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Etiology of stroke and choice of models

Paul R. Krafft¹, Emma L. Bailey², Tim Lekic¹, William B. Rolland¹, Orhan Altay¹, Jiping Tang¹, Joanna M. Wardlaw^{2,3}, John H. Zhang^{1,4,5}, Cathie L. M. Sudlow^{2,6,*}

¹Department of Physiology, School of Medicine, Loma Linda University, Loma Linda, CA, USA

²Division of Clinical Neurosciences, University of Edinburgh, Edinburgh, UK

³SINAPSE Collaboration (Scottish Imaging Network, a Platform for Scientific Excellence), University of Edinburgh, Edinburgh, UK

⁴Department of Anesthesiology, School of Medicine, Loma Linda University, Loma Linda, CA, USA

⁵Department of Neurosurgery, School of Medicine, Loma Linda University, Loma Linda, CA, USA

⁶Centre for Molecular Medicine of Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK

Abstract

Animal models of stroke contribute to the development of better stroke prevention and treatment through studies investigating the pathophysiology of different stroke sub-types and by testing promising treatments before trials in humans. There are two broad types of animal models: those in which stroke is induced through artificial means, modeling the consequences of a vascular insult but not the vascular pathology itself; and those in which strokes occur spontaneously. Most animal models of stroke are in rodents due to cost, ethical considerations, availability of standardized neurobehavioral assessments, and ease of physiological monitoring. While there are similarities in cerebrovascular anatomy and pathophysiology between rodents and humans, there are also important differences, including brain size, length and structure of perforating arteries, and gray to white matter ratio, which is substantially lower in humans. The wide range of rodent models of stroke includes models of global and focal ischemia, and of intracerebral and sub-arachnoid hemorrhage. The most widely studied model of spontaneous stroke is the spontaneously hypertensive stroke-prone rat, in which the predominant lesions are small subcortical infarcts resulting from a vascular pathology similar to human cerebral small vessel disease. Important limitations of animal models of stroke - they generally model only certain aspects of the disease and do not reflect the heterogeneity in severity, pathology and comorbidities of human stroke - and key methodological issues (especially the need for adequate sample size, randomization, and blinding in treatment trials) must be carefully considered for the successful translation of pathophysiological concepts and therapeutics from bench to bedside.

*Correspondence: Cathie L. M. Sudlow, Division of Clinical Neurosciences, University of Edinburgh, Edinburgh EH9 1TE, UK. cathie.sudlow@ed.ac.uk.

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Background

Pathological types and subtypes of stroke

Stroke is the second most common cause of death worldwide (after ischemic heart disease) and is a leading global cause of disability (1). Stroke is a clinical syndrome, with three main pathological types - ischemic (80% in populations of European origin, somewhat less in Chinese and other Asian populations), intracerebral hemorrhage (ICH, 15% in European populations, 30% or more in Chinese and other Asian populations) and sub-arachnoid hemorrhage (SAH, 5% in European populations) (2). There are several subtypes within each of these. Approximately 50% of ischemic strokes are attributed to large-artery atherothrombotic disease, 25% to disease of the small intracranial arteries (resulting in lacunar strokes), 20% to cardiac emboli, and 5% to various rare causes (e.g. extracranial artery dissection) (2). ICH maybe subclassified according to its location (lobar, deep, intraventricular, or combinations of these) or its cause if known (e.g. hypertension, amyloid angiopathy, arteriovenous malformation) (3). About 75% of spontaneous SAH cases are due to a ruptured aneurysm, 20% have no identifiable cause (of which at least half are due to idiopathic or non-aneurysmal perimesencephalic SAH), and the remainder are caused by a variety of rare disorders such as arteriovenous malformations of the brain or spine (4).

Current strategies for treatment and prevention

Of the millions of people worldwide each year who suffer a stroke (5), up to 50% will have died or be dependent on others six-months later (2). The prognosis is worse for hemorrhagic stroke, with a one-month case fatality of around 50% (3,6). Only a few interventions have been shown by large randomized controlled trials to improve outcome after acute stroke: stroke unit care, including early and longer term rehabilitation, is proven to save lives and reduce dependency following ischemic or hemorrhagic stroke; for ischemic stroke, early aspirin is of proven benefit and can be given to most patients, intravenous thrombolysis improves outcome for a small proportion presenting very early after symptom onset, and early hemicraniectomy benefits those very few younger patients who have malignant middle cerebral artery (MCA) territory infarction (7); randomized trials do not yet support routine use of specific interventions for ICH, but surgical evacuation is indicated for a small proportion with infratentorial hemorrhage and declining conscious level (3); and oral nimodipine improves outcome after aneurysmal SAH (4). Even if used appropriately and widely for all stroke patients in the population, these interventions collectively can only reduce the proportion of people dying or becoming disabled as a result of their stroke by <10% (7). Better and more widely applicable interventions for acute stroke are therefore needed if we are to reduce further the burden of poststroke death and disability.

After a stroke or transient ischemic attack (TIA), there is an increased risk of stroke and other serious vascular events, particularly in the early days and weeks. Secondary preventive

strategies are composed of lifestyle changes such as the following: smoking cessation, healthy diet, weight loss, and exercise; blood pressure reduction; cholesterol reduction with a statin and antiplatelet treatment for those at risk of recurrent ischemic events; and carotid endarterectomy for a few patients with recently symptomatic carotid stenosis (8). These are effective when used optimally for individuals, but since only recurrent strokes, or strokes preceded by a TIA, are amenable to this type of prevention, the impact in reducing the number of strokes occurring in the population is limited. In addition, our current understanding of how the effects of these interventions differ between different drugs (e.g. different antihypertensive agents or different cholesterol lowering drugs) and between different stroke subtypes (e.g. the effects of blood pressure reduction following large-artery atherothrombotic or small vessel disease lacunar ischemic stroke) is incomplete. An improved understanding of these will lead to better and more targeted secondary prevention.

Primary prevention, i.e. preventing strokes from occurring in the first place, through targeting individuals at high risk or through mass population approaches could be highly effective. Indeed, modeling exercises suggest that around 70% of strokes could be prevented through optimization at population level of blood pressure, cholesterol levels, alcohol intake, cigarette smoking, diet, overweight, and exercise (9). Such exercises are, however, dependent on several assumptions, and in particular tend not to distinguish between the various different pathological types and subtypes of stroke. To predict the likely effects on stroke of existing primary preventive strategies, and to allow the development of better strategies for the future, a better understanding of the underlying causes of the different subtypes is needed.

Potential uses of animal models of stroke

Improved understanding of etiology

Animal models of stroke can contribute to an improved understanding of the underlying etiology of different subtypes of stroke and so to the development of better prevention and treatment strategies. In considering how animal models can contribute to our understanding of stroke, it is important to appreciate that most animal models of disease, including stroke, do not attempt to model the whole disease process but aim to enable detailed study of specific aspects of this in carefully controlled circumstances. For stroke, different animal models have been developed that model aspects of the underlying vascular pathology, the parenchymal pathology that results from a vascular insult, or sometimes both.

Testing new interventions

Animal models are also useful in testing promising therapeutic interventions, both pharmacological and nonpharmacological (e.g. hypothermia or rehabilitation strategies) before trials in humans are carried out. These are usually experiments testing the effects of interventions in induced stroke models, in particular models of ischemic stroke resulting from large-artery occlusion. Such experiments have considerable potential to inform the design of clinical trials in human stroke, but there is a substantial gap between apparent demonstrations of efficacy of treatments for acute stroke in animal models and evidence for efficacy in human stroke (10). While part of this failure of translation from the laboratory to

the clinic may be explained by shortcomings in the human clinical trials, other explanations include methodological flaws in the design and conduct of animal studies (e.g. lack of proper allocation concealment, randomization, blinding), lack of generalizability of some animal models of stroke (i.e. they do not sufficiently reflect the disease in humans, e.g. due to use of young animals with no comorbidities, and/or use of unrealistic time-to-treatment windows), and publication bias, whereby neutral or negative animal studies are more likely to remain unpublished than neutral clinical trials, leading to the (false) impression that the first are more often positive than the second (11).

Overview of specific animal models

Most animal models of stroke use rodents, but rabbits, pigs, dogs, and primates have also been used (12). The animal models currently used can be usefully divided into two broad types: models in which stroke is induced in animals through artificial means and animals in which strokes occur spontaneously. An essential feature of the former is that they model the consequences of various types of vascular insult through acute vessel injury but do not model the vascular pathology itself. Our focus in this overview is on models in which the animals develop stroke lesions and/or neurological deficits. The table gives a brief summary of induced and spontaneous rodent models of adult stroke together with some important advantages and disadvantages of each (Table 1). Further details of these and of the few nonrodent models are provided later.

Induced stroke models

Various induced models of stroke have been developed in the past decade, mainly in rodents (43–47). These include models of global and focal ischemia, and of both intracerebral and SAH.

Induced cerebral ischemia

Models of global ischemia—These models do not mimic the focal condition of ischemic stroke as defined by the World Health Organization. However, acute global ischemia models may mimic the cerebral damage that occurs after cardiac arrest, while chronic cerebral hypoperfusion models may produce subcortical white matter damage similar to white matter hyperintensities seen on magnetic resonance (MR) brain scans in humans that are thought to be due to underlying cerebral small vessel disease. These models have the potential to be clinically informative since around 50% of all patients that survive sudden cardiac arrest have persistent severe motor and cognitive deficits (48), while white matter hyperintensities in humans are associated with progressive cognitive decline, gait difficulties, and increased risk of stroke and myocardial infarction (49).

Acute global ischemic damage in rodents is commonly induced by permanent occlusion of both vertebral arteries and temporary ligation of the two common carotid arteries (four-vessel occlusion model) (13–15) or by temporary occlusion of the common carotid arteries combined with induced systemic hypotension (two-vessel occlusion model) (16,17,50). Both methods cause extensive bilateral forebrain injury (44). A model of asphyxial cardiac arrest in rats has also been established (18,19). Compared with four- or two-vessel occlusion

models, asphyxial cardiac arrest causes temporary complete ischemia of the whole brain, requiring resuscitation thereafter.

Chronic global hypoperfusion models in rodents include ligation of both common carotid arteries (20) and bilateral common carotid artery stenosis using external microcoils (21). Both models have been shown to produce mainly white matter lesions.

Models of MCA occlusion (MCA)—The majority of ischemic stroke in humans occurs in the vascular territory of the MCA (51), and models of MCAO were developed to mimic the consequences of this clinical situation. MCAO in rodents has been reported to induce long-term sensorimotor and cognitive deficits as well as impairments of postural and sensory reflexes (52–55). This has made MCAO models suitable for assessing the effects of physical interventions to promote recovery (56,57).

Endovascular or surgical approaches are commonly used to produce vessel occlusions (22–26) and may be performed as either permanent or transient vessel blockade. Transient MCAO allows the investigation of ischemia related brain injury as well as cerebral consequences of reperfusion. The late onset and inconsistency of spontaneous reperfusion in stroke patients (27) have led to suggestions that prospective acute ischemic stroke therapies should be tested in transient as well as in permanent MCAO models before entering clinical trials in humans (58).

Thromboembolic occlusion of the MCA or its distal branches is commonly induced via injection of autologous blood clots into the internal carotid artery (ICA) (28,59–62). Although producing a higher variability in lesion size and location than the temporary and permanent vessel occlusion models described earlier, this model allows the investigation of thrombolytic agents or combinations of thrombolytic and neuroprotective drugs (63,64). Other models of induced thromboembolic stroke include delivery of photoactivated thrombogenic agents, which deliver a shower of thromboemboli to a relatively restricted arterial territory producing small foci of cortical ischemia (29–31), and intracarotid injection of sodium dodecyl sulfate (SDS) detergent, resulting in endothelial damage with subsequent thromboemboli, and producing multiple small infarcts in hippocampus, cortex, and thalamus (32).

Models of perforating artery territory ischemic stroke—Several methods of inducing ischemic stroke lesions in the territory of perforating arteries have also been described (12). Occlusion of a small cortical pial artery on the surface of the rat brain with either forceps or photochemical irradiation produces small cortical infarcts. Occlusion by crushing or photocoagulation of thalamoperforators or lenticulostriate arteries in primates produces infarcts resembling striatocapsular ischemic lesions (12). Injection of the powerful vasoconstrictor endothelin-1 into the subcortical tissues of the rat brain, probably affecting several microvessels, has also been used to induce subcortical ischemic lesions (12). Finally, selective occlusions of the anterior choroidal or hypothalamic artery using a precisely placed intraluminal thread in rats or craniotomy and electrocoagulation in the miniature pig have been reported to produce stroke-like neurological signs and subcortical striatocapsular or lacunar-type infarcts (12,65).

Induced SAH

Models of SAH produce intracranial bleeds in the subarachnoid space between the arachnoid membrane and pia mater, most frequently via endovascular perforation of the ICA with a monofilament suture (33,34), or via injection of blood or blood components into the cisterna magna (35,36). The intracisternal injection model allows close control of subarachnoid blood volumes; the perforation model, however, better imitates the actual clinical condition of SAH in humans. Preclinical rodent studies of induced SAH have demonstrated a close correlation between the volume of sub-arachnoid blood, the extent of subsequent ischemic brain injury, and functional impairments (66). Sensorimotor and cognitive impairments have been shown to develop following both blood injection and perforation models of SAH, but only cognitive deficits persisted over a time span of five weeks post-surgery (66–68). This is probably due to the diffuse cortical and subcortical brain injury with lack of direct injury to the descending motor tracts. Analogously, lasting cognitive and psychological deficits are the most important problem for patients surviving SAH (69–72).

Induced ICH

Enzymatically induced vessel rupture as well as intraparenchymal injection of blood or blood products are widespread methods used in mice and rats to mimic the consequences of human spontaneous ICH (37–39). To mimic the effects of hemorrhage in a particular targeted brain area (e.g. the basal ganglia), a small craniotomy (burr hole) is needed, followed by a stereotactically guided injection of bacterial collagenase or blood. Blood injection models are often used to study the pathological mechanisms of hemorrhagic brain damage and edema formation. Hemostatic aspects are, however, better investigated with the collagenase injection model since collagenase disrupts the basal lamina of vessels, causing spontaneous bleeding into the surrounding brain tissue (73,74). Patients with supratentorial ICH involving basal ganglia and thalamus are often left with sensorimotor deficits of varying severities, which can be imitated in rodent models of experimental ICH (75,76). The collagenase injection model, in particular, generates long-term neurofunctional deficits and so has been used more extensively than the blood injection model for studying rehabilitation approaches such as forced running and skilled reaching training after ICH (77–79).

Spontaneous stroke models

Spontaneously hypertensive stroke-prone rat (SHRSP)

Probably the most widely studied spontaneous animal model of stroke is the SHRSP. This rat was selectively bred from a substrain of the spontaneously hypertensive rat (SHR), which was itself developed by selective cross breeding of outbred Wistar Kyoto Rats. While the SHR has elevated blood pressure but rarely shows signs of stroke, the SHRSP develops increasing levels of blood pressure from six-weeks of age, malignant hypertension by 12 weeks, and stroke-like symptoms, including circling, drooling, and limb weakness, by 20 weeks. The SHRSP has mainly been used in MCAO models to produce large MCA territory infarcts. The spontaneous strokes suffered by the SHRSP have been less extensively studied. They include cortical infarcts and cerebral hemorrhages, but the predominant lesions are small subcortical infarcts, which result from pathology affecting the small blood vessels that

appear very similar to the human small vessel disease underlying lacunar ischemic strokes (12,40,80).

The subcortical infarcts suffered by the SHRSP are generally assumed to be a direct consequence of elevated blood pressure. However, a careful systematic review of the relevant literature and a recent immunohistochemical study suggest a primary causative role for endothelial dysfunction, blood-brain barrier (BBB) leakage and inflammation, with evidence of increased BBB permeability in the SHRSP preceding the development of hypertension and well before the appearance of any stroke lesions (40,81), similar to the endothelial dysfunction and BBB leak demonstrated in human small vessel disease (82,83).

Thus, the SHRSP is not only an animal in which strokes may be induced, but also has spontaneous strokes, which seem set to be highly informative for understanding the underlying pathological processes of human stroke. This applies particularly to lacunar ischemic stroke, for which the SHRSP is by far the most relevant animal model (12,40,80), and which has low case fatality, making the small vessel pathology underlying this subtype of stroke very difficult to study in humans. Furthermore, important mechanistic insights may also be obtained from detailed study of the spontaneous cortical infarcts and cerebral hemorrhages suffered by this animal.

Other spontaneous stroke animal models

Male inducible hypertensive rats carry the mouse renin gene on the Y chromosome under the control of an inducible promoter. Induction of the transgene in adult life produces overactivation of the renin-angiotensin system and hypertension. When these induced animals were given 0–9% saline in addition to drinking water, they developed stroke-like signs, with unsteadiness of gait by seven-days. Neuropathological examination of animals sacrificed at 14 days revealed areas of microscopic cerebral infarction scattered throughout the cortex, hemorrhages in association with some areas of tissue necrosis, and hemorrhages on the brain surface in some animals (41). Transgenic mice expressing human renin and human angiotensinogen (R+/A+) treated with high-salt diet and N(omega)-nitro-L-arginine methyl ester have been reported to develop progressively increasing blood pressure, neurological signs, death within 10 weeks, and evidence of ICH in the brainstem, cerebellum and basal ganglia (42). Elderly dogs also sustain spontaneous strokes with neurological deficits (hemiparesis, facial hypalgesia, and hemianopia) but have been far less extensively studied (84,85).

Transgenic models

A number of rare forms of stroke occur in humans as a result of single gene disorders (86). These include several conditions that cause specific vasculopathies of the intracranial small arteries and can give rise to lacunar ischemic strokes and leukoaraiosis on brain imaging. The most common of these is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), resulting from mutations in the *Notch3* gene. Several *Notch3* mutant mouse models have been developed, which reproduce the features of human CADASIL vascular pathology accompanied by progressive white matter damage (but no infarcts) in one of the most recently developed models (87,88). These and other

transgenic models of various other monogenic forms of stroke not only have the potential to provide insights into the disease mechanisms of these rare forms of stroke, but may also prove useful for testing new treatments for the rare monogenic and - perhaps - for some more common subtypes of stroke. A full description and discussion of transgenic mouse models of stroke is beyond the scope of this paper (and so they are not individually listed in the table) (Table 1), yet this is a rapidly developing and exciting area (86).

Choosing the right model

Clearly, the choice of model must depend on the research question being addressed. Acute stroke presents a highly variable clinical condition. Variation in the affected brain area, the severity and preexisting comorbidities contribute to a wide variation in outcomes in patients (89). Animal models, by contrast, tend to eliminate confounding variables in order to prevent misleading interpretations. Additionally, no animal model entirely imitates the complexity of ischemic or hemorrhagic stroke. These limitations must be considered carefully when translating from model to patient.

For preclinical studies of potential acute stroke treatments, several important issues need to be considered. First, it is important to consider for which types or subtypes of human stroke the treatment under investigation may be relevant and ensure that the appropriate model or range of models is used. Second, since the majority of stroke patients are advanced in age and suffer from a range of comorbidities, such as hypertension and diabetes, it may be appropriate to incorporate such conditions into animal models. Third, experimental lesions must be reproducible and operative procedures should be minimally invasive and of short duration. Fourth, it should be possible to monitor physiological parameters and, where appropriate, maintain them within normal range (90). Fifth, a model that allows assessment of a relevant, functional, neurobehavioral outcome is preferable to the sole measurement of a surrogate such as infarct size. Finally, it is important to use a model for which sufficient numbers of animals for adequate statistical power can be assembled at reasonable cost (11).

For better understanding of pathophysiology, the choice of model will depend on its representativeness of the pathology concerned. Spontaneous stroke models will sometimes be most appropriate for such purposes, in particular because they allow the study of the vascular pathology causing stroke lesions as well as the lesions themselves.

Rodent stroke models are frequently the choice both for preclinical studies and pathophysiological investigations. As described earlier, a wide range of rodent stroke models exists that mimic several different pathological stroke subtypes, and rodents with comorbidities are available as well as young, healthy animals. Rats have substantial similarities to humans in terms of cerebrovascular anatomy and physiology (90), and in addition, their reasonably small size allows continuous monitoring of physiological parameters (91). A number of standardized neurobehavioral assessments have been established for rodents, allowing the integration of relevant, functional outcomes, which greatly improves the potential for translation of preclinical stroke studies (75,92–95). Use of rodents allows extensive and comprehensive evaluations of brain specimens at lower cost

compared with larger animals. In addition, the use of rodents is generally considered more acceptable from an ethical perspective than use of larger animals.

There are limitations to the use of rodent models. Notwithstanding the similarities in cerebrovascular anatomy and pathophysiology, there are also important differences, including differences in brain size, length and structure of perforating arteries, and the ratio of gray to white matter, which is substantially lower in humans than in rodents (Fig. 1) (12,80). This last difference is considerable and must be borne in mind particularly for studies of small vessel disease, which in humans causes lacunar stroke lesions predominantly in the deep white matter.

Summary

Animal - and in particular, rodent - models of induced and spontaneous strokes present a plethora of opportunities to investigate pathophysiological mechanisms and responses of stroke and its various pathological types and subtypes. Furthermore, they are used to develop and test acute therapies for ischemic and hemorrhagic brain injury. The addition of neurobehavioral assessments to morphological and mechanistic outcomes has been an important advance in preclinical stroke studies over the past decade (92). However, the limitations of these models must be carefully considered for the successful translation of pathophysiological concepts and therapeutics from bench to bedside (96,97).

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Fig. 1. Approximate brain volume (cm³) and gray : white (G : W) matter ratio in healthy (young) animals shown relative to the human brain.

Table 1

Rodent models of adult stroke

Etiology	Model	Advantages	Disadvantages
Induced stroke models			
Global ischemia - acute	Four-vessel occlusion (13–15)	Reversible forebrain ischemia may be induced in awake animals.	Two-stage operative procedure. Occurrence of seizures. Variable outcomes in different rat strains.
	Two-vessel occlusion (16,17)	One-stage operative procedure. Reversible ischemic forebrain injury.	Necessity of systemic hypotension (exsanguination).
	Asphyxial cardiac arrest (18,19)	Whole brain ischemia followed by related systemic changes such as hypoxemia, acidosis, systemic inflammation, hypercortisolemia, hyperglycemia.	Intensive postsurgery care (assisted ventilation, supplemental fluids). Body temperature during first days of recovery strongly influences outcome.
	Bilateral common carotid artery ligation (20)	Produces white matter changes similar to leukoaraiosis seen on CT and MR brain scans in humans.	Approximately 20% case fatality within one-week of procedure. Damage to visual pathway may compromise neurobehavioral assessment.
Focal ischemia	Bilateral common carotid artery stenosis (21)	Produces milder reduction in cerebral blood flow than ligation model earlier, with white matter lesions but sparing of visual pathways and gray matter	Lesions take two-weeks to develop
	Endovascular MCAO (22–24)	Most frequently used method in rodents. Easy to perform permanent or transient ischemia.	Risk of vessel rupture (SAH). Postsurgical hyperthermia.
	Surgical MCAO (25–27)	Better control of occlusion site and therefore less variability.	Necessity of craniotomy.
	Thromboembolic MCAO (28)	Mimics most common cause of ischemic stroke in humans. Suitable to investigate thrombolytic therapies.	Higher variability in lesion size, location, and occurrence of spontaneous reperfusion.
SAH	Photothrombosis (29–31)	Ability to induce infarct in variable cortical location. Less invasive procedure.	Less relevance to human condition. Occurrence of spontaneous reperfusion.
	Intracarotid injection of SDS detergent (32)	Selective perforating artery occlusion caused by <i>in situ</i> small vessel injury including endothelial damage and fibrin thrombus.	Unpredictable distribution of infarcts.
	Cortical pial artery occlusion (12)	Arterial occlusion with forceps or photochemical irradiation produces small cortical infarcts.	
	Subcortical injection of endothelin-1 (12)	Production of subcortical ischemic lesions in dose-dependent manner.	Several microvessels affected simultaneously - cannot accurately target single perforating vessel.
ICH	Selective intraluminal thread occlusion of anterior choroidal/hypothalamic artery (12)	Production of precise area of subcortical infarction in defined arterial territory	Difficult to place thread accurately - MCAO occurs inadvertently in significant proportion of animals.
	Endovascular perforation (33,34)	Rupture of intracranial vessels reflects the clinical condition of aneurysmal SAH in humans.	Mortality reaches 37–50% within 24 h after SAH induction. Variable severities.
	Intracisternal blood injection (35,36)	SAH severity may be regulated by blood quantity and number of injections.	Nonphysiological blood distribution.
	Intracerebral injection of bacterial collagenase (37)	Rupture of intracerebral vessels mimics ICH in humans. Allows investigations of delayed hematoma expansion and hemostasis.	Bacterial collagenase may induce inflammatory responses. Variability in hematoma size.

Etiology	Model	Advantages	Disadvantages
Spontaneous stroke models			
Focal ischemia	SHRSP (40)	Injection of variable blood compositions. No confounding inflammation.	Matched to hematoma size, neurofunctional deficits resolve more rapidly compared with the collagenase injection model.
	IHR (41)	Similar vascular and parenchymal pathological processes to human small vessel disease and lacunar stroke. Animals given saline rapidly develop scattered microscopic areas of cortical infarction.	Spontaneous strokes do not occur until 20 weeks although can be hastened by high salt diet. Vascular pathology not extensively studied.
ICH	R+/A+ mice (42)	Animals given high-salt diet and L-NAME develop spontaneous intracerebral hemorrhage	Strokes take several weeks to develop. Vascular pathology not much studied.

CT, computed topography; ICH, intracerebral hemorrhage; IHR, inducible hypertensive rat; MCAO, middle cerebral artery occlusion; R+/A+ mice, transgenic mice expressing human rennin and human angiotensinogen; SAH, sub-arachnoid hemorrhage; SHRSP, spontaneously hypertensive stroke prone rat; L-NAME, N(omega)-nitro-L-arginine methyl ester.