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Intracranial Pressure Monitoring In Nontraumatic Intraventricular Hemorrhage Rodent Model

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Abstract

Survivors of intraventricular hemorrhage are often left with significant long-term memory impairment; thus, research utilizing intraventricular hemorrhage animal models is essential. In this study, we sought out ways to measure intracranial pressure, mean arterial pressure, and cerebral perfusion pressure during nontraumatic intraventricular hemorrhage in rodents. The experimental design included three Sprague Dawley groups: sham, standard 200 µl intraventricular hemorrhage, and vehicle control groups. By introducing an intraparenchymal fiberoptic pressure sensor, precise intracranial pressure measurements were obtained in all groups. Cerebral perfusion pressures were calculated with the knowledge of intracranial pressure and mean arterial pressure values. As expected, the intraventricular hemorrhage and vehicle control groups both experienced a rise in the intracranial pressure and subsequent decline in cerebral perfusion pressure during intraventricular injection of autologous blood and artificial cerebrospinal fluid, respectively. The addition of an intraparenchymal fiberoptic pressure sensor is beneficial in monitoring precise intracranial pressure changes.

Introduction

Intraventricular hemorrhage (IVH), a type of intracranial hemorrhage (ICH), is a devastating disease that carries significant mortality and morbidity. IVH is characterized as the accumulation of blood products inside the intracranial ventricles. Isolated IVH in uncommon and typically occurs in adults[1]. It may be associated with hypertensive hemorrhage, ruptured intracranial aneurysm or another vascular malformation, tumors, or trauma[1]. IVH leads to secondary brain injury as well as the development of hydrocephalus[2]. Survivors of IVH are often left with significant functional, memory, and cognitive impairments following their injury. These long-term cognitive and memory deficits are reported in as high as 44% of survivors of ICH[3]. In subarachnoid hemorrhage (SAH), another type of ICH, it is well known that approximately half of the survivors will have memory deficits, and for those who have IVH in addition to SAH, outcomes tend to be significantly worse[4, 5, 6].

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Disclosures

All authors report no conflict of interest.

Underlying mechanisms of memory dysfunction following IVH remain to be elucidated. *In vivo* research utilizing nontraumatic IVH animal models with functional and memory dysfunction is essential in order to discover potential therapeutic targets for such patients. Animal models with more severe memory and functional dysfunction following IVH would be the best to study these changes. The senior author's lab has also been investigating specifically the role of high intracranial pressure (ICP) in the development of memory deficits in IVH rodent models. Hence, methods to precisely measure ICPs during IVH were important to investigate. Herein, we report on methods of precisely measuring ICPs in an IVH rodent model. Although ICP monitoring has previously been used in traumatic ICH as well as subarachnoid hemorrhage animal models, ICP monitoring in spontaneous IVH rodent models is not as commonly reported in the literature[7, 8]. Hence, the experimental design presented herein included three groups of Sprague Dawley rats: sham, standard 200 µl intraventricular hemorrhage, and vehicle control. For IVH group, an autologous intraventricular blood injection model was used. For vehicle control animals, intraventricular injection of sterile Lactated Ringer's solution was used. ICPs, mean arterial pressures (MAPs), and cerebral perfusion pressures (CPPs) were recorded intraoperatively, and results are reported herein.

Protocol

All research methods and animal care/maintenance were performed in compliance with the institutional guidelines at the University of California, Davis. The Institutional Animal Care and Use Committee (IACUC) of the University of California, Davis, approved all animal use protocols and experimental procedures (IACUC protocol #21874).

1. Animal housing

1. Obtain Sprague-Dawley rats of age 8-10 months old. Prior to any experimental procedure, house the rats in a vivarium and allow at least 1 week for general adaptation in their cages following a 12-h light/dark cycle with food and water ad libitum.

2. Anesthesia and pre-operative procedures

1. Anesthetize the rat with 4% isoflurane for 4 min. Hang the rat by its teeth in a supine position on an intubation platform, and intubate endotracheally using an endotracheal cannula and laryngoscope.
2. Place the anesthetized and intubated rat on a ventilator (2% isoflurane and O2/N2 carrier gas). The rat is adequately anesthetized if no response to a painful stimulus such as a hindleg pinch is observed.
3. Insert a rectal thermometer to continuously monitor the temperature.
4. Perform all operative procedures using sterile technique. Clip the hair on the head and the femoral region and prep the skin with Betadine prior to surgery.

5. Aspirate any accumulated respiratory secretions by temporarily removing the rat from the ventilator and aspirating the secretions with PE-50 tubing connected to a 10 mL syringe.
6. Protect the eyes with artificial eye ointment.
7. Inject local bupivacaine (~0.1 mL of 0.25% solution) into the skin and subcutaneous tissues prior to the scalp incision.

3. Surgery protocol

1. Placement of intraventricular needle and intracranial pressure (ICP) monitor
    1. Place the rat in a prone position in a stereotactic frame and ear bar the rat.
    2. Make a 1.5-cm scalp incision along the midline with a 15-blade scalpel.
    3. Apply mild pressure with gauze for hemostasis.
    4. Using a sterile cotton tip applicator, separate the periosteum from the skull until the bregma landmark is visible.
    5. Locate and mark out bregma using stereotaxis and mark out the location of two bilateral burr holes, 1.4 mm lateral and 0.9 mm posterior to the bregma.
    6. Using a hand-held drill, create these two small (up to 2 mm) cranial burr holes in the right and left hemispheres. Irrigate out any excess bone chips with sterile Lactated Ringer's solution.
    7. In the right hemisphere, position a 22-G guide cannula at the level of the burr hole to insert the 28-G needle through the cannula to the depth of the right lateral ventricle (4.6 mm in relation to the bregma) in order to create IVH.
    8. Connect the fiberoptic pressure sensor to the read-out unit. Turn on the read-out unit and ensure the units selected are in mmHg. Then prime the sensor by submerging its tip into a small beaker with Lactated Ringer's solution until the read-out unit reads out zero. Once it is zeroed in the Lactated Ringers solution, it is all set to be inserted.
    9. In the left hemisphere, gently insert the pressure sensor to 2-3 mm depth into the cortex for real-time ICP monitoring.
2. Femoral artery cannulation and insertion of mean arterial pressure (MAP) monitor
    1. After insertion of the ICP monitor, turn the lower trunk of the rat for easy access to the left thigh and groin area.
    2. After sterile preparation and local bupivacaine administration, make a 1.5 cm skin incision over the hindlimb with a 15-blade scalpel.

3. Dissect out the left femoral artery first superficially with a hemostat and then deeper layers using forceps with fine tips under a microscope. Identify the deep blue femoral vein to help locate the adjacent artery.
4. Tie off the distal femoral artery using 3-0 silk suture and place a temporary metal clip on the proximal portion of the femoral artery.
5. Have a second fiberoptic pressure sensor connected to the read-out unit already primed. Insert the pressure sensor into the polyethylene (PE-50) tubing, which is inserted into a Tuohy Borst that is then closed. Connect the Tuohy Borst to a 3-way-stopcock connected to a 1 mL syringe at one end and a 22-G needle with PE-50 tubing at the other end.
6. Under the microscope, make a 2 mm femoral arteriotomy with micro scissors and cannulate it with PE-50 tubing connected to the rest of the setup.

3. Intraventricular injection
    1. Aspirate 500 µL of blood using a 1 mL syringe and turn the 3-way-stopcock to have the pressure sensor read MAP.
    2. Prime the 28-G intraventricular needle connected to PE-50 tubing with the aspirated blood for IVH animals and Lactated Ringer's for the vehicle control animals. Then insert this needle into the guide cannula to the depth of the right lateral ventricle.
    3. Using 100 µL/min rate, inject the blood or sterile Lactated Ringer's solution (200 µL) into the right lateral ventricle by pumping the 1 mL syringe with the thumb. Prior to this and during intraventricular injection, monitor and record ICP, arterial blood pressure, and rectal temperature.
    4. Monitor and record the post-injection ICP and MAP values.
4. Closure
    1. Following completion of the intraventricular injection, withdraw the PE-50 tubing containing the pressure sensor that was inserted into the femoral artery and apply the temporary clip to the femoral artery to prevent bleeding.
    2. Tie off the proximal portion of the femoral artery using the 3-0 silk suture.
    3. Close the femoral incision in an interrupted fashion using 3-0 silk.
    4. Remove the guide cannula with the intraventricular needle and the ICP monitor.
    5. Seal the burr holes with bone wax.
    6. Close the cranial incision with 3-0 silk suture in an interrupted fashion.

7. Apply topical bupivacaine to the incision and inject 0.35 mL of carprofen (5 mg/kg) postoperatively. Do not leave animals unattended until they have regained sufficient consciousness to maintain sternal recumbency.

8. Allow rats to fully recover after surgery under supervision and return them to their home cages with free access to food and water after recovery.

4. Postoperative management

1. Check all postoperative animals daily for seven postoperative days to monitor their recovery, neurological status, behavior, weight, and incisions.

2. Administer 0.35 mL of carprofen (5 mg/kg) by subcutaneous injection at the time of surgery and on the 1st and 2nd postoperative days.

3. Remove the sutures on the 7th postoperative day in a sterile fashion.

Representative Results

Intracranial, mean arterial, and cerebral perfusion pressures

Both ICPs and MAPs were monitored intraoperatively in all animals (Figure 1). Rats were of 8-10 months old with a mean weight of 495 ± 17 g. Real-time ICP graphs were also collected (Figure 2). Excluding the sham group, ICPs increased significantly during intraventricular injection in IVH as well as vehicle control groups (Figure 3). ICPs peaked more in the IVH group (43 mmHg) compared to the vehicle control (36.5 mmHg). The ICPs then quickly decreased and normalized within five min post intraventricular injection in those animal groups. The fiberoptic sensor was successfully used to monitor ICPs and MAPs in real-time. It was observed that MAPs stayed similar throughout the procedure, whereas CPPs decreased during intraventricular injection of either blood or Lactated Ringer's solution (Figure 3).

Discussion

This study investigated mechanisms to measure ICPs, MAPs, and CPPs in a nontraumatic IVH rodent animal model. The results were recorded from the following groups: sham, VH 200 μL, and vehicle control (artificial cerebrospinal fluid intraventricular injection) animals. This experimental design was chosen to investigate how ICPs can be monitored during IVH injection as we hypothesized that the spike in ICPs may contribute to the more significant secondary brain injury and thus memory deficit in IVH animal models.Therefore, the goals of this study were to establish an IVH animal model with objective monitoring of ICPs, MAPs, and CPPs following nontraumatic IVH so that we can apply this further in future experiments that will focus on the effects of ICPs induced by IVH on subsequent memory dysfunction. This pilot study found that ICPs and MAPs can be precisely monitored using a fiberoptic pressure sensor introduced into the left lateral ventricle and femoral artery, respectively. ICPs increase significantly during intraventricular injection of blood

and artificial cerebrospinal fluid. Additionally, the corresponding CPPs decrease during the intraventricular injection.

One of the major concerns for this study was to find a way to monitor and record the very small changes in pressures (ICPs and MAPs) accurately. This was done using a fiberoptic pressure sensor. The fiberoptic sensor had to be small to accurately measure minimal changes in pressure. The fiberoptic sensor that was used is insulated in a cable sheath for its protection. The outer diameter of the sheath is 0.9 mm, and the diameter of the sensor tip itself is 420 μm. We ensured that rat ICP and MAP values could fall into the normal operating range of pressures for this sensor (−50 mmHg to +300 mmHg). Also, the precision of the fiberoptic sensor was ensured to be small, ±1 mmHg (Opsens Solutions).

Majority of current pre-clinical ICH models at this time use rodents with whole blood infusion and collagenase (injection of collagenase enzyme in order to injure the extracellular matrix resulting in IVH) models as the two most common experimental designs[9, 10]. The whole blood infusion model involves infusion of blood via a craniotomy or burr hole and has been reported not only in rodents but also in pigs and primate species.However, no animal model is perfect, and each has its own advantages and disadvantages[9, 10]. With respect to outcomes, behavior, cerebral edema, cell death, and hematoma size are some of the most common endpoints tested in ICH studies. Of the behavioral tests assessing cognitive and memory dysfunction, the majority utilize the Morris water maze test[10]. We have not found studies objectively measuring ICPs in IVH nontraumatic rodent models.

A recent review by MacLellan et al. found many key issues with pre-clinical ICH literature[9]. MacLellan et al. found that an overwhelming majority of the studies report only on positive treatment effects. Many studies with negative results are published in lower-tier journals or not published at all, contributing to not an insignificant publication bias. They also found that many studies do not describe the methodology such as randomization, age and sex of animals, among others. Lack of blinding, lack of reporting physiological variables as well as statistical power are additional weaknesses that were observed in that review. All this makes it challenging for others who attempt to replicate the experiment[10]. Additionally, some studies, such as Hatman et al., demonstrated that the learning and memory deficits tend to be acute and diminish as early as in 8 weeks following experimental ICH in animal models[11]. Hence, these short-term memory effects in animal models might not accurately reflect that long-term memory and cognitive dysfunction that happens after ICH in human subjects.

This study is not without limitations. A major limitation is the low lumber of animals. This was a pilot study, and future animal studies will contain a greater number of animals to solidify the results observed herein. Another limitation of this study is the inability to adequately monitor the MAPs for the duration of the entire surgery as the femoral artery and the Tuohy Borst system both clot off easily despite the use of low heparinized saline to flush the tubing.

In conclusion, herein, we report on methods of precisely monitoring ICPs, MAPs, and CPPs in a nontraumatic IVH rodent animal model. Studies such as this one will pave the

path towards establishing a more consistent IVH animal model and subsequently more rigorous pre-clinical research. Higher quality pre-clinical research on nontraumatic IVH animal models is critical to elucidate potential therapeutic options for IVH survivors in the future.

Acknowledgments

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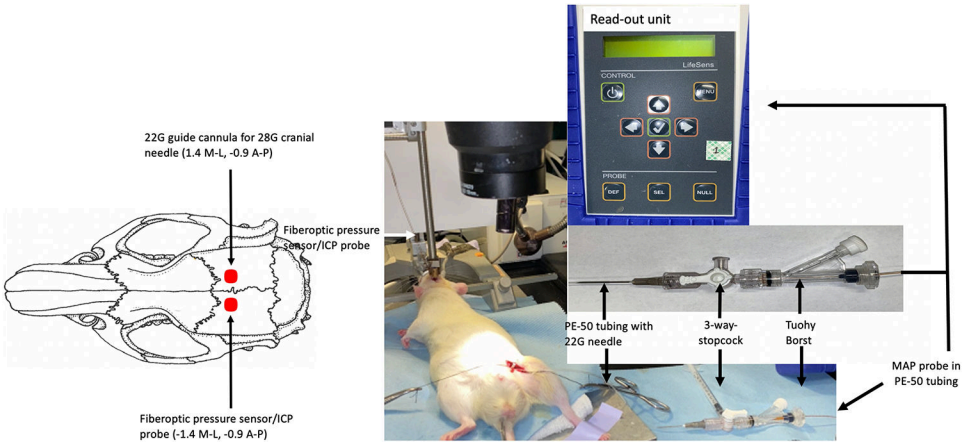

Figure 1: Experimental setup.

(**A**) Location of burr holes. (**B**) Depiction of the entire experimental setup. Abbreviations: A-P, anterior to posterior axis; M-L, medial to lateral axis.

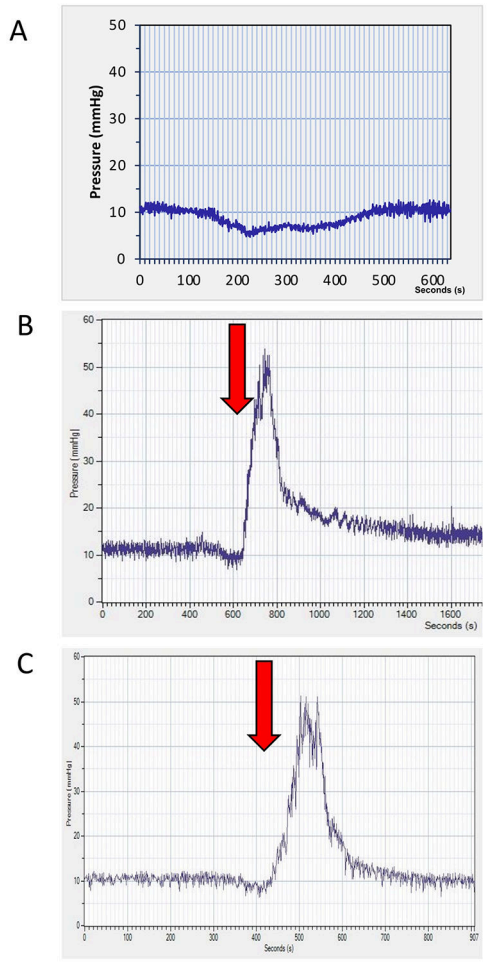

Figure 2: ICP recordings.

Real-time intracranial pressure (ICP) recordings in (**A**) sham, (**B**) IVH, and (**C**) vehicle control animals. Arrow denotes the start of the IVH/LR injection. N=1 in each group.

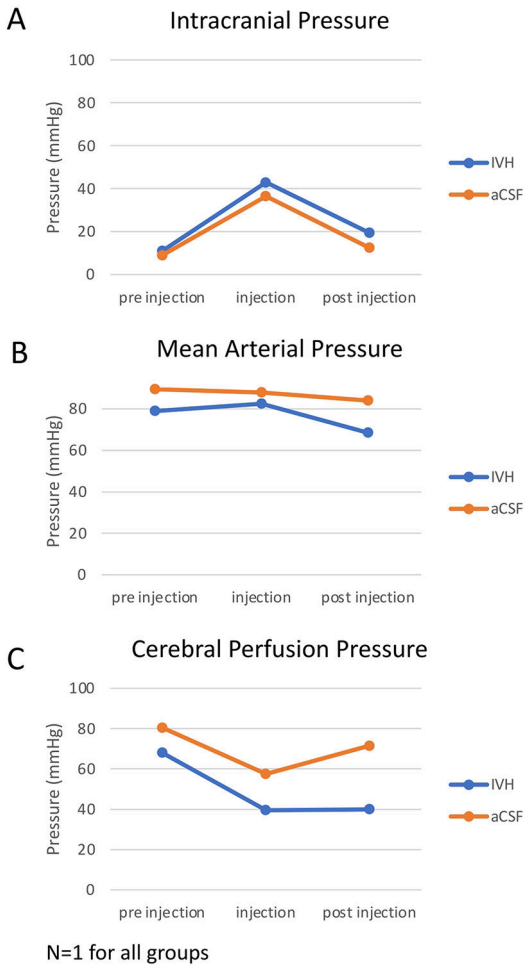

Figure 3: ICP, MAP, and CPP graphs.

(**A**) Mean intracranial pressure (ICP), (**B**) mean arterial pressure (MAP), and (**C**) mean cerebral perfusion pressure (CPP) values pre ventricular injection, during ventricular injection, and post ventricular injection in IVH and vehicle control animals. N=1 in each group.