Experimental Models
Help or Hindrance
Alison E. Willing, PhD

Abstract—Although many potential therapeutics have improved motor and cognitive function in animal models of experimental stroke, very few have been found to have similar beneficial effects in clinical trials. In this review, we examine the advantages and disadvantages of the currently available rodent models in the development of cellular therapies for stroke and how they have been applied. The lack of translation between the animal work and clinical benefits is not because the animal models are not useful. If the recommendations of the Stroke Academic Industry Roundtable are followed, then the studies will produce more clinically relevant information about potential new cell therapies. However, it will also be necessary to design clinical trials in a manner consistent with the preclinical study results. (Stroke. 2009;40[suppl 1]:S152-S154.)

Key Words: behavioral testing ■ cell therapy ■ cerebral ischemia ■ rodent

Stroke was not initially considered a good target for development of a cell therapy. Unlike Parkinson disease in which a single cell type in a specific region of the brain degenerates, the damage induced by a stroke is more all-encompassing with multiple cell types and regions involved. However, there have been a number of studies examining the ability of stem/progenitor-like cells harvested from multiple tissues and developmental stages to treat stroke. For the most part, these experimental therapeutics have not proceeded to clinical trials yet, but issues of the quality of the preclinical studies are important to consider.

Guidelines for Conducting Preclinical Animal Studies
In 2001, Youdim1 wrote “The failure to induce neuroprotection in the clinic versus in the laboratory with currently available drugs would suggest that either the animal models we are employing are not truly representative of the disease state, or that a single drug would not be sufficiently active to do so and thus a cocktail of drugs is to be employed.” There have been ongoing debates about the usefulness of animal models in the stroke field for at least 20 years.2,3 These concerns were formally addressed by the Stroke Therapy Academic Industry Roundtable and recommendations published for the conduct of preclinical stroke studies in animals.4 The candidate drug or treatment should be evaluated in both permanent and reperfusion models of ischemia, and in aged animals of both sexes. Pharmacological parameters such as dosing, route of administration, therapeutic window of efficacy, and toxicity should be determined over an adequate range of doses in small and large animal species. During induction of ischemia, physiological monitoring is essential including demonstration of a reduction in cerebral blood flow. Multiple outcome measures, including both analysis of infarct size and functional outcome, should be used at both short and extended survival times. Furthermore, measures of sensory, motor, and cognitive function should be included in the behavioral analysis. The experiments should be performed blind and be replicated in at least 2 independent laboratories. Finally, the data, both positive and negative, should be published in peer-reviewed journals.

The Stem Cell Therapeutics as an Emerging Paradigm in Stroke Consortium (October 2007) made additional recommendations specific for the development of cell therapies. These included the use of clinically relevant stroke models, development of standardized outcome measures and treatment protocols, use of imaging techniques to verify biodistribution of the cells and evolution of the infarct, establishment and use of safety indices, and finally a demonstrated mechanism of action underlying the therapeutic effect of the cells.5

Clinical Relevance of Common Stroke Models
The remainder of this review limits discussion to focal cerebral ischemia. In an ischemic stroke, blood flow in a vessel is decreased or blocked. This blockage can form locally (thrombus) or a clot can originate elsewhere but become lodged in the smaller vessels of the brain vasculature (embolus).6 An ischemic stroke may also occur because of decreased systemic perfusion pressure producing a decrease

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in blood flow to the brain; this may occur with myocardial infarction, arrhythmia, or trauma accompanied by blood loss. The different types of ischemic stroke, underlying factors, and the vessels commonly occluded are summarized in the Table.

**Animal Models of Focal Ischemia**

There are 6 common models of focal cerebral ischemia and of these, most focus on the middle cerebral artery. The model with the best face validity is the thromboembolic model in which clotted blood is injected. The best results have been obtained with the use of fibrin-rich clots; these clots are more analogous to those occurring in humans and are more resistant to autolysis than platelet-derived clots. Although this approach is ideal for looking at thrombolytic therapies, placement of the thrombi/emboli can be variable. The intraluminal suture model produces reproducible lesions and can be used to produce both permanent and transient middle cerebral artery occlusion. It is possible to study both acute stroke treatments and interventions that target stroke recovery. The disadvantages include a tendency toward hemorrhagic transformation and hyperthermia.7

For microembolization models, microspheres are injected resulting in multiple smaller heterogeneous lesions. Electrocoagulation or ligation of a proximal branch middle cerebral artery is also commonly performed. This produces a well-localized cortical lesion, although the subtemporal craniotomy alters intracranial pressure. Photothrombosis also produces a cortical lesion. A photosensitive dye is injected intravenously and the animal is then irradiated, causing platelet aggregation in local blood vessels. Reperfusion occurs in 0 to 2 hours. Although this is a noninvasive model, its clinical relevance is low. Endothelin is a potent vasoconstrictor that can be administered either stereotaxically or topically. It is possible to precisely localize the lesion with a reversible procedure that does not mechanically damage the blood vessel.8 Astrocytosis is often produced after endothelin administration, however, which may interfere with integration of transplanted cells.

One of the primary advantages of rodent models of stroke is that the consequences of the ischemic insult closely replicate the pathobiology observed in the human brain, making these models very clinically relevant. Where there is a divergence between the rodent model and the human disease is in variability from rat to rat. In humans, strokes vary considerably in severity, location, cause, and extent of reperfusion and are often complicated by other underlying disease processes. In contrast, we can control all of these variables in our experimental models. Other advantages include the well-characterized anatomy and physiology of rodent models and similarities in vasculature to the human.9 Rodent models are cost-effective and the relative ease of genetic manipulation in mice make these models ideal to study the pathophysiology of stroke and the underlying mechanism of recovery.

There are also disadvantages that stem from the differences between the rodent and human. First, the rodent brain is not gyrencephalic like the human brain. Furthermore, white matter makes up a significantly larger proportion of the human brain than the rodent brain.10 Although the nonhuman primate brain is most similar to the human brain, the positive effects observed in the rat or mouse may be lost in other larger animals such as the dog with a gyrencephalic brain.11 The use of a nonprimate large animal may be useful for studying therapeutic efficacy and yet still be more cost-effective than primate studies. The main benefit of the primates is they can better model complex behaviors and recovery, although the primate model should be chosen with care because not all primates have a gyrencephalic brain.

Perhaps the primary disadvantage is that the cell therapy experiments are often carried out across species. Although there will still be issues of rejection with human transplants in humans, they are not as extreme as the issues of rejection with cross-species transplantation in which the donor cells can be rejected within minutes to hours even in an immunosuppressed animal. Therefore, these studies can only provide general information about how human cells will behave in the human.
Standardized Outcome Measures

Most experimental stroke studies measure infarct size, but even this is not standardized. Many studies use 2,3,5-triphenyltetrazolium chloride staining of metabolically active cells; it is fast, reproducible, and inexpensive to perform, but it is insensitive at early and late time points.\(^{12}\) In contrast, Fluoro-Jade histochemistry can highlight specific cells and regions that are compromised as early as 1 hour poststroke.\(^{13}\) Hema-toxylin & eosin or Nissl may also be problematic at early time points poststroke but are effective at longer survival times. None of these assays are clinically relevant.

In clinical trials, pathology is assessed with either CT or MRI. MRI is the method of choice because of greater sensitivity and specificity.\(^{14}\) MRI has not been widely used in rodent experimental models, but it has been shown to accurately reflect the pathology of the infarcted region as determined with histopathology.\(^{15}\)

In addition to measuring infarct size, it is critical to also include measures of functional outcome. Diminished infarct size does not always correlate with functional improvements\(^{16}\) and improved function is the relevant measure of clinical outcome. In many rodent studies, some measure of gross neurological deficit is often used. This can be a useful screening tool, but spontaneous recovery often occurs and the ability of the neurological examination to measure subtle lasting deficits is poor.\(^{17,18}\) To effectively evaluate a cell therapy, longer poststroke survival times are necessary. Ideally, these tests need to be reliable and valid; multiple research groups using the same test in the same model should obtain similar results and the behavior should be relevant to the clinical population. This is easier to achieve with nonhuman primates, but it is also possible with rodents. When performance of normal control subjects and patients with Parkinson disease on a skilled reaching task was analyzed, the basic form of the movements was similar to those observed in rats performing a similar task and the nature of the deficit exhibited by the patients was similar to that observed in the parkinsonian rat.\(^{19}\) For a more thorough discussion of the issues of behavioral testing in stroke, see Kleim et al.\(^{20}\)

Conclusion

It is widely acknowledged that no single animal model perfectly replicates the human stroke. However, research in animal models has led to a wealth of knowledge about the pathophysiology of stroke and a better understanding of the possible targets to treat this disease. The lack of translation between the animal work and clinical benefits does not lie in the animal models, but in how we use the models and how we apply this knowledge to design of clinical trials. We need to carefully choose the stroke model, the outcome measures to use, and when to use them. Perhaps the issue is not that all the variables in these studies are controlled enough, but that they are controlled too much and therefore can never truly represent the patient with stroke in the emergency room. If we do not want to increase variability in our animal studies, then we need to make very careful choices of clinical population to target and when to treat them when we design our clinical trials trying to mirror the animal studies precisely. If heterogeneous populations are still used in clinical trials, then the sample size must be large enough to support this and to allow for powerful post hoc analyses of subpopulations within the sample.

Disclosures

None.

References

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