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Intracranial Pressure Changes in Rat Models of Intracerebral Hemorrhage

by

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DEDICATION

I wish to dedicate my thesis to my parents, George and Li Chang Hiploylee.

ABSTRACT

Intracerebral hemorrhage (ICH) is a devastating subtype of stroke but the role of increased intracranial pressure (ICP) in ICH is unclear - especially since most studies measure ICP in rodents tethered or under anesthesia or restraint. Thus, ICP was measured using telemetry in untethered, awake rats after ICH for 4 days. In PART 1, no pressure differences were found between the site of injury and epidural space. In PART 2, a severe ICH increased ICP for 4 days with modest reductions in cerebral perfusion pressure. In PART 3, moderate to severe ICHs were compared in different models of ICH. When ICH was induced by a collagenase infusion, ICP was increased for 2 days. However, ICP did not increase when the ICH was induced by a whole blood infusion. Lastly, increases in edema correlated with increases in ICP. These findings demonstrate ICH model differences that must be considered when evaluating therapies.

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EPI-ICH – Intracranial pressure monitored in the epidural space of rats with an intracerebral hemorrhage

EPI-SHAM - Intracranial pressure monitored in the epidural space of rats without an intracerebral hemorrhage

BBB – Blood brain barrier

BL - Baseline

BP – Blood pressure

BPM – Beats per minute

CBF – Cerebral blood flow

CBV – Cerebral blood volume

COLLAG – Rats with an intracerebral hemorrhage induced by the collagenase model

CSF – Cerebral spinal fluid

CPP – Cerebral perfusion pressure

GCS – Glasgow coma scale

HR – Heart rate

ICH – Intracerebral hemorrhage

ICP – Intracranial pressure

INTRA-ICH – Intracranial pressure monitored in the intraparenchymal space of rats with an intracerebral hemorrhage

INTRA-SHAM – Intracranial pressure monitored in the intraparenchymal space of rats without an intracerebral hemorrhage

MAP – Mean arterial pressure

SD – Standard deviation

SHAM – Rats without an intracerebral hemorrhage but underwent a similar surgery

TBI – Traumatic brain injury

WB – Rats with an intracerebral hemorrhage induced by the whole blood model

CHAPTER 1

1. Introduction

Stroke is one of the leading causes of disability and second leading cause of death worldwide.[1] About a third of those affected die and another third live with permanent disability.[2] With approximately 50,000 new cases in Canada annually,[3] the associated medical costs, productive years of life lost and burden to caregivers and patients sums to a devastating multi-billion dollar healthcare cost.[2] Though stroke incidence is decreasing in some populations due to advances in medical care, the absolute number of stroke cases continues to rise due to the aging and increasing population.

Strokes can be broadly classified into 2 categories: ischemic strokes and hemorrhagic strokes. Ischemic strokes occur in 80% of all stroke cases and hemorrhagic strokes 20% of the time. However, brain hemorrhages are often seen in severe cases of traumatic brain injury (TBI).[4] Ischemic strokes result from a lack of cerebral blood supply due to embolism (clot builds in brain but originated elsewhere), thrombosis (clot builds and originated at the site of occlusion), or systemic hypoperfusion (overall reduction in blood flow). Hemorrhagic strokes result from a rupture of a cerebral vessel, leading to a buildup of blood called a hematoma. Primary hemorrhagic strokes can occur due to chronic hypertension and amyloid angiopathy, which are the two most common causes. ICH can also occur secondarily to drug abuse, physical activity, coagulation disorders, use of anticoagulants, ischemic transformation, arteriovenous malformation, TBI, tumours or aneurysms.[55] This can occur in the subarachnoid space or in brain

tissue. When bleeding takes place in the brain tissue, this is called an intracerebral hemorrhage (ICH) and occurs twice as often than subarachnoid hemorrhage.[6]

1.1 Intracerebral Hemorrhage

The second-most common stroke is ICH (9-15% of all strokes),[7] resulting in an overwhelming 30-day mortality rate of 40.4%. Devastatingly, half of these patients die in the first 2 days.[8] The chance of survivors to return to independent functioning has been reported from 12-39% however, this does not equate to pre-stroke functioning.[9] In these patients, outlook heavily depends on pre-existing comorbidities, age, hematoma size and location.[8, 10-12] In the acute phase of injury however, 30-day mortality was correlated with low levels of consciousness, as determined by the Glasgow Coma Scale (GCS) (scores range from 3-15; lower scores indicate worse injury) and hematoma volume.[8] These factors along with the presence of intraventricular extension (bleed extending into the ventricular system), age above 80 and an infratentorial origin can collectively predict mortality.[13,14] Based on the aforementioned predictors, patients can be scored on an ordinal grading scale that sums to an ICH score.[13] The ICH score has been validated by others to predict mortality.[14] Otherwise, predicting outcome in ICH patients is difficult and currently no dedicated treatment exists for ICH.

1.1.1 Clinical and Behavioural Manifestation

Clinical presentation in acute ICH depends on location and severity of the stroke. The most common sites for ICH are in the subcortical regions (eg.

thalamus, striatum) particularly in the basal ganglia, which receive inputs from the motor and somatosensory cortex. This will present as motor and sensory deficits.[15] Subcortical hemorrhages are usually associated with hypertension, defined as chronic pressure above 140 mm Hg/90 mm Hg (systolic/diastolic). Chronic hypertension can lead to a small vessel disease called lipohyalinosis which is characterized by the loss of arterial integrity due to the thickening of the arterial wall with the narrowing of the vessel lumen.[16] Hypertensive hemorrhages occur when these vulnerable, small vessels rupture due to the high pressure imposed by the parent vessel.[17] The second most frequent location are in the lobar regions and the common underlying cause is cerebral amyloid angiopathy, in which amyloid (fibrous protein aggregates) deposits are found on the arterial wall. Lobar hemorrhages can present as seizures, weakness or visual problems, depending on the lobe affected.[5]

In the clinical setting, focal neurologic deterioration progresses over a few minutes to hours mainly owing to hematoma expansion (toxicity of blood damages surrounding brain tissue causing rebleeding) and cerebral edema (tissue swelling). This severe mass effect can lead to headache, vomiting and decreased levels of consciousness.

1.1.2 Pathophysiology

Injury after ICH can be broadly classified into primary and secondary injury. Primary injury involves mechanical damage owing to the space-occupying effects of the initial bleed and hematoma expansion.[18-20] Secondary injury involves mass

effect, toxicity of blood, coagulation cascade (involved in blood clotting), neuronal injury, inflammatory response, blood brain barrier (BBB) permeability, seizures and edema.[5, 21-24] These mechanisms of injury will be further discussed below.

The mass effect of cerebral edema and growing hematoma can cause a midline shift of the brain. Maximal hematomal expansion can occur within an hour[19] or up to 2 days and is a significant determinant of mortality and outcome, particularly in the first 24 hours.[25] Also in patients, two waves of mass effect have been described: 1) the first wave is caused by hematoma expansion, in the first two days and 2) second wave is caused by edema progression in the second or third week after onset.[26-28]

Due to the increasing volumes within the skull, mass effect and increases in intracranial pressure (ICP) can compress tissue surrounding the hematoma to ischemic levels.[29] However, perihematomal ischemic regions were not observed in animals[30, 31] or humans[32, 33] and these regions were found to be only hypoperfused. Additionally, reductions in blood volume rebounded 3-5 days after ICH[34] and regional cerebral blood flow (CBF) was even found to rebound within hours.[21, 35] These studies did not find markers of ischemia or resulting structural damage respectively[34, 35] however, the effects of hypoperfusion for these durations is unknown. Furthermore, these drops in regional CBF may be significant if metabolic demand was maintained at 'normal' levels but this was not the case.[36] Additionally, blood pressure (BP) lowering treatments did not reduce CBF.[37, 38]

The debate of whether a perihematomal ischemic region exists remains unresolved.

The presence of red blood cells in brain tissue also plays a significant role in injury. Red blood cells lyse within 24 hours of ICH in response to the activation of the complement cascade (part of the immune system for cleanup) and loss of intracellular energy stores.[39] This of course, contributes to cell death, behavioural deficits, BBB permeability, inflammation, oxidative stress and edema.[22, 24, 40, 41] Furthermore, red blood cells release iron that also contribute to the aforementioned injury and can be found in the brain for at least 3 months after ICH.[42] Thrombin, involved in the coagulation cascade and blood clotting also increases pro-inflammatory factors, reactive oxygen species, BBB permeability and edema.[22, 43] Inflammation can also further increase oxidative stress and edema.[23, 41, 44]

ICH can also lead to seizures, especially after a lobar ICH.[45] Post-ICH seizures occur in 28% of patients in the first 72 hours and were correlated with a midline shift.[46] Interestingly, those with a raised ICP did not present with seizure activity[47] but those that experienced electrographic seizure activity after a TBI also experienced a higher ICP and increased metabolic distress than those who did not. The authors claim increases in ICP were likely to be due to increased CBF and volume.[48] However, research in ICP and seizures remains limited.

1.1.3 Animal Models of ICH

Animal stroke models are used to understand the mechanisms of injury and recovery as well as to evaluate putative therapies for the clinic. However, animal stroke models became questionable after therapies proven to be efficacious in laboratory settings failed to translate to the clinic.[49,50, 51] This brought upon the issues of 1) whether the animal models used were appropriate to evaluate such treatments; 2) clinical and animal study design; and 3) whether the best therapies were selected for clinical trials. A systematic review later demonstrated that the scarce preclinical studies of higher quality (eg. blinded assessments, long-term endpoints) yielded lower efficacy of stroke treatments whereas the numerous, lower quality studies overestimated drug efficacy. The exaggerated promise of these drugs in animal studies led to the inevitable failure of clinical trials.[52] Thus, carefully planned studies of high quality are integral to improving clinical translation.

Problems in successfully translating scientific advances from bench to bedside however, are partly owing to current ICH animal models that do not entirely mimic human ICH pathophysiology. This underscores the importance of studying therapies in more clinically relevant situations (eg. use of aged animals) and in multiple models, as each model has their unique set of advantages and disadvantages. Also, the use of different animals is especially important too as rats have little white matter compared to humans. Pigs have comparable white matter content but such studies are expensive. However, only 5.8% of ICH studies investigated white matter injury in animals which is particularly concerning.[53] Nevertheless, the face validity of these models still requires further elucidation.

Animal models of ICH are primarily used in rodents but dog, cat, porcine and non-human primates are used too.[54] The two most common ICH models are the whole blood model and the collagenase model which will be discussed in detail in the following paragraphs.[4953] Both are capable of triggering cerebral edema, cell injury, an inflammatory response, oxidative stress and BBB permeability.[55] These models may vary slightly from lab to lab and are commonly used to create striatal hemorrhages (the most common site in patients).[15] Cortical, cerebellar and hippocampal ICH have been done too but are far less common.[15] Other ICH models include inflatable balloons to emulate a space-occupying mass,[56] hypertensive stroke-prone rats,[57] or even ischemic-induced hematomas. However, the last time this model was used was in 1976 in non-human primates.[58]

The whole blood model involves injecting blood, usually autologous, into the brain creating a single, monophasic bleed. It was first performed by Ropper, in which 240-280 μl of allogeneic blood was infused into the basal ganglia of unanesthetized rats.[59] Bullock later adapted the model by infusing whole blood under arterial blood pressure to be more clinically relevant. However, a large range in hematoma size was found.[60] A study by Nath *et al.* used controlled blood volumes of 25, 50 and 100 μl in an attempt to create consistent lesions except blood volume did not relate to hematoma shape.[61] Later, Deinsberger *et al.* was successful in hematoma reproducibility by the double injection method. A small amount of blood was first infused and allowed to clot to prevent blood from traveling back up the needle track. The remaining blood was subsequently infused. A total 80 μl blood injection led to a 43.7 μl ± 6.7 (standard error) clot

volume (measured by image analysis on serial stained sections), whereas a 50 µl blood infusion was more successful, resulting to a 41.1 µl ± 10.0 (standard error) clot. However, it was not entirely clear when clot volume was assessed, leading one to believe assessment was performed immediately after ICH.[62] Interestingly, a magnetic resonance imaging study demonstrated a 100 µl blood injection resulted in a hematoma volume of 85.34 µl ± 6.25 (standard error), 12 hours after ICH. This suggests not all blood infused in the brain, stays.[63] This has also been demonstrated by other researchers.[64, 65]

Advantages of the whole blood model include the option to infuse blood under arterial blood pressure rather than an artificial rate of injection and the option to infuse whole blood or its components. On the other hand, precise blood volume control is difficult as not all the blood infused would remain in brain tissue and some even traveling back along the needle tract.[63, 66] Also, ventricular extension,[60] hematoma shape and location inconsistency,[61] quick resolution of both the hematoma and behavioural deficits,[63] hemoglobin crystallization causing an excessive inflammatory response[67] and the absence of a vessel rupture are all problems associated with the model.

The other most commonly used model involves stereotaxically injecting collagenase into the brain. Collagenase is an enzyme that breaks down the basal lamina surrounding cerebral vessels to create a spontaneous bleed. It was developed by Rosenberg *et al.* in which bleeding was seen as soon as 10 minutes, hematoma growth from 1-4 hours and a fully developed hematoma by 4-24 hours.[63, 68] This model is advantageous because it simulates a spontaneous bleed

and hemorrhage size/severity can be easily modified depending on the collagenase dose administered.[69] Hematoma size can also be variable in this model but can generally stay within its intended location depending upon its size. However, it is associated with greater secondary injury when compared to the whole blood model[63, 70] and has shown hemoglobin crystallization too.[67] Greater functional deficits were also detected in collagenase animals up to 28 days whereas whole blood animals returned to baseline levels by 21 days.[63] This study used the Neurologic Deficit Scale to gauge functional recovery though, which was shown to be insensitive to small differences in ICH severity.[71] These features however are not necessarily downfalls of the collagenase model but should be considered during experimental design.

The use of more clinically relevant models (eg. aged, hypertensive animals) is strongly encouraged as frequently used ICH models do not mirror important aspects of ICH such as the time course of cerebral edema which will be briefly discussed in the subsequent section.[53, 72]

1.1.4 Edema

Brain edema is popularly classified as cytotoxic edema or vasogenic edema. Cytotoxic edema is the abnormal accumulation of fluid intracellularly from a toxic agent and cell dysfunction. Vasogenic edema occurs when vessel injury induces vessel fluid to spill into brain tissue.[73] Both of these types occur in ICH and ischemic stroke but the progression, resolution and possible interaction between cytotoxic and vasogenic edema remains unclear. Additionally, cytotoxic

and vasogenic edema are induced by different mechanisms of injury in ICH and ischemic stroke. Thus, antiedema therapies applicable to one stroke may not always be applicable to the other – though, this should usually be the case.

Three waves of edema are described after ICH.[15, 74, 75] The first phase occurs in the initial hours where serum proteins (eg. albumins, fibrinogens) from the hematoma move to adjacent tissue.[21, 34, 36] The next phase occurs over the first couple of days due to thrombin release and the coagulation cascade. Thrombin is released relatively quickly after ICH and causes edema to peak 1-2 days after.[24] Additionally, unheparanized blood infusions induced edema immediately whereas heparinized blood did not, demonstrating that clot formation was necessary to induce edema.[76] This was similarly shown when comparing the edema volume between patients with a spontaneous ICH and patients with an ICH induced by thrombolytics (drugs that dissolve blood clots). Patients with thrombolysis-related ICHs had half of the edema volume experienced by those with a spontaneous ICH. However, computed tomography scans were taken within 3 hours of onset and whether or not those with thrombolysis-related ICHs experienced a delayed edema progression is unclear.[77] Lastly, the third wave of edema is due to blood degradation products after red blood cell lysis.[78] Animal studies of whole blood infusions demonstrate edema to peak later than if thrombin was infused alone, suggesting a delayed wave of edema.[24] Similarly in patients, edema progresses despite hematoma resolution at 10 days after ICH, due to the ongoing clot lysis.[79]

As mentioned, edema peaks on the third day in animal models[80] and has been shown to be resolved by the fifth day[81] - though other studies have found it to be

within 2 weeks instead.[80] In patients, edema growth is highest in the first 48 hours, doubles 7-11 days later and peaks around the end of the second week to the third week.[26-28, 82] However, not all patients with progressive edema showed neurological deterioration.[83] Neurological deterioration was also not clearly attributed to cerebral edema in noncomatose patients and did not occur in all patients with a large hematoma (> 50 ml).[84] During the first 3 days after ICH, both neurological status and edema were shown to worsen, but neurological status improved as edema continued to progress and peak at 14 days.[82] In animal studies however, neurological deficits demonstrate a correlation between edema severity and onset which were both resolved by 2 weeks.[80, 85] This temporal discrepancy between rodents and humans poses as a potential concern for evaluating antiedema treatments. Nevertheless, the clinical impact on edema reducing therapies are not clear on functional outcome.[86]

1.2 Intracranial Pressure

Normal ICP in adult humans is below 15 mm Hg but values change according to body/head position and high elevations can be seen during coughing or sneezing. ICP in children are usually lower and can even be sub-atmospheric in the first few days of life.[87] Pathological levels of ICP are considered to be ≥ 20 mm Hg which can be caused by hydrocephalus, space-occupying masses and brain injury. Space-occupying masses have been observed to cause pressure gradients in patients,[88-90] where higher pressures are found closer to the space-occupying mass. However, Yano *et al.* did not find this in patients with

intracranial lesions[91] and interhemispheric pressure gradients of 30 mm Hg were found even without a intracranial mass-occupying lesion.[92] Pressure gradients are also found in experimental animal models,[93-96] but these included inflatable balloons and not in models of brain injury. Overall, when and how these pressure gradients exist or originate from is not entirely elucidated. More research is required in animal models of brain injury to determine these pressure gradients.

The concept of ICP can be best understood when considering the intracranial components: brain tissue, blood and cerebral spinal fluid (CSF). These incompressible components are encased in a relatively closed skull. When one of the compartments increases in volume, other intracranial components must be displaced in order to compensate for the change (eg. CSF reabsorbed into venous blood outflow). This is brain compliance and healthy individuals can sustain high ICP levels quite well. In CSF infusion studies, ICP levels up to 40 mm Hg were observed in subjects without being symptomatic.[97, 98] This is due to the uninterrupted CSF space in normal individuals that can change after brain injury.[99]

After brain injury however, factors such as obstruction of the ventricular system or introduction of a mass lesion can allow brain compliance to be compromised. Increases in ICP can lead to CSF and/or blood displacement, owing to the aforementioned perihematomal ischemia argument. In situations where brain compliance fails, a given increase in volume causes a greater increase in pressure than if brain compliance was intact. Thus, a nonlinear relationship exists between intracranial volume and pressure. This pressure-volume relationship is

referred to the Monro-Kellie doctrine (Figure 1). Of course, other factors such as CBF and rate of intracranial volume increase can play a part. For example, increases in CBF causes increases in intracranial blood volume and a quickly progressing hematoma may present higher ICP elevations than a slowly progressing hematoma of the same volume.[96]

As such, two individuals can share the same normal ICP value but one can have considerably less brain compliance. In patients with mass lesions, 1 ml of fluid was injected through an intraventricular catheter to determine ICP changes before and after the mass lesion was removed. Changes in ICP were much greater when the mass lesions were present (low compliance) than when the mass lesion was removed (higher compliance). They also found that the larger the brain shift, the higher the pressure changes.[100]

1.2.1 Location and Method of Intracranial Pressure Monitoring

Humans

Guidelines for the Management of Spontaneous Intracerebral Hemorrhage by the American Heart Association in 2010 suggest considering ICP monitoring in ICH patients with intraventricular hemorrhage, transtentorial herniation or a GCS ≤ 8.[101]

The American National Standard for Intracranial Pressure Monitoring Devices recommends technical specifications for the ICP monitor to ensure safety and accuracy.[102] The external ventricular drain is considered to be the gold standard in ICP monitoring. The monitor is inserted into the ventricle and can

drain CSF, if required. Advantages also include its simplicity, relative inexpensive cost, accuracy, capability of recalibration *in situ* and the option of inserting a prophylactic catheter (allows antibiotic administration to reduce infection). However, intraventricular monitoring is invasive and has a reported risk of infection of up to 22%[103] and hemorrhage in 7% of patients in which 0.8% of them were considered clinically significant.[104] In addition, misplacement of the ICP monitor has also been reported. Other methods of ICP monitoring ranked from best to least favourable based on cost, accuracy and reliability are intraparenchymal (accurate but cannot recalibrate), subarachnoid (not very accurate, recalibration possible), subdural (not very accurate, recalibration sometimes possible) and epidural monitoring (not very accurate, recalibration possible).[105] Several studies comparing ICP readings from the subarachnoid, subdural and epidural space to either intraparenchymal or ventricular readings showed a lack in agreement. The discrepancies are unclear as to whether they are real or to the monitor itself. Monitoring in the epidural space however, can be deemed inaccurate due to the possibility of the dura dampening pressure from being transmitted or by blocking the sensor tip from detecting accurate pressure readings.[105]

Non-invasive methods of ICP monitoring are also available and are inexpensive with very low risk. Ocular sonography can be used to measure optic nerve sheath diameter to estimate severity of elevated ICP but these kinds of methods are not well refined and often considered to be inaccurate.[106] Currently though, telemetry is being investigated as a useful tool to monitor ICP in the

hospital and home in patients with hydrocephalus.[107] Nevertheless, ICP monitoring methods vary across hospitals and populations. Guidelines are not universally applicable and even with the aforementioned pressure gradients discussed in patients, whether monitoring near the site of contusion is best or not is currently unknown.[108]

Animals

Like patients, intraventricular,[109, 110] intraparenchymal,[111] subarachnoid,[112] subdural[113] and epidural[114] ICP monitoring have been developed for experimental use in animals. Monitoring further away from the site of injury is considered to produce lesser quality data but this has not been confirmed as quality of data depends on the type of monitor used.

Zwienenberg *et al.* (1999) compared intraventricular, cisterna magnal and intraparenchymal ICP measurements in a subdural hematomal rat model. Measurements in the cisterna magna were lower than the other two and considered less accurate. No difference was found between measurements in the ventricle or parenchyma. However, cortical damage was associated with these invasive methods of monitoring which can interfere with histological and behavioral endpoints.[111] This is especially important when evaluating injury in animal models. The unwanted tissue damage due to the insertion of an ICP monitor can overestimate lesion size and injury and thus, non-invasive methods would be best – given they are as accurate.

Some ICP monitoring in ICH models were done in the mid-1980s that showed that larger volumes of fluid infused into the brain were related to ICP increases.[60, 61] Other than inflatable balloon models, more recent work done by Qureshi *et al.* in mongrel dogs is of interest.[38, 115] Cisterna magna measurements taken 45 and 90 minutes after ICH revealed elevations in ICP. However, the evolution of ICP is not clear and the course of ICP resolution is not known.[115] In another study by Kusaka *et al.* (2004), ICP was measured through the lumbar segment of the spinal cord as well as in the cisterna magna. Both locations of monitoring revealed an increase of ICP to approximately 30 mm Hg during blood infusion into the brain but gradually fell after. Continuous recording was only completed for 1 hour. This may have been due to the drawback of blood observed through the needle track, indicating an incomplete infusion.[66]

Most ICP monitoring methods require the animal to be anesthetized, limiting the time ICP is to be monitored and introduces a potential confounding variable.[116] There may be intrinsic neuroprotective properties of anesthetics.[117] For example, anesthesia has been shown to lower ICP and BP in both animals and humans.[118, 119] In contrast, volatile anesthetics such as halothane increased ICP instead in patients with space-occupying lesions.[120] Additionally, an intentional 1 hour treatment of isoflurane after ICH was sufficient to reduce cell death, improve behavioural deficits and cerebral swelling at 24 hours.[121] It is unfortunate then, that animals are usually continuously monitored under anesthetic for minutes to hours[122] or monitored intermittently (animals undergo anesthesia each time or at the very least have to be immobile or restrained)[109] for longer periods of time.

A few studies, however, examined ICP in unanesthetized horses,[123, 124] non-human primates,[125] dogs,[126] rabbits[109] and goats.[127] These also included rodent models but as mentioned, require the animal to be relatively restrained[118, 128-130] and monitoring is only done for hours.[131] However, a number of tethered systems have been developed. Verlooy *et al.* described a tethered system using a fiberoptic device to measure ICP. They measured within the parenchyma and epidural space and compared them to recordings from the cisterna magna. As intraparenchymal readings correlated well with the cisterna magna, epidural readings did not and showed persistent rises of 10 mm Hg and falls to baseline. They attributed this to a possible mechanical tugging of the dura since the observed artefacts disappeared when the subarachnoid membrane and dura were perforated.[132]

Rooker *et al.* (2003) also used a tethered system to measure ICP. A swivel head was attached atop of the cranium in which sensors turned the animal cage according to rat movement. This allowed the animals to move freely. Measurements were reliably taken in the right parietal cortex for 3 hours in a weight-drop TBI model. The sham group experienced an unexplained progressive increase in ICP from 4-10 hours post-ictus. Whether this was a real ICP rise or an artefact is unclear.[122]

More successful accounts include Sanchez-Valverde *et al.* whom measured ICP in the cisterna magna, lateral ventricle and in the lumbar subarachnoid space of Wistar rats for 3 days in a tethered system. This tethered system had a flexible catheter that allowed animals to move freely.[133] Lastly, Mandell & Zimmerman also used a tethered system to measure in the intraventricular space of Sprague

Dawley rats. It was not clear the period of time the system was capable of monitoring ICP for but it was briefly mentioned that the cannulas used were not compromised until 2 weeks after surgery.[134]

The most recent method was developed by Silasi *et al.* (2009) but in this case, did not involve a tether. Using blood pressure telemetry probes, ICP was successfully recorded for 5 days in freely moving awake rats after ischemic stroke. This method is particularly attractive as it was able to measure continuously in the epidural space without causing any cortical damage.[135] Telemetry was also similarly used by another research group.[136] However, this method is very expensive and like all ICP monitoring systems, can yield pressure drift and artefacts in the data. Also, sudden increases in ICP can be introduced during physical activity or a false reading by the monitor itself.[135] However, it is possible that the monitor is reading the pressure correctly but the tip of the sensor may artificially be reading higher or lower pressures.

1.3 Cerebral Perfusion Pressure

Cerebral perfusion pressure (CPP) is the adequacy in which the brain is perfused with blood. It is defined by mean arterial pressure (MAP) minus ICP. Thus, patients must be simultaneously monitored for MAP and ICP in the intensive care unit in order for CPP to be calculated. ICP opposes CPP so pathological increases in ICP can effectively reduce CPP, possibly to ischemic levels (average threshold 50-60 mm Hg), provided MAP stays the same.[105] CPP drives CBF, which is normally constant at 50 ml per 100 g/min, which is

approximately the same in rats too.[31] This occurs over a MAP range of 60-150 mm Hg in normotensive individuals and 120 to approximately 160 mm Hg in hypertensive individuals.[137-139] This is because of cerebral autoregulation that ensures constant CBF despite changes in BP. The autoregulation curve for hypertensive individuals has higher absolute limits most probably due to the associated thickened arterial wall and narrowed arterial lumen, which in turn, increases cerebrovascular resistance to compensate the increased perfusion. Thus, hypertensive individuals are susceptible to cerebral ischemia since their absolute threshold for ischemia is higher than that of normotensive individuals.[140]

How exactly cerebral autoregulation works is unknown, but it is well accepted that changes in arterial diameter compensate for changes in MAP. For example, increases in MAP are offset by vasoconstriction and decreases in MAP by vasodilation. This is proposed to be done by either a direct response by smooth muscle or triggered by vasoactive factors released from the brain in response to CBF changes.[141] However vasodilation and vasoconstriction can only occur to a certain extent. When the limits of cerebral autoregulation are exceeded, CBF becomes passive to CPP in which CPP determines CBF. This is also the case if cerebral autoregulation becomes impaired after brain injury (Figure 2).

1.4 Therapeutic Management of Elevated ICP

Currently, there is limited data on ICP management in ICH patients and most of what is known is adopted from TBI research. Generally the underlying cause of elevated ICP is treated but when this is not possible, CSF drainage is usually

employed. The next line of defense would be to use osmotic therapies such as mannitol or hypertonic saline solution. Mannitol is an osmotic agent administered as bolus injections and acts by drawing water from tissue into the circulatory system. It acts quickly but can be associated with complications like kidney problems if not used cautiously.[142] In those without an intact BBB, mannitol can theoretically worsen edema via a reversal of the osmotic gradient.[143] Hypertonic saline therapy also acts by drawing water from cells but can be varied on tonicity and volume. A meta-analysis of clinical trials comparing the two therapies revealed that hypertonic saline had a slight advantage in reducing elevated ICP but functional outcome has not been a chief endpoint in most of these studies.[86] Additionally, the tonicity and volume of hypertonic saline therapies are extremely diverse across studies and depend on hospital protocol.

If elevated ICP persists, sedation and hyperventilation can be considered. The safety and efficacy of sedative use in ICP treatment have not been well explored but sedative use is believed to reduce ICP by reducing metabolic demand. A systematic review in 2011 examining the use of sedatives such as ketamine, propofol and benzodiazapene in randomized controlled trials of TBI patients revealed that all sedatives were similar in treating ICP and CPP and improving outcome. However, high doses of these sedatives can be harmful to ICP and CPP.[144] Additionally, hyperventilation has been shown to work extremely well in reducing ICP acutely by inducing vasoconstriction.[145] However, it can lead to respiratory alkalosis (increased blood pH) and possibly secondary ischemia.[146]

Thus, the use of hyperventilation in brain injury is minimal and must be used cautiously.

Barbiturates, neuromuscular blockades, hypothermia or decompressive craniectomy can be chosen to treat refractory ICP (unresponsive to aforementioned treatments). Barbiturate therapy can be complicated with hypotension and an inaccessible neurologic exam. When compared with conventional ICP treatment, pentobarbital was shown to control ICP better by 50% but 30-day mortality did not differ between the two groups.[147] Neuromuscular blockades have similar complications as barbiturates. They are considered to decrease physical movement, resulting in reduced intrathoracic pressure that may have impeded venous outflow, thus decreasing ICP.[148] On the other hand, mild therapeutic hypothermia (35°C) for 10 days after a large ICH successfully prevented perihematomal edema progression with no reported ICP crises (ICP > 20 mm Hg > 15 minutes) versus the 44% ICP crises reported in the historical control group. Additionally, a 72% mortality rate was found in the control group whereas no deaths were reported in the hypothermia group. However, patients treated with mild hypothermia all had pneumonia and 76% cases were found in the control.[149] A follow-up at 12 months demonstrated only 2 of the 12 treated patients died and 50% of them were able to walk unassisted.[150] Of course, this was a small sample size and was compared to a historical control. A review demonstrated that therapeutic hypothermia has great potential to treat ICP but can be complicated during the rewarming phase, where ICP rebound or exacerbation can occur. Slower rewarming can possibly be more beneficial.[151]

Currently the Eurotherm3235Trial is assessing mild hypothermia (32-35°C initiated by infusing refrigerated saline) in TBI patients with ICP as its main endpoint.[152]

Another treatment for refractory ICP is decompressive hemicraniectomy, which involves removing a part of the skull to alleviate pressure. Decompressive hemicraniectomy has been shown to improve brain tissue oxygenation[153] and outcome[154, 155] but this treatment remains to be controversial due to an unclear effect on clinical outcome in both ICH and ischemic strokes. A randomized, prospective trial called RESCUEicp in TBI patients is underway.[156] Hematoma evacuation is an option too but this is done in patients with cerebellar hemorrhages, hydrocephalus from ventricular obstruction or brain stem compression. Superficial supratentorial hemorrhages > 30 ml can be considered for evacuation but the benefits of such surgeries is unclear.[101]

Other general and simple approaches to ICP control include fluid management and a 30° head elevation to encourage venous blood outflow. Caution should be taken to avoid reductions in CPP. In a group of patients with elevated ICP, it was found that for every 10° of head elevation, ICP decreased on average, by 1 mm Hg but CPP was also decreased by 2-3 mm Hg.[157] Although CPP changes were not found in another group of head-injured patients,[158] higher head elevations (60°) was not any better than 30° in reducing ICP.[159]

1.5 ICP vs. CPP

There is debate whether ICP- or CPP-oriented therapies are superior to the other. ICP- and CPP- oriented protocols focus on maintaining ICP and CPP respectively within predetermined ranges. These predetermined ranges however, are not concrete. Whether elevated ICP is really important becomes questionable when mortality is only correlated with ICP at extremely high levels.[160] This is further complicated by the fact that the aging population may be more susceptible to increases in ICP due to their reduced CSF outflow.[161] It is possible that raised ICP is most important when brain stem compression is involved[162] but brain shift and edema can be found without a significant increase in ICP.[162, 163] Though initial ICP was correlated with neurological status, two thirds of those that died had normal ICP.[164] The deepest levels of coma were associated with raised ICP but the time course of ICP did not relate to changing neurological status.[165] Average ICP > 20 mm Hg was significantly correlated with mortality in TBI patients and with 6 month functional outcome. Interestingly, ICP did not correlate with 6 month outcome among survivors.[166] The exact role ICP plays in ICH is not entirely clear.

There is an argument for large, randomized trials to show ICP monitoring is truly beneficial before it is taken into routine practice. Despite ICP monitoring guidelines by the Brain Trauma Foundation, a mere 43% compliance rate was found.[167] Surprisingly, there was no difference when comparing scores on the Glasgow Outcome Scale assessed ≥ 12 months after brain injury and in mortality between a centre that practices ICP monitoring and ICP/CPP oriented therapy and another that did not. In addition, the duration of stay in the intensive care unit was

reported to be longer in the ICP-monitoring centre. However, this study excluded mortality in the first 24 hours (25% in the non-ICP monitoring centre and 15% in the ICP monitoring centre).[168]

Though the clinical importance of ICP can be a debateable issue, whether CPP-focused therapy is a better approach is another. Low CPP was a better predictor than ICP for death and poor outcome in TBI patients[169] and 100% mortality rate was reported in patients who's CPP fell < 40 mm Hg on the first day and < 60 mm Hg on the second day post-ictus.[170] A retrospective study also found CPP < 60 mm Hg was related to poor outcome but this did not improve when CPP was kept > 70 mm Hg.[171] Additionally, CPP > 60 mm Hg had no impact on 6 month outcome and instead, ICP ≥ 20 mm Hg was a robust predictor for neurological deterioration.[172] Despite these discrepancies, the recommended target CPP range is 50-70 mm Hg according to the 2007 Guidelines for the Management of Severe Traumatic Brain Injury. These guidelines however, are mainly based on class II (good quality case control studies to moderate quality randomized controlled studies) and III (poor quality randomized controlled trials) data.[105]

However optimal CPP thresholds may not be as important, as patients with intact autoregulation respond better to CPP-oriented protocols (≥ 70 mm Hg and ICP secondarily < 25 mm Hg) and those with impaired autoregulation respond better to ICP-directed therapies (< 20 mm Hg and CPP secondarily > 60 mm Hg).[173] In contrast to a previous study, there was no correlation between defective

or intact autoregulation with survival. This may be due to the heterogeneous population and small sample size.[160]

A retrospective study calculated optimal CPP based on vascular reactivity for each patient. Patients below their optimal CPP had a higher mortality rate but those above it had a higher disability rate. Good outcome was thus reported when patients were closest to their optimal CPP.[174] Individualized treatment may be best but further research is warranted.

1.6 Rationale

The importance of ICP as an endpoint in ICH is not entirely clear as most of what is known about ICP and ICH is adopted from TBI research. The first step to help elucidate this is to explore ICP in animal models. However as discussed, current rodent models can simulate certain features of ICH pathophysiology but do not in others. Importantly, ICP in the collagenase and whole blood model have not been well explored and methods that currently exist to measure ICP in rodents involve anesthesia, restraint or a tethered system. This can confound accuracy and further the gap between bench and bedside research. Nevertheless in current animal models, reductions in edema are seen as beneficial because they are associated with reductions in ICP despite the fact that ICP was not measured. This is especially important to note as edema is a heavily relied upon endpoint in ICH research.[53]

In the following experiments, telemetry will be used to measure ICP in freely moving, untethered and unanesthetized animals for several days as previously

performed by Silasi *et al.* in ischemic stroke rats.[135] This is the first time telemetry is used to measure ICP in ICH models. ICP will then be correlated with edema to gauge whether ICH animal models mimic human ICH profiles. The collagenase model was primarily used because of its distinct advantage of modifying ICH severity. A severe ICH was initially induced to observe severe ICP and cerebral swelling. We hypothesized that ICP will rise after severe ICH and higher pressures would be detected nearer the site of injury than further away. We also hypothesized lower ICP would be observed in moderate to severe ICH and these ICP rises would be correlated with edema. This work is crucial in establishing the face validity of ICH models.

CHAPTER 2

2. Methods

2.1 Animals

Fifty-four male Sprague Dawley rats (Biosciences breeding colony, University of Alberta; 4-6 months; 350-500 g) were kept on a standard 12 hour light-dark cycle with free access to food and water. All animals were closely monitored and singly housed in 16” × 10” × 8” (L × H × W) polycarbonate cages post-operation. Subjects were given soft mash (Purina rat chow dissolved in distilled water and mixed with Kraft smooth peanut butter and sunflower seeds) until recovered from ICH surgery. After, rats were fed Purina rat chow only. Animals were randomized within each experiment but due to the obvious neurological impairments, the investigator (C.H.) was not blinded to group assignment. However, data collection was automated via a nearby computer. All procedures were performed according to the Biosciences Animal Care and Use Committee at the University of Alberta and the Canadian Council on Animal Care guidelines.

2.2 Experiments

2.2.1 Part 1: Location of ICP monitoring

Part 1 consisted of 2 experiments. In the first experiment, 8 animals were randomly assigned to a severe ICH or sham surgery. Using radiotelemetry, ICP was recorded in the epidural space (EPI-ICH or EPI-SHAM) for 72 hours at which point brain water content was determined. This experiment was repeated

but instead, ICP was recorded in the intraparenchymal space (INTRA-ICH or INTRA-SHAM) at the center of the hematoma.

2.2.2 Part 2: Stroke-induced CPP changes

Baseline readings of heat rate (HR, measured as beats per minute - BPM), activity, MAP, systolic and diastolic pressure were recorded in animals for 1 week using telemetry before the rats were subjected to a severe ICH (collagenase model) or sham surgery. Immediately following surgery, the aforementioned parameters were measured and alternated with ICP recordings every 6 hours for 3 days and every 12 hours for 4 more days. Exactly 7 days after ICH or sham surgery, subjects were euthanized via transcardial perfusion and lesion size was quantified. Owing to signal interference, it was not possible to record with both telemetry probes simultaneously. Thus, they were intermittently turned on and off in an alternating fashion (via magnetic switch).

2.2.3 Part 3: Comparing ICP in different ICH Models

Rats were subjected to a moderate to severe stroke via the collagenase model (COLLAG), the whole blood (WB) model or sham (SHAM) surgery. Surgical procedures were mirrored according to the whole blood model to ensure consistency between all experimental groups. This included taking 100 µl of blood from the tail vein. ICP was recorded until euthanasia at 72 hours when cerebral water content was assessed.

2.3 Telemetry Probes and System

PA-C10 (Data Sciences Int., St. Paul, MN, USA) probes recorded BPM, MAP, systolic and diastolic pressure when the probe's catheter was secured in the femoral artery. The PA-C10 probes were also used in a modified way to measure ICP according to Silasi *et al.*[135] The probes emitted AM radio waves to platform receivers (Model RPC-1, Data Sciences Int.) placed beneath the animal cages. Waveform data was averaged over 5 seconds every minute. This data was then stored using ART software (v. 2.3, Data Sciences Int.).

2.3.1 Pressure Drift

Offset values were calculated to correctly adjust for potential pressure drift during experiments. Telemetry probes were turned on and placed on their respective platform receivers. Special care was taken to allow the catheter head to lie flat on its side. At least 12 hours of data was collected before and after implantation for each animal. The average of both the pre-implant ($\bar{X}_{pre}$) and post-explant ($\bar{X}_{post}$) pressure readings were calculated and subtracted from each other:

$$\bar{X}_{post} - \bar{X}_{pre}$$

This was subsequently divided by the number of data points collected during the experiment. Since the telemetry system collected data every minute, this new value equated to the average pressure increase per minute (x_{min}):

$$[(\bar{X}_{post} - \bar{X}_{pre}) \div \text{number of data points}] = x_{min}$$

A number sequence was then generated:

$$\bar{X}_{pre}, \bar{X}_{pre} + x_{min}, \bar{X}_{pre} + 2x_{min}, \bar{X}_{pre} + 3x_{min} \ldots \bar{X}_{post}$$

Values in this sequence were subtracted from the corresponding data points collected during the experiment. The resultant values were considered to be unbiased values from pressure drift.

2.4 Valsalva Manoeuvre

The Valsalva manoeuvre was used to ensure the closed fluid-filled system to measure ICP was not compromised. It involved gently but firmly squeezing the rat's abdomen. This impeded venous blood flow by briefly increasing thoracic cavity pressure which in turn, caused a transient increase in ICP. The Valsalva manoeuvre was performed immediately after ICP implant and before euthanasia.

2.5 ICP Assembly

2.5.1 Cranial ICP Apparatus

Cranial ICP apparatuses were made out of ordinary lab materials and served to keep the ICP telemetry probes secure atop of the rat head and to protect the probe from damage during the experiment. Cranial apparatuses assembled in this study were slightly modified from Silasi et al.[135] A 5 cc syringe barrel was cut to a ~2.5 cm length with one end flat and the other slightly curved to fit the top of the skull. Near the edge of the curve, tiny holes were drilled along the perimeter of the barrel. Metal screws (MX-080-02, Small Parts Inc., Miami Lakes, FL, USA) were to be inserted in the holes during surgery, to help anchor the cranial apparatus in place (see Figure 3A for full assembly).

2.5.2 Cannulas

Nylon screws (C212SGN, PlasticsOne, Roanoke, VA, USA) with a hole diameter suitable for a 26 G needle were filed such that they will sit flush with the bottom of the skull (i.e., to sense pressure in the epidural space). These modified screws were used to act as cannula guides. Cannulas were constructed according to Silasi et al.[135] Briefly, the needle shaft of a 23 G needle was removed from the hub and filed so that it would either fit tightly inside the screw (to measure ICP in the epidural space) or 6.5 mm deep into the brain (to measure ICP in the centre of the hematoma). A larger needle shaft was chosen to ensure an airtight seal. An approximate 2 cm long PE20 tube (Smiths Medical International Ltd., Kent, UK) was attached to the other end of the cannula. Cannulas were cleaned, soaked in 95% ethanol overnight and filled with sterile saline prior to surgery (see Figure 3B for full assembly).

2.6 Surgery

All rats were anesthetized (4% isoflurane induction, 2% maintenance in in 60% N_2O and balance O_2) for aseptic surgery. During surgery, rectal temperature was maintained at $37^{\circ}C \pm 0.5$. All telemetry probes were sterilized in 2% glutaraldehyde overnight and then repeatedly rinsed in sterile saline prior to surgery.

2.6.1 Intracerebral Hemorrhage

In the collagenase and whole blood model, the dorsal region of the head was shaved and the incision site was injected subcutaneously with Marcaine (0.1 ml; Sanofi Canada, Markham, OT, Canada), treated with 70% alcohol and twice with betadine. With a scalpel blade, a 2-3 cm cut was made in the centre of the head. A hole was drilled 3.5 mm to the right and 0.07 mm posterior of Bregma, to avoid interference with the coronal suture that may potentially affect ICP. A Hamilton syringe (Hamilton, Reno, NV, USA) was lowered 6.5 mm into the brain through the hole and 1 µl of 0.3 U (severe ICH), 0.15 U (moderate to severe ICH) of collagenase (Type IV-S; Sigma, Oakville, OT, Canada), saline or 100 µl of whole blood was injected. Injections in Part 1 and 2 were done over 5 minutes while injections in Part 3 were done over 10 minutes. Five (Parts 1 and 2) or 10 (Part 3) additional minutes were allowed before the syringe was slowly withdrawn to avoid the drawback of liquid up the needle tract. In Part 2, immediately before injection, 100 µl of whole blood was obtained from the tail vein.

2.6.2 ICP implant

After the ICH surgery, the burr hole was plugged with the modified nylon screw described in section 2.5.2 and sealed with cyanoacrylate (Vetbond, 3M Animal Care, St. Paul, MN, USA). Implantation of the ICP assembly was performed in a similar fashion from a previous procedure done by Silasi *et al.*[135] Two additional holes were drilled into the skull, fastened with metal screws and cyanoacrylate. The holes were positioned in such a way that they would border the inner lining of the cranial ICP apparatus. This was so that the screws can

further anchor the entire cranial apparatus in place. At this point, a saline-filled cannula was carefully inserted into the modified nylon screw and again, fixed with cyanoacrylate. The catheter of the PA-C10 probe was inserted into the PE20 tube attached to the cannula until secure. The PA-C10 probe body was threaded through the cranial apparatus and metal screws were inserted into the pre-drilled holes of the barrel. The cranial ICP apparatus was carefully placed on the clean, dry skull surface. Finally, dental cement was dripped into the cylinder, just enough to seal the whole assembly. Dental cement was allowed to dry over ten minutes. A few drops of Marcaine were applied along the cylinder onto the scalp before it was sutured shut. A rubber plunger was used as a lid for the cranial ICP apparatus and the perimeter was secured with dental cement (see Figure 3 for full assembly). Animals were hydrated with 5 cc of sterile saline post-surgery.

2.6.3 MAP surgery

The inner right thigh of the rat was shaved and treated once with 70% alcohol and twice with betadine. Scissors was used to make a ~1.5 cm incision along the femoral artery. By blunt dissection, the femoral artery was isolated. Three silk sutures (Deknatel, 8-S, 136075-0208, Research Triangle Park, NC, USA) were threaded beneath the artery using vessel cannulation forceps so that they lied adjacent to each other. A few drops of lidocaine (00712884, Wyeth Animal Health, Guelph, ON, Canada) were used to dilate the artery. The most distal suture was tied in a knot to block blood flow and gently pulled and held in place. The most proximal suture was also gently pulled in the opposite direction until

visible blanching occurred and held in place. With the bevel bent at a right angle, a 25 G needle was pushed into and along the arterial lumen. This was to help guide the PA-C10 catheter into the femoral artery. The PA-C10 probe was adequately soaked in sterile saline before the catheter was gently pushed into the artery until ~1.5 cm of the catheter remained visible. All three sutures were tied and knotted around the artery to keep the catheter secure. Excess sutures were clipped. The probe body was placed subcutaneously and secured in place with sutures. The surgical site was treated with a few drops of Marcaine immediately before and after the skin was sutured shut.

2.7 Water Content Assessment

Animals were anesthetized with isoflurane (4% isoflurane in 60% N_2O and balance O_2) and decapitated. Brains were extracted and blocked from 4 mm posterior to 2 mm anterior of the injection site. The block was further divided into ipsilateral and contralateral cortical and striatal sections. The cerebellum was also extracted for control. The brain sections were weighed before (wet weight) and after (dry weight) being baked for 24 hours at 100°C. The water content of each sample was calculated by the following formula:

$$[(\text{wet weight} - \text{dry weight}) \div \text{wet weight}] \times 100 = \text{water content (\%)}$$

Brain tissue samples normally contain ~78% water content and higher percentages than this shows the presence of cerebral edema.[175]

2.8 Histological Preparation and Quantification

Animals were overdosed with sodium pentobarbital (DIN 00141704, Bimeda-MTC, Cambridge, ON, Canada, 100mg/kg, i.p) and transcardially perfused through the left ventricle with approximately 100 ml of 0.9% saline and subsequently, 200 ml of formalin. Brains were extracted and allowed to sit in formalin for 1 week and in 10% sucrose in formalin for 2 days before coronally sectioned into 40 μm slices. Sections were mounted on glass slides and stained with cresyl violet.

Lesion size was quantified using ImageJ (v. 1.46, National Institutes of Health) on every tenth section per animal. The lesion size was summed and expressed as mm^3.

2.9 Statistics

All ICP, CPP, BP and HR values were averaged by day and were analyzed using repeated measures ANOVA with simple contrasts comparing each day to baseline (BL) data, when applicable. The last 3 days before ICH manipulation were averaged for BL values. When applicable, post-hoc Tukey tests and simple comparisons were used. Independent samples t-tests were performed to compare water content of appropriate brain regions. Hours spent above an ICP threshold was counted for each animal and regressed with water content. All regression analyses were run using linear regression. Lastly, mortality was analyzed using a Fischer exact test. All statistical analyses were run by SPSS v. 17.0 (Chicago, IL) and were considered significant when $p \leq 0.05$.

CHAPTER 3

3. Results

There were no noticeable changes in ICP between the animals' dark and light cycle and thus, circadian rhythm changes were not expected to confound results. This has also been found by another research group.[136]

3.1 Exclusions

In Part 1, 1 animal from each ICH group were excluded. One animal died prematurely in the ICH-EPI group presumably from a large hematoma (Figure 4) and another had a notably small hematoma despite receiving the same dose of collagenase in the ICH-INTRA group (Figure 5). Six additional animals in Part 2 and another 2 animals in Part 3 were excluded due to technical difficulties, experimenter error and ICH-induced mortality. After exclusions, Part 1 had a total N = 14 (EPI-ICH: n = 3, EPI-SHAM: n = 4; INTRA-ICH: n = 3, INTRA-SHAM: n = 4), Part 2 had a total N = 8 (ICH: n = 5, SHAM: n = 3), and Part 3 had a total N = 22 (COLLAG: n = 8, WB: n = 8, SHAM: n = 6).

3.2 Mortality

Mortality was only observed in severe collagenase-induced ICH (0.3 U) animals. In all of the experiments, a total of 15 animals had a severe ICH, 3 of which died within 24 hours, resulting in a 20% mortality rate. There was no difference in mortality between the two groups, however ($p = 0.224$).

3.3 Part 1: No difference between location of ICP monitoring

Elevated ICP was observed in the EPI-ICH group when compared to EPI-SHAM ($F_{(1,5)}$ = 18.15, p = 0.008) and in the INTRA-ICH group compared to INTRA-SHAM ($F_{(1,5)}$ = 70.05, $p \leq 0.001$). There was no effect of Day during intraparenchymal monitoring ($F_{(2,10)}$ = 0.77, p = 0.489), but an effect was found in the EPI-ICH group ($F_{(2,10)}$ = 4.11, p = 0.05). Also, no interaction between Day × Surgery ($F_{(2,10)}$ = 3.42, p = 0.074; $F_{(2,10)}$ = 2.85, p = 0.105, respectively) was found (Figure 6). Combined data suggests no difference between the two locations of monitoring ($F_{(1,12)}$ = 0.02, p = 0.902), and on average severe ICH increased ICP by 6.59 mm Hg. Thus, data was pooled for water content assessment. Additionally, epidural monitoring was the location of choice for Part 2 and Part 3 to avoid cortical and striatal damage associated with intraparenchymal measurements.

3.3.1 Brain Edema

Water content in the severe ICH group was an additional 8.21% greater in the ipsilateral striatum ($t_{(12)}$ = 16.60, p ≤ 0.001), 2.71% in the ipsilateral cortex ($t_{(12)}$ = 15.57, p ≤ 0.001), 1.4% in the contralateral striatum ($t_{(12)}$ = 3.35, p = 0.006) and 0.7% in the contralateral cortex ($t_{(12)}$ = 2.89, p = 0.014) when compared to SHAM. The cerebellum, used as a control, was not different between ICH and sham groups ($t_{(12)}$ = -0.13, p = 0.899, Figure 7).

3.4 Part 2: Modest reductions in CPP after severe ICH

3.4.1 Heart Rate

HR was not significantly different at baseline ($t_{(6)}$ = -1.11, p = 0.312) between the sham and ICH group. HR was not affected by severe ICH ($F_{(1,6)}$ = 0.76, p = 0.42) over the 1 week period ($F_{(6,36)}$ = 2.35, p = 0.051). Although, there was a trend towards decreasing HR. This can possibly be explained by the different sampling interval between the first 3 days and the last 4 days. There was also no Surgery × Day interaction ($F_{(6,36)}$ = 0.76, p = 0.608, Figure 8).

3.4.2 ICP

An unexplained increase in ICP on days 6 and 7 in the sham group was observed (data not shown). This presumed error resulted in a conservative omission of ICP and BP data recorded from days 5 to 7. Severe ICH increased ICP on average by 7.21 mm Hg ($F_{(1,6)} = 9.7$, $p = 0.021$) with no effect of Day ($F_{(3,18)} = 2$, $p = 0.150$) or Day × Surgery ($F_{(3,18)} = 2.5$, $p = 0.092$, Figure 9).

3.4.3 Blood Pressure

Severe ICH did not significantly change diastolic ($F_{(1,6)} = 0.05$, $p = 0.823$), systolic ($F_{(1,6)} = 0.07$, $p = 0.795$) or MAP ($F_{(1,6)} = 0.00$, $p= 0.957$). However, there was an effect of day ($F_{(4,24)} = 7.42$, $p \leq 0.001$, $F_{(4,24)} = 6.51$, $p= 0.001$, $F_{(4,24)} = 8.42$, $p \leq 0.001$, respectively). There was also no significant Day × Surgery effect for all 3 parameters ($F_{(4,24)} = 1.07$, $p = 0.395$; $F_{(4,24)} = 0.78$, $p = 0.547$; $F_{(4,24)} = 1.23$, $p = 0.325$, respectively, Figure 10).

3.4.4 CPP

CPP was modestly reduced by severe ICH ($F_{(1,6)} = 11.64$, $p = 0.014$), on average by 7.5 mm Hg with no significant interaction between Day × Surgery ($F_{(3,18)} = 0.52$, $p = 0.674$, Figure 11).

3.4.5 Lesion Volume

No linear relationship was found between lesion volume (*M:* 64.55 mm^3 ± *SD:* 21.47) and peak ICP ($F_{(1,4)} = 5.51$, $R^2 = 0.647$, p = 0.101, Figure 12). However, this finding is limited by the small sample size.

3.5 Part 3: ICP rises only found in the collagenase model

3.5.1 Brain Edema

When compared to SHAM, the water content of the ipsilateral striatum of COLLAG was an additional 8.06% and 4.44% additional in the WB ($p \leq 0.001$; p = 0.001, respectively). In the ipsilateral cortex, an additional 2% and 0.88% was found respectively ($p \leq 0.001$; p = 0.010, respectively) when compared to SHAM. However, COLLAG also had higher water content than WB in these regions (3.62% increase, p = 0.003 and 1.12% increase, p = 0.001, respectively). In the contralateral hemisphere, the striatum had increased water content in the COLLAG (1.34% increase, p = 0.001) and WB models (1.23% increase, p = 0.003) but there was no significant difference between ICH models (p = 0.931). There was also no difference between all groups in the contralateral cortex ($F_{(2,19)} = 1.3$, p = 0.296) and in the cerebellum ($F_{(2,19)} = 1.03$, p = 0.375, Figure 13).

3.5.2 ICP

There was an effect of ICH ($F_{(2,19)} = 5.38$, p = 0.014), Day ($F_{(2,38)} = 4.19$, p = 0.023) and Surgery × Day ($F_{(4,38)} = 3.61$, p = 0.014). By simple contrasts, COLLAG increased ICP by 6.01 mm Hg on Day 1 (p = 0.005) and 5.16 mm Hg

on Day 2 (p = 0.009) and a trend on Day 3 (p = 0.068). However, WB did not significantly increase ICP when compared to sham (p = 0.848). In addition, COLLAG increased ICP by 4.51 mm Hg when compared to WB on Day 2 (p = 0.012) and by 5.53 mm Hg on Day 3(p = 0.008) but not on Day 1 (p = 0.215, Figure 14).

3.5.3 Water Content Vs. ICP

Data from Part 1 were combined with Part 3 to examine the relationship between water content and ICP. A significant linear relationship was found between Day 3 ICP and Day 3 water content in ICH animals ($F_{(1,21)}$ = 12.87, R^2= 0.391, p = 0.002, Figure 15).

After data was collected, categorical classes of ICP were developed to correlate with water content. The number of hours each animal spent 1 SD or 2 SD above sham animals were counted. The peak ICP each animal experienced was also noted. All categories were linearly correlated with Day 3 water content (Table 1). An example of one of these categories is demonstrated in Figure 16.

4. Discussion

4.1 Primary Findings

The purpose of our experiments was to first establish the best location of ICP in ICH models of freely moving, unanesthetized animals. Our next goal was to determine the face validity of currently used rodent ICH models in increasing ICP. We found that telemetry was capable of measuring ICP in these rodent models for 3 days with no detectable pressure gradients. Thus, measuring in the epidural space was determined to be the "superior" location of monitoring as it is less invasive. We also found that the collagenase model successfully increased ICP for at least 4 days after a severe ICH and for 2 days after a moderate to severe ICH. ICP did not rise after a moderate to severe ICH induced by whole blood. Lastly, increases in edema correlated with increases in ICP. These findings demonstrate, within the context of our study, that the collagenase model may be a suitable model to evaluate ICP therapies.

4.2 Animal models and ICP

Within our experiments, the whole blood and collagenase ICH model both demonstrated increased water content but the collagenase was the only model that was successful in increasing ICP. It is possible the water content found in the whole blood model in our study was lower than what was found by others. However, it is difficult to compare edema values due to differences in methodology (eg. larger tissue samples may yield lower edema values as edema is expected to lessen further away from the site of injury). Additionally, we injected

100 µl of blood into the brain – twice the volume of what would equate to an average ICH (53.8 ml) in patients.[62, 176] Furthermore, a previous study in our lab showed that a 100 µl blood infusion resulted in a ~85.34 µl hematoma volume 12 hours later.[63]

Theoretically, larger volumes of autologous blood could be injected into the brain but this is technically difficult as blood clots easily in the needle and can back up the needle track.[66] As previously mentioned, 50 µL of blood infusion in rats equates to an average ICH in patients[62, 176] but a 100 µL blood infusion did not significantly increase ICP in our study. In a study with mongrel dogs, ICP rises were observed in the whole blood model. According to the authors, the blood volumes infused were comparable to 55-75 ml in humans.[115] It is possible that rats have a higher brain compliance however in CSF infusion studies, rats had the highest CSF resistance (ie. highest impedance to CSF outflow), followed by dogs and humans[131] which was found to increase with age.[161] Further exploration in comparing volumes of the skull, brain tissue, brain CSF and blood within the skull between species and with increasing age is required to elucidate these pressure-volume relationships. Additionally, after a similar impact-acceleration injury, no mortality was reported in Sprague Dawley rats whereas 50% of Wistar rats died suggesting a higher tolerance in the Sprague Dawley strain.[177]

Thus at least in the context of our study (4-6 month old Sprague Dawley rats for 3 days after ICH), the whole blood model is not a valid model to evaluate ICP. Edema-reducing therapies evaluated in this model become questionable as many assume reductions in edema can be translated into alleviating pathological ICP

elevations and improving CBF. However, antiedema therapies studied in this model can still treat edema and ICP in clinical settings.[86, 115] In the collagenase model on the other hand, ICP increases were found after a moderate-severe ICH for at least 2 days and a severe ICH for at least 4 days. These increases may have been found due to the greater secondary injury associated with the collagenase model[63, 70] and the greater increase in water content found in our study. These ICP increases, however, can possibly serve as a target in evaluating ICP-reducing therapies. Future studies are required to see whether these increases are important in functional outcome.

4.3 The importance of edema

Within the context of our study, the severe increases in water content led to modest increases in ICP. This suggests edema alone is not a valuable endpoint despite the fact that most animal literature uses edema as a sole endpoint.[178-180] Edema in patients has shown to be mainly plasma derived due to the serum proteins extruded from the hematoma[34] – though injections of blood components and thrombin in animals can also induce edema.[24, 78] The space-occupying effects of this edema can cause mechanical damage and increase ICP, but other harmful effects of edema are not clear. Many studies heavily depend on edema to gauge treatment efficacy as it was found to be the second-most common endpoint next to behaviour.[53] This is interesting to note as therapies have shown that modestly reducing edema did not provide functional benefit 30 days after ICH[181] nor any improvements in long-term behavioural or histological endpoints.[81] In contrast, an

inhibitor of carbonic anhydrase (a compound present in erythrocytes) reduced edema by only ~2% in addition to alleviating neuronal death and behavioural deficits.[182] Furthermore, edema severity was correlated with poor behavioural scores; as edema improved, so did behavioural performance. This correlation was found up to 14 days as edema was resolved by this time.[80] Also, a number of therapies have demonstrated decreases in edema in addition to improvements in functional and histological outcomes.[121, 149, 182-185] However, outcome was mostly assessed in the first few days after ICH. Whether improved outcome was a direct result from reductions in edema though, is unclear and warrants further research.

Thus, it is possible long-term endpoints are required to assess the true importance of edema. Only 10% of ICH animal studies looked at chronic functional outcome whereas the remaining studies focused within the first 6 days.[53] Clinical studies also do not provide clear results. No correlation existed between edema volume growth and 3 month functional outcome.[28] Yet, another clinical study found that relative edema was related with better outcome.[186] Thus, more research on edema and its role in long-term functional outcome is required especially since the natural resolution of edema after ICH may contribute to improvements seen in therapies.[53]

It is important to acknowledge that though edema may not have a clear correlation with functional outcome, there appears to be a better relationship with hematoma size – the larger the hemorrhage, the larger one can expect edema volume to be.[187, 188] Larger hematoma evacuations led to greater reductions in edema.[189] In a retrospective study of 404 patients, perihematomal edema volume

growth within the first 72 hours was correlated with death or dependency at 90 days when adjusted for age, gender, and treatment but not when adjusted for initial hematoma volume.[188] It is possible edema can be used as an indicator of ICH severity but whether treatment of cerebral swelling can translate to histological or functional benefits is uncertain.

4.4 Intracranial pressure and edema

Within the context of our experiments, there is no clear threshold in which ICP must exceed to increase edema or vice versa. Our post-hoc exploratory analysis revealed several ICP classifications that may possibly be used for future experiments to predict cerebral swelling. These included Peak ICP and the total number of hours spent 1 or 2 SD above sham animals. From this incipient analysis, it appears that the longer ICP is elevated, the higher one can expect the brain water content to be. However, this was only an exploratory analysis and future experiments are required to appropriately assess these categories with a larger sample size.

However, ICP was increased for 4 days after severe ICH and though not significantly different from each other, ICP was highest in the first 24 hours. As edema peaks on the third day in animal models,[80] one might expect ICP to be highest on this day too. However, hematoma resolution at this time could have diminished the mass effect from edema. Edema has been shown to be resolved by the fifth day in animals,[81] though other studies have found it to be within 2 weeks instead.[80] Unfortunately, our ICP monitoring system failed to measure ICP past 4

days to demonstrate ICP resolution. Also, because of the severity of the ICH used, the edema peak may actually be much later than 3 days. Larger hemorrhages resulted in delayed edema peaks despite similar initial growth of edema when compared with smaller hemorrhages.[190] Within the context of our study though, it would be interesting to note the temporal relationship between edema and ICP. However this relationship may be difficult to define as dramatic edema increases do not similarly increase ICP as previously mentioned.

In patients, edema growth is highest in the first 48 hours and edema peaks around the end of the second week.[26-28, 82] The difference in edema time course between animals and humans is especially important for therapeutic translation as the target critical time window in animals will be different from that of humans. It would thus be important to outline the ICP time course in animal models if ICP-reducing therapies are to be studied.

However, conclusive statements cannot be made until the pressure-volume relationship in the rat is outlined. This is important because we failed to demonstrate the theoretical non-linear pressure-volume relationship as described in Figure 1 but instead, found a linear relationship (Figure 15). Of course, this linear relationship was based on changes in water content only and did not include other volume changes such as lesion volume. This information was limited as animals euthanized for water content assessment could not have been used for histological analyses. However, lesion volume was analyzed in a separate experiment and failed to correlate with Peak ICP. This agreed with findings by Bullock *et al.* in which maximum ICP within 2 hours after ICH did not correlate

with hematoma size. However, hematoma size was calculated as the percentage of the hemisphere which could have been confounded by cerebral swelling that can occur several hours after hemorrhage.[60, 75] Further exploration in determining volumes of rat brain components is required to elucidate these pressure-volume relationships. However, the extent of these ICP rises may depend on lesion location.

In patients with large hematomas, herniation was observed when hematomas were located in the temporal or temporoparietal regions but not when in the frontal lobar or parieto-occipital regions. Patients with the former were at greater risk for brain-stem compression and hematoma volume was correlated with the degree of herniation.[10] In an inflatable balloon study with baboons, ICP responses were observed when balloons were implanted in the supratentorial or in the infratentorial region. Those implanted with the latter experienced a faster reduction in blood flow and larger rise in ICP. Baboons also experienced both high and low levels of CBF at low levels of ICP whereas those with supratentorial balloons experienced low CBF at higher ICP levels. This suggested cerebral autoregulation was impaired much faster in baboons with infratentorial masses.[191] The hemorrhage location in our experiments was not in the infratentorial region and thus, could explain the limited rises in ICP observed. Lesions closer to the brainstem not only suppress vital functioning but also impede blood flow of the major vessels. Brainstem compression is thought to be the cause of Cushing's Triad, a sign of increased ICP, which is described as bradycardia (abnormal slowed HR), respiratory depression and hypertension.[192] However within the

supratentorial region, there was no difference between basal ganglic, thalamic and lobar hemorrhages in mass effect.[26]

4.5 Pressure Gradients

Our results suggested epidural ICP measurements were sufficient without causing cortical damage. No detectable pressure differences were found between the epidural space and in the centre of the hematoma, suggesting the absence of a pressure gradient and thus, no "superior" location of monitoring. Though considered the "gold standard" in clinical settings, intraventricular readings were thus not attempted in this study. Though intraventricular and intraparenchymal monitoring both would have involved an invasive cannula extended into the brain, intraventricular monitoring would have theoretically caused more tissue damage. For example, an immobile cannula laterally positioned to the site of hematoma and edema growth would have caused tissue to push up against the cannula – causing tissue damage and potentially an artificial pressure gradient. In contrast, measuring in the centre of the hematoma would have been less destructive - assuming the mass effect from the hematoma and edema will exert forces outward from the site of the initial bleed.

It is possible the absence of a pressure gradient could have been due to artificially reduced ICP readings in the intraparenchymal monitoring group. The cannula that extended into the hematoma may have provided a pathway for blood to travel upward and be flushed out with CSF. Though, no signs of subarachnoid hemorrhage were noticed in this method. Another explanation could be that the

hematoma provided a pliable space, thus more “give”, than if the cannula were to be placed in the less pliable compressed tissue adjacent to the hematoma. However, this hypothesis cannot be tested as placing the cannula adjacent to the hematoma would be technically difficult and would involve predicting hematoma expansion.

Due to conflicting evidence in current animal literature, the presence of pressure gradients in ICH is undetermined. Thus, we continued to measure ICP in the epidural region exclusively as this method is less invasive than the intraparenchymal method. Future studies can include measuring in the intraventricular space to increase patency and avoid system failure as was seen 6 days after implant.

4.6 Physiological Parameters

Interestingly, HR and BP were not affected by ICH however these experiments were performed in relatively young, healthy, male rats. Most ICH patients are hypertensive upon admission with other comorbidities and experience sustained elevated BP or rapid decline in the first 24 hours - both of which are related to mortality.[193, 194] Though not a large focus of our study, our experiment suggests these BP responses observed in clinic are not likely due to the ICH itself but the vulnerable combination of age and other comorbidities in response to an ICH or the body`s natural response to maintain homeostasis (ie. increasing BP to increase cerebral perfusion). It is also possible acute BP rises are a non-specific (ie. stressful hospital admission experience) or specific (ie. injury to autonomic

areas of the brain) stress response to ICH. Again, our animal model did not have vulnerable cerebral vasculature or any comorbidities that may have contributed to BP responses. Thus, additional work in more clinically relevant models is required (eg. aged, hypertensive animals).

MAP also remained the same after ICH, and so CPP was almost exclusively determined by changes in ICP in our study. These modest reductions in CPP are in favour for the hypoperfused perihematomal region argument and suggest there is likely no ischemia present in these models. These small CPP changes can possibly provide a target for testing CPP-oriented therapies in animals. Whether or not these CPP reductions are even important in functional outcome in these models remains unknown. However, this can likely change if more clinically relevant models were used (eg. aged, hypertensive animals).

4.7 Limitations

The purpose of this thesis was to determine whether measuring ICP using telemetry in the current, commonly-used ICH rat models was feasible and if it were, to evaluate the face validity of these models in mimicking patient ICP. As previously shown by Silasi *et al.*, telemetry was successful in measuring ICP in animal models of ischemic stroke. The authors were able to demonstrate pathological increases when compared to pre-stroke ICP values. Ischemic stroke was induced by blocking blood flow to the carotid arteries that were accessed through the neck.[135] In contrast, the ICH models used in this study required stereotaxic drilling of the skull in order to inject collagenase or whole blood into

the striatum. Pre-stroke ICP values were thus not possible, as the ICP implant would have posed as a physical barrier. Previous attempts have been made to tuck the PA-C10 probe into the nape of the neck instead but artefacts were seen during trunk and neck movement.[135] Presently, installing the ICP assembly atop of the skull was the most appropriate approach to minimize artefacts.

The ICP rises noted in this study were much lower than expected, given the extent of injury. In our study, every 1% increase in water content correlated with a 0.96 mm Hg increase in ICP. The accuracy of our method was thus brought into question especially since the surgical procedure involved drilling a total of 3 holes into the skull. These holes potentially equalized pressure before they were sealed. It is likely that our ICP readings were slightly erroneous during the first day or at least the first few hours after ICH before ICP was allowed to re-stabilize, thus underestimating ICP and overlooking an initial increase in ICP. This could also have been an effect of anesthesia. Additionally, the sham group experienced an unexplained ICP rise on Day 6 and 7 that indicated a possible interruption in the fluid-filled system (e.g., clot or blockage at the tip of the cannula). However, all of these animals responded to the Valsalva manoeuvre on the seventh day. Full patency of the system may have been lost during this time. Despite the aforementioned complications, the ICP values found in our series of experiments for the first 4 days fell into the normal range of 0-12 mm Hg in unanesthetized rats.[123] This range might vary according to rat strains. ICP for Sprague Dawley rats, without anesthesia, approximates ~5 mm Hg which agrees with our findings.[134, 135] Another study found ≤ 7.7 mm Hg.[122] Wistar rats on the other

hand, appear to have a range that is a little higher: ≥ 9.1 mm Hg.[118, 132, 133] However, one research group found ICP to be ≥ 8.39 mm Hg in the Sprague Dawley strain but this is not clear as some of their papers did not report exact values.[128-130] Additionally, ≥ 9.22 mm Hg was found in Sprague Dawley rats using telemetry. The probe was subcutaneously implanted in the dorsal midscapular region and it was not clear whether the catheter was subject to movement that could have caused artefacts during physical activity,[136] as was seen in our lab's previous attempt.[135] Of interest, a study performed in TBI-injured Wistar and Spraque Dawley rats had similar ICP responses, though this was done in anesthetized animals and normal values were not reported nor compared.[177] However, a conclusive statement warrants experiments comparing the two species using the same ICP monitoring approach in awake animals. Lastly, the Valsalva manoeuver was successful in all animals immediately after surgery and prior to euthanasia. The transient spike and fall to baseline in ICP observed in our study was an easy and robust way to confirm our system was responsive although it does not test calibrations.

To evaluate the face validity of ICH rodent models, we first decided to induce a severe ICH using the collagenase model and observe ICP responses. Though mortality was not considered significantly different from the control group, 20% of the ICH-induced animals died in the first 24 hours. The highest ICP reading observed in rats that survived was 37 mm Hg however on average, an increase of 6.94 mm Hg was found across animals. The gap between ICH and sham animals were progressively less in moderate to severe lesions induced by the collagenase

model, followed by the whole blood model. Severe increases in water content were observed in these models but dramatic increases in ICP did not result. Of course, our surrogate measure for edema was brain water content via the wet/dry method, which may not have provided representative values of "true edema". Water content within the hematoma may have confounded these numbers as blood water content is 82.7%[195] and pilot studies in our lab suggest this as well. Extracting perihematomal tissue would be required to circumvent this issue but this poses some challenges. Neatly separating the hematoma from its surrounding tissue without artificially altering the fluid contents of the tissue is technically difficult. Nevertheless, water content in the ipsilateral striatum was above 80% in ICH animals, suggesting cerebral swelling.

4.8 Future Directions

Further attempts to increase the patency of our ICP monitoring system to measure ICP at protracted time points are of interest. Only 3 sham animals were successfully monitored for 7 days in which 1 experienced "pathological" ICP rises (artefact) on Day 6 and 1 more on Day 7. Additional pilot studies are needed to confirm that the ICP system actually fails on the fourth day. Similarly, another study also showed an unexplained increase in ICP in their control group 4 hours after implantation.[122] A future study can involve intraventricular monitoring to increase the patency of the system. The shaft of the nylon screw can also be excised to reduce the risk of tissue obstructing the system. The nylon screw head can be superimposed and fixed over the cranial burr hole to guide the cannula in.

In this case, the cannula will sit flush with the top of the skull instead of the bottom. Of course, this method allows more "space" within the skull and can possibly help alleviate ICP. However, pilot studies confirming whether the original system actually fails on the fourth day is required before such attempts are made.

If these attempts are successful, it would be important to determine the temporal ICP patterns following an ICH. Whether these increases are sustained, resolved or further increased is vital information in evaluating ICP-reducing therapies.[196] In our lab, hypothermia therapy is studied extensively and is of interest in targeting these ICP increases. Hypothermia therapy has shown some promise in reducing ICP.[149, 197-199] The rewarming phase however is concerning as ICP can rebound during this time. It is important that rewarming in ICH models do not coincide with the natural peak in ICP as this can rebound and exacerbate ICP as seen in the National Acute Brain Injury Study: Hypothermia II.[200] Several other studies also noted increases in ICP during rewarming[197, 201] which can even lead to death.[202] A systematic review revealed that fast rewarming not only reverses the protection by hypothermia therapy but also exacerbates injury.[203] Rewarming can cause cardiovascular collapse, reduced CPP and hypoxia, thus increasing ICP.[204] Future experiments can test different rates of rewarming after ICH compared between different methods of cooling (eg. systemic and focal cooling).

Other ICP-reducing therapies can also be applied after ICH such as hypertonic saline solution or mannitol to alleviate ICP. Whether reductions can translate to

an improved outcome can help further validate these ICH models. These therapies can then pose as a positive control for evaluating future experimental treatments that target ICP.

To ensure accuracy of our telemetry ICP system, comparisons to other methods of monitoring can be performed in future experiments. Examples include using a solid state catheter under anesthesia or connecting the animal to a bench top BP monitor. The BP monitor can be attached to a swivel head atop of the cranium, allowing the animal to freely move. These may be cheaper methods in measuring ICP and allow for larger sample sizes. Our study was limited by the expensive cost of telemetry probes and thus, could only run a few animals at a time.

In our lab, we use several other telemetry devices such as ones that measure temperature and EEG activity. One of particular interest is our EEG probe. We recently found seizure activity after ICH animal models (Klahr et al., unpublished data). Post-ICH seizures occur in 28% of patients in the first 72 hours and were correlated with a midline shift.[46] Interestingly, those with a raised ICP did not present seizure activity[47] but those that experienced electrographic seizure activity after a TBI also experienced a higher ICP and increased metabolic distress than those who did not. The authors claim increases in ICP were likely to be due to increased CBF and volume.[48] It would be of interest for future studies to correlate seizure activity with ICP and even temperature.

It is also important to determine skull size and volume of brain components of the species and strain of interest - in our case, the Sprague Dawley. As noted

previously, Sprague Dawley rats appear to differ in injury tolerance and normal ICP values. It would thus be interesting to learn whether these differences could be attributed to higher brain compliance. Volume of brain components can be determined by brain imaging or by extraction methods such as hemoglobin assays for blood volume and the use of draw syringes for CSF collection.[205] Additionally, to locate where current ICH rodent models lie on the pressure-volume curve, bolus or continuous injections of fluid that is immiscible with CSF could be performed. This can help determine whether ICH models currently used are in situations of high or low brain compliance. This can further be elaborated by location of injection or using animals of older age with comorbidities such as hypertension.[49, 66] This would be interesting to note as CSF outflow resistance increases with age.[160] Thus, higher ICP rises may be observed in aged animals and would pose as a more clinically relevant model. This would be of interest for a future study. Older animals were shown to sustain more severe cerebral swelling than younger rats after an injection of 100 µl of blood.[206] However, this can partly be due to changes in blood components with age.[207]

4.9 Conclusions

Telemetry was successful in measuring ICP for at least 4 days after ICH in unanesthetized and untethered rats. ICP was increased after ICH when induced by collagenase but not by whole blood. Thus, these ICP rises observed in the collagenase model can serve as a target to evaluate ICP-reducing therapies.

However, further research is required to elucidate how these ICP increases can be used and translated to help guide therapy in clinical settings.

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Table 1. The total number of hours ICH animals spent 1 SD and 2 SD above sham animals predicted brain water content on Day 3. This was also the case with Peak ICP. However, this was a post-hoc exploratory analysis and future experiments are required to appropriately assess these classifications with a larger sample size.

Category	F - value	R^2	p-value
Hours above 1 SD of sham	22.49	0.529	≤ 0.001
Hours above 2 SD of sham	18.54	0.481	≤ 0.001
Peak ICP	6.4	0.242	0.02

Figure 1. In situations of low brain compliance, a given increase in volume causes a greater increase in pressure than if brain compliance was high. Thus, a nonlinear relationship exists between intracranial volume and pressure. This pressure-volume relationship is referred to the Monro-Kellie doctrine.

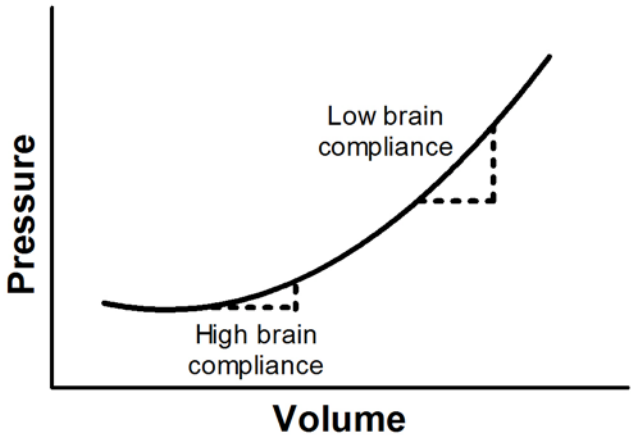

Figure 2. The cerebral autoregulation curve for an injured, normotensive and hypertensive individual. CBF remains constant over a given range in MAP. Outside the limits of cerebral autoregulation, CBF becomes passive to changes in MAP. The cerebral autoregulation curve for a hypertensive individual is shifted to the right. If brain injury occurs, cerebral autoregulation can become impaired.

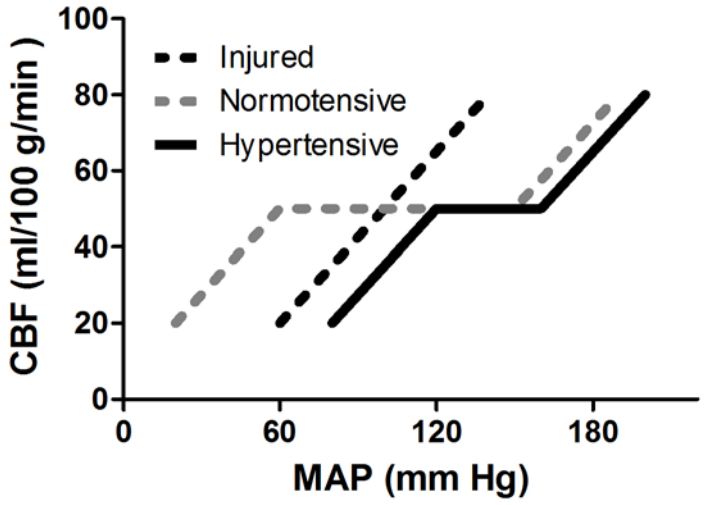

Figure 3. (A) Cranial ICP apparatus fully implanted on animal. The plastic cylinder, made from a 5 cc syringe barrel protects and secures the PA-C10 probe. The whole assembly is secured with dental cement. (B) A filed 23 G needle shaft attached to PE20 tubing (left) fits snug into a modified nylon screw guide (right). Cannula as shown in the picture is used for epidural ICP monitoring. Cannulas extended 6.5 mm below the screw head are used for intraparenchymal ICP monitoring.

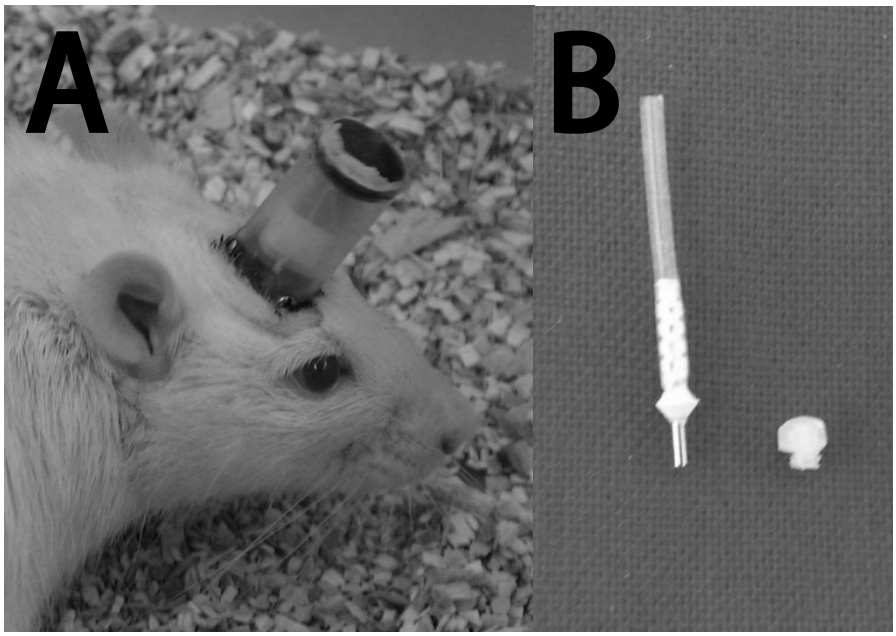

Figure 4. PART 1: Excluded animal from the EPI-ICH group. Solid line represents average ICP per hour. Dashed line represents average activity counts per hour. Graph suggests animal died approximately 24 hours after surgery, suffering from two ICP spikes at 16 hours (28 mm Hg) and 23 hours (21 mm Hg). Animal appeared to be hyperactive around 4 hours after surgery but hypoactive after 8 hours.

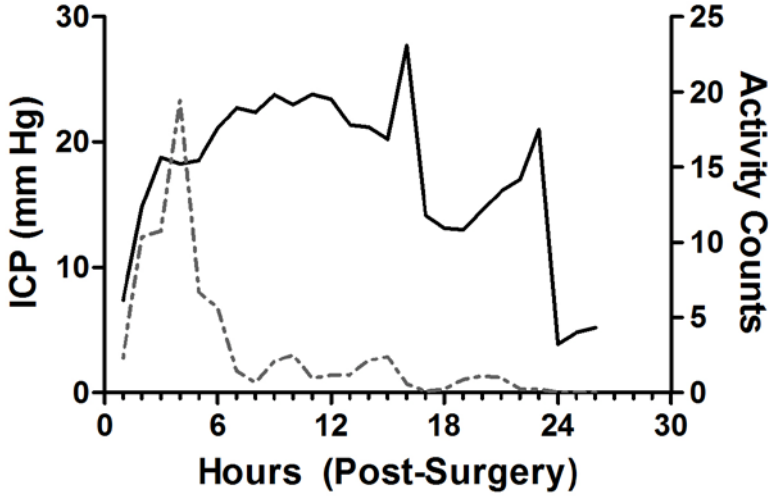

Figure 5. PART 1: Animal was excluded as a result of a notably smaller hematoma than other animals in the ICH groups. All ICH and SHAM data from Part 1 are also plotted for comparison. Excluded animal experienced a relatively higher ICP than SHAM but lower than ICH on the first day. Elevated ICP in this animal gradually fell to normal levels by the third day. This reinforces the sensitivity of the telemetry ICP monitoring system in measuring different sized hematomas. Values expressed as mm Hg (mean ± SD).

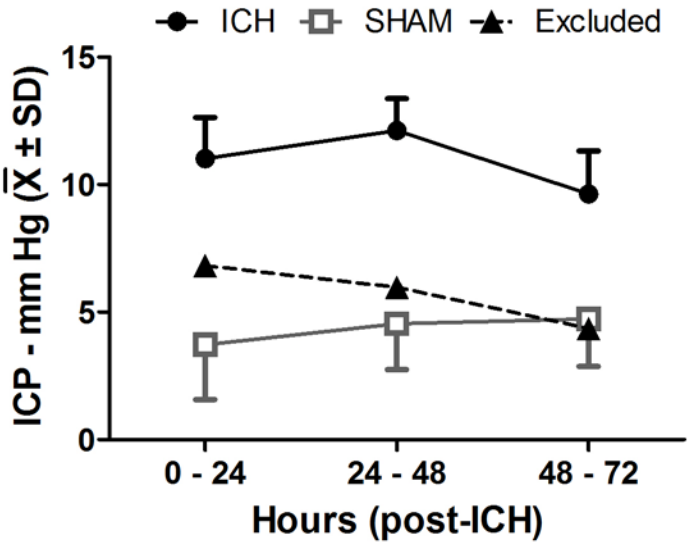

Figure 6. PART 1: Severe ICH elevated ICP when ICP was measured in the intraparenchymal (center of the hematoma) and in the epidural space. No difference was found between the 2 locations of monitoring (p = 0.902). Values expressed in mm Hg (mean ± SD).

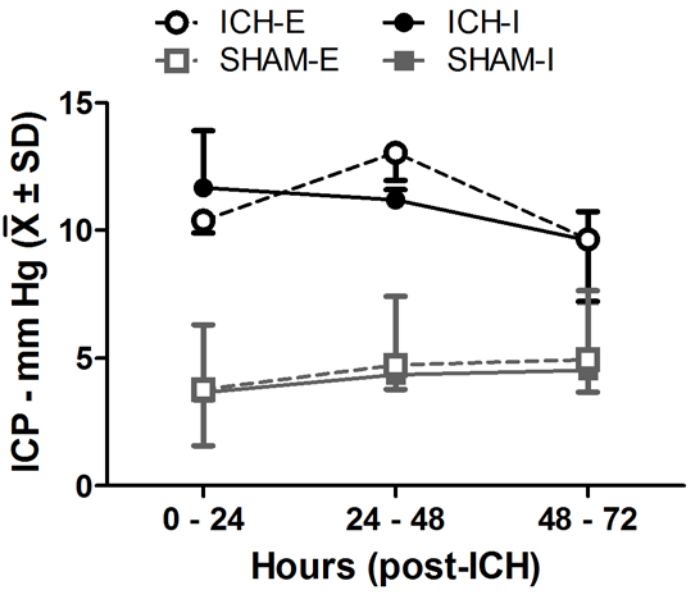

Figure 7. PART 1: Brain water content was increased 3 days after severe ICH in the ipsilateral and contralateral striatum and cortex. Asterisk (*) denotes a significant difference between groups ($p \leq 0.05$). Values expressed as percentage (mean ± SD).

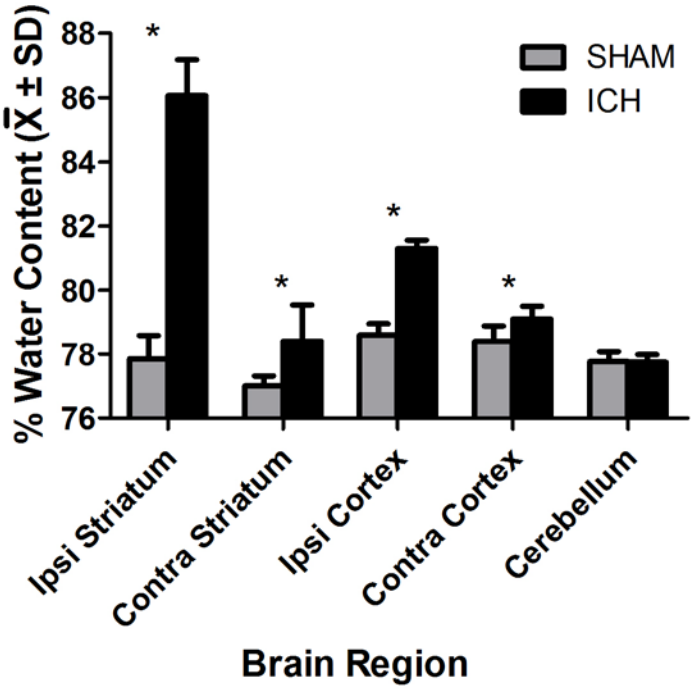

Figure 8. PART 2: Severe ICH did not alter HR for 7 days from BL. Values expressed as percentage of baseline (mean ± SD).

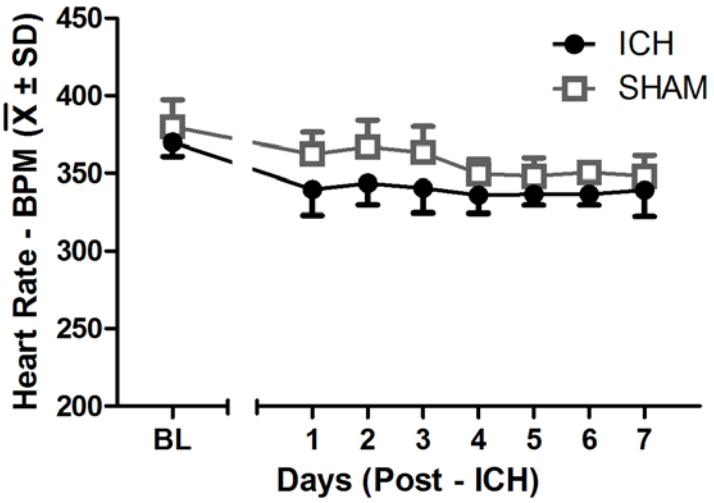

Figure 9. PART 2: Severe ICH increased ICP for 4 days. Values expressed as mm Hg (mean ± SD).

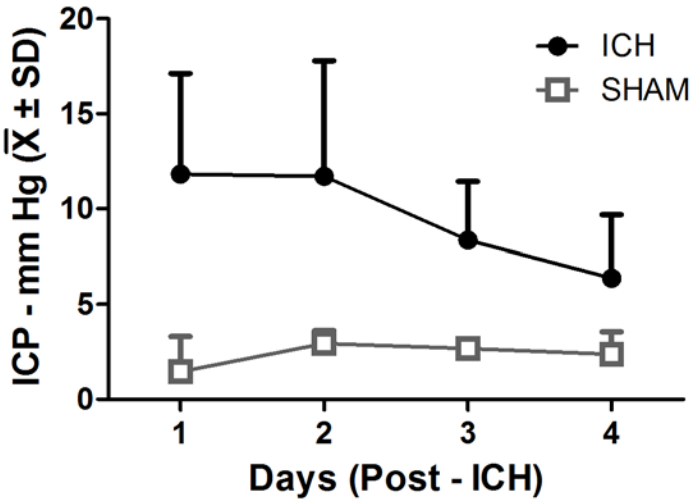

Figure 10. PART 2: Severe ICH did not change systolic, MAP and diastolic pressure for 4 days. Values expressed as mm Hg (mean ± SD).

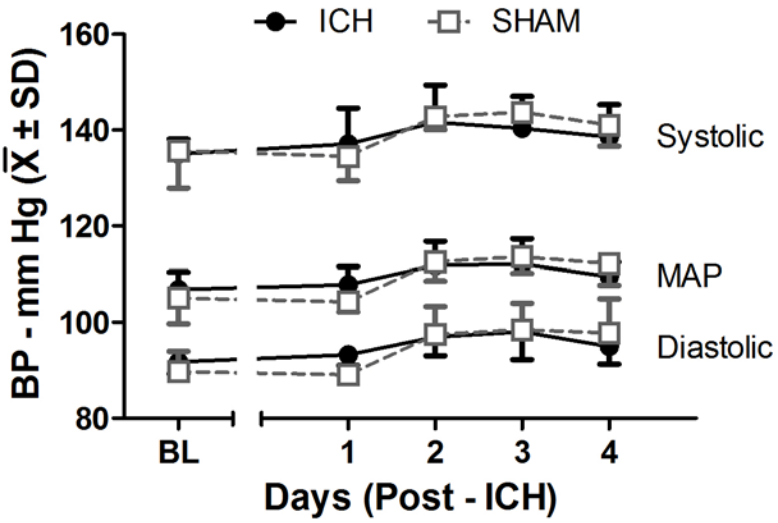

Figure 11. PART 2: Severe ICH modestly reduced CPP on Day 1, 2 and 4. Asterisk (*) denotes a significant difference between groups ($p \leq 0.05$). Values expressed as mm Hg (mean ± SD).

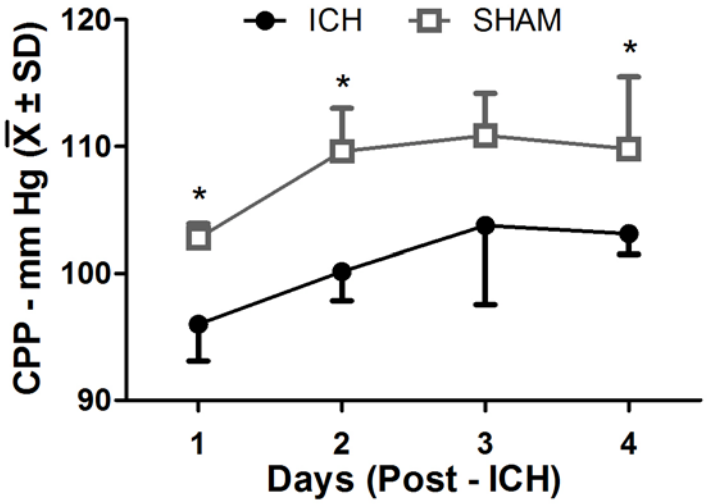

Figure 12. PART 2: There was no relationship between lesion volume on Day 7 (*M:* 64.55 mm^3 ± *SD:* 21.47) and Peak ICP (R^2 = 0.647, p = 0.101) after severe ICH. Confidence bands are plotted with a 95% confidence interval.

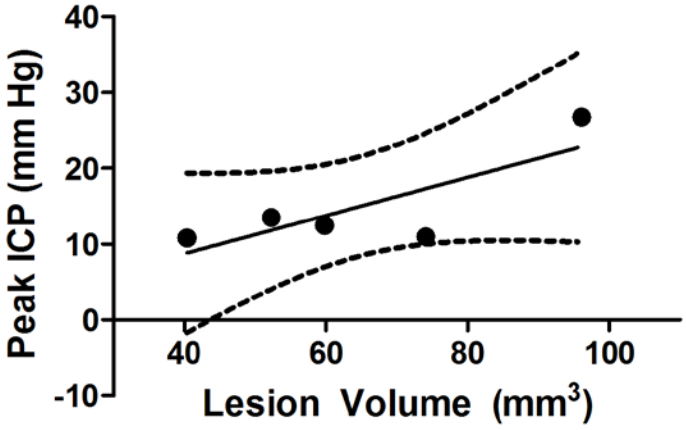

Figure 13. PART 3: Moderate to severe ICH caused an increase in water content in select brain regions on Day 3. In the ipsilateral striatum and cortex, there was a significant difference between all groups. In the contralateral striatum, both methods of ICH (WB and COLLAG) increased water content but were not significantly different from each other. Asterisk (*) denotes a significant difference from SHAM ($p \leq 0.05$). Pound symbol (#) denotes a significant difference from WB ($p \leq 0.05$). Values expressed as percentage (mean ± SD).

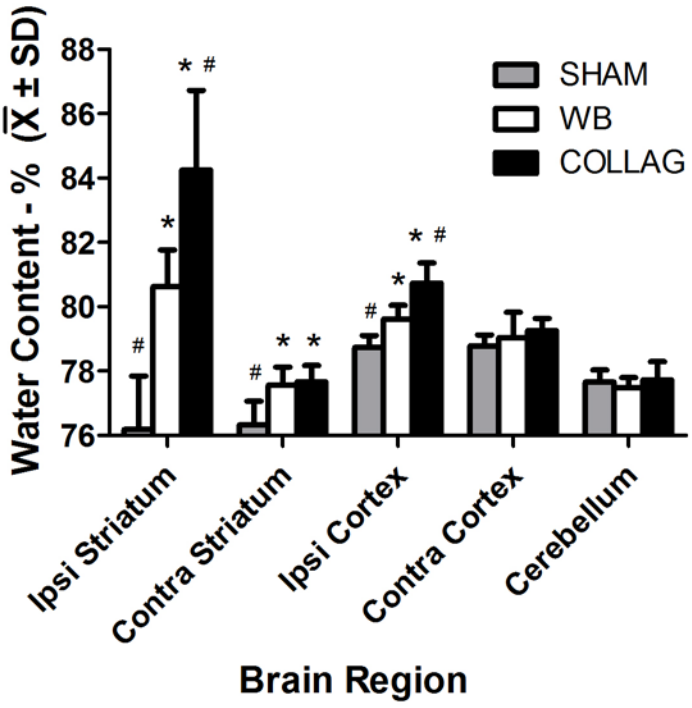

Figure 14. PART 3: COLLAG increased ICP on Day 1 and 2 when compared to SHAM and on Day 2 and 3 when compared to WB. WB did not significantly increase ICP when compared to SHAM. Asterisk (*) denotes significant difference between all groups. Values expressed as mm Hg (mean ± SD).

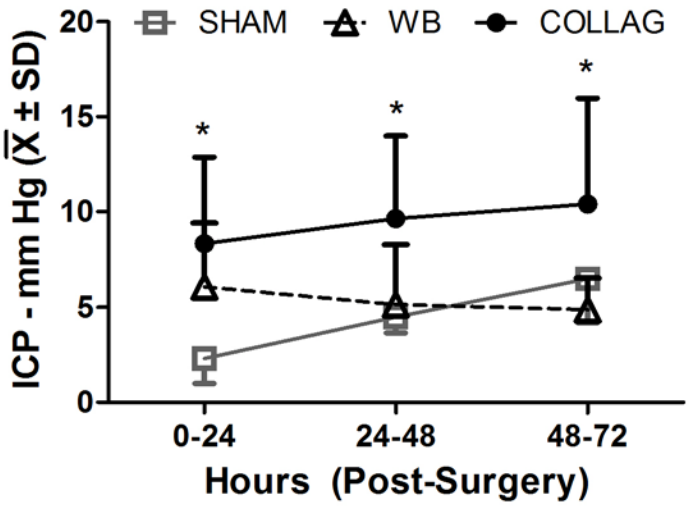

Figure 15. Data from Part 1 were pooled with Part 3. Line of best fit demonstrates a linear relationship between Day 3 water content and Day 3 ICP in ICH animals ($R^2 = 0.343$, $p \leq 0.001$). The 95% confidence bands of the line of best fit are also plotted.

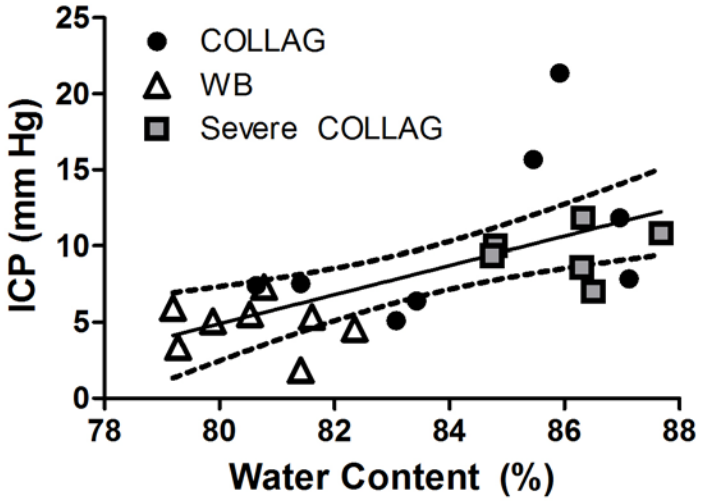

Figure 16. The total number of hours ICH animals spent 1 SD above sham animals was linearly correlated with brain water content on Day 3 ($R^2 = 0.529$, $p \leq 0.001$). The 95% confidence bands of the line of best fit are also plotted. However, this was a post-hoc exploratory analysis and future experiments are required to appropriately assess this with a larger sample size.