Intensive Care Med (2001) 27: 1116–1123
DOI 10.1007/s001340100977

REVIEW

Eloisa Gitto
Russel J. Reiter
Malgorzata Karbownik
Dun Xian-Tan
Ignazio Barberi

Respiratory distress syndrome in the newborn: role of oxidative stress

Received: 3 November 2000
Final revision received: 15 March 2001
Accepted: 6 April 2001
Published online: 15 June 2001

E. Gitto (✉) · R. J. Reiter · D. Xian-Tan
Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio,
Mail Code 7762, 7703 Floyd Curl Drive,
San Antonio, Texas 78229-3900, USA
E-mail: reiter@uthscsa.edu
Phone: +1-210-567 3859
Fax: +1-210-567 6948

E. Gitto · I. Barberi
Institute of Medical Pediatrics,
Neonatal Intensive Care Unit,
University of Messina,
Via Consolare Valeria, Messina, Italy

M. Karbownik
Department of Thyroidology,
Institute of Endocrinology,
Medical University of Lodz,
5 DR. Sterlinga St., 91-425 Lodz, Poland

Abstract Reactive oxygen and nitrogen species are generated by several inflammatory and structural cells of the airways. These oxidant species have important effects on a variety of lung cells as regulators of signal transduction, activators of key transcription factors and modulators of gene expression and apoptosis. Thus, increased oxidative stress accompanied by reduced endogenous antioxidant defenses may play a role in the pathogenesis of a number of inflammatory pulmonary diseases, including respiratory distress syndrome (RDS) in the newborn. There obviously are conflicting reports on the effect of oxygen, ventilation and nitric oxide (NO) on RDS and, thus, the question arises as what the neonatologist should do when confronted with a newborn with RDS. Clearly, utilizing lung protective strategies requires compromises between gas exchange goals and potential toxicities associated with over-distension, derecruitment of lung units and high oxygen concentrations. The results discussed in this brief review suggest rigorous clinical tests with antioxidants which may help to define the mechanisms associated with RDS and which could lead to new treatment strategies.

Keywords Distress respiratory syndrome · Oxidative stress · Antioxidants

Introduction

Respiratory distress syndrome (RDS) is a leading cause of mortality and morbidity in preterm newborns [1]. Acute respiratory distress is a clinical syndrome with various underlying causes but the "primum movens" is a deficiency of surfactant. The condition is characterized by impaired gas exchange, decreased static compliance and a non-hydrostatic pulmonary edema due to loss of the integrity of the alveolocapillary barrier with impairment of normal surfactant function. These changes result in an increased tendency of the alveoli to collapse.

The clinical definition of RDS usually includes a combination of increased difficulty breathing, retractions, tachypnea, cyanosis and intractable apnea, associated with abnormal blood gas results, e.g., $PaCO_2$ levels of 60 mmHg, arterial partial pressure of oxygen (PaO_2) levels lower than 60 mmHg at an inspired oxygen fraction (FIO_2) of 0.60 or greater, and blood pH lower than 7.25 [1]. Although the mechanisms of tissue injury have not been fully elucidated, there is evidence for the involvement of oxidative damage due to reactive oxygen species (ROS) [2].

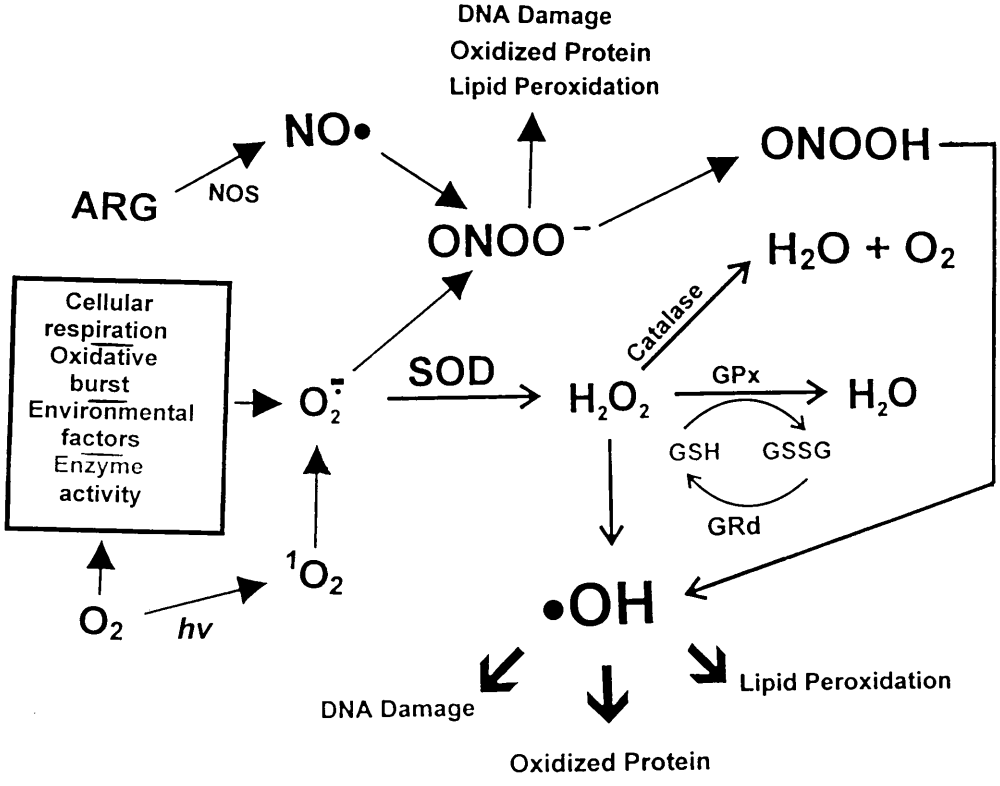

Fig. 1 Free radicals (molecules with unpaired electrons in their outer orbital) and reactive oxygen species (ROS, non-radical by-products of O_2 metabolism) are damaging to a variety of molecules. The most toxic products produced are probably the hydroxyl radical (•OH) and the peroxynitrite anion ($ONOO^-$); these agents indiscriminately damage any molecule, e.g., DNA, proteins and lipids, in the vicinity of where they are produced. Antioxidants protect against such damage by directly scavenging these toxic species or they stimulate enzymes, e.g., catalase, glutathione peroxidase (GPx) and glutathione reductase (GRd), which metabolize reactive species to non-toxic products

Mechanism of respiratory distress syndrome

Experimental and clinical studies have shown that any harmful tissue event (infections, trauma, anoxia) is perceived mainly by the macrophage and monocyte cells which secrete cytokines, among which are interleukin-1 (IL1) and tumor necrosis factor (TNF). These agents stimulate stromal cells with the additional production of cytokines by fibroblasts, endothelium, epithelium and mast cells. The second wave of cytokine production brings about the synthesis of IL1, IL2, IL6 and IL8, which allow for the progression and the amplification of the inflammatory response. Due to the interaction of these mediators, the inflammatory cells activate polymorphonuclear leukocytes (PMN), macrophages/monocytes, platelets and mast cells as well as a variety of humoral systems including complement, coagulation-fibrinolysis and arachidonic acid [3].

The activation of the above-mentioned mechanisms leads to the formation of toxic substances derived from oxygen, e.g., free radicals and ROS (Fig. 1). These oxidizing agents have important effects on a variety of lung cells as regulators of signal transduction, activators of key transcription factors and modulators of gene expression and apoptosis. In experimental models it has been observed that the specific targets of a hyperoxic insult to the lung are the vascular endothelial cells and the epithelial cells of the alveoli. ROS induce ultrastructural changes in the cytoplasm of pulmonary capillary endothelial cells and cause focal hypertrophy and altered metabolic activity. Thus, increased oxidative stress accompanied by reduced endogenous antioxidant defenses may play a role in the pathogenesis of a number of inflammatory pulmonary diseases including respiratory distress in the newborn [4, 5]. A deficit in the precise balance between exposure to oxidants and endogenous antioxidants results in what has been referred to as oxidative stress. The molecular damage caused by oxidative stress appears to be involved in the pathogenesis of a growing number of diseases, including RDS of the newborn [6, 7].

When phagocytes such as neutrophils are stimulated by microorganisms or other stimuli they become activated and increase their oxidative metabolism; as a result, free radicals and ROS are formed. If these oxygen-based products are not inactivated, their high chemical reactivity leads to damage to a variety of cellular macromolecules including proteins, carbohydrates, lipids and nucleic acid. This results in cell injury and may induce cell death [3]. Under these conditions, a surfactant deficiency may be aggravated by inactivation of the small amount of endogenous surfactant that is produced [2]. Furthermore, if exogenous surfactant is given this might also be destroyed [5, 8].

Reactive oxygen species also have been implicated in the molecular damage seen in the bronchoalveolar lav-

age (BAL) fluid of patients with RDS [9, 10]. This hypothesis is supported by several findings: hydrogen peroxide (H_2O_2) is detected in the expired air of RDS patients, and myeloperoxidase and oxidized α-1-antitrypsin have been found in BAL lavage fluid. Increased plasma lipid peroxidation has been noted in critically ill patients and in patients with sepsis and at risk of developing RDS. Also, evidence of augmented levels of oxidized lipids and proteins have been found in the plasma of patients with RDS and ROS and these increased levels also have been implicated in the molecular damage seen in the BAL fluid of patients with RDS. BAL fluid normally contains a large amount of glutathione, but in patients with RDS this is mostly in the oxidized form [2, 11].

Consistent with this, oxidative inactivation of α-1-antiprotease also has been observed in RDS. Elevated concentrations of xanthine and hypoxanthine are present in the plasma and BAL fluid of patients with RDS and have been shown to be a potential source of ROS in the presence of exogenously added xanthine oxidase. Also, increased concentrations of orthotyrosine and metatyrosine in BAL fluid protein imply the formation of the damaging hydroxyl radicals (•OH) in the lungs of these patients, since orthotyrosine and metatyrosine are isomers of tyrosine thought to be formed exclusively by aromatic hydroxylation of phenylalanine by •OH [8]. Chlorotyrosine and nitrotyrosine also have been found in BAL fluid from patients with RDS. Increased concentrations of chlorotyrosine residues in BAL fluid proteins from patients with RDS indicate hypochlorous acid (HClO) production by activated inflammatory cells in the lungs of these patients. Chlorotyrosine is formed by HClO-dependent chlorination of paratyrosine. HClO is a damaging oxidant formed from H_2O_2 and chloride ions by the enzyme myeloperoxidase, present in activated inflammatory cells. HClO has been implicated as possibly being the major damaging species produced by activated neutrophils. HClO itself is a destructive oxidant but it also may interact with low molecular mass iron or superoxide anion radicals (O_2^-•) to produce the •OH.

Nitrotyrosine concentrations also are significantly increased in the BAL fluid protein of patients with RDS [12]. Nitration of tyrosine residues previously has been used as an in vivo marker of the formation of peroxynitrite ($ONOO^-$). $ONOO^-$ is the oxidant formed when nitric oxide (NO•) couples with O_2^-• (Fig. 1). Earlier studies report increased nitrotyrosine concentrations in the lungs of patients with RDS. $ONOO^-$ is capable of damaging lipids, proteins and DNA. Additionally, under acidic conditions it can decompose to form a powerful oxidant with properties similar to •OH. There is, however, another possible explanation for the formation of nitrotyrosine. Recent work shows that nitrotyrosine can arise from the reaction of tyrosine with nitroxyl chloride, an intermediate formed by the interaction of nitrite (the auto-oxidation product of nitric oxide) with HClO. Interestingly, nitrotyrosine concentrations in BAL fluid protein from patients with RDS treated with NO were elevated compared with those found in lung-injured patients not receiving this therapy [13].

Increased nitrotyrosine concentrations may reflect augmented $ONOO^-$ formation in the patients receiving NO. Since the patients receiving inhaled NO are no sicker, in terms of Acute Physiology and Chronic Health Evaluation II score or FIO_2 requirements, than those patients not receiving this therapy, it implies that inhaled NO may react with O_2^-• in these circumstances to form the nitrating agent [14].

Finally, myeloperoxidase concentrations are significantly elevated in the BAL fluid from patients with RDS, indicating lung neutrophil recruitment and activation [15].

Oxidative stress and antioxidants

Preterm infants are thought to be hypersusceptible to oxidative stress because of inadequate antioxidant protection. This is a consequence of low levels of plasma radical scavengers such as α-tocopherol and melatonin, a marked deficiency in metal-binding proteins such as transferrin and ceruloplasmin for the protection of metal-catalyzed free radical reactions, and reduced activities of antioxidant enzymes such as catalase and glutathione peroxidase [4, 13].

Hyperoxic exposure itself, although essential for the survival of RDS infants, probably induces excessive production of reactive oxygen metabolites in the respiratory system. Vento and colleagues [14] evaluated the effects of oxygen exposure during the first 6 days of life on the tracheobronchial aspirate fluid in 16 mechanically-ventilated preterm infants. In newborns ventilated with 100% oxygen, the tracheobronchial fluid contained high levels of uric acid and low total antioxidant capacity. Yigit et al. [15] demonstrated that serum MDA levels were insignificantly higher in infants requiring mechanical ventilation compared to those breathing spontaneously. There exist several potential causes of intracellular and extracellular oxidant stress in preterm newborns with RDS. The high inspiratory concentrations of oxygen required to achieve adequate arterial oxygenation, prooxidant drugs and infections or extrapulmonary inflammation can all promote ROS accumulation and the utilization and depletion of antioxidative factors.

Exposure of premature newborns to hyperoxia is a factor in the development of chronic disease. Chronic lung disease (CLD) of the newborn is one of the definitive factors influencing the mortality and morbidity of very low birth-weight infants [9, 16]. The etiology of

CLD is unknown, but many investigators have suggested that free radicals could play a key role in its development. The exposure of immature lungs to prolonged periods of high levels of inspired oxygen is accepted as an important contributor to the development of CLD through both free radical effects on endothelial and epithelial cell barriers, that lead to pulmonary edema, and trigger mechanisms, that lead to activation and accumulation of inflammatory cells.

Ogihara et al. [4] have suggested a role for oxygen radicals as the trigger for CLD. In addition, their data indicate that the plasma allantoin concentrations and the allantoin/urate ratio may be useful early predictors of the development of CLD. Exposure to hyperoxia commonly occurs during mechanical ventilation of the premature newborn and is a factor in the development of CLD [5]. The most common reason neonates require respiratory support is RDS. It is being increasingly realized that modes of mechanical ventilation that result in end-inspiratory alveolar over-stretching and/or repeated alveolar collapse and re-expansion disturb the normal fluid balance across the alveolocapillary membrane. The effects of this include disturbance in the integrity of the endothelium and epithelium and impairment of the surfactant system; these changes are similar to those seen in acute RDS [17, 18].

Cytokines and other mediators

In 1999 Clark [19] proclaimed that "the concept of ventilator-induced lung injury has come of age". There are many data which suggest that ventilation can cause biotrauma associated with a "mediator storm" (perhaps cytokines) and that it is responsible for distal organ dysfunction, subsequent multiorgan failure and death. Although it has been shown that pulmonary cytokine levels also appear to be elevated in some neonates on assisted ventilation, the exact relationship to neonatal lung injury has yet to be defined. Proinflammatory mediators may be elevated because of fetal exposure to maternal inflammatory mediators, postnatal infections or due to release of mediators from the preterm lung attributable to ventilator-induced injury. The preterm lung is susceptible to injury with the initiation of ventilation because potential lung volumes are small, surfactant may be deficient, the lung matrix is not fully developed and the air spaces contain residual fetal lung fluid. Tidal volume (Vt) during the resuscitation of preterm infants is not monitored, and easily visible chest movements will result in Vt in excess of that routinely needed to ventilate infants [5]. Preterm infants are often hyperventilated and low PCO_2 values after birth correlate with an increased incidence of chronic lung disease [20]. The question as to whether oxygen is the only source of free radicals is unanswered.

Ogihara et al. [12] measured plasma concentrations of aliphatic aldehydes within the first week of life in 13 premature infants who subsequently developed lung disease and 11 infants without CLD. All of the aldehydes measured were increased in these infants with CLD compared with non-CLD infants. There were no significant differences between the infants with and without CLD in terms of gestational age, birth weight or delivery method. In this study fetal distress and the Apgar scores were nearly identical for the two groups. Thus, the difference in aldehyde concentrations between the two groups was not attributable to prenatal or perinatal hypoxia or reperfusion injury. Excessive lipid peroxidation is one consequence of an imbalance between free radical generation and antioxidant defense capacities. However, if excessive free radical formation occurs in CLD infants, this seems not to be due to the hyperoxia itself since the ventilation method and total oxygen supplementation were comparable in the two groups. Since a rapid influx of neutrophils into the lungs is observed at delivery in infants with RDS, activated neutrophils may be a primary source of free radicals which, in turn, induce lipid peroxidation [21]. Perhaps the ventilation effects create the same situation that is observed in RDS, where oxidative stress is due to activated inflammatory cells [18].

Recent reports also suggest a role for NO, which is produced by activation of the various isoforms of nitric oxide synthase (NOS) (Fig. 2) in mediating the inflammatory response of the lung to hyperoxia. Thus, NO may have a role in the development or prevention of bronchopulmonary disease (BPD) [13, 22]. NO, also known as endothelium-derived relaxing factor, has been shown to be an effective vasodilator in experimental and clinical pulmonary hypertension in the newborn. In severe pulmonary hypertension associated with respiratory failure, inhaled NO reduced pulmonary arterial pressure and lowered respiratory failure in the neonates.

Studies in animals have shown that NO, ranging from 5–10 ppm, is a potent pulmonary vasodilator, with the duration of vasodilatation varying from a few minutes to several hours. Clark and co-workers [23] conducted a clinical trial and showed that inhaled NO reduced the extent to which extracorporeal membrane oxygenation is needed in neonates with hypoxemic respiratory failure and pulmonary hypertension. Frostell et al. [24] have shown that NO reverses acute hypoxic pulmonary vasoconstriction. On the other hand, there are conflicting reports on the influence of NO in combination with exogenous surfactant on gas exchange and lung mechanics. Some studies indicate that NO causes surfactant dysfunction, while others find a favorable effect on the surfactant [25].

Depending on the concentration and the presence of other reactive molecules, NO may either activate or in-

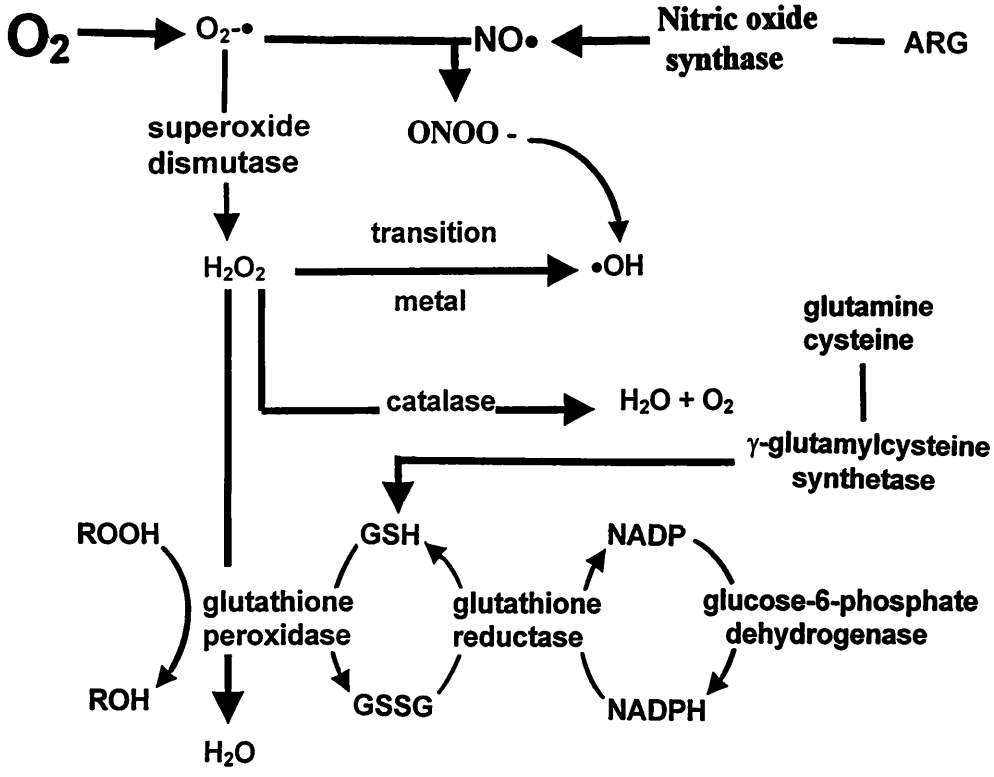

Fig. 2 The peroxynitrite anion ($ONOO^-$) is a highly toxic species which is formed when the superoxide anion radical (O_2^-•) couples with nitric oxide (NO•). There is also evidence that the $ONOO^-$ is metabolized to the hydroxyl radical (•OH) which is also highly reactive. $ONOO^-$ formation is controlled by two enzymes, i.e., nitric oxide synthase [which determines nitric acid (NO•) levels] and superoxide dismutase (SOD) which converts O_2^-• to hydrogen peroxide (H_2O_2). Increased SOD activity reduces steady state concentrations of O_2^-• and thereby lowers $ONOO^-$ generation. Other antioxidative enzymes including catalase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase remove H_2O_2 from cells and help maintain glutathione, an intracellular antioxidant, in its reduced form (GSH). The high concentrations of GSH within cells is determined by the activity of its rate limiting enzyme, γ-glutamylcysteine synthetase

hibit the pulmonary surfactant system [26]. Under conditions of an excess O_2^-• generation, inhaled NO may be oxidized to nitrogen dioxide, which causes acute tracheobronchial injury, vascular endothelial injury and surfactant dysfunction. $ONOO^-$, generated in the presence of NO and a O_2^-•, induces lipid peroxidation, alters proteins and DNA and results in increased vascular permeability and surfactant dysfunction. High doses of NO cause the generation of methemoglobin which binds to surfactant and reduces surface activity. On the other hand, NO may serve as an antioxidant by scavenging lipid peroxides and may provide protection against inactivation of the surfactant complex [14]. Sison et al. [25] have shown that a short period of inhaled NO can be beneficial in the treatment of lung disease associated with pulmonary edema and surfactant dysfunction.

Potter et al. [27] have shown that exposure of rat pups to hyperoxic upregulated both inducible (iNOS) and endothelial nitric oxide synthase (eNOS) and increased NO activity, as measured by intracellular cGMP levels. The increases in both enzymes in lung parenchyma in response to hyperoxic conditions suggest that NO may be involved in the response of the lung to excessive oxygen exposure and that elevations in iNOS may, in part, be caused by neutrophil and alveolar macrophage migration into the lung due to oxygen-induced pulmonary inflammation. Conversely, eNOS upregulation is due to an oxygen-induced over-expression of the enzyme in the vascular endothelium and airway epithelium. These authors also showed that inhibition of NOS reduced cGMP levels in the hyperoxic rat pups; however, it did not seem to reverse the pathologic consequences of hyperoxic exposure, in contrast to a previous study of Pierce et al. [28] wherein it was found that blockade of NO production with L-NAME aggravated pulmonary oxygen toxicity and/or mortality in newborns. There obviously are conflicting early reports on the effect of ventilation and NO and, therefore, the question arises as what the neonatologist should do when confronted with a newborn with RDS.

In clinical trials, inhaled NO has reduced the need for extracorporeal membrane oxygenation. The body of accumulated data indicates that NO may act as an antioxidant as well a prooxidant, depending on a number of known and unknown factors, e.g., the concentration of NO itself and the concentration of other oxidants. In low doses, NO is an antioxidant and in high doses its prooxidant effects are more pronounced. The available evidence suggests the use of doses of inhaled NO beginning at 20 ppm in term newborns with PPHN, since this treatment decreases extracorporeal membrane oxygenation use without an increased incidence of adverse defects. Although brief exposures to higher doses (40–80 ppm) of NO appear to be safe, sustained treatment with 80 ppm NO exaggerates the risk of methemoglobinemia [29].

The concept of combination therapies for the treatment of pulmonary hypertension has substantial merit. The pathogenetic mechanisms responsible for the development and maintenance of pulmonary hypertension are complex and include a deficiency of endothelial prostacyclin and an excess thromboxane production, impaired NO synthesis or increased endothelin-1 production. It is likely that multiple mechanisms interact to produce varying degrees of pulmonary vasoconstriction and vascular remodeling. Although there are a variety of modalities of ventilation that are non-invasive, each ventilatory strategy has a potentially negative consequence. Small Vt ventilation is associated with progressive low volume and surfactant dysfunction [3]. Limiting Vt requires higher levels of end-expiratory pressure and/or FIO_2 to maintain adequate oxygenation. Higher levels of FIO_2 can contribute to oxidant-induced lung injury.

Clearly, utilizing lung protective strategies requires compromises between gas exchange goals and potential toxicities associated with over-distension, derecruitment of lung units and high oxygen concentrations.

Clinical critique

It is well documented that exposure to hyperoxia can result in lung inflammation and damage, subsequently leading to CLD. However, whereas hyperoxia induces oxidative stress, that this is the cause of CLD in newborns who are exposed to hyperoxia has been difficult to prove. Proinflammatory mediators may be elevated because of fetal exposure to maternal inflammatory mediators, post-natal infection or due to the release of these mediators from preterm lungs caused by ventilator-induced injury. Neonatal lungs are still undergoing development and growth; therefore, cytokine responses may be immature and different from those seen in adults.

The most common reason for neonates requiring respiratory support is RDS. In this disease, the pathophysiology is one of progressive loss of lung volume, intrapulmonary shunt and deflation instability. Animal models of RDS have clearly shown that ventilator strategies alter the clinical and pathologic evolution of RDS. In addition, it is claimed that neonates with RDS are susceptible to lung injury and the subsequent development of related conditions. This is not uniformly true in humans, however. Van Zoeren et al. [30] evaluated markers of oxidative stress and antioxidant activity in plasma and erythrocytes for 14 days after birth in infants with neonatal RDS, relative to those of control infants. The results did not provide convincing evidence of augmented oxidative damage and diminished antioxidant defense in preterm infants with neonatal RDS. Moison et al. [31] compared the plasma redox ratios of uric acid in well preterm infants with those with RDS and CLD newborns, and investigated the relationship between these ratios and their respective measurements in tracheal aspirate. Uric acid and ascorbic acid redox ratios were significantly elevated in tracheal aspirates compared to plasma samples, and there was a strong positive correlation between both ratios in RDS and CLD newborns. These markers may be useful in monitoring newborns with RDS.

While there are obviously conflicting findings in this field, it is generally accepted that antecedent lung inflammation or injury makes the lungs more susceptible to volutrauma and oxidant-induced injury. The resulting damage promotes inflammation that is not limited to the lung but that may also affect distant organs, and oxygen, when used at high concentration, can be toxic. Thus, the use of lung protective strategies in the neonate requires proactive decisions that must be specific for disease pathophysiology and lung maturity, and that involve compromises between gas exchange goals and potential toxicities of the treatments.

In clinical studies that have used NO in neonates, children and adults, the outcomes have also not been consistent and no clear consensus exists regarding the appropriate clinical use of inhaled NO. Because numerous clinical studies with encouraging preliminary results exist in the literature, the findings have led to the increasing use of inhaled NO in various clinical situations. Most of these studies have shown, however, that NO administration results in significant changes in acute physiologic variables only. A study by the Neonatal Inhaled Nitric Oxide Study Group suggested a significant reduction in the need for extracorporeal membrane oxygenation in infants with hypoxic respiratory failure. This supposition was based on the lack of adverse hemodynamic effects, low measured levels of NO_2 and normal methemoglobin levels. Despite the evidence that supports the use of inhaled NO, significant concerns regarding its potential toxicity remain. NO binds rapidly to hemoglobin and is carried through the systemic circulation. Not all of its subsequent actions are known. NO is known to couple with the $O_2^{-}\bullet$ to form the highly toxic $ONOO^{-}$ (Fig. 2). The toxicity of $ONOO^{-}$ is still under investigation, but it is probably responsible for surfactant damage and other protein alterations due to the generation of nitrotyrosine residues. These nitrotyrosine compounds have been identified in both animal models and human lung samples. Such surfactant damage/dysfunction has been previously shown in ARDS suggesting that exogenous NO may lead to pulmonary toxicity.

Concluding remarks

Reactive oxygen and nitrogen species are generated by several inflammatory and structural cells of the airways. These oxidant species may have important effects on different lung cells as regulators of signal transduction, activators of key transcription factors and modulators of gene expression and apoptosis. Thus, an increased oxidative stress accompanied by reduced endogenous antioxidant defenses may play a role in the pathogenesis of a number of inflammatory pulmonary diseases including respiratory distress in newborns.

Newborns have less protection against oxidation. In comparison with healthy adults, lower levels of plasma antioxidants such as vitamin E, β-carotene, melatonin and sulfhydryl groups, lower levels of plasma metal-binding proteins such as caeruloplasmin and transferrin, and reduced activity of erythrocyte superoxide dismutase are typical of newborn infants. Furthermore, infants frequently have higher plasma levels of non-transferrin-bound iron and higher erythrocyte free iron levels than do adults. Although antioxidant drugs could play a useful role in the therapy of inflammatory lung diseases, their clinical impact seems relatively modest at present. Rigorous clinical investigation with the existing antioxidants and development of new drugs which improve lung function are not only important but vital to the development of effective treatment strategies in newborns with debilitating respiratory distress.

Acknowledgement E. Gitto was supported by a fellowship of the "Miglierina-Griffini" Foundation.

References

1. Mariani GL, Carlo WA (1998) Ventilatory management in neonates: science or art? Clin Perinatol 25: 33–48
2. Boda D, Nemeth I, Pinter S (1998) Surface tension, glutathione content and redox ratio of the tracheal aspirate fluid of premature infants with IRDS. Biol Neonate 74: 281–288
3. Esteban J, Morcillo JE, Cortjo J (1999) Oxidative stress and pulmonary inflammation: pharmacological intervention with antioxidants. Pharmacol Res 40: 393–404
4. Bhandari V, Mauli KN, Kresch M (2000) Hyperoxia causes an increase in antioxidant enzyme activity in adult and fetal rat type II pneumocytes. Lung 178: 53–60
5. Ikegami M, Kallapur S, Michna J, Jobe AH (2000) Lung injury and surfactant metabolism after hyperventilation of premature lambs. Pediatr Res 47: 398–404
6. Lamb NJ, Gutteridge JMC, Baker C, Evans TW, Quinlan GJ (1999) Oxidative damage to proteins of bronchoalveolar lavage fluid in patients with acute respiratory distress syndrome: evidence for neutrophil-mediated hydroxylation, nitration and chlorination. Intensive Care Med 25: 1738–1744
7. Huertas JR, Palomino N, Ochoa JJ, Quiles JL, Ramirez-Tortosa MC, Battino M, Roblex R, Mataix J (1998) Lipid peroxidation and antioxidant erythrocyte membranes of full-term and preterm newborns. Biofactors 8: 133–137
8. Kim K, Whitin JC, Sukhova NM, Cohen HJ (1999) Increases in extracellular glutathione peroxidase in plasma and lungs of mice exposed to hyperoxia. Pediatr Res 46: 715–721
9. Banks BA, Ischiropoulos H, McClelland M, Ballard PL, Ballard RA (1998) Plasma 3-nitrotyrosine is elevated in premature infants who develop bronchopulmonary dysplasia. Pediatrics 101: 870–874
10. Dellinger RP (1999) Inhaled nitric oxide in acute lung injury and acute respiratory distress syndrome. Inability to translate physiologic benefit to clinical outcome benefit in adult clinical trials. Intensive Care Med 25: 881–883
11. Janssen YMW, Soultanakis R, Steece K, Heerdt E, Singh RJ, Joseph J, Kalyanaraman B (1998) Depletion of nitric oxide causes cell cycle alterations, apoptosis and oxidative stress in pulmonary cells. Am J Physiol 275:L1100–1109
12. Ogihara T, Hirano K, Morinobu T, Kim HS, Hiroi M, Ogihara H, Tamai H (1999) Raised concentrations of aldehyde lipid peroxidation products in premature infants with chronic lung disease. Arch Dis Child Fetal Neonatal Ed 80:F21–F25
13. Metnitz PGH, Bartens C, Fisher M, Fridrich P, Steltzer H, Druml W (1999) Antioxidant status in patient with acute respiratory distress syndrome. Intensive Care Med 25: 180–185
14. Vento G, Mele MC, Mordente A, Romagnoli C, Matassa PG, Zecca E, Zappacosta B, Persichilli S (2000) High total antioxidant activity and uric acid in tracheobronchial aspirate fluid of preterm infants during oxidative stress: an adaptive response to hyperoxia? Acta Paediatr 89: 336–342
15. Yitig S, Yurdakok M, Kilinc K, Oran O, Erdem G, Tekinalp G (1998) Serum malondialdehyde concentrations as a measure of oxygen free radical damage in preterm infants. Turk J Pediatr 40: 177–183
16. Saugstad OD (1998) Chronic lung disease: the role of oxidative stress. Biol Neonate 74 (Suppl 1):21–28
17. Verbrugge SJ, Uhlig S, Negger SJ, Martin C, Held HD, Haitsma JJ, Lachmann B (1999) Different ventilation strategies affect lung function but do not increase tumor necrosis factor-alpha and prostacyclin production in lavaged rat lungs in vivo. Anesthesiology 91: 1834–1843
18. Verbrugge SJ, Lachmann B (1999) Mechanism of ventilation-induced lung injury: physiological rationale to prevent it. Monaldi Arch Chest Dis 54: 22–37
19. Clark RH, Slutsky AS, Gerstmann DR (1999) Lung protective strategies of ventilation in the neonate: what are they? Pediatrics 105: 112–114
20. Gannon M, Wiswell TE, Spitzer AR (1998) Volutrauma, $PaCO_2$ and neurodevelopmental sequelae following assisted ventilation. Clin Perinatol 25: 159–175
21. Bouhas RKL, Jarstrand C (1999) Phagocyte-induced lipid peroxidation of lung surfactant. Pediatr Pulmonol 27: 322–327
22. Robbins CG, Davis JM, Merrit TA, Amirkhanian JD, Sahgal N, Morin FC, Horowitz S (1995) Combined effects of nitric oxide and hyperoxia on surfactant function and pulmonary inflammation. Am J Physiol 269:L545–L550

23. Klark RH, Kueser TJ, Walker MW, Southgate WM, Huckaby JL, Perez JA, Roy BJ, Kinsella JP (2000) Low-dose nitric oxide therapy for persistent pulmonary hypertension of the newborn. New Engl J Med 342: 572–574
24. Frostell C, Fratacci MD, Wain JC, Jonnes R, Zapol WM (1991) Inhaled nitric oxide. A selective pulmonary vasodilator reversing hypoxic pulmonary vasoconstriction. Circulation 83: 2038–2047
25. Sison C, Bry K, Sephus J Hallman M (2000) Effects of inhaled nitric oxide and surfactant treatment on lung function and pulmonary hemodynamics in bronchoalveolar-lavage-induced respiratory failure. Pediatr Pulmonol 29: 202–209
26. Shen YH, Wang XL, Wilken DEL (1998) Nitric oxide induces and inhibits apoptosis through different pathways. FEBS Lett 433: 125–131
27. Potter CF, Kuo NT, Farver CF, Mcmahon JT, Chang CH, Agani FH, Haxhiu MA Martin RJ (1999) Effects of hyperoxia on nitric oxide synthase expression, nitric oxide activity and lung injury in rat pups. Pediatr Res 45: 8–13
28. Pierce MR, Voelker CA, Sosenko IRS, Bustamante S, Olister SM, Zhang XJ, Clark DA, Miller MJS (1995) Nitric oxide synthase inhibition decreases tolerance to hyperoxia in newborn rats. Mediators Inflamm 4: 431–436
29. Kinsella JP, Abman SH (2000) clinical approach to inhaled nitric oxide therapy in the newborn with hypoxemia. J Pediatr 136: 717–726
30. Van Zoeren-Grobben D, Lindeman JH, Houdkamp E, Moison RM, Wijnen JT, Berger HM (1997) Markers of oxidative stress and antioxidant activity in plasma and erythrocytes in neonatal respiratory distress syndrome. Acta Paediatr 86: 1356–1362
31. Moison RM, De Beaufort AJ, Haasnot AA, Dubbelman TM, Van Zoeren-Grobben D, Berger HM (1997) Uric acid and ascorbic acid redox ratios in plasma and tracheal aspirate of preterm babies with acute and chronic lung disease. Free Radical Biol Med 23: 226–234