Video Article

Autologous Blood Injection to Model Spontaneous Intracerebral Hemorrhage in Mice

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Abstract

Investigation of the pathophysiology of injury after intracerebral hemorrhage (ICH) requires a reproducible animal model. While ICH accounts for 10-15% of all strokes, there remains no specific effective therapy. The autologous blood injection model in mice involves the stereotaxic injection of arterial blood into the basal ganglia mimicking a spontaneous hypertensive hemorrhage in man. The response to hemorrhage can then be studied in vivo and the neurobehavioral deficits quantified, allowing for description of the ensuing pathology and the testing of potential therapeutic agents. The procedure described in this protocol uses the double injection technique to minimize risk of blood reflux up the needle track, no anticoagulants in the pumping system, and eliminates all dead space and expandable tubing in the system.

Video Link

The video component of this article can be found at http://www.jove.com/details.php?id=2618

Protocol

1. Preparation of equipment

1. Wipe down the stereotaxic frame and pump with 75% ethanol to minimize bacterial contamination.
2. Sterilize Hamilton syringe and fused silica needle.

   Note: If chemical sterilization is used, be sure to rinse several times in sterile water before use.
3. Wipe surface of paraffin wax paper with 75% ethanol and allow to dry.

2. Preparation of mouse for injection

Note: Have mice delivered to your animal facility at least 7 days before the surgery to allow them to acclimate to the new environment and reduce stress.

1. Weigh mouse for pre-operative baseline.
2. Induce anesthesia with 30% Oxygen, 70% Nitrous oxide, and 4% Isoflurane until unresponsive to tail pinch
3. Inject mouse with buprenorphine 0.1mg/kg intraperitoneally for post-operative analgesia
4. Shave scalp
5. Coat eyes with sterile petroleum jelly
6. Prepare scalp with betadine x 3 wipes, then allow scalp to dry
7. Make 1 cm midline sagittal incision of scalp using sterile surgical scalpel

   Note: A generous incision will allow complete exposure of skull landmarks.
8. Shave 1 cm of ventral surface of tail beginning 1 cm from base and prepare with betadine x 3 wipes
9. Place mouse on stereotaxic frame

   Note: It is important to ensure mouse is secure in the frame with surface of skull parallel with the base of the frame, with excellent exposure of bregma and at least 3 mm to the right of bregma.

3. Intracerebral hemorrhage surgery

Notes: During the entire surgery the mouse is anesthetized with 30% Oxygen, 70% Nitrous oxide, and 1-3% Isoflurane, continuously maintained at 37 ± 0.5°C using a thermistor-controlled heating pad and monitored by rectal thermometer.

1. Attach sterile 27 g needle on 1 cc syringe on frame.
2. Adjust stereotaxic arm until needle is exactly over bregma.
3. Adjust arm so that needle is at +2.5 mm lateral to bregma and lower to surface of skull.
4. Manually rotate syringe to make burr hole on surface of skull while applying gentle downward movement of frame- taking care not to completely perforate skull.
5. Remove needle and complete burr hole manually with syringe/needle

   Note: Completing the burr hole by hand allows for immediate recognition when you have perforated the inner table of skull and minimizes risk of inadvertently pushing needle into brain parenchyma.
6. Make transverse incision on ventral surface of tail using sterile surgical blade and allow 2-3 large drops of arterial blood to fall onto paraffin wax paper. Then quickly stop bleeding with pressure using sterile gauze.
7. Withdraw 17 µL blood into Hamilton syringe and place syringe on pump.
8. Adjust stereotaxic arm to point 5° medially relative to vertical axis.
9. Carefully adjust stereotaxic arm so that tip of the needle is over the burr hole in skull and then lower needle 3.5 mm.
10. Wait 2 minutes then withdraw the needle 0.5 mm (so that tip is 3 mm deep)
11. Wait 5 minutes to allow the brain to re-expand around the needle and minimize risk of blood refluxing up the needle insertion track during injection.
12. Inject blood 1 µL/minute for 7.5 µL.
13. Wait 5 minutes to allow initial blood clotting and for tissue shifts to occur to minimize elevations in intracranial pressure.
14. Inject the remaining 7.5 µL at 1 µL/minute
15. Allow needle to remain in place for 25 minutes to allow for blood clotting

    Note: Failure to wait for blood clotting will result in blood refluxing up the needle insertion site when withdrawing the needle
16. Slowly withdraw the needle and immediately rinse with hot water to prevent any residual blood in the needle from clotting and ensure reusability of needle.
17. Remove the mouse from the frame and close the tail and scalp incisions with veterinary surgical glue.
18. Turn off anesthesia.
19. Allow to mouse to awaken while being continuously warmed with free access to moistened food.
20. Return mouse to cage with littermates when fully awake. Place wet food pellet on the bottom of cage to help animals in access to food.

4. Representative results:

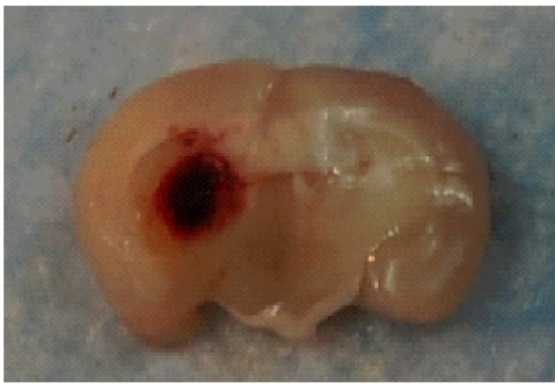

Figure 1. Coronal section of mouse brain 15 minutes after ICH surgery. Immediately after sacrifice the brain was inspected for ICH success based on gross inspection of a coronal section at the needle insertion site. Hemorrhages that tracked down to the base of the brain, up the needle track past the corpus callosum, or into the ventricles were deemed unsuccessful and that mouse was eliminated from all analyses. Overall ICH success rates were 75-85% in 50 mice with 0% mortality.

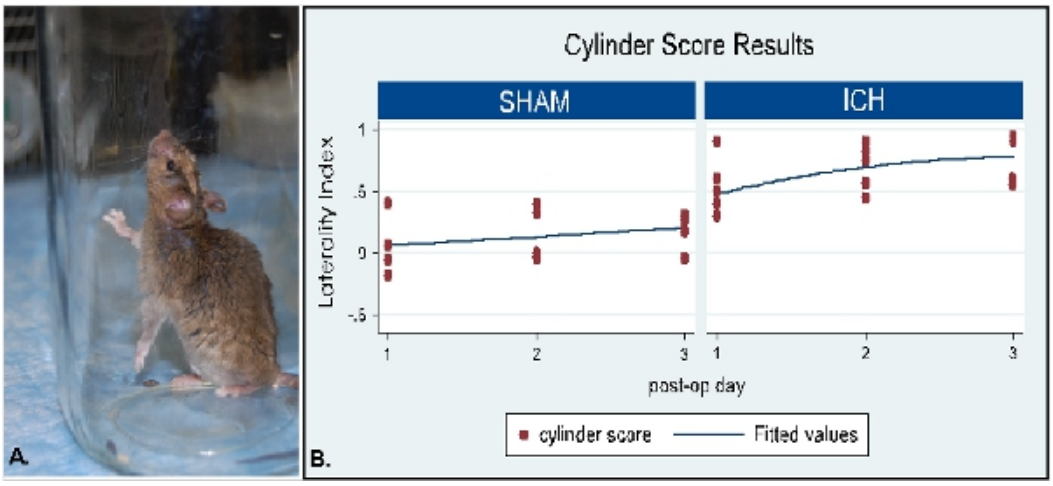

Figure 2. Cylinder testing demonstrates left hemiparesis after right basal ganglia ICH. (A) Sample mouse rear after ICH surgery. Note the placement of only the right forelimb on the wall of the cylinder after left basal ganglia ICH. (B) Graph of cylinder testing [1] results from cohort of mice after ICH surgery (n=5) compared to sham (n=4). Sham mice had all procedures except blood injection (needle was inserted into brain).

Each mouse was placed in a 12-cm diameter clear glass cylinder and observed for 20 rears. The initial placement of the forelimbs on the wall of the cylinder was scored per rear. Subsequent movements (such as lateral exploration) were not scored until the mouse returned to the ground and the next rear scored. The laterality index was calculated as (# right forelimb placements on the side of the cylinder - # left forelimb placements)/(# right + # left + # both), where 0 indicated no forelimb preference and 1 indicated only the right forelimb was used.

Discussion

This surgical model of intracerebral hemorrhage in mice using autologous tail artery blood results in a reproducible model of spontaneous basal ganglia hemorrhage. An ICH model in mice offers the advantage of the availability of transgenic animals to investigate pathophysiology; however, their small size makes neurosurgical procedures more technically difficult than in larger animals.

The collagenase model and the autologous blood injection model are two well-established models of experimental ICH. While the collagenase model offers an easier procedure and a highly reproducible hemorrhage [2], the bacterial protein used to degrade the basement membrane could potentially effect any investigation of innate inflammatory responses. In addition, collagenase-disrupted BBB could unnaturally facilitate drug access to the brain during pharmacological (e.g., neuroprotection) experiments. A warfarin-associated ICH model has also recently been developed [3], which allows investigation of hemorrhage expansion for this subset of patients. The benefits of the autologous blood injection model include presence of mechanical damage associated with mass effect, a sterile system without exogenous proteins, the ability to eliminate anticoagulation in order to investigate the natural coagulation and inflammation pathways after spontaneous hemorrhage, and exquisite control over the size of the hemorrhage. Since all mice have the same hemorrhage size, the effects of therapeutic interventions on both tissue and functional outcome can be studied with precision with relatively small sample sizes.

The surgical procedure described here is similar to other published models using autologous blood injection(4-7), and several steps in our protocol were based on these published protocols. Significant improvements in this technique include the elimination of all expandable tubing and dead space in the system, which could potentially interfere with accurate measurement of the volume of blood injected, elimination of all anticoagulants, and a moderately large hemorrhage volume compared to other models of non-anticoagulated blood. A 15 uL ICH in an average 450 uL adult mouse brain accounts for 3% of brain volume. This is roughly comparable to a 40 mL ICH in man, assuming normal average adult brain volume is 1400 mL. This ICH volume results in measurable neurological deficits that persist over two weeks for the study of recovery while maintaining zero mortality rate, which is of practical importance when using expensive transgenic animals.

Direct visualization of this surgery should eliminate common mistakes and aid in ease of replication. Hopefully this will translate to further investigation into the mechanisms of injury and accelerate the development of potential therapeutics.

Disclosures

No conflicts of interest declared.

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