Review Article

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Oxidative Stress in Preterm Infants

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Abstract

Background: Oxidative stress is caused by an imbalance between the production of Reactive Oxygen Species (ROS) and the ability to detoxify them with the help of antioxidants. The premature infant is especially susceptible to ROS-induced damage because of inadequate antioxidant stores at birth, as well as impaired upregulation in response to oxidant stress. Thus, the premature infant is at increased risk for the development of ROS-induced diseases of the newborn, such as bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis, and periventricular leukomalacia. *Purpose of Review:* This review will examine the unique susceptibility of premature infants to oxidative stress, the role of reactive oxygen species (ROS) in the pathogenesis of common disorders of the preterm infant, and potential for therapeutic interventions using enzymatic and/or non-enzymatic antioxidants. *Methodology:* An extensive search was made on internet as well as Neonatology textbooks were referred for this review. *Summary:* Potential therapies for ROS-induced disease include both enzymatic and nonenzymatic antioxidant preparations. More research is required to determine the beneficial effects of supplemental antioxidant therapy.

Keywords : Reactive Oxygen Species; Antioxidants; Oxidative Stress; Prematurity.

Oxidative Stress in the Fetus and Newborn

Oxidative stress (OS) results from an imbalance between reducing agents and enzymes involved in the removal of free radicals (FR) and/or reactive oxygen species (ROS). The consequence of OS on fetal structure involves the activation of a complex array of genes involved in inflammation, coagulation, fibrinolysis, cell cycle and signal transduction. It is now recognized that ROS are important for fertilization and developing embryos. In moderate quantities and in presence of a good antioxidant capacity, FRs are continuously generated inthe organism and are essential for cell aerobic metabolism and fetal growth, but they are toxic when overproduced, resulting in an attack of all classes of biological macromolecules,polysaccharides, nucleic acid, lipids and proteins [1].

Hypoxia, hyperoxia, inflammation, Fenton chemistry, endothelial damage, arachidonic acid cascade are other mechanisms that lead to the formation of highly reactive products. FR reactions lead to DNA damage (fragmentation, apoptosis, base modifications and strand breaks), to lipid, protein and polysaccharides oxidation and as a consequence FR reactions may induce a wide range of biological toxic effects.The newborn-infant is very susceptible to FR-induced oxidative damage. First, becauseof its immaturity, the infant is frequently exposed to oxygen therapy and hyperoxia. At birth the newborn encounters an environment much richer in oxygen (PO_2 100torr) than the intrauterine environment (20-25 torr). This 4-5 fold increase exposes the newborn to a flood of FR. Second, the antioxidant defense and its ability to be induced during hyperoxic challenge are impaired.6 Third, the preterm infant has an increased susceptibility to infection and inflammation, which increases OS. Finally, free iron is found in the plasma and tissue of premature infants to a greater extent than in the term infants [1].

Normal Fetal and Neonatal Antioxidant Enzyme Maturation

Frank and Groseclose documented the development of antioxidant enzymes in the lungs of

rabbits during late gestation. Enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) are important in scavenging ROS and have been shown to increase 150% during the last 15% of gestation. There are three forms of SOD that have been identified: copper-zinc superoxide dismutase (Cu/ZnSOD), present primarily in thecytoplasm, manganese superoxide dismutase (MnSOD) in the mitochondria, and extracellular superoxide dismutase (EC-SOD) located in the extracellular spaces in adults, but primarily intracellular in newborns. The only known function of SOD is to convert extremely reactive superoxide radicals to hydrogen peroxide and water. Catalase, GPx, and glutathione reductase then convert hydrogen peroxide to water [2].

Antioxidant enzyme expression generally increases in most fetal compartments throughout the progression of pregnancy. Qanungo and colleagues found that SOD, catalase, GPx, and glutathione reductase activities increased with gestational age, as evidence of lipid peroxidation decreased in human placental and fetal tissues .Development of theantioxidant system during fetal life must also include redox signaling in themaintenance of pregnancy through uterine-placental-fetal interactions. There is evidence of regulation ofantioxidant enzymes in the context of local nitric oxide (NO) generation via nitric oxide synthases and downstream NO-dependent signaling in the placenta, critically important to normal vascular development [2].

Preterm Birth and Oxidative Stress

Delivery constitutes a significant oxidative stress and the gestation of the newborn and circumstances of delivery will affect the overall burden. Premature delivery often occurs before the normal upregulation of antioxidant systems and other ROS scavengers, such as glutathione and ceruloplasmin. This is in addition to relatively deficient utero-placental transfer of nutrients important to antioxidant defenses and places the newborn at particular risk of ROS-induced injury. MnSOD mRNA appears to be induced in the fetal membranes following spontaneous labor and in the presence of chorioamnionitis.The effects of inflammatory stimuli may or may not induce placental antioxidant expression, depending on the stage of pregnancy. Antenatal corticosteroids used to accelerate lung maturation in threatened preterm birth might also lead to increased activity of SOD, catalase, andglutathione-S-transferase. This can help to counteract relative deficiencies in antioxidants stemming from preterm birth [2].

Oxidative Stress and Adverse Pregnancy Outcomes

Oxidative stress has been suggested as a causative agent in pregnancy-related disorders, such as recurrent pregnancy loss, pre-eclampsia, preterm premature rupture of membranes (pPROM), intrauterine growth restriction (IUGR), and fetal death. *In utero* stressors contribute to ROS production and resulting tissue damage. Markers of oxidative stress, such as non-protein bound iron, have been measured in cord blood as an indicator of intrauterine ROS production. These markers have been associated with the development of several postnatal disease processes, suggesting that *in utero* oxidative stress is a significant risk factor, especially in premature neonates. Identifying high risk neonates at birth may allow for early treatment with antioxidants and possible prevention of further oxidant damage. It is also reasonable to speculate that maternal deficiencies in antioxidant related micronutrients could contribute to reproductive disorders. Antioxidant deficiencies could induce an imbalance between ROS and diet-dependent antioxidants and supplementation may decrease this effect. However, randomized controlled trials using prenatal supplementation with vitamins C and E have failed to show a reduction in the risk of gestational hypertension or pre-eclampsia, and may in fact increase the risk of premature rupture of membranes. These findings might be due to the timing of supplementation after placentation has already occurred, inadequate dosing, or the fact that other antioxidants may have better function. Further research is needed to identify methods of decreasing oxidative stress *in utero* and reducing ROS induced diseases in the mother and her newborn infant [2].

Prevention of Oxidative Stress

Avoid Oxidative Stress

Increased production of ROS can occur as a result of many conditions affecting the mother (maternal diabetes, maternal drugs, chorioamnionitis, congenital infections), as well as the newborn infant (hyperoxia, reperfusion, inflammation). Prevention and control of maternal diabetes which if not treated, is associated with an increased production of ROS, can minimize the incidence of ROS-induced fetal structural defects. Epidemiologic data have suggested a strong association between chorioamnionitis and the development of bronchopulmonary dysplasia due to increased concentrations of proinflammatory cytokines in human amniotic fluid and fetal cord blood, indicating a systemic inflammatory response during chorioamnionitis. Treatment of maternal

chorioamnionitis may minimize the ROS-induced fetal insult [3].

Obviously, avoidance of neonatal conditions such as asphyxia, hyperoxia, and retinal phototherapy light exposure which can cause excessive release of free oxygen radicals are the best strategy in avoiding the oxidative stress in neonates. It is also important to consider the fact that infection, especially sepsis, is a significant source of oxidative stress. Accordingly, early identification and treatment of sepsis and the concept of optimal oxygenation are considered important preventive strategies. Conventional indications suggest that optimal oxygen tension should be maintained between 50-70 mmHg [3].

A significant decrease in chronic lung disease and retinopathy of prematurity without any difference in mortality were observed in extremely low birth weight infants kept at less than 95% oxygen saturation compared to those kept at more than 95%. Hyperoxia and oxidative stress may occur during neonatal resuscitation with a potential risk is associated with resuscitating those infants, especially preterm infants, with 100% oxygen due to more production of ROS compared to room air. Accordingly, it seems reasonable to suggest avoiding routine neonatal resuscitation with 100% oxygen. To avoid hyperoxia in very pre-term infants (less than, 32 weeks gestation), use of an oxygen blender and pulseoximeter during resuscitation is recommended [3].

Another preventive strategy is avoiding or at least minimizing the barotrauma and volutrauma caused by mechanical ventilation in preterm infants with respiratory distress syndrome. Barotrauma, volutrauma and oxygen toxicity, during mechanical ventilation, are important factors in the pathogenesis of CLD with the release of multiple pro-inflammatory cytokines and increased production ROS and RNS that are destructive to lipids, proteins and DNA, within the pulmonary cells. Avoidance of the use of pure oxygen during resuscitation as well as avoidance of mechanical ventilation with the use of early surfactant and nasal continuous positive air pressure (CPAP) may reduce respiratory tissue damage [3].

Therapeutic Interventions with Antioxidants

Supplementation with enzymatic and/or non-enzymatic antioxidants might have beneficial effects in decreasing injury from excess production of ROS, particularly in disorders such as bronchopulmonary dysplasia, retinopathy of prematurity, periventricular leukomalacia, and necrotizing enterocolitis.

Bronchopulmonary Dysplasia

Although the pathogenesis of BPD is complex, studies do support a role for ROS-mediated damage. Vitamins A, C, and E are important factors in normal physiology as well as antioxidant defense. These vitamins are known to inhibit ROS-induced lipid peroxidation and scavenge ROS. In infants with BPD, plasma β-carotene and vitamin A concentrations are lower, likely reducing antioxidant protection. This may account for higher plasma 3-nitrotyrosine and protein carbonyls in those preterm infants at highest risk for developing BPD . Given that preterm infants are relatively deficient in antioxidant defenses, exogenous antioxidants such as vitamins A, E and recombinant human SOD (rhSOD) have been administered in attempts to prevent BPD.

Although a Cochrane meta-analysis suggests that supplementation with vitamin A reduces BPD,.neurodevelopmental and pulmonary outcomes at 18-22 months corrected gestational age (CGA) were not significantly different [5].

Randomized controlled trials of vitamin E supplementation have also failed to show a reduction in the incidence of BPD. Trace elements, such as copper, zinc, iron, and selenium are also essential for normal antioxidant enzyme function and supplementation with these nutrients could optimize total antioxidant capacity. However, studies examining trace elements as active cofactors in extremely low birth weight infants showed that lower trace element concentrations did not substantially influence antioxidant enzyme concentration or the development of BPD [6]. In addition, typical diseases of prematurity, including BPD were not associated with decreased antioxidant enzyme activities.

Retinopathy of Prematurity

The developing retina in premature infants is particularly susceptible to damage mediated by ROS, as evidenced in many animal studies. Oxygen fluctuations can induce cells to express NADPH oxidase, which leads to increased ROS and apoptosis of endothelial cells, contributing to the avascular retina. N-acetylcysteine (NAC) has been shown to decrease lipid hydroperoxide (LHP) in a rat model, but was not found to significantly reduce avascularity or clock hours of neovascularization. Repeated oxygen fluctuations also increased retinal vascular endothelial growth factor (VEGF) and ROS. Neutralizing VEGF bioactivity reduced

neovascularization and tortuosity, and inhibiting ROS with the NADPH oxidase inhibitor apocynin reduced the avascular retina by interfering with apoptosis [2].

Resveratrol is a phytoalexin produced by a variety of plants in response to stress. Kim and associates investigated resveratrol as a nitric oxide mechanism modulator as well as caffeic acid for retinal neovascularization anti-angiogenic activity and found some protective effects against the development of ROP [7]. Further research demonstrates that early blocking of peroxynitrite-mediated tyrosine nitration and peroxynitrite formation by the use of epicatechin (a green tea extract) as well as NAC could also be considered a new therapeutic target for ischemic proliferative diseases of the retina.

Periventricular Leukomalacia (PVL)

Preterm infants are vulnerable to reperfusion type injury and accompanying oxidative stress due to decreased regulation of cerebral perfusion. PVL is thought to develop after microglial activation leads to an accumulation of markers of oxidation in oligodendrocytes, such as nitrotyrosine and protein carbonyls. ROS have also been implicated in causing neuronal cell death. *In vitro* exposure to hyperoxia induces apoptosis in oligodendroglial cells in a developmentally dependent pattern. This is prevented by inhibition of lipoxygenase, with decreased expression of myelin basic protein *in vivo* in hyperoxia-exposed rat pups. Maternal lipopolysaccharide (LPS) exposure has been shown to stimulate the secretion of pro-inflammatory markers in maternal serum and amniotic fluid of pregnant mice, mimicking maternal infection. LPS-induced peroxisomal dysfunction depletes oligodendrocytes and exacerbates cerebral white matter injury in premature infants. NAC pretreatment attenuates this LPS-induced cerebral white matter injury by replenishment of reduced-GSH, ROS scavenging, and maintenance of peroxisomal proliferation/function via a peroxisome proliferators activated receptor-α (PPAR-α) dependent mechanism. LPS activates microglia cells which induces cell death and greatly impairs oligodendrocyte development, which may underlie selective white matter damage and hypomyelination in PVL [8].

Melatonin has been studied as a neuroprotective agent in PVL in mouse models. In a recent report, agomelatine and melatonin did not prevent the initial appearance of white matter lesions, but they did promote secondary lesion repair. The effects of melatonin were only observed when given within the first two hours following the insult. However, agomelatine was still neuroprotective when administered eight hours after the insult. Although further research is needed, this may represent a promising new therapy for prevention of PVL [9].

Necrotizing Enterocolitis

While the etiology of NEC is multifactorial, inflammation and ROS production appear to play a key role. An increased incidence of NEC has recently been noted in infants who are born to mothers with chorioamnionitis. These findings suggest that prenatal infection/inflammation may predispose the intestine of the preterm infant to the development of NEC. In a neonatal rat model of NEC, LPS administration led to increased susceptibility to intestinal injury. This increase in intestinal injury appears to be mediated in part by inducible nitric oxide synthase (iNOS) and can be attenuated with the selective iNOS inhibitor aminoguanidine.

During the early stages of NEC, NOS uncoupling becomes progressively worse, favoring production of ROS, vasoconstriction, intestinal ischemia, and NEC. It is possible that targeting iNOS or iNOS-derived NO may be of therapeutic benefit in preventing NEC [2].

Enteral glutamine alone or in conjunction with arginine has been shown reduce oxidative stress in juvenile rat models. This occurs not only in hypoxia–reoxygenation, but also in healthy newborn rats. Therefore, enteral glutamine and arginine may be useful for preventing NEC in premature neonates, although further experimental and clinical studies are needed [10].

Potential Antioxidant Therapies in Premature Neonates

Antioxidants are critical inprotecting against ROS-induced injury and several preclinical studies support antioxidant supplementation. Non-enzymatic proteins (transferrin, ferritin, ceruloplasmin), enzymes (superoxide dismutases, catalase, glutathione peroxidase), oxidizable molecules (glutathione, vitamins E, A, C, carotenoids, flavonoids), and trace elements (copper, zinc, selenium) all play a role in maintaining a delicate balance between ROS production and oxidant damage to tissues and organs.

Enzymatic

Enzymatic antioxidants are gestationally

regulated, with premature newborns having decreased expression relative to full term neonates. Multiple models using transformed human alveolar epithelial cells have suggested that overexpression of antioxidants prevents ROS-induced injury. Increased expression of either MnSOD or CuZnSOD reverses the growth inhibitory effects of hyerpoxia in lung epithelial cells. Overexpression of SOD not only reduced ROS production, but also mitigated the activation of the JNK / AP1 pathway which has been implicated in ROS-induced mitochondrial injury and apoptotic cell death. Melatonin is a pineal hormone that exhibits an indirect antioxidant effect by supporting SOD and glutathione peroxidase activity as well as directeffects through lipid peroxidation and scavenging oxygen-induced ROS. In a neonatal rat model, melatonin reduced ROS production and increased antioxidant levels in hyperoxia induced lung damage, indicating a potential protective effect in BPD.

Increasing SOD and catalase (CAT) activities have consistently been associated with protection against oxygen toxicity. Naturally derived commercial surfactants contain both SOD and CAT activity in significant concentrations. By adding additional SOD and CAT to surfactant preparations, the antioxidant effects are also potentiated [11].

Non-enzymatic Antioxidants

Resistance to oxidative stress also relies on non-enzymatic pathways. Non-enzymatic antioxidants are depleted in response to ROS-mediated stress. The effects of vitamin A are likely mediated through its action on retinol-binding protein and the retinoic acid receptor. NAC is a precursor of the antioxidant glutathione and a large multicenter trial showed no reduction in survival or incidence of BPD at 36 weeks CGA or improved pulmonary function at term. Ceruloplasm, transferrin, and ferroxidase all aid in

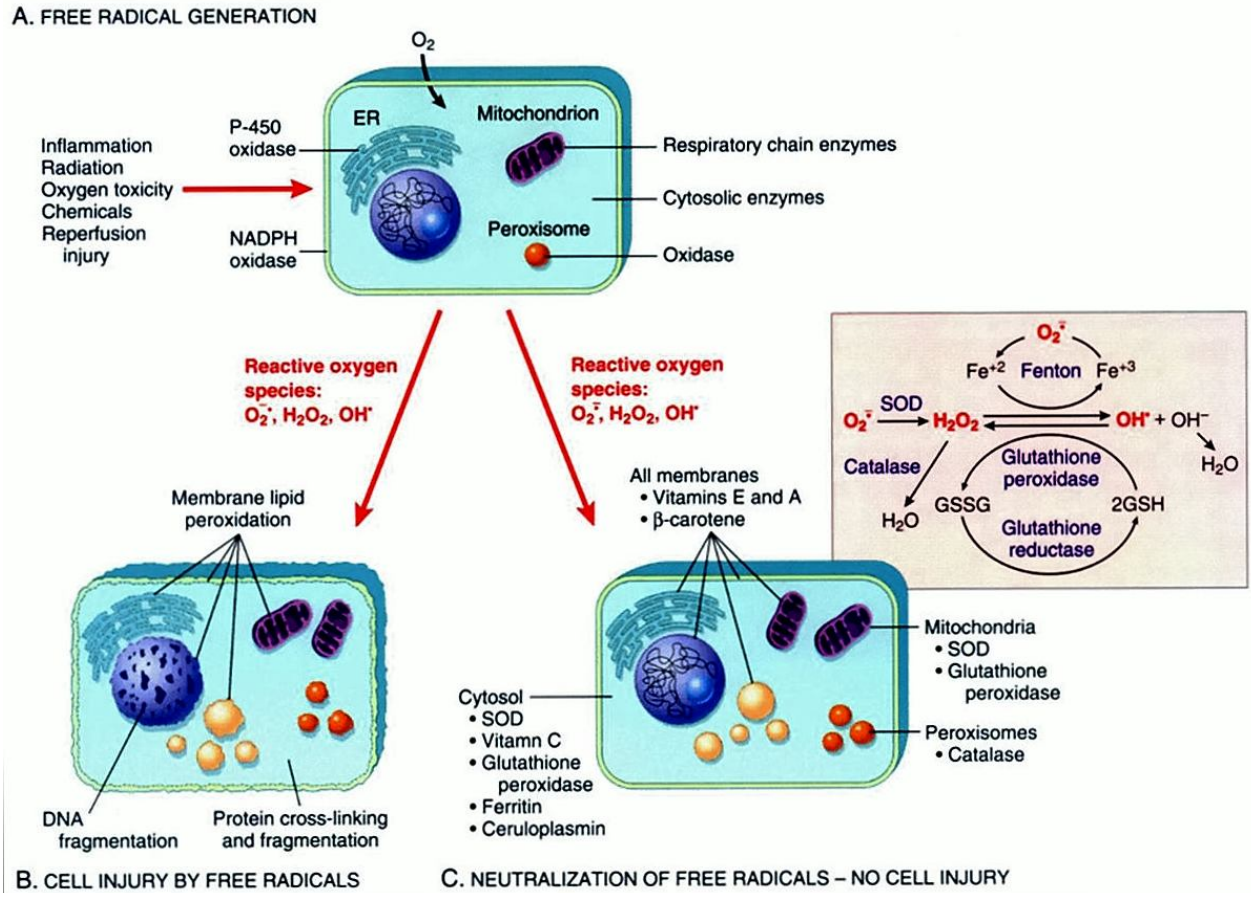

Fig. 1: Disruptions in Oxidant / Antioxidant Balance Can Cause Significant Cell Injury Production of reactive oxygen species can lead to significant cellular damage in the absence of antioxidants.
A–Free radical production occurs after cellular insult resulting from inflammation, radiation, oxygen toxicity, chemicals, or reperfusion injury.
B–Reactive oxygen species cause membrane lipid peroxidation that leads to cell injury through DNA and protein fragmentation.
C–Free radicals in the presence of antioxidants are neutralized and protect the cell from injury.

the metabolism of iron, which can act as a potent oxidizing agent. Diminished function or bioavailability of these proteins may predispose the preterm infant to increased production of ROS [2].

New Antioxidants under Investigation

There are multiple potential therapeutic antioxidants currently under investigation that could benefit premature infants. One protein under investigation, Pon3, was shown in laboratory studies to have antioxidant properties and to be up-regulated in rat, sheep, and human cord blood late in gestation. More research is needed, but Pon3 could serve as a potential therapeutic target in premature infants. Clinical trials involving antioxidants currently registered with the NIH at www.clinicaltrials.gov include supplementation of preterm infants with lactoferrin and cysteine, examination of concentrations of beta-carotene, lutein, and lycopene in preterm infants fed formulas with mixed carotenoids and the effects on the developing eye, early administration of human erythropoietin in very preterm infants, NAC administration to women with intra-amniotic infection and / or inflammation, early enteral administration of vitamin E to extremely premature infants, and multiple trials involving inhaled nitric oxide. The results from these trials may change the way we treat many common neonatal conditions [2].

Key Points

- Premature infants are at increased risk of damage due to ROS due to inadequate antioxidant stores at birth as well as decreased production of antioxidants in response to oxidant stress.
- ROS-induced injury is important in the development of many common disorders of prematurity, such as BPD, ROP, NEC, and PVL.
- Supplementation with enzymatic and / or non-enzymatic antioxidants might have **b**eneficial effects in decreasing injury from excess production of ROS.

Conclusions

A delicate oxidant / antioxidant balance exists in the fetus and newborn. This balance can tip towards oxidant injury in the setting of preterm birth. Antioxidant enzymes are primarily upregulated in the latter part of gestation and preterm birth is associated with an increased generation of ROS. The use of supplemental antioxidants represents a logical strategy to prevent or ameliorate injury from excess production of ROS, but studies in animal models and in preterm infants have yielded mixed results. Caution must be taken since ROS are critical second messengers in various cell signaling pathways that control normal cellular functions, but strategies that maintain normal antioxidant balance may be beneficial to the preterm newborn.

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References

1. Buonocore, G., and S. Perrone. "Biomarkers of oxidative stress in the fetus and newborn." *Hematology Meeting Reports (formerly Haematologica Reports)*. 2009; 2(10).
2. Lee, Jennifer W., and Jonathan M. Davis. "Future applications of antioxidants in premature infants". *Current opinion in pediatrics* 2011; 23(2): 161.
3. Knuppel, R. A., Hassan, M. I., McDermott, J. J., Tucker, J. M., & Morrison, J. C. Oxidative Stress and Antioxidants: Preterm Birth and Preterm Infants. Source: *www.intechopen.com*
4. Ballard PL, Truog WE, Merrill JD, Gow A, Posencheg M, Golombek SG, et al. Plasma biomarkersof oxidative stress: relationship to lung disease and inhaled nitric oxide therapy in prematureinfants. Pediatrics. 2008 Mar; 121(3): 555-61. [PubMed: 18310205].
5. Tyson JE, Wright LL, Oh W, Kennedy KA, Mele L, Ehrenkranz RA, et al. Vitamin A supplementation for extremely-low-birth-weight infants. National Institute of Child Health and Human Development Neonatal Research Network. N Engl J Med. 1999 Jun 24; 340(25): 1962-8. [PubMed: 10379020].
6. Loui A, Raab A, Maier RF, Bratter P, Obladen M. Trace elements and antioxidant enzymes in extremely low birthweight infants. J Trace Elem Med Biol. 2010 Apr; 24(2): 111-8. [PubMed: 20413069].
7. Kim WT, Suh ES. Retinal protective effects of resveratrol via modulation of nitric oxide synthase on oxygen-induced retinopathy. Korean

J Ophthalmol. 2010 Apr; 24(2): 108-18. [PubMed: 20379461].

8. Khwaja O, Volpe JJ. Pathogenesis of cerebral white matter injury of prematurity. Arch Dis Child Fetal Neonatal Ed. 2008 Mar; 93(2): F153-61. [PubMed: 18296574].
9. Gressens P, Schwendimann L, Husson I, Sarkozy G, Mocaer E, Vamecq J, et al. Agomelatine, a melatonin receptor agonist with 5-HT(2C) receptor antagonist properties, protects the developing murine white matter against excitotoxicity. Eur J Pharmacol. 2008 Jun 24; 588(1): 58-63. [PubMed: 18466899]
10. Kul M, Vurucu S, Demirkaya E, Tunc T, Aydinoz S, Meral C, et al. Enteral glutamine and/or arginine supplementation have favorable effects on oxidative stress parameters in neonatal rat intestine. J PediatrGastroenterolNutr. 2009 Jul; 49(1): 85-9. [PubMed: 19503000]
11. Dani C, Buonocore G, Longini M, Felici C, Rodriguez A, Corsini I, et al. Superoxide dismutase and catalase activity in naturally derived commercial surfactants. Pediatr Pulmonol. 2009 Nov; 44(11): 1125-surfactants. [PubMed: 19830697]