Neuroprotective Effect of an Antioxidant in Ischemic Brain Injury
Involvement of Neuronal Apoptosis

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Abstract

The production of reactive oxygen species (ROS) has been implicated in reperfusion injury after cerebral ischemia, and antioxidant enzymes are believed to be among the major mechanisms by which the cells counteract the deleterious effect of ROS after cerebral ischemia. ROS also mediate the mitochondrial signaling pathway that may lead to apoptosis following cerebral ischemia. The recent development and availability of transgenic and knockout mutant rodents that either overexpress or are deficient in antioxidant genes have provided powerful tools for dissecting the molecular and cellular mechanisms of signaling pathways, direct oxidative damage, or both that are involved in ischemic brain injury. This article focuses on the contribution of ROS or an antioxidant system to the molecular pathway of postischemic apoptosis following transient focal cerebral ischemia by using transgenic mice that overexpress the cytosolic antioxidant copper/zinc superoxide dismutase.

Key Words: Focal cerebral ischemia; reperfusion injury; apoptosis; DNA damage; superoxide dismutase.

Introduction

Reperfusion injury is believed to play a critical role in the pathophysiology of cerebral ischemia. Although reperfusion of ischemic tissue with thrombolytic agents after a short period of ischemia is considered to reduce infarction volume, reperfusion at a later period exacerbates ischemic brain damage. The production of reactive oxygen species (ROS) has been implicated in reperfusion injury after cerebral ischemia, and antioxidant enzymes are believed to be among the major mechanisms by which the cells counteract the deleterious effect of ROS after cerebral ischemia and reperfusion (1–3). It has been demonstrated that approximately 2 to 5% of the electron flow in isolated brain mitochondria produces superoxide anion radicals (O2−) and hydrogen peroxide (H2O2) (4). These ROS are scavenged by superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase. Other small molecular antioxidants, such as glutathione (GSH), ascorbic acid, and α-tocopherol, are also involved in the detoxification of free radicals.

During reperfusion, these endogenous antioxidative defenses are likely to be perturbed as a result of overproduction of ROS by cytosolic pro-oxidant enzymes and mitochondria, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in ischemic brain tissue. Accumulating evidence has shown that ROS are directly involved in oxidative damage with cellular macromolecules, including lipids, proteins, and nucleic acids in ischemic tissues, which leads 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. Although the causative role of ROS in ischemic brain injury has not been determined, the recent development and availability of transgenic and knockout mutant rodents that either overexpress or are deficient in antioxidant genes have provided powerful tools for dissecting the molecular and cellular mechanisms of signaling pathways, direct oxidative damage, or both that are involved in ischemic brain injury. This article focuses on the contribution of ROS or an antioxidant system—especially cytosolic antioxidant copper/zinc SOD (SOD1)—to the molecular pathway of apoptosis, which has been assumed to participate in ischemic brain injury, following transient focal cerebral ischemia (FCI).

**Antioxidant Enzymes**

SODs are specific antioxidant enzymes that detoxify O$_2^-$ to H$_2$O$_2$. Three isoforms of SODs, copper/zinc SOD (CuZnSOD, SOD1), manganese SOD (MnSOD, SOD2), and extracellular SOD (ECSeSOD, SOD3) are major antioxidant enzymes based on cellular distribution and localization. CuZnSOD is a major cytosolic enzyme that is constitutively expressed in mammalian cells, with a molecular weight of 32,000. Its gene is located at human chromosome 21 and has been implicated in various central nervous system disorders, including amyotrophic lateral sclerosis and cerebral ischemia. MnSOD is a mitochondrial antioxidant enzyme with a molecular weight of 88,000. ECSeSOD is an isoform that is localized in extracellular space, cerebrospinal fluid, and cerebral vessels (3,6). These three SODs dismutate O$_2^-$ and form H$_2$O$_2$, which is then scavenged by peroxisomal catalase or GSHPx at the expense of GSH. GSH is regenerated from oxidized GSH by GSH reductase in the presence of Nicotinamide adenine dinucleotide phosphate (NADPH).

Other lipid peroxides are also scavenged by GSHPx. Among these SODs, CuZnSOD has been extensively used in experimental studies on cerebral ischemia and reperfusion. The short half-life of CuZnSOD (6 minutes) in circulating blood and its failure to pass the blood–brain barrier (BBB) make it difficult to use this enzyme for the treatment of cerebral ischemia by systemic administration; however, a modified enzyme with an increased half-life, such as polyethylene glycol-conjugated CuZnSOD, successfully reduced infarction volume in rats with FCI (7). Liposome-entrapped CuZnSOD has an increased half-life of up to 4.2 hours, BBB permeability, and cellular uptake and is reported to be protective against cerebral ischemia and traumatic brain injury (8,9).

**Neuroprotective Role of SODs in Cerebral Ischemia**

Among the antioxidant enzymes, CuZnSOD has been shown to be highly protective against ischemia/reperfusion injury after transient FCI (10,11), shown especially by the studies using genetic engineering animals of CuZnSOD. Overexpression of SOD1 in transgenic mice resulted in a reduction of infarction volume, edema formation, and better neurological outcomes after transient FCI (10,12). In contrast, target disruption of SOD1 in mutant mice resulted in a marked exacerbation of cerebral infarction and edema formation after transient middle cerebral artery (MCA) occlusion (11). These results suggest that the constitutively expressed CuZnSOD has a potential role in reducing ischemic brain injury and/or cerebral reperfusion injury.

Target disruption of SOD2 (a mitochondrial isoform) has also been reported to exacerbate ischemic brain injury following both permanent and transient FCI (13,14), whereas mice that overexpress SOD2 showed neuronal protection against oxidative stress after transient FCI (15). Neuronal protection by ECSeSOD also was demonstrated following cerebral ischemia.

Overexpression of SOD3 provides neuronal protection in FCI (16) and in global cerebral ischemia (17), whereas target disruption of SOD3 showed exacerbation of ischemic brain injury (18). Contrary to these observations, immature mice transgenic for CuZnSOD have greater brain injury after hypoxia–ischemia than their wild-type nontransgenic littermates (19). This conflicting result may be caused by the different balance of the antioxidant system between adult and neonatal mice. Because CuZnSOD dismutates superoxide to H$_2$O$_2$, overexpression of SOD1 in the presence of developmentally low activities of the catalytic enzymes GSHPx and catalase leads to an increased production of H$_2$O$_2$ and may explain the increased brain injury observed after hypoxia–ischemia in neonatal SOD1 transgenic mice.

**Cerebral Ischemia and Apoptosis**

Neuronal cell death after FCI has been attributed to passive necrotic processes. However, increasing evidence suggests that an active process similar to programmed cell death or apoptosis may contribute to the death of neurons after FCI (20–28). Fragmented DNA (as shown by agarose gel electrophoresis) was present in rat brains subjected to permanent MCA occlusion (20,21). The DNA fragmentation is known to be associated with increased intranucleosomal endonuclease activity (21) or to be reduced by the protein synthesis inhibitor cycloheximide (20,29). Morphological studies with terminal deoxynucleotidyl transferase-mediated uridine 5’-triphosphate-biotin nick end labeling (TUNEL) demonstrate that the inner boundary zone of the caudate putamen is vulnerable to DNA fragmentation after FCI (23,25) and that the number of DNA-damaged neuron is maximized at 24 to 48 hours following ischemia (30).

To detect the occurrence of intranucleosomal DNA fragmentation (31), we extracted genomic DNA from the ischemic brain and the homologous sample from the contralateral side and analyzed them by gel electrophoresis (Figure 1; ref. 32). Intranucleosomal DNA fragmentation was absent from the control tissue in both the transient and permanent FCI samples (31). A significant amount of DNA laddering appeared at 25 hours but was not detected at 5 hours, after both transient and permanent FCI. At 25 hours, the characteristic laddering was prominent after transient FCI (Figure 1, lane 4), whereas a strong smear background as well as a lesser amount of laddering was observed after permanent FCI (Figure 1, lane 8). These results suggested that apoptosis is more prominent in transient FCI and reperfusion compared to permanent FCI without reperfusion (32). We then compared the amount of DNA laddering between wild-type and SOD1 transgenic mice (33). A significant amount of DNA laddering was detected 24 hours after ischemia and was decreased in the SOD1 transgenic mice (Figure 2), suggesting that CuZnSOD1 has a potential role in preventing apoptosis following FCI (34).