Exciting, Radical, Suicidal
How Brain Cells Die After Stroke
Eng H. Lo, PhD; Michael A. Moskowitz, MD; Thomas P. Jacobs, PhD

It is now widely accepted that not all brain cells die immediately after stroke. Surrounding a core of severe and rapid tissue injury, brain cell death evolves more slowly in a heterogeneous area that has been called the penumbra.1,2 In 1977, Astrup et al provided one of the first experimental demonstrations of the penumbra in a baboon model of cerebral artery occlusion.3 This region of brain in acute ischemic stroke was found to be electrically silent but sufficiently active metabolically to sustain membrane potentials. Neurons within the penumbra are functionally impaired but not yet dead. Without reperfusion, the penumbra collapses, brain cells die, and the lesion expands.

Although the precise timing and cellular pathways involved are not fully understood, it is believed that mechanisms actively promoting cell death are triggered after stroke. Remarkable progress has been made in dissecting these mechanisms over the past 3 decades. Three major pathways have emerged: excitotoxicity, oxidative stress, and apoptosis, and they are inextricably linked. Here, we explore the notion that stroke is most fruitfully investigated by an integrative “systems biology” approach that encompasses cell death and survival signaling within all components of the neurovascular unit.

Cell Death: A Convergence of Factors

How do brain cells die after stroke? A large body of data suggest that it may be exciting (glutamate and excitotoxicity), radical (oxidative stress and free radicals), and suicidal (apoptotic-like pathways).4-6 Simply put, when brain fails to generate sufficient ATP, such as after oxygen and glucose deprivation, energy failure occurs and ionic gradients are lost. Glutamate is released, reuptake processes are impaired, and this excitatory amino acid binds to its postsynaptic receptors and promotes excessive calcium entry and calcium release. Calcium-dependent syntheses and proteases contribute to cell and tissue demise by degrading key cytoskeletal and enzyme proteins, and generating nitric oxide and peroxynitrite. Mitochondrial functions such as oxidative phosphorylation also fail and reactive oxygen radicals are released that further compromise cells by attacking proteins, lipids, and nucleic acids. Families of “executioner” molecules (eg, caspases, AIF) dismantle multiple cell processes in the cytoplasm and nucleus to promote cell death by suicidal mechanisms resembling apoptosis. Necrosis may proceed by an analogous programmed pathway just now being revealed in the nematode c-elegans.7 Ultimately, highly dynamic and cell-specific pathways cause death by necrosis and apoptosis, with their extent depending on duration and depth of the ischemic insult. Despite convincing experimental evidence, none of these 3 major cell death pathways has been successfully exploited for treating acute stroke patients. The reasons are complex, and detailed explanations are beyond the scope of this short editorial.8,9

Just how did these notions about ischemic cell death evolve over the past 30 years? A PubMed search is very revealing. From 1975 to 2003, the number of articles on stroke increased from about 900 to almost 7000 (Figure A). Within this subset (Figure B), those studying excitotoxicity or oxidative stress appeared in the mid 1980s, appeared to peak in the late 1990s, and showed trends of decreasing thereafter. Publications on apoptosis began to increase in 1993 and continued until the present time. Those publications implicating multiple mechanisms (ie, at least 2 out of the 3) only appeared recently and are slowly increasing in number. Based on the complexity of intracellular prolife and prodeath signaling and the death of multiple cell types, we anticipate the trend to continue in this direction.

Although the original model of excitotoxicity emphasized calcium influx through glutamate receptor-coupled ion channels, ionic imbalance may also proceed via other routes. Energy deprivation triggers acidosis, which activates novel classes of acid-sensing ion channels that further perturb sodium and calcium homeostasis.10 Importantly, glutamate receptors are also coupled to multiple intracellular kinase signals that subserve the cell’s stress response repertoire. For example, NMDA receptor activation engages Ras-GRF exchange factors and upregulates ERK and CREB pathways that mediate endogenous prosurvival responses to cellular stress. Double knockout mice lacking both Ras-GRF1 and

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From the Neuroprotection Research Laboratory (E.H.L.), Departments of Radiology and Neurology, Massachusetts General Hospital, and Program in Neuroscience, Harvard Medical School, Charlestown, Mass; the Neurovascular Regulation and Stroke Laboratory (M.A.M.), Departments of Radiology and Neurology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Mass; and the Neural Environment Cluster (T.P.J.), National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Md.

Correspondence to Dr Eng H. Lo, Neuroprotection Research Laboratory, Harvard Medical School, MGH East 149-2401, Charlestown, MA 02129. E-mail Lo@helix.mgh.harvard.edu
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Ras-GRF2 are less resistant to ischemic injury and show increased infarction after focal cerebral ischemia. The net effect of ERK activation, however, may also depend on the severity of ischemia. Whereas ERK may be beneficial after moderate ischemia, sustained overactivation of ERK after severe ischemia may paradoxically promote cell death. Another important mediator that has emerged is Akt, a serine–threonine kinase that phosphorylates and inactivates cell death mediators such as BAD, caspase-9, and glycogen synthase kinase-3. In cultured neurons, Akt protects against a wide array of excitotoxic, oxidative, and apoptotic insults. In a mouse model of focal cerebral ischemia, Akt is rapidly downregulated in the dying ischemic core, whereas increased Akt and neuronal survival in the cortex accompanies SOD1 overexpression, a superoxide radical scavenging protein.

In addition to prosurvival responses, neuronal stress also triggers deleterious intracellular signals. The 2 stress-activated protein kinases, c-jun-N-terminal kinase (JNK) and p38, have been intensely investigated in recent years. JNK phosphorylates Bax and enhances its mitochondrial translocation, where it then augments pro-apoptotic caspase activation. Elevated phospho-JNK colocalizes with TUNEL-positive apoptotic neurons in mouse focal cerebral ischemia and inhibition of JNK protects injured brain. Consistent with these pharmacologic data, ischemic brain injury is reduced in knockout mice lacking the neuron-specific JNK3.
isoform. Because these stress kinase signals play key roles in the cross-talk between multiple cell death pathways, they are attractive targets in the context of stroke where excitotoxicity, oxidative stress, and apoptosis may converge. Perhaps most importantly, such targets may afford a longer therapeutic window. At least in experimental rat and mouse models, JNK and p38 inhibitors are reportedly effective up to 6 hours after ischemic onset. Ultimately, multiple pathways are involved, so targeting the overall imbalance between prodeath versus prolife signaling mechanisms may be required.

**The Neurovascular Unit: More Than Just Neurons?**

Recently, the National Institutes of Neurological Disorders and Stroke (NINDS) engaged the stroke community to identify new research directions through the Stroke Progress Review Group (http://www.ninds.nih.gov/find_people/groups/stroke_prg/04_2002_stroke_prg_report.htm). The priorities reported by this group emphasized the significance of targeting multiple mechanisms in many cell types within the neurovascular unit to advance our knowledge of stroke. To facilitate these studies, the National Institutes of Health has implemented several initiatives, including Novel Targets and Therapy Development for Ischemic Stroke (http://grants.nih.gov/grants/guide/rfa-files/RFA-HL-05-004.html), Neuroprotective CNS Barriers in Neurological Diseases (http://grants2.nih.gov/grants/guide/PA-files/PAS-03-165.html), Neurovascular Mechanisms in Brain Function and Disease (http://grants.nih.gov/grants/guide/PA-files/PAS-04-072.html), and Genetics and Pathobiology of Vascular Cognitive Impairment (http://grants.nih.gov/grants/guide/PA-files/PAS-04-149.html). More recently, a trans-National Institutes of Health Stroke Working Group was also organized to address the multidisciplinary challenges of stroke research. This working group, which has representatives from 8 institutes, highlights the need and opportunity for more collaborative and integrative approaches to advance stroke research.

This fundamental concept of the neurovascular unit emphasizes that neurons participate as part of an integrative brain response in which signaling between cells and between cells and matrix are important determinants of tissue outcome in neurological disorders. How has the stroke literature reflected this concept? Although most publications remain focused on cell death specifically within neurons, there is an emerging and important recognition that glia and endothelial cells are also key players in stroke (Figure C). In this context, an ideal therapeutic target would be one active in multiple brain cell types. For example, the stress-activated protein kinase p38 not only mediates neuronal death but also may augment caspase-3–mediated cell death in cerebral endothelial cells after hypoxic injury. Another multiligand mediator might be the arachidonic acid cascade enzyme 12/15-lipoxygenase, which has recently been shown to contribute to glutamate-induced oxidative cell death in both neurons and oligodendrocytes, thus making it a potential target for both gray and white matter injury in stroke.

These past 2 years witnessed the emergence of erythropoietin (EPO) as a potential cerebroprotectant for stroke. A very limited clinical trial suggested that EPO may be safe and possibly efficacious in acute ischemic stroke. The mechanisms of EPO’s actions are complex, but precisely because it targets multiple pathways of cross-talk signaling in cell death, EPO may become an especially potent therapeutic approach. Importantly, EPO is protective in both neurons and cerebral endothelial cells. EPO may even enhance neurogenesis and angiogenesis during stroke recovery. The recent development of an EPO derivative that is not erythropoietic yet retains its neuroprotective properties may further enhance its potential clinical importance.

In contrast to many failures in trials of clinical neuroprotectors, the only Food and Drug Administration-approved stroke therapy targets the vasculature, ie, thrombolyis, using recombinant tissue plasminogen activator. It makes good sense to develop combination therapies for ischemia that combine vascular and parenchymal actions, especially because preclinical data suggest that many drug combinations improve neuroprotection and extend the therapeutic window in ways not possible with a single agent. It has been proposed that co-administering recombinant tissue plasminogen activator with activated protein C (APC) may be one rational approach. APC may enhance thrombolysis, decrease blood–brain barrier perturbations, and salvage brain. By inactivating factors Va and VIIIa and by binding the tissue plasminogen activator antagonist PAI-1, APC may promote thrombolysis by preventing downstream microvascular thrombosis. APC may also reduce inflammation based on successful experience with recombinant APC (drotrecogin alpha, activated) in severe sepsis. APC appears to downregulate endothelial cell adhesion molecules such as intracellular adhesion molecules and selectins, thereby reducing leukocyte infiltration and secondary tissue injury. APC reduces glutamate and oxidative stress-induced apoptosis in both cultured neurons and cerebral endothelial cells, and decreases recombinant tissue plasminogen activator–associated worsening of infarction and neurological deficits in a mouse stroke model. Taken together, these data suggest that combination approaches that simultaneously target vascular and parenchymal injury might increase the safety and lengthen the time window for effective thrombolytic reperfusion in stroke.

The pathophysiology and treatment of stroke remains a daunting scientific and clinical problem. Despite impressive advances in elucidating the complexity of cell death mechanisms, the way forward may entail deciphering those intracellular signals that mediate cross-talk between multiple pathways, and perhaps between multiple cell types. Targeting prodeath versus prolife signals within all cells of the neurovascular unit may eventually lead us to improved methods to treat salvageable brain tissue after ischemic insult.

**References**


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