Is There a Role for Antioxidant Therapy in Bronchopulmonary Dysplasia?1

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ABSTRACT Bronchopulmonary dysplasia (BPD) is a chronic lung disease first described in 1967 as a complication of therapy for premature infants with hyaline membrane disease, and treatment with high concentrations of oxygen was thought to be a major contributor to its development. Thus, interventions to enhance lung antioxidants to prevent the development of BPD were considered appropriate therapeutic strategies. In the last decades, advances in the acute care of premature infants has reduced the reliance on therapy with high concentrations of supplemental oxygen. Nevertheless, the incidence of BPD has not changed significantly. The changing clinical context in which BPD develops helps the question of whether oxidation is important in the development of BPD and, therefore, whether designing interventions enhancing lung antioxidants is still warranted. This review presents evidence that premature infants that develop BPD have qualitative and quantitative differences in oxidation of lipids and proteins when compared to infants that do not develop BPD. Such differences in oxidation patterns are the most obvious in the first few days of life. The emerging evidence thus supports the concept that the lung injury process leading to the development of BPD occurs within hours to days of delivery and that oxidation is a major contributor to this pathological process. Unfortunately, early attempts at delivery of antioxidants to the lung have not been successful, perhaps because of an inability to deliver antioxidants in a timely manner to the areas in the lung in which deleterious oxidations are occurring. Further research is necessary to determine both the nature and the location of the oxidative events that lead to the development of early lung injury, so that more appropriate and specific antioxidant interventions can be designed. J. Nutr. 131: 947S–950S, 2001.

KEY WORDS: • bronchopulmonary dysplasia • premature infant • oxidative stress • antioxidants

Bronchopulmonary dysplasia (BPD) was first described by Northway in 1967 in patients that were born prematurely, and were treated with high concentrations of supplemental oxygen to relieve the hypoxemia resulting from hyaline membrane disease (Northway et al. 1967). The diagnosis of BPD was made when a premature patient required supplemental oxygen at 28 d of life and had an abnormal chest X-ray. The pathophysiology of BPD was extensively studied over the next several decades and pulmonary oxygen toxicity was believed to play a prominent role in the lung injury process that leads to the development of BPD. Using prematurely delivered baboons as a model, deLemos and colleagues (1987) showed that exposure to 100% oxygen for 6 d resulted in lung lesions that were consistent with those observed in premature humans with BPD (deLemos et al. 1987). Other factors that contributed to the lung injury associated with the development of BPD were structurally immature lungs and an acute early course of hyaline membrane disease (reviewed by deLemos and Coalson 1992).

Given the origins of BPD the most reasonable approach to preventing the disorder should include eliminating the three primary causative factors of BPD: prematurity, hyaline membrane disease and oxygen toxicity. Unfortunately, the inroads made into preventing prematurity by improving prenatal care have been offset by the increase in prematurity as a consequence of an increase in multiple births secondary to treatments for infertility. Major advances in preventing hyaline membrane disease have been achieved by more frequent use of prenatal steroids, which induce maturation of the surfactant system (Anonymous 1995). Also, the treatment of premature infants with exogenous surfactant has markedly reduced mortality and morbidity in premature infants (Ten Centre Study Group 1987, Collaborative European Multicenter Study Group 1988, Bose et al. 1990, Corbet et al. 1991, Long et al. 1991, Hoekstra et al. 1991, Liechty et al. 1991). These advances in the treatment of premature infants have led to marked improvements in the respiratory status in premature infants, so that in the first few days of life premature infants are generally exposed to only low concentrations of supplemental oxygen (Welty 2000).

Despite the improvements in acute respiratory disease, and the lower exposures to supplemental oxygen, the incidence of
BPD in premature infants remains largely unchanged. The minimal effect of improved acute care on the incidence of BPD may be attributed to the impact of this care on greater survival in the most immature infants who otherwise would have probably died. It is these extremely low birth weight infants who develop BPD, even though their acute courses were not marked by severe respiratory distress (Hudak and Egan 1992). The fact that premature infants develop BPD without being exposed to high concentrations of supplemental oxygen begs the question of whether oxidative stress still contributes to the development of BPD. This report reviews the evidence as to whether oxidized molecules are more frequently observed in infants that develop BPD, thus supporting a role for oxidative stress as a causative factor in BPD. It also explores the evidence that interventions designed to augment antioxidant defense mechanisms protect infants from the development of BPD. Finally, current research on efficacious methods to augment antioxidants in patients that are predisposed to develop BPD are reviewed.

Approach to investigations of the possible role of oxidative stress in the development of BPD

Figure 1 reviews the pathways by which oxidative events may occur at the cellular level in patients exposed to oxidant stresses and the possible mechanisms by which cells may be protected by antioxidants. Exposure to increased concentrations of supplemental oxygen increases the formation of superoxide (O$_2^- $), which by itself is a weak oxidant, and this molecule is reduced to hydrogen peroxide (H$_2$O$_2$) in a reaction catalyzed by superoxide dismutase (SOD). Hydrogen peroxide can then be reduced to water in a reaction catalyzed by catalase. The reaction catalyzed by catalase is probably of limited importance because catalase is primarily compartmentalized to peroxisomes (Zhou 2000). Thus, reduction of H$_2$O$_2$ by glutathione (GSH) to water and glutathione disulfide (GSSG) in a reaction catalyzed by glutathione peroxidase is probably more important to limit the accumulation of intracellular H$_2$O$_2$ concentrations. In addition, because elevated intracellular levels of GSSG may be toxic by reacting with protein thiol groups (PSH), GSSG may be reduced to GSH at the expense of NADPH oxidation in a reaction catalyzed by glutathione reductase (GR). This reaction along with transport into extracellular fluids is probably also important to limit oxidative damage by intracellular GSSG accumulation. This antioxidant defense system may also break down when excess O$_2^-$ reacts with Fe$^{2+}$ to produce Fe$^{3+}$, followed by a reaction of Fe$^{3+}$ with H$_2$O$_2$ to produce extremely reactive oxygen radicals, which then oxidize macromolecules and lead to cellular dysfunction and death when oxidations occur to molecules that are crucial for cellular function. In light of these reactions there are several approaches that can be used to determine whether oxidative stress contributes to the development of BPD. One way is to attempt to reduce the oxidative stress by administration of antioxidants. If antioxidant administration reduces the incidence of BPD, it is reasonable to interpret that oxidative stress plays a significant role in its development. Another approach is to identify specific molecular oxidations that occur and are observed more commonly and/or to a greater extent in patients that go on to develop BPD than in patients who do not develop BPD. Finally, an approach that suggests a predilection to oxidative stress would be to determine whether molecules critical in the pathways to detoxify oxidants or in enhancing oxidations are altered in patients that develop BPD vs. those patients that do not.

Measurements of GSH, GSSG and iron in premature infants

It has not been possible to measure antioxidant enzymes in various organs in premature patients, to determine whether antioxidant enzymes are lower or less active in patients who eventually develop BPD than in non-BPD infants. However, measurements of GSH and/or GSSG in plasma samples or lung fluid samples are possible. Low GSH concentrations and high GSSG concentrations would suggest that patients were susceptible, or in fact possibly experiencing a significant oxidative stress. Smith and co-workers (1993) measured plasma GSH and GSSG concentrations in premature infants and found that plasma GSH concentrations were surprisingly low in premature infants and that there was a linear relationship between gestational age and plasma GSH concentrations. However, there was no comparative analysis of plasma GSH and GSSG between BPD and non-BPD subjects. In a similar study, Boda and co-workers (1998) found that GSH concentrations were lower and the GSSG-to-GSH ratio was higher in tracheal aspirate samples in premature infants that had hyaline membrane disease than that in control infants. In addition, there was a significant correlation between low surfactant levels and function with low GSH levels and high GSSG-to-GSH ratios in tracheal aspirate samples in the first 2 wk of life. These
studies provide preliminary evidence that premature infants are biochemically predisposed to oxidant injury, even when being treated with little or no supplemental oxygen, and supports the notion that supporting premature infants by enhancing the glutathione system may provide some protection from BPD. Perhaps such infants are also at risk of other forms of oxidant injury that may lead to complications of prematurity such as intraventricular hemorrhage, retinopathy of prematurity and necrotizing enterocolitis (reviewed by Kelly 1993). Therapeutic strategies to counter a state of oxidative stress might include nutritional or pharmacological treatments that enhance the glutathione system, which may be useful in preventing BPD and other diseases of prematurity resulting from excessive oxidized molecules.

Along with deficiencies in the glutathione system, evidence for “free” iron was previously demonstrated in premature infants. In normal circumstances iron is carefully sequestered in proteins that bind iron, so that iron can be transported to and used in cells for normal cellular function. “Free” nonsequestered iron is traditionally measured by incubation of samples with bleomycin, which chelates nonsequestered iron. Free iron in the form of Fe$^{3+}$ can participate in a reaction that generates hydroxyl radicals, which are potent oxidants. In a study by Evans and co-workers (1992), bleomycin-chelatable iron was not detected in the plasma from adults but was detected in the plasma from some premature and term infants. Similarly, Moison and co-workers (1993) found no bleomycin-chelatable iron in adult blood, whereas 48% of very premature infants and 25% of term infants had bleomycin-chelatable iron in their cord blood samples. In addition, plasma from premature infants stimulated lipid peroxidation while the plasma from term infants and adults inhibited lipid peroxidation. Bleomycin-chelatable iron was also observed in the bronchoalveolar secretions in > 65% of premature infants (Gerber et al. 1999). None of these studies that looked at “free” iron attempted to study the association between detectable iron and evidence for oxidations occurring in the infants to which iron may have contributed. Taken together, the evidence is suggestive of a role for iron and iron-mediated reactions to contribute to the development of BPD. In summary, premature infants are biochemically extremely susceptible to oxidant injury in the lung, leading to BPD, and therapy directed at enhancing antioxidant systems, especially the glutathione system, is attractive. Further investigations to determine whether iron-mediated oxidations play a role in lung injury and the development of BPD will require assessment of blood and tissue samples from premature infants, who are then tracked to determine whether they develop BPD.

**Measurements of molecular oxidations that occur in patients that develop BPD**

To determine whether oxidative stress secondary to deficiencies in antioxidants such as the glutathione system, or secondary to free iron plays a role in the development of BPD, products of lipid oxidation and protein oxidation have been assessed in premature infants. In a study by Ogihara and co-workers (1999), plasma levels of lipid aldehydes were measured in the first week of life in premature infants. Plasma concentrations of Heptanal, 2-nonenal and 4 hydroxynonenal were higher in the first 24 h of life in infants that developed BPD than in those that did not (Ogihara et al. 1999). In another study high exhaled pentane was strongly associated with several adverse outcomes in premature infants (Nycz et al. 1998). There was a striking inverse correlation between gestational age and exhaled pentane on the first day of life, and peak exhaled pentane was associated with an increased risk for death and intraventricular hemorrhage. Also, infants that developed BPD had higher exhaled pentane on the first day of life than did patients that did not develop BPD (Nycz et al. 1998). Such studies of lipid peroxidation must be interpreted with caution because lipid peroxidation pathways are complex and traditional measurements made in human subjects such as exhaled ethane and pentane and plasma aldehydes account for a small fraction of all of the products of lipid peroxidation (reviewed by Smith 1991). However, the accumulated evidence on lipid oxidation products are supportive of the notion that oxidative stress as it pertains to lipid peroxidation pathways may play a role in the development of BPD and other adverse outcomes associated with prematurity.

Protein oxidation was also previously assessed in premature patients and correlated with the development of chronic lung disease. Most frequently, protein oxidation is measured by incubating protein samples with 2,4-dinitrophenylhydrazine (DNPH), which binds covalently to “protein carbonyls” and forms hydrazones that can be detected by measuring absorbance at 365 nm or by Western blotting with antibodies raised against the dinitrophenyl epitope (Levine et al. 1990, Awasthi et al. 1998). In lavage samples from premature infants, Gladstone and Levine (1994) found that infants that had a more severe acute course had higher protein carbonyl contents than did patients that were not as ill, and that anti-inflammatory treatment was associated with a decrease in protein carbonyl contents. However, no attempt was made to determine whether protein carbonyl formation was associated with an increased risk for the development of BPD. There was an association between higher protein carbonyl contents in tracheal aspirates in the first week of life and the development of BPD in a study by Varsila and co-workers (1995). In a recent study Ramsay and co-workers (1998) utilized Western blotting to assess tracheal aspirate samples for DNPH-reactive proteins in a single center in which exogenous surfactant was routinely used to prevent RDS. There were no differences in oxygen requirements of tracheal aspirate contents of total DNPH-reactive proteins between premature infants that did or did not develop BPD (Ramsay et al. 1998). However, infants that developed BPD did have more frequent oxidation of specific proteins than did infants that did not develop BPD. This recent study suggests that identifying specific proteins that are more frequently oxidized in infants that develop BPD may be important in determining specific mechanisms for the development of BPD.

In summary, studies of specific lipid and protein oxidation products suggest that specific oxidations occur more frequently in premature infants that develop BPD than in those that do not. This series of studies also points out that oxidation products measured as early as in the first day of life are strongly associated with the development of BPD. This point is important to recognize because it suggests that the process leading to the development of BPD is set in motion early in the course of premature infants, and that interventions designed to reduce the incidence or severity of BPD should target the injury process as early as possible.

**Antioxidant administration to premature infants**

Despite the prevailing evidence that oxidative stress plays a role in the development of BPD, there has been surprisingly little effort to administer antioxidants to premature infants as a preventative measure. As early as 1984, bovine superoxide dismutase was administered subcutaneously to infants with respiratory distress and was associated with less chest X-ray...
evidence of BPD, fewer physical findings associated with BPD and fewer days on CPAP than those in the control group (Rosenfeld et al. 1994). In a study in which recombinant human superoxide dismutase was administered intratracheally to premature infants, initial results were promising, in that there appeared to be evidence of less injury early in the course of infants that received superoxide dismutase than that in control infants (Rosenfeld et al. 1996). However, there was no effect of superoxide dismutase administration on the incidence of BPD (Davis et al. 2000). Interestingly, despite the lack of efficacy in preventing BPD, superoxide administration was associated with fewer respiratory symptoms in the first year of life. Antioxidant administration studies in premature infants may be difficult to interpret until better ways to deliver anti-oxidants to the appropriate cells and the appropriate subcel- lular compartments are developed.

Summary and final recommendations

Despite the fact that improvements in the acute manage- ment of premature infants has led to a less severe acute course of RDS, thus lowering the need for treatment with high concentrations of supplemental oxygen, there remains sub- stantial evidence that oxidative stress plays a large role in the development of BPD. More rational interventions to prevent deleterious oxidative events that injure the lung await the identification of specific molecular oxidations occurring more frequently in premature infants. Crucial research questions that must be addressed before molecularly specific antioxidant administration is possible include:

1. Identification of specific oxidations that occur more frequently in premature infants that go on to develop BPD.
2. The mechanisms by which these specific oxidations oc- cur. For example, are most of the specific oxidations consistent with iron-mediated oxidations, in which case interventions reducing the levels of free iron would be indicated?
3. In which cells and subcellular compartments are the specific oxidations occurring. The identification of the cells most susceptible to oxidative events should assist researchers in designing studies to deliver antioxidants to the appropriate cell types.
4. The effect of the specific oxidations on the function of the molecule oxidized.

Until these goals are met, antioxidant administration may not prevent or ameliorate BPD because of the inherent lack of specificity of antioxidant administration in the complex disease process that leads to the development of BPD.

LITERATURE CITED
