Video Article

Intrastriatal Injection of Autologous Blood or Clostridial Collagenase as Murine Models of Intracerebral Hemorrhage

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URL: https://www.jove.com/video/51439
DOI: doi:10.3791/51439

Keywords: Medicine, Issue 89, intracerebral hemorrhage, mouse, preclinical, autologous blood, collagenase, neuroscience, stroke, brain injury, basal ganglia

Date Published: 7/3/2014

Citation: Lei, B., Sheng, H., Wang, H., Lascola, C.D., Warner, D.S., Laskowitz, D.T., James, M.L. Intrastriatal Injection of Autologous Blood or Clostridial Collagenase as Murine Models of Intracerebral Hemorrhage. *J. Vis. Exp.* (89), e51439, doi:10.3791/51439 (2014).

Abstract

Intracerebral hemorrhage (ICH) is a common form of cerebrovascular disease and is associated with significant morbidity and mortality. Lack of effective treatment and failure of large clinical trials aimed at hemostasis and clot removal demonstrate the need for further mechanism-driven investigation of ICH. This research may be performed through the framework provided by preclinical models. Two murine models in popular use include intrastriatal (basal ganglia) injection of either autologous whole blood or clostridial collagenase. Since, each model represents distinctly different pathophysiological features related to ICH, use of a particular model may be selected based on what aspect of the disease is to be studied. For example, autologous blood injection most accurately represents the brain's response to the presence of intraparenchymal blood, and may most closely replicate lobar hemorrhage. Clostridial collagenase injection most accurately represents the small vessel rupture and hematoma evolution characteristic of deep hemorrhages. Thus, each model results in different hematoma formation, neuroinflammatory response, cerebral edema development, and neurobehavioral outcomes. Robustness of a purported therapeutic intervention can be best assessed using both models. In this protocol, induction of ICH using both models, immediate post-operative demonstration of injury, and early post-operative care techniques are demonstrated. Both models result in reproducible injuries, hematoma volumes, and neurobehavioral deficits. Because of the heterogeneity of human ICH, multiple preclinical models are needed to thoroughly explore pathophysiologic mechanisms and test potential therapeutic strategies.

Video Link

The video component of this article can be found at https://www.jove.com/video/51439/

Introduction

Intracerebral hemorrhage (ICH) is a relatively common form of cerebrovascular disease with approximately 40–50% of afflicted patients dying within 30 days [1]. Unfortunately, little improvement has been made in the mortality rate over the last 20 years [2]. Reports from the National Institutes of Health [3] and guidelines from the American Heart Association [4] stressed the importance of developing clinically relevant models of ICH to extend the understanding of pathophysiology and develop targets for new therapeutic approaches.

Several models exist to mimic human ICH [5]. As understanding of ICH pathophysiology matures, it has become evident that a variety of models may be used to examine different aspects of the disease. Previously used models include murine amyloid angiopathy [6], intraparenchymal microballoon insertion and inflation [7], and direct arterial blood infiltration [8,9]. Lobar hemorrhage from amyloid angiopathy has been modeled with the use of transgenic mice and represents a distinct ICH subtype. Microballoon models mimic acute mass effect from hematoma formation but fail to capture the brain's cellular response to the presence of blood. Finally, direct arterial blood infiltration subjects the brain to arterial pressures from the femoral artery. Thus, this model mimics arterial pressures and the presence of blood but does not subject the brain to microvascular injury from small blood vessel rupture. Further, this model has inherently high variability. Interestingly, spontaneously hypertensive rats [10] develop spontaneous ICH as they age. Study of these animals after ICH development may mimic the disease in the presence of one of the major comorbidities predisposing humans to ICH. While these other models exist, intrastriatal injection of Clostridial collagenase [11] or instrastiatal injection of autologous whole blood [12] are, currently, the two most common models used in preclinical ICH research.

ICH model selection should be made based on the objective of the experimental question, including species selection and method of inducing hematoma formation. For instance, pigs are large animals with relatively large white matter brain volumes compared to mice. Thus, porcine models are suited to study white matter pathophysiology following ICH. In contrast, rodent brains are largely gray matter, but transgenic systems make rodents useful to assess molecular mechanisms of injury and recovery after ICH. Each model has its inherent strengths and weaknesses (**Table 1**), which should be carefully considered prior to experimentation.

The following protocols demonstrate the autologous blood and collagenase injection models in mice. These models have each been translated from models originally developed in rats [13,14] and allow the use of widely available transgenic technology to explore molecular mechanisms associated with cell death after ICH. Both represent distinctly different injury mechanisms from human ICH, and both have distinctly different expected outcome in terms of behavioral and histological measures. Thus, certain hypotheses may lend themselves to one model over the other, but many ideas may require validation in both models.

Table 1. Comparison of characteristics of collagenase- and autologous blood injection intracerebral hemorrhage models.

	Collagenase Injection	Blood Injection
Ease of Use	+++	++
Reproducibility	++	++
Control of Hemorrhage Size	++	+++
Blood Reflux	+	++
Simulates Human Disease	+	-
Simplicity	++	++
Use in Multiple Species	++	++

Protocol

Ethics Statement: This protocol has been approved by the Duke University Institutional Animal Care and Use Committee and follows all the guidelines for the ethical use of animals.

1. Preparation of Equipment

1. Autoclave the surgical tools prior to surgery.
2. Disinfect the stereotactic apparatus with 70% ethanol.
3. Turn on water bath and keep water temperature at 42 °C.
4. Dissolve Type IV-S clostridial collagenase in normal saline at a concentration of 0.075 U per 0.4 µl.

2. Collagenase Injection Model

1. Weigh the mouse.
2. Anesthetize the mouse in an induction chamber with 5% isoflurane in 30% O_2/70% N_2. Adequate anesthesia is signaled after approximately 2 min when mouse respirations have slowed to 1 per second.
3. Intubate the trachea with a 30 mm 20 G intravenous catheter.
4. Connect the catheter to a rodent ventilator and mechanically ventilate the lungs with 1.6% isoflurane in 30% O_2/70% N_2 at a rate of 105 breaths per minute with a delivered tidal volume of 0.75 ml.
5. Shave the scalp with an electronic shaver. Once the mouse is anesthetized and intubated, move it to a different work station for shaving and then returned to the surgical bench.
6. Secure the head in a stereotactic frame, and level the head with both coronal and sagittal suture as reference points.
7. Apply ophthalmic ointment to eyes.
8. Insert a rectal temperature probe. Maintain rectal temperature at 37.0 ± 0.2 °C using an underbody circulating waterbed.
9. Wipe the surgical area with betadine followed with 70% ethanol and repeat 3 times.
10. Make a 1 cm midline scalp incision and wipe periosteum laterally with a sterile cotton-tipped applicator to expose bregma.
11. Drill 1 mm diameter burr hole 2.2 mm left lateral to bregma with a water-cooled drill.
12. Rotate collagenase vial 5 times, then wash a 0.5 µl syringe with 25 G needle (attached to stereotactic frame) with 0.5 µl collagenase solution 5 times (Leave 0.5 µl of collagenase solution in syringe after last wash).
13. Align needle tip with burr hole then expel 0.1 µl from syringe and wipe needle bevel with razor to discard.
14. Using a micromanipulator, advance the needle 3 mm deep to cortex and leave motionless for 30 sec.
15. Inject 0.4 µl over 90 sec.
16. Decrease isoflurane to 1% and leave needle motionless for 5 min.
17. Withdraw needle slowly.
18. Apply 1 - 2 drops of 0.25% bupivacaine subcutaneously and suture the skin.
19. Turn off isoflurane vaporizer and remove mouse from the stereotactic frame.
20. Allow mouse to recover spontaneous ventilation with subsequent tracheal extubation.
21. Return mouse to a clean cage and allow free access to food and water.

3. Autologous Blood Injection Model

1. Follow the steps 2.1 - 2.11 for the collagenase injection model.
2. Draw 50 µl of sterile normal saline into a 30 G 50 µl syringe.
3. Connect the microliter syringe with a 70 cm PE10 tube.
4. Expel all the normal saline from microliter syringe into PE10 tube to completely de-air tubing.

5. Pull the microliter syringe piston out 1 mm to make an air bubble at the distal opening of the PE10 tube-microliter syringe apparatus to avoid mixture of saline and blood during later procedures.
6. Wipe the distal central tail artery region of the mouse with 70% ethanol, and cut the artery with a razor at 0.5 to 1 cm to the tail tip.
7. Collect 40 µl of blood from the tail cut into the PE10 tube-microliter syringe apparatus. Note: that heparin is not used in the needle, tubing, or mouse.
8. Attach the microliter syringe onto the injection pump.
9. Connect the metal cannula portion of a 27 G needle to the end of the PE10 tube, and secure the needle to a micromanipulator on the stereotactic frame.
10. Expel 2 µl of blood out of 27 G needle and wipe needle bevel with razor to discard.
11. Align needle tip with burr hole and insert needle 3 mm deep to cortex.
12. Inject 35 µl of autologous blood at a rate of 2 µl per min.
13. Decrease isoflurane to 1% and leave needle motionless for 10 min.
14. Withdraw needle over 30 sec.
15. Apply 1 - 2 drops of 0.25% bupivacaine subcutaneously and suture the skin.
16. Turn off isoflurane vaporizer and remove mouse from the stereotactic frame.
17. Allow mouse to recover spontaneous ventilation with subsequent extubation.
18. Return mouse to a clean cage and allow free access to food and water.

4. Sham Operation

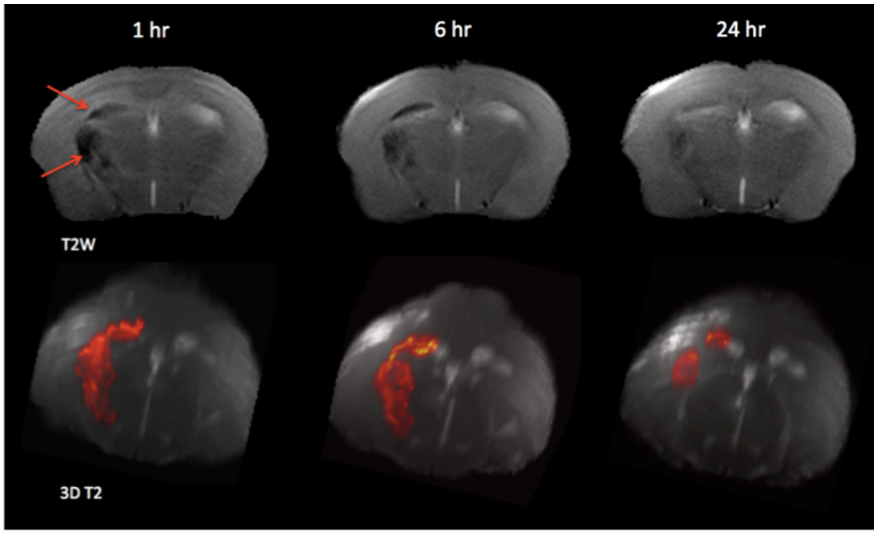
1. Follow the same procedures for collagenase injection model, except without injection after needle insertion.

5. Post-surgery Care

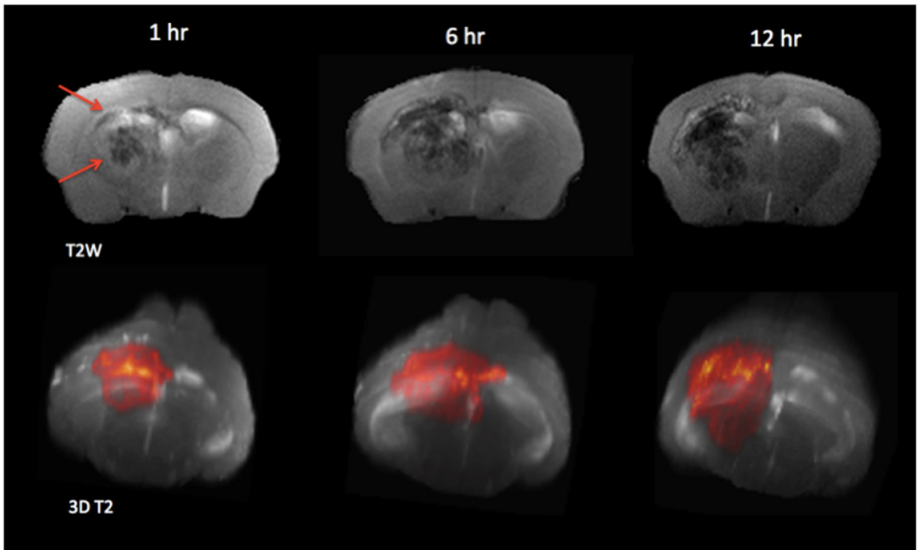
1. Inject 0.5 ml of normal saline subcutaneously in the evening of the surgical procedure at the back of the animal's neck.
2. Provide softened food with water and gel food in small plastic cups placed on the floor of the cage. Replace the food daily for 7 days.
3. Check for weight loss, wound healing, and signs of discomfort daily for 7 days.
4. If recovery intervals of greater than 7 days are required, suture removal may be performed under light inhaled anesthesia (approximately 1% isoflurane in 30% O_2/70% N_2), if necessary.

Representative Results

Due to differences in hematoma formation (**Figure 1**), ipsilateral turning is shown immediately after wake up for autologous blood injected mice and within 2 - 4 hr after collagenase injection, as hematoma expansion occurs (**Figure 2**). Absence of ipsilateral turning should raise concern for absence of significant injury. On the first post injury day, mice in both models should demonstrate significant neurological deficits (**Figure 3**). At 24 hr after injection, ipsilateral hemispheres show stable hematoma volumes (**Figure 4**); further, at 24 hours after injection, brain water content should be expected to be 79.8 + 0.34% in collagenase-injected mice and 79.3 + 0.23% in autologous blood-injected mice. Mortality should be expected to occur between 10 - 25% of collagenase-injected mice and less than 10% of autologous blood-injected mice. Unavoidable death due to hematoma volume, cerebral edema, and increased intracranial pressure usually occurs within the first 24 - 48 hr after intrastriatal injection. Death occurring after 72 hr may often be avoided with proper post-injury care (*e.g.*, ready access to softened food and water). Functional recovery generally begins by post injury day 2 with autologous blood-injected mice recovering significantly faster than collagenase-injected mice.



A.



B.

Figure 1. Serial magnetic resonance imaging of mouse brains comparing autologous blood and collagenase injection models of intracerebral hemorrhage. After intracerebral hemorrhage induction via left intrastriatal injection of 35 µl autologous blood **(A)** or 0.075 U Type IV-S clostridial collagenase **(B)** in 10 - 12 week old C57/Bl6 male mice, serial magnetic resonance imaging demonstrates hematoma expansion in the collagenase-injected mice compared to stable hematoma formation in the autologous blood-injected mice. Hematoma volumes are 10.1, 23.1, 29.9 mm^3 at 1, 6, & 12 hr after collagenase injection, respectively, and 7.0, 5.8, 3.2 mm^3 at 1, 6, and 24 hr after whole blood injection, respectively. Please click here to view a larger version of this figure.

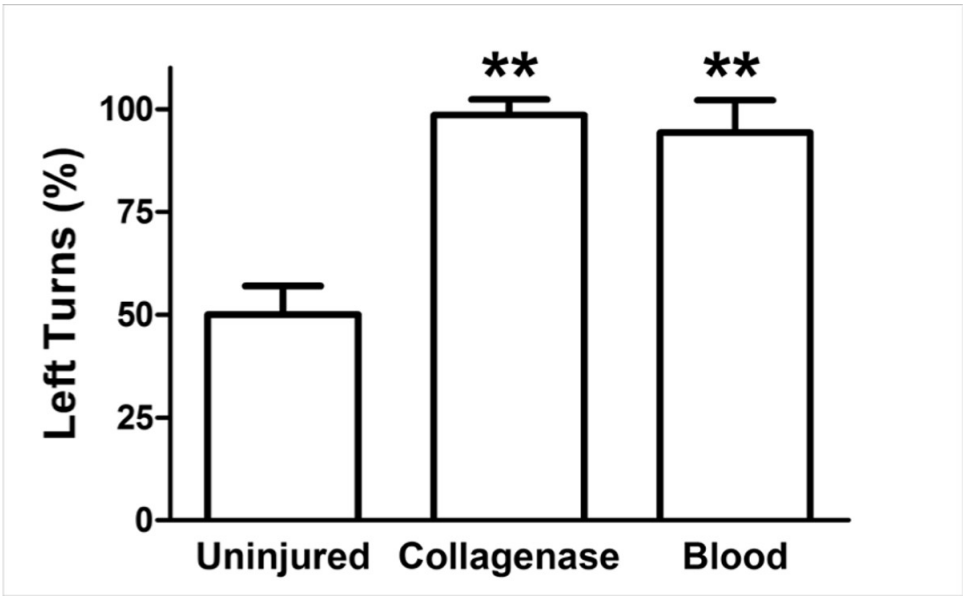


Figure 2. Corner turn test in mice 24 hr after intracerebral hemorrhage. Taken immediately after intrastriatal collagenase injection into the left basal ganglia, presence of expected ipsilateral turning response in 10 - 12 week old C57/Bl6 male mice denotes adequate injury. This turning should occur immediately after significant injury in mice injected with autologous blood and within 2 - 4 hr in collagenase-injected mice. Mice in both models showed more left turns after injury compared to uninjured mice (**p <0.01; one-way ANOVA with post-hoc Scheffe's test; n = 10/group).

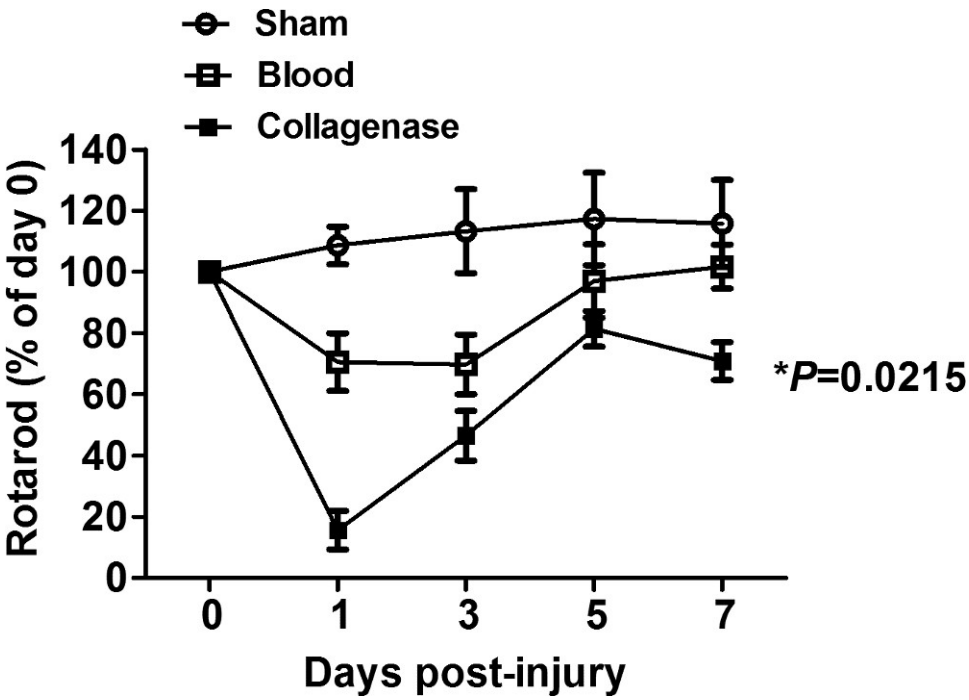


Figure 3. Rotarod performance after intracerebral hemorrhage in mice. Baseline and post injury rotorod latencies of 10 - 12 week old C57/Bl6 male mice for one week after left intrastriatal 35 µl autologous blood-, 0.075 U Type IV-S clostridial collagenase-injection, or sham operation (*p = 0.022; repeated measures ANOVA with post-hoc Scheffe's test; F-value = 12.726; n = 10/group). Mice are assessed via rotorod testing every other day after injury to avoid significant training bias.

Figure 4. Hematoxylin and eosin stains of mouse brain after intracerebral hemorrhage. Photomicrographs of 10 - 12 week old C57/Bl6 male mouse brains at 24 hr after left intrastriatal injection of 35 µl autologous blood (right) or 0.075 U Type IV-S Clostridial collagenase (left). Hematoma volumes are 20.2 mm^3 after collagenase injection and 6.4 mm^3 after whole blood injection. Please click here to view a larger version of this figure.

Discussion

Despite emerging preclinical research and resultant large clinical trials for promising therapeutics [15-18], there are no pharmacological interventions demonstrated to improve outcome in ICH, and care remains largely supportive. Lists of possible therapies may be generated by high throughput technologies, such as transcriptomic and proteomic work. While these technologies continue to advance our knowledge of potential therapeutic targets, forward and backward translation of promising targets may be best examined through use of clinically relevant preclinical models [19-22]. Such models are useful because they allow rapid throughput of selected candidates, examination of mechanisms *in vivo*, inexpensive investigation of dosing, therapeutic window, and other parameters germane to developing clinical trials [23-25]. While obvious advantages exist in using preclinical models, modeling should occur in the most clinically relevant but logistically feasible system available. While models exist for 'higher' order animals such as primates, use of mice to model human disease provides an inexpensive, high-throughput, and powerful technology to examining pathologic mechanisms and therapeutic effects. Incorporating transgenic systems allows for an even more robust evaluation of mechanistic pathways and cell populations involved.

Currently two murine models are in common use: intrastriatal autologous blood or collagenase injection. Both models are versatile and easy to use, relative to other stroke models. Both models can induce ICH in different brain areas [26], allowing evaluation of regional responses; hematoma volume can be controlled and changed, allowing for evaluation of mild, moderate, and severe injury; and clinically relevant physiology (*e.g.*, blood pressure, temperature, *etc.*) can be controlled. Finally, while each model was originally developed in the rat, both have since been translated into mice to allow the use of transgenic systems [21,24,25,27]. However, each model lends itself to the study of different aspects of ICH, as each represents distinctly different components of ICH. Autologous blood-injection may recreate the brain's response to intraparenchymal blood exposure. Thus, initial mass affect and shear forces, mild inflammatory changes, apoptosis, and blood resorption may all be studied [10,28]. Further, recent modifications to this model have resulted in the ability to mimic hematoma expansion [29,30]. However, this model does not invoke the component of vascular injury and/or hematoma expansion found in the human disease. In contrast, collagenase-injection adds the elements of vascular rupture, early hematoma expansion, and enhanced neuroinflammatory effect. While obvious concerns exist about artifactual contribution of collagenase to this inflammatory effect, there is a lack of hard evidence for this [31], and our own (unpublished) data suggest that collagenase in isolation does not induce a marked inflammatory response in cell culture.

From a procedural standpoint, both models require limited skill with microsurgery and, thus, are easily learned so as to obtain reproducible effects. Pitfalls to be avoided include: 1) invasion of the dura or creating thermal brain injury when drilling, 2) or penetration of the ventricular system with needle insertion. Dural injury allows for reflux of injectant, and intraventricular injection results in little to no intraparenchymal hematoma formation. Further, care must be taken upon needle withdrawal to not disrupt newly formed/forming hematoma. Mortality is to be expected in a certain percentage of mice but is directly related to hematoma size and degree of injury desired; thus, this outcome may be titrated by injectant volume/concentration.

As with all models, protocols will be optimized for use by specific operators. Due to inherent variability in all *in vivo* systems, experience with a particular model as a key factor for success cannot be overstated. A model's distinctive characteristics, operator experience with a given model, outcome metrics of interest, and logistical factors must all be taken into account when selecting the best possible experimental model.

Disclosures

Lei, Sheng, Wang, Lascola, Warner, and Laskowitz have no conflicts of interest to declare. James received grant funding by American Heart Association, National Institutes of Health, and Cephalogics.

Acknowledgements

This work was supported by the American Heart Association Scientist Development Grant and the Foundation for Anesthesia Education and Research (MLJ). We would like to thank Talaignair N. Venkatraman PhD for his assistance with magnetic resonance imaging.

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