Intracerebral hemorrhage (ICH) is the second-most common stroke with a high mortality and morbidity rate (Sacco et al., 2009). The hematoma mass effect and progressive edema can lead to life-threatening elevations in intracranial pressure (ICP) arising from mass effect of the hematoma and edema. According, edema is a common endpoint to gauge treatment efficacy in rodent ICH models. Despite widespread reliance on edema, its relationship with ICP and cerebral perfusion pressure (CPP) is unknown. Blood pressure (BP) and ICP were measured by telemetry devices in rats after collagenase (“severe” – 0.3 U, and “moderate” – 0.15 U doses) or blood infusion (100 µl) into striatum (vs. saline infused shams). We compared epidural and intraparenchymal ICP readings (collagenase), evaluated CPP (collagenase), and compared models. Moderate (9.46 mm Hg ± 4.72 SD, 3 day average) and severe collagenase ICHs (10.79 ± 3.50) significantly increased ICP versus shams (4.02 ± 2.09), whereas blood infusion did not (5.37 ± 0.55). The two monitoring locations gave similar readings after severe collagenase ICH. Increased ICP reduced CPP by ~7.5 mm Hg for days after the larger collagenase infusion. CPP averaged from 103–112 mm Hg in shams. Edema occurred in all ICH models and predicted ICP. However, ICP and CPP were only modestly changed even after severe ICH and edema. Thus, small changes in edema typically reported in the literature, which often use smaller bleeds than presently used, likely minimally affects ICP and CPP. Further research into the face validity of these models, endpoints, and their ability to evaluate therapeutics is needed.
Tysebotn, 1997) while also reducing edema, brain injury and neurological impairments after ICH (Khatibi et al., 2011; Ma et al., 2009), and telemetry does not require restraint or tethering.

Thus, we used telemetry in rats to measure ICP in the whole blood and collagenase ICH models. Several experiments were conducted to: evaluate the optimal location of monitoring, assess ICP and CPP changes after severe ICH, compare ICP changes in the collagenase and whole blood models, and correlate edema with ICP. We hypothesized that higher ICP would follow more severe insults, which would correlate with edema. We also expected higher ICP nearer the site of injury than farther away.

Materials and methods

Animals

Fifty-four male Sprague Dawley rats (Biosciences, University of Alberta, 350–500 g, ~3–5 months old) were on a standard 12 h light–dark cycle with free access to food and water. Animals were closely monitored and singly housed in polycarbonate cages. Subjects were given soft food (rat chow, peanut butter dissolved and mixed in distilled water with additional sunflower seeds) for 1–2 days after surgery and regular rat chow thereafter (LabDiet 5001, St. Louis, MO). Animals were randomized, but due to obvious neurological impairments (abnormal movements, turning bias, diminished contralateral limb use, etc.) the investigator (C.H.) was not blinded to stroke assignment. However, data collection was computerized. The University of Alberta’s Biosciences Animal Care and Use Committee, which follow the Canadian Council on Animal Care guidelines, approved all procedures.

Experiments

Experiment 1: location of ICP monitoring

Experiment 1 had 2 parts in which 8 animals were randomized to severe ICH (0.3 U of collagenase) or sham surgery (SHAM) in each part. ICP was recorded in the epidural space (ICH-E or SHAM-E) in the first and intraparenchymally (ICH-I or SHAM-I) in the second experiment. ICP was recorded for 72 h and brain water content was assessed at this time of presumed peak edema (Fingas et al., 2007; Xi et al., 1998).

Experiment 2: stroke-induced CPP changes

Baseline heart rate (HR), body movement (arbitrary units), MAP, systolic and diastolic pressure were recorded in 14 animals for 1 week before they were subjected to severe ICH (0.3 U of collagenase) or SHAM surgery. These parameters were then measured and alternated with ICP recordings every 6 h for 3 days and then every 12 h for 4 days. Animals were euthanized 1 week after surgery to determine lesion volume.

Experiment 3: comparing ICP in different ICH models

Twenty-four rats were subjected to a SHAM procedure or a moderate ICH induced by infusing 0.15 U of collagenase (ICH-CO) or 100 μL of whole blood (ICH-WB). Surgical procedures were mirrored according to the blood model to ensure consistency among groups (e.g., surgical procedures and duration of anesthesia). ICP was recorded until euthanasia at 72 h when water content was assessed.

Surgery

For aseptic surgery, rats were anesthetized with isoflurane (4% induction, 2% maintenance in 60% N2O and balance O2) while rectal temperature was maintained at 37 °C. Surgical sites were shaved, treated with 70% alcohol and dovidine (Laboratoire Atlas Inc., Montreal, Canada) before cutting. Wounds were treated with bupivacaine (Hospira, Montreal, Canada). All telemetry probes were first sterilized in 2% glutaraldehyde and rinsed several times in sterile saline.

BP monitoring

The PA-C10 (Data Sciences, St. Paul, MN) telemetry probes were used to measure BP as well as ICP (below). For BP, the right femoral artery was isolated and the PA-C10 catheter was inserted and secured with sutures. The probe was secured subcutaneously and the wound was treated with bupivacaine before it was sutured. After implantation, rats were monitored by receivers (Model RPC-1) placed under animal cages. Waveform data was averaged over 5 s every minute. The data was then stored using Dataquest ART software (v. 2.1). Due to signal interference, physiological parameters were recorded from one probe per animal at a time and thus, simultaneous recording from multiple probes in one animal was not possible (DeBow and Colbourne, 2003).

ICH surgery

This surgery was performed as previously done (MacLellan et al., 2008). Rats were placed in a stereotaxic frame and a hole was drilled 3.5 mm to the right and 0.07 mm posterior of Bregma avoiding the cor- onal suture. A 26 G syringe (Hamilton, Reno, NV) was lowered 6.5 mm from the skull surface into the stratum for infusion of: 1 μL of 0.3 U of collagenase (severe ICH, type IV-S; Sigma, Oakville, Canada), 1 μL of 0.15 U of collagenase (moderate ICH), 1 μL of sterile saline, or 100 μL of blood obtained from the tail vein. Injections were done over 5 (Experiments 1 and 2) or 10 min (Experiment 3). An additional 5 (Experiments 1 and 2) or 10 min (Experiment 3) elapsed before the needle was removed to avoid drawback of liquid.

ICP implant

This apparatus was built similarly to our previous work (Silasi et al., 2009). The shaft of a hollow nylon screw (C2125GN, PlasticsOne, Roanoke, VA) was cut to the thickness of a rat’s skull. A 23 G needle shaft (cannula), which was later inserted through the hollow screw, was cut to fit either in the epidural space or at 6.5 mm deep into the brain. A ~2.5 cm long cylinder (5 cm 3 syringe) was also made to protect the ICP system from damage.

Immediately after collagenase, blood or saline infusion the hollow screw was inserted into the burr hole and further secured with Vetbond (3M Animal Care, St. Paul, MN). Two metal screws were also inserted into the skull so that they would border the inner lining of the plastic cylinder, which centered on the hollow screw and sat vertically on the skull and was secured with dental cement. The cannula was inserted into the nylon screw and secured with Vetbond. The epidural or intraparenchymal cannula was connected to the PA-C10 probe’s catheter by a small length of saline-filled PE20 tubing. All of this was placed inside the cylinder and a rubber plunger tip sealed the apparatus. The incision was sutured and animals were hydrated subcutaneously with 5 mL of sterile saline.

Pressure offsets were taken prior to and after probe use. As well, the integrity of the ICP system was tested via an abdominal compression maneuver before and after ICP monitoring (Silasi et al., 2009).

Water content assessment

Rats were anesthetized with isoflurane and decapitated. The brain was removed and blocked from 4 mm posterior to 2 mm anterior of the infusion. This tissue was divided into ipsilateral and contralateral cortical and striatal sections. The cerebellum served as a control. Brain tissue was weighed before (wet weight) and after (dry weight) being baked for 24 h at 100 °C (Fingas et al., 2007; Xi et al., 1998). The % water content was calculated as (wet weight − dry weight) / wet weight) × 100.
Histological preparation and quantification

Animals were overdosed with pentobarbital (100 mg/kg i.p.) and transcardially perfused with saline then 10% neutral buffered formalin. Brains were post-fixed and subsequently immersed in 10% sucrose in formalin for 2 days before coronal sectioning at 40 μm. Sections were taken every 200 μm from before, through and beyond the injured zone and were stained with Cresyl Violet. Normally, total tissue loss is calculated by subtracting the affected ipsilateral hemisphere from the contralateral hemisphere. However, due to the severity of ICH used in these experiments, hemispheric swelling was expected on Day 7. Thus, total lesion volume (mm³) was quantified for each animal (ImageJ v. 1.46, NIH) (MacLellan et al., 2008).

Statistics

Group sizes were based upon our methods paper that used a focal ischemia model (Silasi et al., 2009). There were no significant differences in ICP between the dark and light cycles so daily averages of ICP were analyzed using repeated measures ANOVA with simple contrasts when applicable (SPSS v. 17.0, Chicago, IL). The CPP, BP and HR data were similarly analyzed. Significant interactions were analyzed using one-way ANOVAs on each day with post-hoc Tukey tests. The last 3 days before ICH were averaged for baseline (BL) values. Water content was evaluated with t-tests.

Lesion volume was regressed with peak ICP (highest ICP averaged over 1 h) and Day-3 water content with Day-3 ICP. Hours spent 1 or 2 standard deviations (SD) above the average SHAM values were regressed (linear and quadratic) with water content (Experiments 1 and 3). Mortality was analyzed using a Fisher exact test. Comparisons were significant when p ≤ 0.05. All data are given as mean ± SD and 95% confidence bands are plotted when applicable.

Results

Exclusions and mortality

Two animals in Experiment 1, 6 in Experiment 2, and 2 in Experiment 3 were excluded due to technical difficulties, experimenter error and mortality. Mortality occurred only after the 0.3 U collagenase infusion (severe ICH). In total, 15 animals had a severe ICH, 3 of which died within 24 h (p = 0.224 vs. SHAM). Two died quickly after ICH. However, sufficient ICP data was collected for 1 animal (Fig. 1A), and this rat’s data suggests that ICP values exceeding 25 mm Hg put animals at a high risk of spontaneous death. After exclusions, Experiment 1 had 14 rats (ICH-E: n = 3, SHAM-E: n = 4, ICH-I: n = 3, SHAM-I: n = 4), Experiment 2 had 8 rats (ICH: n = 5, SHAM: n = 3), and Experiment 3 had 22 rats (ICH-CO: n = 8, ICH-WB: n = 8, SHAM: n = 6).

Experiment 1: no difference between locations of ICP monitoring

Intraparenchymal and epidural data from Experiment 1, which used the 0.3 U collagenase infusion, were analyzed in a 3-way ANOVA to assess ICP monitoring location (Fig. 1B). There were no effects of Location, Day × Location and Day × ICH × Location (p ≥ 0.223). Thus, the epidural

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Fig. 1. (A) Rat with two ICP (solid line) spikes at 16 h and 23 h, and death at ~24 h after collagenase induced ICH (0.3 U). This rat’s activity (dashed line) was high initially but hypoactive after 6 h. The activity readings are a relative measure of activity determined by the telemetry probe’s movement across the receiver (detected by changes in signal strength). All animals that died were excluded from analysis and were not included in graphs B–D. (B) Severe ICH (0.3 U) increased ICP for 3 days (daily averages). No difference was found between epidural and intraparenchymal monitoring. (C) Hourly ICP readings are shown for the combined SHAM and combined ICH groups from Experiment 1 to illustrate group differences and variability over time. Significantly higher readings occurred in the ICH animals at almost all times. (D) Water content in the ipsilateral and contralateral hemisphere increased 3 days after severe ICH (*p ≤ 0.05).
and intraparenchymal readings were not different and these animals were pooled for edema assessment. Additionally, epidural monitoring was used for Experiments 2 and 3 to avoid injury from intraparenchymal measurement. These epidural and intraparenchymal data from Experiment 1 were also separately analyzed. ICP was greater in ICH-E and ICH-I (p ≤ 0.008 vs. their SHAMs). There was no effect of Day with epidural monitoring (p = 0.489), but there was a Day effect with epidural monitoring (p = 0.050). There was no ICH × Day effect for either experiment (p ≥ 0.074). Hourly averages (72 data points) are shown for the combined SHAM and the combined ICH groups (Fig. 1C). A repeated-measured ANOVA on these data revealed significant Group, Time and Group × Time effects (p < 0.001) with further one-way ANOVAs showing significantly higher ICP readings in the ICH group for 68 of the 72 h with most of these being p ≤ 0.001. Group variability was similar with only one significant Levene’s test (for heterogeneity) out of 72 (hourly) comparisons.

Severe ICH substantially increased water content in the ipsilateral striatum and cortex as well as modestly increasing edema in the contralateral striatum and cortex (p ≤ 0.014, Fig. 1D). The cerebellum was not different between ICH and SHAM (p = 0.899). In all cases, we visually confirmed that an ICH had occurred in collagenase treated rats, as expected with this model, and that there was no obvious bleed from the SHAM procedure.

**Experiment 2: severe ICH modestly reduced CPP**

An unexplained increase in ICP (e.g., up to 26 mm Hg) occurred on Days 6 and 7 in 2 of 3 SHAM rats (not shown) presumably from a technical problem (e.g., blocked cannula). Accordingly, this cast doubt on the validity of these later readings and we conservatively omitted ICP readings from Days 5 to 7. As in Experiment 1, severe ICH (0.3 U of collagenase) increased ICP (p = 0.021) with no effect of Day (p = 0.150) or Day × ICH (p = 0.092, Fig. 2A).

Severe ICH did not change diastolic, systolic or MAP (p ≥ 0.778, Fig. 2B). For these, there was an effect of Day with slightly increasing pressure after surgery (p ≤ 0.001), and no significant Day × ICH interaction (p ≥ 0.296). Severe ICH reduced CPP (p = 0.014, Fig. 2C) with an effect of Day (p ≤ 0.001) and no Day × ICH interaction (p = 0.674). Thus, CPP was modestly reduced in ICH rats by 7.5 ± 1.32 mm Hg each day.

There was no significant effect of ICH on HR but it was slightly reduced after severe ICH by 6.7 BPM (p = 0.062, not shown). A Day effect was observed with an average reduction of 28.4 BPM ± 14.47 over the days (p ≤ 0.021 vs. BL), but there was no ICH × Day effect (p = 0.630).

SHAM animals only had a needle track whereas all ICH rats had substantial striatal injury with some cortical and corpus callosum damage (Fig. 3A). There was no significant linear (p = 0.101, r = 0.804, Fig. 3B) or quadratic relationship (p = 0.127, r = 0.934) between lesion volume (64.55 mm³ ± 21.47) and peak ICP. Similarly, the relationships between average daily ICP and lesion size did not reach statistical significance (e.g., Day 1 average ICP with lesion volume: p = 0.061, r = 0.861).

**Experiment 3: increased ICP occurred in collagenase-induced ICH only**

All of the ICH-CO (0.15 U collagenase dose) and ICH-WB (100 μL) rats were visually confirmed to have had an ICH on Day 3 (during tissue processing), as expected for these models. Both ICH-CO and ICH-WB increased water content in ipsilateral striatum and cortex (p ≤ 0.010 vs. SHAM, Fig. 4A). However, ICH-CO had higher water content than ICH-WB rats (p ≤ 0.003). The contralateral striatum had increased water content in ICH-CO and ICH-WB (p ≤ 0.003), which were not different (p = 0.931). There was also no difference among groups in the cerebellum or contralateral cortex (p ≥ 0.296).

There was an effect of ICH, Day, and ICH × Day on ICP (p ≤ 0.023, Fig. 4B). Increased ICP in the ICH-CO group occurred on Days 1 and 2 (p ≤ 0.023 vs. SHAM) but did not reach significance on Day 3 (p = 0.157). The ICH-CO had higher ICP than ICH-WB on Days 2 and 3 (p ≤ 0.033) but not significantly on Day 1 (p = 0.422). The ICH-WB did not significantly increase ICP (p = 0.848 vs. SHAM), although there was a modest transient rise.

**ICP predicts edema**

Significant linear (r = 0.625, p = 0.002, Fig. 5) and quadratic relationships (r = 0.625, p = 0.009) were found between Day 3 water content and Day 3 ICP in all ICH animals from Experiments 1 and 2. Similarly, there was a significant linear (r = 0.727, p ≤ 0.001) and quadratic (r = 0.760, p ≤ 0.001) relationship between hours spent 1 SD above SHAM and Day 3 brain water content. The same held for hours spent 2 SD above SHAM (not shown).

**Discussion**

We are the first to show that telemetry can be used in freely moving rats to continually measure ICP for several days following ICH. Our data thus confirm the utility of this method for ICH. We found that moderate (0.15 U) and severe (0.3 U) collagenase-induced hemorrhaging elevated ICP for several days (same results in 4
separate experiments) whereas infusing 100 μL of blood did not. The severe collagenase insult also caused a small but significant reduction in CPP. Overall, ICP predicted edema, which was present in all models but greatest after the high collagenase dose. Finally, we observed similar ICP readings with epidural and intra-hematoma locations. Thus, at least for severe ICH in rat, monitoring location did not matter as no pressure gradient was found.

Generally, our findings indicate that the collagenase model is better suited to studying ICP/CPP changes than the standard model of infusing 100 μL (or less) of blood. Furthermore, one might argue that the modest amount of edema observed in many ICH studies, especially those using the whole blood model, likely has little impact on ICP/CPP. The corollary of this is that reductions in edema in these settings can have little to no meaningful impact on ICP/CPP. Thus, neuroprotective treatments that improve behavior or lessen injury in rodent studies are probably not working through edema-related changes in ICP/CPP. Nonetheless, one would expect edema to be a reasonable surrogate marker for other deleterious processes (hematoma size, blood brain barrier damage, etc.), and thus changes in edema may still be a useful measure of treatment efficacy (e.g., functional outlook) in the short and perhaps long term. However, the latter argument needs to be experimentally verified.

The higher ICP rise after a collagenase infusion likely stems from the greater amount of edema present in the collagenase model and probably not from a larger mass effect. Further work is needed to prove this, but our previous study showed less intraparenchymal blood after our moderate collagenase dose than what remained in the brain after 100 μL of blood was infused (~85 μL at 12 h post-ICH) (MacLellan et al., 2008). That study also showed greater blood brain barrier injury and considerably more tissue destruction in the collagenase model, which may account for the greater edema. Of course infusing larger volumes of blood would cause greater ICP elevation, but at least some of the additional blood would travel up the needle track. A potential solution is to use the double injection model, which may result in somewhat greater ICP. Notably, injecting 100 μL is already roughly like injecting twice the equivalent blood volume than the average ICH in patients (Volpin et al., 1984). Instead, it is perhaps easier for an investigator to use larger doses of collagenase to readily produce greater ICP elevations. Thus, by using a severe-ICH collagenase model in rats, one could study ICP/CPP therapies, such as hypothermia (Fingas et al., 2007; Kollmar et al., 2010; MacLellan et al., 2006b; Staykov et al., 2013). Expectedly, mortality may be of concern with these severe insults.

One might use such arguments to favor one model. However, we strongly encourage the use of multiple ICH models owing to other important differences and because no one knows which model(s) or endpoint(s) best predicts clinical findings (MacLellan et al., 2012). Nonetheless, we are concerned about the common use of edema as an efficacy endpoint (stated or implied) in animal studies (MacLellan et al., 2012). The current findings in rat show that even very large hematomas causing considerable edema only modestly affect ICP/CPP, which is unlikely to cause injury such as through inducing peri-hematoma ischemia. As well, a number of therapies have reduced edema without improving outcome (MacLellan et al., 2006b). Thus, it seems that one should not rely solely upon edema...
to gauge treatment efficacy. Furthermore, the inability to attenuate edema may not necessarily reflect lack of neuroprotection.

Our telemetry method, like other approaches to measuring ICP, is not perfect. First, pre-stroke ICP data were not collected because the implant would have physically blocked the collagenase or blood infusion. The delay in implanting the probe and for pressure to normalize after opening the cranium (screw holes that were later sealed) also means that we may have missed initial ICP changes. For example, we would have expected a greater, but transient spike in ICP after the 100-μl blood infusion compared to the progressive bleed caused by collagenase (MacLellan et al., 2008; Rosenberg et al., 1990). Second, we were able to obtain clear data for at least 4 days, but this is not enough to track the complete course of ICP changes in all settings or animals. Further methodological refinements or delayed probe implantation may circumvent this problem. Third, slight errors created by head orientation, movements or other forms of ‘noise’ (e.g., electrical interference) cannot be easily excluded beyond that already done by the software and the present experimental design. Note that the placement of the probe on the head (sensor at same fixed level with respect to cannula tip) minimized errors, such as would occur with rearing (Silasi et al., 2009). Regardless, these minor errors are not expected to have biased our findings (ICH vs. SHAM), which for the sham and severe collagenase rats were reproducible across four separate experiments. Our ICP readings in sham animals are also comparable to those previously found in normal rats (Mandell and Zimmermann, 1980; Silasi et al., 2009; Zwienenberg et al., 1999), but it is important to note that there is considerable variability in resting ICP among rodent studies. As well, we have previously shown that telemetry findings are comparable to a non-telemetry method in anesthetized rats (Silasi et al., 2009) (Colbourne, unpublished data), at least in our hands. Regardless, different methods (e.g., placement of lead tip with respect to the sensor) can certainly affect ICP readings thereby necessitating the use of appropriate controls, as presently done (SHAMs), and an evaluation of stroke-induced changes relative to those controls (not just using raw values). Fourth, owing to signal interference we were not able to measure ICP and BP simultaneously. It is possible then that we missed some important moment-to-moment changes in CPP.

Our experimental design also had some limitations. First, we did not assess intracranial compliance in our ICH studies as we did not want to influence edema or histological endpoints. Future studies could evaluate changes in ICP with acute injections of cerebrospinal fluid into the contralateral ventricle. We would expect to see greater increases in ICP in rats when brain compliance is compromised, perhaps such as after severe ICH. Second, our results in rat will vary somewhat from other species including humans owing to differences in amount (and placement) of injury, edema, brain compliance, and of course the measurement techniques used. Similarly, other factors, such as advanced age (Gong et al., 2004; Kirkman et al., 2011; Wasserman et al., 2008), must be considered. We used adult rats because they are most commonly used in ICH research (MacLellan et al., 2012), but further research is clearly needed. Third, although we did see that a hematoma was present in all of our ICH rats, we did not quantify the volume of blood, which would have been an interesting addition to our work. Additional studies or methods would have been required to document both edema and hematoma size in the same animals. As well, earlier survival times would be needed (e.g., 12–24 h) as hematoma size continuously declines with time (i.e., hematoma resolution) thereby confounding the assessment of hematoma size at 3 and especially our 7-day survival time. Hematoma size is a key predictor of outcome in humans, and is expected to predict both edema and ICP in the rat models. However, as discussed above, it is clearly not the only factor when comparing rodent models. Fourth, we did not assess functional recovery because it is well known that rats recover considerably in days following ICH (Hua et al., 2002; MacLellan et al., 2006a) and we wished to avoid any unnecessary stressors from handling and testing, as well as the unavoidable loss of data with rats being away from telemetry data recorders. Of many factors contributing to impairment and recovery, our data suggests that edema and raised ICP may be more important in the collagenase model we used than the standard blood infusion model, but further work is needed to prove this. Finally, we did not precisely determine the threshold for mortality in our studies as this was not our intent, nor did we have enough spontaneous mortality to precisely define that threshold. Nonetheless, based upon our limited data here and in our previous work with ischemic stroke (Silasi et al., 2009), it appears that those rats with ICP values exceeding 25 mm Hg are at significant risk of death, but of course this will vary with the timing and duration of ICP changes as well as model and animal characteristics.

In summary, the collagenase but not the blood infusion model caused a significant but modest rise in ICP lasting several days. Thus, the collagenase model may be more appropriate for studying therapies that target edema, such as hypothermia, and especially its effects on ICP/CPP in rodents. Additional research is needed to further explore the face validity of these models (e.g., aged hypertensive rats) and to test therapies that influence ICP in an effort to assess face validity that ultimately works towards improved translational success.

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