Published in final edited form as:

Future Neurol. 2014 September ; 9(5): 541–551. doi:10.2217/fnl.14.44.

Animal models of stroke: translational potential at present and in 2050

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Abstract

Translation from basic science bench research in ischemic stroke to bedside treatment of patients suffering ischemic stroke remains a difficult challenge. Despite literally hundreds of compounds and interventions that provide benefit in experimental models of cerebral ischemia, efficacy in humans remains to be demonstrated. The reasons for failure to translate the extensive positive basic science findings to successful clinical trials have been the focus of discussion for years. Some attribute the failure to flaws in clinical trial design, others question the predictive value of current animal models and some question the quality of preclinical data. It is likely that a combination of all these shortcomings have ultimately led to the failure. The purpose of this review is to analyze the commonly used animal models used in the field today, provide a framework for understanding the current state of basic science research in the ischemic stroke field and discuss a path forward.

Keywords

animal models; basic science; clinical trial; ischemic stroke; neuroprotection

The remarkable number of neuroprotective agents that showed promise in preclinical studies and failed in clinical trials has created some doubt regarding the validity of current animal models as predictive tools. It is important to note that many attribute the failure to translate such promising pre-clinical data to successful clinical trial to poor clinical trial design and inappropriate generalization of preclinical data [1,2]. It is likely a combination of poor trial design and flawed animal data has contributed to the many failed trials. The fact of the matter is that the more recent clinical trial designs are quite good, in general, compared with the clinical trial designs of 10–30 years ago. Thus, for the purpose of the current discussion, we focus our attention on the state of animal modeling. The problem concerning so many

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Financial & competing interests disclosure: The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

negative clinical trials was clear enough that a task force was organized to tackle the problem, and in 1999 the Stroke Therapy and Academic Industry Roundtable (STAIR) published guidelines for improved preclinical evaluation of potential neuroprotective drugs [3]. The STAIR recommendations provided important guidelines to improve preclinical study quality, including performing experiments in a blinded, randomized fashion, coupled with histological and functional outcome assessments. In addition to study quality, it was recommended that the initial studies be performed in rats with independent replication in at least two independent laboratories. The STAIR committee also emphasized the need for careful attention to the therapeutic time window of opportunity, dose–response relationships, route of administration and pharmacokinetics of the drug being tested (Table 1). Despite the publication of the initial STAIR recommendations 15 years ago and several subsequent STAIR committee meetings [2,4,5], an alarming number of studies do not adhere to these methodological recommendations. A comprehensive analysis of experimental stroke studies performed in 2006 analyzed 1026 studies and observed highly variable adherence to the STAIR criteria and less than impressive experimental data for most compounds that were tested in animals prior to progressing to clinical trials [6]. Several recent studies have further confirmed this observation that preclinical study quality remains suboptimal [7–10]. Indeed, the SAINT II trial of NXY-059 was based upon preclinical data that did not fully meet the STAIR criteria. Many of the animal studies were not blinded and the ability of NXY-059 to cross the blood–brain barrier and gain access to its target site remains in question [11]. Thus, while the STAIR committee has put forth rigorous standards, it remains a guideline that is often ignored in the haste to move toward a clinical trial. The improvement of study quality mandated by the STAIR recommendations, in combination with more appropriate use of existing models and further refinement of animal models to mimic clinical complexities are all required to ultimately improve the predictive value of animal models of ischemic stroke. Nonetheless, it remains possible that animal models of stroke are too dissimilar to the human stroke condition and that even complete implementation of the STAIR recommendations would not be sufficient to lead directly to successful clinical trials in stroke. Furthermore, successful translation will require continued refinement of animal models in combination with improvements and innovations in clinical trial design. Indeed, multiple more recent STAIR committee meetings have focused on strategies to improve clinical trial design, including utilization of new imaging methods, improved statistical analyses, refinements in patient selection, extended therapeutic windows and the possibility of combination therapies [1,4–5,12]. Therefore, we believe that enhanced quality of preclinical testing in concert with ongoing improvements in clinical trial design has the potential to lead to successful stroke therapies in the future.

Ischemic stroke models currently in use

Several methods are used to generate focal cerebral ischemia currently, in a variety of species. We will briefly discuss these methods (see Table 2 for summary of models discussed). The use of craniotomy to gain direct access to cerebral arteries is the foundation for several inter-related models. With access to cerebral arteries, vessel occlusion can be achieved mechanically using surgical clips [13,14], ligating sutures [15] or internally sealed by a photothrombotic lesion using a combination of Rose Bengal dye and light [16].

Generally, these occlusion models are used to produce permanent vessel occlusion, although it is sometimes possible to reperfuse. The major advantage of these methods is they are amenable to use with animals of varying sizes. Thus, these methods have been used to study ischemic stroke not only in the more standard laboratory animals such as mice, rats and rabbits, but also in larger animals such as dogs, cats, pigs and nonhuman primates. The larger species have advantages over rodents due to the gyrencephalic cortices that have gray to white matter ratios that more closely mimic the human brain. In addition to the capability of monitoring white matter damage following ischemic stroke in large animals, increased body size allows for sophisticated physiological monitoring such as blood gases, blood pressure, hemoglobin measurements, evoked potential monitoring and electroencephalography. Another advantage of large animals is the ability to perform pharmacokinetic and pharmacodynamics analysis. In addition, large animals are amenable to brain imaging methods used in humans, such as MRI, functional MRI and nuclear magnetic resonance spectroscopy. These imaging advantages have been overcome in recent years with the ability of many of these imaging methods to be applied to smaller animals, particularly mice and rats. The advantage of access to larger gyrencephalic brains, such as nonhuman primates, is countered by the disadvantage of required craniectomy. Most significantly is the inability to model the increased cerebral intracranial pressure experienced following ischemia, as evidenced by the observation that craniectomy has been shown to improve recovery following large strokes [17]. In addition, small animals offer the advantage of being far more cost effective to obtain and maintain and have the additional advantage of being genetically homogeneous and modifiable, specifically mice. The use of inbred strains of mice has exploded in recent years due to the increased availability of genetically engineered mice. The homogenous genetic background has improved reproducibility compared with out-bred strains of mice, rats and larger animals that have increased injury variability compared with inbred mice. However, this observation coupled with reports that different inbred strains of mice have different ischemic sensitivities [18] points to the shortcomings of the use of inbred mice, as the human population has very high genetic variability. Nonetheless, it is optimal to perform initial mechanistic studies using inbred mouse and rat models, such as the intraluminal filament and thromboembolic models described below.

The most commonly used method of inducing stroke without craniectomy is the intraluminal filament model [19], which uses a thread to occlude the middle cerebral artery (MCAO), modeling the site most commonly occluded in humans. This method involves introducing a nylon thread into the extra cranial internal carotid artery and advancing the tip of the thread until it occludes the origin of the middle cerebral artery (MCA), generally determined by the concurrent use of laser Doppler flowmetry to assess relative cortical perfusion. This method is routinely used in mice and rats. Translating this technique to larger species has been uncommon, although balloon catheters have proven useful in primates [20]. The most significant advantage of the intraluminal occlusion model is that the thread can be removed at any time resulting in reperfusion. This allows two distinct advantages; first, injury is able to be finely tuned based upon varying the ischemic duration; and second, this model allows the study of ischemia–reperfusion injury. Importantly, this intraluminal filament model has been demonstrated to produce injury that expands with time, modeling the ischemic

penumbra observed in humans. Thus, this model has been the model of choice for the majority of neuroprotection studies and remains the gold standard for mechanistic studies. Indeed, the use of the intraluminal filament model in rodents has facilitated the discovery of a multitude of mechanisms that contribute to ischemic injury. Detailed description of these various injury mechanisms can be found elsewhere [21], such as excessive glutamatergic transmission (excitotoxicity), programmed cell death (apoptosis), oxidative stress, vascular damage and injury to the blood–brain barrier and inflammatory responses. An interesting new endogenous mechanism of protection is the extravasation of microemboli to cause revascularization and restoration of blood flow [22]. Compounds targeting each of these mechanisms (and others) have been shown to provide protection in preclinical studies and have progressed to clinical trials. While the details regarding the various mechanisms identified are beyond the scope of the current review, it is important to note that interaction between these mechanisms in combination with comorbidities is likely a factor that has contributed to failed translation.

However, it is important to point out that while the intraluminal MCAO model has several advantages and has provided the field with important mechanisms of injury following ischemic stroke, it does not model the mechanism of occlusion observed in people – that is, thromboembolism. Indeed, most of the large ischemic strokes observed in humans are caused by thromboembolism and the only US FDA-approved treatment is the use of the thrombolytic agent tissue plasminogen activator (tPA), which, while not a true neuroprotectant, can improve outcome via enhanced reperfusion. For this reason, embolic stroke models have been developed. The most reliable thromboembolic model currently in use involves introducing a large thromboembolic clot via catheter close to the origin of the MCA, thus producing a large embolic MCAO similar to that observed using the intraluminal filament method [23]. It is important to consider the method of generating the clots as it has been reported that thrombin-induced clots are more resistant to the effects of tPA than clots generated using unmodified arterial blood allowed to spontaneously clot [24]. Thus, in order to take full advantage of the embolic stroke method and test neuroprotection strategies that may be combined with tPA, it is important to use clots amenable to reperfusion using tPA. The major drawback to the embolic stroke models is the variability of the rate and timing of reperfusion and the lack of experimental control regarding the exact duration of ischemia. Thus, this model is a good proxy for the human condition, as the use of autologous blood clots in these embolic models are currently the best model of cardioembolic strokes. However, this is a difficult experimental model to assess complex temporal mechanisms.

By contrast, the intraluminal filament model has greater temporal control, but less faithfully mimics the human clinical condition. We believe that the future of translational research will take advantage of these two models sequentially, using the intraluminal filament model to initially assess mechanisms and determine doses of lead compounds. Progress towards the clinic will then move the treatment determined via the intraluminal filament model to the embolic model. Finally, it must be considered that the vast majority of experimental studies performed to date use healthy, young adult animals, in stark contrast to the human population that are aging with a variety of comorbid clinical conditions. We anticipate that successful translation will require careful consideration of these comorbidities, some of which can be modeled in animal models.

Comorbidities & experimental stroke models

A major question in the experimental stroke field remains whether the neuroprotection observed in young, healthy animals can be extrapolated to the human population, which is generally relatively sick and aging. It is important to consider the impact of comorbidities, such as hypertension and diabetes, that are highly associated with ischemic stroke [5,25–26]. Various other risk factors have been observed, such as obesity, smoking and excessive alcohol consumption [26]. In addition to these important modifiable risk factors, it is important to consider age and gender when developing models to test new treatments for stroke. Figure 1 illustrates that animal models of stroke require consideration of various modifiable (hypertension, diabetes and obesity) and nonmodifiable (age, gender and genetics) risk factors, which all interact with each other to add to the complexities of stroke.

Age, gender & comorbidities

It is somewhat concerning that we perform almost all of our stroke studies in young, healthy animals, with little consideration of the fact that age is the most significant single risk factor for stroke [27]. The most likely reason for not studying aging animals is that it is extremely costly and time consuming. However, a few studies have addressed this issue and generally observe that aged animals have larger infarcts than young animal controls following experimental stroke [28–30]. Indeed, a few studies have observed altered responses in aged brains to treatments, demonstrating the importance of considering this important factor when testing new compounds. For example, testosterone replacement in aging animals is protective [31], compared with the generally detrimental effects of testosterone observed in young adult animals. Aging is associated with several factors that likely contribute to altered ischemic sensitivity and may alter the response of candidate therapies. Aged animals and humans exhibit evidence of increased oxidative stress, inflammation and vasculature changes that are consistent with the increased vulnerability to ischemia observed in the population [32]. Thus, we need to move our animal models towards inclusion of aging animals with one or more of the most common comorbidities (hypertension and/or diabetes/hypertension) in addition to the use of both male and female animals. This complex modeling is time consuming and costly, but clearly is necessary to more accurately model the human patient population. Another major roadblock to such complex model systems is the current model of basic science funding and grant review, which currently values reductionist research to answer very limited cellular and molecular mechanistic questions. Thus, the progression in the translational research community must embrace the complexities of diverging signaling cascades and the various health/genetic factors, which may all contribute to more reliable models of the human condition.

Hypertension

Hypertension, defined as elevated blood pressure >140/90 mmHg, significantly increases the risk of stroke, with studies estimating that between 30 and 52% of strokes are associated with hypertension and elevated blood pressure [26,33]. Importantly, hypertension is generally controlled with medication and the recent American Heart Association/American Stroke Association guidelines for the prevention of stroke in patients recommends the reduction of blood pressure, although the absolute target level remains in question [34].

The selectively bred spontaneously hypertensive rat (SHR) has been extensively studied to assess the role of hypertension in stroke [35]. The SHR rat develops hypertension within the first few months of birth and exhibits elevated blood pressure that appears to be maximal at approximately 200 mmHg. The stroke-prone SHR (SHRSP) rat was developed by inbreeding the offspring of SHR rats that died of stroke [36]. These two strains of rats have been extensively used and the induction of focal ischemia results in larger infarcts compared with control normotensive rat strains [37]. Chronic hypertension is associated with several changes that likely contribute to enhanced ischemic vulnerability, including structural changes to the cerebral vasculature [38–40], inflammation [41–43] and chronic oxidative stress [44]. Not surprisingly, antihypertensive drugs are effective in reducing cerebral ischemic injury in hypertensive animals, with the greatest effect being observed when the antihypertensive agent was administered immediately prior to stroke onset, with less protection observed when administered postischemia [35]. However, few studies have considered the effect of long-term treatment with antihypertensive drugs, as generally found in the human population. It is important to note that several other models of hypertension are available, some of which have been used in stroke studies. For example, hypertension can be induced by a high salt diet, and the Dahl salt-sensitive rat has been demonstrated to develop hypertension and have high mortality rates after experimental stroke [45]. In addition, hypertension can be induced in healthy young animals by renal artery clipping and/or kidney removal, again resulting in exacerbated injury following experimental stroke [46].

While the vast majority of neuroprotectant agents have been tested only in healthy normotensive animals, several neuroprotectants have been tested in both normotensive and hypertensive rats. In general, neuroprotectants have been observed to be less effective in hypertensive animals [35]. Interestingly, NXY-059 was less protective in hypertensive compared with healthy animals [11]. This is surprising considering the antioxidant effects of NXY-059. Indeed, a commonly investigated consequence of hypertension is increased expression of the enzyme NADPH oxidase, which appears to be the primary source of reactive oxygen species in the vasculature [47]. The decreased efficacy of antioxidant treatment in hypertensive animals may be due to genomic changes that occur as a consequence of chronic hypertension and oxidative stress [48–51]. Studies have indicated that hypertensive rats have genomic changes consistent with altered metabolism, oxygen handling, excitability, blood–brain barrier function and inflammation. Additionally, a recent study demonstrated that neurons obtained from SHRSP rats have enhanced sensitivity to ischemia and exhibited a greater percentage of apoptotic neurons following *in vitro* ischemia (oxygen–glucose deprivation) [52]. Thus, it is critical to thoroughly study cell death and protection mechanisms in combination with chronic and treated hypertension to unravel these complex interactions. The NXY-059 example again emphasizes the importance of improved study quality and analysis of comorbidities prior to initiation of clinical trials [11].

Neuroprotective drugs that aim to progress to clinical trials should be tested for efficacy in the context of hypertension, among other comorbidities. In addition to the genetically inbred SHR rats, use of nongenetic models of hypertension are available and should be used to test new compounds prior to clinical trials. Most importantly, the human patient populations generally do not have untreated hypertension, rather humans have medically controlled

hypertension to bring blood pressure towards the normal range. Unfortunately, the interaction between chronic normalization of blood pressure in hypertensive animals and ischemic stroke outcome has not been reported. It is strongly recommended that future neuroprotectants be tested in hypertensive animals with controlled blood pressure in order to more closely mimic the clinical situation. In addition, it is important to perform similar studies in aging, hypertensive animals. However, such studies are very time-intensive and costly and thus likely should be reserved for the testing of compounds that show promise in the more feasible animal models.

Diabetes, hyperglycemia & obesity

Hyperglycemia and diabetes are extremely common in stroke patients, with estimates of up to 25% of stroke patients having a history of diabetes [53] and an even larger proportion presenting with hyperglycemia at the time of admission for stroke [54]. Similarly, obesity dramatically increases the risk for stroke, and is associated with diabetes and hyperglycemia. Thus, like hypertension, it is critical to test the effects of diabetes and obesity on stroke injury and its interaction with neuroprotectants. Several animal models of diabetes and hyperglycemia are available, including toxin-induced Type 1 diabetes (alloxan and streptozotocin) as well as genetic animal models of Type 2 diabetes (db/db mouse, nonobese diabetic mouse and Zucker diabetic fatty rat, among others) [55]. Importantly, diabetic animal models, like humans, exhibit both hyperglycemia and obesity. Interestingly, obesity and chronic hyperglycemia cause similar mechanistic changes that are associated with increased ischemic sensitivity. In addition to models of diabetes, acute hyperglycemia has been used in combination with stroke to determine interactions. Elevated blood glucose using intraperitoneal injection of dextrose has been observed to cause increased infarct size following experimental stroke in mice, rats, rabbits and dogs [56–61]. Similarly, mice and rats with genetically induced diabetes exhibit increased injury [62]. The mechanism of hyperglycemia-enhanced injury has been the focus of intensive research, with a clear role for acidosis [63,64] and alterations in the vasculature being described. Hyperglycemic/diabetic animals have been observed to have worse blood–brain barrier damage following ischemia compared with control animals [57,65–67]. Several mechanisms have been linked to vascular dysfunction following stroke in hyperglycemic and diabetic animals, including increased inflammatory infiltration [68,69], increased oxidative stress and MMP-9 activation [66], impaired nitric oxide signaling [70] and vascular remodeling, which reduces elasticity and alters resistance has been observed in diabetic animals [71,72] and, more recently, in humans [73].

Interestingly, attempts have been made to determine whether glycemic control of diabetic animals alters stroke outcome, with one group observing no benefit of normoglycemia in diabetic rats [74]. By contrast, insulin treatment to reduce blood glucose has been reported to reduce infarct volume [75–77]. Insulin neuroprotection in diabetic animals was recently attributed to normalization of endothelial nitric oxide signaling [78]. However, it should be noted that insulin is also neuroprotective in healthy normoglycemic animals [79], via various signaling pathways downstream of brain insulin receptors, including PI3K/AKT activity [80,81], enhanced synaptic plasticity/decreased excitability [82–84] and improved cerebral blood flow [81,85]. Thus, the complex interaction between treatments to normalize blood

glucose and ischemic sensitivity need to be further investigated before translational potential can be determined. As many diabetic humans routinely take insulin to treat Type 1 and Type 2 diabetes, it is critical to modify experimental stroke models to test the effects of new potentially protective agents in combination with insulin-treated diabetes. Similarly, the explosion of Type 2 diabetes throughout the world has led to increasing numbers of people using various glycemic compounds to control blood glucose levels. Future studies of neuroprotectants should take advantage of the various diabetes models to assess the interaction between diabetes, stroke and promising therapeutic agents.

Gender

Epidemiological data indicate that stroke rates are strikingly sex-specific, exhibiting incidence rates that are high in men compared with women, regardless of ethnicity [86]. Stroke risk increases with age in both sexes, eventually normalizing at ages >60–75 years. As women have longer life expectancy than men, despite the reduced rate of incidence, it is expected that more women than men will suffer stroke during their lifetimes and emerging evidence indicates that outcome may be worse in aged women compared with men [87]. Experimental stroke data reliably show that female brains suffer relatively smaller injury compared with males. Most studies have utilized rodent models of focal ischemia and focused on ischemic injury and the role of female sex steroids in protecting the female brain. Extensive research has focused on the mechanism of relative protection in female animals and several studies have observed the enhanced sensitivity to injury in males with various comorbidities, such as diabetes [62,74] and hypertension [88]. While these studies have analyzed outcome differences in the male and female brain, an important exception is the study by Yamori and colleagues that demonstrated earlier incidence of stroke in male SHRSP rats compared with age matched females [89]. The extensive epidemiological data and experimental literature demonstrating sex differences in incidence and outcome makes it important to consider sex in preclinical models of neuroprotectants (STAIR 1999). The simplest explanation for the ongoing use of only male animals in experimental stroke studies is the doubling of animal costs as a consequence of using both male and female animals in each study, along with the lack of estrus cycle in male animals, which may reduce variability. Nonetheless, while expensive and time consuming, it is critical that neuroprotectants be tested in both male and female animals prior to considering clinical trial design, as there is extensive literature indicating that some protective strategies are effective in only one gender [90,91].

Plasticity & repair

It is critical to consider long-term functional recovery, rehabilitation and repair of neuronal networks when designing new treatments for brain injury. A vast majority of preclinical stroke studies report acute outcomes and generally focus on histological injury within the first 24–72 h after stroke. However, analysis of longer-term outcomes (weeks to months) is critical. Lesion size in humans and infarct size in rodents do not always correlate well with functional deficits and recovery. Interestingly, it was recently demonstrated that aging animals suffer smaller infarcts compared with young adult animals, while functional recovery was worse in the aged animals [92]. Functional recovery is one of the major end points of all clinical trials and thus should be incorporated into experimental studies.

Researchers have spent decades developing robust neurobehavioral tests that are reliably used in rodents and should be taken advantage of in future experimental stroke studies [93]. Additionally, in light of the failure of so many neuroprotectants to translate into significant clinical benefit, it is important to begin to investigate repair mechanisms that may be utilized to augment current rehabilitation strategies. In order to improve functional recovery, it is important to consider the effects of ischemia on the brain's networks. Clinical and basic science research has demonstrated impaired plasticity following ischemia, defined as diminished ability of neurons to alter synaptic strength in response to stimuli. An emerging understanding of the long-term imbalance between excitatory and inhibitory circuits has provided a new direction for research aimed at improving functional recovery of the brain. Several reports have observed diminished excitatory and enhanced inhibitory neurotransmission weeks to months after experimental stroke [94] and most excitingly, that targeted increases in excitatory drive or inhibition of inhibitory networks can improve motor function after cortical strokes [95,96]. Thus, emerging animal models will have the opportunity to interrogate the effects of treatments on brain circuits with a view towards restoring balance and thus functional connectivity in the injured brain.

Conclusion & future perspective

Thousands of experimental studies have demonstrated neuroprotection using hundreds of promising new therapeutic interventions. Unfortunately, despite a multitude of large, time-consuming and extremely expensive clinical trials, to date, there have been no successful trials of neuroprotectants. This review summarizes the most commonly used animal models currently available to study stroke and described some of the shortcomings that are likely to be addressed in the coming years. Most important is the full implementation of the STAIR committee recommendations to improve preclinical study quality, such as more rigorous experimental design, use of functional outcome measures and improved statistical analyses. It is true that complete implementation of the STAIR recommendations may not be sufficient to lead directly to successful clinical trials in stroke, nonetheless, we firmly believe that enhanced quality of preclinical testing to include consideration of comorbidities coupled with ongoing improvements in clinical trial design has the potential to lead to successful stroke therapies in the future. We suggest that the current intraluminal filament model of ischemic stroke in rodents represents the optimal first step in the discovery of mechanisms of injury and to interrogate molecular mechanisms of new neuroprotectants. A stepwise progression towards high-quality preclinical data is recommended to form the basis for future clinical trials. Following initial testing using the filament MCAO model, testing new neuroprotectants in combination with tPA in an embolic rodent model is suggested, unless the new agent to be tested is a thrombolytic, in which case it is not necessary to test in combination with tPA. Optimal preclinical modeling of the complexity of stroke observed in the patient population will require consideration of the multitude of risk factors observed in the clinic. Thus, testing agents in aging rodents with various comorbidities is the next step, followed ultimately by use of nonhuman primate models to test safety and efficacy in a gyrencephalic brain. Finally, Phase I safety studies should include imaging to determine pharmacokinetics and blood-brain barrier permeability before proceeding to large and costly Phase II/III trials. This stepwise process will be time-intensive and very costly. However,

the enormous cost, time and effort to perform a clinical trial far outweigh the cost, time and effort to performing preclinical studies with comorbidities. In conclusion, the stroke research community currently has at its disposal all the necessary experimental tools to more reliably model the stroke patient population and with improved study quality and careful consideration of various comorbidities there remains reason to be hopeful that neuroprotectants will eventually be translatable in order to improve outcomes in patients following stroke.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The project is funded by NIH grants NS046072, NS080851 and the Walter S and Lucienne Driskill Foundation.

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Executive Summary

Ischemic stroke models currently in use

- Photothrombotic stroke useful for localized stroke models; however, it is a permanent occlusion that requires craniectomy.
- Intraluminal filament MCAO is the most commonly used model that has the benefit of not requiring craniectomy, being easy to control ischemic duration and allowing reperfusion. However, MCAO not suitable for testing combined therapies with tissue plasminogen activator.
- Thromboembolic stroke models have the strength of mimicking human embolic strokes and good for testing in combination with thrombolytic treatments. Difficult to assess complex temporal changes and not easy to reperfuse are downsides to this model.

Comorbidities & experimental stroke models

- An introduction to the importance of incorporating comorbidities into animal modeling for preclinical ischemic stroke research.
 - Age, gender and comorbidities
 - Hypertension
- Provides background on role of hypertension in stroke prevalence and a discussion of the animal models available to study ischemic stroke in animals with hypertension.
 - Diabetes, hyperglycemia and obesity
- Provides a background on the role of diabetes and hyperglycemia in stroke prevalence and a discussion of the animal models available to study ischemic stroke in animals with hypertension.
 - Gender
- Discusses the epidemiological data demonstrating the sexually dimorphic nature of ischemic stroke and animal models that recapitulate this important epidemiological data.
 - Plasticity and repair
- Discusses the importance of considering rehabilitation and repair of neuronal networks when modeling ischemic stroke and developing new therapeutic approaches.

Figure 1.
Schematic of the complex interaction between animal models and comorbidities.

Table 1

Overview of recommendations for standards regarding preclinical drug development put forward by the Stroke Therapy Academic Industry Roundtable (STAIR).

STAIR criteria	Recommendations
Rodent models	Improve blinding and randomization Perform behavioral outcome measures in combination with histopathology Independent replication in at least two laboratories
Primate studies	Extension of promising studies in rodents
Drug administration	Appropriate route must be considered and optimal CNS levels of experimental compound determined
Toxicology	Toxicology performed in several species, in both intact and animals with stroke Sex differences should be considered for protection and toxicology studies
Dose–response	Careful dose–response relations to be performed in preclinical models before clinical studies
Time window	Determination of therapeutic window critical for appropriate clinical trial design

Data taken from [3].

Table 2

Overview of advantages and disadvantages of experimental stroke models.

Experimental stroke model	Advantages	Disadvantages
Photothrombotic	Amendable to animals of varying sizes Specific anatomical localization	Permanent occlusion Requires craniectomy
Intraluminal filament MCAO (transient or permanent)	No craniectomy Control of ischemic duration/magnitude Easy to assess temporal progression of postischemic signaling Reperfusion	Filament does not mimic human occlusion Not suitable to test combined therapies with tissue plasminogen activator
Thromboembolic	Blood clot mimics human embolic stroke Good model for testing thrombolytic combined with neuroprotectants	Not easy to reperfuse Difficult to assess complex temporal changes