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The Role of Genetic Polymorphisms in Antioxidant Enzymes and Potential Antioxidant Therapies in Neonatal Lung Disease

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Abstract

Significance: Oxidative stress is involved in the development of newborn lung diseases, such as bronchopulmonary dysplasia and persistent pulmonary hypertension of the newborn. The activity of antioxidant enzymes (AOEs), which is impaired as a result of prematurity and oxidative injury, may be further affected by specific genetic polymorphisms or an unfavorable combination of more of them. ***Recent Advances:*** Genetic polymorphisms of superoxide dismutase and catalase were recently demonstrated to be protective or risk factors for the main complications of prematurity. A lot of research focused on the potential of different antioxidant strategies in the prevention and treatment of lung diseases of the newborn, providing promising results in experimental models. ***Critical Issues:*** The effect of different genetic polymorphisms on protein synthesis and activity has been poorly detailed in the newborn, hindering to derive conclusive results from the observed associations with adverse outcomes. Therapeutic strategies that aimed at enhancing the activity of AOEs were poorly studied in clinical settings and partially failed to produce clinical benefits. ***Future Directions:*** The clarification of the effects of genetic polymorphisms on the proteomics of the newborn is mandatory, as well as the assessment of a larger number of polymorphisms with a possible correlation with adverse outcome. Moreover, antioxidant treatments should be carefully translated to clinical settings, after further details on optimal doses, administration techniques, and adverse effects are provided. Finally, the study of genetic polymorphisms could help select a specific high-risk population, who may particularly benefit from targeted antioxidant strategies. *Antioxid. Redox Signal.* 21, 1863–1880.

Oxidative Stress and Antioxidant Enzymes in the Newborn

NEWBORNS, ESPECIALLY IF preterm, encounter an elevated oxidative burden, as a consequence of hyperoxic events, leading to increased reactive oxygen species (ROS) production, mechanical ventilation, and inflammatory and infective complications (28, 29, 32, 92, 115, 147–149). Oxidative stress (OS), resulting from an unbalance between oxidant factors and antioxidant defenses, significantly contributes to different newborn pathologies, which are grouped as ''oxygen radical diseases of neonatology'' (149). According to this hypothesis, the main complications of prematurity, such as bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), intraventricular hemorrhage (IVH), and periventricular leukomalacia, can be considered organ-specific manifestations within a single spectrum of OS-related injury (21, 29, 32, 55, 58, 78, 147, 149). Significant OS also occurs in persistent pulmonary hypertension of the newborn (PPHN), resulting in cardiopulmonary transition failure from fetal to extrauterine life (164).

Preterm newborns and depressed term newborns may experience sustained hyperoxia during resuscitation and respiratory support, especially if the fractional inspired oxygen (FiO_2) is inappropriately targeted. In fact, resuscitation of term infants with ambient air relative to FiO_2 100% is associated with reduced mortality and shorter need for resuscitation (122, 125, 126, 145, 146, 149, 169, 177–179), and resuscitation of preterm newborns with a low oxygen strategy significantly reduces the need for respiratory support, the

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incidence of BPD and OS markers (180). Indeed, a significant correlation was established between cord levels of total hydroperoxides, advanced oxidation protein products, non-protein bound iron, and the risk of free-radical-related diseases of prematurity, including BPD (116), suggesting that a very early activation of oxidative pathways significantly impacts long-term outcomes. In addition, it is well known that mechanical ventilation for respiratory distress syndrome (RDS) behaves similar to a double-edged sword in preterm newborns, as it is a major activator of inflammation and remodeling processes in the developing lung (28, 29, 78, 147).

In addition, neonates with PPHN encounter significant OS, as treatment with high oxygen concentration increases lung ROS burden, which can directly damage pulmonary parenchyma and vessels (84, 85). As inhaled nitric oxide (iNO) is administered, ROS can propagate lung damage through the formation of reactive nitrogen species (RNS), leading to further oxidative damages and NO depletion, with worsening of arterial pulmonary vessels constriction (130, 153).

In these settings, antioxidant enzymes (AOEs) activity is expected to play a crucial role in the resultant outcome. Several observations demonstrated that superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reductase (GRd) increase during gestation, paralleling the maturation of the surfactant system (11, 12, 49, 50, 110, 112, 121, 152, 183). After preterm birth, newborns lack proper levels of antioxidants, due to placental-fetal transfer interruption and insufficient endogenous production (20, 32, 55, 115). Indeed, preterm newborns exhibit significantly lower erythrocyte levels of SOD until 20 days of life and of CAT until 100 days if compared with term controls (107). Moreover, while term newborns own the capacity to induce AOEs in case of OS, as fetal distress (87) or resuscitation with FiO_2 100% (179), preterm animal models fail to induce SOD and GPx in response to oxidative challenges (48, 104), even if this aspect shows significant differences between animal species (11–13, 50).

SOD2 is strictly necessary for surviving in room air, as demonstrated in knockout models, and it is induced under hyperoxic conditions in order to cope with mitochondrial ROS overproduction (89). The study of SOD2 expression profile in human lung postmortem materials demonstrated a differential location according to gestational age, probably reflecting the different role of SOD2 during fetal lung development (11), although the total amount of SOD2 did not consistently increase during gestation. Such an observation is in agreement with other data obtained from human specimens (12, 165), but it is discordant from the results obtained in preterm newborns and baboon models (41, 104). Lung specimens of RDS or BPD patients did not show different SOD2 expression relative to age-matched controls, suggesting the inability of preterm newborns to induce lung SOD2 in response to OS (11).

AOEs are also crucial for the establishment of proper feto-placental-uterine interactions, as their expression profile shows spatiotemporal developmental peculiarities at the feto-maternal interface (33). SOD2 is particularly abundant in the fetal villous endothelium, reflecting a preferential role in the trophism of chorionic villi (65); while SOD3 is mainly intracytoplasmic and located in the cyto- and syncytiotrophoblast in the first trimester, but it is later relocated to the extracellular matrix of peri-villous and peri-vascular spaces, probably taking part in placental vasculogenesis (65).

According to a recent pathogenetic model of the complications of prematurity, genetic factors play a central role in determining the entity of the inflammatory response to specific stimuli occurring in the fetus and in the preterm newborn (68). It has been proposed that newborns with hyperresponsive phenotype inducing exaggerated inflammatory cytokines and ROS production are at a high risk of free-radical-related complications, while newborns with hyporesponsive phenotype show a high risk of extensive infective invasion, tissue destruction, and mortality. Instead, the induction of proper levels of inflammatory response is associated with higher probability of survival without major complications. Among other factors, genetic predisposition may include not only specific polymorphisms of mediators that are involved in inflammatory pathways, which have been extensively studied, but also polymorphisms which are involved in antioxidant defense, as preliminarily demonstrated (59, 118).

Genetic Polymorphisms and Lung Diseases

A single nucleotide polymorphism (SNP) is a modification in the DNA that consists of a substitution or a deletion of a single nucleotide from the original sequence, occurring every 100–300 base pairs and present, by definition, in at least 1% of the population (42). The study of SNPs represents a useful tool in the investigation of the genetic susceptibility to develop a certain disease, as it consists of the sequencing of particular regions of interest within a candidate gene, which is certainly or putatively involved in the pathogenesis of the disease (42).

SNPs produce different effects on the resultant protein according to their location within a gene. When located in the promoter region, an SNP can alter the amount of the resultant protein, while it can alter the protein function if located in the coding regions. On the contrary, if an SNP is located within a non-coding region, it will usually not affect the product. However, some SNPs occurring in non-coding regions actually interfere with protein expression, particularly affecting post-translational control and mRNA stability (40, 42). Microarray chips are provided and may contain approximately 500,000 SNPs, in order to screen contemporarily for multiple SNPs associated with a specific disease (42).

SNPs of AOEs

In the adult population, SNPs of AOEs have been extensively investigated and have been linked to the risk of several different diseases in cardiovascular, respiratory, oncologic, ophthalmologic, and neurodegenerative fields. With regard to lung pathologies and respiratory outcomes in adults, specific SNPs in *SOD2* were found to influence the risk of asthma (77, 91); while SNPs in *SOD3* were observed to affect the risk of obstructive lung disease (95, 192), and were associated with reduced pulmonary function (30) without influencing the risk of asthma (77). Moreover, SNPs of *CAT* (69, 91) and inducible heme-oxygenase (69) were proved to influence the risk of asthma.

On the contrary, in the population of preterm newborns, SNPs of AOEs have been poorly studied. However, based on the pathogenesis of the major complications of prematurity which definitely involves oxidative injury, it is

reasonable to speculate that SNPs of AOEs likely affect the outcomes of prematurity. The SNP Val105Ile in the subclass pi of Glutathione-*S*-transferase (GST), which encodes for a low-activity variant of the enzyme, was positively linked to the risk of BPD in a small population of preterm newborns (93). Such observations can be explained while considering the dual role of GST in the lung, which consists not only of the enhancement of glutathione (GSH) recycle, thus incrementing antioxidant potentials, but also of the modulation of the synthesis of eicosanoids and other inflammatory mediators (51).

Despite the small amount of data about SNPs of AOEs in preterm newborns, they may play a key role in the pathogenesis of the complications of prematurity, similar to several adult diseases. This may be a more crucial point than the demonstration of the involvement of SNPs in pro-inflammatory and oxidant mediators in the occurrence of FRDs of prematurity. In fact, the observation of SNPs with deleterious effects on AOEs activity may pose the basis for specifically targeted therapeutic interventions. Therefore, we studied a group of 10 SNPs in *SOD* and *CAT* genes in a cohort of 152 preterm newborns ≤ 28 weeks of gestational age, in order to assess their possible correlation with RDS requiring mechanical ventilation, BPD, ROP, and IVH. According to multivariate logistic regression analysis, the SNP rs2536512 in *SOD3* showed a trend toward a protective association with BPD, and the SNP rs8192287 in *SOD3* was proved to be an independent protective factor for IVH (59, 118). The *SOD3* rs2536512 SNP is located in the region expressing the enzyme N-terminal domain (23) and results in reduced SOD3 activity (168). This SNP was previously associated with the risk of hypertension and non-insulin-dependent diabetes mellitus in adults (142, 168); while in pregnant women with pre-eclampsia, it was linked to the risk of intrauterine growth restriction (136). Instead, the rs8192287 SNP is located in the 5′ untranslated region but probably intervenes in the control of mRNA stability; however, the effects of this SNP on enzymatic activity are not fully elucidated (30). Such an SNP was previously related to adverse respiratory outcomes in adults (30) but has never been linked to intracranial bleeding events. However, SOD3 over-expression in the animal model was proved to confer protection against focal stroke and global cerebral ischemia; while SOD3 knockout animals exhibited worse cerebrovascular outcomes (154–156). This observation is concordant with the protective role of SOD3 in the endothelial cells, where the scavenging of superoxide both prevents oxidative damage to the endothelium and the nearby cells of arterial walls and hinders the superoxide-induced inactivation of NO-mediated signaling pathways, thus preventing endothelial dysfunction (43). Haplotype reconstruction analysis was performed in order to assess the effect on adverse outcomes of combinations of different SNPs of one enzyme. The risk of RDS requiring mechanical ventilation was reduced by specific haplotypes of *SOD1*, *SOD2*, and *SOD3*; while the risk of BPD was increased by a specific haplotype of *SOD2*, and it was increased by a haplotype in *SOD3* (59, 118). Moreover, specific haplotypes of *SOD1* and *SOD2* were also proved to reduce the risk of IVH and ROP, while a haplotype of *SOD3* was shown to reduce the risk of IVH alone (Table 1) (59, 118). It is reasonable to speculate that protective haplotypes are associated to increased antioxidant activity in preterm infants, resulting in lower incidence of free-radical-related diseases, while unfavorable haplotypes may be associated with impaired antioxidant defense. However, data on SNP effects on proteomics are highly incomplete (Table 2).

Interestingly, the analysis of genotype distribution showed that homozygous patients for the SNP rs4880 in *SOD2* had significantly lower gestational age and birth weight than heterozygous and homozygous wild-type infants; while homozygous patients for the SNP rs5746136 in *SOD2* exhibited significantly lower gestational age. The SNP rs4880 is located in a coding region, while SNP rs5746136 is located in

Table 1. Haplotypes of Superoxide Dismutase and Catalase Significantly Associated with Adverse Outcomes

Gene symbol	*Outcome*	*Haplotypes*	*Frequency in subjects without adverse outcomes*	*Frequency in subjects with adverse outcomes*	*Coef.*	*SE*	p
CAT							
	RDS	CTC	0.122	0.085	−0.18	0.09	0.0430
	SNP1 = rs1001179; SNP2 = rs1049982; SNP3 = rs769217						
SOD1							
	IVH	GG	0.005	<0.0001	−0.05	0.01	<0.0001
	ROP	GG	0.004	<0.0001	−0.08	0.005	<0.0001
	RDS	GG	0.007	<0.0001	−0.29	0.01	<0.0001
	SNP1 = rs17880135; SNP2 = rs204732						
SOD2							
	BPD	GT	NA	0.014	0.57	0.001	<0.0001
	IVH	GT	0.005	<0.0001	−0.23	0.001	<0.0001
	ROP	GT	0.004	0.00003	−0.08	0.001	<0.0001
	RDS	GT	0.007	<0.0001	−0.46	0.001	<0.0001
	SNP1 = rs4880; SNP2 = rs5746136						
SOD3							
	BPD	TGC	0.116	0.058	−0.21	0.084	0.0153
	IVH	TGC	0.119	0.033	−0.18	0.083	0.0263

SNP1 = rs8192287; SNP2 = rs2536512; SNP3 = rs1799895.

BPD, bronchopulmonary dysplasia; CAT, catalase; IVH, intraventricular hemorrhage; RDS, respiratory distress syndrome; ROP, retinopathy of prematurity; SOD, superoxide dismutase; SNP, single nucleotide polymorphism.

Table 2. Main Characteristics of Single Nucleotide Polymorphisms of Superoxide Dismutase and Catalase

Gene	*dbSNP*	*Region*	*Nucleotide substitution*	*Aminoacid variation*	*Effects on AOE activity*
SOD1	rs17880135	3′ UTR	T > G	Not applicable	Unknown
	rs204732	3′ near gene	G > A	Not applicable	Unknown
SOD2	rs4880	Exon	G > A	Ala16Val (missense)	Unknown
	rs5746136	3′ UTR	C > T	Not applicable	Less efficient transport into mitocondria; possible reduction of activity but not fully elucidated (157)
SOD3	rs8192287	5′ near gene	G > T	Not applicable	Probably relevant for translational control and mRNA stability; unknown effects on activity (30)
	rs2536512	Exon	A > G	Thr40Ala	Reduced activity (168)
	rs1799895	Exon	C > G	Arg213Gly (missense)	Increased circulating levels (144)
CAT	rs1001179	5′ near gene	−262C > T	Not applicable	Unknown
	rs1049982	5′ UTR	−20T > C	Not applicable	Unknown
	rs769217	Exon	C > T	Asp389Asp (synonymous)	Unknown

Data regarding SNP localization and effects on nucleotide and amino-acid sequences were extracted from www.ncbi.nlm.nih.gov/SNP/ accessed on 2013/07/15.

AOE, antioxidant enzyme; UTR, untranslated region.

the mitochondrial targeting sequence (157); however it is unclear whether the resultant enzymatic activities are increased or reduced. These observations may be explained speculatively with a possible interference of SNPs of SOD2 with the activity of the enzyme at the feto-maternal interface, finally affecting the pregnancy outcome. In fact, AOEs are believed to participate in the formation of proper vascular connections and in determining adequate local tissues trophism and functionality, thus playing a pivotal role in the maintenance of pregnancy (33). At the interface, SNPs that decrease the activity of AOEs would likely result in oxidative unbalance, favoring local oxidative damage and preterm birth. Our observations are concordant with previous reports of several SNPs in mediators of inflammation or remodeling involved in the pathogenesis of preterm birth if present in the fetus and/or in the mother (68), such as tumor necrosis factor-alpha (TNF-α) (7, 103, 132), interleukin (IL)-6 (184), IL-4 (74), IL-1 receptor antagonist (53), matrix metalloproteinase (MMP)-9 (46), MMP-1 (52), and Fas (73). These data suggest that SNPs which enhance inflammation and OS or reduce antioxidant defenses may putatively participate not only in the development of the complications of prematurity, but also in the pathogenesis of prematurity itself.

SNPs of inflammatory mediators and remodeling molecules

Since the pathogenesis of BPD involves a complex mixture of alveolar and vascular developmental arrest, inflammation, and oxidative damage to the developing lung (78, 147, 149), several studies investigated the role of SNPs of genes promoting inflammation. Particularly, SNPs of *TNF* have been extensively investigated with conflicting results. *TNF* 308A polymorphism, which is linked to TNF-α overproduction, was observed to increase the risk of BPD (76), but the following studies did not confirm this observation (1, 18) and a recent meta-analysis excluded this association (166). *IFNG* +874T SNP, which induces interferon-γ overproduction, was associated with a shorter need for supplemental oxygen and mechanical ventilation and resulted a protective factor against BPD in preterm infants (17). Mixed results were reported for SNPs of ILs (17, 176, 191), and only SNP −634 of *IL6* resulted in association to prolonged oxygen therapy in a large cohort of very low-birth-weight infants (176). The toll-like receptors (TLRs) family, which is believed to contribute to lung development by enhancing pathogen removal and regulating tissue repair, has also been investigated. The TLR5 (1174C > T) variant resulted in association with the risk of severe BPD, and SNP (2054C > T) of the toll-IL-1 domain containing adaptor protein, which is involved in the TLR4 signaling pathway, also resulted in association with the risk of BPD (143); moreover, *TLR6* rs5743827 SNP was recently proved to reduce the risk of BPD (188). Among the factors regulating inflammatory cells' recruitment, the SNP −173C of macrophage migration inhibitory factor, which was associated with increased expression, resulted in an independent protective factor for BPD (120); while L-selectine Ser213 allele was associated with an increased risk of BPD (39). MMP are key regulators of the extracellular matrix remodeling process, which is of primary importance for a proper alveolarization of the preterm lung (78). Two SNPs of *MMP-16* (rs2664352 and rs2664349), which are associated with reduced levels of *MMP16* and MMP-2 in the tracheal aspirates, were found to be protective against BPD (62). Finally, since NO may be a source of RNS, especially in a hyperoxic environment, SNPs of nitric oxide synthase (NOS) were also studied. Different SNPs of inducible and endothelial nitric oxide synthase were associated to the risk of ROP (139), likely affecting retinal vascularization at different stages (19, 22, 24) and to the risk of cerebral palsy (108); however, in contrast to the adult population (66, 67, 80, 167), SNPs of NOS have not been assessed with regard to respiratory outcomes in the preterm newborn.

Antioxidant Strategies

Based on the evidence that OS plays a pivotal role in the pathogenesis of BPD and PPHN, it appears reasonable to include antioxidant strategies in the prevention and treatment of such conditions. New potential treatments for newborn

lung diseases are a matter of great importance, as current therapies are far from optimal (78). Several lines of evidence suggest the beneficial effects of antioxidant therapies in the prevention of hyperoxia-induced lung injury and in the treatment of PPHN *in vitro* and in animal models (Tables 3 and 4). Despite this, antioxidant treatments cannot be regarded at present as a part of the routine equipment for such indications, as clinical data are insufficient or inconsistent. Therefore, future research in this field should focus on transferring experimental studies to clinical settings, in order to provide possible evidence that supports the clinical use of antioxidant treatment. The study of SNPs of AOEs could be of great importance from this perspective. In fact, the assessment of SNPs in AOEs or a combination of more SNPs with unfavorable effects on antioxidant activities could be considered a helpful tool to identify a specific subpopulation of newborns with a particularly high risk of adverse outcomes, who could maximally benefit from antioxidant strategies.

AOEs replacement

BPD and hyperoxic lung injury. Several animal studies proved that SOD administration is effective in reducing lung injury induced by hyperoxia and mechanical ventilation (15, 37, 113, 174), in accordance with the observation that absent SOD3 activity is associated with increased hyperoxia-induced lung injury; while SOD2 over-expression in type II alveolar cells is associated with prolonged survival in hyperoxic conditions (27, 189).

Endotracheal administration of recombinant human SOD (rhSOD) has been proved to reduce lung injury in preterm newborns receiving mechanical ventilation for RDS (36, 135). After one administration of rhSOD soon after birth, SOD levels in serum, tracheal aspirates, and urine significantly increased with regard to placebo-treated controls. Neutrophil chemotactic activity in tracheal aspirates resulted in significantly lower SOD-treated newborns for the first week of life, suggesting that rhSOD administration reduces the activation of lung inflammation. The duration of such an increase was dose dependent, lasting for 3 days for the lowest administered dose and 4 days for the highest dose (135). However, the duration of increased SOD levels in body fluids was likely too limited to affect the development of BPD (135). Repeated endotracheal administration of SOD, started within 2 h of surfactant treatment for a total of seven doses, induced a four- to seven-fold increase of SOD activity, in

Table 3. Studies of Exogenous Administration of SOD/CAT or SOD Overexpression in Respiratory Distress Syndrome Patients or Hyperoxic Lung Injury Models

Patients or models	*Administration details*	*Principal outcomes*	*Ref.*
Rats, hyperoxic	IV liposome-encapsulated bovine SOD and CAT	Increased survival rate Reduced pleural effusion	(174)
Rats, hyperoxic	ET liposome-encapsulated bovine SOD and CAT	Increased survival rate Reduced lung histological abnormalities	(113)
Piglets, hyperventilated and hyperoxic	ET rhSOD 5 mg/kg	Reduced neutrophils chemotactic activity, total cell count, elastase activity, and albumin in TA	(37)
Rabbits, lungs challenged with xanthine and xanthine oxidase	ET liposome-encapsulated bovine SOD and CAT	Preserved lung filtration coefficient	(15)
Preterm infants ($n = 26$) BW 750–1250 g < 24 h of life	ET rhSOD 0.5 or 5 mg/kg within 30 min of surfactant, single dose	Reduced neutrophil chemotactic activity and albumin in TA (high dose)	(135)
Preterm infants ($n = 33$) BW 700–1300 g < 24 h of life	ET rhSOD 2.5 or 5 mg/kg within 2 h of surfactant, up to 7 doses	Reduced neutrophil chemotactic activity and albumin in TA Reduced wheezing requiring bronchodilators or steroids at 1 year follow up	(34, 36)
Transgenic mice, hyperoxic	Lung-targeted SOD3 overexpression	Reduced lung neutrophils and oxidized GSH Increased alveolar surface and lung volume density	(4)
Transgenic mice, hyperoxic	Lung-targeted SOD3 overexpression	Preserved alveolar and bronchiolar epithelium proliferation Reduced DNA damage Preserved apical protein expression in type I cells	(14)
Rabbit, hyperoxic	Transfection with hSOD3 containing plasmid, AER	Increased lung cGMP Decreased lung NF-κB expression	(3)
Transgenic mice, hyperoxic	Lung-targeted SOD3 overexpression + IP Antileukinate for 7 days	Reduced lung neutrophils, 8-isoprostane and oxidized GSH Reduced myeloperoxidase activity in TA	(102)

TA, tracheal aspirate; IV, intravenous; ET, endotracheal; IP, intraperitoneal; AER, aerosolized; GSH, glutathione; rhSOD, recombinant human SOD; NF-κB, nuclear factor kappa B.

Table 4. Studies of Exogenous Administration of SOD/CAT or SOD Overexpression in PPHN Models

Models	*Administration details*	*Principal outcomes*	*Refs.*
Piglets, hyperoxic and iNO	ET rhSOD 0.5 mg/kg single dose or 5 mg/kg, 2 doses or AER rhSOD 10 mg/kg, single dose	Reduced neutrophil chemotactic activity, total cell count, and protein concentration in TA Reduced lung malondialdehyde	(131)
PPHN lambs	ET rhSOD 5 mg/kg + iNO 5 or 80 ppm	Reduced PPA and PVR *vs.* iNO or rhSOD alone	(162)
PPHN lambs	ET rhSOD 5 mg/kg + iNO 20 ppm	More rapid increase in oxygenation and reduced mean airway pressure *vs.* rhSOD or iNO alone Reduced nitrotyrosine in PA *vs.* iNO alone	(86)
Premature RDS lambs	ET rhSOD 2.5 or 5 or 10 mg/kg, single dose + iNO 5 ppm	No increase in oxygenation *vs.* iNO alone	(79)
PPHN lambs	ET rhSOD 5 mg/kg or iNO 10 ppm	rhSOD or iNO reduced lung ROS content *vs.* untreated rhSOD, but not iNO, restored eNOS function and increased BH4 in PA *vs.* untreated	(45)
PPHN lambs	ET rhSOD 5 mg/kg or iNO 20 ppm	rhSOD or iNO reduced PDE5 activity and increased cGMP in PA *vs.* untreated	(44)
PPHN lambs	ET CAT 20,000 U/kg	Increased oxygenation *vs.* untreated	(187)
PPHN transgenic mice	Lung-targeted hSOD3 overexpression	Reduced RV pressure, RV mass, and pulmonary vasculature wall thickness *vs.* PPHN wild type	(5)
PPHN lambs	Isolated PA cells transfected with SOD2 containing vector	Reduced SOD2 Tyrosine nitration, increased eNOS activity, and relaxation of PA *vs.* non-transfected	(2)

PA, pulmonary artery; PPA, pulmonary arterial pressure; PVR, pulmonary vasculature resistance; RV, right ventricle; eNOS, endothelial nitric oxide synthase; PDE5, phosphodiesterase 5; PPHN, persistent pulmonary hypertension of the newborn; ROS, reactive oxygen species; iNO, inhaled nitric oxide.

serum, urine, and tracheal aspirates, which lasted for 14 days. Despite prolonged action and significant reduction in lung inflammatory markers observed with such regimen, no differences in BPD occurrence were detected (36). A one-year follow up of this cohort demonstrated that rhSOD-treated newborns had a lower frequency of repeated wheezing episodes requiring bronchodilators or corticosteroids administration (34), suggesting that early reduction of oxidative lung injury may prevent subsequent long-term pulmonary damages. Such beneficial effects were particularly evident for the subgroup of rhSOD-treated newborns < 27 weeks of gestational age, who experienced a 55% reduction in wheezing episodes, a 55% decrease in emergency department accesses, and a 44% reduction in hospitalization (34). RhSOD was well tolerated, and treated newborns did not differ from controls with regard to the incidence of short-term and long-term complications (35, 36).

Following these encouraging results, the enhancement of AOEs activity in the prevention of hyperoxia-induced lung injury has been further investigated. Based on the observation that SOD3 overexpression reduced lung adhesion molecules and inflammatory cytokines in the adult models exposed to hyperoxia (47), lung-targeted human SOD3 overexpression was studied in the newborn mice model of hyperoxia-induced lung injury (4). Transgenic mice overexpressing SOD3 showed significantly lower lung content of neutrophils and oxidized GSH after 7 days of hyperoxia and preserved higher alveolar surface area and lung volume density after 21 days in comparison with wild-type animals, suggesting that the beneficial effects on alveolarization may be due to the early reduction of OS and inflammation. Since SOD3 is a secreted extracellular protein, it has been hypothesized to protect from superoxide-induced damages in compartments that cannot be reached by intracellular AOEs, such as the surface of alveolar epithelium and alveolar hypophase (25). However, in the newborn, SOD3 is also represented in the intracellular space, suggesting that SOD3 overexpression possibly affects the redox state of both cells and the extracellular environment. The authors also suggested that SOD3 would exert beneficial effects not only because of scavenging activity but also through the formation of hydrogen peroxide, which is involved in processes that are fundamental to tissue development and remodeling, such as apoptosis and cell proliferation (4). Lung-targeted human SOD3 overexpression was further demonstrated to exert beneficial effects on lung development in preterm animal models under hyperoxic conditions during the transition from saccular to alveolar stages (14). Transgenic mice showed proper proliferation of alveolar and bronchiolar epithelial cells at 3 days, which was significantly impaired in wild-type hyperoxic animals, and they also presented less DNA damage and preservation of specific apical protein expression in type I cells (14).

It is well known that hyperoxic damage to the lung is the result of a double mechanism, involving both the direct toxic effects of ROS and the accumulation of inflammatory cells and their mediators (70, 90). Alveolar macrophages are believed to control the initial inflammatory response through the secretion of pro-inflammatory mediators, such as IL-1β and TNF-α, which are responsible for the recruitment of further inflammatory cells, perpetuating the activation of inflammatory and oxidative cascades (90). Antileukinate, a potent inhibitor of CXC chemokines receptors 1 and 2, which specifically suppresses neutrophils chemotaxis, was actually demonstrated to reduce lung inflammations in several models (64, 88, 99). Based on the beneficial effects of SOD3 *per se* on neutrophil recruitment in the lung, it has been recently studied in combination with Antileukinate in a mice neonatal model of hyperoxia-induced lung injury (102). Targeted lung overexpression of human SOD3 and antileukinate treatment showed synergistic activity, as transgenic mice receiving the treatment showed a lower content of neutrophils in the lung and of myeloperoxidase in the bronchoalveolar lavage as well as lower levels of OS markers, such as 8-isoprostane and oxidized GSH, in comparison with wild-type treated animals; alveolar density was also improved in the former group (102). Moreover, the administration of a plasmid containing human SOD3 *via* aerosol to animal models under hyperoxic conditions was associated with increased levels of lung cGMP relative to untreated controls at 3 and 7 days of hyperoxia, indicating a significant preservation of NO bioavailability in treated animals (3), which is of fundamental importance in RDS patients, in order to prevent further impairment of the ventilation/perfusion ratio. Interestingly, such effects were associated with reduced nuclear factor kappa B (NF-κB) concentration in treated animals, thus indicating a protective role of SOD3 in the downstream inflammatory pathways (3).

Persistent pulmonary hypertension of the newborn. The study of AOEs in the treatment of PPHN is based on the observation that elevated ROS and RNS burden in this condition, as a result of the administration of high concentrations of oxygen along with iNO, may be involved in the extensive lung damage observed in this population (130, 153). The assessment of genetic variants of AOEs and NOS could be a crucial point in properly directing antioxidant treatments in PPHN.

Based on the hypothesis that SOD could reduce the formation of peroxynitrite thanks to the scavenging of superoxide in newborns with PPHN, rhSOD treatment was preliminary assessed in newborn piglets receiving high FiO_2 and iNO, and was demonstrated effective in reducing inflammatory cell count and neutrophil chemotactic activity in bronchoalveolar fluid, and malondialdehyde in lung tissue, both in case of endotracheal administration or nebulization (131). It was later demonstrated that endotracheal rhSOD administration not only mitigates oxidative injury, but also potentiates the beneficial effects of iNO on lung vascular tone, resulting in additive benefits on lung hemodynamic measures (162). The combination of iNO and endotracheal rhSOD induced a significantly greater decrease in pulmonary arterial pressure and pulmonary vascular resistance than iNO or rhSOD alone, and the ratio of pulmonary arterial pressure/aortic pressure remained significantly lower in the former group throughout the study period. The addictive effects of the treatments could be probably attributed both to the scavenging of superoxide with RNS reduction and to the resultant increased iNO bioavailability with consequent enhancement of iNO effects. This hypothesis is concordant with the observation that *in vitro* SOD enhances pulmonary arterial vessel relaxation induced by an NO-donor compound and that this effect is not abated by CAT (2, 16), excluding the fact that it is mediated by increased H_2O_2, which is known to induce vasorelaxation (22). Further animal experiments demonstrated that the combined treatment of iNO + rhSOD in a single dose produced a more rapid improvement in oxygenation than the treatment with rhSOD or iNO alone and was associated with a need for lower medium airway pressure (86). Such promising results were partly mitigated by the observation that the alveolar/arterial oxygen ratio did not significantly improve in the group treated with rhSOD + iNO in comparison with either intervention alone. However, endotracheal rhSOD abated lung levels of 3-nitrotyrosine, a marker for peroxynitrite formation, suggesting a key role in preventing iNO-associated lung injury, probably through a multiple doses strategy, in order to achieve a more stable enzymatic action. Endotracheal rhSOD administration was also studied in a lamb model of RDS of prematurity (79), with analogous results on pulmonary hemodynamics, as iNO + rhSOD treatment did not improve oxygenation with regard to iNO alone. However, early treatment with high-dose rhSOD was associated with improved oxygenation in comparison to the control group not receiving iNO; while no beneficial effects were observed on lung edema and compliance. Delayed rhSOD administration (2 h after birth) did not produce any beneficial effect in this study, but it was reported to be effective in another study (4 h after birth) performed with term animal models, suggesting a probable differential response depending on gestational age, with more premature lungs resulting in being more vulnerable to OS and ventilation-induced injury (86).

More recent insights into the activity pathways of SOD (Figs. 1 and 2) provided evidence for the possible mechanisms

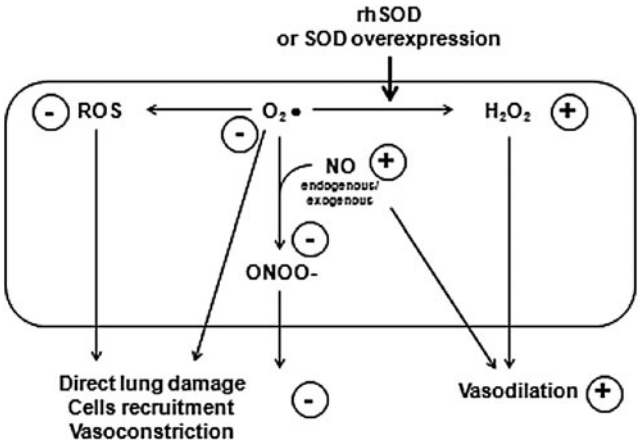

FIG. 1. Effects of rhSOD or SOD overexpression on ROS, RNS, and NO availability in pulmonary arterial smooth muscle cells. Resultant effects are highlighted in circles. NO, nitric oxide; ROS, reactive oxygen species; RNS, reactive nitrogen species; rhSOD, recombinant human superoxide dismutase.

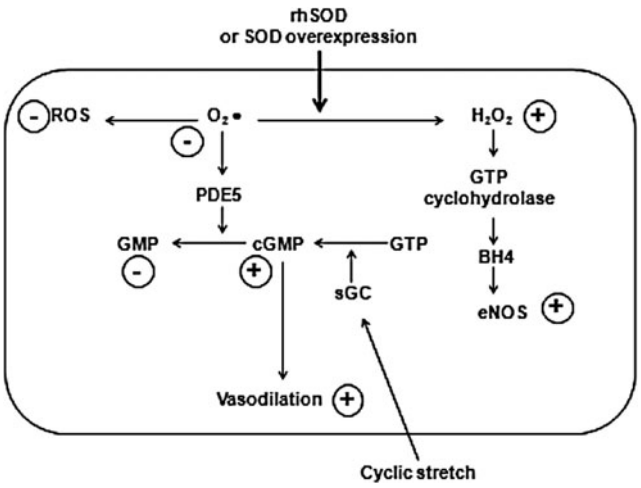

FIG. 2. Effects of rhSOD on PDE5 and eNOS. Resultant effects are highlighted in circles (44, 45, 86, 151). eNOS, endothelial nitric oxide synthase; PDE5, phosphodiesterase 5.

responsible for the observed beneficial effects of SOD on lung vasculature in PPHN (44, 45, 151). The activity of endothelial NOS (eNOS) in pulmonary vasculature increases after birth as a consequence of relative hyperoxia and mechanical forces on lung vasculature in both healthy and ventilated animals (16, 44, 45, 164), taking part in the feto-neonatal transition, but such an increase is suppressed in PPHN models (45). Endotracheal rhSOD or iNO, in addition to ventilation with FiO_2 100%, induces a significant eNOS mRNA increase in PPHN models with regard to PPHN animals receiving only ventilation with FiO_2 100%, but rhSOD was proved more effective in increasing protein expression, and either rhSOD or iNO reduced lung ROS accumulation in PPHN (45). As demonstrated by the contractile response to noradrenaline in the presence of an eNOS inhibitor, rhSOD, but not iNO, restored eNOS functionality, as rhSOD restored the contraction response to the inhibitor; while iNO did not influence the test. Interestingly, rhSOD, but not iNO, significantly increased lung levels of tetra-hydro-byopterin (BH4), which is an essential cofactor for eNOS activity, and also increased the rate-limiting enzyme for the synthesis of BH4 (45). These observations demonstrate that SOD and iNO may exert additive but also partially different effects on lung vasculature in PPHN, thus possibly serving as adjunctive or alternative treatments for such a condition. Furthermore, while guanilate cyclase (GC) is overexpressed after birth in healthy models and phosphodiesterase 5 (PDE5) is down-regulated, resulting in increased cGMP relative to the fetus and relaxation of pulmonary vasculature, in the PPHN animals both GC and PDE5 are overexpressed, with lung cGMP content comparable to fetal levels (43a). Either iNO or endotracheal rhSOD decreased PDE5 activity and restored normal postnatal levels of cGMP (44). These data are concordant with the observation that ROS may compromise GC activity (161) and activate PDE5 expression (45, 106), while rhSOD would prevent such detrimental effects and are also in agreement with clinical studies indicating the usefulness of PDE5-inhibitors, as Sildenafil, in the treatment of PPHN (105, 163). A recent study also reported that cyclic stretch is associated with increased OS in pulmonary artery smooth cells along with increased GC expression (151). In this setting, rhSOD administration abated OS markers, but preserved induced GC levels (151).

Moreover, the development of antioxidant treatments that are capable of influencing SOD3 activity definitely deserves further investigation, as SOD3 appears abundantly expressed within lung tissue and smooth muscle (112), serving as a key regulator of vasodilation (71) and of antioxidant status in PPHN (187). In pulmonary arterial smooth muscle cells of PPHN lambs, SOD3 was suppressed relative to controls and hyperoxia or H_2O_2 addition similarly suppressed SOD3 in control cells. In agreement with these data, SOD3 activity was lower in PPHN animals than in controls. Interestingly, the addition of CAT to cultured control cells incubated with H_2O_2 restored proper SOD3 activity, while endotracheal administration of CAT to PPHN lambs increased SOD3 lung activity and oxygenation and reduced lung superoxide levels (187). Therefore, H_2O_2 formation during PPHN has been hypothesized to impair SOD3 activity, resulting in excessive superoxide load and vasoconstriction, which would be abated by specific treatments that are aimed at increasing SOD3 lung activity. A recent experiment of SOD3 transfection in mice pulmonary microvascular endothelial cells was recently associated with reduced intracellular ROS and xanthine oxidase accumulation after hypoxia exposure, and transgenic mice with lung targeted human SOD3 overexpression showed significantly lower signs of PPHN after prolonged hypoxia relative to PPHN wild-type animals, as demonstrated by lower right ventricle systolic pressure and mass and lower pulmonary vascular wall thickness, indicating less vascular remodeling (5). Transfection of PPHN wild-type animals with plasmid containing DNA encoding human SOD3 significantly reversed the indexes of PPHN, thus suggesting that SOD3 activity enhancement not only prevents PPHN development but could also be effective in treating already established cases, with possible crucial clinical implications (5).

Since reduced SOD2 expression in pulmonary arterial cells of PPHN lambs was proved to contribute to OS and endothelial dysfunction (2), it could be regarded as a further useful therapeutic target for antioxidant treatments. SOD2 activity was increased in PPHN cells relative to controls, while SOD2 tyrosine nitration was increased. SOD2 viral transduction in PPHN cells resulted in reduced superoxide mithocondrial content with increased H_2O_2, increased eNOS expression, and enhanced relaxation of pulmonary arteries, suggesting multiple roles of SOD2 in modulating vascular reactivity and OS in PPHN.

Finally, since NADPH oxidase was proved a major source of superoxide anions in pulmonary arteries in different models of PPHN (38, 60) and the treatment with NADPH oxidase inhibitor was proved effective in reducing ROS in pulmonary arteries of PPHN models (186), the associated therapy of rhSOD and NADPH oxidase may offer additive benefits and, therefore, deserves to be investigated for this purpose.

With regard to SOD2, the possibility to develop a recombinant protein may be likely challenged by the large size and high complexity of the enzyme (119). A possible tool to overcome this issue may be offered by SOD mimetics, which are low-molecular-mass compounds with specific catalytic activity developed to avoid the problems of dimensions, stability, immunogenicity, and delivery of natural enzymes

(119, 141). SOD mimetics include Mn-based metalloporphyrins and Mn-salen complexes, which also possess minor CAT activity, and Mn-containing macroyclics, which selectively mimic SOD activity (141). SOD mimetics have been studied in animal cell models of different adult disease states that are characterized by OS and inflammation, such as neurological diseases, cancers, and ionizing radiation-induced injury (119, 141). These antioxidants have not yet been studied in models of neonatal lung diseases; however, they represent a possible future research topic, as they could also offer the theoretical advantage of enhanced penetration into the lung tissues in comparison with native or recombinant SOD.

Surfactant administration in RDS: potential antioxidant contribution

Endotracheal surfactant administration is one of the milestones of RDS treatment, as it was proved to reduce mortality, mechanical ventilation need, and air leaks, although it did not affect the occurrence of BPD (159). Besides the well-known beneficial effects on alveolar surface tension and mechanics (123, 124), the surfactant was also proved to possess anti-inflammatory and antioxidant properties (31, 96, 97), possibly contributing to the benefits of surfactant administration in RDS. The exogenous surfactant not only replaces the pre-existing surfactant partially inactivated by ROS (97), but also naturally contains specific proteins and minor lipids with antioxidant activities (Table 5). Both enzymatic and non-enzymatic antioxidants would contribute to reduce alveolar ROS accumulation, preventing local damage and ROS diffusion (97, 137). Indeed, lower levels of OS markers were found in tracheal aspirates of surfactant-treated preterm neonates relative to untreated preterm controls (101).

AOEs and other antioxidant molecules are commonly found in the epithelial lining fluid of the normal human lower airways (25, 26). Natural calf lung surfactant was proved to contain measurable amounts of SOD and CAT and to exert scavenging activity against H_2O_2 (97). Endotracheal administration of the surfactant also induced a significant increase of SOD content in alveolar type II cells, demonstrating the occurrence of enzyme uptake *via* liposome during the surfactant recycle process (97). On the contrary, a surfactant extract containing 1% proteins was shown to contain no significant amount of AOEs and did not exert any scavenging activity (97), strengthening the concept that protein compounds are of crucial importance to surfactant activities, which should be strongly considered in the setting of synthetic surfactant development. A recent study confirmed that four natural surfactants contain definite amounts of SOD and CAT, which have been detailed for Poractant, Beractant, Calfactant, and Bovactant (31). Poractant provided the highest content of SOD per ml, while Calfactant was proved to contain the highest amount of CAT. Moreover, Poractant provided the major amount of SOD per recommended dose for clinical use, while Calfactant was proved to contain the major amount of CAT per dose. Poractant also showed the highest scavenger activity when incubated with H_2O_2 (31). Despite relevant results obtained *in vitro*, the role of surfactant AOEs *in vivo* remains to be established. Moreover, the assessment of the role of surfactant AOEs in the prevention of ROS-mediated lung injury in the newborn would require specific measurements of alveolar lining fluid, AOE content, and antioxidant capacity after natural and synthetic surfactant administration in model systems.

Besides AOEs, natural surfactants also contain non-enzymatic antioxidants, such as plasmalogens and polyunsaturated phospholipids (PUPLs) (137). Plasmalogens were proved to work synergistically with cholesterol, SP-B, and SP-C in the formation of the surfactant reservoir and in the spreading of dipalmitoylphosphatidylcholine; in the experimental models, plasmalogens addiction to the surfactant achieved a further reduction of surface tension and viscosity in comparison to the surfactant alone (124, 137). Plasmalogens were also recently demonstrated to confer consistent antioxidant protection against ultraviolet light-induced lipid peroxidation within cell membranes (193) and also to exert antioxidant functions in low-density lipoproteins (72). The content of plasmalogens resulted in being consistently higher

Table 5. Antioxidant Compounds in Natural Surfactants

	Curosurf poractant	*Survanta beractant*	*Alveofact bovactant*	*Infasurf calfactant*
Doses				
mg of PLs/kg	200	100	100	100
ml of surfactant/kg	2.5	4	2.2	2.86
SOD				
U/mg of PLs	0.396[a]	0.474	0.027[b]	0.383[c]
U/ml of surfactant	31.7	11.9	1.21	13.4
U/dose per kg	73.3	47.6	2.6	38.3
CAT				
nmol/min/ml of PLs	0.81[d]	2.60	1.58	3.23
nmol/min/ml of surfactant	64.80	65.00	71.10	113.10
U/dose per kg	149.80	260.00	157.80	323.50
Plasmalogens				
mol% of total PLs	3.8 ± 0.1	1.5 ± 0.2	0.9 ± 0.3	n.a.
PUPLs				
mol% of total PLs	26 ± 1	6 ± 1	11 ± 1	n.a.

[a] $p = 0.019$ *versus* Survanta.
[b] $p < 0.0001$ *versus* Survanta.
[c] $p = 0.003$ *versus* Survanta.
[d] $p < 0.0001$ *versus* Infasurf.
PLs, phospholipids; PUPLs; polyunsaturated phospholipids; n.a., not available.

for Poractant than Beractant and Bovactant (124, 137). Poractant presents the highest concentration of PUPLs among natural surfactants, which are considered one of the main substrates for lipid peroxidation in the lung surfactant (137). Plasmalogens and polyunsatured fatty acids in tracheal aspirates at birth in preterm neonates resulted in being significantly lower in patients who developed BPD in comparison to non-BPD controls (138).

AOE addition to surfactants is based on the rationale to vehiculate them into the alveolar epithelial cells. The incubation of lung epithelial cells with an emulsion of Beractant plus SOD and CAT resulted in significantly higher SOD and CAT activity relative to cells incubated with enzymes or Beractant alone (109). These results were confirmed in lung homogenates obtained from animal models after endotracheal administration of Beractant plus SOD and CAT, suggesting that surfactant supplementation with AOEs is an efficacious way to increase antioxidant defenses in the alveolar environment (109). Liposome encapsulation of SOD and CAT was also studied to enhance intracellular delivery of the enzymes, and such a method was proved effective in increasing AOEs activity in the alveolar cells of preterm animal models of hyperoxia-induced lung injury (182). *In vitro*, the addition of SOD to natural surfactants resulted in increased scavenger activities for Poractant, Beractant, and Bovactant; while CAT addition resulted in increased scavenger activity for Poractant, Bovactant, and Calfactant (31). The addition of both SOD and CAT induced a further increase in scavenger activity in comparison with SOD or CAT addition alone, suggesting a possible synergic activity (31). Our recent data, which are still unpublished, demonstrate that SOD and CAT addition to natural surfactants reduces lung OS markers in preterm lambs with RDS.

In addition to direct antioxidant properties, the surfactant also exerts a complex web of anti-inflammatory and immune-modulating activities, which indirectly contribute to lower OS. Surfactant proteins A and D (SP-A and SP-D), which belong to the family of collectins, are involved at multiple levels in the immune response to several pathogens (61). SP-A and SP-D have the property to bind to alveolar pathogens, favoring their clearance (61, 98). SP-A was recently demonstrated to down-regulate inflammation in the presence of LPS, suppressing the pro-inflammatory pathways mediated by NF-κB (190), and both SP-A and SP-D down-regulate specific immune responses through the inhibition of pro-inflammatory cytokine secretion and the modulation of lymphocyte proliferation (61). The role SP-D may be of particular importance, as lower levels of SP-D were detected in tracheal aspirates of preterm newborns who subsequently developed BPD relative to age-matched non-BPD controls (82). Furthermore, SP-D modified by RNS was proved to lose aggregating activity (98), and a genetic variant of SP-D was recently found to be over-represented in RDS patients, relative to non-RDS controls (140).

Melatonin and other antioxidants

Several antioxidant therapies different from direct AOEs replacement were studied in preterm newborns for the prevention of respiratory morbidities. Vitamins A, E and *N*-acetyl-cysteine failed to reduce the occurrence of BPD in preterm newborns (175, 185), and intravenous GSH did not improve acute respiratory outcomes in preterm baboons (160). In contrast to previous observations (6), a recent study in the animal model of hyperoxic resuscitation after perinatal asphyxia suggested a potential role for *N*-acetylcysteine in lung protection, as it was proved to reduce lung and systemic levels of OS and inflammatory markers (111). Moreover, pentoxifillyne, a phosphodiesterase inhibitor with immunomodulatory and antifibrotic features, has been investigated in the animal model of hyperoxia, and it was proved to reduce lung edema and neutrophils influx, while it increased lung activity of SOD, CAT, and GPx (9), in agreement with previous reports of reduced inflammatory cytokines in tracheal aspirates (173) and lung protection against mechanical ventilation-induced injury (81, 158). Besides antioxidant activity, pentoxyfilline also induces VEGF expression and enhances pulmonary vasculature development (9), which could be a major issue for the prevention of BPD-associated vascular abnormalities.

Melatonin (MT), which is secreted by the pineal gland as a circadian rhythm transducer and presents multiple antioxidant properties, was proved effective in the prevention of different OS-related processes in adults, such as aging and neurodegenerative diseases (8, 75, 127, 128, 134, 150, 181). In neonatal medicine, MT is at present one of the most promising treatments for hypoxic-ischemic encephalopathy in addition to hypothermia (133). With regard to respiratory outcomes, MT administration was studied in a small cohort of preterm newborns with RDS, and it was shown to reduce the nitrite/nitrate ratio and the circulating levels of IL-6, IL-8, and TNF-α, which are typically over-represented in the lungs of BPD patients (57). The same group of authors reported 110 preterm newborns $\leq$32 weeks of gestational age ventilated with different strategies, who were randomly assigned to receive an MT treatment course or placebo (56). The MT-treated group showed significantly lower ventilator parameters and lower circulating levels of IL-6, IL-8, and TNF-α (56). These data, which preliminarily assess the efficacy and safety of MT administration in preterm newborns, suggest a possible role for MT in the prevention of BPD, as one of the crucial points in the pathogenesis is the secretion of inflammatory cytokines and inflammatory cell recruitment in the lung (78). Unfortunately, data on long-term respiratory outcomes of the studied cohorts were not provided. These clinical observations are in agreement with the demonstration in the mouse model of BPD that MT treatment reduces lung myeloperoxidase and nitrite/nitrate ratio and increases GPx, SOD and CAT activity (114). MT-treated animals showed significantly higher number of alveoli and less fibrotic aspects, suggesting that antioxidant effects of MT may positively influence alveolization and lung development under stressful conditions after preterm birth. MT was also proved to reduce circulating markers of lipid peroxidation in a small cohort of septic newborns as soon as 1 h after the first dose, with regard to the untreated septic newborn (54). Cytokines levels were not assessed in this group; however, since infectious events in both prenatal and postnatal life are clearly related with the risk of BPD (78), MT appears as a possible prevention tool, as it lowers both OS markers and inflammatory cytokines.

The antioxidant action of MT influences multiple pathways (134). MT possesses direct antioxidant properties (117, 171), as it works as a powerful scavenger of ROS, particularly

hydroxyl radical, with the formation of metabolites, which, in turn, shows scavenging activity (63, 94, 170, 172). Beyond this, MT also up-regulates AOEs, particularly GPx, GRx, SOD, and CAT (10, 83, 100, 129, 134). MT, after binding to specific receptors MT1 and MT2, would activate different signaling pathways starting off from G-proteins and involving protein kinase A, phospholipase C, extracellular regulated kinase, and Jun N-terminal kinase, which would then result in the modulation of gene transcription. Moreover, MT could directly interact with the calcium-calmodulin complex, further affecting transcription processes, and, because of ROS reduction, it would also affect the activity of NFκB and would prevent ROS-mediated enzyme inactivation (134). Putatively, MT could offer clinical benefits in newborns with SNPs related to the impaired activity of AOEs. The study of MT treatment may be of particular interest in those newborns who present SNPs related to the impaired activity of AOEs, as it could putatively compensate for the antioxidant deficit. We suggest that the beneficial effects of MT treatment in newborns may occur as a consequence of MT anti-inflammatory and scavenging activities, particularly in case of favorable effects on OS markers observed very early after MT administration (54). On the contrary, MT-induced AOE overexpression has never been measured in newborns; neither as enzymatic activity nor as mRNA production. However, we can speculate that this aspect could be of major importance in determining the long-term effects of MT administration, particularly in case of a prolonged treatment course. The reliable route of administration and the reassuring safety profile (56, 57) makes MT a valuable candidate for further studies on BPD prevention.

Conclusions

OS stress plays a pivotal role in the pathogenesis of BPD and other complications of prematurity, such as IVH, PVL, and ROP, because of reduced antioxidant capacity of preterm newborns and high oxidative burden. OS also significantly occurs in PPHN, as a result hyperoxic ventilation and iNO administration. Genetic predisposition to induce excessive levels of inflammatory and oxidative mediators may be associated with increased risk of OS-related complications. In preterm newborns, specific SNPs of AOEs and inflammatory mediators were found to affect the risk of respiratory morbidities, as RDS requiring mechanical ventilation and BPD.

Since the treatment options for lung diseases of the newborn are far from optimal, new preventive and treatment strategies should be urgently assessed. Recent evidence suggests potential benefits of AOE supplementation or hyperexpression in the prevention and treatment of BPD, RDS, and PPHN. Moreover, surfactant replacement for RDS may exert antioxidant effects, thanks to AOEs and non-enzymatic antioxidants that are naturally present in animal surfactants, and such antioxidant potentials could be usefully enhanced by the addition of AOE to surfactants. Since the very main part of available data regarding antioxidant options comes from animal models and *in vitro* experiments, clinical trials are mandatory in order to assess the therapeutic potentials of these strategies. Among non-enzymatic antioxidants, MT was investigated for the prevention of adverse respiratory outcomes in the preterm newborns, with preliminary positive results, which should be further addressed.

A more extensive assessment of SNPs involved in the pathogenesis of newborn lung diseases may be particularly helpful to identify specific populations with a particularly high risk of adverse outcome, who may maximally benefit from antioxidant therapies, and the clarification of the effects of SNPs on proteomics may pose the basis for an individually targeted AOE supplementation.

Finally, antioxidant supplementation or induction during pregnancy should be studied as a potential strategy to reduce fetal and neonatal adverse outcomes in models of OS-related complications of pregnancies, such as pre-eclampsia.

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Author Disclosure Statement

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Abbreviations Used

AER = aerosolized
AOE = antioxidant enzyme
BPD = bronchopulmonary dysplasia
CAT = catalase
eNOS = endothelial nitric oxide synthase
ET = endotracheal
FiO_2 = fractional inspired oxygen
GC = guanilate cyclase
GPx = glutathione peroxidase
GRd = glutathione reductase
GSH = glutathione
GST = glutathione-*S*-transferase
IFN-γ = interferon-gamma
IL = interleukin
iNO = inhaled nitric oxide
IP = intraperitoneal
IV = intravenous
IVH = intraventricular hemorrhage
MMP = matrix metalloproteinase
MT = melatonin
n.a. = not available
NF-κB = nuclear factor kappa B
NO = nitric oxide
NOS = nitric oxide synthase
OS = oxidative stress
PA = pulmonary artery
PDE5 = phosphodiesterase 5
PLs = phospholipids
PPA = pulmonary arterial pressure
PPHN = persistent pulmonary hypertension of the newborn
PUPLs = polyunsaturated phospholipids
PVR = pulmonary vasculature resistance
RDS = respiratory distress syndrome
rhSOD = recombinant human SOD
ROP = retinopathy of prematurity
ROS = reactive oxygen species
RNS = reactive nitrogen species
RV = right ventricle
SNP = single nucleotide polymorphism
SOD = superoxide dismutase
TA = tracheal aspirate
TNF-α = tumor necrosis factor-alpha
TLR = toll-like receptor
UTR = untranslated region