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ORIGINAL PAPER

Improving the translation of animal ischemic stroke studies to humans

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Abstract Despite testing more than 1,026 therapeutic strategies in models of ischemic stroke and 114 therapies in human ischemic stroke, only one agent tissue plasminogen activator has successfully been translated to clinical practice as a treatment for acute stroke. Though disappointing, this immense body of work has led to a rethinking of animal stroke models and how to better translate therapies to patients with ischemic stroke. Several recommendations have been made, including the STAIR recommendations and statements of RIGOR from the NIH/NINDS. In this commentary we discuss additional aspects that may be important to improve the translational success of ischemic stroke therapies. These include use of tissue plasminogen activator in animal studies; modeling ischemic stroke heterogeneity in terms of infarct size and cause of human stroke; addressing the confounding effect of anesthesia; use of comparable therapeutic dosage between humans and animals based on biological effect; modeling the human immune system; and developing outcome measures in animals comparable to those used in human stroke trials. With additional study and improved animal modeling of factors involved in human ischemic stroke, we are optimistic that new stroke therapies will be developed.

Keywords Stroke · Animal models · Clinical trial · Cerebrovascular disease

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Introduction

The translation of acute ischemic stroke therapies from animals to patients with ischemic stroke has been challenging. With over 1,026 agents tested in models of ischemic stroke and 114 tested in humans, only one medical therapy, tissue plasminogen activator (tPA), has received approval by the FDA for the acute treatment of ischemic stroke (O'Collins et al. 2006)(Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group 1995). The reasons for this failure to translate has been the subject of numerous meetings (STAIR recommendation) and reviews (Stroke Therapy Academic Industry 2001; Fisher and Stroke Therapy Academic Industry 2003; Fisher et al. 2005; Fisher et al. 2007; Fisher et al. 2009; Saver et al. 2009; Albers et al. 2011; Lapchak et al. 2013; Feuerstein et al. 2008). In addition, the NIH/NINDS have published criteria regarding the rigor of animal stroke studies (Landis et al. 2012; Lapchak et al. 2013). Proposed recommendations to improve the translation of acute stroke therapies have included evaluation of agents in the therapeutic time window of ischemic stroke, need to assess dose response, need to assess aged animals of both sexes with vascular risk factors, therapy not acting on the intended therapeutic target, importance of randomization and blinding, defining inclusion and exclusion criteria, ensuring studies are adequately powered, replication of studies by independent groups, declaration of conflicts of interests, and need to evaluate therapies in multiple animal models of stroke including gyrencephalic species.

The goal of the current commentary is not to review prior recommendations with which we agree, but to discuss additional aspects that may be important to the translational success of ischemic stroke therapies. Frequently many of the aspects incorporated in the design of a human stroke trial are not incorporated into animal studies of acute ischemic stroke.

However, modeling features of a clinical stroke trial in animals may be essential to determine whether a therapy will ultimately translate to human ischemic stroke. Presented below is a discussion of several factors that may advance preclinical stroke models, including use of tissue plasminogen activator, modeling ischemic stroke heterogeneity in terms of infarct size and cause of human stroke, addressing the confounding effect of anesthesia, dosing therapeutics based on biological effect, modeling the human immune system, and developing methods to assess animal stroke outcomes similar to measures used in human stroke trials.

Implications of tPA for animal studies

tPA is standard of care for patients acute ischemic stroke (Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group 1995). Thus, in human clinical trials evaluating new treatments for acute ischemic stroke, patients treated with tPA are likely to be enrolled. As a result, animal stroke models evaluating acute stroke therapies should also include a group of animals treated with tPA. The inclusion of a tPA treated animal group has several advantages. It permits evaluation of the interaction between the new treatment and tPA, including assessing the impact on thrombolytic activity and adverse events. In addition, tPA treated animals can be used as a positive control as discussed below.

Compounds being considered for clinical trials may benefit from having preclinical animal data comparing the compound of interest to tPA. The rationale is that tPA is the only drug where the observed beneficial effect in animal ischemic stroke has translated to a benefit in human ischemic stroke. If a test compound can demonstrate a beneficial effect similar to the effect of tPA in animals, then it may have an increased probability to be effective in humans. For example, a lead compound could be compared to tPA and vehicle control in a blood clot embolic model. To move forward, the test compound should be as effective as tPA in treated animals. The blood clot ischemic stroke model is suggested because of its prior success in translating tPA to stroke patients, and thrombosis/clot embolism is an important feature of all three human stroke subtypes (Hsia et al. 2003; Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group 1995).

Use of anesthesia

In patients with ischemic stroke anesthesia is generally not used, and most strokes do not occur while a patient is anesthetized. However, in the majority of animal stroke models cerebral ischemia is induced while animals are under anesthesia. Though some studies have suggested anesthesia may not affect outcomes, this may depend on type of anesthetic and possibly the cause of stroke (Gelb et al. 2002; Head and Patel 2007; Kirsch et al. 1996; Koerner and Brambrink 2006; Wang et al. 2008; Zivin et al. 1985). Stroke models without anesthesia are needed to evaluate lead compounds being considered for clinical stroke trials. Alternatively, one might question whether anesthesia should be used in patients with acute ischemic stroke. Anesthesia may have neuroprotective effects and may be an important factor to help translate certain compounds with identified benefit in animal stroke models to patients (Gakuba et al. 2011). However, the routine use of anesthetics in patients with ischemic stroke has a number of limitations, including loss of neurological exam to monitor response to therapy, effects on blood pressure and cerebral perfusion, association with worse outcomes in endovascular treated patients, and need for intensive care that is not widely available in all centers treating patients with ischemic stroke (Froehler et al. 2012; Flexman et al. 2012; Davis et al. 2012; Abou-Chebl et al. 2010).

Importance of stroke cause

Ischemic stroke in humans is heterogeneous. Clinical stroke trials generally enroll stroke patients of diverse age, gender, ethnicity, and importantly, stroke cause. The value of modeling age and gender in animal stroke studies has been addressed in the STAIR criteria. However, the importance of modeling stroke cause warrants further consideration, as it too may influence the translational success of an acute stroke therapy. Given most stroke trials include multiple causes of stroke, having preclinical data demonstrating effectiveness in multiple animal stroke models that emulate the major causes of human ischemic stroke may be important to translational success.

The three major causes of human ischemic stroke are large vessel atherosclerosis, cardioembolism, and lacunar small vessel disease. Each has a different pathogenesis. Large vessel stroke is due to atherosclerotic plaque rupture and embolization; cardioembolic stroke is due to cardiac clot formation and embolization; and lacunar stroke is due to a disease of the small penetrating cerebral vessels. It is not known if the mechanisms of brain injury differ between these three major causes of human stroke. However, different prognosis, mortality, and stroke prevention treatment highlight that differences among cardioembolic, large vessel, and lacunar stroke exist and potentially may influence acute ischemic stroke treatment. We have found molecular differences in inflammation and gene response among the three major causes of ischemic stroke in humans (Jickling et al. 2011; Jickling et al. 2010). This suggests that aspects of the immune response are different for each major cause of stroke. Though further study is required, it is possible that the molecular response to

brain ischemia and neuroinflammation may differ for each cause of stroke. Thus, certain acute stroke treatments may have greater therapeutic effect for certain causes of stroke.

If cause of stroke is important to the treatment of acute ischemic stroke, then animal studies should be performed in models of the three major causes of human stroke (large vessel atherosclerotic, cardioembolic, and small vessel lacunar). Currently most preclinical animal studies use a filament stroke model, which models cerebral ischemia well but does not replicate any of the three major causes of human stroke. This is emphasized by the fact that very few genes regulated in the blood of rats following filament induced stroke are regulated in the blood of human ischemic stroke (Tang et al. 2006; Tang et al. 2001; Stamova et al. 2010). Different patterns of infarct evolution have also been observed in embolic compared to filament stroke models in rodents (Henninger et al. 2006). Furthermore, Hossmann has argued that the filament model is a poor model of human stroke that should be abandoned to test therapies for human ischemic stroke because the pathophysiology of ischemic brain injury induced by mechanical vascular occlusion is different from the thromboembolic occlusion that occurs in humans (Hossmann 2012).

A therapy shown to be effective in the filament stroke model should have additional evaluation performed in stroke models that have greater similarity to the human causes of cerebral ischemia. The blood clot/embolic stroke model emulates aspects of human cardioembolic and arterial embolism. This model has been applied in rodents and rabbits, and as discussed below has the benefit of producing variable infarct size (Lapchak 2010; 2009; Meyer et al. 2013; Chapman et al. 2001). Large vessel atherosclerotic stroke has been modeled in apolipoprotein E deficient mice and in rabbits fed a high fat diet (Zhang et al. 2010; Daugherty et al. 2009; Verbeuren 2006; Russell and Proctor 2006; Yanni 2004). Small vessel ischemic lacunar stroke has been modeled using the Spontaneous Hypertensive Rat (SHR), though additional models of cerebral small vessel disease are needed (Bailey et al. 2009, 2011a, b; Yao and Nabika 2012; Hainsworth and Markus 2008). In both atherosclerotic and SHR stroke models, the occurrence of cerebral ischemia is spontaneous and requires monitoring for ischemic stroke occurrence. There are many other stroke models not mentioned here, including a photocoagulation model, the in situ thrombin administration model (Orset et al. 2007), and the ferric chloride application model (Karatas et al. 2011). The relationship of these models to three major causes of stroke in humans is requires further study.

Infarct size, is heterogeneity desirable in animal models

Infarct size of patients enrolled in clinical stroke trials is usually quite variable, ranging from small infarcts causing minor disability to very large infarcts that result in marked disability and death (Lansberg et al. 2011, 2012; Kidwell et al. 2013). In contrast, in animal stroke studies an infarct of consistent size is usually sought as this facilitates determination of infarct size reduction in response to treatment (Takano et al. 1997; Belayev et al. 1996). Given the variability in human infarct size, animal stroke studies may better translate to patients if treatments are assessed across a range of infarct sizes. Such a design would require a larger numbers of animals and thus be more difficult and expensive. However, given the expense of human trials these larger animal studies that mimic clinical stroke trials may be warranted. This might be considered over modeling but strokes of different volume may have different degrees of blood brain barrier damage, inflammatory response, and potentially therapeutic response. The clot stroke model is one model that does produce variable infarct size. In the rabbit blood clot model cerebral infarcts of different sizes have been used to construct treatment response curves to assess therapeutic effect (Lapchak et al. 2002a, b).

What is the appropriate behavioral outcome in animal stroke studies

In clinical studies of acute ischemic stroke the modified Rankin Scale (mRS) is used to assess functional status at 90 days following the index event (Huybrechts and Caro 2007; Banks and Marotta 2007). Frequently it is the primary outcome measure in clinical stroke trials, thus the features assessed by the mRS should also be important in animal studies of ischemic stroke (Kidwell et al. 2013; Hacke et al. 2008; Ginsberg et al. 2013; Broderick et al. 2013). The mRS is a simple six point scale that does not provide a comprehensive assessment of functional status but does provide an index of a person's disability related to the stroke (Fearon et al. 2012; Weisscher et al. 2008). This simplicity permits reasonable inter-rater reliability, which is essential in multicenter clinical trials (Banks and Marotta 2007; Quinn et al. 2009; Zhao et al. 2010). Furthermore, the mRS is validated and familiar to stroke investigators worldwide. Thus, the mRS is likely to remain an important outcome measure in clinical trials of ischemic stroke.

How the mRS scale relates to functional outcomes in animal stroke models remains unclear. A mRS score ≤ 2 is generally considered a good outcome; a score >2 is a poor outcome with a score of 6 indicating death. The mRS evaluates disability, with a focus on ability to walk, attend to bodily needs, and carry out activities of daily life. These functions are a reflection of impairment in various aspects of the motor, sensory, visual and cognitive systems. Using behavioral measures in animal stroke models that are reflective of the mRS in humans may help improve therapeutic translation in stroke. Determining which features to assess in animal stroke models that are most reflective of features assessed by the mRS requires further

investigation. In a mouse MCAO stroke model, Rosell et al. found that no specific behavioral test reliably assessed long-term functional outcome (Rosell et al. 2013). The optimal time to assess outcome in animal stroke also warrants consideration. Human trials often assess mRS at 90 days after the onset of ischemic stroke. Thus, treatment effects in animals should also be evaluated at time points beyond 24 h. Given the shorter lifespan of rodents, a timeframe of 21–28 days has been suggested, though sustained benefit at longer time points may be desirable in some studies. Outcome assessment of these later time points permits identification of treatment effects that may be transient or decline with time and would thus be less relevant to the ultimate stroke outcome.

Another difference between human clinical trials and animal studies is the analysis of mortality as an outcome. Mortality is an important outcome in human studies of ischemic stroke. However, in experimental stroke studies animals that die are often excluded from analysis (Sicard and Fisher 2009). To improve preclinical stroke studies, mortality should be among the outcomes measured. Furthermore, stroke severity could be modulated so that animal mortality is similar to that expected in a human stroke trial.

Immune differences between animal and human ischemic stroke

The immune system is increasingly viewed as important in acute ischemic stroke. Humans and rodents have a number of differences in their immune systems that may be relevant to acute ischemic stroke and the evaluation of certain therapies. In humans, 70 % of circulating leukocytes are neutrophils, whereas in rodents only 20–30 % of leukocytes are neutrophils (Fox 1985). Thus, the baseline number and types of immune cells are very different. Immune responses to ischemic stroke may also differ between rodents and humans. We have found the circulating leukocytes have a very different gene expression profile in rodent MCAO stroke compared to that in patients with acute ischemic stroke (Stamova et al. 2010; Tang et al. 2001; Tang et al. 2006). Primates and rabbits have a leukocyte composition closer to that of humans. Potentially, these or other stroke models may be preferred in the evaluation of certain stroke therapies in which the immune system is deemed important.

How to translate dose from animal models to humans

Knowing how to translate the dose of a therapy used in an animal stroke model to an effective dose in patients with ischemic stroke is challenging. A variety of methods and formulas have been developed to aid in this process (Reagan-Shaw et al. 2008; Singh 2006). Many of these methods do not address biological differences between rodents and humans that may be very important to drug delivery and activity on its therapeutic target.

One possible solution is to develop an assay to measure a drug's effect on its biological target. Dosage could then be based on target response and calibrated to achieve a similar response in humans as achieved in animal stroke models (Liou et al. 2012; Plenge et al. 2013). A surrogate blood biomarker of biological effect may be very useful in this regard. Drug levels may also be useful, though a drug level alone may not necessarily indicate biological activity. In some cases evaluation of cerebral spinal fluid may necessary if entry into the central nervous system is required for biological activity. Importantly, this should be performed in both animal and human studies, as a compound's ability to cross the blood brain barrier in rodent ischemic stroke does not necessarily imply it can cross the blood brain barrier of a patient with ischemic stroke.

Conclusions

Animal stroke studies are performed for a variety of reasons. Many are proof of concept studies evaluating aspects of ischemic stroke biology or identifying treatment targets for further investigation. This is important work that is essential to better understand and develop novel approaches to treat stroke. However, given the many studies that have not translated to humans, we need to be mindful that promising results in proof of concept stroke studies require additional evaluation prior to human study. For the field to advance and translate more therapies to human stroke we need new ideas, stroke models, and approaches. In this commentary we have emphasized several differences between experimental animal stroke and human clinical stroke trials. Though the suggestions made may be contentious, discussion is essential to determine the factors and experimental protocols needed to better model human ischemic stroke in animals and improve the translation of therapies to patient with ischemic stroke.

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