Published in final edited form as:
Acta Neurochir Suppl. 2011 ; 111: 3–8. doi:10.1007/978-3-7091-0693-8_1.

History of Preclinical Models of Intracerebral Hemorrhage

Qingyi Ma,
Department of Physiology and Pharmacology, Loma Linda University, School of Medicine, Risley Hall, Room 219, Loma Linda, CA 92354, USA

Nikan Khatibi,
Department of Anesthesiology, Loma Linda University, School of Medicine, Loma Linda, CA, USA

Hank Chen,
Department of Physiology and Pharmacology, Loma Linda University, School of Medicine, Risley Hall, Room 219, Loma Linda, CA 92354, USA

Jiping Tang, and
Department of Physiology and Pharmacology, Loma Linda University, School of Medicine, Risley Hall, Room 219, Loma Linda, CA 92354, USA

John H. Zhang
Department of Physiology and Pharmacology, Loma Linda University, School of Medicine, Risley Hall, Room 219, Loma Linda, CA 92354, USA and Department of Anesthesiology, Loma Linda University, School of Medicine, Loma Linda, CA, USA and Department of Neurosurgery, Loma Linda University, School of Medicine, Loma Linda, CA, USA

Abstract

In order to understand a disease process, effective modeling is required that can assist scientists in understanding the pathophysiological processes that take place. Intracerebral hemorrhage (ICH), a devastating disease representing 15% of all stroke cases, is just one example of how scientists have developed models that can effectively mimic human clinical scenarios. Currently there are three models of hematoma injections that are being used to induce an ICH in subjects. They include the microballoon model introduced in 1987 by Dr. David Mendelow, the bacterial collagenase injection model introduced in 1990 by Dr. Gary Rosenberg, and the autologous blood injection model introduced by Dr. Guo-Yuan Yang in 1994. These models have been applied on various animal models beginning in 1963 with canines, followed by rats and rabbits in 1982, pigs in 1996, and mice just recently in 2003. In this review, we will explore in detail the various injection models and animal subjects that have been used to study the ICH process while comparing and analyzing the benefits and disadvantages of each.

Keywords

ICH; Animals; Microballoon; Collagenase

Correspondence to: Jiping Tang.
jtang@llu.edu.

Conflict of interest statement We declare that we have no conflict of interest.

Introduction

Intracerebral hemorrhage (ICH) is a devastating disease accounting for roughly 15% of all stroke types. As many as 50,000 individuals are affected annually in the United States, with a large number of those individuals facing chronic morbidities and early mortalities.

Over the years, basic science research has focused on reducing and/or blocking the cascade of harmful events in ICH with the goal of improving clinical outcomes. But in order to effectively study the mechanisms behind these events, proper modeling is needed that can mimic pathophysiologic processes in humans. Studies on various animal models began in 1963 with canines, followed by rats and rabbits in 1982, pigs in 1996, and mice just recently in 2003. These animal subjects have been thoroughly studied individually and compared to human models looking for a parallel between the two groups.

As important as it is to find an animal subject that will mimic processes in the human brain, creating the actual hematoma is another challenge. Currently there are three models of injections that are being used to induce an ICH in subjects. They include the microballoon model introduced in 1987 by Dr. David Mendelow, the bacterial collagenase injection model introduced in 1990 by Dr. Gary Rosenberg, and the autologous blood injection model introduced by Dr. Guo-Yuan Yang in 1994 [1-3].

In this retrospective review, the history behind the development of the ICH model will be presented and discussed. Furthermore, the advantages and disadvantages of each model type and animal subject will be evaluated.

ICH Models

Microballoon Model

In 1987, Sinar et al. [2] made a microballoon insertion model in rats as a way to study the mass effects of ICH. A microballoon mounted on a no. 25 blunted needle was inserted into the right caudate nucleus after a burr hole had been created on the skull. The microballoon was inflated to 0.05 mL over a period of 20 s and was kept inflated for 10 min before being deflated. At the end of the study, the authors looked at brain histology, intracranial pressure, and cerebral blood flow. They found the microballoon model to be successful in producing an effective brain lesion with an extensive area of ischemic damage noted on the right caudate nucleus. Additionally, there was a reduction in cerebral blood flow and an increase in intracranial pressure at the site of damage.

The advantage of the microballoon model, according to Rojas et al. [4], is that it mimics the space-occupying aspect of the hematoma. The disadvantage is it fails to address the potential effects of blood and subsequent substances released by the clot formation. This could potentially be the reason why there is a smaller degree of ischemia in this model versus what would be expected with an equivalent volume of blood [5, 6].

Collagenase Injection Model

Collagenases are proteolytic enzymes that degrade the basement membrane and interstitial collagen [7]. Additionally, they have been shown through immunocytochemical studies to surround blood vessels [8]. As a result, in 1990, Rosenberg et al. made a new model for spontaneous ICH using bacterial collagenase injections directly in the brains of Sprague-Dawley rats [1]. In this model, male rats were placed in a stereotactic apparatus, and 2 μl of saline containing 0.01–0.1U bacterial collagenase (type XI or VII) was infused into the left caudate nucleus over 9 min. Bleeding occurred as early as 10 min after collagenase injection, with edema also seen at the site of hemorrhage [1]. Other modifications to this

model were made, including: injection site changes, adjustments to collagenase concentration, injection rates/volumes, and heparinization. This model conceptually integrates small vessel breakdown to produce hemorrhage and allows a controllable amount of variability in hemorrhage size [9]. The advantage of this model is its ability to mimic spontaneous intraparenchymal bleeding in humans while avoiding the technical difficulties with handling blood [10]. It also mimics the hematoma expansion of continuous bleeding that occurs naturally in ICH patients [11, 12]. The disadvantages of this model are related to bacterial collagenase's ability to introduce a significant inflammatory reaction [10].

Blood Injection Model

The blood injection ICH model has become the standard for experimental ICH. The first recorded publication using arterial blood as a single injectable agent was conducted by Ropper et al. in 1982 [13]. Using a 27-gauge cannula, fresh blood from the ventricle of a donor rat was infused over 1 s into the right caudate nucleus of the subject ICH rat. This method did not account for key sources of variability. Hence, in 1984, a variation of Ropper's model was performed by Bullock et al. [14] to study the changes in intracranial pressure and cerebral blood flow. Instead of using donor blood, Bullock used a 22-gauge needle that bridged the right caudate nucleus to the femoral artery. For the first time, the study was able to look at ICH under arterial pressure – thus effectively evaluating the pathophysiology of ICH. One of the main disadvantages of this method was the lack of reproducibility because of the potential variations in blood pressure.

This is why in 1994 a study at the University of Michigan by Yang et al. [3], discovered that the use of a microinfusion pump could address the concerning issues that had confronted previous authors. Using a microinfusion pump, a constant rate of autologous blood (extracted from the femoral artery) was infused into the right caudate nucleus, creating a controllable and reproducible hematoma. This single blood injection model has been applied to most of the recent ICH studies. Unfortunately, one of the major issues with Yang's technique was the reflux of blood up the needle tract and into the ventricular system or extension into the subdural space with a more rapid injection rate. Additionally, the inability of this technique to reproduce the systemic arterial pressure that can influence the hematoma size is also seen as a slight disadvantage. Finally, the use of the femoral artery created problems down the road when it came time to assess neurobehavioral deficits.

To address the concern of blood reflux up the needle tract, a double injection model was created just 2 years later. In 1996, a study led by Deinsberger et al. [15] at the Justus Liebig University in Germany modified the single arterial blood injection model originally designed by Yang and instead used a double arterial blood injection model. What Deinsberger and his team proposed was injecting 5 μl of fresh autologous blood into the caudate nucleus and waiting 10 min to allow for clot formation. This way, when it was time to inject the rest of the autologous blood to mimic the hematoma, the chances of reflux would be minimized significantly. This was the main advantage of the double blood injection model over the single injection model – it minimized blood reflux through the needle tract. The disadvantages, however, were the obvious difficulties with infusion and increased potential of clot formation because of the time lag between injections.

Large Animal Models

Monkeys

Chimpanzees and rhesus monkeys share over 90% of their DNA with humans in addition to physiologic, structural and size similarities, making them ideal candidates for preclinical study. In 1982, Segal et al. [16] used macaque monkeys to demonstrate the effects of local therapy on hematoma formation using the thrombolytic urokinase. Their treatment was

given after injection of 6 mL autologous blood into the right internal capsule. Additionally, in 1988, Bullock et al. [17] used adult Vervet monkeys to demonstrate and quantify a 90–120 min decrease in the regional cerebral blood flow (rCBF) following an ICH. A key refinement to the primate model made by Bullock was the use of a catheter that infused blood directly from the femoral artery into the right caudate, thereby keeping the infusion pressure closer to arterial pressure and reducing complications from blood handling and delay.

The experiments using monkeys were costly, and the various levels of restrictions and regulations were concerning [4]. Hence, their use in ICH modeling was quickly discontinued.

Canines

Like monkeys, canines have long been the subject of medical research across the same range of fields, most notably having contributed to cardiovascular physiology [18]. But like monkeys, use of canines in research involves similarly stringent criteria and cost. In 1963, Whisnant et al. [19] developed a model for experimental ICH by performing single injections of 0.5-1.5 mL of fresh autologous venous blood into the basal nuclei or deep white matter region in canines – producing varying sizes of ICH. In 1975, Sugi et al. [20] used canines to develop a single autologous arterial blood injection model, noting lactate elevations in CSF after injection. In 1999, Qureshi et al. [21] made single autologous blood injections (7.5 mL) over 20–30 min under arterial pressure into the deep white matter adjacent to the basal ganglia of canines. The needle was pointed 20° lateral to the vertical axis. The complications encountered in this study were the increased frequency of transtentorial herniation. Hence, they then used smaller injection volumes ranging from 2.8 to 5.5 ml, which successfully induced formation of ICH with fewer complications [22]. In 1999, Lee et al. [23] made use of an infusion pump for injection of 3–5 mL of non-heparinized autologous arterial blood into the temporo-parietal cortex. This method took 8 min in canine subjects and allowed for the formation of consistently sized clots.

In 1985, the microballoon method was used by Takasugi et al. [24] who modified this method by injecting venous blood directly into the balloon as an attempt to minimize reflux. This model mimicked both the increased pressure and blood volume following ICH. Using this model, Takasugi was able to classify the chronological stages after ICH and concluded that the increased repair time after ICH was correlated to the degree of histologic injury to the surrounding tissue rather than to the size of the hematoma itself.

Pigs

Known for their large, gyrated brain and well-developed white matter, the large hematoma volume in pigs post-ICH enables a closer examination of the area compared to other animal species [25]. In 1996, Wagner et al. [25] developed a lobar hemorrhage model in pigs where 1.7 mL of autologous arterial blood was slowly injected using an infusion pump into the frontal white matter. The slow injection reduced the likelihood of ventricular rupture or leakage of blood along the needle track. Compared with rapid infusions at high pressures, this method more closely modeled ICH in humans where bleeding generally originates from small intraparenchymal arteries. In 2000, Kuker et al. [26] injected 0.5–2.0 ml of venous blood with a blood reservoir into the anterior frontal lobe to study the characteristics of hematomas using magnetic resonance imaging. This study used Takasugi's method of prior microballoon catheter insertion to reduce needle pathway reflux. A different study in 2002 led by Rohde et al. [32] modified this model into a double-injection procedure (with a main injection of 2–3 ml of autologous venous blood with blood reservoir in their study) to better prevent post-injection reflux.

Pigs were also used in collagenase injection models. Collagenase infusions of 10 μl by micro-infusion pump over 20–30 min were made into the right somatosensory cortex by Mun-Bryce et al. [27] in 2001. This study examined tissue excitability following ICH and evaluated the outcomes using magnetic resonance imaging in addition to electro- and magneto-encephalography. Use of collagenase, which is released from injured cells [5], does address the clinically relevant phenomenon of vasogenic edema following ICH. The levels of collagenase, however, are far above those encountered in clinical ICH and therefore correlations must take this into account.

Small Animal Models

Rabbits

Rabbits were first used by Kaufman et al. in 1985 [28] in a single autologous blood injection model. The study failed to yield conclusive results, and in fact, the rabbit died shortly after injection. A decade later, arterial blood was injected using an infusion pump by Koeppen et al. [29]. Arterial blood extracted from the ear was injected into the right thalamus and, to minimize reflux, needle withdrawal was delayed. The study found that subjects exhibited a reduction in neurobehavioral deficits.

Compared to larger animal models, use of rabbits is less costly, meets a higher success rate, and allows for an extended period of study with less mortality. Qureshi et al. in 2001[30] modified this model in order to look at patterns of cellular injury. In this model, a 30-gauge needle penetrated the brain, while autologous arterial blood was infused into the white matter of the left frontal lobe. Instead of using arterial blood, Gustafsson et al. in 1999 [31] used autologous venous blood that was injected manually in the brain. Although a hematoma did form, the use of venous blood differs from what is seen in humans.

Rats

The earliest rat model using a single arterial blood injection method was conducted in 1982 by Ropper et al. [13]. The study reported that blood, not the mass effect as was previous postulated, was responsible for the changes in regional cerebral blood flow. Unfortunately, because of the nature of the design, certain outcomes could not be evaluated – the disadvantage of using donor blood introduces various immune reactions, while the lack of arterial pressure fails to mimic a true human ICH experience. Additionally, variability in outcomes was an issue because of the potential for reflux up the needle tract and the potential for blood volume discrepancies. Several of these issues were addressed later by Bullock et al. in 1984 [14]. For instance, blood infusion was conducted more rapidly under arterial pressure and in a smaller time window (10 s); however, it was difficult to reproduce reliably. The blood pressure variations from animal to animal resulted in different injury volumes, and the small time window required significant technical mastery. This was followed by development of a method that instead held the rate of infusion constant using microinfusion pumps [6]. The use of microinfusion pumps allowed production of a controllable and reproducible lesion with a slower injection rate and a lower pressure than in an arterial pressure model (100 mmHg). A remaining shortcoming was that a more rapid injection rate resulted in a variable reflux of blood along the needle track and poorly reproducible lesions.

To address the issue of needle tract reflux, the double injection method was developed by Deinsberger et al. in 1996 [15]. In this model, a smaller volume of blood was first infused, allowing for clot formation and a reduction in blood reflux. While technically challenging, this technique met with great success in reproducibility and minimization of pathway reflux. The use of venous blood in the single blood injection model was addressed by Masuda et al. in 1988 [33]. While they described a significant success rate in the production of

intraparenchymal hematomas, the use of venous blood did not faithfully replicate the conditions in the major form of clinical ICH, which involves rupture of an arterial vessel.

In 1990, Rosenberg et al. established a new model for spontaneous ICH using bacterial collagenase infused directly into the brain in Sprague-Dawley rats [1]. This model was especially popular because it was the closest mimicker of spontaneous ICH in human beings [10].

Mice

The ICH model in mice was derived from experiments in rats. A single arterial blood injection into the right basal ganglia in mice was described by Nakamura et al. in 2004 [34]. This study compared the effects of autologous arterial blood, donor whole blood, and saline injections on brain edema development. The study found that donor blood injection was associated with a significantly greater increase in edema in the ipsilateral cortex compared to an autologous blood injection model.

In 2003, Belayev et al. [35] placed a cannula into the left striatum and injected 5 μl of heparinized cardiac blood from a donor mouse. Following the injection, 7 min were given for clotting to occur, and a final injection of blood was given (10 μl). Double injection methods, such as this one in mice, were met with great success because of their consistency. Soon after, in 2006, a triple injection method in mice was developed by Ma et al. using venous blood [36]. Two infusions of 5 μl of blood were separated by a 7-min pause for clot formation. After the second infusion, a 1-min pause was given for additional formation, followed by a 20-μl infusion of blood. This method also produced consistent outcomes.

In 2008, Rynkowski et al. [37] published a protocol for a modified double blood injection model in mice. In this model, 30 μl of autologous blood from the central tail artery was injected directly into the right stratium in two steps as previously described [35]. In both the double and triple injection mouse models, potential immunoreactive blood from other mice was used, and although heparinized to minimize clot formation, it prevented proper study of pathologic processes associated with the hematoma formation.

In 1997, Choudri et al. [38] first utilized the previously established rat model for collagenase injection in mice by infusing 1 μl of bacterial collagenase into the right basal ganglia over 4 min. This was followed by Clark et al. in 1998 [39] who performed a 2-min injection of 0.5-μl-volume collagenase into the right caudate and globus pallidus, followed by a 3-min delay to reduce tract reflux. Additionally, Clark's group performed a 28-point neurobehavioral evaluation at 24 and 48 h. Neurobehavioral scoring has since been adopted by other groups as a way to follow functional differences with administration of various substances meant to worsen or improve the injury in ICH [6, 39, 40].

Both rats and mice have been widely used in research because of their feasibility and ease with which to anesthetize them compared to larger animals. Transgenic systems exist primarily in mice, making this the optimal model for studying genomic effects on ICH and secondary mechanisms of injury. However, the relatively small size makes them difficult to implement techniques that are used in larger animals.

Conclusion

In this review, we looked at the three main models that have been developed to understand the physiology behind ICH – microballoon infusion, collagenase injection, and the autologous blood injection model. Additionally, we compared the various animals species that have been used to conduct these experiments, including monkeys, canines, pigs, rabbits,

mice, and rats. Although there are no ideal subjects or models that can mimic the natural process in humans, each model can be used to study certain aspects of the pathophysiological process behind an ICH. In the future, the ideal ICH model should have characteristics that can model spontaneous intracerebral hemorrhage in humans and allow for effective studies on physiology, procedural interventions, and mechanisms of secondary brain injury.

Acknowledgments

This study is partially supported by NIH NS053407 to J.H. Zhang and NS060936 to J. Tang.

References

1. Rosenberg GA, Mun-Bryce S, Wesley M, Kornfeld M. Collagenase-induced intracerebral hemorrhage in rats. Stroke. 1990; 21:801–807. [PubMed: 2160142]
2. Sinar EJ, Mendelow AD, Graham DI, Teasdale GM. Experimental intracerebral hemorrhage: effects of a temporary mass lesion. J Neurosurg. 1987; 66:568–576. doi:10.3171/jns.1987.66.4.0568. [PubMed: 3559723]
3. Yang GY, Betz AL, Chenevert TL, Brunberg JA, Hoff JT. Experimental intracerebral hemorrhage: relationship between brain edema, blood flow, and blood-brain barrier permeability in rats. J Neurosurg. 1994; 81:93–102. doi:10.3171/jns.1994.81.1.0093. [PubMed: 8207532]
4. Rojas H, Zhang JH. Animal models of spontaneous intracerebral hemorrhage. In: Zhang JH (ed) Advancements in neurological research. Research SignPost. 2008:39–89. ISBN:978-81-308-0225-1.
5. Nath FP, Jenkins A, Mendelow AD, Graham DI, Teasdale GM. Early hemodynamic changes in experimental intracerebral hemorrhage. J Neurosurg. 1986; 65:697–703. doi:10.3171/jns.1986.65.5.0697. [PubMed: 3772459]
6. Wu B, Ma Q, Khatibi N, Chen W, Sozen T, Cheng O, Tang J. Ac-YVAD-CMK decreases blood-brain barrier degradation by inhibiting caspase-1 activation of interleukin-1beta in intracerebral hemorrhage mouse model. Transl Stroke Res. 2010; 1(1):57–64. [PubMed: 20596246]
7. Harris ED Jr, Krane SM. Collagenases (third of three parts). N Engl J Med. 1974; 291:652–661. [PubMed: 4368516]
8. Montfort I, Perez-Tamayo R. The distribution of collagenase in normal rat tissues. J Histochem Cytochem. 1975; 23:910–920. [PubMed: 172556]
9. James ML, Warner DS, Laskowitz DT. Preclinical models of intracerebral hemorrhage: a translational perspective. Neurocrit Care. 2008; 9:139–152. doi:10.1007/s12028-007-9030-2. [PubMed: 18058257]
10. Andaluz N, Zuccarello M, Wagner KR. Experimental animal models of intracerebral hemorrhage. Neurosurg Clin N Am. 2002; 13:385–393. [PubMed: 12486927]
11. Fujii Y. Studies on induced hypothermia for open heart surgery. II. Adequate flow of hypothermic perfusion in the dog. Nippon Geka Hokan. 1972; 41:149–159. [PubMed: 4675241]
12. Kazui S, Naritomi H, Yamamoto H, Sawada T, Yamaguchi T. Enlargement of spontaneous intracerebral hemorrhage. Incidence time course. Stroke. 1996; 27:1783–1787. [PubMed: 8841330]
13. Ropper AH, Zervas NT. Cerebral blood flow after experimental basal ganglia hemorrhage. Ann Neurol. 1982; 11:266–271. doi:10.1002/ana.410110306. [PubMed: 7092179]
14. Bullock R, Mendelow AD, Teasdale GM, Graham DI. Intracranial haemorrhage induced at arterial pressure in the rat. Part 1: description of technique, ICP changes and neuropathological findings. Neurol Res. 1984; 6:184–188. [PubMed: 6152312]
15. Deinsberger W, Vogel J, Kuschinsky W, Auer LM, Boker DK. Experimental intracerebral hemorrhage: description of a double injection model in rats. Neurol Res. 1996; 18:475–477. [PubMed: 8916066]
16. Segal R, Dujovny M, Nelson D, et al. Local urokinase treatment for spontaneous intracerebral hematoma. Clin Res. 1982; 30:412A.

17. Bullock R, Brock-Utne J, van Dellen J, Blake G. Intracerebral hemorrhage in a primate model: effect on regional cerebral blood flow. Surg Neurol. 1988; 29:101–107. [PubMed: 3336844]
18. Fujii Y, Tanaka R, Takeuchi S, Koike T, Minakawa T, Sasaki O. Hematoma enlargement in spontaneous intracerebral hemorrhage. J Neurosurg. 1994; 80:51–57. doi:10.3171/jns.1994.80.1.0051. [PubMed: 8271022]
19. Whisnant JP, Sayre GP, Millikan CH. Experimental intracerebral hematoma. Arch Neurol. 1963; 9(6):586–592.
20. Sugi T, Fujishima M, Omae T. Lactate and pyruvate concentrations, and acid-base balance of cerebrospinal fluid in experimentally induced intracerebral and subarachnoid hemorrhage in dogs. Stroke. 1975; 6:715–719. [PubMed: 822]
21. Qureshi AI, Wilson DA, Hanley DF, Traystman RJ. No evidence for an ischemic penumbra in massive experimental intracerebral hemorrhage. Neurology. 1999; 52:266–272. [PubMed: 9932942]
22. Qureshi AI, Wilson DA, Traystman RJ. Treatment of elevated intracranial pressure in experimental intracerebral hemorrhage: comparison between mannitol and hypertonic saline. Neurosurgery. 1999; 44:1055–1063. discussion 1063-1054. [PubMed: 10232539]
23. Lee EJ, Hung YC, Lee MY. Anemic hypoxia in moderate intracerebral hemorrhage: the alterations of cerebral hemodynamics and brain metabolism. J Neurol Sci. 1999; 164:117–123. doi:S0022-510X(99)00068-4 [pii]. [PubMed: 10402021]
24. Takasugi S, Ueda S, Matsumoto K. Chronological changes in spontaneous intracerebral hematoma – an experimental and clinical study. Stroke. 1985; 16:651–658. [PubMed: 4024177]
25. Wagner KR, Xi G, Hua Y, Kleinholz M, de Courten-Myers GM, Myers RE, Broderick JP, Brott TG. Lobar intracerebral hemorrhage model in pigs: rapid edema development in perihematomal white matter. Stroke. 1996; 27:490–497. [PubMed: 8610319]
26. Kuker W, Thiex R, Rohde I, Rohde V, Thron A. Experimental acute intracerebral hemorrhage. Value of MR sequences for a safe diagnosis at 1.5 and 0.5 T. Acta Radiol. 2000; 41:544–552. [PubMed: 11092473]
27. Mun-Bryce S, Wilkerson AC, Papuashvili N, Okada YC. Recurring episodes of spreading depression are spontaneously elicited by an intracerebral hemorrhage in the swine. Brain Res. 2001; 888:248–255. doi:S0006-8993(00)03068-7 [pii]. [PubMed: 11150481]
28. Kaufman HH, Pruessner JL, Bernstein DP, Borit A, Ostrow PT, Cahall DL. A rabbit model of intracerebral hematoma. Acta Neuropathol. 1985; 65:318–321. [PubMed: 3976368]
29. Koeppen AH, Dickson AC, McEvoy JA. The cellular reactions to experimental intracerebral hemorrhage. J Neurol Sci. 1995; 134(Suppl):102–112. doi:0022510X9500215N [pii]. [PubMed: 8847540]
30. Qureshi AI, Ling GS, Khan J, Suri MF, Miskolczi L, Guterman LR, Hopkins LN. Quantitative analysis of injured, necrotic, and apoptotic cells in a new experimental model of intracerebral hemorrhage. Crit Care Med. 2001; 29:152–157. [PubMed: 11176176]
31. Gustafsson O, Rossitti S, Ericsson A, Raininko R. MR imaging of experimentally induced intracranial hemorrhage in rabbits during the first 6 hours. Acta Radiol. 1999; 40:360–368. [PubMed: 10394862]
32. Rohde V, Rohde I, Thiex R, Ince A, Jung A, Duckers G, Groschel K, Rottger C, Kuker W, Muller HD, Gilsbach JM. Fibrinolysis therapy achieved with tissue plasminogen activator and aspiration of the liquefied clot after experimental intracerebral hemorrhage: rapid reduction in hematoma volume but intensification of delayed edema formation. J Neurosurg. 2002; 97:954–962. doi:10.3171/jns.2002.97.4.0954. [PubMed: 12405387]
33. Masuda T, Dohrmann GJ, Kwaan HC, Erickson RK, Wollman RL. Fibrinolytic activity in experimental intracerebral hematoma. J Neurosurg. 1988; 68:274–278. doi:10.3171/jns.1988.68.2.0274. [PubMed: 3339444]
34. Nakamura T, Xi G, Hua Y, Schallert T, Hoff JT, Keep RF. Intracerebral hemorrhage in mice: model characterization and application for genetically modified mice. J Cereb Blood Flow Metab. 2004; 24:487–494. doi:00004647-200405000-00002 [pii]. [PubMed: 15129180]
35. Belayev L, Saul I, Curbelo K, Busto R, Belayev A, Zhang Y, Riyamongkol P, Zhao W, Ginsberg MD. Experimental intracerebral hemorrhage in the mouse: histological, behavioral, and

hemodynamic characterization of a double-injection model. Stroke. 2003; 34:2221–2227. doi: 10.1161/01.STR.0000088061.06656.1E. [PubMed: 12920264]

36. Ma B, Zhang J. Nimodipine treatment to assess a modified mouse model of intracerebral hemorrhage. Brain Res. 2006; 1078:182–188. doi:S0006-8993(06)00158-2 [pii]. [PubMed: 16492378]
37. Rynkowski MA, Kim GH, Komotar RJ, Otten ML, Ducruet AF, Zacharia BE, Kellner CP, Hahn DK, Merkow MB, Garrett MC, Starke RM, Cho BM, Sosunov SA, Connolly ES. A mouse model of intracerebral hemorrhage using autologous blood infusion. Nat Protoc. 2008; 3:122–128. doi:nprot.2007.513 [pii]. [PubMed: 18193028]
38. Choudhri TF, Hoh BL, Solomon RA, Connolly ES Jr, Pinsky DJ. Use of a spectrophotometric hemoglobin assay to objectively quantify intracerebral hemorrhage in mice. Stroke. 1997; 28:2296–2302. [PubMed: 9368579]
39. Clark W, Gunion-Rinker L, Lessov N, Hazel K. Citicoline treatment for experimental intracerebral hemorrhage in mice. Stroke. 1998; 29:2136–2140. [PubMed: 9756595]
40. Thiex R, Mayfrank L, Rohde V, Gilsbach JM, Tsirka SA. The role of endogenous versus exogenous tPA on edema formation in murine ICH. Exp Neurol. 2004; 189:25–32. doi:10.1016/j.expneurol.2004.05.021. [PubMed: 15296833]