Neuronal Death/Survival Signaling Pathways in Cerebral Ischemia

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Summary: Cumulative evidence suggests that apoptosis plays a pivotal role in cell death *in vitro* after hypoxia. Apoptotic cell death pathways have also been implicated in ischemic cerebral injury in *in vivo* ischemia models. Experimental ischemia and reperfusion models, such as transient focal/global ischemia in rodents, have been thoroughly studied and the numerous reports suggest the involvement of cell survival/death signaling pathways in the pathogenesis of apoptotic cell death in ischemic lesions. In these models, reoxygenation during reperfusion provides a substrate for numerous enzymatic oxidation reactions. Oxygen radicals damage cellular lipids, proteins and nucleic acids, and initiate cell signaling pathways after cerebral ischemia. Genetic manipulation of intrinsic antioxidants and factors in the signaling pathways has provided substantial understanding of the mechanisms involved in cell death/survival signaling pathways and the role of oxygen radicals in ischemic cerebral injury. Future studies of these pathways may provide novel therapeutic strategies in clinical stroke. **Key Words:** Cerebral ischemia, apoptosis, signaling pathway, oxidative stress.

INTRODUCTION

Many studies have shown that reactive oxygen radicals play important roles in the pathogenesis of various neurological disorders, such as ischemia, trauma, and degenerative disease. They damage cellular macromolecules, such as lipids, proteins and nucleic acids and lead to cell injury and death.[1,2] Besides these direct injuries, recent studies have shown that oxygen radicals are also involved in cell death/survival signaling pathways.[1,3–5] As for ischemic injury, it is generally accepted that apoptosis plays a pivotal role in cell death *in vitro* after hypoxia. Recent studies also suggest that apoptotic cell death occurs *in vivo* in cerebral ischemia models.[1,6] Experimental ischemia and reperfusion models, such as transient focal/global ischemia in rodents, have been thoroughly studied and the cumulative evidence suggests the involvement of cell survival/death signaling pathways in the pathogenesis of apoptotic cell death in the ischemic lesions.[7–11] In these models, reoxygenation during reperfusion provides a substrate for numerous enzymatic oxidation reactions.[1,2] In this review, the mechanisms of cell death/survival signaling pathways after ischemia and the involvement of oxygen radicals in these pathways will be discussed.

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ISCHEMIC CELL DEATH SIGNALING PATHWAY

Mitochondrial pathway of apoptosis

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 (FIG. 1, Table 1).[12] Cytochrome *c* is translocated from mitochondria to the cytosolic compartment after transient focal cerebral ischemia in rats,[13] in brain slices that are subjected to hypoxia-ischemia,[14] and in vulnerable hippocampal CA1 neurons after transient global cerebral ischemia.[9] Mitochondria are known to be involved in both the necrosis and apoptosis pathways, which depend on the severity of the insult or the nature of the signaling pathways.[7,15–17] In most instances, severe cerebral ischemia renders the mitochondria com-

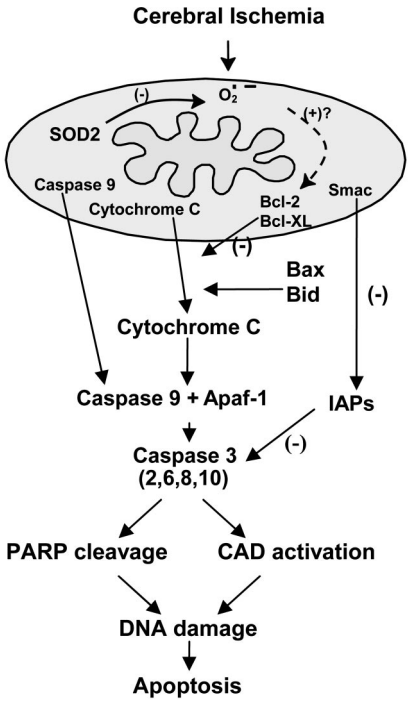

FIG. 1. Mitochondria as targets for oxidative stress signaling after cerebral ischemia. Cerebral ischemia and reperfusion generate ROS within mitochondria, which then signal the release of cytochrome *c* by mechanisms that may be related to the Bcl-2 family proteins, Bcl-2, Bcl-X_L, Bax, and Bid. Cytochrome *c*, once released, binds to Apaf-1 followed by caspase-9 to form a complex that subsequently activates caspase-3 and other caspases, such as caspase-2, -6, -8, and -10. The IAP family suppresses apoptosis by preventing the activation of procaspases and also inhibits the enzymatic activity of active caspases; Smac is also released by apoptotic stimuli and binds IAPs, thereby promoting activation of caspase-3. Activated caspase-3 is known to cleave many nuclear DNA repair enzymes, such as PARP and to activate CAD, which then leads to nuclear DNA damage without repair, resulting in apoptosis.

pletely dysfunctional for ATP production, which ensures necrotic cell death. *In vitro* studies demonstrate that various cellular or biochemical signaling pathways involve mitochondria in apoptosis by releasing cytochrome *c* to the cytoplasm. Cytochrome *c* interacts with the CED-4 homolog, Apaf-1, and deoxyadenosine triphosphate, forming the apoptosome and leading to activation of caspase-9.[18–21] Caspase-9, which is presumably an initiator of the cytochrome-*c*-dependent caspase cascade, then activates caspase-3, followed by caspase-2, -6, -8, and -10 activation downstream.[22] 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.[10,23,24] Caspase-11 is also a critical initiator of caspase-1 and -3 activation, and caspase-11 knockout (KO) animals have shown reduced apoptosis after focal ischemia.[25] Since caspase-11 is an upstream activator of caspase-1 in cytokine maturation, involvement of cytokines in apoptosis should also be considered after cerebral ischemia. The downstream caspases cleave many substrate proteins including poly(ADP-ribose) polymerase (PARP).[23,24,26] 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. Consistent with these notions, PARP KO mice showed decreased infarct after transient middle cerebral artery occlusion (MCAO).[27] In contrast, there are proteins that can prevent caspase activation in the cytosol. The inhibitor-of-apoptosis protein (IAP) family suppresses apoptosis by preventing the activation of procaspases and also by inhibiting the enzymatic activity of

TABLE 1. *Transgenic and Knockout Studies of Proapoptotic and Antiapoptotic Proteins*

Study	Insult	Findings	Reference
Bid−/−	Transient MCAO	Decreased infarct (−67%)	Plesnila et al.[35]
Bcl-2 Tg	Permanent MCAO	Decreased infarct (−50%)	Martinou et al.[34]
Bcl-2 Tg	Global ischemia	Decreased injury	Kitagawa et al.[79]
Bcl-2 Tg	Permanent MCAO	No protection	Wiessner et al.[80]
Bcl-2 Tg	Permanent MCAO	Decreased injury	de Bilbao et al.[81]
Bcl-2−/+, −/−	Transient MCAO	Increased infarct	Hata et al.[36]
Bcl-X_L Tg	Permanent MCAO	Decreased infarct (−21%)	Wiessner et al.[80]
Caspase-1 NM	Transient MCAO	Decreased infarct (−44%)	Hara et al.[82]
Caspase-1 NM	Permanent MCAO	Reduced injury	Friedlander et al.[83]
Caspase-1−/−	Permanent MCAO	Reduced injury	Schielke et al.[84]
Caspase-1−/−	Transient MCAO	Decreased infarct	Liu et al.[85]
Caspase-11−/−	Permanent MCAO	Reduced apoptosis	Kang et al.[25]
PARP−/−	Transient MCAO	Decreased infarct	Eliasson et al.[86]
PARP−/−	Transient MCAO	Decreased infarct in chronic stage	Goto et al.[87]
Fas NM	Transient MCAO	Decreased infarct	Rosenbaum et al.[39]
TNFR(p55&75)−/−	Transient MCAO	Increased injury	Bruce et al.[41]
TNFR(p55&75)−/−	Transient MCAO	Increased injury	Gary et al.[88]
P53−/+, −/−	Permanent MCAO	Decreased infarct (−27%, −15%)	Crumrine et al.[89]

NM, negative mutant: TNFR, TNF-receptor-α.

active caspases.[28,29] The second mitochondria-derived activator of caspase (Smac), is also released by apoptotic stimuli and binds IAPs, thereby promoting activation of caspase-3.[30] 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.[10]

The Bcl-2 family proteins have one or more Bcl-2 homology domains and play a crucial role in intracellular apoptotic signal transduction by regulating permeability of the mitochondrial membrane.[31] Although still controversial, many researchers believe that mitochondrial cytochrome *c* is released through the permeability transition pore (PTP), and that Bcl-2 family proteins directly regulate the PTP.[32] Among these proteins, Bax, Bcl-X_S, Bak, Bid, and Bad are proapoptotic. They eliminate the mitochondrial membrane potential by affecting the PTP and facilitating the release of cytochrome *c*.[33] Conversely, Bcl-2 and Bcl-X_L function to conserve the membrane potential and block the release of cytochrome *c*. As expected, after focal cerebral ischemia, decreased infarct was observed in Bcl-2 overexpressing transgenic (Tg) mice[34] and in Bid KO animals,[35] whereas Bcl-2 KO mice showed an increased infarct.[36] These findings, especially in the studies using proapoptotic/antiapoptotic protein-Tg/KO animals (Table 1), suggest the importance of mitochondrial permeability regulation and Bcl-2 family proteins in ischemic cerebral injury.

Receptor-mediated pathway of apoptosis

The death receptor pathway of apoptosis is initiated by members of the death receptor family, such as the Fas receptor and the tumor necrosis factor (TNF) receptor (FIG. 2, Table 1). For example, in the Fas receptor pathway, the extracellular Fas ligand (FasL) first binds to a receptor and an adaptor molecule, Fas-associated death domain (FADD) protein, then activates procaspase-8.[37] Subsequently, caspase-8 activates caspase-3, and this effector caspase cleaves PARP and activates CAD, leading to DNA damage and cell death. In the middle of this pathway, caspase-3 uses downstream caspases as in the mitochondrial pathway.[38] Caspase-8 is also able to truncate and activate one of the Bcl-2 family proteins, Bid, and to initiate the mitochondrial pathway of apoptosis. Increased expression of Fas and FasL was observed in the ischemic region after focal cerebral ischemia and the loss of Fas receptor function in negative mutant mice, resulting in a smaller infarct.[39] In addition, Fas and FasL mRNA were induced, caspase-10 was activated and FADD was up-regulated in the vulnerable hippocampal CA1 subregion after global ischemia. Furthermore, caspase-3 and FADD were colocalized with caspase-10[40]; this evidence strongly suggests involvement of the Fas receptor pathway of apoptosis after cerebral ischemia. Unlike Fas-receptor KO animals, TNF-receptor p55 and p75 KO mice[41] showed increased injury after transient focal ischemia, suggesting the neuroprotective effect of the TNF receptor. Results of these death receptor-KO studies also provide evidence that these receptors play an important role in cell death after ischemia (Table 1); however, the relationship between oxidative stress and receptor ligation is unknown and requires further studies.

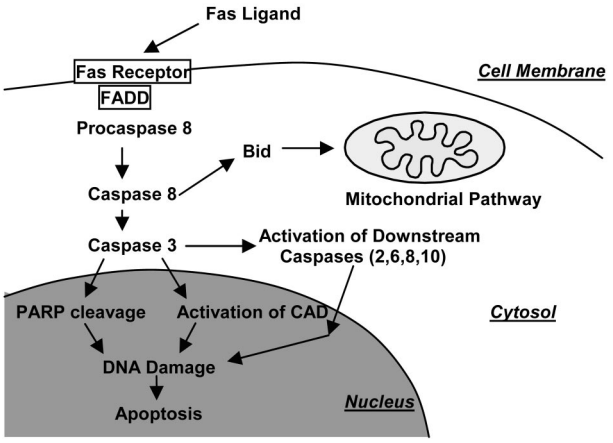

FIG. 2. Fas receptor pathway of apoptosis. The extracellular Fas ligand first binds to a receptor, and then an adaptor molecule, FADD protein, activates procaspase-8. Then, caspase-8 activates caspase-3 and this effector caspase cleaves PARP and activates CAD, leading to DNA damage and cell death. In the middle of this pathway, caspase-3 uses downstream caspases as in the mitochondrial pathway. Caspase-8 is also able to truncate and activate one of the Bcl-2 family proteins, Bid, and initiates the mitochondrial pathway of apoptosis.

ISCHEMIC CELL SURVIVAL SIGNALING PATHWAY

Bad as a target of cell survival signaling

Bad is an important proapoptotic member of the Bcl-2 family that links the upstream cell survival signaling pathway and downstream pathway to inactivate antiapoptotic Bcl-2 family proteins.[42] *In vitro* studies show that Bad resides in an inactive complex with the molecular chaperone 14-3-3 via the phosphorylation of four serine residues (Ser-112, -136, -155, and -170).[43] With apoptotic stimuli, Bad is dephosphorylated, dissociated from 14-3-3, and translocated to the outer membrane of mitochondria, where it subsequently dimerizes with Bcl-X_L and promotes mitochondrial cytochrome *c* release.[43] Ser-155 residue is important for direct interaction between Bad and Bcl-X_L and its phosphorylation is regulated by several upstream signaling pathways. After cerebral ischemia, dephosphorylation and translocation of Bad from the cytosol to the mitochondria are observed and dimerization of Bad progresses with Bcl-X_L in the early stages after MCAO.[11] These results suggest the pivotal function of Bad in ischemic cell death (FIG. 3).

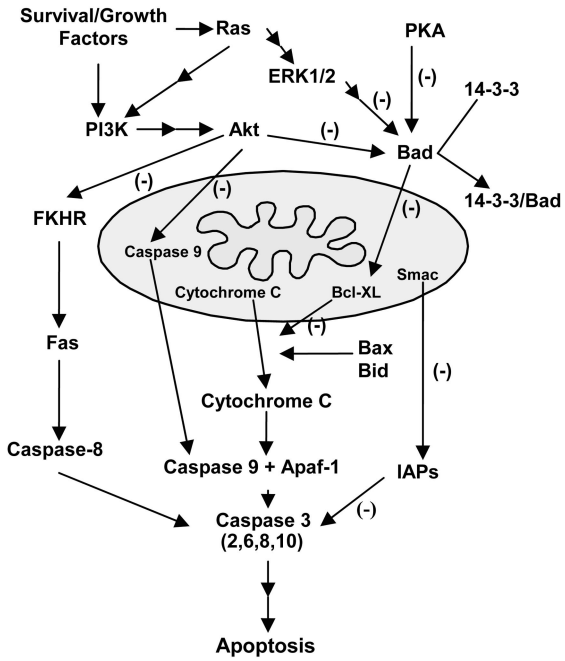

FIG. 3. Cell survival signaling pathways. Three major pathways that inhibit Bad are shown. Bad is an important proapoptotic member of the Bcl-2 family that links cell survival and apoptosis pathways. Bad promotes the release of cytochrome *c* by inhibiting the antiapoptotic effects of Bcl-X_L. The phosphatidylinositol-3 kinase (PI3K) pathway is activated by survival/growth factors and leads to activation of Akt. Akt inhibits the Forkhead family of transcription factors (FKHR), caspase-9, and Bad, and ultimately leads to inhibition of both mitochondrial and Fas receptor pathways of apoptosis. ERK1/2 activation through active Ras and PKA also inhibit Bad and thereby block cytochrome *c* release by Bcl-X_L inhibition.

Upstream survival signaling pathways for Bad inhibition

There are several pathways to the inhibition of the proapoptotic function of Bad. Ras is considered to play a central role in signaling for growth-factor-mediated resistance to apoptosis.[6] Recent studies have shown that pharmacological blockade of Ras results in an inhibition of the protective effects of ischemic preconditioning in primary cultures, and conversely, overexpression of Ras by transfection provides protection for cultured ischemic neurons.[44] Ras can directly activate phosphatidylinositol-3 kinase, an upstream effector for activation of Akt. Akt is an initiator of the downstream pathways that inhibit the apoptotic pathways. Akt phosphorylates Bad and obviates its inhibitory effects on Bcl-X_L, ultimately inhibiting the release of cytochrome *c* by blocking the channel formation on the mitochondrial membrane by Bax.[6] Akt also inhibits proteolytic activity of caspase-9 by phosphorylating it on Ser-196.[45] 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.[46] Mitogen-activated protein kinase (MAPK) family members play a critical role in the regulation of cell growth, differentiation, and cellular response to cytokines and stress.[47] One MAPK family member, extracellular signal-regulated kinase (ERK), has two isoforms (ERK1/2), which are constitutively expressed in the normal brain[48] and are activated by MAPK/ERK kinase 1/2. In this pathway, Ras recruits the main effector, Raf-1, to activate MAPK/ERK kinase 1/2.[49] Active ERK1/2 inactivates Bad through phosphorylation of 90-kDa ribosomal S6 kinases.[50] 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.[51] Phosphorylation of ERK1/2 is thought to be involved in apoptosis and cell death after transient MCAO.[8] Phosphorylation of the Ser-155 residue in Bad is regulated by protein kinase A (PKA) in studies *in vitro*.[52] In rodent focal cerebral ischemia models, intraventricular injection of H89, a PKA inhibitor, effectively suppressed PKA activity[53] and dimerization of Bad/Bcl-X_L and subsequent apoptotic cell death.[11] This cumulative evidence suggests that Akt, ERK1/2, and PKA pathways inhibit Bad function as cell survival signaling pathways after cerebral ischemia.

OXIDATIVE STRESS AS A MOLECULAR SWITCH FOR ISCHEMIC CELL DEATH/SURVIVAL SIGNALING

Generation of oxygen radicals and clearance pathways

Many studies have shown that reactive oxygen radicals play important roles in the pathophysiology of various neurological disorders.[1,2,54,55] Experimental ischemia and reperfusion models, such as transient focal/global ischemia in rodents, have been thoroughly studied and the cumulative evidence suggests involvement of oxygen radicals in the pathogenesis of ischemic lesions. In these models, cerebral blood flow is reduced by occluded vessels in brain regions that are supplied with oxygen. Reoxygenation during reperfusion provides a substrate for numerous enzymatic oxidation reactions. Mitochondria produce superoxide anion radicals and hydrogen peroxide (H_2O_2) under normal physiological conditions.[56] These constantly produced reactive oxygen species (ROS) are scavenged by superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase. SOD specifically processes superoxide anion (O_2^-) and produces H_2O_2, which is then detoxified by catalase or GSHPx, and finally changed to water and superoxide. Hydroxyl radicals (^-OH) may be generated from H_2O_2 through the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow HO + Fe^{3+} + {}^-OH$). Other small molecular antioxidants, including glutathione (GSH), ascorbic acid, and α-tocopherol, are also involved in the detoxification of free radi-

TABLE 2. *Transgenic and Knockout Studies of Superoxide Dismutases and Glutathione Peroxidase*

Study	Insult	Findings	Reference
SOD1 +/−	Permanent MCAO	Decreased cortical infarct (−35%)	Kinouchi et al.[63]
SOD1 +/−	Permanent MCAO	No protection	Chan et al.[90]
SOD1 +/−	Transient MCAO	Decreased infarct	Yang et al.[91]
SOD1 +/−	Transient MCAO	Sustained hsp70 mRNA expression	Kamii et al.[92]
SOD1 +/−	Transient MCAO	Sustained c-fos mRNA expression	Kamii et al.[93]
SOD1 +/−	Global ischemia	Induction of hsp70	Kondo et al.[94]
SOD1 +/−	Transient MCAO	Decreased injury (−50%)	Kamii et al.[95]
SOD1 +/−	Neonatal hypoxia	Increased injury in neonates	Ditelberg et al.[96]
SOD1 +/−	Neonatal hypoxia	Increased injury in neonates	Fullerton et al.[97]
SOD1 +/−	Global ischemia	Decreased injury (−50%)	Chan et al.[64]
SOD1 +/−	Global ischemia	Decreased injury (−50%)	Murakami et al.[69]
SOD1 +/−	Transient MCAO	Decreased DNA fragmentation	Fujimura et al.[98]
SOD1 +/−	Transient MCAO	Decreased cytochrome *c* release	Fujimura et al.[7]
SOD1 +/−	Transient MCAO	Down-regulation of nuclear factor-κB	Huang et al.[99]
SOD1 +/−	Transient MCAO	Decreased activation of activator protein-1	Huang et al.[100]
SOD1 +/−	Global ischemia	Decreased active caspase-3, -9	Sugawara et al.[10]
SOD1 +/−	Transient MCAO	Decreased ERK activation	Noshita et al.[8]
SOD1 +/−	Transient MCAO	Decreased Bad activation	Saito et al.[11]
SOD1 −/−	Transient MCAO	Increased infarct (+40%)	Kondo et al.[67]
SOD1 −/−	Transient MCAO	Increased lesion size and edema	Kondo et al.[68]
SOD1 −/−	Global ischemia	Increased cell death	Kawase et al.[66]
SOD1 +/−, −/−	Permanent MCAO	No increase in infarct volume	Fujimura et al.[101]
SOD2 +/−	Transient MCAO	Decreased injury	Keller et al.[71]
SOD2 −/+	Permanent MCAO	Increased infarct (+66%)	Murakami et al.[69]
SOD2 −/+	Permanent MCAO	Increased active caspase-9	Fujimura et al.[70]
SOD2 −/+	Transient MCAO	Increased cytochrome *c* release	Noshita et al.[102]
SOD2 −/+	Permanent MCAO	Increased superoxide production	Kim et al.[103]
ECSOD +/−	Transient MCAO	Decreased infarct (−28%)	Sheng et al.[72]
ECSOD +/+	Global ischemia	Decreased injury (−48%)	Sheng et al.[74]
ECSOD −/−	Transient MCAO	Increased infarct (+81%)	Sheng et al.[73]
GSHPx-1 +/+	Transient MCAO	Decreased infarct	Weisbrot et al.[76]
GSHPx-1 −/−	Transient MCAO	Increased apoptosis	Crack et al.[77]

cals. Reperfusion after ischemia causes overproduction of ROS in mitochondria, and consumption of endogenous antioxidants by these radicals may lead to a dramatic rise in intracellular ROS. It has been demonstrated in numerous studies that ROS are directly involved with cellular macromolecules such as lipids, proteins, and nucleic acids in oxidative damage in ischemic tissues, which leads to cell death. Recent studies have provided evidence that indirect signaling pathways mediated by ROS can also cause cellular damage and death in cerebral ischemia and reperfusion.

Antioxidant enzymes and studies using Tg and KO animals

SODs are specific antioxidant enzymes that detoxify O_{2^-} and produce H_2O_2. Three SODs, copper/zinc SOD (SOD1), manganese SOD (SOD2), and extracellular SOD (ECSOD), are major antioxidant enzymes based on cellular distribution and localization (Table 2). SOD1 is a major cytosolic enzyme with a level constituted at approximately 0.1% of total proteins in mammalian cells. SOD2 is a mitochondrial enzyme, whereas ECSOD is an isoform that is localized in extracellular space, cerebrospinal fluid, and cerebral vessels.[57] All three SOD isoforms dismutate O_{2^-}, forming H_2O_2, which is scavenged by catalase or GSHPx at the expense of GSH. GSH is generated from oxidized GSH by GSH reductase in the presence of reduced nicotinamide adenine dinucleotide phosphate. Other lipid peroxides are also scavenged by GSHPx. SOD1 has been extensively used in experimental studies involving cerebral ischemia and reperfusion. Unfortunately, mixed and confusing results were obtained when free non-modified SOD1 was used. The extremely short half-life of SOD1 (6 min) in circulating blood and its failure to pass the blood-brain barrier and be taken up intracellularly, make it difficult to use for enzyme therapy in cerebral ischemia.[58] However, a modified enzyme with an increased half-life, polyethylene glycol-conjugated SOD1, has been successfully used to reduce infarct volume in rats that were subjected to focal cerebral ischemia.[59] Liposome-entrapped SOD1 has an increased half-life (up to 4.2 h), blood-brain barrier permeability and cellular uptake, and has been proven to be an effective treatment for reducing the severity of ischemic and traumatic brain injuries.[60,61]

Numerous studies using genetically modified mice that either overexpress or are deficient in SODs have been published (Table 2). In SOD1-overexpressing Tg mice, a three-fold increase in SOD1 activity has been observed in all brain regions in heterozygous mice, whereas in homozygous mice, a five-fold increase in SOD1 activity was achieved.[62] In these mice, a 35% decrease in infarct volume was observed after permanent focal ischemia involving coagulation of the distal MCA and in bilateral common carotid artery occlusion.[63] In global ischemia, SOD1 overexpression is neuroprotective, with a 50% reduction in hippocampal CA1 cell death,[64,65] and this protection is probably partly due to blocking of the mitochondrial pathway of apoptosis.[10] The role of SOD1 in cerebral ischemia is further confirmed by the use of SOD1-deficient mice. These SOD1 KO mice had increased cell death and edema after transient MCAO and global cerebral ischemia.[66–68] The importance of mitochondrial production of oxygen radicals and the protective role of SOD2 after permanent cerebral ischemia have been demonstrated in SOD2 KO mice. These mutant mice show exacerbated infarct volume after permanent MCAO,[69] and increased mitochondrial cytochrome *c* release and subsequent DNA fragmentation after permanent focal cerebral ischemia.[70] However, mice that overexpress SOD2 showed neuronal protection against oxidative stress after transient focal cerebral ischemia.[71] The ECSOD level in the brain is much lower than in other organs, but recent studies have demonstrated that overexpression of this protein provides protection after focal and global ischemia, whereas KO animals showed a larger infarct after focal ischemia.[72–74] Results from pharmacological trials and studies using Tg/KO rodents provide strong evidence to support the importance of SODs and superoxide in the pathophysiology of ischemic brain injury.

As described, superoxide generated in mitochondria was processed by SODs as a first step in its clearance pathway. This step generates H_2O_2, which is still a harmful ROS. Catalase and GSHPx catalyze the reduction of H_2O_2 to water and oxygen. Since constitutive catalase expression is at a low level in neurons compared with other organs,[75] GSHPx is especially important for detoxifying H_2O_2 after cerebral ischemia and reperfusion. There are at least five mammalian GSHPx isoenzymes; GSHPx-1 is the most ubiquitous form, and localizes in the cytosol and mitochondria in most tissues. Neuronal injury in GSHPx-1 Tg and KO mice has been examined after focal ischemia (Table 2). Overexpression of human GSHPx-1 in Tg mice reduced the infarct volume by 48% after transient MCAO.[76] Conversely, in GSHPx-1 KO mice, infarct volume was increased threefold and caspase-3 expression was present at earlier time points compared with wild-type animals.[77] More recently, Crack et al.[78] used a crossed SOD1 Tg mouse and GSHPx-1 KO mouse model. These SOD1 Tg/GSHPx-1−/− crossed mice showed a larger infarct compared with wild-type mice. This study may suggest that despite a high level of SOD1, GSHPx activity needs to be maintained at a basal or higher level so that H_2O_2 and lipid peroxides, which are also generated during cerebral ischemia and reperfusion, can be eliminated.

CONCLUSIONS

From numerous results accumulated over the past decade, it is clear that oxidative stress is involved in cell death after cerebral ischemia. More recent studies strongly suggest the involvement of cell death/survival signaling pathways. Genetic manipulation of factors in the signaling pathways has provided substantial progress in understanding the mechanisms of apoptotic cell death and survival signaling pathways. Future studies of these pathways may provide novel therapeutic strategies in clinical stroke.

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