Interactions of Oxygen Radicals with Airway Epithelium

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Reactive oxygen species (ROS) have been implicated in the pathogenesis of numerous disease processes. Epithelial cells lining the respiratory airways are uniquely vulnerable regarding potential for oxidative damage due to their potential for exposure to both endogenous (e.g., mitochondrial respiration, phagocytic respiratory burst, cellular oxidases) and exogenous (e.g., air pollutants, xenobiotics, catalase negative organisms) oxidants. Airway epithelial cells use several nonenzymatic and enzymatic antioxidant mechanisms to protect against oxidative insult. Nonenzymatic defenses include certain vitamins and low molecular weight compounds such as thiols. The enzymes superoxide dismutase, catalase, and glutatione peroxidase are major sources of antioxidant protection. Other materials associated with airway epithelium such as mucus, epithelial lining fluid, and even the basement membrane/extracellular matrix may have protective actions as well. When the normal balance between oxidants and antioxidants is upset, oxidant stress ensues and subsequent epithelial cell alterations or damage may be a critical component in the pathogenesis of several respiratory diseases. Oxidant stress may profoundly alter lung physiology including pulmonary function (e.g., forced expiratory volumes, flow rates, and maximal inspiratory capacity), mucociliary activity, and airway reactivity. ROS may induce airway inflammation; the inflammatory process may serve as an additional source of ROS in airways and provoke the pathophysiologic responses described. On a more fundamental level, cellular mechanisms in the pathogenesis of ROS may involve activation of intracellular signaling enzymes including phospholipases and protein kinases stimulating the release of inflammatory lipids and cytokines. Respiratory epithelium may be intimately involved in defense against, and pathophysiologic changes invoked by, ROS. —Environ Health Perspect 102(Suppl 10):85–90 (1994)

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Introduction

Reactive oxygen species (ROS) including free radicals (e.g., hydroxyl radical [OH•], superoxide anion [O_2^-]) and nonradicals (e.g., hydrogen peroxide [H_2O_2], hypochlorite [HOCl]) have been implicated in the pathogenesis of diseases ranging from cataracts to cancer. ROS may damage tissues through peroxidation of cell lipids, DNA strand breakage, alteration of amino acids in either structural or functional proteins, or alterations of cellular metabolism. Epithelial cells lining the respiratory airways are in a uniquely vulnerable position regarding potential for oxidative damage. In addition to potential oxidant exposure from normal aerobic cellular metabolism, these cells are exposed to relatively high oxygen tensions and are often exposed to air pollutants, phagocytic cells, catalase negative bacteria, and reactive xenobiotic-drug metabolites. Not unexpectedly, ROS have been specifically implicated in the pathogenesis of a variety of respiratory diseases including adult respiratory distress syndrome (ARDS), asthma, emphysema, and asbestosis (*1,2*).

The respiratory tract is exposed to ROS from a wide variety of sources, both exogenous and endogenous. Inhaled pollutants such as ozone (O_3), nitrogen dioxide, automobile exhaust, and cigarette smoke contain numerous oxidants (*3*). Aerobic bacteria produce ROS; those which are catalase negative (e.g., *Mycoplasma pneumoniae*) can be an additional source of exogenous H_2O_2 in infected airways (*4*). Phagocytic cells, a first line of defense against microorganisms, produce superoxide anions and related products (H_2O_2, HOCl, OH•)(*5*). While these ROS are necessary for defense of the host, they secondarily expose host tissues to damage as well (*1*). Superoxide anion also is formed nonenzymatically in epithelial cells, as in all aerobic cells, through autooxidations of mitochondrial electron transport chain constituents (*6*). Peroxisomes and other organelles contain many cellular oxidases that produce ROS (e.g., xanthine oxidase, galactose oxidase, indoleamine dioxygenase, etc.) (*7*). Hyperoxia provokes increased endogenous production of superoxide and H_2O_2 in lung cells (*8,9*); 100% O_2 increases oxygen tension in the lung to approximately 700 mm Hg, while oxygen tension in other tissues changes minimally (*1*). Finally, several xenobiotics are believed to damage lungs through production of oxygen free radicals as the compounds are metabolized (i.e., paraquat, bleomycin, nitrofurantoin) (*10–12*).

Since proteins, lipids, carbohydrates, and DNA are susceptible to oxidation, there are a number of mechanisms whereby ROS can harm cells and tissues. Cells are subject to damage through oxidation of structural or functional proteins, such as elastin, collagen, and polysaccharides (*1*). Enzymes like α-1 protease inhibitor may be altered, profoundly affecting function of the cell (*13*). Certain membrane functions may be disturbed also; oxidation of membrane sulfhydryl groups alters a variety of functions such as K^+ pumping capacity or amino acid transport ability (*14*). H_2O_2 decreases Na^+,K^+-ATPase activity independent of H_2O_2-induced ATP depletion (*15*). Membrane receptors (e.g., β-adrenoceptors) are functionally damaged after

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exposure of airway tissue to ROS *in vitro* (*16*). Oxidants also may injure cells by causing peroxidation of cellular lipids. Lipid peroxidation involves initial abstraction of a hydrogen from a polyunsaturated fatty acid by a potent oxidant, leaving a carbon with a single unpaired electron. The lipid radical then reacts with oxygen to form lipid peroxyl radicals (also strong oxidants) that then extract a second hydrogen from another methylene carbon. This reaction continues in a chainwise manner converting carbons of membrane phospholipids to hydroperoxides (*17*). This type of reaction would certainly damage cell membranes and also produce reaction products like aldehydes, which are themselves toxic to cells (*6*). H_2O_2 can be especially harmful as it can rapidly permeate cells and inhibit ATP synthesis through glycolytic and oxidative phosphorylation pathways (*18*). Oxidants also harm cells through damage to DNA (*19*). Oxidants may induce base hydroxylation and strand breaks that lead to cell death or malignant transformation (*18*). ROS can also exacerbate inflammation. They can produce potent chemoattractants to recruit inflammatory cells that in turn produce more ROS (*20*). ROS also increase local production of inflammatory eicosanoids (*21*). These effects can lead to epithelial cell shedding and loss (*13,22,23*).

Respiratory tract tissues are more vulnerable to the deleterious effects of ROS than are many other tissues. The respiratory system is designed for gas exchange; air flows from the nasal passages to the large conducting airways, then to the smaller airways, and finally into alveoli where the actual exchange takes place. All of these surfaces are lined by epithelial cells. Respiratory epithelial cells are exposed to high oxygen tensions, and they are the first cells to contact inhaled oxidants. The epithelial cells also are exposed to ROS generated by inflammatory reactions, xenobiotic metabolism, and endogenous production. For these reasons epithelial cells may be a first line of defense for the respiratory tract in protection from oxidants. When the normal balance between oxidants and antioxidants is upset, oxidant stress ensues and subsequent epithelial damage may be a crucial component in the pathogenesis of several respiratory diseases.

Antioxidative Protection by Airway Epithelium

As exposure to potentially damaging ROS is unavoidable for aerobic organisms, a complex set of antioxidant defenses have developed. Cells contain many antioxidants, some of which are nonenzymatic radical scavengers, others that prevent radical formation, and others that enzymatically transform ROS. The most important antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione cycle enzymes such as glutathione peroxidase (GPO). SOD catalyzes the dismutation of superoxide anion to oxygen and hydrogen peroxide (H_2O_2); this same reaction also occurs spontaneously, but the rate is greatly increased by SOD. The latter two enzyme systems are used in cell defense against the common oxidant, H_2O_2. Catalase actually converts H_2O_2 to water and oxygen. The glutathione cycle lets H_2O_2 (or organic hydroperoxides) react with reduced glutathione in the presence of glutathione peroxidase to form water (or an alcohol) and glutathione disulfide.

Nonenzymatic Antioxidant Defenses

Respiratory epithelium may use several antioxidant strategies. Nonenzymatic epithelial antioxidants include both small and large molecular weight compounds. The low molecular weight antioxidants include vitamins E and C, β-carotene, uric acid, thiols, and taurine (*24*). Vitamin E is a lipophilic chain-breaking antioxidant; it acts by stopping the chain reaction involved in lipid peroxidation (*17*). Carotenoids and vitamin C are more hydrophilic and may quench radicals in the nonlipid cellular compartments (*17*) [vitamin C can also act as a prooxidant (*2*)]. The hydrophilic molecule urate has both chain-breaking and preventive (by stabilizing vitamin C) antioxidant activity (*17*). Thiols and taurine are largely hydrophilic chain-breaking antioxidants, protecting critical sulfhydryl groups on proteins (*17*). Larger molecular weight antioxidants include lactoferrin, albumin, ceruloplasmin, and transferrin (*25*). Several of these compounds act by binding heavy metals, making them unavailable for production of free radicals (i.e., participation in the Fenton reaction to produce hydroxyl radical) or participation in lipid peroxidation.

Enzymatic Antioxidant Defenses

Tracheal, bronchial, and alveolar epithelium possess enzymatic antioxidants. These cells are capable of rapidly scavenging exogenous H_2O_2. In both tracheal and alveolar epithelial cells, CAT seems quantitatively the more important of the H_2O_2 scavenging enzymes at concentrations of $H_2O_2 > 10^{-4}$ M [(*26–28*); LA Cohn et al., submitted]. Inhibition of CAT significantly inhibits the ability of tracheal and alveolar epithelial cells to consume H_2O_2, while inhibition of GPO does not. This does not mean that GPO is not an important epithelial protector: inhibition of GPO does potentiate the damage inflicted on epithelial cells by exogenous H_2O_2 (*26,28–30*). Epithelial cells also possess two forms of SOD. The Cu,Zn form of SOD is the more potent of the two and is primarily cytosolic and nuclear in location; this form is constitutive in expression (*31*). The manganese form of SOD is located in the mitochondria and is inducible in expression (*31*). There is evidence that mitochondrial Mn-SOD can be up-regulated by ROS or cytokines such as TNF, interleukin 1 (IL-1), and IL-6 (*32–36*). The ability to increase levels of this cellular antioxidant may protect epithelium in situations where inflammation and subsequent increases in ROS are likely (*37*). Increases in Mn-SOD would more likely be protective in situations where endogenous ROS pose more of a threat than exogenous ROS, such as hyperoxia (*38,39*).

Epithelium-associated Antioxidants

Materials closely associated with airway epithelial cells may contribute to antioxidant defense. Epithelial lining fluid (ELF) is a thin layer of locally secreted substances and plasma ultrafiltrate that bathe the epithelial surface. This fluid has marked antioxidant protective effects, mostly due to CAT, released by the normal turnover of airway epithelium (*40*). Other antioxidants in ELF include SOD, GPO, GSH, vitamin C, vitamin E, transferrin, and ceruloplasmin (*40–42*). Airway submucosal gland secretions also contain antioxidants; in human secretions uric acid is especially important (*43*). Mucus also is known to have antioxidant capabilities (*44*). The sugar moieties in mucus may act similarly to mannitol and glucose in scavenging hydroxyl radical and H_2O_2 (*45,46*). Mucus glycoproteins react with H_2O_2; in the process both H_2O_2 and glycoproteins are degraded (*44,47,48*). In our studies with guinea pig primary tracheal epithelial cells (GPTE), removal of the apical mucus layer reduced the efficiency of H_2O_2 scavenging (LA Cohn et al., submitted). The extracellular matrix of the airway epithelium also may contribute to epithelial antioxidant protection. Matrix material remaining on collagen-coated filters after GPTE cells were removed retained a marked ability to scavenge exogenous H_2O_2 (LA Cohn et al., submitted). Extracellular

antioxidant enzymes could be contained within the ECM. For example, Coursin et al. used immunostaining to show dense deposits of GPO in lung connective tissue (*49*), and lung extracellular matrix contains SOD as well (*50*). H_2O_2 could also be degraded through interactions with low molecular weight molecules like GSH which may be localized to the ECM, or it could be consumed by nonspecific interactions with matrix molecules such as glycosaminoglycans, laminin, heparin sulfate proteoglycan, etc.

Functional Responses of Airway Epithelium to Oxidant Stress

This section describes the effects of oxidant stress (a shift in the prooxidant/antioxidant balance toward oxidation) on airway epithelial cells. ROS have been shown to elicit effects on the lung by decreasing overall pulmonary function. Respiratory airway inflammation incited by oxidant stress may promote the pathogenesis of pulmonary dysfunction. Second messenger products and feedback pathways may mechanistically link ROS to the observed functional responses at molecular and cellular levels.

Pulmonary Function

Oxidant stress, especially due to oxidant air pollutants, has been observed to profoundly affect pulmonary physiology. Many of these pathophysiologic effects may be mediated by products released from airway epithelial cells either by pathophysiologic stimuli or by cell damage. O_3 induces transient changes in human lung function, including decreased forced expiratory volumes and flow rates (*51*), stimulation of the tracheobronchial mucociliary system (*52*), increased nonspecific airway reactivity, increased specific airway resistance, increased respiratory frequency on exercise, and an involuntary reduction of maximal inspiratory capacity (*53*). It should be noted that O_3 decreases mucociliary transport in sheep (*54*) and other species in contrast to the human data mentioned above. Hazucha and associates (*53*) explain the measured responses and increases in airway reactivity to bronchoconstrictor challenge by O_3 stimulating, and maybe sensitizing, airway sensory fibers.

Hypersecretion of respiratory mucus is associated with the pathogenesis of several airway diseases including asthma, acute and chronic bronchitis, and cystic fibrosis (*55*). Oxidant stress can increase secretion of respiratory mucin and depress ciliary beating efficiency, diminishing the ability of the mucociliary system to clear potentially pathogenic agents. O_3 and sulfur dioxide provoke mucus hypersecretion in the respiratory airways (*55*). Studies in our laboratory have shown that enzymatically generated oxidants (purine/xanthine oxidase [P/XO]) provoke hypersecretion of respiratory mucins in GPTE cells [(*56*); CM Li, unpublished observation]. Ciliated epithelial cell necrosis has been observed after O_3 exposure in rats (*57*). Sulfur dioxide causes airway hyperreactivity (*58*) and has been reported to damage ciliated airway epithelial cells and decrease mucociliary transport in dogs (*59*). Nitrogen dioxide decreases mucociliary transport and functional residual capacity in dogs (*60*). H_2O_2 reversibly inhibits airway ciliary activity (*61*).

Pulmonary dysfunction associated with exposure to ROS may involve direct effects on airway epithelial cells causing release of mediators, or may result from damage to these cells. Epithelial-derived mediators may act through autocrine or paracrine pathways to affect neighboring cells and tissues, i.e., mediators may produce the mechanical responses by acting on airway smooth muscle or nerve endings. Damage to, or destruction of, ciliated epithelial cells would obviously decrease mucociliary clearance function.

Airway Inflammation

A complex sequence of events is involved in the inflammatory process, each step of which can have detrimental effects on the lung and its host defense functions (*62*). Although pulmonary function changes are transient, there is strong evidence that airway inflammation has a more prolonged time course. Airway inflammation, assessed by several parameters (including inflammatory lipid mediators and cytokines), has been well documented in studies of bronchoalveolar lavage fluid (BALF) of humans and animals exposed to O_3. Human BALF (obtained by several different O_3 exposure protocols) exhibits increases in number of neutrophils, eicosanoid production [prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, and thromboxane B_2 (TxB_2)], indices of airway permeability (protein, albumin, and immunoglobulin G), fibrogenic process activation (tissue factor, Factor VII, urokinase plasminogen activator, and fibronectin), the cytokine IL-6, and the complement component C3a (*62,63*). O_3 induces transient increases in the expression of IL-6 and IL-8 mRNAs and increases release of fibronectin, IL-6, and IL-8 in virally transformed human bronchial epithelial (BEAS-2B) cells *in vitro* (*64*). Mineral dust particles such as quartz, asbestos, and silica induce pulmonary inflammation and activate immune cells to produce ROS. Some mineral dusts even contain endogenous radicals (*65*).

Airway inflammation resulting from diverse stimuli provokes hypersecretion of mucus (*55*). P/XO stimulates mucus hypersecretion by a mechanism involving cyclooxygenase metabolism of arachidonic acid (AA) to $PGF_{2\alpha}$ (*56*). Decreased epithelial barrier function causes edema that may contribute to hypersecretion of mucus by exposing airway secretory cells to plasma secretagogues (*55*). Increased epithelial cell paracellular permeability (decreased barrier function) has been reported in *a*) pulmonary epithelium of dogs exposed to sulfur dioxide (*59*) and nitrogen dioxide (*60*); *b*) Madin Darby canine kidney (MDCK) epithelial cells exposed to H_2O_2, glucose/glucose oxidase, and xanthine/XO (*66*); *c*) ferret tracheal epithelium exposed to xanthine/XO (*67*); and *d*) rat alveolar epithelium exposed to H_2O_2 (*15*). P/XO also induces permeability edema in isolated perfused rabbit lungs (*68*).

Airway inflammation can be induced by exposure to ROS or alternatively ROS can be produced by resident (epithelial cells and macrophages) or nonresident (neutrophils) pulmonary cells during inflammatory events. Inflammatory processes in the airways may incite some or all of the changes in pulmonary function discussed in the previous section.

Second Messenger Pathways

On a more fundamental level, the mechanism(s) of oxidant toxicity may involve activation of second messenger pathways in pulmonary target cells. Exposure of airway epithelial cells to oxidant stress can affect synthesis and release of lipid mediators, such as eicosanoids and platelet activating factor (PAF), by a mechanism involving activation of cellular phospholipases: phospholipase A_2 (PLA_2), phospholipase C (PLC), and phospholipase D (PLD).

Eicosanoids and PAF are bioactive substances with potent proinflammatory actions. They may activate other cells by binding to cell surface receptors and inducing second messenger formation (*69*). *t*-Butyl hydroperoxide (*t*-BOOH) activates PLA_2, without requiring new protein synthesis, and stimulates cyclooxygenase metabolism of the released AA in bovine

pulmonary endothelial cells (*70*). AA and eicosanoids are released by BEAS-2B cells after *in vitro* O_3 exposure (*71,72*). O_3 exposure also has been shown to increase release of PAF by several different pulmonary cell types (*72*). H_2O_2 liberates saturated and unsaturated fatty acids, activates PLA_2 and PLC, and impairs incorporation of unesterified fatty acids into complex lipids (ATP-dependent reacylation) in bovine pulmonary endothelial cells (*73*). *t*-BOOH regulates the activity of cyclooxygenase and lipoxygenases in rabbit platelets: low concentrations enhance formation of TxB_2 and hydroxyheptadecatrienoic acid (HHT), while relatively higher concentrations inhibit their formation (*74*).

PLC-mediated second messenger pathways regulate a large variety of cellular processes including metabolism, secretion, contraction, neural activity, and cell proliferation (*75*) and may also mediate chemotaxis and other aspects of inflammation. ROS generated by P/XO increase the synthesis of $G\alpha_{q/11}$, a GTP-binding ("G") protein connected with phosphoinositide-specific PLC (PLCβ) signaling (H Li, unpublished observation). H_2O_2 increases intracellular calcium, and activates PLA_2 and PLC in rat alveolar epithelial cells (*76*). O_3 activates PLA_2, PLC, and PLD and increases release of PAF by GPTE cells (DT Wright et al., submitted) and P/XO activates PLA_2 and PLC [(*56*); DT Wright, unpublished observation].

Protein kinase C (PKC) plays a critical role in many aspects of cell metabolism, activation, and proliferation (*77*). PAF stimulates mucus secretion through a lipoxygenase-dependent pathway in GPTE cells (*78*) and a PKC-zeta-mediated pathway in feline tracheal epithelial cells (*79*). GPTE cells exposed to PAF (or phorbol esters) release H_2O_2 through a mechanism involving activation of PKC (*80*). P/XO stimulates PKC translocation and activation in GPTE and BEAS-2B cells (B Fischer, unpublished observations). PKC can be activated by ROS (produced upon UVB radiation) without prior activation of PLC or PLA_2 in human platelets (*81*). H_2O_2 has been shown to reversibly inhibit ciliary activity in sheep airways by a mechanism involving activation of second messenger pathways including PKC (*61*). H_2O_2 increases epithelial permeability in MDCK cells via a mechanism that involves activation of PLC and consequently PKC (*82,83*)

It has been well documented that several cell types possess PLD activity that can be stimulated by various inflammatory mediators (*84–86*). H_2O_2 and linoleic acid hydroperoxide activate PLD, independent of PKC activity, in bovine pulmonary endothelial cells (*87*).

ROS may exert their effects on airway epithelial cells by a mechanism involving either direct oxidation of phospholipases (perhaps even associated cell surface receptor proteins or G proteins), or peroxidative changes in membrane lipids surrounding these signal transducing elements. Phospholipases, especially the acyl hydrolase phospholipase A_2 and the phosphodiesterases PLC and PLD, are ubiquitous membrane lipid-hydrolyzing enzymes that are critical elements of stimulus-response coupling. Alterations in membrane phospholipid metabolism may be central to oxidant-mediated cell injury as the liberation of AA and eicosanoids has been observed frequently and lysophospholipids act as biological detergents which are lytic to membranes (*88*). Second messengers generated by these phospholipase pathways in response to oxidant stress may act through autocrine or paracrine mechanisms to evoke numerous cellular alterations including inflammation (increased epithelial permeability, eicosanoid and PAF release, and infiltration of inflammatory cells) and functional responses such as increased secretion of mucus.

Conclusions

Airway epithelial cells are in a uniquely vulnerable position regarding exposure to ROS from both endogenous and exogenous sources. They are equipped with a complex array of nonenzymatic and enzymatic antioxidant defenses to combat these constant oxidative insults. When the delicate balance between oxidant burden and antioxidant defense is overwhelmed, oxidant stress ensues. Effects of this oxidant stress on airway epithelium are diverse. Significantly, second messenger systems appear to be activated, relating to increased airway inflammation and possibly leading to numerous pulmonary function impairments. Oxidant stress therefore may be important in the pathogenesis of numerous respiratory diseases.

Mechanistically, oxidant stress may contribute to initiation and/or propagation of lung disease. Pulmonary injury may be mediated by sustained hyperoxia (*1*), oxidant air pollutants (gaseous and particulate) (*7*), and certain xenobiotic toxins (e.g., paraquat) (*7*). Other pulmonary diseases whose pathology is strongly associated with ROS (but that are not necessarily initiated by ROS) include ARDS (*89*), emphysema (*1*), and idiopathic pulmonary fibrosis (*24*). ROS also may propagate the overall respiratory disease process in conditions such as asthma (*13*), cystic fibrosis (*24*), and human immunodeficiency virus infection (*90*).

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