Pathophysiology of acute ischemic stroke

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A variety of pathophysiologic events occur following a stroke, and knowledge of these events can lead to potential therapeutic strategies that may reverse or attenuate injury. Events that occur following stroke include accumulation of excitatory amino acids, alterations in the genomic response, mitochondrial injury, and secondary injury, often in the setting of reperfusion. This review will focus on these areas, with an emphasis on identifying potential strategies to reverse or limit ischemic damage to the brain.

EXCITOTOXIC HYPOTHESIS AND CALCIUM TOXICITY

It is well recognized that a significant portion of ischemia-induced neuron damage is mediated by excessive accumulation of excitatory amino acids, leading to toxic increases in intracellular calcium and other ions. This increase in calcium activates various signaling pathways, ultimately leading to cell death. Soon after cessation of cerebral blood flow, energy-dependent pumps fail, resulting in the flow of ions down their concentration gradients. This results in cellular swelling and depolarization. Calcium (Ca^{++}) enters the cell through voltage-dependent ion channels and activation of ligand-gated receptors, resulting in activation of a number of proteases, kinases, lipases, and endonucleases, ending in cell death. Glutamate, which is the major excitatory neurotransmitter in the brain, accumulates in the extracellular space and activates its receptors, some of which are also calcium-permeable.

There are four major types of glutamate receptors. The ionotropic receptors include the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA), and kainate (KA) receptors. The fourth receptor class is the metabotropic receptor (mGluR), which is further subdivided into eight subtypes. This latter receptor class appears to be G-protein-coupled and involves phosphoinositide hydrolysis, with some mGluR subtypes bearing protective properties and others being damaging.

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EARLY GENE EXPRESSION AND CEREBRAL ISCHEMIA

Although it has long been held that protein synthesis and gene expression cease after ischemia onset, recent work has shown that quite the opposite is true. In fact, the brain has been shown to upregulate many genes and their corresponding proteins in response to injury. It is now recognized that some of these genes may actually protect the brain from a variety of stresses, including stroke. One such gene that is increased following stroke is the 70-kD inducible heat shock protein (HSP70). HSP70 performs chaperone functions by assisting in the proper folding of newly synthesized proteins. Following ischemia, it is thought to perform this function and to prevent protein aggregation. The availability of gene chips permitting the simultaneous study of thousands of genes will provide the ability to screen for other genes not previously identified, and could lead to the discovery of novel treatment strategies.

REACTIVE OXYGEN AND NITROGEN SPECIES

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been implicated in exacerbating ischemic brain injury by reacting with macromolecules, and they may activate apoptosis and inflammation.

When tissue becomes reperfused, high levels of oxygen lead to the generation of ROS and lead to direct tissue damage. ROS are thought to be generated by injured mitochondria, especially during reperfusion, but other pro-oxidant systems in the brain are activated due to increased intracellular

calcium (eg, xanthine oxidase, cyclooxygenase). ROS can also be generated by activated microglia and peripheral leukocytes via the NADPH oxidase system. ROS are involved in activating several pathways involved in cell death, such as apoptosis and inflammation. Administration of antioxidants or genetic overexpression of endogenous antioxidants (eg, superoxide dismutase or glutathione peroxidase) can limit the extent of ischemic damage. Ebselen, a glutathione peroxidase mimic, has been studied in clinical trials with some promising results.

RNS include species such as nitric oxide (NO) and peroxynitrite (ONOO, formed from NO and superoxide, and especially damaging to DNA). NO is generated from L-arginine through one of several nitric oxide synthase (NOS) isoforms. The neuronal form (nNOS) is activated via NMDA receptor stimulation, whereas inducible NOS (iNOS) is largely produced in inflammatory cells such as microglia and monocytes. These two isoforms are, for the most part, damaging to the brain under ischemic conditions. Both pharmaceutical nNOS and iNOS inhibitors and genetic mutant mouse models have shown that inhibition of these isoforms improves outcome in experimental stroke. A third isoform found in endothelial cells (eNOS) is believed have vasodilatory properties and may play a beneficial role, as it may ultimately improve local blood flow. A class of cholesterol-lowering agents, the statins, is thought to increase local eNOS generation.

■ INFLAMMATION

During reperfusion, inflammatory cells may also gain access to necrotic regions. Although the inflammatory response accompanying ischemia has long been thought to be involved in recovery and repair, this response is also known to potentiate damage, particularly in the acute to subacute phases. This is not entirely surprising, since leukocytes are capable of producing a variety of reactive species and toxic proteases when stimulated. An endogenous inflammatory response also occurs in the brain as microglia are activated in the presence of ROS. Interfering with these processes by preventing leukocyte infiltration (anti–adhesion molecule treatments), microglial activation (minocycline), or leukocyte generation of reactive species (aminoguanidine to inhibit iNOS or apocynin to inhibit NADPH oxidase) has been shown experimentally to reduce ischemic injury. Although two anti–adhesion molecule strategies have already been studied in clinical trials, these studies were flawed or were never completed to the point of offering meaningful conclusions on whether this approach may be useful in humans.

■ APOPTOSIS

Recent studies have defined an orderly "programmed" cell death referred to as apoptosis. Apoptosis normally occurs in many organisms as a part of development, but it is now recognized to contribute to cell death in a variety of pathologic states, including stroke. Apoptosis is an energy-dependent process leading to DNA fragmentation. Although much of ischemic injury has been presumed to be necrotic, apoptosis has been detected in penumbral brain regions and after mild ischemic insults.

Central to most forms of apoptosis are the caspases, a family of proteases whose name stems from the fact that they are cysteine proteases. Caspases are proenzymes that must be cleaved to their active form and that ultimately lead to DNA cleavage and chromatin condensation (hallmarks of apoptosis).

There are several classes of caspases, including the initiator (eg, caspase 8) and effector (eg, caspase 3) classes. The intrinsic, or mitochondria-dependent, pathway has been the most studied in ischemia models. This model proposes that less severe ischemic insults result in damage to the mitochondria due to prolonged depolarization, exposure to ROS, or both. This leads to the formation of a permeability transition pore that allows release of cytochrome *c* from its inner membrane. Cytosolic cytochrome *c* complexes with procaspase 9 and Apaf-1, forming the so-called apoptosome, leading to activation of caspase 9. Caspase 9 then activates effector caspases, including caspase 3, leading to DNA fragmentation and chromatin condensation, all hallmarks of apoptosis. Caspase antagonists and inhibitors of cytochrome *c* release (eg, cyclosporin A) have been shown to reduce cerebral ischemic injury. Apoptosis may also proceed independent of caspases via mitochondrial release of apoptosis initiating factor (AIF), which translocates from the mitochondria into the nucleus, leading to morphologic changes characteristic of programmed cell death. This latter pathway may be especially relevant in stroke, as the demonstration of caspase activity in ischemia models has been variable

despite morphologic evidence of apoptosis.

The Bcl-2 family of proteins possess both pro- and anti-apoptotic functions. Bcl-2 and Bcl-X_L are "death suppressors" that act by preventing cytochrome *c* and AIF release. Bak, Bid, Bcl-X_S, and Bax are "death enhancers" that are capable of forming the permeability transition pore. Studies in rodents in which Bcl-2 or Bcl-X_L is overexpressed show that these proteins are neuroprotective.

Other apoptotic pathways have also been implicated during ischemia. The extrinsic, or receptor-mediated, pathway may also be involved since some studies have detected the presence of tumor necrosis factor α (TNF-α) and Fas ligand (FasL) in ischemic brain. These factors bind to their respective receptors (TNFR1 and Fas), resulting in activation of caspase 8, which directly activates caspase 3 independent of cytochrome *c* release.

■ CONCLUSIONS

The molecular biology of ischemic injury is a rapidly growing field that may lead to the identification of novel stroke therapies. To date, no pharmacologic neuroprotectant has been found efficacious in humans. However, recent studies using mild hypothermia to prevent neurologic damage in patients suffering cardiac arrest are promising. Because hypothermia may target multiple cell death pathways, it may someday prove to be the ultimate neuroprotectant.

■ FURTHER READING

Zheng Z, Lee JE, Yenari MA. Molecular mechanisms and potential targets for treatment. Curr Mol Med 2003; 3:361–372.

Han HS, Yenari MA. Cellular targets of brain inflammation in stroke. Curr Opin Investig Drugs 2003; 4:522–529.