



Pathophysiology of Neuronal Cell Death After Stroke

16

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Abstract

Stroke is a leading cause of death around the world and results in a drastic reduction in the quality of life. Thus, molecular mechanisms underlying stroke-related neuronal cell death such as necrosis, necroptosis, apoptosis, and autophagy have been extensively investigated in the past 30 years. In the ischemic stroke brain, depletion of ischemic energy leads to increased cytosolic Ca^{2+} through pump failure and cell depolarization, activating phospholipase A2. Phospholipases liberate arachidonate, causing a burst of free radicals in the peri-infarcted lesion. Free radicals lead to apoptotic cell death, and play an important role in the pathological process of ischemic stroke. Concurrently, the free radical scavenger, edaravone, was developed from translational research, mainly using the animal stroke model, and was approved in April of 2001 in Japan for the treatment of acute cerebral infarction, as a neuro-brain protection drug.

In this chapter, we review the molecular mechanisms underlying neuronal cell death in strokes and the development of edaravone and its application to clinical settings, while incorporating our recent related findings.

16.1 General Principles of Cell Death Mechanisms: Necrosis, Necroptosis, Apoptosis, and Autophagy

Necrosis is the term currently used to describe non-programmed cell death or accidental cell death [1, 2], and is generally considered to be a passive process because it does not require new protein synthesis, with minimal energy requirements. This accidental and passive cell death, necrosis, is morphologically characterized by cell and organelle swelling, as well as membrane rupture, followed by the uncontrolled loss of intracellular contents (Table 16.1, Fig. 16.1). Necrosis is usually induced by noxious stimuli including infectious agents, hypoxia, and extreme environmental conditions, including heat and radiation. After exposure to noxious stimuli, the depletion of energy leads to an increase in cytosolic Ca^{2+} through pump failure and cell depolarization. An acute increase in the intracellular calcium concentration activates a calcium-dependent cysteine protease, calpain, that leads

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Table 16.1 Characteristics of necrosis, necroptosis, and apoptosis

		Necrosis	Necroptosis	Apoptosis
Morphological change	Swelling of organelles	+	+	–
	Cell swelling	+	+	–
	Cell membrane rupture	+	+	–
	Release of cell content	+	+	–
	Membrane blebbing	–	–	+
	Cell shrinkage	–	–	+
	Nuclear fragmentation	–	–	+
	Chromatin condensation	–	–	+
Molecular biological change	Phosphatidylserine (PS) exposure on the cell membrane	–	–	+
	Caspase activation	–	–	+
	RIPK1/RIPK3 activation	–	+	–

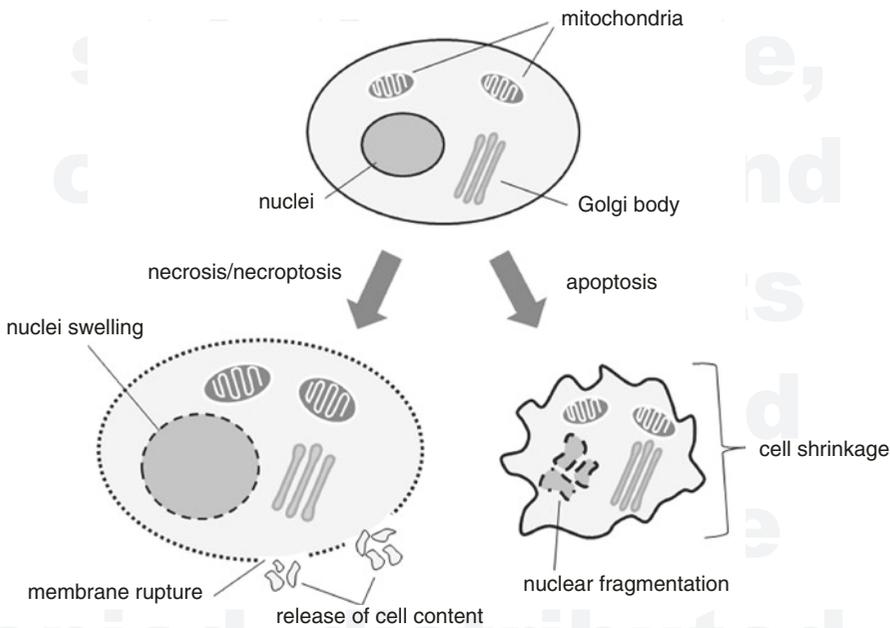


Fig. 16.1 Comparison of morphological changes between necrosis/necroptosis and apoptosis. In necrosis/necroptosis, the cell swells, becomes leaky and the cell membrane is disrupted. Finally, the cell releases its con-

tents into the surrounding tissue resulting in inflammation. On the other hand, apoptotic cells shrink, chromatin condenses and cells are phagocytosed without triggering inflammatory processes

to cleavage of the cytoskeletal protein. Finally, extracellular fluid enters into cells through the ruptured cell membrane, causing cell swelling and cell death [3].

As mentioned above, necrosis has historically been regarded as unregulated cell death that is induced by nonphysiological stress. However,

accumulating evidence suggests that several types of programmed necrosis, including necroptosis [2], ferroptosis [4], parthanatos [5], pyroptosis [6], NETosis [7], and transcriptional repression-induced atypical death (TRIAD) [8], can be executed by a regulated mechanism. In TRIAD, general transcriptional repression

induces slowly progressive atypical cell death associated with a shift in the balance between YAPdeltaCs as prosurvival factors and activated p73, which promotes cell death. Our lab and other labs found that TRIAD occurs and takes part in pathological processes in neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Huntington's disease [8, 9]. As a type of programmed necrosis, necroptosis was originally defined as cell death by necrotic cell death morphology and dependency on the function of receptor-interacting serine/threonine-protein kinase 1 (PIPK1) [2, 10] (Table 16.1). Necroptosis occurs following the activation of PIPK1, in response to the ligation of tumor necrosis factor-receptor (TNF-R). PIPK1 activates PIPK3, which gains the ability to phosphorylate and activates mixed lineage kinase domain-like protein (MLKL), leading to cell death (Table 16.1).

Apoptosis is currently considered as caspase-mediated programmed cell death [11, 12] and is morphologically characterized by plasma membrane blebbing, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation (Table 16.1, Fig. 16.1). Apoptotic cells also exhibit biochemical changes such as the exposure of phosphatidyl-l-serine on the plasma membrane [13]. These apoptosis-related morphologic features result from the activation of caspases by either death receptor

ligation or the release of apoptotic mediators from the mitochondria. In other words, apoptosis involves a complex cascade of reactions that are regulated by specific protease termed caspases. Dying by apoptosis requires energy in the form of ATP. Finally, apoptotic bodies are recognized and removed by phagocytic cells, thus apoptosis basically does not induce inflammation around dying cells.

Autophagy is a self-degradative process in response to various stresses, especially nutrient deficiency. In other words, autophagy is regarded as the process by which a cell consumes itself during periods of starvation. The process of autophagy involves four steps including initiation, nucleation, fusion of the autophagosome and lysosome, and hydrolyzation [14] (Fig. 16.2). Firstly, a double membrane vesicle forms in the cytosol encapsulating whole organelles and bulk cytoplasm, and these vesicles are referred to as autophagosomes. Autophagosomes then fuse with the lysosome, where the contents are degraded to be recycled. The formation of an autophagosome is induced by class 3 phosphoinositide-3-kinase, Atg6 and ubiquitin or ubiquitin-like modifications of the target proteins. Autophagy plays a degradative role during which cells degrade dysfunctional and unnecessary cellular components for the turnover of both damaged organelles and long-lived proteins.

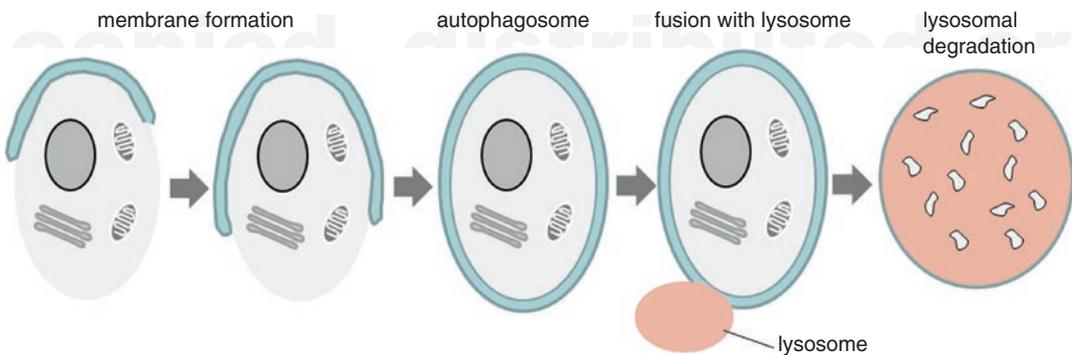


Fig. 16.2 Schematic diagram of autophagy. Autophagy initially begins with the formation of an isolation membrane. Secondly, expansion of the membrane forms an

autophagosome. Thirdly, the outer membrane of the autophagosome fuses with a lysosome. Finally, the sequestered material is degraded and recycled

16.2 Mechanism of Cell Death Caused by Ischemic Stroke Versus Hemorrhagic Stroke

In the ischemic stroke brain, depletion of ischemic energy leads to increased cytosolic Ca^{2+} through pump failure and cell depolarization, activating phospholipase A2. Phospholipases liberate free fatty acids, particularly arachidonate, from cell membranes. This freed arachidonate causes a burst of free radicals in the ischemic penumbra, which is a therapeutic target area, and free radicals are drastically increased after reperfusion [15]. The free radicals directly or indirectly lead to apoptotic cell death, and play an important role in the pathological process of ischemic stroke (Fig. 16.3a).

On the other hand, in a hemorrhagic stroke, the intracerebral hematoma is a key component of pathological processes. This parenchymal accumulation of blood causes tissue disruption causing a mass effect such as primary brain

injury. With large hematomas, the mass effect may increase intracranial pressure, and decrease cerebral blood flow resulting in peri-hematoma ischemia. However, the extent to which the peri-hematoma ischemia takes place remains controversial [16]. In secondary brain injury of a hemorrhagic stroke, thrombin is the main player. Thrombin is essential for blood coagulation and becomes activated within 1 hour after intracerebral hemorrhage. The activated thrombin breaks down the blood–brain barrier (BBB), leading to brain edema, and directly induces neuronal damage. The lysis of hematoma within the first day after intracerebral hemorrhage causes the release of hemoglobin, which is then converted into neurotoxic components such as heme and iron. Heme and iron are major contributors to secondary brain injury as a result of abundant free radical production (Fig. 16.3b) [17]. Of note, free radicals are a common key player and common therapeutic target both in ischemic and in hemorrhagic strokes.

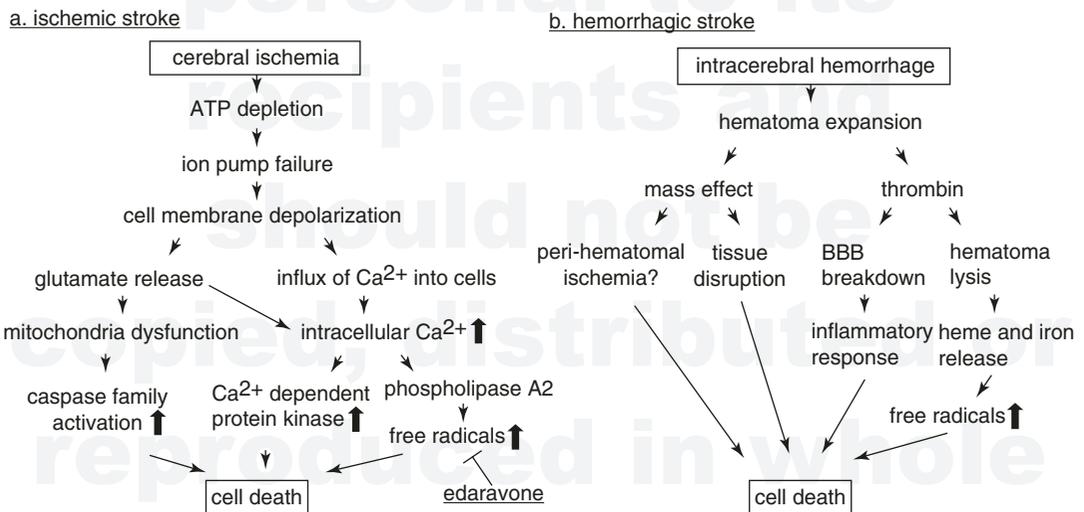


Fig. 16.3 Comparison of pathological mechanism of cell death between ischemic stroke (a) and hemorrhagic stroke (b). Free radicals are a common key player and common

therapeutic target both in ischemic stroke and in hemorrhagic stroke

16.3 Excitotoxic Cell Death

Excitotoxic cell death is triggered by the release of glutamate or related excitatory amino acids under certain conditions, for example, cerebral ischemia. Glutamate is a major excitatory neurotransmitter in the central nervous system, acting through both 1) ligand gated ion channels such as the N-methyl-D-aspartate (NMDA) receptor, the α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, and the kainate receptor, and 2) G-protein coupled (metabotropic) receptors. Cerebral ischemia increased the extracellular concentrations of glutamate, which are mainly released from acidemic glial cells [18]. This results in widespread stimulation of both synaptic and extra-synaptic NMDA receptors, causing a massive influx of calcium into cells, and activation of intracellular enzymes, causing cell death (Fig. 16.3a). Therefore, glutamate receptor antagonists have attracted much attention as potential neuroprotective agents, but randomized clinical trials have failed to show any beneficial effect of those drugs in acute ischemic stroke patients [19].

16.4 Apoptosis Induced by Stroke

Neurons in the ischemic core die through necrosis whereas apoptosis is the main contributor to neuronal cell death in peri-infarct lesions called the penumbra. Numerous studies have shown that excessive intracellular calcium increases via the activation of the glutamate receptor, especially the NMDA receptor, and can cause alterations to the mitochondrial structure and calcium-dependent opening of the mitochondrial permeability transition (MPT) pore, allowing the release of soluble proteins such as apoptosis-inducing factor and cytochrome c [20, 21]. Cytochrome c combines with Apaf-1 to promote caspase-9 activation, which in turn activates effector caspase to trigger an ensuing cascade of proteolytic events, leading to cell death [22].

16.5 Free Radicals as a Therapeutic Target

As described above, arachidonate causes a burst of free radicals in the ischemic penumbra, and free radicals are drastically increased after reperfusion [15]. Many researchers have tried to discover a free radical scavenger without side-effects such as narcotizing or suppressing cerebral metabolism [23]. In the early stages of investigations, edaravone was found to have a promising effect by quenching the hydroxyl radical (OH) and by inhibiting both OH-dependent and OH-independent lipid peroxidation. To evaluate the effect of edaravone on brain edema in the post-stroke brain, we administered edaravone in the transient middle cerebral artery occlusion (tMCAO) rat model. In this model, water content, which reflects disruption of the BBB, significantly increased after 3 and 6 hours of ischemia, and a further increase was found after 3 hours of ischemia following 3 hours of reperfusion. Therefore, we concluded that edaravone markedly suppressed ischemic and post-ischemic brain swelling [24] (Fig. 16.4). In addition, post-ischemic treatment with edaravone significantly decreased the size of cerebral infarcts and improved neurological deficits 1 day after tMCAO [25]. Another research group reported that edaravone markedly suppressed the accumulation of a product of nucleic acid oxidation, 8-oxo-2'-deoxyguanosine (8-oxodG), and sequential inflammatory responses at the peri-infarct lesion in the mouse stroke model [26]. In addition, we recently reported that edaravone showed strong neuroprotection after cerebral ischemia, which was confirmed by in vivo and ex vivo optical imaging for the apoptosis marker, annexin V, while also reducing cerebral infarct (Fig. 16.5) [27]. In a clinical trial, edaravone attenuated the resulting disability in humans 90 days after acute ischemic stroke without serious adverse effects [28] and it has been used clinically in Japan as a neuroprotective agent for acute stroke patients since 2001.

Fig. 16.4 Effects on brain edema in the rat MCA occlusion or occlusion–recirculation model (revised from Abe et al. 1988). Rat MCA occlusion or recirculation was obtained by insertion or removal of a nylon thread, respectively. MCA: middle cerebral artery. * $p < 0.05$ vs. control (by Dunn's multiple comparison test) [24]

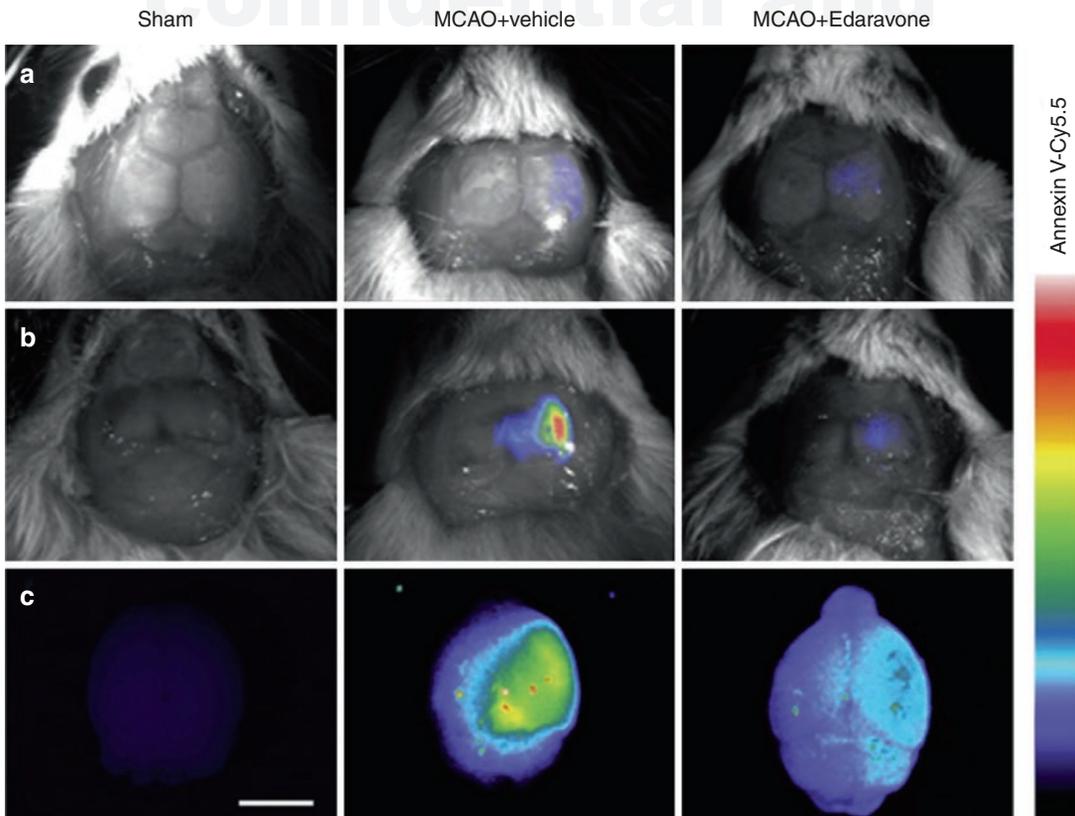
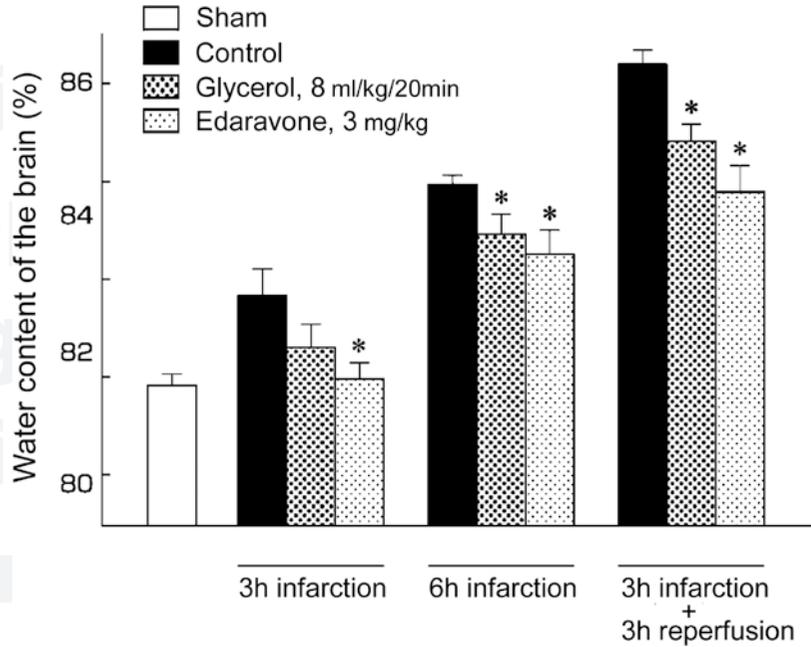


Fig. 16.5 In vivo imaging of Annexin V-Cy5.5 (a) with removal of head skin, (b) removal of the skull bone, and (c) ex vivo imaging of the brain (revised from Liu et al.

2011). This optical imaging method successfully demonstrated that edaravone treatment suppressed apoptosis in post-stroke mice brains 48 hours after tMCAO [27]

16.6 Conclusion

In this chapter, we briefly highlighted the pathophysiological mechanism of neuronal cell death in the ischemic and hemorrhagic strokes. From the results of basic research, free radicals are regarded as a key regulator of disease progression in not only the ischemic stroke but also in the hemorrhagic stroke. Therefore, in the near future, the free radical scavenger edaravone may be widely applied to the therapy of various kinds of diseases.

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