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Novel Stroke Therapeutics: Unraveling Stroke Pathophysiology and Its Impact on Clinical Treatments

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Stroke remains a leading cause of death and disability in the world. Over the past few decades our understanding of the pathophysiology of stroke has increased, but greater insight is required to advance the field of stroke recovery. Clinical treatments have improved in the acute time window, but long-term therapeutics remain limited. Complex neural circuits damaged by ischemia make restoration of function after stroke difficult. New therapeutic approaches, including cell transplantation or stimulation, focus on reestablishing these circuits through multiple mechanisms to improve circuit plasticity and remodeling. Other research targets intact networks to compensate for damaged regions. This review highlights several important mechanisms of stroke injury and describes emerging therapies aimed at improving clinical outcomes.

Pathophysiology of Stroke

The lack of blood flow during a stroke results in an intricate pathophysiological response resulting in neural injury, as depicted in Figure 1 (Hossmann, 2006). Multiple mechanisms, including excitotoxicity, mitochondrial response, free radical release, protein misfolding, and inflammatory changes, lead to neural cell loss, but many of these pathways ultimately pave the way for recovery. Injury and death of astrocytes, as well as white matter injury, also contribute to cerebral damage. The delicate balance between detrimental or beneficial effect often relies on the timing and the magnitude of the factors involved. The inflammatory response is a prime example of a system that both propagates ischemic injury and helps promote recovery. Inflammation initially contributes to cellular injury through the release of cytokines and harmful radicals but eventually helps to remove damaged tissue, enabling synaptic remodeling. Glial cells also serve dual roles, helping to regulate the blood-brain barrier, promoting angiogenesis and synaptogenesis, but conversely forming the glial scar that may prevent further plasticity (Gleichman and Carmichael, 2014). The goal for this review is to provide a brief overview of the pathophysiology of stroke followed by a discussion of the current state of stroke recovery research with an emphasis on those approaches that target multiple mechanistic pathways. Many of these therapies are aimed at upregulating pathways that enhance recovery while reducing the deleterious pathways triggered by the initial ischemic insult. Further understanding and optimizing this delicate balance may facilitate development of effective stroke therapeutics.

Excitotoxicity

CNS ischemia results in a deficiency of glucose and oxygen leading to the inability of neuronal cells to maintain normal ionic gradients. Depolarization of these neurons leads to excessive glutamate release resulting in the intracellular influx of calcium, triggering cell death pathways such as apoptosis, autophagocytosis, and necrotic pathways (Lipton, 1999). This process has been termed excitotoxicity and is mediated largely through the glutamatergic pathways involving N-methyl-D-aspartate receptors (NMDARs), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptors (AMPARs), and kainate receptors (Dirnagl et al., 1999; Moskowitz et al., 2010). The role of calcium in excitoxicity also remains complex and has numerous effects in the ischemic environment. The intracellular increase in calcium triggers mitochondrial dysfunction and activation of free radicals, phospholipases, and proteases, which lead to cell death or injury (Szydlowska and Tymianski, 2010). Interestingly, the interplay between the cells is also critical to the spread of injury after ischemic insults. Blockage of the gap junctions between cells in the adult brain reduces neuronal death (Wang et al., 2010), potentially indicating the important interactions that occur between cells during neuronal damage. These processes also promote cerebral edema, which has clinical import in the first few days after a stroke. Numerous therapeutic approaches have centered on interrupting pathways triggered by excitotoxicity to improve stroke recovery, and while often successful in animal models (Yenari et al., 2001; Namura et al., 2013), translation of these findings into the clinic remains challenging.

Mitochondrial Alterations

The mitochondria play a critical role in cell energy homeostasis and are thus prominently involved during ischemia when the energy balance is disrupted and ATP synthesis is altered. The rapid influx of calcium experienced with excitoxicity leads to excess accumulation in the mitochondria, causing dysfunction, which leads to mitochondrial permeability transition pore (mtPTP) opening and cytochrome c release (Liu et al., 1996; Murphy et al., 1999). These events create mitochondrial swelling and membrane collapse, initiating cell death cascades such as

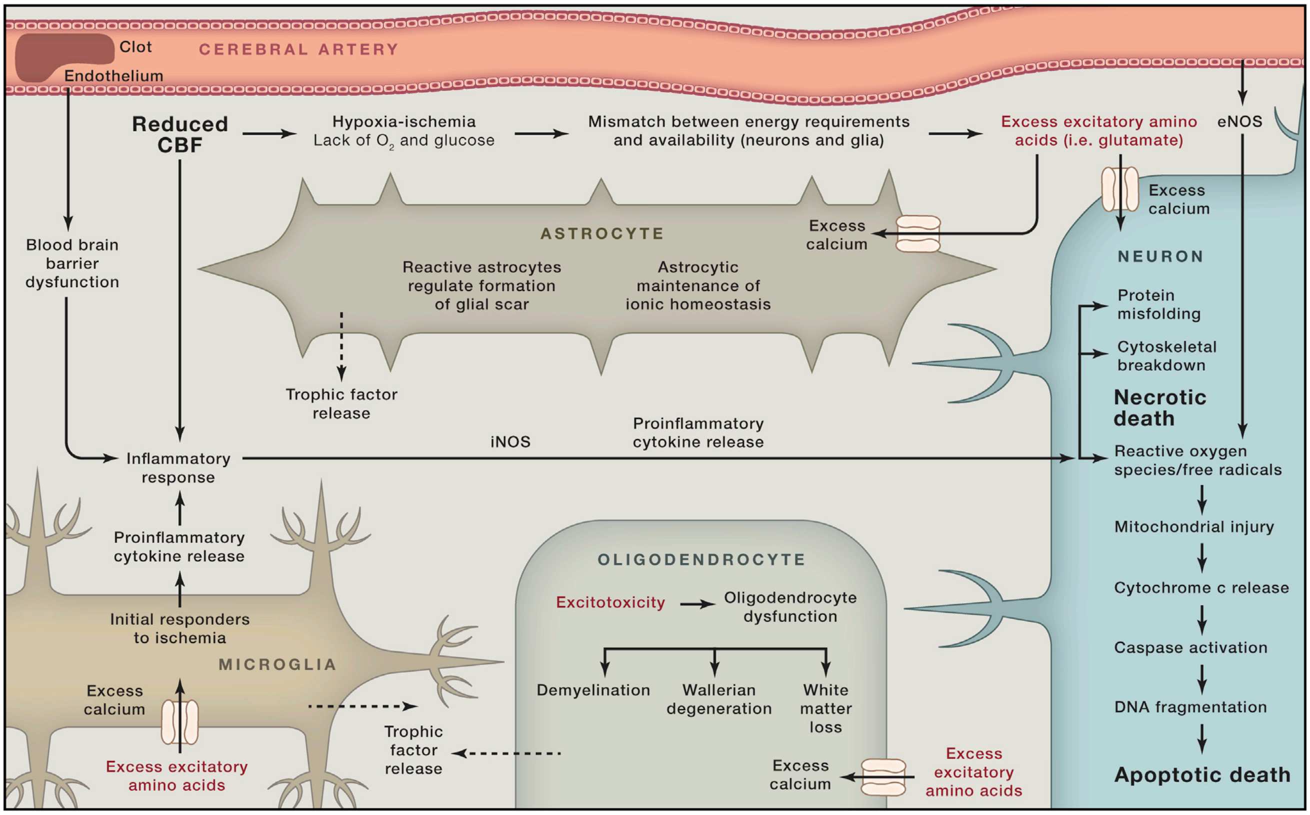

Figure 1. Pathophysiology of Stroke

apoptosis (Liu et al., 1996). The reactive oxygen species (ROS) created by the mitochondria also play a prominent role in reperfusion injury and cell death in the ischemic environment (Kalogeris et al., 2014). Maintaining mitochondrial integrity and limiting their induction of apoptotic and oxidative stress pathways in the cell are important avenues to preventing widespread cell toxicity from an ischemic insult.

Free Radicals

Brain ischemia also triggers free radicals, which contribute to the oxidative stresses on neural tissue. The influx of calcium triggers nitric oxide (NO) production by nitric oxide synthase (NOS) that leads to injury through the formation of oxygen free radicals and the production of peroxynitrite (ONOO−) (Iadecola, 1997). The mitochondria undergo dysfunction during ischemia, leading to further oxidative stress (Kalogeris et al., 2014). NADPH oxidase also plays a critical role in ROS production in the setting of excitotoxicity and ischemia (Moskowitz et al., 2010). Furthermore, chimeric bone marrow studies have shown that inflammation contributes with neutrophils releasing inducible NOS (iNOS), which leads to toxic levels of NO (Garcia-Bonilla et al., 2014; Moro et al., 2004). Free radicals trigger the PI3-kinase/Akt pathway as well as upregulate the transcription factor NF-κB. Interestingly, the timing and environment of activation of this pathway likely determine whether stroke recovery is improved or impeded by this signaling cascade (Crack and Taylor, 2005). Other pathways of interest are the transient receptor potential (TRP) channels. TRP channels, TRPM7 specifically, are linked to free radicals in ischemia and likely contribute to increasing the influx of calcium and cellular toxicity experienced during decreased oxygenation (Sun et al., 2009). Not only do free radicals contribute to initial toxicity, they also prevent recovery, which makes them an important post-stroke therapeutic target (Miyamoto et al., 2013). Numerous methods have reduced the oxidative stress from free radicals in ischemic injury and shown neurologic improvement in preclinical models. Combining the regulation of these pathways with other ischemic injury mechanisms may lead to novel therapeutics.

Protein Misfolding

The largest stores of intracellular calcium reside in the endoplasmic reticulum (ER), an organelle that regulates protein synthesis and responds to protein misfolding (Zhang et al., 2014). These processes are largely affected by ER stress induced by ischemic injury (Roussel et al., 2013). As excitotoxic changes occur in neural cells, the sarcoplasmic/ER calcium ATPase (SERCA) pump fails due to energy depletion and adds to the occurrence of cell death (Szydlowska and Tymianski, 2010). The increased accumulation of misfolded proteins also trigger the protein kinase-like ER kinase (PERK) pathway regulating eIF2α kinase activation, which halts new protein synthesis (Althausen et al., 2001). The phosphorylation of eIF2α has been explored as a means to alter damage in cerebral ischemia. Inositol requiring enzyme 1 (IRE1) is another protein involved in the misfolding of proteins that has been shown to induce apoptotic pathways during periods of ER stress (Morimoto