

Oxidant/antioxidant imbalance in smokers and chronic obstructive pulmonary disease

An imbalance between oxidants and antioxidants has been proposed in the pathogenesis of chronic obstructive pulmonary disease (COPD).¹ Although much research has focused on the protease/antiprotease theory of the pathogenesis of COPD, particularly emphysema, less attention has been paid to the role of the oxidant/antioxidant imbalance in this condition. This is surprising since cigarette smoke, which is the major risk factor for the development of COPD, contains 10^{17} oxidant molecules per puff of which 10^{14} are oxygen free radicals.² There is a delicate balance between the toxicity of oxidants and the protective function of the intracellular and extracellular antioxidant defence systems which is critically important for the maintenance of normal pulmonary cellular functions.³ In this editorial we will examine the oxidant/antioxidant imbalance in smokers and patients with COPD to determine if there is enough evidence to warrant therapeutic trials with antioxidant therapy in patients with COPD.

Cigarette smoke is a complex mixture of over 4700 chemical compounds including high concentrations of oxidants and free radicals present in both the gas phase and the tar phase of cigarette smoke.^{2,4} Those in the gas phase are both organic and inorganic including reactive oxygen species and free radicals, aldehydes, peroxides and oxides of nitrogen. In the tar phase radicals are stable and predominantly organic, such as semiquinone which can react to produce superoxide anion (O_2^-).

Short lived radicals in the gas phase of cigarette smoke may be quenched immediately in the epithelial lining fluid (ELF); however, redox reactions in cigarette smoke condensate may produce reactive oxygen intermediates for a considerable time.⁵ In normal lungs there are 50-70 inflammatory cells per alveolus; more than 80% are alveolar macrophages and less than 1% neutrophils.⁶ In cigarette smokers the numbers of alveolar macrophages increase by at least 2-4 times and neutrophils by 10 times.⁷ In vitro studies have shown that alveolar leucocytes and macrophages from cigarette smokers spontaneously release increased amounts of oxidants such as O_2^- and hydrogen peroxide (H_2O_2) compared with non-smokers.^{8,9} It has also been suggested that neutrophils sequestered in the pulmonary circulation are primed to release oxidants in cigarette smokers.^{10,11} Passive cigarette smoking has also been associated with increased peripheral blood leucocyte counts which show enhanced release of oxidants.¹²

Oxidants, whether inhaled or generated from leucocytes, can inactivate the major antiprotease in the airways - alpha₁-proteinase inhibitor (α_1 PI) - by oxidation of its active site.^{13,14} This diminishes the binding of α_1 PI to elastase, hence reducing its inactivation and allowing it to bind to and destroy elastin, leading to emphysema.¹⁵ Although this hypothesis is supported by in vitro studies,¹³ it has been more difficult to demonstrate convincingly oxidative inactivation of α_1 PI in vivo.¹⁴ Other antiproteases which may have a protective role against proteolytic attack and hence may prevent the development of emphysema, such as antileucoprotease, can also be inactivated by oxidants.¹⁶ Components of the lung matrix - for example, elastin and collagen - can be directly damaged or fragmented by oxidants in cigarette smoke.¹⁵ Cigarette smoke-derived oxidants also decrease the deformability of circulating neutrophils¹⁷ leading to increased neutrophil sequestration in

the pulmonary microvasculature.¹⁰ Inhalation of cigarette smoke in hamsters activates neutrophils, increasing their adhesion to the endothelium of both arterioles and venules.¹⁸ This increased neutrophil adhesion is mediated by superoxide anion in cigarette smoke, since it is inhibited by pretreatment with copper/zinc superoxide dismutase.¹⁸ Neutrophils sequestered in the pulmonary circulation following cigarette smoke inhalation in the rabbit show increased expression of CD18 integrins,¹⁹ although this effect has not been demonstrated following in vitro exposure.²⁰ Thus, by several mechanisms involving oxidants, cigarette smoke causes neutrophil sequestration in the pulmonary microcirculation¹⁰ and hence their migration into the airspaces in smokers. These migrated cells remain primed to release more reactive oxygen intermediates.^{11,12}

All tissues are vulnerable to oxidant damage but, by virtue of its location, the airspace epithelium is particularly vulnerable.²¹ Reactive oxygen species may influence airway cells in a number of ways. Oxygen radicals generated close to a cell membrane oxidise membrane phospholipids (lipid peroxidation), a process which may continue in a chain reaction. Lannan and colleagues²² showed an oxidant-mediated cytotoxicity of human alveolar epithelial cells with decreased epithelial cell adherence and increased detachment and lysis following exposure to cigarette smoke condensate. Li *et al*²³ demonstrated that the deleterious effects of cigarette smoke condensate on epithelial permeability in vitro in epithelial cell monolayers and in vivo in rat lungs were associated with changes in the homeostasis of the antioxidant glutathione. These in vitro and animal studies are supported by human studies of increased epithelial permeability in smokers, shown by increased technetium-99m labelled diethylenetriamine pentaacetate (^{99m}Tc-DTPA) lung clearance.²⁴ Preliminary data by Morrison and coworkers showed a further increase in DTPA clearance following acute smoking.²⁵

There are technical difficulties in measuring specific markers of oxidative injury. The only definitive way to demonstrate excessive free radical activity is by electron spin resonance which cannot be applied to the study of tissues at present. Instead, investigators rely on indirect measurements of free radical activity in biological fluids such as measurements which assess oxidative damage to lipids, proteins, or DNA. These markers indicate that oxidative damage has occurred, but not that this event is involved in the pathogenesis of the condition.

A major site of free radical attack is on polyunsaturated fatty acids in cell membranes, producing lipid peroxidation which generates hydroperoxides and long lived aldehydes. The end products of these reactions are malondialdehyde, ethane, and pentane. Levels of lipid peroxides in plasma and bronchoalveolar lavage fluid, measured as thio-barbituric acid reactive substances (TBARS), are significantly increased in healthy smokers and patients with acute exacerbations of COPD compared with healthy non-smokers.²⁶⁻²⁸ There is, however, a problem with the specificity of the assays used to measure the metabolites of lipid peroxide reactions.

Evidence for increased oxidative stress in COPD is emerging. Patients with COPD have increased numbers of activated intravascular and airspace neutrophils and macrophages which release more O_2^- than those from

healthy controls.²⁹⁻³¹ Postma and co-workers³² showed a correlation between O₂⁻ release by peripheral blood neutrophils and bronchial hyperreactivity in patients with COPD. Rahman and colleagues²⁶ found increased superoxide anion production from peripheral blood neutrophils obtained from patients with acute exacerbations of COPD. The presence of inflammatory cells capable of inducing an oxidative stress may therefore have a role in the inflammation and airway injury in patients with COPD.

Preliminary data by Rahman and colleagues²⁴ used an assay which compared the antioxidant capacity of plasma with the vitamin E analogue Trolox to measure the Trolox equivalent antioxidant capacity (TEAC). They found that the plasma TEAC was significantly decreased in smokers one hour after smoking and in patients with acute exacerbations of COPD when compared with plasma from age-matched non-smoking controls. Albumin, which has one sulphhydryl group per molecule, may form albumin-thiol radicals or albumin-SH group conjugates with electrophils present in cigarette smoke. Thus, a profound depletion of plasma protein sulphhydryls was shown following cigarette smoke exposure *in vitro*³³ which may account for the fall in antioxidant capacity in plasma after smoking. Furthermore, a relationship has been shown between a deficiency in the antioxidant capacity of plasma and the presence of a family history of lung disease.¹

Many investigators have measured the major plasma antioxidants (ascorbic acid, α -tocopherol, uric acid, and sulphhydryls) in smokers.^{34,35} These studies show a depletion of ascorbic acid, vitamin E, β -carotene, and selenium in the serum of chronic smokers. However, circulating red blood cells from cigarette smokers contain increased levels of superoxide dismutase and catalase but similar glutathione peroxidase activity and are more capable of protecting endothelial cells from the effects of hydrogen peroxide than cells from non-smokers.³⁶ Galdston and coworkers³⁷ found that an indirect measurement of the serum antioxidant activity was decreased in cigarette smokers. They also showed that increased levels of the antioxidant ceruloplasmin occurred in the serum of cigarette smokers. However, these levels did not prevent the inhibition of the antiprotease activity of α_1 PI in smokers.

Cross and coworkers³³ demonstrated that exposure to the gas phase of cigarette smoke *in vitro* caused loss of plasma protein sulphhydryls, bilirubin, and ascorbic acid, whereas uric acid and α -tocopherol were not affected. The depletion of plasma antioxidants was associated with increased levels of protein carbonyls and lipid peroxides.³⁷ Ascorbate may be a particularly important antioxidant in plasma since whole cigarette smoke induces lipid peroxidation in plasma *in vitro* which is inhibited by ascorbate.³⁸ It has also been shown that protein thiols and ascorbic acid inhibit protein carbonyl formation in plasma following cigarette smoke exposure, whereas bilirubin, uric acid, and α -tocopherol do not.³³ Nitric oxide, which is present in the gas phase of cigarette smoke, reacts with tyrosine to form nitrotyrosine products of proteins in plasma *in vitro*.³⁹ These products can interfere with cell signalling pathways involving tyrosine phosphorylation. Products of nitrotyrosination have been implicated in many lung diseases.⁴⁰

There is limited information on the antioxidant defences of the respiratory tract epithelial lining fluid in smokers and even less for patients with COPD. The important thiol antioxidant glutathione (GSH) is increased in the epithelial lining fluid in the airways of chronic smokers⁴¹ which is related to humeral markers of inflammation.⁴² The reason for the increase in concentration of GSH in the bronchoalveolar lavage fluid of smokers is not known, but it may be due to cell rupture leading to passive release of

GSH into the extracellular space, specific triggers to cells such as type II pneumocytes or macrophages to increase synthesis and release of GSH, or to plasma exudation as a result of increased alveolar-capillary permeability due to inflammation. Despite the twofold increase in GSH concentrations found in the bronchoalveolar lavage fluid of chronic smokers, GSH may not be present in sufficient quantities to deal with the excessive oxidant burden during acute smoking when acute depletion of GSH may occur.^{23,25}

Rahman and colleagues⁴³ studied the acute effects of cigarette smoke condensate (CSC) on GSH metabolism in a human alveolar epithelial cell line *in vitro* and *in vivo* in rat lungs after intratracheal CSC instillation. They found a dose and time-dependent depletion of intracellular soluble GSH, concomitant with the formation of GSH conjugates, without a significant increase in levels of oxidised GSH (GSSG); these results are supported by studies *in vivo* in animal lungs.⁴⁰ The activities of glutathione synthesis and redox system enzymes such as glutathione peroxidase, gamma-glutamylcysteine synthetase, and glucose-6-phosphate dehydrogenase were transiently decreased in alveolar epithelial cells and in rat lungs after exposure to CSC, possibly as a result of the action of highly electrophilic free radicals on the active site of the enzymes.

Pacht and coworkers⁴⁴ showed reduced levels of vitamin E in the bronchoalveolar lavage fluid of smokers compared with those in non-smokers. By contrast, alveolar macrophages from smokers have both increased levels of ascorbic acid and augmented uptake of ascorbate.⁴⁵ Similarly, Bui and colleagues⁴⁶ found a marginal increase in vitamin C in the bronchoalveolar lavage fluid of smokers compared with non-smokers. Enhanced activities of the antioxidant enzymes superoxide dismutase and catalase in alveolar macrophages from young smokers has also been demonstrated.⁴⁷ However, Kondo and coworkers⁴⁸ showed that the increased superoxide generation by alveolar macrophages in elderly smokers was associated with decreased antioxidant enzyme activities compared with non-smokers. The activities of copper/zinc superoxide dismutase, glutathione-S-transferase, and glutathione peroxidase are also decreased in alveolar macrophages from elderly smokers. These lower activities were not associated with decreased gene expression but were due to modification at the post-translational level.⁴⁸

The apparent discrepancies between these studies of different antioxidants in epithelial lining fluid may be due to different smoking histories in chronic smokers, particularly the time relationship to the last cigarette. Activities of superoxide dismutase and glutathione peroxidase are also higher in the lungs of rats exposed to cigarette smoke.⁴⁹ McCusker and Hoidal⁴⁷ demonstrated enhanced alveolar macrophage antioxidant enzyme activities following cigarette smoke exposure which resulted in reduced mortality when the hamsters were subsequently exposed to >95% oxygen. They speculated that mammalian alveolar macrophages undergo an adaptive response to chronic oxidant exposure that may ameliorate potential damage to lung cells from oxidant stress. The mechanisms of the induction of antioxidant enzymes in erythrocytes,³⁴ alveolar macrophages,⁴⁷ and lungs⁴⁹ by cigarette smoke exposure are not known. However, the induction of anti-oxidant genes is presumed to be involved. We have recently shown in preliminary studies that cigarette smoke increased the expression of gamma-glutamylcysteine synthetase mRNA in human alveolar epithelial cells.⁵⁰

Based on accumulating evidence that oxidants/free radicals play an important part in the pathogenesis of COPD, enhancing the pulmonary antioxidant capacity may therefore be of potential therapeutic benefit in this condition. This can be achieved in two ways – either by enhancing

endogenous antioxidant enzymes to limit the generation of free radicals or by increasing non-enzymatic antioxidants which can detoxify reactive oxygen species once they are formed. A further possible intervention is to reduce the recruitment or the activation of inflammatory cells in the lungs, so limiting the production of reactive oxygen intermediates. However, it would be premature to recommend antioxidant therapy in COPD before we have a greater knowledge of the nature of free radicals/oxidants involved and their mechanisms in the pathogenesis of COPD. Additionally, it is important to determine the mechanism of antioxidant depletion and, in particular, to establish which antioxidants are depleted in biological fluids in patients with acute COPD to enable a logical strategy for antioxidant therapy to be developed.

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