Mechanisms and challenges in translational stroke research

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ABSTRACT
Translating basic science advances into clinical meaning has been challenging for stroke research. This does not, however, mean that the investigation of basic mechanisms is irrelevant. Translation is difficult because the underlying mechanisms are complex and ill-defined. The original focus on neuroprotection has now evolved into a broader consideration of the role of non-neuronal cells in stroke pathophysiology. The neurovascular unit may provide a conceptual framework within which interactions between neural, glial, and vascular cells comprise a basis for function and dysfunction in the central nervous system. Importantly, these cell–cell signaling pathways are also biphasic in nature, that is, mechanisms that are deleterious in the acute phase may surprisingly be required for neurovascular remodeling and plasticity during stroke recovery. Furthermore, injury-into-repair gradients are significantly influenced by a host of modifying factors and comorbidities. Rigorous dissection of these complex and recursive mechanisms should be required before they can be rationally targeted for stroke.

INTRODUCTION
Stroke is a major health problem. It continues to be an important cause of mortality and morbidity. With an aging population, the incidence of stroke will surely continue to increase, with significant implications for both cerebrovascular disease and dementia. Therefore, the search for widely applicable and effective treatments for diverse patient populations remains an urgent unmet need.

In carefully selected patients with acute ischemic stroke, rapid thrombolysis with tissue plasminogen activator is a proven Food and Drug Administration (FDA) approved approach. Recently, a number of clinical trials now suggest that mechanical reperfusion with thrombectomy devices may also be efficacious in selected patient cohorts. This may represent a major advance in acute ischemic stroke care. Importantly, this may also represent an opportunity to further pursue combination therapies. Initial analyses suggest that there remains a 20–30% mismatch between effective recanalization and clinical improvements, and there may also be up to 60–90 min worth of ‘usable time’ when the patient is already in the hospital before recanalization actually occurs. These scenarios may provide opportunities for further improving outcomes in a wider range of patients if one can design targeted therapies to further protect brain tissue while mechanical recanalization is taking place.

NOT JUST NEUROPROTECTION
The initial impetus for stroke therapeutics was triggered in part by the definition of excitotoxicity. After cerebral ischemia, energetic perturbations lead to uncontrolled neuronal depolarization, disruption in glutamate transmitter homeostasis, and overactivation of N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and metabotropic glutamate receptors that ultimately lead to ionic imbalance and neuronal death. Furthermore, ischemic injury also perturbs mitochondrial function, leading to the generation of reactive oxygen and nitrogen species, all of which underlie oxidative stress and neuronal dysfunction. Finally, at least in some experimental model systems, cerebral ischemia may also lead to an upregulation of various programed cell death pathways, comprising apoptosis, autophagy, and necroptosis. In cell culture models of oxygen-glucose deprivation and animal models of focal cerebral ischemia, blockade of these various excitotoxic, oxidative stress, and cell death mechanisms appear to significantly reduce neuronal injury. However, none of these neuroprotective strategies have been proven effective in clinical trials.

The lack of effective neuroprotection has been widely analyzed and debated. Perhaps the treatment time windows were overoptimistic in early clinical trials. Perhaps patient populations were not optimized in many of these prior ‘take-all-comers’ design. Perhaps the standard dichotomized χ²-modified Rankin score was not sensitive enough. Perhaps some of these neuroprotective compounds did not effectively penetrate the blood–brain barrier. Perhaps cell culture and animal models may not accurately capture all aspects of clinical stroke in humans. There will surely be many reasons involved.

Nevertheless, the lack of effective translation does not mean that the basic molecular and cellular mechanisms are incorrect or irrelevant. Perhaps the difficulty in translation means that the mechanisms of stroke pathophysiology are complex and remain ill-defined. Reversing an initial insult by restoring blood flow is an ‘upstream’ method with large effects...
in the right patients. However, the subtleties of ‘downstream’ neuroprotection may be more difficult to detect.

Over the past decade, the concept of the neurovascular unit has provided a framework for dissecting stroke mechanisms.6 13 In its essence, the concept is simple. The central nervous system (CNS) works not just because neurons are firing action potentials. The whole system works because of intricate cell–cell signaling loops between multiple cells from neuronal, glial, and vascular compartments (figure 1). For example, proper neurotransmission requires a balance between neuronal release and glial reuptake mechanisms. The blood–brain barrier function requires crosstalk between the cerebral endothelium and pericytes and astrocytic end-feet. Renewal and homeostasis in white matter require crosstalk between endothelial cells and oligodendrocyte precursors. Even the revolutionary functional MRI method itself is predicated on crosstalk between neuronal activity and hemodynamic response. Similarly, dysfunction and disease are also mediated by crosstalk between all cell types in the brain. Hence, the pathophysiology of stroke (and perhaps all CNS disorders) cannot be effectively investigated without careful consideration of the entire neurovascular unit.14

INJURY AND REPAIR
The ischemic penumbra provides an important theoretical target in stroke.15 Within the ischemic core, blood flow deficits are severe and brain cells die rapidly. However, the penumbral areas surrounding this core are thought to be transiently sustained by collaterals, so the more moderate levels of ischemia allow some brains cells to temporarily survive. Of course, over time, the penumbra will collapse and brain cells will die if proper treatments are not provided. The penumbra may represent a meta-stable state where recovery versus cell death are in dynamic and precarious balance.16 Importantly, the penumbra is both dying as well as actively trying to recover.17 The mammalian brain possesses remarkable endogenous mechanisms for compensation, plasticity, and remodeling. Stroke mechanisms may be interpreted under a general rubric of inflammation and wound healing. Many mediators in the pathophysiology of stroke and CNS disease come from the larger families of damage-associated molecular pattern (DAMP) and pathogen-associated molecular pattern (PAMP) signals.18 Hence, the numerous pathways that are triggered after stroke may possess biphasic properties. For example, over-activation of NMDA receptors will cause excitotoxicity and neuronal death in the acute phase, but without proper NMDA signaling, recovery cannot take place when patients move into rehabilitation units.17 Uncontrolled oxidative stress is damaging but homeostatic levels of free radicals are required for angiogenesis and endothelial progenitor cell function.19 Programmed cell death pathways may also be essential for maintaining functional cell subsets, and deletion of autophagy genes may induce neurodegeneration.20 Microglia are not always damaging; M1-like subsets are deleterious, whereas M2-like subsets may promote tissue repair.21 Hence, targeting the penumbra in stroke and, perhaps more broadly, treatment of any CNS disorder may require careful attention to the signals involved as the brain transitions from initial injury into subsequent endogenous modes of neurovascular remodeling and recovery.22

COFACTORS AND COMORBIDITIES
Stroke pathophysiology is complex because it involves more than just metabolically dying neurons. Crosstalk between neuronal, glial, and vascular compartments plays a critical role.6 Recently, however, it is recognized that the brain is not an ‘isolated organ’, so an ‘extended’ neurovascular unit has been proposed.11 Experimental and clinical data strongly suggest interactions between the CNS and peripheral responses from immune, hormonal, and cardiac systems. The spleen, bone marrow, and circulating immune cells are all activated after stroke.23 Changes in the brain are correlated with changes in the heart.24 Crosstalk between the CNS and peripheral response may provide the potential signals and substrates that permit the influence of many cofactors and comorbidities (figure 2). Genome-wide-association-studies (GWAS) suggest that many stroke genes may represent cardiovascular pathways.25 Hypertension and diabetes have long been known to be significant factors in stroke.26 In fact, many of these modifying factors alter stroke risk, as well as significantly contribute to the acute process of brain injury and the delayed process of brain repair. For example, it has been suggested that diabetic vascular systems may suffer
from impaired angiogenic programs that might influence stroke recovery. From the experimental side, model systems will have to be adapted to incorporate these important modifying factors. From the clinical side, stroke trials may have to more carefully identify and separate patient cohorts depending on how the mechanisms being targeted are affected by various risk factors. Ultimately, a systems biology approach may be helpful. For example, mapping the transcriptome of reactive astrocytes should offer insight into how deleterious versus pro-recovery gliosis may be regulated for therapeutic benefit. Recent attempts to map the brain vasculome should also provide a rich database for dissecting new mechanisms and stroke targets in susceptible patient populations.

LOOKING AHEAD

A challenging problem is not necessarily intractable. The recent success of endovascular trials suggests that we can indeed design clinical trials that select patients for treatment in a timely manner. Once patients are in this system, time windows do exist for combination therapies to be contemplated. The completion of the Field Administration of Stroke Therapy–Magnesium (FAST-MAG) trial further suggests that prehospital treatments may even be considered under the right circumstances. Advances in neuroimaging suggest that rapid methods to separate responders from non-responders may perhaps be feasible in the near future. Ultimately, rigorous attempts to define and link mechanisms across all cell, animal, and human model systems will be essential. Stroke remains a difficult disease with heterogeneous patient populations. However, with a rigorous dissection of causal mechanisms, translation may still be possible.

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