Delivering antioxidants by zip code

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Primary lung disease, the associated inflammatory response, and treatment with supplemental oxygen all result in the generation of increased amounts of reactive oxygen species (ROS), which can cause significant damage to the lung. The susceptibility of the lung to oxidative injury depends largely on the ability to up-regulate protective ROS scavenging systems. Unfortunately, both intracellular and extracellular antioxidants are expressed at relatively low levels in the human lung and are often not acutely induced when exposed to oxidative stress such as hyperoxia. Premature infants are especially sensitive to oxidant injury due to a relative lack of antioxidant defenses and an excess of ROS (even room air is supraphysiological). For example, bronchopulmonary dysplasia (BPD) affects ~20–60% of premature infants and is strongly associated with significant morbidity and mortality. Increasing evidence suggests that oxidative injury is intimately involved in the pathogenesis of this acute and chronic lung injury (10). Chang and colleagues (1) have shown that hyperoxia and the generation of ROS are responsible for many of the pathological features observed in a premature baboon model of BPD. In addition, animal models and clinical studies of adults with acute respiratory distress syndrome (ARDS) reveal biochemical evidence of significant ROS-induced lung injury (7). Thus developing therapeutic strategies to supplement endogenous antioxidant enzyme (AOE) activities to scavenge excess ROS represents a rational approach to minimize lung injury due to oxidant stress.

AOE such as superoxide dismutase (SOD; converting toxic superoxide anion into potentially less toxic H₂O₂) and catalase (converting H₂O₂ into water) are appropriate agents for augmentation of antioxidant defenses in the lung. Over the past two decades, many investigators have delivered AOE to the lung by liposomal- or viral-mediated genetic constructs or through protein supplementation in an attempt to prevent oxidant injury (5). Ilizarov and colleagues (9) have shown that pulmonary epithelial cells overexpressing AOE have significantly improved survival when exposed to oxidative stress such as hyperoxia and parquat. Animals genetically engineered to overexpress AOE and adenovirus-mediated transfer of AOE cDNA also protect the lung against hyperoxic injury (3, 4). However, although gene transfer may provide more stable and prolonged increases in AOE activities, this method would not be efficacious in acute situations, when protective intervention is immediately required. In addition, gene delivery methods have been associated with the development of significant toxicity.

Alternatively, the delivery of AOE proteins has the advantage of providing an immediate therapeutic benefit. Several groups have shown in both animal models and human trials that lung injury may be ameliorated by administration of one of these antioxidants, specifically SOD (4). Our group has studied prophylactic, intratracheal administration of recombinant human CuZnSOD (rhSOD) in premature infants to prevent oxidant-induced lung damage and BPD. Inflammatory markers were significantly reduced in tracheal aspirate samples of infants receiving rhSOD, and clinical outcome was significantly improved at a median of 1-yr corrected age (4, 6). Critically ill infants treated with rhSOD at birth had up to a 60% decrease in episodes of severe pulmonary illness (asthma, respiratory infections), emergency room visits, and hospital readmissions by 1-yr corrected age, suggesting that AOE supplementation is effective in preventing oxidant-induced pulmonary injury. It was unclear whether the antioxidant was uniformly distributed to the lung and which cell type was primarily affected.

Pulmonary endothelial cells perform vital functions and are also vulnerable to oxidant injury. Intratracheal administration of AOE may not provide adequate protection to this site. Although endothelial cells are normally exposed to circulating AOE, SOD and catalase are rapidly cleared from the bloodstream when delivered intravenously, which could compromise attempts to fully protect the vascular endothelium against oxidative stress. Coupling AOE to polyethylene glycol or using liposome encapsulation can prolong the half-life of the active enzymes in vivo, increase bioavailability, and enhance the protective effect (8). However, concerns with toxicity have precluded the routine use of these administration strategies.

Attempts to maximize efficacy and minimize toxicity have led to the concept of vascular immunotargeting over the past decade. Investigators have conjugated AOE with antibodies directed against endothelial surface antigens such as angiotensin-converting enzyme and adhesion molecules [ICAM-1 or platelet/endothelial cell adhesion molecule (PECAM)-1]. After intravascular administration to animals, the antibody/AOE complex binds to the endothelium, enters endothelial cells, and augments their antioxidant defenses (8). Vascular immunotargeting represents a promising approach for site-specific delivery of AOE.
The report from Christofidou-Solomidou et al., one of the current articles in focus (Ref. 2, see p. L283 in this issue), provides the first in vivo evidence of the protective effect of vascular immunotargeting of AOE to endothelium in the context of pulmonary vascular oxidative stress. The investigators present novel and provocative data regarding a more specific and efficient way to deliver AOE to pulmonary endothelial cells and prevent oxidative injury. They first developed an anti-PECAM/catalase conjugate that increased catalase activity when administered to cells in vitro. They then extended these experiments to a mouse model of acute oxidant (H₂O₂) injury and found that this antibody/catalase complex localized rapidly and primarily to the pulmonary vasculature, prevented lung damage, and significantly reduced mortality by 80%, even in the most severe forms of lung injury. The animal model used in their study has many clinical and pathological features of acute lung injury/ARDS in humans and is directly produced by oxidant stress. This study also provided compelling evidence on the protective effects of AOE in oxidant-induced lung injury. The potential of using specific immunotargeting may not only be beneficial in preventing lung injury, but may also be beneficial in other forms of ROS-induced damage from ischemia-reperfusion, hyperoxia, inflammation, and environmental oxidants.

Caution must be exercised in interpreting the clinical utility of these results. The limitation of this model concerns the method of inducing oxidant injury using glucose oxidase conjugated with a thrombomodulin antibody (anti-TM/GOX), which causes damage primarily in endothelial cells. Most forms of acute and chronic lung injury in infants, children, and adults involve many cell types in the lung, including airway and alveolar epithelial cells, macrophages, and fibroblasts as well as endothelial cells. It is not clear if the anti-PECAM/AOE complex would provide similar protection for these cells. In addition, the anti-PECAM/catalase complex was administered simultaneously with anti-TM/GOX. This prophylactic administration strategy would be expected to be beneficial for those conditions in which exact onset of oxidant stress is known, such as hyperoxia and mechanical ventilation, radiation injury, and lung transplantation. As the authors point out, further studies are required to elucidate the potential of these conjugates for treating existing lung diseases such as ARDS.

As the pharmacokinetic studies demonstrate, the half-life of the anti-PECAM/catalase complex in the lung is ~1–2 h. Multiple doses may be required to prevent repeated oxidant insults, such as exposure to prolonged hyperoxia in critically ill patients. Although single-dose administration does not appear to cause pathological alterations in the lung within 2 wk, toxicity of multiple doses needs to be carefully evaluated in future in vivo studies. This is particularly important since the potential role of H₂O₂ as a second messenger in mediating growth-signaling pathways has recently been suggested. It would also be interesting to determine in future animal studies whether other anti-PECAM/AOE complexes could provide more complete protection against other forms of oxidant injuries such as hyperoxia, since cell culture models suggest that catalase alone does not prevent hyperoxic injury (9).

Although it is too early to accurately predict clinical applications, immunotargeting strategies seem to be ushering in a new “golden” era of AOE supplementation therapy.

REFERENCES


