iron and thrombin also cause brain injury after ICH. In a response to ICH-related damage, the complement cascade is activated, followed by red blood cell lysis. Erythrocyte lysis takes up to a week to occur in humans, but begins within 3 days in rodents and is completed by day 4 (Wagner et al., 2003). This process also corresponds to heme-oxygenase -1 (HO-1, degrades heme) expression which may be a double-edged sword (Wang et al., 2011). Deficiency of HO-1 in human and in a mouse knockout model leads to increased vulnerability to oxidant stress and inflammation. However, overexpression of HO-1 may be cytotoxic when the concentration of iron exceeds antioxidant capacities (Qing et al., 2009; Wang et al., 2011). Animal models of ICH have shown substantial elevation in iron. For instance, non-heme iron was 2-4 times higher in brain tissue samples up to 28 days after a striatal infusion of blood, and the ferric iron concentration was most prominent within and near the hematoma in rodents (Wu et al., 2003). Likewise, it was previously shown that the total iron in the injured hemisphere increases for up to 28 days after ICH, and the highest concentration was found in the area adjacent to the hematoma (Auriat et al., 2012). Although, haptoglobin (a hemoglobin-binding protein) expression has been shown to increase over the course of a week after ICH (Zhao et al., 2009), these proteins are likely overloaded by the iron concentration in the perihematomal tissue (Wu et al., 2003, Zhao et al., 2009). Labile iron from degraded hemoglobin may contribute to oxidative damage after ICH by catalyzing the Fenton reaction, which produces highly reactive hydroxyl radicals (Wang, 2014). These radicals proceed to damage macromolecules such as DNA, lipids, and proteins. However, mitochondrial dysfunction and inflammation also increase free radical production (Testai et al., 2008; Wang et al., 2010).

Nevertheless, several animal studies have shown that the iron chelators such as deferoxamine and bipyridine attenuate brain edema, tissue damage and neurological deficits in a rat ICH model (Nakamura et al., 2003; Nakamura et al., 2006; Okauchi et al., 2009). On the contrary, when we evaluated the ability of bipyridine and deferoxamine to reduce iron-induced injury after ICH, we did not find an effect on non-heme iron levels, behavioral impairments or edema after bipyridine treatment (Caliaperumal et al., 2013). Similarly, deferoxamine failed to attenuate lesion volume, neurological and behavioural deficits, lesion volume, or edema after collagenase-induced ICH. These findings suggest that this drug is not neuroprotective in the collagenase model, in sharp contrast to positive results in whole blood studies (Huang et al., 2002; Hua et al., 2006; Okauchi et al., 2009). However, as recommended by the STAIR report (STAIR, 2009), evidence should come from multiple stroke models across several labs before assuming efficacy of neuroprotectants in ICH (Cui et al., 2015).

Thrombin, an essential serine protease of the coagulation cascade, is released immediately following ICH. After injury to a blood vessel, a series of events occur to plug the site of injury and prevent bleeding. Prothrombin (coagulation factor II) is proteolytically cleaved to form thrombin, which converts soluble fibrinogen into insoluble fibrin, forming a protective seal (Lee et al., 1997; Xi et al., 2006). There is evidence that thrombin mediates brain injury in human ICH. In a study by Hamada et al., 2000, systemic administration of argatroban (a thrombin inhibitor) lessened brain water content in the perihematomal region. Interestingly, treatment was delayed for 24 hours after ICH to prevent re-bleeding, suggesting that some thrombin is still present for at least 24 hours. Either some of the thrombin produced during coagulation is contained within the hematoma and slowly released into the perihematomal space (Mutch et al., 2001), or prothrombin from plasma may enter the brain through the damaged BBB

and be converted into thrombin. Animal studies show that low concentrations of thrombin are neuroprotective (Vaughan et al., 1995; Masada et al., 2000), while evidence both *in vivo* (Nishino et al., 1993; Lee et al., 1997; Xi et al., 1998) and *in vitro* (Striggow et al., 2000; Jiang et al., 2002) suggests that high concentrations of thrombin within brain tissue can be harmful.

Iron and thrombin may also interact to worsen brain injury after ICH through thrombin-induced upregulation of iron uptake into cells. Hua et al., 2003 showed that thrombin upregulates the transferrin (a plasma protein iron transporter) receptor in the ipsilateral basal ganglia 1 and 3 days after intracerebral thrombin infusion. Further, infusion of holo-Tf (transferrin bound to ferric iron) did not increase intracellular iron levels, but co-administration of holo-Tf and thrombin significantly increased cellular iron levels (Hua et al., 2003). Thus, thrombin likely upregulated brain Tf receptor levels, which allows to uptake of iron-bound Tf into brain cells (Hua et al., 2003; Nakamura et al., 2005), but it unclear whether the extent of this interaction may be amenable to therapeutic intervention. Altogether, the mechanisms of secondary cerebral injury after ICH are complex and overlapping. Delineating specific pathways of protection and injury may allow for careful intervention in ICH.

1.3 Intracranial Pressure

1.3.1 Importance of ICP

The adult brain is an 'intracranial vault' encasing brain tissue, blood and cerebral spinal fluid (CSF). An increase in any of these components is reciprocated by a change in other intracranial components (e.g. CSF and/or blood displacement). These compensatory mechanisms maintain brain compliance and describe the Monroe-Kellie doctrine (Dunn, 2002). When brain compliance is exhausted, intracranial pressure (ICP) rises, which can lead to compression of vital

brain structures (herniation), reduced cerebral perfusion pressure (CPP = mean arterial pressure (MAP)-ICP), and in some cases hypoperfusion of the perihematoma region, resulting in perihematoma ischemia and eventual death (Venkatasubramanian, 2011). Therefore, in some cases ICP monitoring and management is necessary.

Normal ICP in adult humans is ≤ 15 mmHg, while an ICP ≥ 20 mmHg can be fatal (Dunn, 2002). Clinical studies report disagreement between ICP recordings from different brain regions and recording instruments, due to cost, accuracy, and reliability. Likewise, preclinical ICP recordings vary widely (4-47 mm Hg) across rodent studies (Uldall et al., 2014), indicating the importance of accurate ICP monitoring methods and sites. A novel method developed in our lab (Silasi et al., 2009), allows continuous measurement of ICP in the epidural space of awake, freely moving ischemic stroke rats over several days. Hiploylee and Colbourne 2014 recently adapted this model to assess ICP changes and edema in ICH models (collagenase vs. whole blood model). In this study, moderate and severe ICHs significantly increased ICP versus shams (10 versus 5 mm Hg) for several days, whereas a 100 µl blood infusion (whole-blood model) did not. Overall, ICP predicted edema, notably in the collagenase model (Hiploylee and Colbourne, 2014). Thus, ICP monitoring in rodents also seems warranted (e.g. to test efficacy of anti-edema therapies)

1.3.2 Monitoring Intracranial Pressure

Across several studies (Raboel et al., 2012), there are differences in ICP readings from the intraparenchymal, subdural, epidural, or ventricular space, which might be a reflection of the method itself. ICP monitoring methods can be classified based on invasive and non-invasive methods. Some of the invasive methods include lumbar puncture, ventricular catheter, or an

external transducer. The external ventricular drainage (EVD) technique, where a catheter is guided through a burr hole in the skill and inserted into the ventricle to drain cerebrospinal fluid (CSF) is the current gold standard in ICP monitoring (Raboel et al., 2012). This method can also be used to administer medicine or antibiotics. Other advantages include accuracy, simplicity, inexpensive cost, and capability of recalibration *in situ*. However, EVD is invasive with hemorrhagic and infectious complications occurring due to surgical placement difficulties. Hemorrhagic complications have been reported in an average of 5.7% of cases, in which 0.61% required surgical intervention, caused neurological deficits or fatality (Binz et al., 2009). Selection of a particular method is dependent on the technology applied and the location of ICP measurement.

Due to the complication of invasive methods of ICP monitoring, there is an increasing shift towards non-invasive methods such as transcranial Doppler sonography (TCD), tympanic membrane displacement, and telemetry methods. Telemetry methods offer an alternative to traditional ICP monitoring without the use of a cable connected to a monitor. Instead, the pressure values are transferred to a receiver, and does not limit patient mobility. Thus, telemetry ICP monitoring techniques are currently under investigation for hospital use in hydrocephalus patients (Kiefer et al., 2012).

ICP monitoring has also been investigated in animal studies. Monitoring away from the site of injury has been thought to produce less accurate recordings, but a recent investigation in rodents reported no difference in ICP recordings between intraparenchymal, and epidural ICP measurements (Hiploylee and Colbourne, 2014). However, cortical damage has been reported with invasive ventricle or parenchyma ICP recordings, which might overestimate ICP recordings. Greater damage due to invasive methods may also interfere with accurate injury

assessments, such as lesion volume. Apart from invasive techniques, many existing ICP monitoring methods are largely confounded. Many methods require animals to be anaesthetized or restrained, which does not allow for long ICP assessments (e.g. days). Anaesthetics have also been reported to lower ICP and BP in animals and humans (Gyring et al., 1984). An alternative practice is to monitor ICP under anaesthesia intermittently over days, but similar to human, even a relatively brief anesthetic induction (e.g. 1 hour) post-ICH may reduce cell death and improve behavioural deficits. However, care must be taken with certain anesthetics such as ketamine as it increases CBF, and ICP (Jenett et al., 1969; Bazin, 1997)

Great efforts have been put into developing ICP monitoring methods that do not require animals to be anesthetized or restrained for long periods. Tethered systems have been investigated for several years. Verlooy et al., 1990 introduced a tethered system that used a fiber-optic device to measure ICP in the parenchyma, epidural space, and cisterna magna. Intraparenchymal but not epidural readings were comparable to the cisterna magna. Epidural readings had consistent rises of 10 mm Hg and then returned to baseline. The authors attributed this to possible interference from the dura since these artefacts disappeared when the dura was perforated (Verlooy et al., 1990). In 1991, Kotwica et al. measured ICP in ischemic stroke rats intermittently for 15 days. However, the ICP monitoring system failed 56% of animals. Similarly, Rooker et al., 2003 used a tether system to measure ICP in the right parietal cortex of rats for 3 hours in a weight-drop traumatic brain injury (TBI) model. However, an unexplained increase in ICP from 4 to 10 hours post-insult rendered the system unreliable. In contrast, Sanchez-Valverde et al., 1995 successfully modified the tethering technique by using a flexible catheter to allow free movement of animals. ICP measurements were taken in the cisterna magna, lateral ventricle, and lumbar subarachnoid space of Wistar rats for 3 days. In a recent

method developed by Silasi et al., 2009, ICP was successfully recorded in the epidural space of freely moving awake untethered rats for 5 days after ischemic stroke. This was achieved using blood pressure telemetry probes, and therefore avoided several complications typical of past methods: cortical damage, anaesthesia, and tethering and stress. Despites these advantages, this method is very expensive and the pressure catheter probe is highly sensitive. For example, Hiploylee and Colbourne, 2014 found an unexplained ICP rise in sham animals. They attributed this artefact to a possible clot or blockage at the tip of the cannula. Air bubbles, blood components, or foreign materials in the pressure-sensing region of the probe catheter may also cause inaccurate pressure readings. System integrity can be confirmed by performing a brief abdominal compression, causing a transient ICP spike (Silasi et al., 2009). However, the advantages outweigh the disadvantages that are common of any ICP monitoring system. Using this method, ICP has also been successfully measured in an ICH rat model (Hiploylee and Colbourne, 2014). The authors found that that a severe collagenase-induced ICH increased ICP for 4 days, but ICP did not increase after a whole blood infusion. They also found that increases in edema correlated with increases in ICP. Thus, the collagenase model can be used to evaluate ICP-reducing therapies.

Although, ICP monitoring has gained a place in intensive care units (ICU), there is still a clinical debate about the importance of ICP (Gupta et al., 2006). Narayan et al., 1982 reported a 53-63% incidence of raised ICP in patients with severe closed head injury with abnormal CT scans while patients with normal CT scan had a 13% incidence of raised ICP. The correlation between high ICP and poor outcomes after TBI has been well reported by many authors, and reported to improve mortality (Gupta et al., 2006). However, a recent randomized controlled trial (RCT) did not show a difference in outcome benefit in patients monitored with a treatment

threshold of 20 mm Hg versus patients that were not monitored. Similarly, a retrospective comparison of two trauma centres that monitored ICP, revealed no association between an increase in therapy levels and outcome benefit (Cremer et al., 2005). Altogether, there is no evidence in RCTs to support the use of ICP monitoring in TBI to target ICP thresholds. With respect to ICP monitoring in patients with large hemispheric infarcts, patents often deteriorate without clinical evidence of ICP elevation or CPP reduction (Frank, 1995). When herniation occurs in patients, it often presents without an elevation in ICP. In addition, ICP measurements may be less reliable in patients with malignant infarction who undergo decompressive hemicraniectomy (Singh and Edwards, 2013). In our hands, we found that a large middle cerebral artery occlusion (MCAO) ischemic stroke induced prolonged ICP elevation in rats. The highest peak ICP of 50 mm Hg was observed in one rat who died prematurely, although we cannot conclude that elevated ICP contributed directly to its death (Silasi et al., 2009). Similarly, Beard et al., 2015 recently showed that ICP rises transiently 24 hours after a MCAO in rats. They correlated ICP elevation to collateral flow reductions, and regarded it as a possible explanation for collateral failure (Beard et al., 2015). Thus, despite the lack no Class I evidence, ICP monitoring is useful since information can be derived from ICP and may allow prediction of prognosis.

1.3.3 Cerebral Edema and Intracranial Pressure

Cerebral edema refers to the swelling within a tissue due to the accumulation of fluid, which may contribute to ICP elevation. Brain tissue can become edematous after almost any injury (e.g. traumatic injury, hypoxic ischemia, meningitis, brain tumours and abscesses, etc.). There are two main types of edema – vasogenic, and cytotoxic. Vasogenic edema occurs when

the blood brain barrier is disrupted and vessel fluid fills the brain tissue. This type of edema can spread to regions distant from the site of injury. Cytotoxic (cellular) edema is described as the cellular swelling of brain cells due to cellular dysfunctions, and is more commonly associated with ischemic or hypoxic injury (Woo and Broderick, 2002; Thiex et al., 2007).

There are three proposed temporal phases of edema after ICH (Ziai, 2013). In the first few hours after ICH, serum proteins move to adjacent tissue. Over the following 24 to 48 hours, the coagulation cascade is activated. Thrombin is released relatively quickly and causes edema to peak 1-2 days after ICH (Xi et al., 2002; Ziai, 2013). It appears that clot formation is necessary to induce edema since unheparinized blood infusions induced edema immediately, whereas heparinized blood did not (Xi et al., 1998). Similarly, when an ICH was induced by thrombolytics (drugs that intentionally dissolve blood clots) in patients, only half of the edema volume was observed in comparison to patients with spontaneous ICH (Gebel et al., 2001). The last phase of edema formation is due to hematoma lysis and an inflammatory reaction to blood degradation products (Huang et al., 2002; Wu et al., 2006).

Cerebral edema is a common endpoint in many neuroprotection studies in ICH (MacLellan et al., 2012). However, temporal discrepancies between rodent and humans make it difficult to assess the impact of edema reducing therapies. In most patients (Sansing et al., 2003), edema peaks between days 5 and 6, but has also been shown to accumulate rapidly in brain tissue over the first 48 hours, and peak around the end of the second to the third week (Staykov et al., 2011). However, rodent studies have shown edema to peak 3 days after the onset of injury, and resolve within a week (Hua et al., 2002). In addition, rodent studies show a correlation between neurological deficits and edema severity, which both resolved at 2 weeks (Hua et al., 2002; Kuroiwa et al., 2008). However, there can be improvements in neurological deficits, but the

animals still have lasting impairments (just not gross deficits). In noncomatose patients, neurological deterioration was not associated with cerebral edema (Mayer et al., 1994). Thus, edema may not be an independent predictor of outlook after ICH.

There is also the question of whether perihematomal edema is vasogenic in origin. It was hypothesized that edema adjacent to the hematoma was cytotoxic and indicative of an ischemic process. Although, several MRI studies demonstrate hypoperfusion in perihematomal studies, diffusion-weighted imaging studies have reported conflicting results (Schellinger et al., 2003; Butcher et al., 2004; Pascal et al., 2007). Olivot et al., 2010 previously found that perfusion in the perihematomal zone was significantly delayed in ICH patients, and the presence of cytotoxic edema may be related to toxic factors such as thrombin and iron in the mass effect. However, perihematomal edema is unrelated to decreases in cerebral blood flow (Wada et al., 2007). In fact, it is hypothesized that edema originates from fluid extravasation from the hematoma, and not vascular in nature. This is supported by evidence of conservation of BBB permeability in the perihematomal zone (McCourt et al., 2015). Similarly, MacLellan et al., 2008 reported greater BBB damage within the hematoma in a rodent collagenase model. Nevertheless, excessive accumulation of fluid in the brain tissue may have a significant influence on intracranial volume and brain compliance. Thus, if edema is primary motivator for ICP rises after ICH, anti-edema therapies should also reduce ICP.

1.3.4 Therapeutic Management of ICP

It is the best practice of ICP management to target the specific processes responsible for the rise in ICP including the surgical removal of mass lesions, hemicraniectomy, control of hydrocephalus, and dexamethasone treatment for edema (Dunn, 2002). However, elevated ICP

can easily be a result of several changes within the cranial vault. In fact, any abnormal changes in physiological status may adversely affect ICP or cerebral perfusion. For example, care is taken after ICH to reduce the risk of fever because it can in some cases predict poor outcome (Schwarcz et al., 2000; Szczudlik et al., 2002). Other studies indicate that even small degrees of hyperthermia can worsen brain injury by exacerbating excitotoxic neurotransmitter release, free radical production, BBB damage, and apoptosis (Minamisawa et al., 1990; Baena et al., 1997). However, hyperthermia has not consistently worsened outcome after experimental ICH (MacLellan et al., 2005; Penner et al., 2011), and clinical studies yield conflicting results (Schwarz et al., 2000; Szczudlik et al., 2002). Elevation of the head of the bed has also been reported to worsen ICP when decreased below 15º, or increased above 30º (Moraine et al., 2000). In adult patients with TBI, positioning the patient's head midline encourages jugular venous outflow and lowers ICP. However, if a patient is hypovolemic, this might result in a decrease below optimal CPP thresholds (60-70 mm Hg), and subsequent cerebral ischemia and possible death (Durward et al., 1983; Ng et al., 2004).

Several pharmacological agents for elevated ICP management must be considered. Analgesics or sedation, and in some cases neuromuscular blockades may be used in the intensive care unit to lower ICP. However, these strategies have not shown a beneficial effect of mortality or outcome (Dunn et al., 2002; Ennis and Brophy 2011). In contrast, osmotic agents such as mannitol are widely used to lower elevated ICP in adult TBI patients. Mannitol is an osmotic diuretic that decreases blood viscosity, causes vasoconstriction, and decreases cerebrovascular volume. The net result is a fast decrease in ICP within 15 to 30 minutes. In patients with intracranial hypertension, a bolus of mannitol is administered intravenously (IV) over 20 to 30 minutes to reduce ICP in less than 5 minutes. In contrast, mannitol is administered intermittently

(every 2 to 6 hours) in patients requiring prolonged ICP reduction (Rangel-Castillo et al., 2007; Ennis and Brophy, 2011). Since mannitol draws fluid from the brain parenchyma to be excreted in the kidney, it is contraindicated in hypovolemic patients. Other potential adverse effects include abnormal renal function or rebound ICP after discontinued treatment. Similarly, hyperventilation is a useful emergency intervention as it decreases ICP by causing vasoconstriction, which decreases CBF. However, aggressive hyperventilation therapy may compromise cerebral perfusion and cerebral autoregulation. Thus, brief hyperventilation (15 to 30 minutes) is only recommended to treat acute elevations in ICP (Haddad and Arabi, 2012). Nevertheless, the ICP management techniques mentioned above typically target specific processes. Since ICH is a heterogeneous condition, a multifactorial treatment strategy may be best suited for adequate ICP management.

1.4 Therapeutic Hypothermia

Therapeutic hypothermia (TH) is an intentional drop in body and brain temperature, and has been clinically successful in treating cardiac arrest and neonatal hypoxia/ischemia (Bernard et al., 2002; Gluckman et al., 2005; Arrich 2007; Azzopardi et al., 2008). Early applications of this treatment included trauma, schizophrenia, and cancer (Fay, 1958). In the late 1700s, Dr. James Currie carried out the first successful hypothermia experiments in patients to determine the effects of cooling on body temperature, pulse and respiration (Wang et al., 2006). In the early 1800s, Russians frequently applied hypothermia by covering people with snow to resuscitate them (Varon et al., 2008). In 1892, Sir William Osler reported a decline in mortality when patients with typhoid fever were treated with hypothermia (Wang et al., 2006). In 1958, William and Spencer treated four cardiac arrest patients with hypothermia. The outcome was reduced

neurological deficits, and prevented mortality in all patients (William and Spencer, 1958). Benson et al., 1959 also reported favourable neurologic recovery in 50% of cardiac arrest patients, compared to 14% treated with normothermia (Benson et al., 1959). It was later found that a mild hypothermia (32-35ºC) was neuroprotective after ischemia and trauma in animals (Colbourne et al., 1997; van der Worp et al., 2007). There is overwhelming evidence in animal models of global and focal ischemia that TH is neuroprotective, which has supported the translation of this treatment strategy to clinical trials (van der Worp et al., 2007; MacLellan et al., 2009). The cooling for Acute Ischemic Brain Damage (COOL-AID) group completed 2 clinical trials of hypothermia in ischemic stroke using surface cooling (Krieger et al., 2001) and endovascular cooling (De Georgia et al., 2004). Both trials demonstrated feasibility for cooling methods. The recently completed ICTuS trial Phase II has demonstrated that TH is safe and feasible in ischemia – no difference in mortality of 90-day outcomes was apparent. Two ongoing clinical trials are assessing the efficacy of therapeutic hypothermia in acute ischemic stroke – the ICTuS 2/3 Phase II Trial (Lyden et al., 2014), and the EURO-HYP-1 Phase 3 Trial (van der Worp et al., 2014). Localized TH has also been applied in clinical ICH (Feng et al., 2002; Abdullah et al., 2011; Su et al., 2015). For instance, Kollmar et al., 2010 treated 12 patients with large ICH with hypothermia (35ºC) for 10 days, and found that cooling prevented an increase in edema compared to non-treated patients. Based on these findings, the ongoing Cooling in Intracerebral hemorrhage (CINCH) trial aims to provide greater insight into hypothermia management of ICH (Kollmar et al., 2012). Despite not knowing the entire set of mechanisms by which TH mitigates cell death after ischemia (Yenari and Hemmen, 2010), it is expected that some protection should occur against ICH because ischemia and hemorrhage share overlapping

mechanisms of injury (Keep et al., 2012). Hypothermia has several mechanisms of action, and a few which are presumed to be of greatest important are discussed below.

1.4.1 Mechanisms of Hypothermia

1.4.1.1 Metabolism

Earlier, it was assumed that the neurological protection of hypothermia was due to a reduction in energy metabolism alone – for every 1ºC reduction in body temperature, cerebral metabolism is reduced by 6% to 10% (Hagerdal et al., 1975; Schmitt et al., 2014). It is now understood that a reduction in metabolism is beneficial when oxygen supply is limited or impaired (Schmidt et al., 2014). Hypothermia may also improve brain glucose utilization evidenced by a lower lactate-pyruvate ratio in cooled TBI patients compared to controls (Wang et al., 2007). Animal studies have shown that as cerebral oxygen consumption decreases, CBF declines in parallel when body temperature decreases from 38ºC to 28-30ºC (Hagerdal et al., 1975; Lafferty et al., 1978; Busija and Leffler, 1987) . This suggests that autoregulation is preserved at low temperatures. However, when Ehrlirch et al., 2002, compared cerebral vascular resistance at several temperatures in a swine model, they found significant residual metabolic activity at 18ºC. Temperatures below 18ºC achieved greater metabolic suppression but it may be associated with a greater loss of cerebral autoregulation (Ehrlirch et al., 2002). Recently, Stretti et al., 2014 showed that cerebral blood flow velocity in patients decreases in parallel when temperature decreases. When brain compliance is reduced, modest temperature changes can affect ICP. Impaired CBF also causes an immediate reduction in ATP, which triggers a switch to anaerobic glycolysis and subsequent intracellular acidosis and calcium accumulation. This

eventually leads to mitochondrial dysfunction and activation of apoptosis (Lee et al., 2000; Samaniego, 2013).

1.4.1.2 Inflammation

After ICH or any brain tissue injury, the inflammatory cascade is activated during which leukocytes; microglia, macrophages, and plasma proteins target the site of injury. Hypothermia has been shown to have an anti-inflammatory effect by reducing neutrophil infiltration and microglial activation (Wang et al., 2002; Deng et al., 2005). Mild TH also reduces inflammatory mediators such as nitric oxide and cytokines (Han et al., 2002; Scumpia et al., 2004; Meybohm et al., 2010), by possibly inhibiting inflammatory transcription factors (Han et al., 2003). Necrotic tissue and ROS can stimulate the inflammatory response, so it is hypothesized that the mechanism of hypothermia may be upstream of necrosis (Deng et al., 2003). However, anti-inflammatory effect of hypothermia have been observed without cell death, suggesting that TH has a direct effect on the immune response (Deng et al., 2003; Han et al., 2003). In patients, TH has been shown to reduce pro-inflammatory cytokine production in monocytes following brain injury (Abiki et al., 1999; Kimura et al., 2002).

1.4.1.3 Edema and ICP

The integrity of the BBB may be compromised following brain injury, which allows the development of brain edema. As discussed earlier, edema contributes to the mass effect after ICH and is critical in large ICH, and frequently leads to pathological ICP rises and brain herniation. Both animal and clinical data support the use of TH as an anti-edema therapy (Kawanishi et al., 2008; Kollmar et al., 2010; Staykov et al., 2011), and presumably a promising

candidate for ICP management. Mild hypothermia has been shown to reduce vascular permeability and cytotoxic edema after ischemia and TBI respectively (Oz et al., 2001). In a pilot study by Kollmar et al., 2010, TH was shown to prevent the progression of perihemorrhagic edema (PHE) and improve outcome in patients with large supratentorial ICH. In a follow up by the same group, Staykov et al., 2013 similarly described a reduction in perihemorrhagic edema volume in TH-treated ICH patients compared to historical controls. One potential mechanism of edema management is that TH inhibits matrix metalloproteinases (MMPs) and preserve the basal lamina of blood vessels after stroke (Karibe et al., 1994; Wagner et al., 2003). This is evidenced by experimental studies, which demonstrate lower levels of MMPs in rodents treated with mild TH vs normothermic controls (Hamann et al., 2004; Lee et al., 2005). Therefore, the mechanism underlying ICP reduction after TH may be linked to reduction in edema volume or impaired progression of edema, and presumably reduced CSF (Murtha et al., 2015). Neurocritical diseases may have different primary insults and cascades of secondary brain injury, suggesting the use of a different cooling approach and regimen to provide therapeutic benefit. This calls into question whether the proposed benefits of induced cooling outweigh the risks associated with current cooling methods.

1.4.2 Methods of Cooling

1.4.2.1 Focal versus Systemic Cooling

One of the key studies demonstrating survival in cardiac arrest patients treated with therapeutic hypothermia used “conventional surface cooling” which refers to cooling with ice packs or air-circulating cooling blankets/mattresses (HACA, 2002). Presently, cooling methods can be broadly divided into surface cooling (e.g. water-circulating wrapping blankets, cold water

immersion, cooling pads) and core cooling devices using metal intravascular catheters or saline-filled balloons, and cold fluid infusions. Intravascular balloons filled with cold saline have been reported to induce cooling relatively quickly (~ 1.5°C/hour) and easily maintained (Polderman and Herold, 2009). However, this invasive procedure increases the time to reach target temperature, which is clinically important, as "time is brain" (Saver, 2006). Systemic (whole body) surface cooling is the most common method of inducing hypothermia in both experimental and clinical studies of trauma, focal and global ischemia (Diller and Zhu, 2009), but it is not without risks. A reduction in core body temperature may influence several temperature-dependent systems and cause cardiovascular, coagulation, metabolic and immunologic complication. For example, skin thermoreceptors may induce peripheral vasoconstriction and shivering in response to temperature changes, which results in an increase in blood pressure and cardiac output and potentially increases ICP (Polderman and Herold, 2009). Coagulation factors are also sensitive to temperature changes (Watts et al., 1998). Valeri et al. 1987 have shown that hypothermia impairs clotting factors and platelet function when temperature is reduced to below 33°C and 35°C, which likely influences hemostasis. Blood vessel injury triggers a cascade of events involving contraction of vessel wall, platelet aggregation to form a platelet plug, and recruitment of coagulation factors the site of injury to stop bleeding. Cooling may slow down this process, and prolong active bleeding. Along with the risk of complications with systemic hypothermia, this method is associated with a slow cooling rate because of the increase in thermal resistance (Krieger et al., 2001). Therefore, it takes longer to reach target core temperature, which may compromise treatment efficacy. In contrast, localized brain hypothermia protocols (i.e. cooling helmets, metal cooling coils), are expected to reduce such deleterious cardiac cardiovascular effects (Clark and Colbourne, 2007; Auriat et al., 2012). Clinically,

localized cooling is also considered advantageous over systemic hypothermia (Wang et al., 2004; Qiu et al., 2006; Abou-Chebl et al., 2011). For example, a cooling helmet using technology developed by NASA has been shown to reduce brain temperature to ~35°C within 24 hours after stroke and improved neurological outcome (Wang et al., 2004). No deleterious effects (e.g. rebound hyperthermia or ICP, infection, coagulopathy) was observed. Traditionally, localized cooling can be achieved at various depths: mild hypothermia (32-35°C), moderate hypothermia (30-32°C), and deep hypothermia (< 30°C) (Polderman, 2009).

1.4.2.2 Hypothermia in Animals

Several methods have been used to induce hypothermia in animals. Systemic cooling can be achieved in anesthetized of awake animals, by placing animals on a cooling blanket, in a cold room, or inducing hypothermia pharmacologically (Babcock et al., 1993; Dowden et al., 1999). Brain-selective cooling can be produced in anesthetized animals using a cooling blanket (Nurse and Corbett, 1994), a coil wrapped around the head (Ding et al., 2004), or intraarterial cold saline infusion (Chen et al., 2013). However, long-term cooling is not ideal in anesthetized animals because of the increased risk of anesthetic overdose, and mortality (Clark and Colbourne, 2007). Prolonged cooling can be investigated using drug-induced hypothermia (Katz et al., 2012) or fan and water sprays in awake rodents (Colbourne et al., 1996), which requires constant temperature monitoring. Focal cooling methods would have the advantage of invasive and inexpensive temperature recordings. In 2002, Tooley et al. induced selective hypothermia in anesthetized newborn pigs with hypoxic ischemic injury. An external cooling cap was used to achieve deep brain temperature to 31.4ºC, with no significant changes in core temperature. A similar method by Ferreiro et al., 2005, encouraged the use of selective brain hypothermia in hypoxic-ischemic

encephalopathy. The authors used a cooling cap to demonstrate reduced mortality (55% versus 66% randomized to conventional care). A simple and effective experimental method developed by Clark and Colbourne, 2007 involves surgically placing a small metal coil adjacent to the skull and flushing the coil with cold water. This allows the induction of local brain hypothermia in awake, freely moving rats, and offers investigators the ability to easily control cooling depth, and longer treatment durations.

1.4.2.3 Hypothermia in Patients

Conventional cooling methods are non-invasive and more applicable on a larger scale, but maintaining temperature may be more difficult with surface cooling and patient discomfort and shivering is difficult to prevent (Sessler, 2009). Patients have been cooled using fans, convective air blankets, water mattresses, and alcohol bathing. However, these methods are slow to achieve target temperature and labor intensive (van der Worp et al., 2010). Alternatively, endovascular catheters inserted via the femoral vein in the inferior vena cava have been used in stroke patients to provide cooling (Georgiadis et al., 2001; Kreiger et al., 2001). Chilled intravenous fluids (e.g. saline at 4ºC) may be infused at the onset of endovascular cooling for a rapid induction of hypothermia. This technique has been tolerated well in SAH and TBI patients (Bernard et al., 2003; Polderman et al., 2005). In 2009, Kollmar et al. performed a small study to demonstrate that ice-cold saline infusion is safe for induction of mild hypothermia in stroke patients. The Cooling for Acute Ischemic Brain Damage (COOL-AID) trial demonstrated feasibility of an endovascular cooling device, but was not powered enough to comment on efficacy (Hemmen et al., 2010). With respect to ICH, the current Cooling in Intracerebral

Hemorrhage (CINCH) trial using an endovascular cooling method may validate the use of hypothermia in ICH patients (Kollmar, 2012).

The ideal cooling technique would selectively cool the brain and avoid systemic side effects of whole body hypothermia. Selective cooling methods include the use of helmets, head caps, and neckbands. These methods have been tested in sedated patients with TBI (Wang et al., 2004; Qui et al., 2006) and after cardiac arrest (Wandaller et al., 2009). However, it is unclear whether these methods will be feasible in awake stroke patients because it requires invasive monitoring to control target temperature throughout cooling. Testing in this patient population is essential. A modeling study showed that temperatures below 36ºC could not be reached in the deep brain tissue without using additional neckbands covering the carotid bifurcation (Keller et al., 2009). In addition, the authors noted that temperature sensors had to be applied at least 2 cm below the cortical surface to give values representative for deep brain tissue (Keller et al., 2009).

1.4.3 Limitations of Cooling Protocols

For the purposes of achieving robust neuroprotective effects of TH, it is important to set therapeutic standards for cooling parameters such as optimal intervention time, method of cooling, temperature, and duration. The neurologic outcomes of TH may depend on the initiation of cooling after injury. For instance, when systemic (whole-body) cooling was induced soon after a collagenase-induced lesion, prolonged mild TH (33-35ºC for 48 hours) failed to reduce lesion size. Conversely, outcomes were better when cooling began 12 hours after the onset of injury (MacLellan et al., 2004). However, an acute increase in systemic blood pressure, thereby promoting further bleeding negating improved functional outcome. For instance, in animal models, moderate hypothermia has been associated with reversible platelet dysfunction and

prolonged bleeding (Valeri et al., 1987; Staab et al., 1994). However, in many rodent studies, TH is initiated soon after the onset of insult in contrast with clinical setting where most patients receive treatment several h post-insult. Consequently, early initiation of cooling may markedly increase the anti-coagulatory effect of hypothermia, thereby aggravating bleeding in a variety of injury settings (i.e. ICH, hemorrhagic transformations after ischemia). To date, longer intervention delays of brain-selective hypothermia in collagenase-induced ICH models have not been investigated. It is disconcerting that adverse effects of mild hypothermia, such as interfering with enzyme kinetics in the coagulation cascade (Polderman, 2012), may counteract the beneficial effects on cooling if cooling is introduced before clotting is accomplished, or cause re-bleeding.

1.4.4 Rewarming

It is equally important to consider rate of rewarming post-hypothermia. Unfavourable rewarming rates may increase ICP, leading to a reduction in CPP, secondary ischemia and edema, and subsequent death. Both experimental and clinical literature regard the post-hypothermic rewarming rate a crucial factor in assuring neuroprotection after brain injury (Polderman, 2009). Although several studies allude to this potential confound, clinical evaluations of rewarming rates after hypothermic interventions are scarce (Povlishock and Wei, 2009). Rather, it is inferred from limited experimental data that fast rewarming after induced hypothermia may reverse the protective effects of TH or even exacerbate damaging processes after injury. Consideration of rewarming rates has largely been a consequence of studies or reviews assessing hypothermic intervention after severe TBI, where fast rewarming may cause sudden vasodilation, reduced CPP and hypoxia, and rebound increases in ICP (Algaza et al.,

2006). Therefore, in ICH settings where rebound ICP may mask the potential neuroprotective effect of TH, but exacerbate traumatically induced pathology and its neurological consequences, experimental evidence in this area is essential.

Experimental studies of rewarming after hypothermia suggest that slow rewarming is superior to fast rewarming. For example, when Berger et al., 2007 compared fast rewarming (20 minutes) and slow rewarming (2 hours) following mild hypothermia starting 2 hours after temporary MCAO in rodents, the authors found that slow rewarming reduced infarct volume and edema (measured by aquaporin expression). However, a slow rewarming protocol allows for a longer cooling duration, which presents an experimental confound. Fast rewarming might also have consequences on the biochemical and physiological responses, at least in rodents. Eshel et al., 2002 previously demonstrated that when anesthetized rats were cooled for 20 minutes and rewarmed over 30 minutes (fast) or 2 hours (slow), there was no effect on biochemical changes. However, there was a sharp and early increase in cardiac output and heart rate due to systemic vasodilation in fast rewarmed animals. Sudden vasodilation could also be an effect on the periphery where rewarming causes vasodilation and changes in blood circulation versus central changes in BP and heart rate/volume. This may have further consequences in patients with already high ICP, and potentially compromise survival. Of course, it is unknown whether these acute changes will occur in un-anaesthetized rodents, but these results suggest a worrisome effect of fast rewarming that should not be ignored.

Clinically, the rewarming period has also been observed to be a critical phase (Schwab et al., 1998; Shiozaki et al., 1999). With respect to ICP, Steiner et al., 2001 correlated slower increase in ICP elevations and slower decrease in CPP with slower rewarming (0.1ºC-0.2ºC over 2-4 hours) in stroke patients. Slowing the speed of rewarming may decrease the restart of

metabolic changes interrupted or slowed down by hypothermia (Dempsey et al, 1987; Dietrich et al., 1991). It is important to note that it is unclear how slow patients should be rewarmed to avoid deleterious events. Studies suggest that faster rate of 0.25ºC to 0.33°C/hour can be tolerated in patients without ICP-related complications (Ginsberg et al., 1992; Krieger et al., 2001; Krieger et al., 2004). However, no clear boundary between slow and fast rewarming rates has been established to definitively define fast and flow rewarming rates after hypothermia. This, of course, limits our investigations both experimentally, and in the clinic.

1.5 Rationale

This thesis aims to use telemetry methods to evaluate the hypothermic management of ICP in adult rats, by measuring and comparing ICP and edema changes in the collagenase-induced ICH model whereby bacterial collagenase is injected into the striatum to break down the basal lamina of surrounding blood vessels, and induce bleeding over several h. This model is advantageous because the ICH severity can be easily modified to better reflect the insult severity in patients likely to be treated with TH. In addition, we have previously shown that distinct ICP changes can be observed with larger injuries (Hiploylee and Colbourne, 2014). Since focal cooling methods are expected to minimize detrimental effects associated with systemic cooling (i.e. shivering, cardiovascular complications, etc.), an extracranial cooling coil apparatus developed in our lab will be used to achieve targeted hemispheric temperature (Clark and Colbourne, 2007). This thesis will also directly assess differential rewarming rates associated with focal cooling to determine a safe protocol. Collectively, these assessments will be the first to date to evaluate the influence of TH on ICP in an ICH model, and increase our understanding

of hypothermic ICP-reducing therapies to avoid potentially downregulating various protective mechanisms.

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CHAPTER 2

Localized Hypothermia Aggravates Bleeding in the Collagenase Model of Intracerebral Hemorrhage

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A version of this chapter has been accepted for publication. John RF, Williamson MR, Dietrich K, Colbourne F (2014). Therapeutic Hypothermia and Temperature Management.

2.1 Introduction

Intracerebral hemorrhage (ICH) is a devastating subtype of stroke accounting for 15% of all strokes and it has a high risk of mortality and morbidity. The initial primary damage results from mechanical trauma as blood pushes through the brain parenchyma. This is followed by secondary mechanisms of injury, such as inflammation, edema, and oxidative stress, among many other contributors (Keep et al., 2012). Despite advances in our understanding of ICH pathophysiology, treatments for ICH in rodent studies have failed to translate to patient care, in part, because they often target a single step in a complex cascade of events leading to cell death, among other reasons (van der Worp et al., 2007; Frantzias et al., 2011; MacLellan et al., 2012). In contrast to drug therapies, therapeutic hypothermia (TH), a multimodal treatment strategy, has translated in some cases (e.g., cardiac arrest), and there is considerable interest in using cooling for stroke, trauma, and other conditions (Polderman, 2008).

Despite not knowing the entire set of mechanisms by which TH mitigates cell death after ischemia (Yenari and Han, 2012), it is expected that some protection should occur against ICH because ischemia and hemorrhage share overlapping mechanisms of injury (Kirkman et al., 2011; Keep et al., 2012). For example, TH may help against ICH by targeting secondary components of injury such as inflammation, edema, and raised intracranial pressure (Kollmar et al., 2010; van der Worp et al., 2010). However, the effectiveness of TH has varied widely among animal ICH studies (MacLellan et al., 2009). Likely, this stems from differences among studies with regard to the choice of species, models, insult severity, endpoints, and treatment protocols. For instance, the two commonly used models involve injecting either autologous blood or collagenase into the brain. Infusing blood mimics a single large bleed, whereas collagenase causes the enzymatic breakdown of blood vessels and causes protracted bleeding, which mimics

the bleeding profile of some ICH patients (MacLellan et al., 2008, 2012; Kirkman et al., 2011). Use of the whole body (systemic) TH causes hematoma expansion in the collagenase (MacLellan et al., 2004), but not whole blood model of ICH in rat (MacLellan et al., 2006b). Systemic hypothermia increased blood pressure in that collagenase study (MacLellan et al., 2004), and this effect along with coagulopathy (van der Worp et al., 2010) likely worsened the hematoma size. This effect is a significant clinical risk for treating hemorrhagic and traumatic insults (Polderman, 2009, 2012), because hematoma size directly determines the extent of primary and secondary injury. This is why clinical studies would attempt to exclude patients with active bleeding.

Selective brain cooling methods, such as cooling helmets, are expected to have fewer deleterious cardiovascular effects (van der Worp et al., 2010). Therefore, we developed a method to induce localized brain hypothermia in rat and have shown that it does not appreciably affect blood pressure, heart rate, etc. (Clark and Colbourne, 2007; Auriat et al.,2012). So, one might expect this method to be more effective against ICH; however, this has not been the case (MacLellan et al., 2009). As one method is not superior to the other at this time, it suggests that focal cooling shares similar mechanisms of action, weaknesses, and side effects as systemic cooling. In this study, we evaluated the hypothesis that focal brain hypothermia worsens hemorrhaging after a collagenase-induced striatal ICH in rat. We created a large hematoma to better reflect the insult severity in patients likely to be treated with TH (vs. those with small bleeds who recover well on their own). We compared treatment delays of 6 hours (HYPO-6), 12 hours (HYPO-12), and 24 hours (HYPO-24) to reflect the range in intervention delays expected in clinical usage (vs. a normothermic control group—NORMO) and commonly used in animal studies. Such delays are inevitably due to the time required to get ICH patients to the hospital

and assessed, to initiate cooling, etc., and because TH may be initiated to treat delayed elevations in intracranial pressure. We also evaluated our hypothesis in the thrombin-infusion model. Thrombin is a serine protease that is generated after ICH to initiate clotting, but injections of thrombin alone (reductionist model) also causes blood–brain barrier disruption, cell death, perihematomal edema (Keep et al., 2012), and has been recently shown to paradoxically cause cerebral hemorrhaging by activating protease-activated receptor-1 (PAR-1) (Cheng et al., 2014). This latter effect concerns us as it is possible that TH would aggravate bleeding in this simplified model of ICH, and this might then explain why TH failed to mitigate thrombin-mediated injury in our recent study (Wowk et al., 2014). If this is the case, then TH may aggravate bleeding after ICH, in part, due to worsening thrombin-mediated hemorrhaging.

2.2 Methods

2.2.1 Subjects

All protocols followed the Canadian Council of Animal Care Guidelines and were approved by the Biosciences Animal Care and Use Committee at the University of Alberta. A total of 96 male, Sprague Dawley rats (~275–600g, ~12– 24 weeks old) were obtained from the University of Alberta Biosciences colony. All animals were individually housed after surgery with ad libitum access to food and water. Housing rooms were temperature, humidity, and light (12-hour light–12-hour dark cycle) controlled. Animals were randomly assigned to groups. Independent studies assessed the brain and core temperature during focal brain cooling that followed an ICH. The hematoma size was assessed by a hemoglobin based spectrophotometric assay.

2.2.2 Brain Temperature Probe Implantation

Brain temperature probes were implanted (n = 5) as previously described (Clark and Colbourne, 2007; Fingas et al., 2007). Rats were anesthetized with isoflurane (4% induction, 2% maintenance in 60% N_2O and remainder O_2) and placed in a stereotaxic frame. A rectal temperature probe connected to a heated water pad maintained core temperature at ~37°C. After collagenase injection (described below), three holes were drilled surrounding the original burr hole. Metal screws were inserted into the holes and secured with a small amount of dental cement. A plastic cylinder (cut from a 5-mL syringe) was centered on the hollow screw to house the brain temperature probe (model XM-FH-BP; Mini-Mitter Co.). Dental cement was used to secure the cylinder to the metal screws and the skull. The probe was inserted into place so that the thermocouple sensor was placed inside the striatum and slightly dorsal to the collagenase injection site. Brain temperature was recorded for 10 hours after surgery. Brain temperature was not measured in other studies (described elsewhere in the Methods section) to avoid potential confounds of inserting a probe into the brain.

2.2.3 Core Temperature Probe Implantation

Core temperature telemetry probes were implanted as previously described (MacLellan et al., 2004; Fingas et al., 2007). Under isoflurane anesthesia, a sterilized calibrated probe (model TA10TA-F40; Data Sciences Int.) was inserted through an abdominal incision (~3 cm). Muscle and skin were sutured, closed, and Marcaine (Sigma) was applied. Animals were allowed a 4-day recovery period before collagenase infusion. Temperature data for 5 NORMO and 5 HYPO-6 (6-hour hypothermia delay) rats were collected (Data Sciences Int.; A.R.T. v 2.3). Their blood was

used to create a standard curve for the hemoglobin assay (data not shown). Core temperature probes were not used in the other experiments.

2.2.4 Striatal Injection Surgery

Rats were anesthetized and 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). A 26 G Hamilton syringe was then lowered 6.5mm below the surface of the skull into the striatum and either 1.2 µL of 0.2U/ µL bacterial collagenase in saline (Type IV-S; Sigma), 1U of rat thrombin in 30 µL saline (product T5772; Sigma), or 1.2 µL of saline (sham surgery) was injected over 5 minutes (Wowk et al., 2014).The needle was left in place for an additional 5 minutes and then slowly removed to prevent backflow. A solid metal screw was then inserted into the burr hole, except for those with brain temperature probes, for which a hollow plastic screw was used (to guide the brain probe). Following these procedures a cooling coil was implanted as described below.

2.2.5 Local Brain Hypothermia

Immediately following collagenase, thrombin, or sham surgery, an 8-mm wide cooling coil was implanted between the skull and the temporalis muscle on the right side (Clark and Colbourne, 2007; Fingas et al., 2007). The coil was attached to a metal spring encasing PE-50 tubing, which allowed for ice chilled water to be flushed through the coil (for HYPO groups).The apparatus was held in place with dental cement. Tethers and tubing were attached to overhead swivels (model 375/20; Instech) to allow animals to move freely. Based on the current and published data, a flow rate of 96–120mL/hour, that we presently used, caused cooling (31°C-

34°C) at the injection site (Clark and Colbourne, 2007; Fingas et al., 2007; Auriat et al., 2012). The NORMO controls were similarly treated, except that no water perfused through the cooling device.

Cooling was initiated in HYPO animals by perfusing cold water through the coil beginning at 1, 6, 12, or 24 hours post-injection depending on the treatment group and experiment. Rats were cooled until euthanasia, except in the brain temperature experiments where rats were gradually rewarmed over 2 hours. Hematoma size was measured in three NORMO and three HYPO-6 sham animals 24 hours after surgery. For the collagenase experiments, hematoma size was determined at 24 hours post-ICH in 5 HYPO-6 and 5 NORMO rats, and 15 NORMO and 15 HYPO-12 rats. Hematoma size was also measured at 36 hours post-ICH for 9 HYPO-24 and 9 NORMO rats. For thrombin infused animals, we assessed the hematoma size in 4 NORMO rats at 24 hours, and we compared 6 NORMO and 7 HYPO-1 rats at 48 hours after infusion.

2.2.6 Euthanasia and Hemoglobin Assay

The spectrophotometric hemoglobin assay, which measured blood volume, was adapted from previous work (Choudhri et al., 1997; MacLellan et al., 2004). Briefly, animals were anesthetized with isoflurane and immediately decapitated. The brain was removed and dissected into left (contralateral) and right (ipsilateral) hemispheres, and the cerebellum. Olfactory bulbs and spinal cord were removed. Each region was weighed and transferred to a tissue homogenizer (7mL; Pestle ‘‘B,’’ Kimble Chase). Distilled water was added in a 1:4 tissue to water ratio (weight: volume) prior to homogenization. Tissue homogenates were incubated on ice for 7 minutes to allow for additional osmotic lysing of red blood cells. Homogenate samples (in

duplicate or triplicate) were transferred to 1.5 mL Eppendorf tubes and centrifuged at 15,800 g for 35 minutes. Subsequently, 100 µL aliquots of hemoglobin-containing supernatant were mixed with 600 µL of diluted Drabkin's reagent (Sigma) in 1.5mL cuvettes and allowed to react for 15 minutes. Absorbance was measured at 540 nm with a spectrophotometer (Model 4001/4; Thermo Fisher Scientific), and compared with a previously generated standard curve to determine total (intra- and extravascular) blood volume in each brain region. Standard curves were generated from cardiac blood samples taken from several animals and were made for each batch of Drabkin's solution.

2.2.7 Statistical Analysis

Data are presented as mean–standard deviation and were analyzed using the SPSS (v. 21; SPSS, Inc.). Independent samples t-tests that did or did not assume equal variances (in case of significant heterogeneity) were used to make group comparisons, including between hemispheres.

2.3 Results

2.3.1 Brain Temperature

A total of 3 rats were excluded from the brain temperature data because of technical problems (e.g., blocked cooling coil). A brief cooling (1 hour) and rewarming (2 hour) period was used in the remaining 2 rats. As expected, our method was effective in decreasing striatal temperature (Fig. 2-1A) from a normothermic range to 32.8°C-34.2°C. Additional animals were used to measure core temperature before and during HYPO-6 treatment, which lasted 18 hours (until euthanasia). These measurements confirmed that focal brain cooling did not appreciably

influence body temperature as the average group temperature was less than 0.5°C different from the NORMO group (Fig. 2-1B).

2.3.2 Intraparenchymal Blood Volume

There were no significant group differences ($p \geq 0.105$; data not shown) between NORMO and HYPO-6 treatments for rats infused with saline (SHAM). However, there was slightly more blood in the ipsilateral hemisphere overall (19.35 μL vs. 15.20 μL in the contralateral side) indicating that the needle insertion and/or saline infusion caused slight bleeding ($p = 0.031$).

Two animals were excluded from the HYPO-12 study, and one animal from the HYPO-24 study, due to technical errors. In all collagenase experiments, there were no significant group differences in blood volume for the contralateral hemisphere and the cerebellum ($p \geq 0.160$). As expected, the injured hemisphere had significantly ($p \leq 0.002$) more blood (i.e., hematoma) than the contralateral hemisphere (no hematoma). The HYPO-6 ($p = 0.018$; Fig. 2-2A) and HYPO-12 ($p = 0.042$; Fig. 2-2B) treatments significantly worsened bleeding at 24 hours post-injection (vs. NORMO groups). This was not the case for the HYPO-24 animals that showed no increase in hematoma size in the ipsilateral hemisphere compared with NORMO animals at 36 hours post-injection ($p = 0.258$; Fig. 2-2C). Therefore, the timing of initiating TH was an important factor in determining whether bleeding was aggravated after collagenase infusion.

Blood volume in the ipsilateral hemisphere was slightly increased in NORMO rats ($p = 0.236$ vs. contralateral side, Fig. 2-3A) at 24 hours after thrombin injection (thrombin infused rats did not get collagenase), but this increase was not significantly significant. By 48 hours thrombin caused a small but statistically significant increase in hematoma size in NORMO ($p =$

0.002, Fig. 2-3B) and HYPO-1 rats (p = 0.038) compared with the contralateral hemisphere. However, as the increase in hematoma volume was similar to that found with saline infusion (SHAM), it suggests that thrombin alone did not induce additional cerebral bleeding. Regardless, no significant (p = 0.130) differences in blood volume were observed between NORMO and HYPO-1 groups among brain regions. Three rats were excluded from the HYPO-1 group because of technical problems (e.g., blocked cooling coil)

2.4 Discussion

Our primary finding was that mild localized TH markedly aggravated cerebral bleeding after a large collagenase-induced ICH in rats. This effect depended upon the intervention delay because 6- and 12-hour delayed cooling worsened bleeding, but 24-hour delayed cooling did not. Unlike collagenase-induced ICH, delayed TH did not aggravate bleeding after an intrastriatal infusion of thrombin or saline. In our hands, thrombin did not appear to cause additional hemorrhaging (at the 24- and 48-hour survival times) beyond that caused by an intraparenchymal needle insertion. Together, these findings show that localized TH protocols may worsen bleeding depending upon the brain insult and treatment parameters. For collagenase-induced ICH, aggravated bleeding may be a significant confound in published studies.

Data from animal models of ischemia strongly support the notion that TH loses efficacy with greater intervention delays (Yenari and Hemmen, 2010), which makes sense based upon the pathophysiological events occurring after stroke and cardiac arrest (i.e., ‘‘time is brain’’ concept). Similar arguments can be made for ICH, which is why one would normally seek to intervene as early as possible. However, in one study, the opposite pattern was observed; that is, systemic cooling provided greater protection as intervention delay increased from 1 to 12 hours

after collagenase infusion (MacLellan et al., 2004). In that study, early cooling exacerbated bleeding, which likely occurred because cooling increased blood pressure, which itself worsens bleeding (Benveniste et al., 2000), and cooling may have also slowed clotting. As our focal cooling method does not notably affect blood pressure or body temperature (Clark and Colbourne, 2007; Auriat et al., 2012), we had hoped that this method would be a safer alternative to systemic cooling. However, focal cooling clearly worsened bleeding in the collagenase model, which suggests too that the effect is likely mediated by a coagulation defect. Another possibility is that cooling caused re-bleeding. This might be the case for immature clots (e.g., collagenase model out to 12 hours) as a recent thromboelastography study demonstrated that a mature clot is seemingly not influenced by cooling (Ruzicka et al., 2012). Finally, one must consider the temperature sensitivity of collagenase and its deactivation in vivo. For instance, it is possible that the half-life of collagenase was slowed by cooling, thereby resulting in more prolonged bleeding. Without further experimentation, we do not know how focal cooling worsened bleeding (e.g., effects on initial clotting, fibrinolysis and/or collagenase's activity).

One limitation of our study is that we did not provide any histological or behavioral evidence to demonstrate worsened outcome because of increased bleeding. While hematoma size clearly determines these outcomes (Venkatasubramanian et al., 2011), TH may also have additional neuroprotective effects that would likely complicate the relationship between hematoma volume and brain injury. Regardless, it is only logical that the significantly enhanced bleeding presently observed would be harmful, and be easily discernable if experimentally tested on its own. This could be evaluated by intentionally varying either the dose of blood or collagenase infused to show hematoma-injury relationships, as previously done (MacLellan et al., 2006a). Another limitation is that we did not vary the depth of cooling. Coagulation would be

less affected with milder TH protocols, which convey some protection with less risk (van der Worp et al., 2007; Polderman, 2012). We did not assess initial severity, which would be interesting given that the effects of TH would likely diminish with smaller doses of collagenase (e.g., less or no hematoma expansion after smaller bleeds). Finally, our results likely apply to other cooling methods, such as intentional drug induced TH (Wei et al., 2013) or unintended cooling (e.g., drug side effect).

Findings in the collagenase model may not apply to other ICH models, as discussed. Indeed, we would not expect focal cooling to worsen bleeding in the blood infusion model because systemic hypothermia does not affect bleeding in this model (MacLellan et al., 2006b), likely because there is little spontaneous bleeding (MacLellan et al., 2008). So, we tested our hypothesis in the thrombin reductionist model because a recent study showed that infusing 0.5U of rat thrombin into the striatum of mice resulted in hemorrhaging as measured days later (Cheng et al., 2014). In our study, however, 1U of rat thrombin did not increase bleeding in rats beyond that caused by a needle insertion, and when assessed at either 24 or 48 hours. This was surprising, but it might simply be due to differences between these studies (e.g., rats vs. mice). Regardless, the early induction of hypothermia did not cause additional bleeding in our thrombin model. Therefore, it appears that cooling-induced bleeding did not confound our recent investigation of thrombin-mediated injury (Wowk et al., 2014) where cooling failed to provide benefit against edema, cell death, and behavioral impairment. Note that we used the same model in that study as presently used.

2.5 Conclusions

In summary, we report that mild brain-selective TH significantly worsens bleeding when initiated up to 12 hours after a collagenase-induced ICH whereas no effect was observed after saline or thrombin infusion. Thus, these findings may partly explain the negative data in some animal studies that used TH after collagenase-induced ICH. For this model, our data also clearly demonstrate the need for prolonged temperature measurement and control, such as in neuroprotection studies where unintended drug-induced cooling and aggravated bleeding may limit the compound's potential efficacy. Finally, these findings support clinical suspicions that the beneficial effects of TH (e.g., reducing edema and intracranial pressure) could be easily lost or greatly diminished in patients if such complications are not identified and managed or avoided (e.g., use of delayed TH to target late elevations in intracranial pressure) (Staykov et al., 2013). In our opinion, side effects such as aggravated bleeding and the fact that mild TH does not seem to protect against key mechanisms of secondary degeneration after ICH, notably thrombin (Wowk et al., 2014) and iron toxicity (Wowk et al., 2014), are significant road blocks to the successful translation of TH as a neuroprotective (cell saving) treatment for ICH. Of course, this does not mean that TH will not reduce morbidity and mortality in patients with severe ICH such as through lowering perihemorrhagic edema and intracranial pressure. Despite repeated observations that TH lessens edema (total brain water content) in rodent ICH studies, we suspect that this is of little benefit given that the mild to moderate insults used would not likely raise intracranial pressure to the point where it is of real concern in animal studies (Hiploylee and Colbourne, 2014).

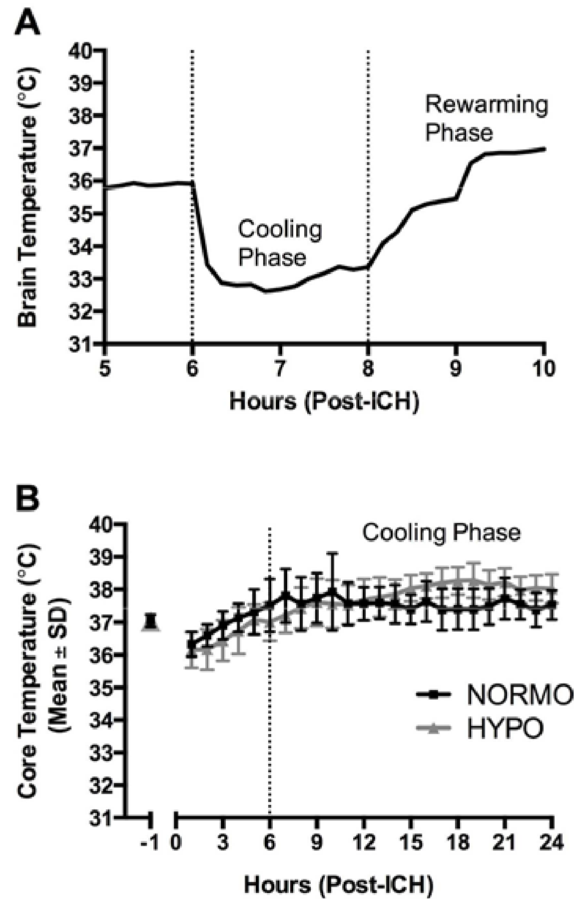

FIG. 2-1. (A) Brain temperature was measured by a telemetry probe in a rat treated with TH at 6 hours after collagenase infusion. Brain temperature quickly decreased upon flushing the cooling device with cold water. In this case rewarming began 8 hours post-ICH and was continued over 2 hours. This experiment was done to simply illustrate the depth of hypothermia produced by our cooling system. Longer durations of TH were used in the rest of the study. Temperature readings were recorded every minute and averaged every 10 minutes. **(B)** Core temperature was measured by telemetry probes in 5 NORMO and 5 HYPO-6 treated rats previously given a collagenase infusion. In this case, TH started at 6 hours and continued for 18 hours. Measurements were taken every minutes and averaged every 30 minutes. ICH, intracerebral hemorrhage; TH, therapeutic hypothermia.

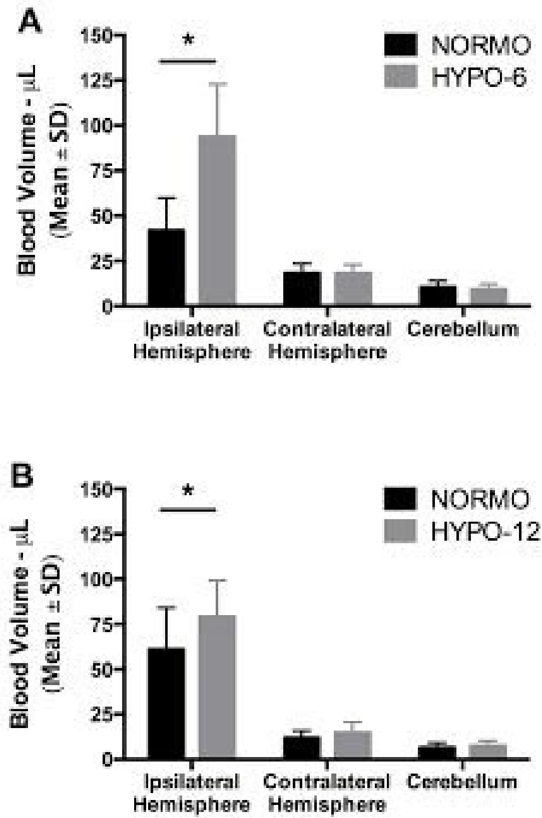

FIG. 2-2. Intraparenchymal blood volume, which includes intra- and extravascular blood, in the collagenase model where treatment was initiated at **(A)** 6 hours (5 NORMO and 5 HYPO-6 rats with 24-hour survival), **(B)** 12 hours (14 NORMO and 13 HYPO-12 rats at a 24-hour survival). The hematoma size in the ipsilateral hemisphere significantly increased with the HYPO-6 (p = 0.018) and HYPO-12 (p = 0.042). An asterisk denotes $p < 0.05$. As expected, focal TH treatment did not affect bleeding volume in the control regions (contralateral side and cerebellum).

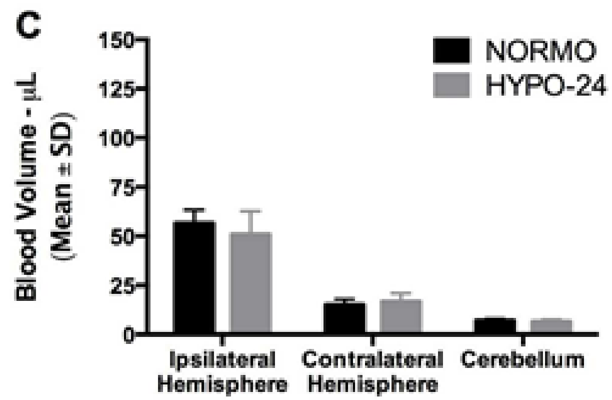

FIG. 2-2. (C) 24 hours 8 NORMO and 9 HYPO-24 rats at a 36-hour survival. The hematoma size in the ipsilateral hemisphere did not significantly increased 24 hours post-injection (p = 0.258). As expected, focal TH treatment did not affect bleeding volume in the control regions (contralateral side and cerebellum).

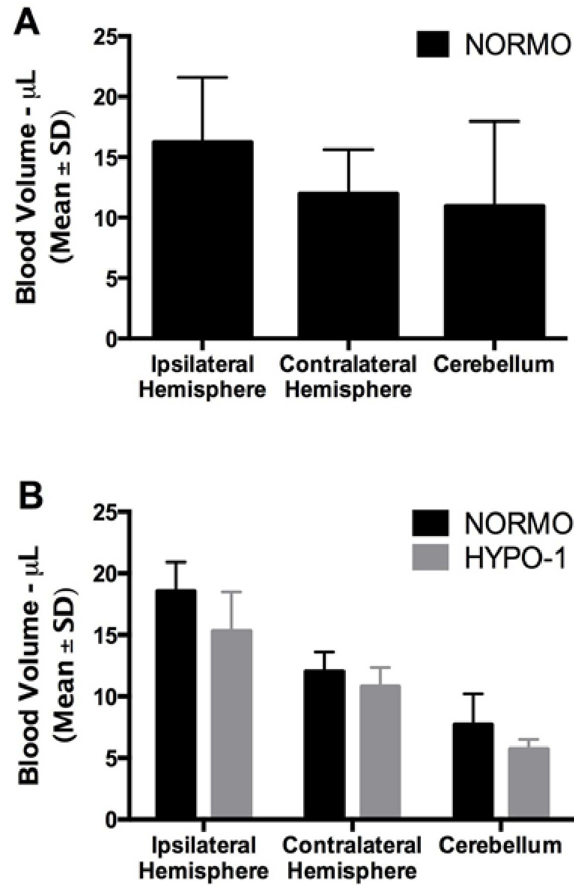

FIG. 2-3. (A) An intrastriatal infusion of thrombin in saline slightly but nonsignificantly increased intraparenchymal blood volume at 24 hours post-infusion compared with the normal side (n = 4). **(B)** At 48 hours, this effect was statistically significant (ipsilateral vs. contralateral hemisphere). However, at both times the difference between hemispheres in these thrombin experiments was comparable to that produced by a saline infusion suggesting that the procedure and not thrombin caused a small hematoma. Regardless, bleeding was not different ($p \geq 0.130$) between groups (7 NORMO vs. 4 HYPO-1 rats) in any of the brain regions analyzed at 48 hours post-injection. Note that the Y-axis scale differs from **FIG. 2-2.** owing to the fact that there was considerably less blood after an infusion of just thrombin than in the collagenase model of ICH.

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CHAPTER 3

Localized Hypothermia Reduces Intracranial Pressure Following Collagenase-induced Intracerebral Hemorrhage in Rat

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A version of this chapter has been submitted for publication. John RF and Colbourne F (2015). Experimental Neurology.

3.1 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% (Broderick et al., 1993, Qureshi et al., 2005). 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, 2012). 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 (Steiner et al., 2001). Thus, it is thought that reducing edema will lessen 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 infusing 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 found after infusing a roughly comparable volume of blood into the striatum of rat (Hiploylee and Colbourne, 2014). The course of edema is similar between these models with a peak occurring around the third day and complete resolution within about a week (Hua et al., 2003; Fingas et al., 2007). In patients,

edema growth is highest in the first 48 hours and peaks up to 2-3 weeks, but not all patients with progressive edema show neurological deterioration and it seems that edema itself may not independently predict outlook (Arima et al., 2009). 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 (Hiploylee 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., 2002; 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 a comparison to historical controls, Kollmar et al., 2010 reported that mild (35ºC) endovascular cooling for up to 10 day prevented the progression of edema and improved outlook 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 et al., 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/hour (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 (Hiploylee 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; Hiploylee 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 hour 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 et al., 2011; Rincon et al., 2014; Su et al., 2015). However, treatment onset was delayed for 24 hour because localized TH can aggravate intraparenchymal hemorrhaging even when administered up to 12 hours after collagenase infusion (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).

3.2 Materials and Methods

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-hour 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.

Experiments

Experiment 1: Effects of ICH on ICP and Edema

A total of 7 rats were given a striatal ICH (ICH) and 8 had a sham surgery (SHAM). Epidural ICP was recorded for 72 hours after this surgery and which time they were euthanized to assess peak brain edema (Fingas et al., 2007).

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 hours after surgery and this treatment lasted for 3 days. The NORMO controls were not cooled. All animals were euthanized 1 week after surgery, and hematoma volume was measured using a hemoglobin spectrophotometric assay (Choudri et al., 1997; John et al., 2014) to determine whether TH caused additional bleeding.

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 hours (HYPO-s, n = 17). Cooling began 24 hour 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.

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 hour 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.

ICH Surgery

All animals were anesthetized with isoflurane (4% induction, 2% maintenance in 60% N_2O and balance O_2) 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 minutes. The needle was left in place for an additional 5 minutes 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.

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 23G 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; Hiploylee 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 minutes, and stored using ART software (DataSciences Int., A.R.T. v 2.3). In all experiments, room pressure recordings were collected at least 2 hours prior to surgery to allow for the correction of pressure-offset errors.

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 hours after collagenase infusion. This was followed by rapid (water flow turned off) or gradual rewarming (step reductions in flow over 6 hours) depending on the experiment. Based on published data, a flow rate of 96-120 mL/hour, 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 behaviours (e.g., rest vs. active). Finally, body temperature remains normothermic during cooling (Clark and Colbourne, 2007; Auriat et al., 2012).

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 minutes to allow for additional osmotic lysing of red blood cells. Homogenate samples were then transferred to 1.5 mL Eppendorf tubes and centrifuged at 15 800 g for 35 minutes. 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 minutes. 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.

Water Content Assessment

Animal were anesthetized with isoflurane and immediately decapitated. Brains were blocked from 2 mm anterior to 4 mm posterior to the injection. Ipsilateral and contralateral

cortex and striatum, and cerebellum (control) were separated and weighed before (wet weight) and after (dry weight) being baked for 24 hours at 100ºC in an oven. Normal striatal water content is ~78% (Fingas et al., 2007).

Statistical Analysis

All data was analyzed using SPSS (v. 21, SPSS Inc., Chicago, IL). ICP data were analyzed using ANOVA and when needed additional post-hoc Tukey tests. Mortality was analyzed using a point biserial correlation (Pearson r) and multiple linear regressions. Comparisons were considered significant when $p \leq 0.05$. Data are presented as mean, and standard deviation bands are plotted where applicable.

3.3 Results

Mortality and Exclusions

Of 107 animals, 23 were excluded because of technical problems (e.g., faulty cooling device) or experimenter error. One additional animal was euthanized early because of feeding difficulties and excess weight loss. Nine others spontaneously died within 18 hours post-insult. Their ICP data suggests that at least some of these resulted from ICP spikes. Indeed, in a multiple regression analysis, average and peak ICP (from the time of ICH up to the point of death) predicted mortality ($r = 0.656$, $p < 0.001$). Of the two, 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 hours, 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.

Experiment 1: ICH-induced ICP and Edema Changes

Compared to SHAMs that had a 3-day average ICP of 6.5 mm Hg, those with an ICH averaged 18.0 mm Hg (p = 0.007, Figure 3-1A). Although not formally assessed, a hematoma was seen during tissue processing for all collagenase-treated rats.

The SHAM group had normal water content in striatum (Figure 3-1B) whereas the ICH group had substantially more (from 78% to 84%, $p < 0.001$). The cerebellum control samples were normal and not different between groups (p = 0.346).

Experiment 2: 24-hour intervention delay does not worsen bleeding

The large dose of collagenase caused substantial bleeding (~61 µL) within striatum as determined 7 days later versus natural blood volume in the brain of 10 - 20 µL (John et al., 2014). Inducing TH starting 24 hours after collagenase infusion did not affect hematoma volume (p = 0.481 vs. NORMO, Figure 3-2). The volume of blood in cerebellum control samples was similar between groups (p = 0.866).

Experiment 3: TH reduced ICP but not edema

As in experiment 1, the ICH rats had above normal ICP values on day 1. As the groups were not treated differently at this time, the average (p = 0.527, Figure 3-3A) and peak ICP values (p = 0.686, Figure 3-3B) were similar among groups. Although there was a trend for the HYPO groups to have lower average and peak ICP values on day 2, which is the first 24-hour 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 day 3 and 4 ($p \leq 0.042$). Tukey post-hoc comparisons were done to identify those comparisons that were significant as illustrated in Figure 3-4. For instance, on day 3 (2nd day of cooling), both HYPO groups significantly reduced peak ICP ($p \leq 0.047$ vs. NORMO). Similarly, peak ICP remained lower in both HYPO groups upon rewarming (day 4, $p \leq 0.047$).

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 day 1-3). The average ($p = 0.819$) and peak ICP ($p = 0.837$) comparisons on day 1 were not significantly different, which was expected because all groups were normothermic during the first 24 hours. On day 2, the first day of TH, there were non-significant trends for cooling to reduce average ($p = 0.143$) and peak ICP ($p = 0.127$). In contrast, TH significantly lessened average ($p = 0.009$) and peak ICP ($p = 0.002$) on day 3, the 2nd day of cooling.

The HYPO-f and HYPO-s groups did not significantly differ for any of the above ICP post-hoc comparisons ($p \geq 0.233$). As there were potentially more transient effects that would be missed in the above analyses, we examined the hourly data starting 6 hours prior to rewarming or the equivalent time in the NORMO group until 12 hours later (Figure 3-3C). 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, Figure 3-4A). 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 ($p = 0.967$). A linear correlation analysis showed no relationship between either

peak ICP (day 4) and edema (r = 0.078, $p = 0.654$) or average ICP (day 4) and edema (r = 0.057, $p = 0.747$) (all three groups included, Figure 3-4C). Likewise, there were no significant relationships for each group ($p \geq 0.092$).

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 (Figure 3-4A), we examined whether edema was established by day 1, and whether cooling lessened edema when assessed in rats euthanized at 72 hours post-ICH. Significant edema was found on day 1 in untreated ICH rats (Figure 3-4B; $p = 0.001$ for comparison with SHAMs from Experiment 1). Edema after normothermic ICH significantly increased from day 1 to 3 ($p < 0.001$). Nonetheless, TH, which started at 24 hours post-ICH, did not lessen edema on day 3 ($p = 0.801$). These animals were subjected to TH up to a few minutes prior to euthanasia. Cerebellum control samples were normal in all cases.

3.4 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 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 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 did result in 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). Several animals died within the first 18 hours after ICH in this study, and ICP data revealed that these spontaneous deaths were related to transient ICP spikes. Several studies reporting on the high mortality rate for ICH patients indicate that 20-50% of these deaths occur within the first 2 days (Broderick et al., 1993; 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 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 and perhaps make it even more difficult to 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; Kollmar et al., 2010), and presumably a promising candidate for ICP management. Our animal data strongly support previous clinical observations (Kollmar et al., 2012) as discussed, and extends them to show that brain-selective TH can significantly reduce ICP even when initiated 24 hours after ICH begins. Interestingly, in our study TH lowered ICP even though there was no reduction in cerebral

edema. 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 presumed mechanisms (e.g., 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.

While the majority of animal studies cool for < 6 hours (van der Worp et al., 2007), several ischemia studies suggest that prolonged TH is more effective than brief TH after 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 hours after injury (John et al., 2014) likely due to the protracted nature of bleeding in this model (Rosenberg et al., 1990; MacLellan et al., 2008). Thus, it is likely 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 caused rebound edema, which surprisingly did not affect ICP. Regardless, elevated edema suggests a harmful effect, which should be avoided by using slower rewarming. Our data suggests that a 6-hour period is sufficiently slow, but additional study is needed (after varying depths and durations of cooling, young and old subjects, etc.).

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 (Clark and Colbourne, 2007; Fingas et al., 2007; John et al., 2014). Second, we did not assess histological or behavioural 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 (Ghabriel et al., 2010; Murtha et al., 2015) and it is likely that this applies to ICH as well, but further study is needed.

3.5 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 causes 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-hour delayed TH protocol. Although TH is a promising strategy, at least for ICP control after ICH, further pre-clinical work is needed to optimize its effectiveness and to better understand mechanisms of action.

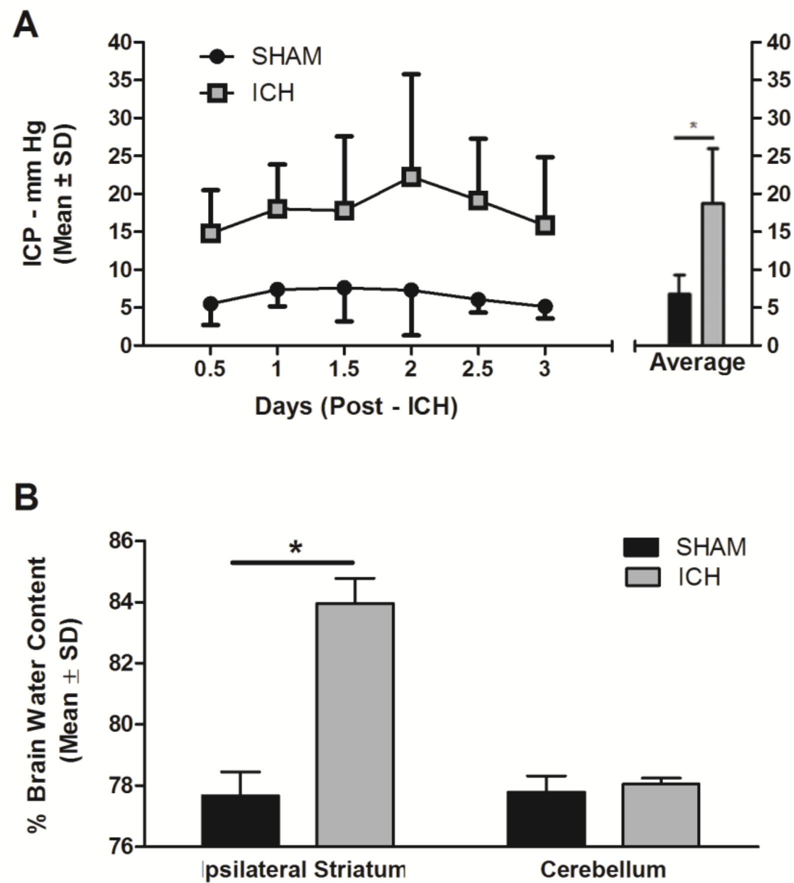

FIG. 3-1. (A) The ICH increased ICP for 3 days (* denotes a significant difference of $p = 0.007$ vs. SHAM). Values are in mm Hg (mean ± SD). **(B)** Water content increased in select brain regions on day 3. There was a significant difference between groups in brain water content in the ipsilateral striatum (* $p < 0.001$). Values expressed as percentage (mean ± SD).

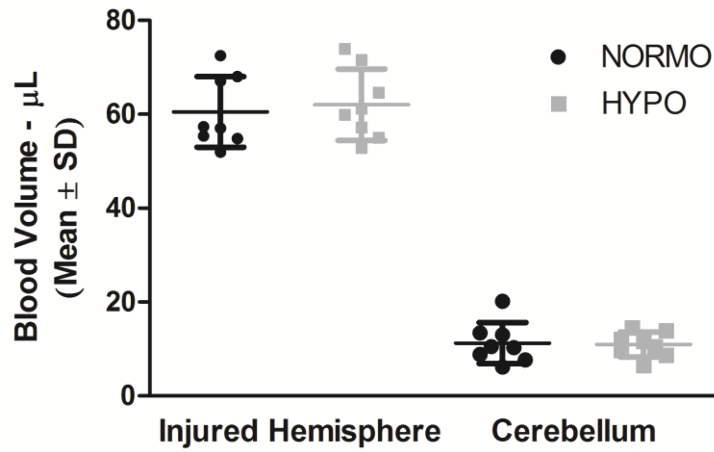

FIG. 3-2. Cooling was started at 24 hours after collagenase infusion (HYPO), and animals were cooled for 3 days. Normothermic controls (NORMO) were not cooled. All animals were euthanized 1 week post-ICH. Localized cooling did not significantly impact bleeding volume in the ipsilateral hemisphere ($p = 0.481$ vs. NORMO group) or in the cerebellum control region ($p = 0.866$).

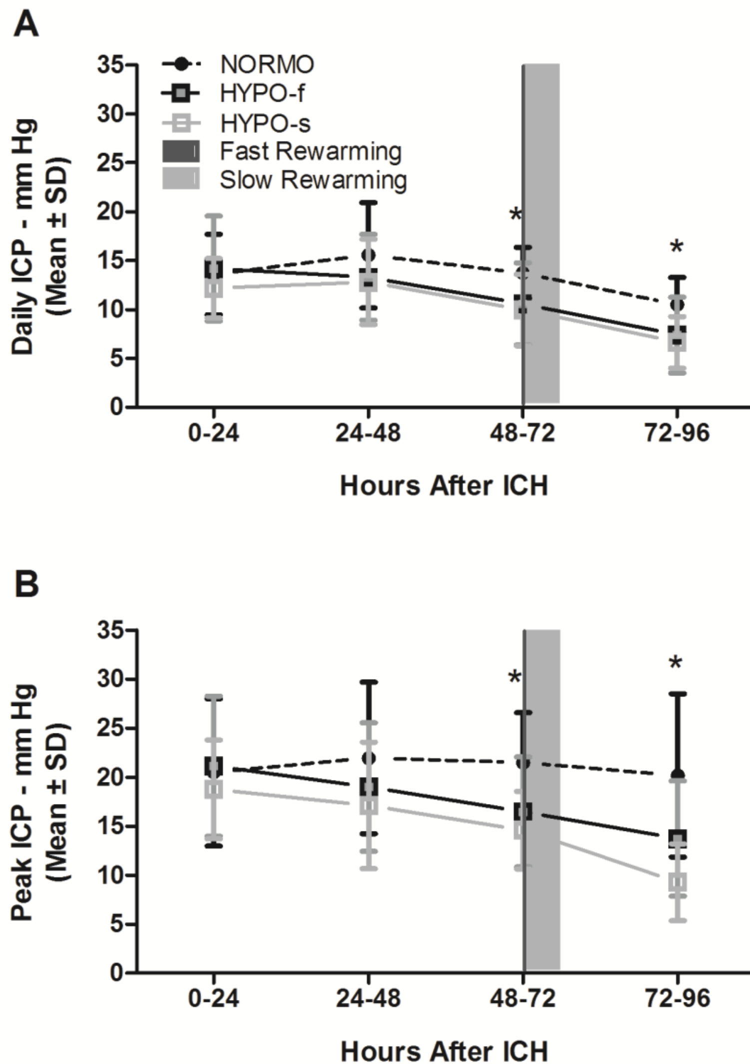

FIG. 3-3. (A) Epidural ICP measurements were recorded every min and averaged every 30 minutes. Cooling was initiated at 24 hours, followed by either fast (i.e. instantaneously, HYPO-f) or slow rewarming over 6 hours (HYPO-s) starting after 48 hours of cooling. There was no effect of localized TH on mean ICP until day 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 ($p < 0.05$).

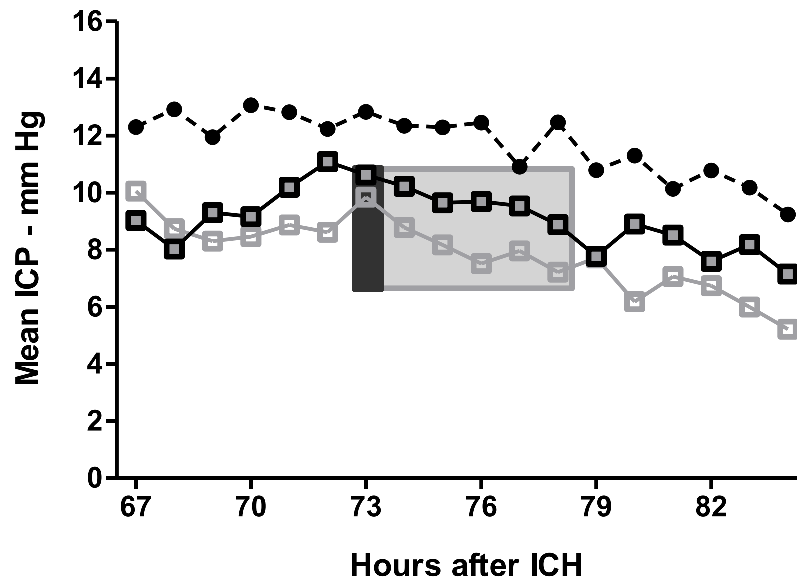

FIG. 3-3. (C) This graph depicts the ICP data starting from 6 hours prior to rewarming or a comparable time in controls. There were no obvious spikes in ICP, such as at the start of rewarming in either the HYPO-f or HYPO-s groups.

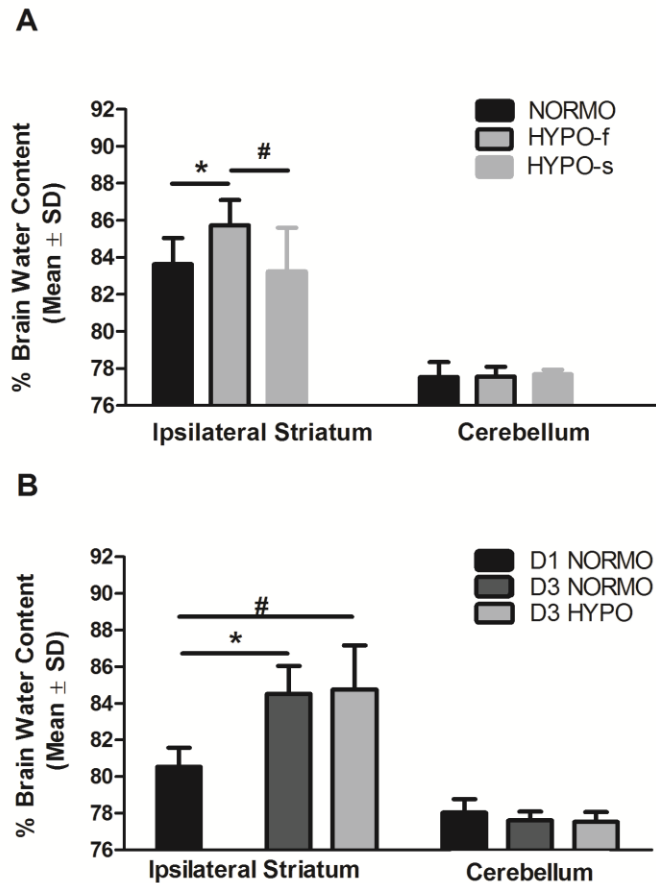

FIG. 3-4. **(A)** Cooling did not reduce day 4 brain water content in either treatment group. In fact, fast rewarming actually worsened edema (*$p \leq 0.016$ vs. NORMO, #$p \leq 0.010$ vs. HYPO-f). Water content in the cerebellum was not statistically different among groups ($p = 0.818$). **(B)** In a separate experiment, significant edema was found on day 1 in untreated ICH rats (Figure 4B; $p = 0.001$ for comparison with SHAMs from Experiment 1). Edema after normothermic ICH significantly increased from day 1 to 3 (*$p < 0.001$). Edema after hypothermic ICH was also significantly increased (#$p \geq 0.010$ vs. day 1 NORMO). Nonetheless, TH, which started at 24 hours post-ICH, did not lessen edema on day 3 ($p = 0.801$ vs. day 3 NORMO).

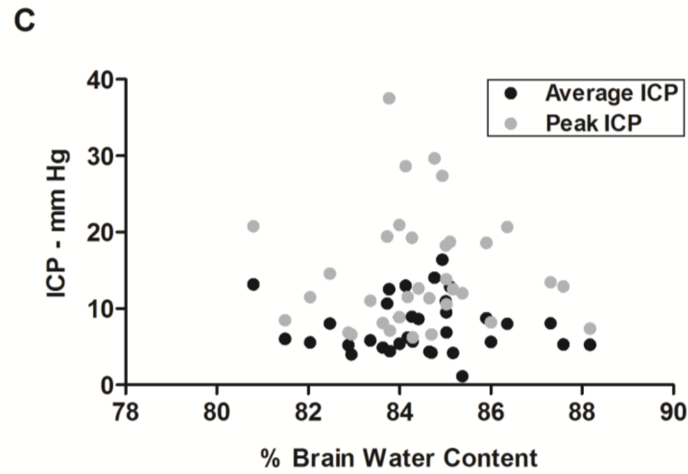

FIG. 3-4. **(C)** There were no significant linear relationships between day 4 water content (ipsilateral striatum) and average ICP (r = 0.057, *p* = 0.747) or peak ICP (r = 0.078, *p* = 0.654, all three groups included). Similarly, there were no significant relationships for each group.

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CHAPTER 4

4.1 Primary Findings

The intent of this thesis was to investigate the influence of localized mild TH on ICP in a rat model of collagenase-induced ICH. In Chapter 2, we compared three treatment intervention delays and showed hypothermia treatment after ICH must be delayed to at least 24 hours after injury to prevent aggravated bleeding. These findings may partly explain the negative data in some experimental studies that induced TH soon after collagenase-induced ICH. Using a 24-hour intervention delay, we evaluated the effect of this treatment on ICP and edema in Chapter 3. We found that severe ICH significantly elevated ICP. Delaying cooling for 24 hours delayed cooling reduced average and peak ICP but did not reduce edema. Fast rewarming after cooling markedly increased edema on day 4, which had an insignificant effect on average or peak ICP. Finally, edema did not predict mean or peak ICP. These findings demonstrate four main conclusions: a) early TH after ICH can worsen hemorrhaging, b) mild TH reduces average and peak ICP but does not reduce edema c) fast rewarming worsens edema and potentially causes more harm, and d) edema does not always predict mean or peak ICP.

4.2 Hypothermia Worsens Bleeding after ICH

In Chapter 2, we varied treatment initiation (6, 12, 24 hour) following experimental ICH to compare the efficacy of delayed hypothermia. We found that inducing hypothermia earlier than 24 hours after stroke worsens hemorrhaging. In fact, the longer the intervention delay the less of an effect hypothermia had on bleeding. However, we know from ischemia studies that the efficacy of hypothermia decreases with longer intervention delays (Dietrich et al., 1993; Yenari and Hemmen, 2010). The present study was not designed to evaluate neuroprotection but

worsened bleeding is intuitively not protective. Clinically, there is a ≥ 33% growth in the volume of parenchymal hemorrhage during the first 24 hours after ICH in at least 38% of patients, and most of the ongoing bleeding occurs within the first 3 to 4 hours after hemorrhage onset (Brott et al., 1997). This was also confirmed by several small retrospective computed tomography (CT) series of ICH (Chen et al., 1989; Broderick et al., 1990; Fujii et al., 1994). If bleeding stops within 6 hours why does hypothermia almost double the bleeding volume when initiated 6 hours after ICH? There are several possibilities. Bleeding in the collagenase model may be more extensive than what occurs in patients. For instance, collagenase may cause bleeding from many more vessels and over a much longer time than what happens in most patients. Temperatures below 35°C can induce mild platelet dysfunction or a mild decrease in platelet count in some patients (Polderman, 2009). With deeper hypothermia (e.g. ≤ 33ºC), the coagulation cascade is further affected through disrupted synthesis and kinetics of clotting enzymes and plasminogen activator inhibitors (Polderman, 2009). In addition, earlier studies (Michenfelder and Milde, 1991; Lanier 1995; Mrozek et al., 2012) have shown a linear relationship between cerebral metabolism and temperature, with a 6 to 10% change in metabolism per degree Celsius. This results in a proportional reduction in CBF, leading to decreased vascular volume and ICP (Mrozek et al., 2012). However, some studies suggest that cerebral metabolic coupling may induce a nonlinear relationship between cerebral metabolism and blood flow (Sakoh and Gjedde, 2003; Lin et al., 2010; Mrozek et al., 2011). In line with this theory, hypothermia may reduce metabolism without a concomitant decrease in blood flow, thus exaggerating bleeding. In addition, secretion of vasoactive agents such as thromboxane A_2 and prostaglandin I_2 are important to maintain blood homeostasis (Milde et al., 1992). Brain injury can disrupt this production, and therefore perfusion. There is evidence of hypothermia attenuating these

disruptions (Busto et al., 1989; Aibiki et al., 2000; Moore et al., 2011), but this is influenced by cerebral autoregulation. With large injuries, as in the present study, cerebral autoregulation may be so impaired that damaging results to perfusion are irreversible, and bleeding continues. Another possibility is that cooling caused re-bleeding. This might be the case for immature clots, as a recent thromboelastography study demonstrated that a mature clot is seemingly not influenced by cooling (Ruzicka et al., 2012). However, we do not know when clotting is 'mature' after collagenase-induced ICH.

Significant bleeding problems have not been reported in patients with severe TBI, stroke, or cardiac arrest; but these studies excluded actively bleeding patients. Thus, many studies report minor or near absent risks of severe bleeding associated with mild hypothermia (Polderman, 2012). For example, Storm et al., 2008 found that the risk of bleeding in cardiac arrest patients did not increase in those treated with mild hypothermia. However, there was a trend toward more red blood cell units being required to reach target hematocrit in patients who developed bleeding complications. Since neurological outcomes were better in treated patients compared to matched historical patients treated with thrombolysis only, the authors concluded that the bleeding risk did not pose a severe threat to withhold treatment. However, such conclusions are cautioned because a mildly elevated bleeding risk may be magnified in actively bleeding patients. If we assume that the proportion of patients experiencing a growth in hemorrhage volume within the first 24 h are those with severe ICHs, these are also the patients likely to be treated with hypothermia. As such, careful consideration should precede the use of hypothermia in patients who are actively bleeding, or who are at high risk of bleeding.

4.3 Hypothermia and ICP Management

Using a 24-hour intervention delay, we evaluated whether brain-selective mild, hypothermia can mitigate edema and manage ICP after ICH in Chapter 3. The finding that cooling reduces peak and mean ICP but does not significantly lessen edema even on Day 3 (peak edema), suggests that edema reduction is not the likely mechanism of ICP control. This hypothesis is further supported by our observation that fast rewarming exacerbated edema, but did not cause any noticeable changes in ICP. Several animal experiments and clinical data support the use of TH as an anti-edema therapy and promising candidate for ICP management (MacLellan et al., 2006b; Kollmar et al., 2010, Staykov et al., 2011). A recent investigation in TBI has also confirmed TH ability to reduce ICP despite different cooling methods (Flynn et al., 2015). Kollmar et al., 2012 previously assessed the ability of mild TH (endovascular cooling to 35ºC) to target PHE after clinical ICH. The authors reported that prolonged (10 days) TH effectively mitigated edema and prevented ICP elevation in patients with large ICH (> 25 ml). All patients were rewarmed slowly by 0.1°C/hour, and no evidence of rebound edema was reported in sharp contrast to the present study. However, patients were treated with an endovascular catheter-cooling device and for a much longer period. Cooling for longer periods may depress the rebound effect during rewarming, and prevent worsened edema. Generally, discrepancies in findings between animal and clinical data might be explained by the fact that we have not been able to set clear therapeutic standards for cooling parameters such as optimal initiation, duration, and rates of rewarming.

4.3.1 Parameters of Cooling

Within the context of our study design, it is possible that a longer treatment duration would have caused an even further reduction in ICP because we did not observe a therapeutic effect on ICP until the second day of cooling (Day 3 after injury). The optimal duration of hypothermia after ICH is unclear, but prolonged hypothermia (≥ 48 hours) in rodent and clinical studies of TBI and ischemia has been shown to improve functional recovery and reduce injury (Colbourne et al., 2000; Clark et al., 2008; Yenari et al., 2008; Kee Ng et al., 2009). In the largest study to compare short-term and long-term cooling durations in severe TBI patients with intracranial hypertension, Jiang et al., 2006 found that outcome at 6 months significantly improved in 43.5% of cases compared to 29.0% of cases in the short-term mild hypothermia group. In a meta-analysis of 12 randomized trials of hypothermia for TBI, McIntyre et al., 2003 reported that 48 hours of cooling in TBI patients was more effective than short-term hypothermia in reducing mortality and improving neurological outcome. This data was also consistent with animal data that suggested that cooling for longer than 48 hours in some TBI cases might be necessary since cerebral edema may last for up to 4 and sometimes 7 days after TBI (Markgraf et al., 2001). However, the treatment duration may also depend on the time of initiation. We have previously shown that when mild, systemic prolonged hypothermia was delayed for 12 hours and maintained for 48 hours in rodents, ICH lesion volume and behavioural deficits were significantly reduced. In contrast, early and brief treatment (cooled for 7 hours beginning 1 hour after collagenase-infusion) resulted in significantly more bleeding at 12 hours (MacLellan et al., 2004). This adverse effect of cooling may be a result of the surface cooling method which is associated with coagulopathy-related complications (Kirkpatrick et al., 1999; Schubert 1995), but in Chapter 2 we confirmed that early induction using focal cooling methods may also cause

bleeding complications (John et al., 2014). At least in the context of the collagenase-induced ICH model, we are limited by this bleeding confound and possibly reducing our treatment efficacy with longer intervention delays. Time might also be critical in clinical application of hypothermia, as it may take long for patients to reach the hospital and be stabilized prior to treatment, during which patients may potentially miss a theoretical therapeutic window.

4.3.2 Edema Management for ICP

Recognizing that edema peaks in several rodent studies on Day 3 (Hua et al., 2003; Fingas et al., 2007), and previous findings that brain-selective cooling attenuates brain edema after ICH and ischemia (Kim et al., 2013; Fingas et al., 2007; Fingas et al., 2009), we expected TH to have an effect on edema in the present study. Instead, mild TH did not reduce edema on Day 3 or 4, which obfuscates the relationship between ICP and edema. We can infer from previous data that even a modest amount of edema in the whole blood model does not impact ICP (Hiploylee and Colbourne, 2014). It is unclear whether there is a threshold that edema must exceed to increase ICP. The present study was not designed to evaluate this but it is possible that the longer ICP is elevated, the greater edema will be. In Chapter 3, we observed a growth in brain water content between Day 3 and Day 4. Due to the severity of the ICH, the edema peak may actually be much later than 3 days. When we combined all the data, edema did not correlate with peak or mean ICP, suggesting that there must be mechanisms other than edema contributing to ICP elevation and its attenuation by hypothermia.

A recent study by Murtha et al., 2015 revealed that even in small strokes, animals with little edema had a significant increase in ICP. This is important because several of the studies that have declared a causative relationship between edema and ICP have been done in patients

with high edema volumes (Ropper et al., 1984; Schwab et al., 1998; Morley et al., 2002). The opposite could also be true and explains why fast rewarming worsened ICP but not edema in Chapter 3; brain water content can be significantly worsened in the absence of a dramatic ICP response. Based on the Monroe-Kellie doctrine, an increase in the volume of any one of the intracranial components (e.g. blood, brain, and CSF) must be associated with a rise in ICP if brain compliance is exhausted. It has been speculated that in the initial stages after brain trauma, the CSF volume (slowed production, increased drainage) counters maladaptive changes in ICP (Ghabriel et al., 2010; Murtha et al., 2015). Since we did not measure CSF, we cannot address this component. However, in severe ICHs where integrity of the blood-CSF barrier is strongly compromised, CSF management may provide further insight into the control of ICP elevation after ICH.

4.3.3 Impact of Rewarming on ICP

The impact of post-hypothermia rewarming on treatment efficacy in ICH has been discussed as a potential confound, but scarcely investigated (Povlishock et al., 2009). In fact, consideration of rewarming rates has largely been a consequence of reviews assessing hypothermic intervention after severe TBI, where fast rewarming may cause sudden vasodilation, reduced CPP and hypoxia, and rebound ICP (Algaza et al., 2006). Ultimately, these changes may mask the beneficial effects provided by TH. Thus, the present study found it essential to experimentally compare fast and slow rewarming rates in an ICH setting to provide further insight into hypothermia management of ICP. In Chapter 3, we demonstrated that fast rewarming (i.e. instantaneously) caused rebound edema, while no effect on edema was observed after slow rewarming over a period of 6 hours. Interestingly, the edema volume in the slow

rewarmed grouped was comparable to the normothermic animals (~83%). This is not an insignificant amount since normal brain water content is 78% (Fingas et al., 2007; Clark et al., 2008; Hiploylee and Colbourne, 2014; John et al., 2014), and implies that animals may need to be rewarmed for longer periods to further depress edema. Clinically, post-hypothermia rewarming is normally done at a slow controlled rate. Many clinical studies have adopted slow rewarming rates within the range of 0.3-1.7 ºC/hour to normothermic temperature (Kollmar et al, 2010; Hemmen et al., 2010; van der Worp et al., 2014). Note that abnormal ICP responses most often occur or are mostly reported in patients who were cooled for a relatively short period (Povlishock and Wei, 2009). This present a confound since short re-warming means rewarming occurs sooner after the insult whereas longer cooling means rewarming later after the insult is which is also confounded by the length of cooling. In a large RCT by Jiang et al., 2006, the authors compared short-term (2 days) and long-term (5 days) systemic hypothermia in TBI patients. All patients were rewarmed to 36.8ºC ± 0.5ºC at a rate of 1 ºC/hour, and neurological outcome on the Glasgow Outcome Scale (GOS) was assessed 6 months after the injury. The authors reported that rewarming after short duration hypothermia led to rebound ICP and poor long-term outcome compared to long-term hypothermia. They concluded that early rewarming might cause rebound ICP. Adelson et al., 2005 have also found rebound ICP in the systemic hypothermia group for up to 12 hours after rewarming in pediatric TBI patients if cooling lasted less than 48 hours. These findings are not limited to TBI patients. Since ICP often increases progressively, then remains high for several days following TBI (Jiang et al., 2006), in severe ICH patients where hypertension is also an important factor, cooling longer may be more beneficial. On the other hand, systemic cooling methods were used to induce hypothermia in the studies mentioned above. Given the complications associated with these methods (e.g. BP,

adverse cardiovascular events), similar ICP responses might not occur with focal cooling methods in ICH. The current Cooling in Intracerebral Hemorrhage (CINCH) trial has adopted a rewarming rate of 0.05 ºC/hour for approximately ≤ 40 hours after 8 days of endovascular cooling (Kollmar et al., 2012). The results of this trial will influence future therapy for patients with large ICH.

4.4 Limitations

A major limitation of this work is the rodent ICH model, which may not have the same bleeding profile as humans. As mentioned earlier, many patients stop bleeding within a few hours (Ovesen et al., 2014). Here, we have a severe collagenase-induced ICH that mimics the extreme cases of prolonged bleeding. Our results, therefore should best predict the outcome in those patients. However, if cooling is beneficial for ICP reduction in severe ICH, it should also provide benefit in patients that allow for early intervention. No direct comparisons between ICH severities were made in this study. It would be interesting and valuable to directly contrast the efficacy of early intervention of hypothermia and ICP responses in both small and large ICH injuries. This could be achieved by varying the dose of collagenase infused to show hematoma-injury relationships as previously done (MacLellan et al., 2006a).

Clinically, various methods exist to focally cool the brain such as cooling helmets, and neckbands. However, these methods require several hours to reach target temperature (Wang et al., 2004). The cooling method used in this study removes heat from the surface of the skull and creates a temperature gradient in the brain. Thus, cortical structures may undergo greater cooling than the striatum (Clark and Colbourne, 2007). Furthermore, this method would not be suited for large animals (e.g. pigs) or humans due to thicker skulls and larger brains.

Another limitation is that we did not measure brain temperature during our TH intervention. From earlier work (Clark and Colbourne, 2007; John et al., 2014) we infer that the ipsilateral striatum of rats would be cooled to 34.7ºC ± 0.2ºC. Precise control with this method is not easily achieved and the use of brain temperature probes in the present study is problematic because it may cause additional injury and overestimate ICP responses. In addition, animals were slowly rewarmed in a stepwise manner where small changes in the rewarming cause almost immediate changes in brain temperature (data not shown). Given the difficulty in controlling the rewarming rate, it is possible that fluctuations in brain temperature occurred while attempting to achieve the optimal rewarming rate, which may be injurious to the protection initially provided by hypothermia. Our telemetry system does not allow simultaneous measurement of ICP and brain temperature due to signal interference. Further methodological refinements may circumvent this problem.

Finally, we did not provide any histological or behavioural evidence to demonstrate worsened outcome because of rebound edema or benefit because of reduced ICP. Since several studies have shown the degree of brain edema is associated with poor outcome (Zazulia et al., 1999; Xi et al., 2006), it is likely that the significant increase in brain edema observed after fast rewarming would be harmful. Correlating behavioural deficits with worsened outcome after fast rewarming and rebound edema in the collagenase-induced ICH model may be useful in efficacy studies of TH.

4.5 Future Directions

Of interest, it would be important to determine the pressure-volume relationship in rat. We have previously found a linear relationship (Hiployee and Colbourne, 2014) in contrast to

the non-linear pressure-volume relationship described by the Monroe-Kellie Doctrine. However, that linear relationship did not account for lesion volume, which is likely magnified in a severe ICH. Brain compliance is another important consideration. Different rodent strains might differ in injury tolerance. An impact-acceleration injury study reported no mortality in Sprague-Dawley rats whereas 50% of Wistar rats died. This suggests that the Sprague-Dawley strain has a higher brain compliance (Engleborghs et al., 1998). Further exploration may be required to elucidate these pressure-volume relationships. An option is to continuously infuse artificial CSF to determine when brain compliance is compromised in severe ICH, and how that might be affected with temperature changes.

We have previously attempted to measure ICP at protracted time points, but the patency of the ICP monitory system after 4 days is worrisome owing to severe ICP rises (likely an artefact) on Day 6 and Day 7 in sham animals (Hiploylee and Colbourne, 2014). Other studies have also shown unexplained increases in ICP in their control groups 4 h after implantation (Rooker et al., 2003). If we can successfully monitor ICP for longer periods, that will allow us to investigate the relationship between longer cooling periods and ICP changes within the study design. Alternatively, we might be able to determine temporal ICP patterns following an ICH and evaluate TH protocols accordingly. As seen in the National Acute Brain Injury Study: Hypothermia II (Clifton et al., 2011), when rewarming coincides with the peak of ICP, this can cause rebound ICP, and in some cases lead to death (Schwab et al., 2001). Therefore, elucidating ICP progression and resolve in ICH is vital to evaluating targeted temperature ICP-reducing therapies such as hypothermia.

The results of our study are not limited to ICP, and may have implications for seizure activity. ICH can also lead to seizures with raised ICP likely due to increased CBF and volume

(Vespa et al., 2007). We have previously found seizure activity after ICH using an electroencephalography (EEG) probe (Klahr et al., 2014). Seizures occurred in 66% of the collagenase group between 10 and 36 hours (Klahr et al., 2014), which coincides with the period of mortality (18 hours) in the present study. It does not seem like seizures explain the edema spike but more data is needed to see if seizures cause ICP to rise. Further work is required to determine whether the underlying cause for proposed mortality-related ICP spikes was due to seizure activity. In addition, we recently hypothesized that TH may reduce seizure activity in rats. Preliminary findings show that brain selective cooling reduced the number of rats undergoing seizure activity from 63% (normothermic animals) to 33%, and the number of seizures by 66%, but did not reach significance (Klahr et al., 2015). These findings are encouraging, and demonstrate the potential for TH to reduce seizure activity after ICH, but additional animals may be necessary to detect a significant reduction in seizure incidence.

4.6 Conclusions

This thesis evaluated the effects of brain selective cooling on ICP and edema in the rat brain. Our findings confirm that the delay in treatment onset is a critical factor to prevent worsened hemorrhaging and promote therapeutic benefit. We demonstrated that mild, brain selective cooling can significantly reduce ICP, but edema did not predict ICP. These findings challenge the popular hypothesis that ICP elevation is primarily caused by cerebral edema. Furthermore, we demonstrated that rewarming considerations are important in hypothermia protocols, since fast rewarming worsened edema. Taken together, these findings provide a strong impetus to further elucidate ICP responses to hypothermia therapy in experimental ICH, as it has clear implications for future TH protocols in clinical settings.

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