CURRENT TOPIC

Antioxidants in neonatal lung disease

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Chronic lung disease of prematurity (CLD) has risen in importance since the advent of effective care for preterm babies. Factors contributing to CLD include anatomical immaturity, surfactant deficiency, mechanical ventilation, and oxygen therapy.¹ Current modes of treatment are only marginally successful in reducing its incidence.²

Reactive oxygen species and other free radical metabolites have been implicated in the pathogenesis of numerous diseases in both adults and neonates. Toxic effects in the lung, which are presumed to be mediated by free radicals, include: cytotoxic effects on endothelial and epithelial cells of the lung parenchyma,³ and on alveolar macrophages⁴; inhibition of surfactant synthesis⁵; inhibition of the pulmonary vascular response to hypoxia⁶; inhibition of normal lung repair by fibroblasts, thus resulting in altered collagen deposition⁷; and inhibition of normal lung development resulting in decreased alveolisation.⁸ Acute lung disease in preterm infants is accompanied by pronounced inflammatory cell migration into the lungs.⁹⁻¹⁰ In the presence of activated inflammatory cells,⁹⁻¹¹ together with high concentrations of inspired oxygen,¹² the relative immaturity of most host defence mechanisms, and appropriate conditions for reperfusion lung injury,¹² such as re-expansion of collapsed segments, the potential exists for the local generation of reactive oxygen species.

We remain ignorant of the mechanisms which lead to the resolution of respiratory distress syndrome in some infants, while others develop CLD. Bronchoalveolar lavage specimens taken from neonates who have subsequently developed CLD show a persistently increased cell count (mainly neutrophils) in comparison with those who recover without CLD.⁹ This accumulation of polymorphonuclear cells within the lung may lead to an increase in toxic oxygen metabolites.¹³ Animal models based on guinea pigs,¹⁴ mice,¹⁵ rats,¹⁶ rabbits¹⁷ and premature baboons¹⁸ have shown that lung injury comparable with that seen in human CLD can be produced by hyperoxic exposure. These studies suggest that reactive oxygen species have an important role in CLD. However, animal models vary in their biochemical response to hyperoxia.¹⁹⁻²⁰ Thus, although a role for free radicals in lung injury is well established, little is known about the importance of oxygen toxicity in preterm infants.

The observation that the lowest birthweight, least mature infants are most prone to develop

CLD is consistent with the idea that immature oxidant defence mechanisms contribute to the onset of CLD. If this is true, an effective means of augmenting the antioxidant defences of the lung could lead to a reduction in the incidence of CLD. We consider the evidence to support these statements.

Production of reactive oxygen species and free radicals

Free radicals are very reactive unstable molecular species which can initiate chain reactions to form new free radicals. Although formed as a result of a wide range of normal biochemical processes, they are potentially damaging, and several host defence mechanisms are in place to neutralise their effects. Reactive oxygen species are free radicals, but there are other biologically important free radicals, such as thiyl and peroxyl radicals (fig 1).

Reactive oxygen species are formed during normal cellular metabolism. Stepwise reduction of molecular oxygen to water in the mitochondrial electron transport chain generates the superoxide radical, hydrogen peroxide, and the hydroxyl radical. The cytochrome oxidase system, however, achieves a one step reduction of oxygen to water accounting for more than 90% of oxygen reduction and avoiding major free radical formation. These reactive oxygen species are also produced in activated leucocytes²¹ involved in inflammatory processes, as a result of phagocytic bactericidal mechanisms²² which are important in host defence, and as a result of eicosanoid metabolism.³

In the presence of free iron H_2O_2 (hydrogen peroxide) reacts to form the damaging hydroxyl radical in the Haber-Weiss reaction.³ Newborn babies have lower, but highly saturated, plasma transferrin concentrations than adults, resulting in higher circulating non-protein bound iron.²³ Leakage of this iron-replete plasma into the alveolar spaces could induce peroxidation of surfactant²⁴ as well as contribute to the reactive oxygen species damage of alveolar cells.²⁵ Ferrous iron (Fe^{2+}) cannot bind to plasma transferrin unless it is oxidised to the ferric form (Fe^{3+}) by caeruloplasmin, levels of which are also low in infants.²⁶ This is further confounded by high concentrations of vitamin C (cord blood concentrations three times higher than in adults),²⁷ a powerful reducing agent that can antagonise the ferroxidase activity of caeruloplasmin.

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The hypoxanthine-xanthine oxidase enzyme system results in the formation of the superoxide radical.³ This reaction has a role in ischaemia-reperfusion induced lung injury and may account for the development of persistent pulmonary hypertension of the newborn in the baby who has sustained birth asphyxia.

Inhaled nitric oxide (NO) is a selective pulmonary vasodilator, potentially useful in the treatment of pulmonary hypertension²⁸ and ventilation/perfusion mismatch in the newborn. NO exists in the body in redox inter-related forms.²⁹ Peroxynitrite, formed as a result of the reaction of NO with the superoxide radical, is a potent free radical initiating lipid peroxidation, catalysing DNA strand scission, oxidising sulfhydryls and methionine, and it can lead to the hydroxylation, nitrosylation, or nitration of biomolecules.³⁰ Post mortem studies in adults with acute respiratory distress syndrome have shown that they have increased staining for nitrotyrosine, produced as a result of nitration of tyrosine by peroxynitrite. This suggests that there is an imbalance between oxidant production and antioxidant protection.³¹ The formation of nitrosothiols by NO may afford protection from superoxide toxicity because nitrosothiols are relatively resistant to reaction with superoxide and therefore formation of peroxynitrites is prevented.³² However, preliminary studies in preterm infants suggest that they may be deficient in the most abundant thiol in the lungs, glutathione,³³ and consequently may not have this protection. The relative steady state concentrations of superoxide and NO, and their sites of production, will influence whether NO attenuates or exacerbates superoxide cell or tissue injury.³⁴ Until further research clarifies whether, and under which conditions, NO prevents or promotes tissue injury, caution should attend its administration.

Effects of reactive oxygen species and free radicals (fig 1)

Free radicals can denature proteins such as cytosolic and membrane-embedded enzymes, including α -1 proteinase inhibitor³⁵ which normally protects the lung and surfactant

proteins from neutrophil proteases. Oxygen toxicity is associated with the release of chemotactic factors, producing an influx of polymorphonuclear leucocytes that are stimulated to release toxic oxygen metabolites and elastase.³⁶ In neonates with RDS the α -1 proteinase inhibitor concentration in broncho-alveolar lavage is maximal at 96 hours, the time of peak polymorphonuclear leucocyte numbers as well as peak elastase activity. Those who go on to develop CLD are found to have low α -1 proteinase inhibitor concentrations in the face of prolonged polymorphonuclear leucocyte influx and high elastase activity,⁹ possibly as a result of reactive oxygen metabolites generated by polymorphonuclear leucocytes.

Free radicals can also denature protein and DNA and initiate lipid peroxidation, resulting in an increase in membrane permeability³ and inactivation of surfactant.²⁴ These reactions provide markers of free radical activity and oxidative damage. Lipid peroxidation generates hydroperoxides, endoperoxides, aldehydes (such as 4-hydroxynonenal) and the end products malondialdehyde, ethane, and pentane. Oxidative damage to proteins and DNA results in the formation of protein carbonyls and 8-hydroxydeoxyguanosine, respectively. The only definitive way of demonstrating excessive free radical activity in vivo is by electron spin resonance study of tissues. Unfortunately, this cannot be used at present in clinical practice because of the nature of the spin traps.

In very low birthweight infants a poor clinical outcome is associated with an increased production of breath hydrocarbons which are the products of lipid peroxidation, although the site of origin of breath hydrocarbons is unlikely to be merely within the lungs,³⁷ as there is a correlation between the blood concentration of malondialdehyde-thiobarbituric acid and respiratory outcome.³⁸ Tissue injury by any preceding cause is believed to accelerate lipid peroxidation reactions in the disrupted tissue. This may be extremely relevant in those neonates in whom barotrauma and insufficient defences against lipid peroxidation could be acting synergistically in the pathogenesis of CLD.

Figure 1 Reactive oxygen species and other free radicals: some effects and host defence mechanisms.

Figure 2 Intracellular localisation of antioxidants. Cu-Zn-SOD, copper-zinc SOD; Mn-SOD, manganese SOD; G6PD, glucose-6-phosphate dehydrogenase. Extracellular fluid contains a number of antioxidants: catalase, SOD, glutathione, glutathione reductase, caeruloplasmin, transferrin (from Pulmonary oxygen toxicity, Hazinski TA, Kennedy KA. In: Hilman B, ed. Pediatric respiratory disease: diagnosis and treatment, with permission).

Antioxidant defences

Antioxidants are localised to specific intracellular structures as well as being found in the extracellular matrix and fluid (fig 2). The antioxidant enzymes superoxide dismutase (SOD), catalase, and enzymes of the glutathione redox cycle (glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase) are regarded as the primary defence system of the cell against oxidative stress (fig 1). The secondary defence system is provided by a diverse range of substances.³⁹ The enzyme systems eliminate reactive oxygen species and other hydroperoxides generated during normal cellular metabolism. Experimental models of oxygen tolerance have demonstrated an association between protection from severe pulmonary oxygen toxicity and the ability to increase antioxidant enzyme activities in response to high oxygen challenge.¹⁹ The enzyme systems function mainly intracellularly, but glutathione concentrations 100-fold those of plasma exist in one particular extracellular compartment, the epithelial lining fluid of adult lungs.⁴⁰ Preliminary evidence indicates that infants who develop chronic lung disease may have lower glutathione concentrations in their lungs on the first day of life.³³ A recent study has demonstrated a negative gradient for reduced glutathione across the lungs of babies exposed to hyperoxia, suggesting lung uptake of glutathione.⁴¹ Additional non-enzymatic antioxidants present in the epithelial lining fluid in low concentrations include caeruloplasmin, transferrin, ascorbate, ferritin, uric acid and other serum proteins.³⁹

Vitamin E is a lipid soluble antioxidant that represents the principal defence against oxidant induced membrane injury.³⁹ A meta-analysis of eight randomised controlled trials demonstrates that prophylactic vitamin E supplementation does not prevent CLD.² There are several possible explanations for this: the amount of vitamin E that can enter cellular lipid membranes may be limited by the membrane lipid composition rather than its supply; administered vitamin E accumulates slowly, taking weeks to achieve small increments in tissue concentrations.⁴²

The fetus undergoes an abrupt transition

from an hypoxic to a relatively hyperoxic environment at birth. In preparation for this, the late gestational maturation of the antioxidant enzyme system is a normal aspect of fetal lung development in mammals.⁴³ A single small study in human lungs suggests that this may also be the case in the human fetus.⁴⁴ The maturation process parallels that of surfactant. It therefore seems likely that the antioxidant defences of the immature lung will be prepared neither for the hyperoxic environment nor the inflammation found in association with acute respiratory distress.

Prevention of injury due to reactive oxygen species

Oxygen induced lung injury may be minimised by a reduction in oxygen exposure, reducing lung inflammation and maximising the antioxidant defence potential of the lung.

Pregnant rats treated prenatally with dexamethasone give birth to prematurely born pups with increased lung antioxidant enzymes.⁴⁵ Antioxidant enzyme induction by prenatal steroids may also occur in human infants, and is suggested by recent studies which show a reduced incidence of CLD in newborns treated prenatally with dexamethasone.²

The additive effect of undernutrition and hyperoxia on the course of pulmonary oxygen toxicity has been repeatedly demonstrated.⁴⁶ Studies of the effect of protein deficiency on rats exposed to hyperoxia show that the increased mortality is due to a deficiency of sulphur containing amino acids, rather than a result of general protein deficiency.⁴⁷ The rats with supplemented cysteine were able to increase concentrations of total lung glutathione in response to hyperoxia, whereas the glutathione concentrations in the unsupplemented group remained unchanged. Premature infants may be deficient in cysteine⁴⁸ and therefore might benefit from supplementation. However, additional cysteine added to non-deficient diets does not significantly increase tolerance to oxygen.⁴⁷

Low selenium concentrations have been documented in premature infants.⁴⁹ Selenium is an important cofactor for glutathione peroxidase, and low activity of this enzyme has been demonstrated in selenium deficient animals.⁵⁰ These animals also had an increased susceptibility to oxidative lung injury. Premature infants may also benefit from selenium supplementation.⁵¹

Studies in rats suggest that there is a potential for parenterally administered polyunsaturated fatty acids (PUFA) to serve as free radical scavengers and protect against oxygen induced pulmonary injury.⁵² This is compatible with Dormandy's theory that 'intracellular PUFA, located in non-critical nonmembrane sites and immediately replaced after their own oxidation, could serve to avidly scavenge excess oxygen radicals and prevent their interaction with critical membrane PUFA'. However, a randomised controlled trial in infants with RDS who were assigned to receive parenteral nutrition that included a lipid

infusion had a significantly greater duration of mechanical ventilation and of supplemental inspiratory oxygen than infants who received parenteral nutrition without lipid.⁵³ Because extrapolation from animal models to people is unreliable, additional well designed studies are necessary.

Vitamin A may be particularly important in the regeneration of damaged epithelial cells. One study has shown a beneficial effect if premature infants are supplemented with vitamin A.⁵⁴

Attention to the nutritional provision of premature infants may therefore improve their outcome. As many of the proteins, vitamins, and minerals take weeks to achieve adequate tissue concentrations, maternal nutrition during pregnancy may also be important.

Augmentation of the antioxidant defence system of the lung (table)

The target for therapeutic intervention is the epithelial lining fluid and the epithelium of the airways and alveoli. The systemic route may be successful but is more likely to have unwanted side effects. Topical treatment can lead to high concentrations in the epithelial lining fluid, although technical aspects of aerosol administration to newborn babies are formidable. Cellular uptake may be enhanced by delivering agents in lipo-somal form which results in the intracellular release of the contents of the vesicles after endocytosis.

The incorporation of vitamin E into liposomes can increase the α tocopherol content of cells 12-fold in six hours.⁵⁵ In future this finding may permit rapid augmentation of cell and organ vitamin E during the course of oxidant mediated diseases.

N-Acetylcysteine (NAC) has the capacity to react directly with electrophiles, and thus has antioxidant potential. NAC is readily de-acetylated to form thiol metabolites, including cysteine and glutathione, both of which are antioxidants. Oral and intravenous administration of NAC increase plasma and red cell glutathione concentrations in adults,⁵⁶ and observational studies suggest a beneficial effect in acute respiratory distress syndrome.⁵⁷ However, there are conflicting results concerning the effect of oral NAC on epithelial lining fluid glutathione concentrations.^{56 58} Recent animal studies suggest that NAC ameliorates oxidative lung injury by scavenging free radicals rather than by increasing glutathione concentrations.⁵⁹ There are no reported studies on the effects of nebulised NAC on oxidative lung injury.

Glutathione aerosol therapy has been studied in idiopathic pulmonary fibrosis, where it seems to reduce the oxidant burden at the alveolar epithelial surface.⁶⁰ Tracheal administration of reduced glutathione in liposomes, but not reduced glutathione alone, delays the onset and decreases the severity of lung damage in fasted mice exposed to oxygen.⁶¹ However, drug-free liposomes also have a beneficial effect, for reasons that are not clear.

The administration of SOD in various forms has been studied. In a double-blind trial Rosenfeld *et al* treated 45 premature infants with bovine SOD and detected reduced oxygen dependency in the treated group.⁶² Liposome-encapsulated SOD is taken up by lung cells in vitro and in vivo and is effective in protecting rats and lung cells in culture from oxygen toxicity.⁶³ Recent studies of SOD attached to a heparin-like molecule and given parenterally, show selective binding to heparin receptors on the surface of vascular endothelial cells.⁶⁴ SOD localised at these sites in the lung could be quite effective in retarding the attack on endothelial cells by oxygen radicals released by marginating inflammatory cells.

Potentially, it should be possible to modify antioxidant gene expression for therapeutic advantage. However, the associations between transcription, translation, and cytosolic control of antioxidant enzymes are complex.⁶⁵

Potential adverse effects of antioxidant supplementation

As many of the reactions between antioxidants and reactive oxygen species result in the formation of new free radicals which have the potential to cause further tissue damage unless sequestered by another antioxidant, the potential exists for an imbalance between antioxidants to cause increased tissue damage. For example, in the presence of decreased caeruloplasmin relative to ascorbic acid, copper induced oxidation of ascorbate produces hydrogen peroxide and hydroxyl radicals, leading to the degradation of DNA and protein inactivation. Similarly, in the absence of ascorbic acid uric acid reacts with the hydroxyl radical forming urate peroxy radicals which can inactivate α -1 antiproteinase. Thiyl radicals produced as a result of the reaction between reduced glutathione and the hydroxyl radical can also cause biological problems, but are reduced by ascorbate, when present in adequate concentrations. This shows how under certain circumstances supplementing single antioxidants could be detrimental.⁶⁶

Antioxidants with therapeutic potential

Antioxidant	Function	Possible therapeutic intervention
Superoxide dismutase	Reduces superoxide to H ₂ O ₂	Liposome-encapsulated SOD; heparin-bound SOD
Glutathione peroxidase	Reduces lipid hydroperoxides and H ₂ O ₂	Dietary selenium supplementation*
Glutathione	Substrate for the glutathione redox cycle; reacts directly with hydroxyl radical and organic free radical	Dietary cysteine supplementation*; intravenous or aerosol N-acetylcysteine; liposomal glutathione (aerosol)
Vitamin E	Membrane free radical scavenger	Liposomal vitamin E (aerosol); dietary vitamin E*
Vitamin A	Membrane free radical scavenger	Dietary supplementation*

*Beneficial only if deficient.

Conclusion

The lung depends on a delicate balance between oxidant and antioxidant systems to maintain normal cellular function. Although progress has been made recently in understanding the role of reactive oxygen species in lung disease in a variety of animals, many questions are yet to be answered. What is the association between cellular redox state and normal metabolic function, and the role of oxidant species in communication between cells? Which of the antioxidants has the greatest role in the neonatal lung? Are antioxidants important in preventing the initiating factors in disease, or only in limiting secondary processes such as neutrophil recruitment, thromboxane generation, lipid peroxidation and protease inactivation? Will pharmacological augmentation of antioxidant defence disturb the regulatory control of important homeostatic functions such as phagocytic bactericidal mechanisms or eicosanoid metabolism? Can excess of individual antioxidants lead to enhanced free radical generation, with adverse consequences?

Antioxidants clearly have an important role in the defence against free radical induced lung injury. Further work is needed to understand these processes in the preterm human lung directly before clinical trials are carried out to evaluate supplementation of high risk infants with antioxidants for preventing CLD.

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