Future Targets and Cascades for Neuroprotective Strategies

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Abstract—Cumulative evidence suggests that apoptosis plays a pivotal role in neuronal death after cerebral ischemia in various experimental animal models. The time-dependent molecular and biochemical sequelae that lead to apoptotic cell death after the interruption of cerebral blood flow have been established. Many neuroprotective agents that target cell death pathways have been failures, and alternative strategies need to be considered. One such strategy is to target the neuronal survival signaling pathway, which involves the phosphatidylinositol 3-kinase (PI3-K)/Akt (protein kinase B) pathway. It has been demonstrated that PI3-K/Akt and downstream phosphorylated Bad and proline-rich Akt substrate survival signaling cascades are upregulated in surviving neurons in the ischemic brain that overexpresses copper-zinc superoxide dismutase activity. These studies provide an impetus for novel therapeutic targets in neuroprotective strategies in stroke. (Stroke. 2004;35[suppl I]:2748-2750.)

Key Words: apoptosis ■ cell survival signaling ■ mitochondria ■ neuroprotection ■ stroke

Ischemic cell death signaling pathways have recently been demonstrated as intrinsic mitochondria-dependent and extrinsic receptor-mediated pathways of apoptosis.1 The mitochondria-dependent pathway of apoptosis has been thoroughly investigated in experimental animal models of cerebral ischemia. This cell death pathway also includes apoptosis inhibitors that can inhibit activation of caspase.

Apoptosis Signaling Involving Mitochondria in Cerebral Ischemia

The cell death signaling pathway in mitochondria has recently been demonstrated in the ischemic brain with the release of mitochondrial cytochrome c, a water-soluble peripheral membrane protein of mitochondria and an essential component of the mitochondrial respiratory chain (Figure 1). Cytochrome c is translocated from mitochondria to the cytosolic compartment after transient focal cerebral ischemia (tFCI) in rats,2 in brain slices subjected to hypoxia/ischemia,3,4 and in vulnerable hippocampal CA1 neurons after transient global cerebral ischemia.5 Mitochondria are known to be involved in both the necrosis and apoptosis pathways, which depend on severity of the insult or the nature of the signaling pathways.6 In most instances, severe cerebral ischemia renders mitochondria completely dysfunctional for ATP production, which ensures necrotic cell death. In contrast, various in vitro studies demonstrated that cellular or biochemical signaling pathways involve mitochondria in apoptosis by releasing cytochrome c to the cytoplasm. Cytochrome c interacts with the CED-4 homologue Apaf-1 and deoxyadenosine triphosphate, forming the apoptosome and leading to activation of caspase-9, which in turn initiates the cytochrome c–dependent caspase cascade, then activates caspase-3, followed by caspase-2, -6, -8, and -10 activation downstream. Caspase-3 also activates caspase-activated DNase (CAD) and leads to DNA damage. In cerebral ischemia studies, caspase-3 and -9 have also been shown to play a key role in neuronal death after ischemia.7–9 The downstream caspases cleave many substrate proteins, including poly(ADP-ribose) polymerase (PARP).8–10 Substrate cleavage causes DNA injury and subsequently leads cells to apoptotic cell death, but excessive activation of PARP causes depletion of nicotinamide-adenine dinucleotide and ATP, which ultimately leads to cellular energy failure and death (Figure 1). Consistent with these ideas, PARP knockout mice showed a significantly decreased infarction volume after transient middle cerebral artery occlusion.11 A recent study has further demonstrated the role of PARP in the release of apoptosis-inducing factor from mitochondria and subsequent translocation to the nucleus for DNA damage and apoptosis.12 Conversely, there are proteins that can prevent caspase activation in the cytosol. The inhibitor-of-apoptosis protein (IAP) family suppresses apoptosis by preventing activation of procaspases and also by inhibiting the enzymatic activity of active caspases. The second mitochondria-derived activator of caspase (Smac) is also released by apoptotic stimuli and binds IAPs, thereby promoting activation of caspase-3. A recent study showed that mitochondrial release of cytochrome c and Smac preceded caspase activation after global ischemia, suggesting the importance of IAP inhibition as well as caspase activation.7 It is essential to point out that these cell death signaling pathways are regulated by reactive oxygen species and the redox state of the cell during cerebral ischemia.
Akt phosphorylates Bad and obviates its inhibitory effects on Bcl-X̂, ultimately inhibiting the release of cytochrome c by blocking channel formation by Bax on the mitochondrial membrane. Akt also inhibits proteolytic activity of caspase-9 by phosphorylating it on Ser-196. In addition, Akt can translocate into the nuclei and inactivate a proapoptotic member of the Forkhead family of transcription factors by phosphorylation, thereby inhibiting activation of the Fas pathway of apoptosis. Mitogen-activated protein kinase (MAPK) family members, including extracellular signal–regulated kinase (ERK), play a critical role in the regulation of cell growth, differentiation, and cellular response to cytokines and stress. In this pathway, Ras recruits the main effector, Raf-1, to activate MAPK/ERK kinase 1/2. Active ERK 1/2 inactivates Bad through phosphorylation of 90-kDa ribosomal S6 kinases. Transforming growth factor-β1 has been shown to suppress Bad activity by phosphorylation of Bad at the Ser-112 site via activation of the ERK pathway in both in vivo cerebral ischemia models and in vitro studies. Phosphorylation of ERK 1/2 is involved in apoptosis and cell death after transient middle cerebral artery occlusion. Phosphorylation of the Ser-155 residue in Bad is regulated by protein kinase A (PKA) in studies in vitro. In rodent focal cerebral ischemia models, intraventricular injection of a PKA inhibitor, H89, effectively suppressed PKA activity and dimerization of Bad/Bcl-X̂, and subsequent apoptotic cell death. This cumulative evidence suggests that Akt and PKA pathways inhibit the function of Bad as a cell survival signaling pathway after cerebral ischemia.

Besides Bad survival signaling, PI3-K/Akt is also involved in many other survival signaling pathways. One such pathway includes MDM2/p53. In addition, a novel proline-rich Akt substrate (PRAS) was recently detected and found to be involved in apoptosis. We have found that PRAS is phosphorylated by Akt in surviving cortical neurons and that phosphorylated PRAS (pPRAS) and the binding of pPRAS phosphorylated Akt (pPRAS/pAkt) to 14-3-3 (pPRAS/14-3-3) were altered, and their expression was briefly decreased in mouse brains after tFCI. Liposome-mediated pPRAS cDNA transfection induced overexpression of pPRAS, promoted pPRAS/14-3-3, and inhibited apoptotic neuronal cell death after tFCI. Expression of pPRAS, pPRAS/pAkt, and pPRAS/14-3-3 increased in nerve growth factor–treated mice but decreased with inhibition of PI3-K and the nerve growth factor trkA receptor after tFCI. These results suggest that PRAS phosphorylation and its interaction with pAkt and 14-3-3 might play an important role in neuroprotection mediated by nerve growth factor in antiapoptotic neuronal cell death after tFCI. Further studies have also shown that oxidative stress is also involved in modulating the expression of pPRAS and pPRAS/pAkt and of pPRAS/14-3-3 binding, again suggesting that the PI3-K/Akt survival signaling pathway is upregulated by SOD1 overexpression (Figure 2).

We propose that mitochondria and the PI3-K/Akt signaling pathway are determinants for the control of proapoptosis and antiapoptosis in ischemic neurons during stroke. Further studies of the survival signaling pathways may provide novel therapeutic strategies for clinical stroke.
Figure 2. Life and death signaling in ischemic neurons involving mitochondria and the PI3-K/Akt pathway. PKB indicates protein kinase B.

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References


