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REVIEW

Are Underlying Assumptions of Current Animal Models of Human Stroke Correct: from STAIRs to High Hurdles?

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Abstract Animal models of acute ischemic stroke have been criticized for failing to translate to human stroke. Nevertheless, animal models are necessary to improve our understanding of stroke pathophysiology and to guide the development of new stroke therapies. The rabbit embolic clot model is one animal model that has led to an effective therapy in human acute ischemic stroke, namely tissue plasminogen activator (tPA). We propose that potential compounds that demonstrate efficacy in non-rabbit animal models of acute ischemic stroke should also be tested in the rabbit embolic blood clot model *and*, where appropriate, compared to tPA prior to investigation in humans. Furthermore, the use of anesthesia needs to be considered as a major confounder in animal models of acute ischemic stroke, and death should be included as an outcome measure in animal stroke studies. These steps, along with the current STAIRs recommendations, may improve the successful translation of experimental therapies to clinical stroke treatments.

Keywords Cerebral ischemia · Stroke · Animal models · Neuroprotection · Tissue plasminogen activator · Rabbit

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Abbreviations

ApoE	Apolipoprotein E
FDA	Food and Drug Administration
HDL	High-density lipoprotein
ICAM-1	Intercellular adhesion molecule 1
LDL	Low-density lipoprotein
NXY-059	Free radical scavenger
RSCEM	Rabbit small clot embolism model
rt-PA	Recombinant tissue plasminogen activator
SAINT I/II	Stroke Acute Ischemic Stroke NXY-059 Trial
STAIR	Stroke Therapy Academic Industry Roundtable
tPA	Tissue plasminogen activator

Introduction

The uncertainty as to why animal studies of acute cerebral ischemia fail to translate into human stroke treatments continues with NXY-059 in the SAINT II trial [1, 2]. The Stroke Therapy Academic Industry Roundtable (STAIR) recommendations [3–8] have identified important issues in the experimental modeling of ischemic stroke and have sought to promote the translation of animal studies to successful human stroke trials. The STAIR recommendations outline suggestions to optimally pre-clinically assess potential neuroprotective and restorative drugs for the treatment of acute ischemic stroke. Briefly, they include recommendations for drug dose, therapeutic window, choice of animal model, physiological monitoring, outcome measures, and sex differences, amongst others. However, despite such recommendations successful experimental to clinical translation has yet to be achieved. This suggests that further modifications and changes may be required to experimental paradigms in order to achieve such results.

Rather than assess these criteria, or address the many excellent reviews in the literature [9–12], we instead seek to highlight features of stroke models that may have been underestimated, and some assumptions that have been made in the development of animal models of acute ischemic stroke and the testing of neuroprotective agents. The goal is to promote discussion and possible modification of models to ultimately improve the translation of experimental stroke studies to the clinical setting [9, 10, 13–20].

The inconsistency amongst animal models, in addition to the lack of a clear progression of testing to humans has proved a major obstacle in the translation of treatments from animal models to human stroke. One experimental model which has not been adequately considered in experimental stroke research is the rabbit embolic clot model [21]. To date, this is one of the only models which has yielded/predicted a treatment approved for use in acute ischemic stroke in humans—tissue plasminogen activator (tPA) [22]. Notably, however, tPA was first shown to be efficacious in rodent stroke models [23–30]. Thus, there may be some value in comparing the two models and addressing whether one is "better" than the other particularly since the rabbit model is seldom used. Even though tPA improves outcomes in both the rabbit and rodent models, this does not mean that either model actually would translate to humans. For example, just because hundreds of neuroprotectants work in rodent stroke models, this does not mean they translated to humans. If one assumed, for the sake of argument, that it is the results in the rabbit model that predicted translation to humans, the rabbit model should be used for translational studies. Factors that might account for translation in rabbits and not rodents might include: the rabbit immune system is different from the rodent; the particular blood clot model used; the clot—endothelial cell interaction is closer to humans in the rabbit model; rabbit lipid metabolism is closer to humans; the use of death as an outcome measure in the rabbit model; the absence of anesthesia in the rabbit model; and other factors discussed below. Thus, the rabbit results—and possibly not the rodent results—predicted tPA to be a clinical stroke treatment [21, 31–33].

Since it is not possible at present to decide whether the rabbit or the rodent models are "best" for translational studies, we propose that it may be beneficial for compounds that have been found to be effective in non-rabbit models (rat, mouse, non-human primate, and others) to also demonstrate efficacy in the rabbit embolic blood clot model before proceeding to clinical testing. Clearly, it may be useful, more convenient and cost-effective to initially test compounds in rodent blood clot embolic models [34]. Indeed, as highlighted by the STAIR recommendations [3–8], testing potential therapeutics in a second species, and importantly in a species already shown to predict improved outcome in acute ischemic stroke, would improve the likelihood of successful translation. Compounds successful in such a setting should then be given a high priority for evaluation in humans. In contrast, those compounds demonstrating efficacy in only one animal model or species are less likely to be successfully translated into human stroke. We propose that compounds that prove effective in a given model also be tested in the rabbit blood clot embolic model.

This concept also extends to neuroprotection and combination therapy with tPA. Accordingly, when compounds are tested in the rabbit embolic model, they could be evaluated on their own compared to vehicle, but more importantly, where appropriate, they should be compared directly to tPA. A compound shown to improve acute ischemic stroke outcomes comparable to tPA may be more likely to translate to human stroke. This concept would apply to all compounds proposed to improve acute ischemic stroke, including new thrombolytiics and neuroprotectants. That is, if the neuroprotection provided by a "neuroprotectant" is similar to or better than that obtained with tPA, then the "neuroprotectant" is more likely to translate to human stroke. A second consideration is the combination of compounds with tPA. Several neuroprotectants when co-administered with tPA have been identified to improve outcomes in non-rabbit models of acute ischemic stroke [35]. Evaluating such agents further in conjunction with tPA in the rabbit embolic model would provide additional support for potential clinical efficacy.

Acute Ischemic Stroke Models in the Rat and Mouse are not Equivalent to the Rabbit

Certainly, the rabbit model of ischemic stroke has been used to a lesser extent than rodent models. This may be in part due to the increased cost associated with rabbit experiments. Nevertheless, the predictive power of these animal models may differ considerably. Although tPA improves outcomes in both rat and mice blood clot embolic models [25, 36–38], as well as in the rabbit embolic clot model [21], it does not necessarily follow that since tPA works in both rodents and rabbits that the predictive power of the rabbit model is shared by that of the rodent model. Ideally, one should show efficacy in the rodent and the rabbit clot embolic models. Thus, the rabbit becomes the confirmatory "second animal model" as suggested by the STAIRS recommendations.

Human stroke generally involves progression of underlying vascular disease, and is associated with increasing age, hypertension, diabetes, hyperlipidemia, smoking, and heart disease [39, 40]. Accordingly, models that incorporate such factors are more likely to be predictive of clinical efficacy than those that do not. None of the animal models

truly replicate human stroke. They are simply models of stroke. However, choosing the most appropriate animal models for the research question is essential for the success of both experimental and clinical testing. Accordingly, there may be several explanations for the efficacy of the rabbit model and indeed several advantages of this model.

Atherosclerosis

Rodents and rabbits differ in the development of atherosclerosis. Rabbits develop atherosclerosis and share many aspects of human lipoprotein metabolism. For example, the composition of lipoproteins, production of Apoβ100-containing VLDLs by liver, cholesteryl ester transfer activity, and high absorption of dietary cholesterol are similar in humans and rabbits [41, 42]. Transgenic rodents are available which have increased plasma cholesterol and triglycerides as well as low high-density lipoprotein levels [42]. Rabbits, however, rapidly develop atherosclerosis on hypercholesterolemic diets (0.5–4%/weight) where dietary cholesterol supplementation leads to the development of fatty streaks [41]. Though rodents may also develop atherosclerosis, they are inherently much more resistant than rabbits [43]. Atherosclerosis may be induced in rodents with dietary and genetic manipulations, such as apolipoprotein E (ApoE; −/−) and low-density lipoprotein (LDL; −/−), although they develop very unstable atherosclerotic lesions [44–46]. Furthermore, the cholesterol metabolism of rodents is more geared towards HDL, rather that LDL, like that in both humans and rabbits [45]. This is of clinical relevance as the expression of ApoE and LDL receptors differs in young adult versus old rats following cerebral ischemia [47]. Atherosclerosis is an important risk factor in patients with ischemia [48]. Therefore, consideration of this pathology in animal stroke models is crucial. The simple fact that rabbits develop atherosclerosis whereas normal rodents do not may make the rabbit a better model in which to test compounds for stroke due to the lipid metabolism and/or endothelial differences in rabbits versus rodents. Rabbits are commonly used for the study of atherosclerosis and for cardiovascular diseases for these very reasons [49, 50].

The Immune System

Another issue that has not been considered in animal models of stroke is whether the immune system of the rodent is appropriate for modeling that of human stroke. The immune system is critical in human stroke [51–53] as exemplified by the enlimomab clinical trial [54] where anti-ICAM-1 antibody significantly worsened stroke outcome [55]. The composition and type of immune response are important factors in modeling human stroke remains to be seen [56]. The rodent immune cell composition is remarkably different from that of rabbits and humans. Specifically, rodents have a lymphocyte predominance with a 1:5 ratio of neutrophils to lymphocytes. In contrast, rabbits have a 1:1 ratio of neutrophils to lymphocytes, which is similar to the immune system in humans who have a 2:1 ratio of neutrophils to lymphocytes [57, 58]. Rodents and humans also differ significantly in the systemic immune cell gene expression response to ischemic stroke [59–63]. Although, this represents only one aspect of the immune system, it suggests that the way the rodent immune system responds to cerebral ischemia may not reflect that of the human immune response to acute ischemic stroke. It is not known whether the rabbit immune response to cerebral ischemia is similar to humans or not.

Anesthesia

The majority of patients with ischemic stroke are not anesthetized. The effect of a stroke or response to stroke may differ greatly with anesthesia. Accordingly, anesthesia may markedly confound experimental stroke studies. However, there are animal models of stroke that are able to induce an ischemic stroke in conscious animals [21, 64–66]. Such models are likely to model human stroke more closely than those models using anesthesia during the induction of stroke. Specifically, the rabbit blood clot embolism model [21] involves the preparation of animals under anesthesia and then later, the stroke is induced in awake, un-anesthetized animals. Moreover, numerous studies have demonstrated anesthetic agents afford a degree of protection from cerebral ischemia [67–70]. Even light surgical anesthesia may substantially reduce infarct size following stroke [71]. Accordingly, the contribution of anesthesia to the experimental paradigm and potential neuroprotection requires careful consideration, and ideally the confounding effects of anesthesia must be eliminated from animal stroke models.

tPA Effectiveness in Human Stroke Provides Insight into Animal Models

Thrombolysis with tPA is effective in humans in cardioembolic, large vessel thromboembolic, and small vessel lacunar stroke [22, 72]. This finding has important implications for animal models of stroke and for testing of drugs to treat human stroke. Since cardioembolic stroke is due to embolic blood clot, then animal models of embolic autologous clot likely models this type of stroke. There are

no accepted animal models that mimic large vessel thromboembolic stroke and small vessel lacunar stroke in humans [73, 74]. However, since tPA improves outcome in all three human ischemic stroke subtypes, this suggests that all three types of human stroke are caused at least in part by clots that are acted upon by tPA. For cardioembolic strokes, the clots would usually come from the heart. For large vessel strokes the clots would come from the parent vessels likely related to atherosclerosis. For lacunar strokes, clots might form because of abnormalities in the vessel wall or platelet vessel wall interactions. Thus, the blood clot embolic animal models—be they rabbit or rodent—could be viewed as reasonable mimics of human cardioembolic, large vessel and lacunar causes of human stroke. Thus, animal models in which tPA was effective might also be considered as potential models of important aspects of the pathophysiology of these three human stroke subtypes. Addressing treatments for all three causes of human stroke is essential for translation to human stroke trials because few human stroke trials to date, with the exception of cardioembolic stroke, have considered the cause of stroke when choosing subjects to treat.

Outcome Measures in Animal Models—What is the Best One?

In animal studies, a "neuroprotective" compound is typically compared to a "saline or vehicle control." The common outcome measures are either a statistically significant decrease in infarct volume or an improvement in a given behavior [34]. However, these outcome measures in animals may not translate to improving outcomes in human stroke. Thus, alternate outcomes need to also be considered. The original rabbit clot embolic model used death as an outcome measure. This is in contrast to most other animal stroke models. In fact, most rodent studies discard animals that die from as a result of ischemic stroke [75]. Indeed, survival studies are becoming increasingly important as death is an important outcome measure in human studies and may be one of the reasons that the rabbit embolic clot model was predictive of efficacy in humans. Therefore, the inclusion of survival rates in experimental stroke studies is critical in determining the true efficacy of a potential therapeutic agent as excluding animals that have died from the study significantly skews the results so that findings are biased towards surviving animals [34, 75]. In the various trials of tPA, the drug decreased morbidity and mortality [76]. Even if one accepted standard behavioral measures short of death, the behaviors in rats, mice, rabbits and even primates that predict clinical efficacy of therapies for stroke in humans are unknown. Additionally, outcome measures are evaluated at short times following ischemia and not at the times used in clinical trials. Thus, if nothing else death provides at least one additional outcome measure. Using death as the outcome measure in the rabbit embolic clot model likely means that other behavioral assays do not have to be performed and there would be no need to find the behaviors that translate to humans. Moreover, since death was the major behavioral outcome used to demonstrate tPA efficacy in the rabbit blood clot embolic stroke model, this same behavioral endpoint may be useful when testing new neuroprotectants and/or thrombolytics in the rabbit embolic clot model and probably should be included in rodent stroke models.

Concluding Remarks

To date, there has been a lack of translation of stroke treatments from animals to humans. The rabbit blood clot model of embolic stroke, in which tPA improved stroke outcomes, may be a relevant animal model for predicting efficacy of a drug in human stroke. We suggest that blood clot embolic models should be the primary ones evaluated, and head to head comparison with tPA should be performed in the rabbit model and other models. The contribution of the immune system and atherosclerosis must be included in experimental models along with the other STAIR recommendations. Additionally, death needs to be included as an outcome measure. Ultimately, the goal of translational stroke research is to find the simplest yet most predictive animal model of ischemic stroke so that potential treatments can be successfully and rapidly moved to the clinic and improve the care of patients with stroke.

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