FORUM REVIEW ARTICLE

Developmental Regulation of Antioxidant Enzymes and Their Impact on Neonatal Lung Disease

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Abstract

Significance: Deficient antioxidant defenses and compromised ability to respond to oxidative stress burden the immature lung. Routine neonatal therapies can cause increased oxidative stress with subsequent injury to the premature lung. Novel therapeutic approaches to protect the premature lung are greatly needed. **Recent Advances:** Live cell imaging with targeted redox probes allows for the measurement of subcellular oxidative stress and for comparisons of oxidative stress across development. Comprehension of subcellular and cell-type-specific responses to oxidative stress may influence the targeting of future antioxidant therapies. **Critical Issues:** Challenges remain in identifying the optimal cellular targets, degree of enzyme activity, and appropriate antioxidant therapy. Further, the efficacy of delivering exogenous antioxidants to specific cell types or subcellular compartments remains under investigation. Treatment with a nonselective antioxidant could unintentionally compromise cellular function or impact cellular defense mechanisms and homeostasis. **Future Directions:** Genetic and/or biomarker screening may identify infants at the greatest risk for oxidative lung injury and guide the use of more selective antioxidant therapies. Novel approaches to the delivery of antioxidant enzymes may allow cell type- or cellular organelle-specific therapy. Improved comprehension of the antioxidant enzyme regulation across cell type, cell compartment, gender, and developmental stage is critical to the design and optimization of therapy. *Antioxid. Redox Signal.* 21, 1837–1848.

Introduction

BRONCHOPULMONARY DYSPLASIA (BPD) is the most common complication of prematurity, affecting one-third of the infants born under 1250 g and nearly half of those under 750 g (44, 69, 110). These infants are at a high risk, with a 10%–15% associated mortality rate in the first year of life (38). Infants who survive can suffer long-term pulmonary sequelae as well as neuromotor, cognitive, and behavioral disabilities (43, 99, 100). Whereas advances in neonatal care, including the routine use of antenatal steroids and surfactants, have resulted in improved survival of premature infants, data from the National Institutes of Child Health Neonatal Research Network indicate lack of progress in the overall reduction of BPD (102). This finding is due, in part, to inadequate therapeutic options as well as limitations in our understanding of basic mechanisms contributing to neonatal lung injury. Novel interventions that protect the immature lung are greatly in need.

Treatment with exogenous antioxidants represents a compelling therapeutic approach as oxidative stress contributes to neonatal lung injury, and the immature lung possesses compromised antioxidant defenses. However, animal and human antioxidant trials to date demonstrate variable and limited success in preventing BPD. Multiple challenges remain in identifying the appropriate cellular targets as well as the optimal antioxidant (or combination) and enzyme activity. Further, limitations exist in the delivery of antioxidant therapy as immunogenicity, poor cell penetrance, and short half-life impact efficacy.

This article reviews normal development of the antioxidant defense system across gestation and the disruption of this complex system by preterm birth. Factors that impact the generation of oxidative stress are explored, highlighting findings that the developmental stage, cell type, and subcellular organelle all influence this complex response. The current status of antioxidant therapy for oxidative lung injury is reviewed, and novel advances are highlighted. Ultimately,

improved comprehension of antioxidant deficiencies and the neonatal oxidative stress response will allow appropriate tailoring of therapy to the unique needs of the immature lung.

Reactive Oxygen and Nitrogen Species Generation and Lung Injury

Although reactive oxygen and nitrogen species (ROS and RNS, respectively) are by-products of normal oxygen metabolism, their enhanced formation, especially when the cell's antioxidant capacities are limited, can result in damage to proteins, enzymes, lipids, and DNA (Fig. 1) (45). These species include the superoxide, hydrogen peroxide, hydroxyl radical, peroxynitrite, nitric oxide (NO), and nitrogen dioxide as well as other free radicals and free radical precursors. Whereas reactive species are generated by multiple cellular sources, including mitochondria, endoplasmic reticulum, peroxisomes as well as by nitric oxide synthases (NOS) and NADPH oxidases (NOX), the majority of ROS are produced by the electron transport chain *via* oxidative phosphorylation (5, 57). Excess ROS produced by the mitochondria can alter the enzyme function and damage the mitochondrial DNA, which may impact the mitochondrial efficiency and potentially amplify the mitochondrial ROS production (19, 28). Increased mitochondrial oxidative stress triggers programmed cell death and apoptosis through BAX activation and cytochrome C release (23, 124). In addition to direct cell injury, ROS are capable of promoting inflammation, resulting in the release of inflammatory mediators and secondary tissue damage (8, 11, 90, 123). Similarly, excess RNS generation leads to protein nitration, impacting downstream protein function and altering normal cellular homeostasis. Notably, NO produced by NOS enzymes can react with ROS, such as superoxide, to produce peroxynitrite, a highly reactive and damaging nitrogen species. In addition, under certain pathologic conditions, uncoupling of endothelial NOS can result in the production of excess superoxide directly (47, 74). The end result of excess ROS and RNS generation is diffuse lung injury and, in the immature human and animal lung, a phenotype of compromised alveolarization and vascular development, paralleling that seen clinically in BPD (7, 20, 33, 114).

Whereas ROS and RNS can cause cellular injury and compromise lung growth *via* altered cell proliferation, oxidative and nitrative stress is also essential to normal cell function (62). Protection against microorganisms relies on

the increased oxidative stress generated by granulocytes and macrophages (50). Drug metabolism and detoxification involves redox responses by the liver (94). Redox reactions can also play a role in normal metabolic pathways, impacting the production of growth and signaling factors (62, 70). NO generated by NOS enzymes also plays a critical role in regulating both the lung development *in utero* and pulmonary vascular tone at baseline and immediately after birth. These normal cellular reactions must be considered, as therapies designed to reduce ROS and RNS generation may result in unintended, off-target effects.

Antioxidant Deficiencies in the Immature Lung

A complex system of both enzymatic and nonenzymatic antioxidants exists and regulates free radical production within the lung. The most important antioxidant defenses include the superoxide dismutases (SOD); glutathione (GSH), thioredoxin (Trx), and their respective peroxidases and reductases; heme oxygenases, catalases, and small molecular weight antioxidants, including vitamin C and E (6) (Fig. 2). Antioxidant defenses are themselves regulated by the presence of ROS, although the ability to respond to increased oxidative stress can be influenced by species, gender, and age (52, 108, 122). The immature lung may be uniquely susceptible to oxidant injury as studies demonstrate that both baseline antioxidant capacity and ability to adapt to oxidative stress are compromised in the developing lung.

Multiple studies confirm a dramatic maturation of pulmonary antioxidant defenses during the third trimester (6, 12, 52, 53, 104). This *in utero* adaptation has been attributed to the preparation for an oxygen-rich postnatal environment, as the fetal lung is exposed to a partial pressure of oxygen of only 3.3 kPa *in utero*, well below that experienced after birth (39).

Animal studies

The earliest description of the maturation of pulmonary antioxidant defenses during lung development was in fetal and newborn rabbits (53). This study demonstrated an increased antioxidant enzyme activity in the late third trimester and after delivery. Levels of the glutathione peroxidase (GPx), SOD, and catalase activity before birth were 100%–200% greater than those earlier in gestation without evidence of change in *in utero* oxygen consumption (Fig. 3A, B).

FIG. 1. Host defense mechanisms, reactive oxygen species and other free radicals, and damaging effects on lipids, proteins, and DNA. Reprinted from Fardy and Silverman (45), with permission from the British Medical Journal.

FIG. 2. The most important intracellular (I) and extracellular (E) sources of free radicals and enzymatic and nonenzymatic antioxidant defense mechanisms. CAT, catalase; CuZnSOD, copper/zinc superoxide dismutase; Cys, cysteine; EC-SOD, extracellular superoxide dismutase; γ -GCL, gamma glutamate-cysteine ligase; Gly, glycine; G6PDH, glucose 6-phosphate dehydrogenase; GPx, glutathione peroxidase; GR, glutathione reductase; GS, glutathione synthetase; GSH, reduced glutathione; GSSG, oxidized glutathione; γ -GT, gamma glutamyl transpeptidase; H_2O_2 , hydrogen peroxide; HO-1, hemeoxygenase 1; MnSOD, manganese superoxide dismutase; NADP, nicotinamide adenine dinucleotide phosphate; NO, nitric oxide; O_2^- , superoxide; OH $\cdot$, hydroxyl radical; ONOO $^-$, peroxynitrite; TPx, thioredoxin peroxidase; TR, thioredoxin reductase; Trx, thioredoxin; Trx-S $_2$, oxidized thioredoxin; XOR, xanthine oxidoreductase; *in phagocytic cells; **in neutrophils. Reprinted from Asikainen and White (6), with permission from Elsevier.

Maturation of antioxidants during lung ontogenesis was subsequently confirmed in additional animal models, including mice, rats, guinea pig, lamb, and baboons (6, 55, 104). Whereas variation by species and specific antioxidant enzymes exist, these studies, in general, support late gestational maturation of antioxidant capacities with a concomitant deficiency in the immature or preterm lung (6).

Additional cellular and biological mechanisms, including subcellular localization, post-translational modification, and inadequate placental transport, contribute to compromised antioxidant capacities in the immature lung. For example, studies in rabbits identify that the extracellular SOD (EC-SOD or SOD3) transport and secretion into the extracellular space increase with age (86). Developmental regulation of manganese SOD (MnSOD or SOD2) also occurs through the increased activity of mRNA binding proteins in prenatal and neonatal rat lung, potentially impacting the mRNA and protein expression (49). Inadequate maternal-fetal transfer of nonenzymatic antioxidants, including vitamins A and E, further contributes to decreased antioxidant capacities with evidence of age-dependent deficiencies (13, 39, 98). In these

respects, functional deficits further exacerbate antioxidant deficiencies in the immature lung.

Human studies

However, the relevance of these findings in animals to human disease remains unclear. Whereas studies of fetal and newborn lung tissue attempt to determine if similar gestational changes occur in the human lung, the results are less consistent and remain limited by the clinical and ethical challenges of tissue collection. Fetal samples suggest poor correlation between gene transcription and the ultimate levels of protein expression and/or enzyme activity. Specifically, mRNAs for pulmonary copper-zinc SOD (CuZnSOD or SOD1), MnSOD, and catalase increase as the infant approaches full term and, after delivery, only an increased catalase enzyme activity has been demonstrated (4, 76, 103). Immunohistochemical analysis suggests that changes in expression may be cell-type specific, with age-related increases of both CuZn and MnSOD protein expression in peripheral airways, although the finding was not reproduced in larger

FIG. 3. Antioxidant enzyme activity in lungs of fetal and neonatal rabbits expressed as percentage increase in activity above the baseline value. Mean values for superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GP) show pattern of marked increases during the last few days of gestation (A). Oxygen consumption in lung slices from fetal and neonatal rabbits demonstrates no pattern of progressive increase during the last several days before birth (B). * $p < 0.05$ vs. previous age point. Reprinted from Frank and Groseclose (53), with permission from the International Pediatric Research Foundation.

studies (3, 42). Pulmonary Trx increases during human gestation, although not specifically in the third trimester (68).

Evaluation of the nonpulmonary antioxidant function further supports that premature birth results in compromised antioxidant defenses. Comparison of erythrocytes, plasma, and bronchoalveolar lavage fluids from preterm and term infants identified GSH, GPx, and SOD deficiencies that correlated with the degree of prematurity (66, 85). Deficiencies of nonenzymatic antioxidants, including alpha-tocopherol and vitamin A, have also been documented in serum samples from premature infants (46, 64, 98). Finally, indirect evaluation of antioxidant capacities using fetal tissue and serum samples from premature infants confirms elevated markers of oxidative stress and a reduced antioxidant potential (31, 41, 58, 77, 95).

Collectively, these data support that maturation of the antioxidant defense system also occurs during human lung development. Infants born early are therefore burdened by compromised antioxidant capacities, with the degree of prematurity correlating to the relative deficit. In this respect, antioxidant deficiencies parallel susceptibility to pulmonary disease, as risks of pulmonary morbidity, including respiratory distress syndrome, BPD, and chronic lung disease, are greatest in the most premature infants (48, 69).

Response to Oxidative Stress in the Immature Lung

Induction of antioxidant capacities in response to a wide range of oxidants, including (but not limited to) ozone, oxygen, mineral dust, inflammation, and paraquat, has been explored in the term lung (73, 96, 113). As hyperoxia likely represents the greatest oxidant challenge to premature infants, developmental comparisons of the oxidative stress response and efficacy of antioxidant therapy have most often been investigated in this model. In addition, studies in neonatal animal models identify that exposure to supraphysiological oxygen alone results in histopathological changes consistent with BPD, further rationalizing the use of this model for developmental studies (114).

Responses in the term lung

Studies in animal models at term gestation demonstrate appropriate adaptation to oxidative stress *via* induction of antioxidant enzyme capacities. Exposure to physiologic oxygen at birth enhances the EC-SOD activity and increases transcription of Trx and peroxiredoxins (Prxs) in rabbit and baboon lung, respectively (36, 37, 86). Paradoxical to their relative antioxidant deficiencies at birth, newborn mice, rats, and rabbits demonstrate improved survival in hyperoxia as compared with adults. This tolerance may, in part, be attributed to enhanced induction of the antioxidant enzyme expression when challenged by increased oxidative stress (29, 51, 52, 122). Additional adaptive mechanisms described in the term lung include prolongation of antioxidant enzyme mRNA half-life and post-translational modification of antioxidant enzymes with hyperoxic exposure (5, 29).

Responses in the preterm lung

In contrast, preterm animals exposed to hyperoxia demonstrate a limited ability to respond and adapt to oxidative stress. Studies in premature rabbits, lambs, and baboons identify that susceptibility to oxygen-induced lung injury parallels an inability to increase antioxidant expression or activity (56, 81, 92). Studies of pathological specimens from premature infants similarly suggest an inability to induce the MnSOD expression in response to hyperoxia (5). These data suggest that preterm birth disrupts normal development of antioxidant regulatory capacities. Delivery before completion of this developmental process therefore results in compromised ability to respond to the postnatal environment.

Compromised antioxidant defenses, due to both baseline deficiencies as well as an inability to increase the antioxidant function in response to oxidative stress, predispose premature infants to increased ROS and RNS generation. Premature infants encounter multiple environmental challenges associated with the routine provision of neonatal care, including exposure to hyperoxia, hypoxia, ventilation, infection,

inflammation, and ROS-generating therapies (*e.g.*, total parenteral nutrition and dopamine). Whereas each of these factors individually contributes to the generation of oxidative stress, the collective exposure influences the development of BPD (25, 27, 62, 88, 115, 117). The common path of ROS-mediated lung injury shared by these exposures highlights the need for novel strategies to alleviate the burden of oxidative stress.

Developmental Regulation of ROS Generation

Comparisons of ROS generation from immature and adult lung cells have demonstrated inconsistent results. Teng *et al.*, found increased superoxide production in pulmonary artery endothelial cells isolated from fetal lambs compared with term (105). However, Villamor saw no significant age-related difference in the superoxide presence in intrapulmonary arteries and veins of neonatal and older piglets (109). Seminal work by Ischiropoulos *et al.*, identified increased production of superoxide in microsomes and mitochondria isolated from adult compared with neonatal rat lung in both 21% and 100% oxygen (65). The differences in these findings may be, in part, due to variation in the species or cell type studied.

More recent work utilized cytosolic- and mitochondrial-targeted ratiometric redox-sensitive probes to assess subcellular responses to acute hyperoxia during live cell imaging of intact murine lung slices. Consistent with findings in cellular models, hyperoxia induced increased mitochondrial oxidative stress within minutes of exposure without comparable changes in the cytosol. Moreover, the mitochondrial oxidative stress response is exaggerated in neonatal alveolar epithelial cells compared with adult, implying greater hyperoxia-induced ROS production in the immature lung (16) (Fig. 4A). However, the oxidative stress response in pulmonary arterial smooth muscle cells (PASM) of lung slices showed no age dependence with brief hyperoxia (Fig. 4B). This difference may be attributed to the proximity of epithelial cells, but not PASM cells to high oxygen concentrations. These *in vitro* studies highlight the importance of cell-type- and subcellular compartment-specific oxidative responses in the immature lung. Future studies using transgenic mice expressing targeted redox-sensitive optical probes would allow the characterization of developmental responses *in vivo* (61). A better understanding of this process in the intact animal is vital to extrapolation of findings to human neonates.

Oxidative Stress in BPD

The contribution of oxidative stress to the pathogenesis of BPD is supported by multiple studies in premature infants. Perrone *et al.*, evaluated the association between BPD and increased *in utero* oxidative stress due to antenatal inflammation and/or hypoxia. Increased cord blood levels of hydroperoxides, oxidized proteins, and nonprotein-bound iron correlated with the risk of free radical disease, including BPD, intraventricular hemorrhage, necrotizing enterocolitis, and retinopathy of prematurity (ROP). Of these biomarkers, nonprotein-bound iron, which enters the Fenton reaction to produce hydroxyl radical, was the best predictor of the disease (93). Postnatal exposures also contribute to an increased oxidative stress, with evidence that elevated urine 8-hydroxydeoxyguanosine (8-OHdG), plasma 8-isoprostane, and protein carbonylation levels during the first week are all associated with a greater risk of BPD (14, 67). Chessex *et al.*,

FIG. 4. Thirty minutes of hyperoxia induces exaggerated oxidative stress response in AECs of immature mouse lung slices compared with adult. The redox-sensitive protein sensor RoGFP was expressed in the mitochondrial matrix of AECs of lung slices generated from P5–7, P10–12, and 8-week-old adult mice. Superfusion with a hyperoxic medium resulted in increased oxidation in the mitochondrial matrix within minutes of exposure. Younger AECs demonstrated exaggerated mitochondrial oxidation compared with adult. Significant increase above baseline was reached at 11, 17, and 25 min of hyperoxia for P5–7, P10–12, and 8-week-old AECs, with $*p \leq 0.05$, $**p \leq 0.05$, and $^{\dagger}p \leq 0.05$, respectively (A). In contrast, 30 min of hyperoxia induces comparable increase in mitochondrial oxidative stress in P7 and adult pulmonary arterial smooth muscle cells (PASM) (B). Reprinted from Berkelhamer *et al.* (16), with permission from Elsevier.

further identified that the whole blood GSH redox potential at 7 days correlated with the severity of BPD (27) (Fig. 5). Similarly, concentrations of nitrotyrosine, a marker of peroxynitrate formation, are elevated in the bronchoalveolar lavage of premature infants with pulmonary disease (59). Whereas these studies support an association between oxidative and nitrative stress and BPD, they cannot prove a causal relationship.

An ideal biomarker would provide a direct measurement of ROS and RNS generation through noninvasive measures. Advances in electronic-nose and NMR spectroscopy

FIG. 5. Whole blood redox potential correlates with severity of later bronchopulmonary dysplasia (BPD) in infants born <28 weeks and 1000 g. Samples were obtained at day 7 (S1) and day 10 $\pm$ 24 h (S2). * p < 0.005 for mild vs. moderate or severe BPD. Reprinted from Chessex *et al.* (27), with permission from Elsevier.

technologies represent a novel option as direct quantification of pulmonary oxidative stress can be obtained from exhaled breath and monitored serially for change with clinical interventions (79, 80, 120). In a small study, peak exhaled pentane levels in premature infants correlated with the risk of ROP, but not BPD (87). Alternative volatile compounds may prove better biomarkers, for example, exhaled 8-isoprostane (15, 72, 78). Electronic-nose and NMR spectroscopy of the exhaled breath condensate, which are reproducible and validated techniques (18, 82), could be used to identify infants at a higher risk of lung injury and to personalize a pharmacologic approach to prevention and treatment of disease.

Antioxidants as Therapy for BPD

As oxidative stress contributes to the pathogenesis of BPD and preterm infants demonstrate compromised antioxidant defenses, it has been hypothesized that exogenous antioxidant therapy may be protective in premature infants. Some common neonatal therapies are recognized to enhance antioxidant capacities. As an example, glucocorticoids accelerate the maturation of antioxidant enzymes, while surfactants themselves demonstrate antioxidant properties (21, 54, 108). Vitamin A supplementation reduces rates of BPD, but is variably used, potentially due to the need for intramuscular administration and limited long-term benefit (34, 106). Efforts to identify effective antioxidant therapies have proved challenging. The obstacles include antigenicity, short half-life, poor cell penetrance, difficulty of delivery to a specific cell type and/or intracellular organelle as well as limited knowledge of the clinical pharmacology of antioxidants (5).

Multiple approaches have been explored to optimize the efficacy of antioxidant therapy. These have included the compounding of antioxidants (*i.e.*, SOD and catalase), coupling with polyethylene glycol or heparin, encapsulation in liposomes, and immunotargeting to specific cell populations (83). Intratracheal administration of aerosolized therapies has proven to be a viable means of delivering antioxidants despite challenges of homogenous distribution, concerns for airway (rather than alveolar) administration, and the potential for a shortened drug lifespan in alveoli. However, a major limitation of intratracheal delivery remains in the failure to reach the pulmonary endothelium, a key modulator of oxidative

lung injury. As systemic administration of antioxidants requires mega doses to protect the lung, immunotargeting to the pulmonary endothelium has been explored. Selective delivery of antioxidants to the rat pulmonary endothelium has been achieved *via* conjugation to angiotensin-converting enzyme antibodies (84). Emerging technologies also include the use of nanoparticle complexes for improved drug delivery. As an example, *in vivo* studies demonstrate an enhanced efficiency and an improved cellular internalization of antioxidants when coupled to chitosan derivatives (26). Finally, antioxidants targeted specifically to the mitochondria have attenuated cell injury in oxidative stress-induced disease models (71, 112).

Animal studies

Animal studies utilizing models of oxygen-mediated lung injury have been used to evaluate antioxidant therapies *in vivo*. Administration of high doses of nonspecific exogenous antioxidants can paradoxically result in the generation of ROS, and adverse effects of prolonged treatment with the antioxidants have been described in animal models (17). As an example, chronic N-acetylcysteine (NAC) administration resulted in dysregulated NO signaling and pulmonary hypertension in mice as well as the amplification of lung injury and pulmonary morbidity in hyperoxia-exposed rats (91, 107). In contrast, intraperitoneal, intratracheal, and aerosolized administrations of specific antioxidant enzymes, including MnSOD, CuZnSOD, and catalase, have demonstrated benefit in adult mice, rats, and baboons exposed to supraphysiological oxygen (23, 89, 118, 119). However, some controversy exists regarding the ability of MnSOD to mitigate lung injury in hyperoxia. Studies of MnSOD transgenic mice have found variable results, with protection when overexpression was driven by a surfactant protein C (SPC), but not a β -actin promoter (63, 121). Protection with intratracheal and aerosolized administration of MnSOD as well as in the SPC-driven transgenic, suggests that mitochondrial superoxide scavenging in the pulmonary epithelium plays an important role in modulating hyperoxic lung injury.

As antioxidant defenses and ROS generation are developmentally regulated, these findings from antioxidant trials in adult models may not apply to neonatal lung disease. Administration of therapies in preterm rodent models is limited by challenges of drug administration. However, studies in neonatal transgenic mice and larger newborn species provide insight. Overexpression of EC-SOD protects lung development in hyperoxia-exposed neonatal mice, even when specific to type II alveolar cells (1, 10). Similarly, catalytic antioxidants with both SOD and peroxidase activity improve lung development in hyperoxia-exposed preterm rabbits and in the preterm baboon BPD model (24, 111). However, not all antioxidant trials have proved beneficial and the optimal balance of antioxidant expression to ROS remains unclear. As an example, Stabler *et al.* found that intravenous administration of GSH to preterm baboons exacerbates pulmonary disease (101). In addition, treatment with SOD alone may aggravate oxidative stress if inadequate detoxification of hydrogen peroxide occurs (83). Finally, restoration of normal nitrogen species signaling, either through inhaled NO (iNO) or ethyl nitrite, appears to prevent hyperoxia-induced alveolar simplification in both premature baboons and neonatal rats, respectively (9, 75). However, studies in premature infants have been less convincing and the use of iNO for BPD prevention has been discouraged (30).

TABLE 1. SUMMARY OF TRIALS EVALUATING ANTIOXIDANT THERAPY FOR PREVENTION OF BRONCHOPULMONARY DYSPLASIA

Antioxidant	Neonatal antioxidant trials			
	Mode of delivery	Duration of therapy	Outcome	Reference
Bovine SOD	SC	Until off respiratory support	Fewer days on CPAP, no difference in O ₂ at 36 weeks, less clinical evidence of BPD	Rosenfeld <i>et al.</i> (97)
Cimetidine	IV	10 days	No benefit	Cotton <i>et al.</i> (32)
Recombinant human SOD	IT	4 weeks ^a	No difference in O ₂ at 36 weeks, decrease in late pulmonary morbidity	Davis <i>et al.</i> (40)
NAC	IV	6 days	No benefit	Ahola <i>et al.</i> (2)
Selenium	IV, PO	Until 36w CGA	No benefit	Darlow <i>et al.</i> (35)
Vitamin A	IM	4 weeks	Decreased O ₂ at 36 weeks	Tyson <i>et al.</i> (106)
Vitamin E	PO	4 weeks	No benefit	Watts <i>et al.</i> (116)

^aRecombinant human SOD was administered for 4 weeks or until extubated.

Antioxidant therapies have been studied in premature infants with variable outcome. Routes of administration include subcutaneous (SC), intratracheal (IT), intravenous (IV), intramuscular (IM), and oral, (PO).

CGA, corrected gestational age; CPAP, continuous positive airway pressure; NAC, N-acetylcysteine.

Human studies

A limited number of antioxidant therapies have progressed from animal studies to human trials (Table 1). Whereas vitamin A administration resulted in a slight decrease in the risk of BPD, supplementation with vitamin E and selenium failed to demonstrate benefit (22, 35, 106, 116). A 6-day course of the antioxidant NAC by intravenous administration did not reduce the rates of BPD or death in infants born < 1000 g (2). Similarly, a relatively brief (10-day) course of the cytochrome P450 inhibitor, cimetidine, did not alter the pulmonary morbidity or rates of BPD (32).

Perhaps, the most promising findings have been with administration of exogenous SOD. Treatment of a small cohort of preterm infants with subcutaneous bovine SOD resulted in a decreased need for respiratory support, improved radiologic findings, and a decreased evidence of wheezing. However, no difference was noted in days on oxygen therapy (97). In subsequent studies, 4 weeks of intratracheal recombinant CuZnSOD (rhSOD) did not reduce early rates of death or BPD, but decreased pulmonary morbidities at 1 year, including the need for medications, emergency room visits, and rehospitalization (40).

Targeting antioxidant trials to infants at the greatest risk may impact the efficacy of candidate therapies. This could be achieved through an early biomarker or genetic screening. As suggested, emerging technologies, including the use of an electronic-nose, could assist in determining the degree of pulmonary oxidative stress present and likelihood of benefit from the antioxidant therapy. Routine genetic screening could also guide clinical management by identifying infants at the greatest risk. As an example, studies have identified that haplotype reconstruction in the genes involved in oxidative stress, including CuZnSOD, MnSOD, EC-SOD, and catalase, results in a greater risk of lung disease and BPD (60).

Conclusion

A complicated balance between antioxidant capacities and oxidative stress exists in the newborn lung. Premature delivery potentially shifts this balance toward excess ROS and RNS

generation as the pulmonary antioxidant capacities and responses to oxidative stress are compromised with early birth.

Whereas compromised antioxidant capacities imply a role for exogenous antioxidants in the prevention of BPD, candidate therapies have met mixed success. Improved comprehension of cell-type- and organelle-specific responses to oxidative stress will influence the future design of therapeutic strategies. Novel approaches to targeting delivery will allow the effect of tissue- or cell-type-specific therapy to be explored. Biomarker and genetic screening may aid in the identification of infants at the highest risk, allowing candidate therapies to be targeted to those most likely to benefit. The ideal antioxidant therapy would restore the appropriate antioxidant balance without compromising the normal role of ROS and RNS signaling in premature infants and the developing lung.

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Abbreviations Used

8-OHdG = 8-hydroxydeoxyguanosine
 BPD = bronchopulmonary dysplasia
 CuZnSOD = copper–zinc superoxide dismutase
 ECSOD = extracellular superoxide dismutase
 GPx = glutathione peroxidase
 GSH = glutathione
 iNO = inhaled NO
 MnSOD = manganese superoxide dismutase
 NAC = N-acetylcysteine
 NO = nitric oxide
 NOS = nitric oxide synthase
 NOX = NADPH oxidase
 PASMC = pulmonary arterial smooth muscle cells
 Prx = peroxiredoxin
 rhSOD = recombinant CuZnSOD
 RNS = reactive nitrogen species
 ROP = retinopathy of prematurity
 ROS = reactive oxygen species
 SOD = superoxide dismutases
 SPC = surfactant protein C
 Trx = thioredoxin