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Forum Review

Reactive Oxygen Radicals and Pathogenesis of Neuronal Death After Cerebral Ischemia

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ABSTRACT

Reactive oxygen species have been implicated in brain injury after cerebral ischemia. These oxidants can damage proteins, lipids, and DNA, and lead to cell injury and necrosis. Oxidants are also initiators in intracellular cell death signaling pathways that may lead to apoptosis. The possible targets of this redox signaling include mitochondria, death membrane receptors, and DNA repair enzymes. Genetic manipulation of intrinsic antioxidants and the factors in the signaling pathways has provided substantial progress in understanding the mechanisms in cell death signaling pathways and involvement of oxygen radicals in ischemic brain injury. Future studies of these pathways may provide novel therapeutic strategies in clinical stroke. *Antioxid. Redox Signal.* 5, 597–607.

INTRODUCTION

MANY STUDIES have shown that reactive oxygen radicals play important roles in the pathophysiology of various neurological disorders (8, 67, 95). Experimental ischemia and reperfusion models, such as transient focal/global ischemia models in rodents, have been thoroughly studied, and the accumulated evidence suggests the involvement of oxygen radicals in the pathogenesis of their 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 oxygen as a substrate for numerous enzymatic oxidation reactions. In this review, the mechanisms of formation/clearance and signaling pathways of oxygen radicals after cerebral ischemia/reperfusion will be discussed.

GENERATION OF OXYGEN RADICALS AND THEIR CLEARANCE PATHWAYS

Mitochondria are known to produce superoxide anion radicals (O_2^-) and hydrogen peroxide (H_2O_2) under normal physiological conditions (4). These constantly produced reactive oxygen species (ROS) are scavenged by superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (Fig. 1). SOD specifically processes O_2^- and produces H_2O_2, which is then detoxified by catalase or GSHPx and finally changed to H_2O and O_2. Hydroxyl radicals (OH^-) may be generated from H_2O_2 through the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}HO + Fe^{3+} + OH^-$). Other small molecular antioxidants, including glutathione (GSH), ascorbic acid, and α-tocopherol, are also involved in the detoxification of free radicals. 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, and lead to cell death. Recent studies have provided evidence that indirect signaling pathways by ROS can also cause cellular damage and death in cerebral ischemia and reperfusion.

INVOLVEMENT OF ANTIOXIDANT AND PROOXIDANT ENZYMES IN NEURONAL DEATH AFTER ISCHEMIA

SODs

SODs are specific antioxidant enzymes that detoxify O_2^- and produce H_2O_2. Three SODs, copper/zinc SOD (CuZnSOD,

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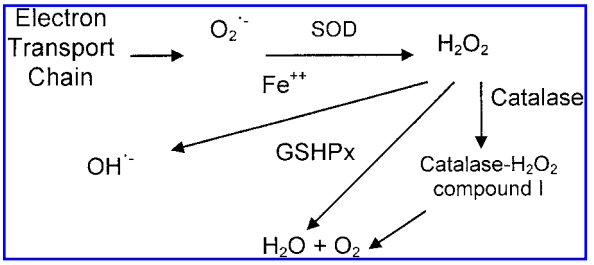

FIG. 1. Oxygen radicals and clearance pathways. SOD specifically processes O_2^- and produces H_2O_2, which is then detoxified by catalase or GSHPx and finally changed to H_2O and O_2. OH^- may be generated from H_2O_2 through the Fenton reaction ($H_2O_2 + Fe^{2+} \rightarrow {\cdot}HO + Fe^{3+} + OH^-$).

SOD1), manganese SOD (MnSOD, SOD2), and extracellular SOD (ECSOD), are major antioxidant enzymes based on cellular distribution and localization. SOD1 is a major cytosolic enzyme with a level constituted at ~0.1% of total proteins in mammalian cells. SOD1 is a mitochondrial enzyme, whereas ECSOD is an isoform that is localized in extracellular space, cerebrospinal fluid, and cerebral vessels (73). 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 NADPH. 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 have been obtained when free nonmodified 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 (11). 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 (42). 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 (9, 53).

Numerous studies utilizing genetically modified mice that either overexpress or are deficient in SODs have been reported (Table 1). In SOD1-overexpressing transgenic (Tg) mice, a threefold increase in SOD1 activity has been observed in all brain regions in the heterozygous SOD1 Tg mice, whereas in the homozygous SOD1 Tg mice, a fivefold increase in SOD1 activity was achieved (12). In these mice, a 35% decrease in

TABLE 1. TRANSGENIC AND KNOCKOUT STUDIES OF SOD AND GSHPX

Study	*Insult*	*Findings*	*Reference*
Superoxide dismutases			
SOD1 +/−	Permanent MCAO	Decreased cortical infarct (−35%)	62
SOD1 +/−	Permanent MCAO	No protection	10
SOD1 +/−	Transient MCAO	Decreased infarct	104
SOD1 +/−	Transient MCAO	Sustained hsp70 mRNA expression	56
SOD1 +/−	Transient MCAO	Sustained c-fos mRNA expression	55
SOD1 +/−	Global ischemia	Induction of hsp70	57
SOD1 +/−	Transient MCAO	Decreased injury (−50%)	57
SOD1 +/−	Neonatal hypoxia	Increased injury in neonates	23
SOD1 +/−	Neonatal hypoxia	Increased injury in neonates	34
SOD1 +/−	Global ischemia	Decreased injury (−50%)	13
SOD1 +/−	Global ischemia	Decreased injury (−50%)	78
SOD1 +/−	Transient MCAO	Decreased DNA fragmentation	31
SOD1 +/−	Transient MCAO	Decreased cytochrome *c* release	32
SOD1 +/−	Transient MCAO	Down-regulation of nuclear factor-κB	46
SOD1 +/−	Transient MCAO	Decreased activation of activator protein-1	45
SOD1 +/−	Global ischemia	Decreased active caspase-3, -9	98
SOD1 −/−	Transient MCAO	Increased infarct (+40%)	65
SOD1 −/−	Transient MCAO	Increased lesion size and edema	66
SOD1 −/−	Global ischemia	Increased cell death	59
SOD1 −/−	Permanent MCAO	No protection	33
SOD2 +/−	Transient MCAO	Decreased injury	60
SOD2 −/+	Permanent MCAO	Increased infarct (+66%)	78
SOD2 −/+	Permanent MCAO	Increased active caspase-9	30
SOD2 −/+	Transient MCAO	Increased cytochrome *c* release	82
SOD2 −/+	Permanent MCAO	Increased superoxide production	61
ECSOD +/−	Transient MCAO	Decreased infarct (−28%)	90
ECSOD +/+	Global ischemia	Decreased injury (−48%)	92
ECSOD −/−	Transient MCAO	Increased infarct (+81%)	91
Glutathione peroxidase			
gSHPx-1 +/+	Transient MCAO	Decreased infarct	100
gSHPx-1 −/−	Transient MCAO	Increased apoptosis	16

infarct volume was observed after permanent focal ischemia involving coagulation of the distal middle cerebral artery and bilateral common carotid artery occlusion (62). In global ischemia, SOD1 overexpression is neuroprotective with a 50% reduction in hippocampal CA1 cell death (13, 77), and this protection is probably partly due to the blocking of the mitochondrial pathway of apoptosis (98). The role of SOD1 in cerebral ischemia is further confirmed by the use of SOD1-deficient mice. These SOD1 knockout mice had increased cell death and edema after transient middle cerebral artery occlusion (MCAO) and global cerebral ischemia (59, 65, 66). The importance of mitochondrial production of oxygen radicals and the protective role of SOD2 after permanent cerebral ischemia have been demonstrated in SOD2 knockout mice. These mutant mice show exacerbated infarct volume after permanent MCAO (78), and increased mitochondrial cytochrome *c* release and subsequent DNA fragmentation after permanent focal cerebral ischemia (30). However, mice that overexpress SOD2 showed neuronal protection against oxidative stress after transient focal cerebral ischemia (60). The ECSOD level in the brain is much lower than that in other organs, but recent studies have demonstrated that overexpression of this protein provides protection after focal and global ischemia, whereas knockout animals showed larger infarct after focal ischemia (90–92). Results from pharmacological trials and studies using Tg/knockout rodents provide strong evidence to support the importance of SODs and superoxide in the pathophysiology of ischemic brain injury.

GSHPx

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. As constitutive expression of catalase is at a low level in neurons compared with other organs (21), 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 of GSHPx-1 Tg and knockout mice has been examined after focal ischemia (Table 1). Overexpression of human GSHPx-1 in Tg mice reduced the infarct volume by 48% after transient MCAO (100). Conversely, in GSHPx-1 knockout mice, infarct volume was increased threefold and caspase-3 expression was present at earlier time points compared with wild-type animals (16). More recently, Crack *et al.* (17) utilized a crossed SOD1 Tg mouse and GSHPx-1 knockout mouse model. These SOD1 Tg/GSHPx-1 −/− crossed mice showed a larger infarct compared with wild-type mice. Taken together, GSHPx plays an essential role in detoxifying noxious ROS, and increased H_2O_2 in the brain may be an initiator of apoptosis after ischemia and reperfusion.

Nitric oxide synthases (NOSs)

Many prooxidant and antioxidant enzymes participate in oxidative stress-induced signaling and injury in cerebral ischemia. Based on oxidant products, there are three major classes of prooxidant enzymes: (a) NOSs; (b) cyclooxygenases, xanthine dehydrogenase, xanthine oxidase, and NADPH oxidase; and (c) myeloperoxidase and monoamine oxidase. NOSs use arginine and O_2 as substrates and produce nitric oxide (NO) as an oxidant product. Three isoforms of NOS exist in CNS parenchyma: neuronal NOS (nNOS, NOS1); an isoform that is induced (iNOS, NOS2) in microglia/macrophages, astrocytes, and endothelial cells; and a constitutive form that is localized in the endothelium (eNOS, NOS3). nNOS and eNOS activities are Ca^{2+}-dependent, whereas iNOS is Ca^{2+}-independent. NO produced by nNOS and iNOS has been implicated in both *in vitro* cell culture injury and ischemic brain damage, whereas NO produced by eNOS is known to be neuroprotective because of its vasodilative effects. Interestingly, nNOS-containing neurons are resistant to ischemic injury, and NO produced by nNOS-containing neurons can kill surrounding non–nNOS-containing neurons (19). The rapid expression of eNOS in cerebral microvessels after MCAO in rats suggests that increased expression of eNOS may protect neurons by increasing cerebral blood flow in the penumbra area (108).

Cerebral ischemia studies of NOS knockout rodents are shown in Table 2. The differential role of nNOS and eNOS in NO generation in the brain after cerebral ischemia has recently been demonstrated (99). Other studies indicate that NO produced by iNOS in nonneuronal cells may contribute to cerebral ischemic damage (50). Inducible NO expression peaks 24–48 h after ischemia occurs in infiltrating neutrophils and cerebral vascular cells (51). Recent cell culture studies have demonstrated that the induction of iNOS and the formation of 3-nitrotyrosine under oxygen-glucose deprivation kill cerebral endothelial cells by apoptosis (103), suggesting the injurious role of iNOS expression in an ischemic setting. Cyclooxygenase-1, cyclooxygenase-2 (COX-2), xanthine dehydrogenase, xanthine oxidase, and NADPH oxidase belong to the second prooxidant group. They are constitutively expressed, except for COX-2, which is highly inducible. It has been reported that NO produced by iNOS enhances COX-2 activity in the ischemic brain and that iNOS-positive neutrophils are in close proximity to COX-2-positive neurons (81). The third group of prooxidant enzymes, myeloperoxidase and monoamine oxidase, generate hypochlorous acid and H_2O_2 as main oxidants in leukocytes and in parenchymal cells, respectively. One interesting note is that expression of the prooxidant enzymes is cell-specific, in contrast to the subcellular site specificity of antioxidant enzyme expression. Homozygous knockout mice of nNOS and eNOS were developed by Huang *et al.* (47, 72). A substantial reduction in lesion size was observed after permanent and transient focal cerebral ischemia in nNOS knockout mice (25, 38, 39, 48, 94, 107). The role of eNOS and NO in maintaining local blood flow is likely responsible for the increased lesion volume in eNOS knockout mutants (27, 49).

PROGRAMMED CELL DEATH AFTER ISCHEMIA

Mitochondrial pathway of apoptosis

ROS signaling in mitochondria has recently been demonstrated in the ischemic brain with the release of mitochondrial

TABLE 2. KNOCKOUT STUDIES OF NOSs

Study	*Insult*	*Findings*	*Reference*
nNOS −/−	Permanent MCAO	Decreased infarct (−40%)	48
nNOS −/−	Transient MCAO	Decreased infarct	38
nNOS −/−	Global ischemia	Decreased cell death	83
nNOS −/−	Permanent MCAO	Decreased infarct	107
nNOS −/−	Transient MCAO	Decreased infarct	39
nNOS −/−	Permanent MCAO	Decreased infarct (−40%)	94
nNOS −/−	Permanent MCAO	Decreased injury	25
eNOS −/−	Permanent MCAO	Increased infarct (+20%)	49
eNOS −/−	Permanent MCAO	Alterations in blood flow	72
eNOS −/−	Transient MCAO	No protection of HMB-CoA reductase	27
iNOS −/+	Permanent MCAO	Decreased infarct (−14%)	109
iNOS −/−	Permanent MCAO	Decreased infarct	52
iNOS −/−	Transient MCAO	Interaction with COX-2	81
iNOS −/−	Transient MCAO	Interaction with COX-2	79
iNOS −/−	Transient MCAO	Increased nitrotyrosine formation	44
iNOS −/−	Permanent MCAO	Decreased infarct (−29%)	109

HMB-CoA, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase.

cytochrome *c*, a water-soluble peripheral membrane protein of mitochondria and an essential component of the mitochondrial respiratory chain (5). It has been demonstrated that cytochrome *c* is translocated from mitochondria to the cytosolic compartment after transient focal cerebral ischemia in rats (32), in brain slices that are subjected to hypoxia–ischemia (84), and in vulnerable hippocampal CA1 neurons after transient global cerebral ischemia (97). Mitochondria are known to be involved in both the necrosis and apoptosis pathways, which depend on the severity of the insults or the nature of the signaling pathways (1, 3, 32, 37, 85). In most instances, severe cerebral ischemia renders the mitochondria completely 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 (Fig. 2). It interacts with the CED-4 homologue Apaf-1 and deoxyadenosinetriphosphate, leading to the activation of caspase-9 (68, 70, 105, 110). Caspase-9, which is presumably an initiator of the cytochrome *c*-dependent caspase cascade, then activates caspase-3, followed by caspases-2, -6, -8, and -10 activation downstream (96). In cerebral ischemia studies, caspases-3 and -9 have also been

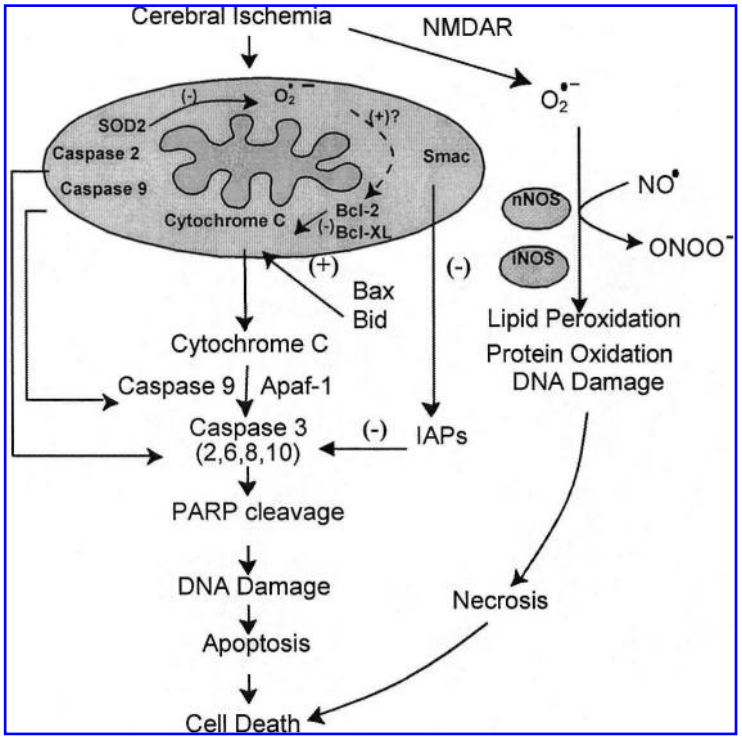

FIG. 2. Mitochondria as targets for oxidative stress signaling after cerebral ischemia. Cerebral ischemia and reperfusion generate ROS within mitochondria, which then signal the release of cytochrome *c* by mechanisms that may be related to the Bcl-2 family proteins, Bcl-2, Bcl-XL, Bax, and Bid. Cytochrome *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 −2, −6, −8, and −10. The IAP family of proteins 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, which then leads to nuclear DNA damage without repair, resulting in apoptosis. The activation of the *N*-methyl-D-aspartate receptor (NMDAR) and formation of O_2^- and NO by neuronal NO (nNO) may directly signal the mitochondrial release of cytochrome *c* or formation of peroxynitrite ($ONOO^-$), and subsequent OH^- production can directly damage lipids, proteins, and DNA and lead to cell death, most likely necrosis.

shown to play a key role in neuronal death after ischemia (15, 80, 98). It has recently been reported that caspase-11 is a critical initiator for activation of caspases-1 and -3, and caspase-11 knockout animals showed reduced apoptosis after focal ischemia (58). As 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) (15, 26, 80). 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 this hypothesis, PARP knockout mice showed decreased infarct after transient MCAO (25). In contrast, there are proteins that can prevent caspase activation in the cytosol. The inhibitor of apoptosis (IAP) family of proteins suppresses apoptosis by preventing the activation of procaspases and also by inhibiting the enzymatic activity of active caspases (22, 76). Second mitochondria-derived activator of caspases (Smac) is also released by apoptotic stimuli and binds IAPs, thereby promoting activation of caspase-3 (7). 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 (98).

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 (106). 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 (93). Among these proteins, Bax, Bcl-XS, Bak, and Bid are proapoptotic proteins. They eliminate the mitochondrial membrane potential by affecting the PTP and facilitating the release of cytochrome *c* (75). Conversely, Bcl-2 and Bcl-XL 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 Tg mice (74) and in Bid knockouts (86), whereas Bcl-2 knockout mice showed an increased infarct (41). These findings suggest the importance of regulation of mitochondrial permeability 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 tumor necrosis factor (TNF) receptor. For example, in the Fas receptor pathway (Fig. 3), the extracellular Fas ligand (FasL) first binds to a receptor, and an adaptor molecule Fas-associated death domain (FADD) protein then activates procaspase-8 (43). Then caspase-8 activates caspase-3, and this effector caspase cleaves PARP and activates caspase-activated DNase, leading to DNA damage and cell death. In the middle of this pathway, caspase-3 uses downstream caspases as in the mitochondrial pathway (69). 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 loss of function of the Fas receptor in negative mutant mice, resulting in a smaller infarct (88). 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 (54). This evidence strongly suggests the involvement of the Fas receptor pathway of apoptosis after cerebral ischemia. Unlike Fas receptor knockouts, TNF receptor knockout mice (6) showed increased injury after transient focal ischemia, suggesting the neuroprotective effect of the TNF receptor (Table 3). These results provide evidence that these death receptors play an important role in cell death after ischemia, however, a future study is needed to clarify the relationship between oxidative stress and receptor ligation.

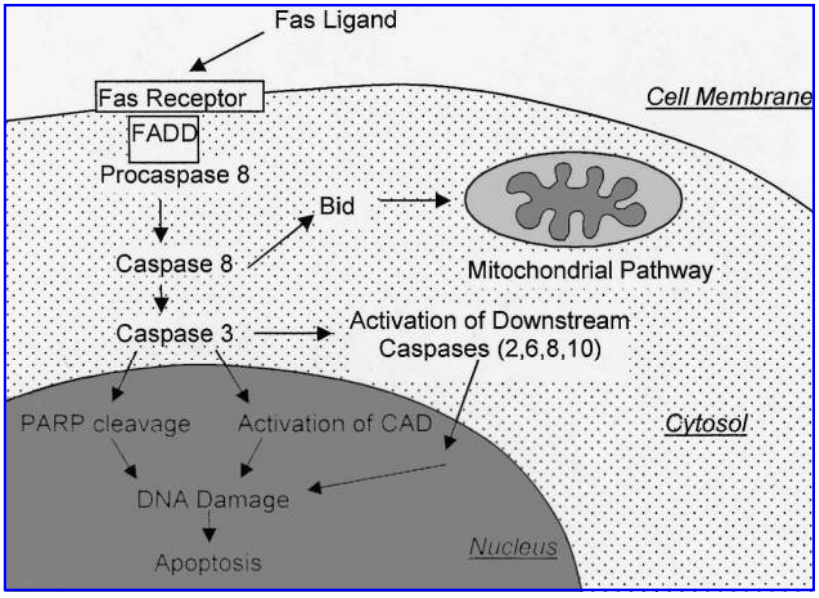

FIG. 3. Fas receptor pathway of apoptosis. Extracellular FasL first binds to a receptor, and an adaptor molecule, FADD protein, activates procaspase-8. Then caspase-8 activates caspase-3, and this effector caspase cleaves PARP and activates caspase-activated DNase (CAD), leading to DNA damage and cell 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.

TABLE 3. TRANSGENIC AND KNOCKOUT STUDIES OF PROAPOPTOTIC AND ANTIAPOPTOTIC PROTEINS

Study	*Insult*	*Findings*	*Reference*
Bid −/−	Transient MCAO	Decreased infarct (−67%)	86
Bcl-2 Tg	Permanent MCAO	Decreased infarct (−50%)	74
Bcl-2 Tg	Global ischemia	Decreased injury	63
Bcl-2 Tg	Permanent MCAO	No protection	101
Bcl-2 Tg	Permanent MCAO	Decreased injury	20
Bcl-2 −/+, −/−	Transient MCAO	Increased infarct	41
Bcl-XL Tg	Permanent MCAO	Decreased infarct (−21%)	101
Caspase-1 NM	Transient MCAO	Decreased infarct (−44%)	40
Caspase-1 NM	Permanent MCAO	Reduced injury	28
Caspase-1 −/−	Permanent MCAO	Reduced injury	89
Caspase-1 −/−	Transient MCAO	Decreased infarct	71
Caspase-11 −/−	Permanent MCAO	Reduced apoptosis	58
PARP −/−	Transient MCAO	Decreased infarct	24
PARP −/−	Transient MCAO	Decreased infarct in chronic stage	36
Fas NM	Transient MCAO	Decreased infarct	88
TNFR −/−	Transient MCAO	Increased injury	6
TNFR −/−	Transient MCAO	Increased injury	35
p53 −/+, −/−	Permanent MCAO	Decreased infarct (−27%, −15%)	18

NM, negative mutant; TNFR, TNF receptor.

DNA repair enzyme as a target for oxygen radical signaling

APE/Ref-1, a constitutive multifunctional protein mainly expressed in the nucleus, is known to be involved in DNA base excision repair by removing the oxygen radical-induced AP site and by regulation of many other transcriptional factors, such as AP-1, that are sensitive to redox regulation (2). Although there is no direct evidence linking AP site repair and apoptosis, incomplete repair of AP sites has been reported to cause mutagenesis and genetic instability. APE is known to be associated with oxidative stress, and in some cases down-regulation of APE expression is associated with apoptosis in cells of the myeloid lineage (87). The levels of constitutively expressed APE are rapidly reduced in neurons after transient focal ischemia (29). Early reduction of APE in the ischemic brain after photothrombotic cerebral ischemia can be prevented by treatment with the antioxidant 21-aminosteroid, suggesting that redox signaling may play a role in APE reduction after cerebral ischemia (14). APE/Ref-1 is also known as a redox factor for AP-1 transcription factors (102). Further studies are required to elucidate the mechanisms of the redox signaling of APE and DNA repair in cerebral ischemia. It is of special interest to note that nuclear factor-κB and AP-1, transcription factors that affect many downstream gene expressions, are overexpressed in the ischemic brain. However, overexpression of both these factors is reduced in Tg mice that overexpress SOD1, suggesting the involvement of oxidative signaling in the activation of these transcription factors (45, 46).

CONCLUSIONS

Numerous reports from the past decade strongly suggest the involvement of oxidative stress in cell death after cerebral ischemia. Genetic manipulation of prooxidant and antioxidant enzymes served as especially useful tools for dissecting the molecular mechanisms of the cell death pathway in ischemia/reperfusion injury. Besides direct oxidative injury to proteins, lipids, and DNA, the intracellular redox signaling of ROS has recently drawn much attention. The possible targets of redox signaling include mitochondria, death membrane receptors, and DNA repair enzymes, and these signaling cascades are thought to lead cells to apoptotic death. Future studies of these cascades and apoptotic cell death after ischemia may provide novel therapeutic strategies in clinical stroke.

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ABBREVIATIONS

COX-2, cyclooxygenase-2; CuZnSOD and SOD1, copper/zinc superoxide dismutase; ECSOD, extracellular superoxide dismutase; eNOS and NOS3, endothelium nitric oxide synthase; FADD, Fas-associated death domain; FasL, Fas ligand; GSH, glutathione; GSHPx, glutathione peroxidase; H_2O_2, hydrogen peroxide; IAP, inhibitor of apoptosis; iNOS and NOS2, induced nitric oxide synthase; MCAO, middle cerebral artery occlusion; MnSOD and SOD2, manganese superoxide dismutase; nNOS and NOS1, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; O_2^-, superoxide anion; OH^-, hydroxyl radical; PARP, poly(ADP-ribose) polymerase; PTP, permeability transition pore; ROS, reactive oxygen species; Smac, second mitochondria-derived activator of caspases; SOD, superoxide dismutase; Tg, transgenic; TNF, tumor necrosis factor.

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