

Oxidative stress and the antioxidant enzyme system in the developing brain

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Preterm infants are vulnerable to the oxidative stress due to the production of large amounts of free radicals, antioxidant system insufficiency, and immature oligodendroglial cells. Reactive oxygen species (ROS) play a pivotal role in the development of periventricular leukomalacia. The three most common ROS are superoxide ($O_2^{\bullet-}$), hydroxyl radical (OH^{\bullet}), and hydrogen peroxide (H_2O_2). Under normal physiological conditions, a balance is maintained between the production of ROS and the capacity of the antioxidant enzyme system. However, if this balance breaks down, ROS can exert toxic effects. Superoxide dismutase, glutathione peroxidase, and catalase are considered the classical antioxidant enzymes. A recently discovered antioxidant enzyme family, peroxiredoxin (Prdx), is also an important scavenger of free radicals. Prdx1 expression is induced at birth, whereas Prdx2 is constitutively expressed, and Prdx6 expression is consistent with the classical antioxidant enzymes. Several antioxidant substances have been studied as potential therapeutic agents; however, further preclinical and clinical studies are required before allowing clinical application.

Key words: Reactive oxygen species, Antioxidants, Brain injuries, Premature infant

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Introduction

Premature infants are especially vulnerable to reactive oxygen species (ROS)-induced injury¹ because of their insufficient ability to synthesize antioxidant enzymes and the resulting deficiency of antioxidant enzymes. The imbalance between ROS production and antioxidant defense may lead to ROS-induced diseases such as bronchopulmonary dysplasia (BPD), periventricular leukomalacia (PVL), or retinopathy of prematurity (ROP)².

Free radicals are highly reactive molecules that contain one or more unpaired electrons, and radicals containing oxygen are referred to as ROS³. Under normal physiological conditions, a balance is maintained between ROS production and the antioxidant enzyme system. However, if this balance breaks down, ROS oxidize lipids, proteins, and polysaccharides and can damage DNA and RNA^{4,5}.

Aerobic organisms have developed antioxidant defenses. Superoxide dismutase (SOD); catalase; glutathione peroxidase (GPx); vitamins A, C, and E; and glutathione are common antioxidants⁶. Majority of the studies on the antioxidant system in the developing brain have focused on the physiological functions of classical antioxidant enzymes such as manganese-containing SOD (Mn-SOD), copper- and zinc-containing SOD (CuZn-SOD), GPx, and catalase, under conditions of oxidative stress⁷⁻⁹. The recently discovered antioxidant enzyme family, peroxiredoxins (Prdxs), was first identified in yeast as a 25-kDa enzyme¹⁰. The peroxidase activities of Prdx1 and 2 in the 2-Cys Prdx group control the reduction-oxidation status during normal oxidative metabolism and in the presence of oxidative

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stress¹¹. Prdx6 is the only member of the 1-Cys Prdx group and catalyzes the reduction of phospholipid hydroperoxide^{12,13}.

In this article, we review oxidative stress and the antioxidant enzyme system in the fetus and preterm infant.

ROS

The three most common ROS are superoxide ($O_2^{\bullet-}$), hydroxyl radical (OH^{\bullet}), and hydrogen peroxide (H_2O_2). $O_2^{\bullet-}$ is produced when molecular oxygen gains an additional electron. $O_2^{\bullet-}$ can develop secondary ROS, H_2O_2 , and OH^{\bullet} ^{14,15}. Intracellularly, ROS are produced by the mitochondrial respiratory chain reaction. Mitochondrial activity reduces oxygen to water via cytochrome C oxidase. Mitochondria can also produce antioxidant enzymes such as SOD, GPx, and glutathione reductase¹⁶. SOD leads to the generation of H_2O_2 from $O_2^{\bullet-}$, which is then dissociated by catalase or GPx into water and molecular oxygen¹⁷. Increased $O_2^{\bullet-}$ production or an inadequate antioxidant system causes H_2O_2 accumulation. H_2O_2 is also produced in response to extracellular responses such as cytokines, neurotransmitters, peptide growth factors, and hormones^{18,19}. H_2O_2 affects the functions of proteins, including those of transcription factors, phospholipases, and protein kinases^{19,20}. H_2O_2 is considered an important intracellular messenger under physiological concentrations, but under pathological conditions, H_2O_2 can react with Fe^{2+} via Fenton reaction to produce the highly reactive OH^{\bullet} ¹.

Nitric oxide (NO) is a relatively weak oxygen free radical produced by nitric oxide synthase (NOS). NO itself has important roles in vessel dilation and neurotransmitter release. However, a reaction between NO and $O_2^{\bullet-}$ leads to the formation of peroxynitrite, a potent free radical causing lipid peroxidation^{15,21}.

Development of antioxidant enzymes during the perinatal period

1. Classical antioxidant enzymes

The main antioxidant enzymes are SOD, catalase, and GPx. There are three forms of SOD: CuZn-SOD, which is mainly located in the cytoplasm, Mn-SOD, which is mainly located in the mitochondria, and extracellular SOD (EC-SOD), which is located in the intracellular spaces in neonates but in the extracellular space thereafter. The only known function of SOD is to convert $O_2^{\bullet-}$ to H_2O_2 . Catalase and GPx catalyze the conversion of H_2O_2 into oxygen and water^{7-9,17}.

The developing human brain needs protective antioxidant enzymes against the oxidative stress that suddenly occurs at birth, due to the hyperoxia caused by transfer from an anaerobic in utero environment to an oxygen-rich environment. The

expressions of SOD, catalase, and GPx are known to increase by 150% during the last 15% of the gestation period²². Development of the antioxidant enzyme system during the fetal period is associated with redox signaling for the maintenance of pregnancy through utero-placental-fetal interactions^{22,23}. In addition, the regulation of antioxidant enzymes associated with local NO generation via NOS and downstream NO-dependent signaling in the placenta are important for normal vascular development^{22,24}. The exact timing of the acquisition of adult levels of these antioxidant enzymes is obscure. Mn-SOD seems to be important for the protection of oligodendroglial (OL) cells in the presence of high levels of iron, which can lead to generation of OH^{\bullet} ²⁵. Previous studies showed that the expression of CuZn-SOD dramatically increases during the highly metabolic period of myelin sheath synthesis and that the quantity of catalase-containing peroxisomes increases during active myelin sheath formation in the postnatal rat^{26,27}. Accordingly, these major classical antioxidant enzymes are thought to be associated with myelinogenesis.

2. Prdx

Prdx was initially discovered in yeast as a 25-kDa enzyme that protects against oxidative damage. Prdx is a widely distributed superfamily of nonselenium GPx, which directly reduce H_2O_2 and alkyl hydroperoxides. There are six mammalian Prdx isoforms: 2-Cys Prdx group (Prdx1-4), atypical 2-Cys Prdx group (Prdx5), and 1-Cys Prdx group (Prdx6)^{10,11}. The 2-Cys Prdx group reduces H_2O_2 by using the electrons provided by thioredoxin. Group members Prdx1 and 2 play roles as scavengers of H_2O_2 and effectors of signaling cascades, in which H_2O_2 acts as a second messenger to regulate cellular responses^{10,11}. On the other hand, Prdx6, the only member of the 1-Cys Prdx group, has been suggested to use glutathione as an electron donor. Its localization to both cytoplasm and lysosomes and its ability to catalyze the reduction of phospholipid hydroperoxide suggest that Prdx6 has functional roles in phospholipid metabolism in a variety of biological systems^{12,13}.

In our recent perinatal rat brain study, the expressions of both Prdx1 and 6 were deficient during the early gestational period and were elevated in the late gestational period. These expression patterns are similar to those of other classical antioxidants. Prdx1 and 6 expressions might be increased against oxidative stress that suddenly occurs at birth. It is likely that the observed increase in the expressions of Prdx1 and 6 is in response to the sudden oxidative stress that occurs at birth. Prdx1 protein expression reached peak level after birth, and then, it gradually decreased to the adult level. Prdx6 expression gradually increased from the late gestation period to the adult level. In contrast, Prdx2 was largely expressed during the gestational period and was constitutive during the perinatal period. We also observed

these expression patterns in our perinatal rat lung studies²⁸⁻³⁰. Prdx6 expression parallels those of SOD and catalase³¹. Prdx1 expression can be induced by specific stimulations occurring at birth, and it is predominantly expressed in OL cells³². Because immature OL cells are vulnerable to free radicals, Prdx1 might have an important role to play in the protection of the brain from perinatal oxidative stress.

Oxidative stress and brain injury in preterm infants

Preterm infants are vulnerable to perinatal insults such as PVL, because of vascular immaturity, impaired cerebrovascular autoregulation, and maturation-dependent vulnerability of OL precursor cells¹. There are two types of PVL: a focal type induced by localized necrosis that is expressed as cystic formation in ultrasonography, and the diffuse type that is more common and is induced by diffuse OL precursor cell apoptosis¹. The diffuse type PVL can be detected by diffusion-weighted magnetic resonance imaging (MRI). It was reported that free radicals are more toxic to OL precursor cells than to mature OL cells by using cell culture under cystine-deprived medium, which results in glutathione depletion, thereby leading to a condition of free radical attack³³. Intraventricular hemorrhage (IVH) provides a source of free iron, which can generate OH[•] by Fenton reaction³⁴.

Hypoxia plays a primary role in perinatal insults. During hypoxia, accumulation of intracellular Ca²⁺ due to activation of N-methyl-D-aspartate receptors can lead to free radical generation, cell apoptosis, and necrosis by various mechanisms. Phospholipase A2 and protease are activated by intracellular Ca²⁺. Increased phospholipase A2 leads to free radical generation from cyclooxygenase and lipoxygenase pathways. Activated protease induces the conversion of xanthine dehydrogenase to xanthine oxidase, resulting in increased free radical generation. In addition, NOS is more activated and easily generates NO, which can react with O₂^{•-} to form peroxynitrite, a potent free radical³⁵.

Preterm infants are susceptible to free radical attack because of several characteristics. Neuronal membranes in preterm infants are rich in polyunsaturated fatty acids, which provide a source of peroxidation¹⁶. OL precursor cells, which are mainly present in the immature nervous system in preterm infants, are vulnerable to free radical attack. Furthermore, OL precursor cells tend to accumulate iron for maturation purposes, and IVH also provides a source of free iron, which facilitates the Fenton reaction. The conversion of H₂O₂ to OH[•] by Fenton reaction increases cytotoxicity in the immature nervous system^{1,16,34}. In fact, free radicals and OL precursor cells are the primary players in the pathogenesis of brain injury in preterm infants. In conclusion, preterm infants are sensitive to ROS because of an antioxidant enzyme deficiency and a tendency to produce large amounts of ROS.

Antioxidant therapies

Several substances are considered as therapeutic candidates for oxidative stress; however, further preclinical and clinical studies are required before clinical application of these substances. Melatonin (5-methoxy-N-acetyltryptamine) is secreted predominantly in the pineal gland and has potent antioxidant and anti-inflammatory activities. Melatonin acts as a direct antioxidant by scavenging free radicals, including OH[•], O₂^{•-}, H₂O₂, and peroxynitrite. Melatonin acts as an indirect antioxidant by increasing the levels of antioxidant enzymes such as GPx, glutathione reductase, SOD, and catalase^{36,37}. Currently, melatonin is not available as a formulation for neonates, but it seems likely that in the near future, it will be available for the treatment of ROS-induced neonatal diseases. Allopurinol, the xanthine oxidase inhibitor, can reduce free radical formation and works as a free radical scavenger or iron chelator at high dosages^{38,39}. Although clinical data about the use of allopurinol are insufficient to determine its efficacy in neonates under oxidative stress, animal studies have provided evidence of its neuroprotective capabilities¹⁴. Vitamins C (ascorbic acid) and E (tocopherols and tocotrienols) are also considered important antioxidants. Vitamin E can stabilize biological membranes and protect against lipid peroxidation^{37,40}. Vitamin C works as a free radical scavenger and can regenerate reduced tocopherol⁴¹. In one study, cotreatment with vitamins C and E was found to exhibit a synergistic antioxidant effect⁴². However, the study failed to show that these antioxidant vitamins significantly reduce ROS-associated injury. Vitamin A is an effective antioxidant known to prevent BPD in preterm infants. In fact, it can reduce BPD incidence but does not affect long-term outcome⁴³. Recombinant human SOD (rhSOD) was tested for the prevention of BPD or ROP in preterm infants^{44,45}. However, the effect of rhSOD was controversial, not conclusive.

Conclusions

The balance between ROS production and the antioxidant system is of particular importance in fetuses and newborns. Preterm infants produce large amounts of ROS and have a deficient antioxidant system. In particular, OL precursor cells, which are mainly present in the immature nervous system in preterm infants, are vulnerable to oxidative stress. Classical antioxidant enzymes such as SOD, GPx, and catalase are deficient in the early gestational period, but their expressions are upregulated during the late gestational period as a response to ROS exposure at birth, which is due to relative hyperoxia upon air exposure. The recently discovered antioxidant enzyme, Prdx, is an important scavenger of H₂O₂. Currently, several potential antioxidants are being studied for clinical applications, and it

is hoped that these efforts will result in suitable antioxidant therapies for preterm infants in the near future.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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