# Potential Molecular Targets for Translational Stroke Research

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The stroke research community is currently at a crossroads, and a shift in focus is necessary to propel basic research forward to develop clinically effective therapeutics. In-depth analysis of the past failures and imposing more stringent standards on future basic research experiments will greatly improve the success of translational research. The purpose of this review is to outline proposed revisions in basic research criteria and offer attractive molecular candidates to mitigate brain damage after cerebral ischemia.

A major pitfall encompassing translational stroke research is that a majority of clinical trials conducted have focused on agents involving recanalization of vessels and on excitotoxicity to reduce neuronal death in the penumbra.[1] Recanalization using tissue plasminogen activator only modestly improves patient outcome, and inhibiting excitotoxicity has shown no clinical benefit.[2] The short duration of excitotoxicity after ischemia does not provide an adequate time window for effective stroke therapy in clinical practice because the exact onset of stroke is often indeterminate and a majority of patients do not seek medical treatment for many hours after the insult. A more reasonable therapeutic window, and hence a greater potential for clinical success, is likely to be attained by placing emphasis on ameliorating the effects of the synergistic processes of programmed cell death (PCD) and inflammation that are active for hours to days after ischemia.[3]

In addition, it is necessary to identify molecular mediators of ischemic neuroprotection suitable for translation to human clinical trials. Accordingly, more stringent criteria for selection of targets are required. The following criteria will facilitate development of translatable targets: (1) target multiple injurious components; (2) demonstrate long-term neuroprotection in animal models; (3) measure neurological improvement in addition to neuroprotection; (4) exhibit cross-species efficacy; and (5) establish efficacy in aged animals.

## Molecular Components of Neuronal Injury

Molecular targets for potential neuroprotection should incorporate mediators of PCD. There are, however, many components of PCD, and a judicious selection of targets is necessary. Death pathways can be divided into 3 categories: (1) upstream death and survival factors such

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as c-Jun N-terminal kinase (JNK) and AKT, respectively; (2) effectors of mitochondrial membrane disruption, including Bax and Bid; and (3) death-execution signals downstream of mitochondrial damage, including caspase-dependent and -independent mechanisms. Targeting death effectors downstream of mitochondria by inhibiting either caspases or caspase-independent pathway effectors such as apoptosis-inducing factor only provide short-term neuroprotection. This is because compensatory mechanisms act as a fail-safe upstream of these molecules to carry out PCD in the event of a lethal insult.

Analysis of factors regulating the mitochondrial pathway reveals a redundancy in molecules. Individual knockdown of prodeath Bcl-2 proteins results in no to modest decreases in infarct volume after focal ischemia in mice. However, double knockout Bax/Bid mice and triple knockout Bax/Bim/PUMA mice display robust decreases in infarct volume lasting at least 14 days after ischemia (authors' unpublished results). Thus, there is a potential for targeting factors directly involved with mitochondrial pathways, and it is necessary to investigate multiple targets.

Because progressively greater neuroprotective efficacy is gained by targeting effectors of mitochondrial membrane disruption than by targeting downstream death effectors, it is reasonable to focus on inhibition of upstream neuronal death mediators or augmentation of neuronal survival mechanisms. Strategies that have the greatest promise include: (1) inhibiting the ASK1/JNK/p38 pathway; (2) activating endogenous prosurvival signaling; and (3) activating peroxisome proliferator-activated receptor $\gamma$-dependent anti-ischemic signaling. Together, these pathways influence death signaling, inflammation, and genomic response after cerebral ischemia.

One recognized mediator of ischemic death is JNK. Activation of JNK activates Bax, PUMA, and Bim and activates the transcription factor c-Jun to actuate a PCD genomic response. An inhibitor of JNK resulted in robust and long-term neuroprotection and improved neurological function after both focal and global ischemia even when administered 6 hours after the insult. [4] Recently, JNK was found to be downstream of apoptosis signaling kinase 1 (ASK1). Multiple cell stressors such as reactive oxygen species and DNA damage activate ASK1, which induces neuronal death by JNK signaling and activation of p38 in microglia. Indeed, ASK1-mutant mice are deficient in stress-induced JNK and p38 activation and are highly resistant to ischemic injury. Targeting ASK1 provides not only neuroprotection, but also more comprehensive protection of brain tissue. Recent evidence indicates that small molecule inhibitors of JNK, ASK1, and highly specific oligopeptide inhibitors of kinases downstream of ASK1 are potential neuroprotective agents.[5]

Another potential neuroprotective strategy is activation of multiple endogenous prosurvival signals. Examination of the signaling pathways activated by prosurvival molecules such as leptin, erythropoietin, insulin, and nerve growth factor reveals a redundancy in the involvement of intracellular molecules, including CREB, AKT, and ERK. Although activation of all of these pathways has been shown to be neuroprotective, concomitant administration of 2 or more of these agents may synergistically activate intracellular prosurvival signaling and provide more robust neuroprotection than any single agent.

The nuclear receptor peroxisome proliferator-activated receptor $\gamma$ is a promising target for clinically effective neuro-protection. It is expressed in every type of brain cell, including vasculature, microglia, and astrocytes. In addition to its ubiquitous expression, peroxisome proliferator-activated receptor $\gamma$ has multiple mechanisms that make it a particularly enticing target. Activation of peroxisome proliferator-activated receptor $\gamma$ has direct neuroprotective effects by inhibition of PCD, it decreases cerebral inflammation, it promotes antioxidant enzymes expression, and it decreases transmigration of inflammatory cells across vasculature.

Administration of peroxisome proliferator-activated receptor $\gamma$ agonists, thiazolidinediones, decreased infarct size, and improved neurological outcome after focal ischemia in rodents.[6] Another advantage of thiazolidinediones is that they are currently approved for treatment of type 2 diabetes. Indeed, patients with diabetes taking the thiazolidinediones pioglitazone had a significantly lower risk of death, myocardial infarction, and stroke.[7] Due to their multiple neuroprotective mechanisms and their established use in patients, thiazolidinediones are promising agents for use in acute stroke.

## Long-Term Neuroprotection

To effectively translate neuroprotective strategies, long-term neuroprotection must be demonstrated. Criteria for long-term protection are decreased infarct volume 2 to 3 weeks after focal ischemia and increased CA1 neurons at least 30 days after global ischemia.

## Neurological Improvement

Neurological outcome is the single most important outcome measure after stroke in humans; however, assessment of neurological improvement in animal models is frequently omitted. For potential translation from basic research to clinical use, it is imperative to show neurological improvement in animal models. Furthermore, it is necessary to develop more sophisticated measurements with greater predictive value to detect neuroprotection.

## Cross-Species Efficacy

Given the heterogeneity of brain structure, metabolism, and vascular anatomy in various species, it is necessary for potential therapeutics to show efficacy in multiple species. This ensures that a therapeutic has greater potential to be extrapolated to human use. Ultimately, efficacy should be shown in nonhuman primates because they are phylogenetically closest to humans.

## Efficacy in Aged Animals

One of the major criticisms of animal models of stroke is that young, healthy animals are studied, whereas patients with stroke are often older with premorbid conditions. Older animals are more susceptible to brain damage and functional impairment caused by stroke than younger animals.[8] Thus, translation of neuroprotective efficacy would be more realistic if neuroprotection were shown in aged animals. Moreover, an agent that is neuroprotective in aged, spontaneously hypertensive rats may be more likely to show efficacy in human patients with stroke.

## Conclusions

The past decades have been wrought with numerous unsuccessful trials of stroke therapeutics despite promising neuro-protective evidence found in animal models. Nevertheless, regardless of the past failures, the stroke research community must have an optimistic outlook on the potential for future stroke therapeutic development. Implementation of the basic research criteria outlined here should increase the translatability of future therapeutics.

## Sources of Funding

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