

# Advances in Our Knowledge of Stroke Mechanisms and Therapy

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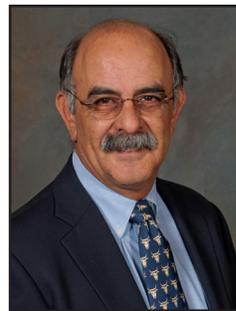
**S**troke, the most common medical emergency, is a cerebrovascular accident that can cause death and long-term disability. Over 80 percent of all strokes are ischemic strokes, when a clot lodges in an artery supplying blood to the brain and the blood flow is reduced or blocked. A small percentage, caused by rupture of brain blood vessels, are called hemorrhagic strokes. Stroke risk factors are well known and include high blood pressure, elevated lipids, diabetes, smoking, heavy drinking, coronary artery disease, heart diseases, *etc.*

Both ischemic and hemorrhagic strokes may cause a variety of neurologic symptoms, including sudden death, loss of consciousness, one-sided numbness, weakness, paralysis, sudden deafness, visual impairment, or difficulty in speaking, thinking, or understanding speech. Specific symptoms correspond with the location of stroke injury. For example, stroke damage in the brain stem (a pivotal center in the regulation of cardiac and respiratory function) may cause sudden death; a clot or bleeding in middle cerebral artery (MCA) territory (including the motor cortex and the underlying caudate-putamen), may cause numbness, weakness or paralysis of the contralateral side of the body.

A computerized tomography (CT) scan or Magnetic Resonance Imaging (MRI) is required to distinguish ischemic and hemorrhagic strokes. One of these images is required for clinicians to decide on the therapeutic strategies for patients when a stroke occurs. There are different mechanisms and treatments for ischemic and hemorrhagic strokes. Here we focus on the description of our current knowledge of the mechanisms and current treatments for ischemic stroke.



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## Current Treatments

A stroke requires immediate medical attention and urgent care because stroke damage to the brain evolves every second. To date, intravenous thrombolytic agents (which pharmacologically dissolve clots) and surgical strategies (which retrieve clots by mechanical means) are used to treat acute ischemic stroke. Tissue plasma activator (tPA) is the only FDA approved medication for acute ischemic stroke and must be used within 4.5 hours from the time of the onset of symptoms of stroke. Other medical thrombolytic drugs such as streptokinase and aspirin, show higher mortality or disability, and a higher risk of brain hemorrhage. Surgical interventions should be performed within 6 hours of acute stroke symptoms, and only after a patient receives tPA. Considerable improvements in stroke outcome are seen with both tPA and surgical clot removal when used within their allowable time windows.

## Mechanisms in Ischemic Stroke

Experimental animal stroke models and cell culture models were developed for stroke studies several decades ago and rigorous scientific investigations have been undertaken. Some models mimic pathophysiological changes in human stroke and address many puzzles in understanding stroke mechanisms, which are essential in developing effective therapeutic strategies for stroke patients.

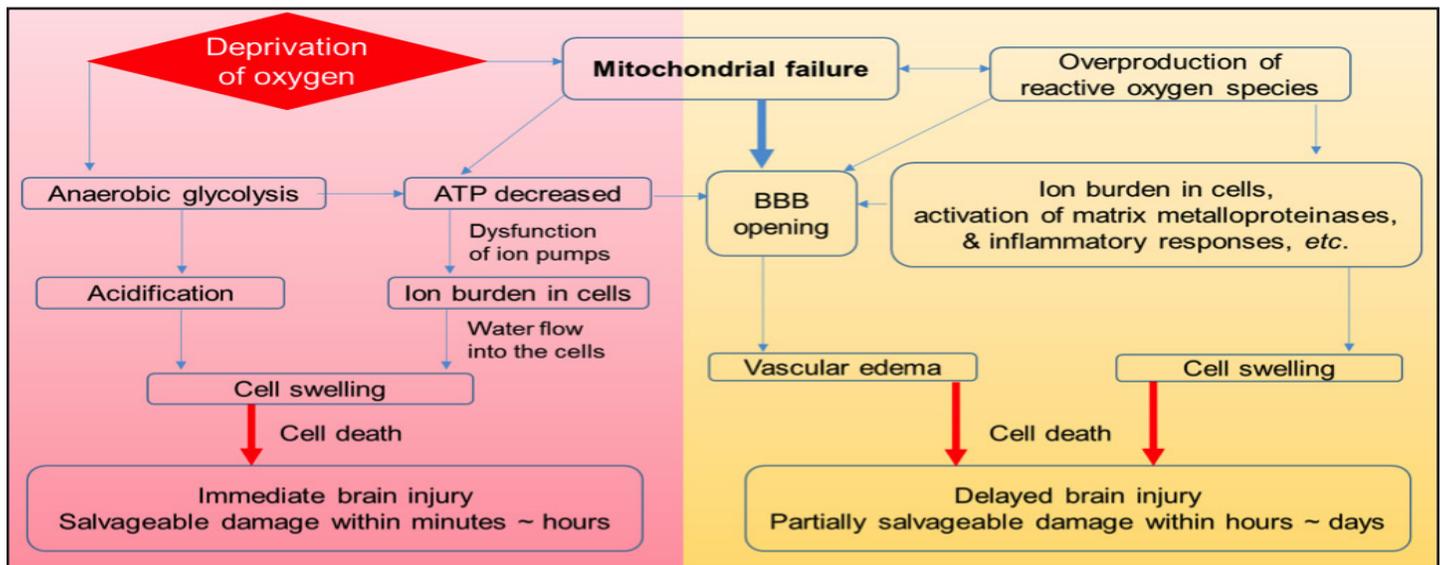
**Figure 1** describes our current understanding of stroke mechanism: during the first few hours, the primary ischemic stroke injury is due to the occlusion of the blood vessels. The occlusion leads to an insufficient energy supply to the brain tissue and causes neuronal death and neurological damage. Reduced oxygen supply compromises mitochondrial function and causes

anaerobic glycolysis (transforms glucose to lactic acid when an insufficient amount of oxygen is available). Elevated levels of lactic acid make the brain tissue acid and results in decreased ATP production and reduced ion pumps activity on cell membrane. As a consequence, concentrations of protons, calcium, and sodium ions increase in the cells. This results in water influx into the cells, cell swelling, death, and formation of core infarction in primary stroke injury. This series of events evolve quickly in the primary stroke site, but might be salvageable by treatment within minutes to hours. Secondary stroke damage is initiated by post-stroke disruption of the blood-brain barrier (BBB), initiating a cascade damaging molecular processes. BBB is formed by endothelial cells surrounding the brain's vasculature, which restrict access of molecules in the general circulation of the brain (See **Figure 1** and following text).

### ***Mitochondrial failure and BBB disruption***

BBB opening is a key stage in secondary stroke injury and has been linked to increased stroke damage and vascular edema in animal models of stroke. Several groups have observed a biphasic BBB breakdown after transient stroke in animal models. The first BBB disruption occurs within several hours of the onset of ischemia and a second phase of BBB opening may happen after several days of ischemia. Recently, using an experimental model of stroke, our group has shown that mitochondria play a critical role in the first BBB opening<sup>1</sup>. Due to lack of oxygen, mitochondrial failure decreases ATP production in the endothelial cells surrounding the blood vessels in the brain, causing a breakdown of the BBB and further exacerbating stroke damage<sup>2</sup>.

We have also shown that lipopolysaccharide



**Figure 1. Mechanisms in Ischemic Stroke Damage.** Ischemia causes immediate brain damage in primary stroke injury and delayed brain damage in secondary stroke injury. Mitochondrial failure plays a critical role in BBB opening that involves in vascular edema following stroke.

(LPS, an exotoxin extracted from bacteria and used to mimic bacterial infection) worsens stroke outcomes by the same mechanisms of compromised mitochondria in the endothelial cells<sup>1-3</sup>. We have also found that a food preservative, tBHQ, interferes with mitochondrial function and worsens stroke outcome as well<sup>4</sup>. This evidence suggests that mitochondrial dysfunction plays a critical role in the first BBB disruption and acute stroke damage.

It is not known yet how the BBB is repaired after the initial first opening. There is a debate as to whether the second BBB opening contributes to brain damage or brain repair after stroke. More investigation is needed to resolve this issue.

### ***Reactive oxygen species (ROS) and stroke<sup>5</sup>***

About 95 percent of ROS, such as superoxide ( $O_2^-$ ) and hydrogen peroxide ( $H_2O_2$ ) are derived from electron leakage during the activity of the mitochondrial respiratory chain, which generates ATP. These ROS are scavenged by manganese-superoxide dismutase (Mn-SOD or SOD2), catalase and glutathione peroxidase. Ischemic stress triggers a disturbance of the mitochondrial membrane potential and the normal proton gradient, which results in excessive ROS generation via electron transfer for the ATP synthesis.

The overproduction of ROS overwhelms oxidant scavenging capacity and establishes a vicious positive feedback loop in which increased ROS-induced ROS production. ROS also damage mitochondrial DNA, induce activation and secretion of matrix metalloproteinases (MMPs), activate inflammatory responses, and lead to cell swelling and death. This process causes BBB damage and secondary injury in acute stroke. However, ROS may also be involved in tissue remodeling and angiogenesis (new blood vessel formation) in the late phase of stroke.

### ***Matrix metalloproteinases (MMPs) and stroke<sup>6</sup>***

MMPs, a large family of proteolytic enzymes, are present in various cells in latent forms and are capable of degrading all components of the extracellular matrix. ROS may induce activation and secretion of MMP-2 in astrocytes, MMP-3 and MMP-9 in endothelial cells and in neutrophils. The activated MMPs breakdown and degrade tight junctions, a critical component of the BBB, and contribute to BBB disruption and hemorrhagic transformation in acute stroke. Interestingly, evidence also suggests that MMPs

participate in brain repair in the stroke late phase.

### ***Inflammatory responses in the ischemic brain<sup>7</sup>***

Our immune system protects the body from infections. We did not anticipate that the immune system would play a negative role in stroke by attacking brain tissue as it were “foreign”. However, this does happen in most of cases of acute strokes. Inflammatory responses are initiated in the clots causing the stroke. Platelets and white blood cells (WBCs) are trapped in the clot fibrin due to hypoxia and shear stress in small blood vessels. MMPs are released by leukocytes and facilitate the BBB opening. Following the BBB disruption, neutrophils, lymphocytes, and monocytes from peripheral blood infiltrate into the brain tissue. Pro-inflammatory cytokines<sup>8</sup> and chemokine released by WBCs may destroy brain neurons directly or indirectly. The inflammatory cells currently being actively investigated include:

### ***Neutrophils***

Neutrophils, an essential part of the innate immune system, are one of the first peripheral responding WBCs to launch a defense against all classes of infections. Vesicles that contain various enzymes, including oxidase and MMPs are stored in neutrophils. Hypoxia induces the secretion of chemokines by neurons, which attract the migration of neutrophils; meanwhile hypoxia-induced ROS over-production activates the release of the MMPs from neutrophils that can attack BBB, permitting neutrophils to migrate into the ischemic brain tissue via the degraded BBB. From there, neutrophils phagocytose dying neurons and release a large amount of granules and ROS that may kill both dying and living neurons.

### ***Antigen Presentation Cells (APCs)***

APCs are classified as professional APCs and non-professional APCs. The former includes macrophages, microglia, dendritic cells, B cells, and

monocytes. From our basic immunology book, we know that professional APCs express major histocompatibility complex II (MHCII) on their surfaces and present antigens to T-cells. Non-professional APCs express MHC class I molecules on their surface. Following BBB disruption and the release of brain antigens into the periphery, these APCs present brain antigens to T-cells in the late stage of ischemic stroke, and they may be partially responsible for ischemia-induced dementia. However, in acute stroke, APCs may produce pro-inflammatory cytokines, such as TNF- $\alpha$  and IL-1 $\beta$  that contribute to post-stroke inflammation; they may also produce anti-inflammatory cytokines IL-10 and TGF- $\beta$ , which promote healing and reduce inflammatory responses.

### ***Lymphocytes***

Lymphocytes, including T-cells and B-cells belong to the adaptive immune system that involves both cellular and humoral immunity against antigens. Growing evidences indicate that T-cells and B-cells participate in the progression of ischemic stroke. Elevated antibodies (produced by B-cells) against brain antigens, and increased cytokines (secreted by T-cells and other WBCs) are detected in stroke, suggesting cellular and humoral responses to ischemic brain damage. Using the experimental stroke animal model, we have previously demonstrated that B-cells are protective in acute strokes by secreting anti-inflammatory cytokine IL-10. Another group found that B-cells could impair cognitive behavioral outcomes and contribute to post-stroke dementia in the late phase. Researchers are striving to identify the role of T cells in stroke. Tolerated T-cells that induce a TH2 cytokine response, including IL-4 and IL-10 secretion, reduce stroke infarction. TH1 cells, secreting pro-inflammatory cytokines (such as IL-1 $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$ .) exacerbate stroke. Mice, which have the recombination activation gene (RAG1) inactivated are deficient in T-cells and B-cells and are protected from ischemia. However, transfer of naive T-cells worsens

stroke infarction following 24 hours reperfusion, suggesting that T-cells are detrimental early in stroke. It has been demonstrated that  $\gamma\delta$ T cells (a unique T-cell subtype that links adaptive and innate immune system) contribute to brain damage by secreting a pro-inflammatory cytokine IL-17.

### ***Other mechanisms***

Scientists have also investigated other mechanisms in stroke, such as, glutamate and other excitatory neurotransmitters released by dying neurons which cause exotoxic damage to healthy neurons. MicroRNAs, a hot topic in life sciences, are changed in stroke animals and patients, and might be involved in brain damage and recovery as well. Other studies have also revealed that nitric oxide (NO) and peroxynitrite (ONOO $^-$ ) produced simultaneously with superoxide in stroke, are detrimental to brain neurons.

### ***Future perspectives***

Despite the benefits afforded by tPA and surgical treatments, there is a desperate need to develop more effective therapies for stroke because very few stroke patients can meet the brief time window necessary for effective clot removal. While there are still debates as to whether we have successfully traveled from clot removal in early stroke to effective treatments in the second stroke injury, it is clear that we have gained more knowledge in the past two decades than any in our prior history. The failure of several clinical trials on some potential drugs raises questions about our complete understanding of the mechanisms in stroke. Given the complicated mechanisms revealed so far, drugs that target a single mechanism may not be able to stop the progression of post-stroke injury. However, if properly timed, we believe that preserving mitochondrial function in the BBB might be able to restrict the damage of stroke. We anticipate that a cocktail of compounds will be needed to preserve mitochondrial function representing a novel strategy for stroke treatments.

## Further reading

- 1 Doll, D. N. *et al.* Mitochondrial crisis in cerebrovascular endothelial cells opens the blood-brain barrier. *Stroke; a journal of cerebral circulation* **46**, 1681-1689, doi:10.1161/STROKEAHA.115.009099 (2015).
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- 3 Doll, D. N. *et al.* Lipopolysaccharide exacerbates infarct size and results in worsened post-stroke behavioral outcomes. *Behavioral and brain functions : BBF* **11**, 32, doi:10.1186/s12993-015-0077-5 (2015).
- 4 Sun, J., Hu, H., Ren, X. & Simpkins, J. W. Tert-butylhydroquinone compromises survival in murine experimental stroke. *Neurotoxicology and teratology* **54**, 15-21, doi:10.1016/j.ntt.2016.01.004 (2016).
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- 6 Turner, R. J. & Sharp, F. R. Implications of MMP9 for Blood Brain Barrier Disruption and Hemorrhagic Transformation Following Ischemic Stroke. *Frontiers in cellular neuroscience* **10**, 56, doi:10.3389/fncel.2016.00056 (2016).
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- 8 Doll, D. N., Rellick, S. L., Barr, T. L., Ren, X. & Simpkins, J. W. Rapid mitochondrial dysfunction mediates TNF-alpha-induced neurotoxicity. *Journal of neurochemistry* **132**, 443-451, doi:10.1111/jnc.13008 (2015).