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Can inhibition of apoptosis rescue ischemic brain?

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Editorial

Recent data indicate that insults to the brain, including cerebral ischemia and trauma, can activate programmed cell death pathways in injured neurons (1–4). Pathological activation of apoptosis may, in fact, contribute to neurodegeneration in a broad range of acute and chronic neurological disorders. Therefore, precise delineation of the mechanisms that regulate apoptosis may greatly enhance our understanding of neurodegenerative processes (5). Recent studies have also highlighted many potential links between the apoptotic and necrotic pathways of cell death that could contribute to irreversible neuronal damage after acute brain injury (4). Mitochondrial dysfunction and oxidative injury are among the pivotal functional mechanisms linking these processes (for review see reference 4). These novel insights, coupled with the recognition that apoptotic neuronal death is a relatively slow and multistep process, has now led to the emergence of a new direction for cerebral ischemia research: Evaluation of whether therapeutic intervention with inhibitors of apoptosis can rescue the ischemic brain.

Several complementary approaches have yielded evidence that acute ischemic brain injury activates apoptotic pathways. Morphological, histochemical, and biochemical assay methods have been used to document injury-induced apoptosis in ischemic brain. Although there is growing awareness of the potential limitations of these methods when used alone (in particular the lack of specificity of histochemical assay of terminal deoxynucleotidyl transferase mediated dUTP-biotin nick end labeling; reference 4), there is strong evidence of ischemia-induced apoptosis, particularly in experimental models that elicit relatively mild ischemic injury in the adult brain (6), and in the neonate (7).

Inhibition of apoptosis is an effective neuroprotective strategy in clinically relevant cerebral ischemia models. The first successful example was a strategy that used in vivo treatment with cycloheximide (1), a global inhibitor of protein synthesis that could block synthesis of endogenous anti-apoptotic proteins such as bcl-2 and/or of pro-apoptotic degradative enzymes. Although the precise cellular mechanisms of neuronal apoptosis are incompletely understood, a group of cysteine proteases known as caspases (cysteine proteases cleaving after an aspartate residue) clearly play a key role as downstream effectors of apoptotic cell death. In the context of brain injury, attention has focused on asking whether activation of two specific caspases, caspase-3 (CPP32) and caspase-1 (interleukin-1 converting enzyme [ICE]), mediate apoptotic neuronal death (3, 6–8). In this issue of *The Journal*, Cheng et al. (7) report that intracerebroventricular or systemic treatment with a pan-caspase inhibitor [boc-aspartyl(OMe)-fluoromethylketone] markedly attenuated hypoxic–ischemic brain injury in a neonatal rodent model of stroke, whereas a caspase-1 inhibitor did not confer protection; they also demonstrated that there was increased caspase-3 functional activity in tissue extracts from hypoxic-ischemic brain. These findings suggest that pathological activation of caspase-3 contributed to tissue injury and that inhibition of its activity could limit tissue injury. Perhaps most exciting, because of potential clinical applications, was the finding that the neuroprotective efficacy of the caspase inhibitor was retained even if treatment was initiated 3 h after the insult.

In other models, inhibition of caspase-1 (ICE) activity has also conferred neuroprotection; thus, ascribing dependence of apoptosis to individual caspases may be premature. An important factor that must be considered in interpreting data generated from this type of investigation is whether these protease inhibitors exert biologically important effects on other substrates. For example, ICE activates pro–IL-1β to generate mature IL-1β, and this proinflammatory cytokine is also a mediator of ischemic brain injury. Schielke et al. (8) reported recently that in mutant mice deficient in the ICE gene, the severity of brain injury induced by a focal ischemic insult was significantly reduced, as compared with wild-type controls. Although the underlying mechanism was uncertain, these results indicated that pharmacological inhibition of ICE could be a useful treatment for stroke.

The potential impact of specific anti-apoptotic proteins on susceptibility to ischemic injury has also been evaluated in studies using viral vectors for in vivo gene transfer into the brain. Tagami et al. (9) hypothesized that overexpression of the anti-apoptotic protein bcl-2 would confer neuroprotection in a stroke model; herpes virus vectors that transduced bcl-2 (HSVbcl2) or *Escherichia coli* lacZ (HSVlac) were injected into rat cerebral cortex 24 h before induction of neocortical focal ischemia. Viable tissue was significantly preserved at the injection sites in ischemic-HSVbcl2–treated animals in comparison with HSVlac-injected controls, indicating that bcl-2 expression could protect neurons from ischemic injury in vivo. Another recent study evaluated the impact of neuronal apoptosis inhibitory protein (NAIP) a protein implicated in the pathogenesis of neurodegeneration in spinal muscular atrophy (10). In that study, Xu et al. found that in a transient forebrain ischemia model in adult rat brain, levels of NAIP were selectively elevated in neurons that were resistant to ischemia, and that intracerebral injection of an adenovirus vector overexpressing NAIP reduced ischemic damage in the rat hippocampus.

In addition to a role in mediating neuronal injury, apoptosis also represents a pivotal mechanism regulating nervous system development (11). A substantial fraction of differentiating CNS neurons are destined to die as the brain matures. Transgenic mice deficient in caspase-3 have markedly abnormal brain development, and a major feature of the brain dysgenesis is neuronal hyperplasia (12). Cheng et al. (7) raised the appealing hypothesis that apoptosis may be more readily acti-

1. *Abbreviations used in this paper:* ICE, interleukin-1 converting enzyme; NAIP, neuronal apoptosis inhibitory protein.

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vated in pathological conditions that occur during the period of brain development, and, as a result, apoptotic mechanisms may play a particularly important role in mediating neurodegeneration in the neonatal brain. Currently, it is not feasible to test this hypothesis for ischemic injury directly because no established experimental cerebral ischemia models yield pathophysiologically equivalent insults over a broad range of maturational stages. Yet, it is intriguing to note that distinctive maturational stage–determined patterns of selective vulnerability to hypoxic-ischemic injury have been identified in human neonates. For example, periventricular oligodendroglia are highly vulnerable in premature infants, whereas in term infants the basal ganglia are particularly susceptible to ischemic injury. Whether maturational stage-specific propensity for pathological activation of apoptosis could contribute to these patterns of ontogenetic vulnerability is a compelling question for future research.

Several factors could limit the clinical utility of anti-apoptotic therapies. It is unclear whether CNS function would be improved by preventing the death of what may well be irreparably damaged cells; perhaps anti-apoptotic agents could ultimately be most efficacious in concert with other treatments to enhance functional recovery. In addition, nonspecific inhibition of programmed cell death could, in fact, have deleterious effects, particularly in the neonate in whom the risks of apoptosis inhibitors could be amplified if critical developmental events were disrupted. As more is learned about the regulation of apoptosis, it will hopefully become feasible to selectively target pathological activation of apoptosis.

Although many therapeutic agents prevent ischemic brain injury in experimental animals, progress has been frustratingly slow in translating experimental neuroprotective efficacy data into clinical practice. Yet, the remarkable advances in our understanding of the pathophysiology of ischemic brain injury and the development of novel neuroprotection strategies, such as those reported by Cheng et al. (7), provide the basis for optimism that effective neuroprotective therapies ultimately will be incorporated into clinical practice, and will improve long-term neurological outcome both in children and adults.

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