Oxidative stress in asthma and COPD: Antioxidants as a therapeutic strategy

Paul Kirkham *, Irfan Rahman

Respiratory Diseases, Novartis Institutes for Biomedical Research, Horsham, West Sussex, RH12 5AB, UK
Department of Environmental Medicine, Division of Lung Biology and Disease, University of Rochester Medical Center, Rochester, NY, USA

Abstract

Asthma and chronic obstructive pulmonary disease (COPD) are inflammatory lung diseases that are characterized by systemic and chronic localized inflammation and oxidative stress. Sources of oxidative stress arise from the increased burden of inhaled oxidants, as well as elevated amounts of reactive oxygen species (ROS) released from inflammatory cells. Increased levels of ROS, either directly or via the formation of lipid peroxidation products, may play a role in enhancing the inflammatory response in both asthma and COPD. Moreover, in COPD it is now recognized as the main pathogenic factor for driving disease progression and increasing severity. ROS and lipid peroxidation products can influence the inflammatory response at many levels through its impact on signal transduction mechanisms, activation of redox-sensitive transcriptions factors, and chromatin regulation resulting in pro-inflammatory gene expression. It is this impact of ROS on chromatin regulation by reducing the activity of the transcriptional co-repressor, histone deacetylase-2 (HDAC-2), that leads to the poor efficacy of corticosteroids in COPD, severe asthma, and smoking asthmatics. Thus, the presence of oxidative stress has important consequences for the pathogenesis, severity, and treatment of asthma and COPD. However, for ROS to have such an impact, it must first overcome a variety of antioxidant defenses. It is likely, therefore, that a combination of antioxidants may be effective in the treatment of asthma and COPD. Various approaches to enhance the lung antioxidant screen and clinical trials of antioxidant compounds are discussed.
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* Corresponding author. Respiratory Diseases, Novartis Institutes for Biomedical Research, Horsham, West Sussex, RH12 5AB, UK. Tel.: +44 1403 323844; fax: +44 1403 323837.
E-mail address: paul.kirkham@novartis.com (P. Kirkham).

1. Introduction

For many years, the science of free radicals was the preserve of physical and inorganic chemists. However, when it came to the impact of free radicals in biology, many at first did not foresee the impact it could have on disease pathology. It is only relatively recently, in the last 20 years, that free radicals in the form of reactive oxygen species (ROS) have become increasingly recognized as playing a major role in many disease processes. ROS such as superoxide anion ($O_2^{\bullet-}$) and the hydroxyl radical ($^{\bullet}OH$) are unstable molecules with unpaired electrons, capable of initiating oxidation. This can result in the oxidation of proteins, DNA, and lipids that may cause direct tissue injury or induce a variety of cellular responses, through the generation of secondary metabolic reactive species. The lung exists in a high-oxygen environment and together with its large surface area and blood supply is highly susceptible to injury mediated by oxidative stress. Consequently, the lung contains many antioxidant defenses in order to protect itself from oxidant-induced tissue damage.

ROS can be generated either endogenously by metabolic reactions, such as from mitochondrial electron transport during respiration or during activation of circulating inflammatory cells or phagocytes, and exogenously from air pollutants or cigarette smoke. As a result, increased levels of ROS have been shown to affect the extracellular environment impacting on a variety of physiological processes (Richter et al., 1995; Gutteridge & Halliwell, 2000). In addition, ROS can initiate inflammatory responses in the lungs through the activation of redox-sensitive transcription factors (Guyton et al., 1996; Rahman & MacNee, 1998). It is proposed that ROS produced by phagocytes that have been recruited to sites of inflammation is a major cause of the cell and tissue damage associated with many chronic inflammatory lung diseases including asthma and chronic obstructive pulmonary disease (COPD) (Hatch, 1995; Rahman & MacNee, 1996; Rahman & MacNee, 1999; Dworski, 2000; Rahman & MacNee, 2000). However, the composition of inflammatory cell types varies widely in asthma and COPD and this could account for the differences in ROS production as well as the pathophysiology between these 2 diseases (Jeffery, 1998; Saetta, 1999; Barnes, 2000).

This review discusses the impact of ROS and the role it plays in the pathogenesis of asthma and COPD. Moreover, it also highlights the antioxidant mechanisms in place to protect against the damaging affects of ROS. Finally, it goes on to explore possible therapeutic approaches that involve the use of a variety of antioxidants.

2. Reactive oxygen species: from production to molecular impact

2.1. Production

There are essentially 2 sources of reactive oxygen species (ROS) that the lungs are exposed to, environmental and cellular (Fig. 1). Environmental-derived ROS consists of both gaseous and particulate air pollution. This ranges from cigarette smoke and oxidant gases, such as ozone, nitrogen dioxide, and sulphur dioxide, to airborne particulate matter <10 μm (PM10) from diesel car exhaust fumes that can promote ROS production in situ (Donaldson et al., 1997). As will be seen later, of these, the single most important etiological factor in driving the pathogenesis of COPD is cigarette smoke. It contains over 4700 chemical compounds and high concentrations of oxidants (10^{14} molecules/puff) and 3000 ppm NO/puff (Church & Pryor, 1985). The nature of ROS found within cigarette smoke varies from short lived oxidants, such as the superoxide radical ($O_2^{\bullet-}$) and the nitric oxide molecule (NO), to long lived organic radicals, such as semiquinones that can undergo redox cycling within the epithelial lining fluid of smokers for some considerable period of time (Nakayama et al., 1989; Zang et al., 1995).

Whereas environmental-derived ROS is by definition from an external source, cellular-derived ROS is enzymatically produced by inflammatory and epithelial cells (Rochelle et al., 1998) within the lung as part of an inflammatory-immune response towards a pathogen or irritant. Several sources for ROS production exist within a cell and include mitochondrial respiration, NADPH oxidase, and xanthine/xanthine oxidase system, of which the principle ROS generator is NADPH oxidase (Fig. 1). This results in the release of $O_2^{\bullet-}$, by activated cells, such as macrophages or eosinophils for example. Here the $O_2^{\bullet-}$ radical can either react with NO to form highly reactive peroxynitrite molecule ($ONOO^-$) or alternatively be rapidly converted to H_2O_2 under the influence of superoxide dismutase (SOD). This in turn can result in the non-enzymatic production of the more damaging hydroxide radical ($^{\bullet}OH$) from H_2O_2 in the presence of Fe^{2+} through the Fenton reaction (see Fig. 2). This leads to the oxidation of Fe^{2+} to Fe^{3+}, which in turn can also generate $^{\bullet}OH$ direct from $O_2^{\bullet-}$ and regenerate Fe^{2+} through the Haber–Weiss reaction (Halliwell & Gutteridge, 1990a). Redox cycling of Fe^{2+} and Fe^{3+} can therefore rapidly result in the formation of the more damaging hydroxide radical ($^{\bullet}OH$) from the initial supply of $O_2^{\bullet-}$. This can be particularly relevant to COPD where smokers have been found to have significantly higher levels of iron in their lungs thereby increasing the potential ROS burden (Ghio et al., 1997). Macrophages also employ other enzymes to produce ROS.
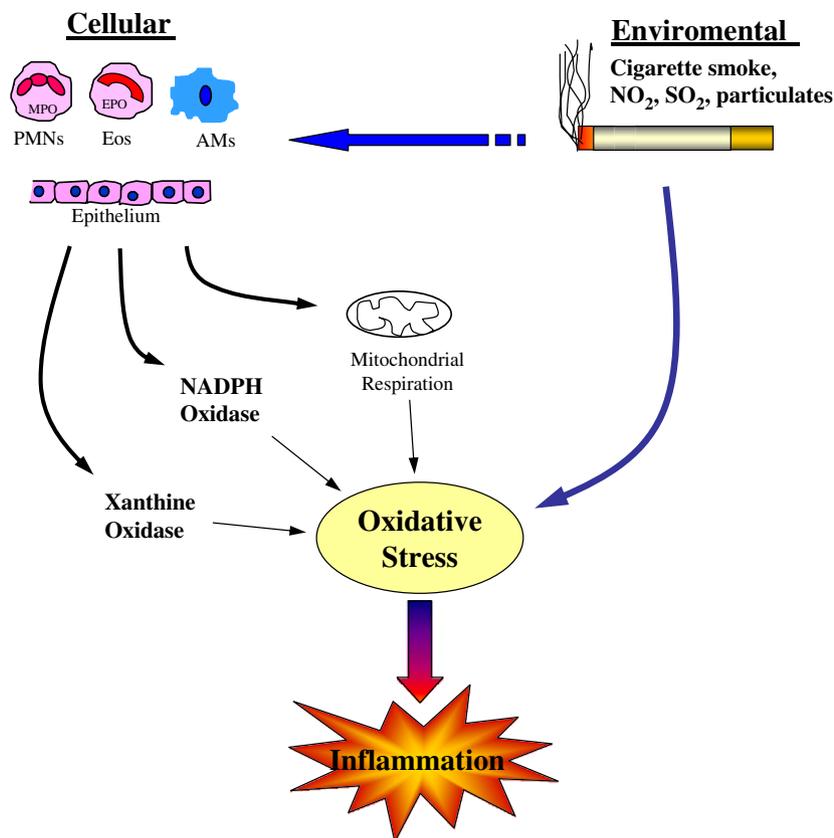
Fig. 1. Sources of oxidative stress. Oxidative stress derived from either environmental or cellular origins results in inflammation. Cellular-derived oxidative stress can be produced through mitochondrial respiration, the NADPH oxidase system, or the xanthine oxidase system. Inflammation itself can have a feed-forward effect, triggering inflammatory cells to produce yet more oxidative stress, exacerbating and intensifying the inflammatory response. PMNs, polymorphonucleocytes; Eos, eosinophils; AMs, alveolar macrophages.

This involves the activity of the heme peroxidases, myeloperoxidase, or eosinophil peroxidase, which are also found in neutrophils and eosinophils, respectively. These enzymes catalyse the formation of the potent and very damaging oxidants hypochlorous acid (HOCl) and hypobromous acid (HOBr) from H_2O_2 in the presence of chloride (Cl^-) and bromide (Br^-) ions, respectively (see Fig. 2). Once produced, ROS can interact with a wide variety of molecules through electron donation in biological systems. This can result in lipid peroxidation and enzyme dysfunction and enhancement of pro-inflammatory cell signaling, thereby profoundly altering cellular function within inflammatory lung diseases (Gutteridge, 1995; Poli et al., 2004).

2.2. Cellular and molecular impact

Reactive oxygen species when generated close to cell membranes oxidize membrane phospholipids (lipid peroxidation), a process that may continue as a chain reaction, generating many lipid hydroperoxide molecules within the cell membrane (Gutteridge, 1995). This can impair membrane function, inactivate membrane-bound receptors and enzymes and increase tissue permeability, processes which have been implicated in the pathogenesis of many forms of tissue injury (Gutteridge, 1995). The product of lipid peroxidation can result in the formation of reactive aldehydes and other bioactive molecules, such as the isoprostanes and platelet-activating factor mimetics. Acrolein and 4-hydroxy-2-nonenal (4-HNE) are two examples of reactive aldehydes. They are highly diffusible end products of lipid peroxidation and are able to induce various cellular events, such as proliferation, apoptosis, and activation of signaling pathways (Parola et al., 1999; Uchida et al., 1999). Both acrolein and 4-hydroxy-2-nonenal have a high affinity towards cysteine, histidine, and lysine residues. These reactive aldehydes can form adducts with both intracellular proteins, such as histone deacetylase HDAC-2 (Marwick et al., 2004), and extracellular proteins, such as collagen and fibronectin (Kirkham et al., 2004), altering their function that in turn can then impact on cell function (Kirkham et al., 2003; Kirkham et al., 2004). In contrast, the isoprostanes are ROS-catalyzed isomers of arachidonic acid and are stable lipid peroxidation products, which circulate in plasma and are excreted in the urine (Reilly et al., 1996; Pratico et al., 1998). It is because of their relative stability in vivo that they have been used as markers of oxidative stress in both asthma and COPD (Morrow & Roberts, 1997). However, one isoprostane member, 8-isoprostane, has been shown to possess a very potent biological activity. It has been demonstrated that 8-isoprostane is a very potent stimulus for smooth muscle contraction through triggering the thromboxane A_2 receptor and this could be one of the many causes of small
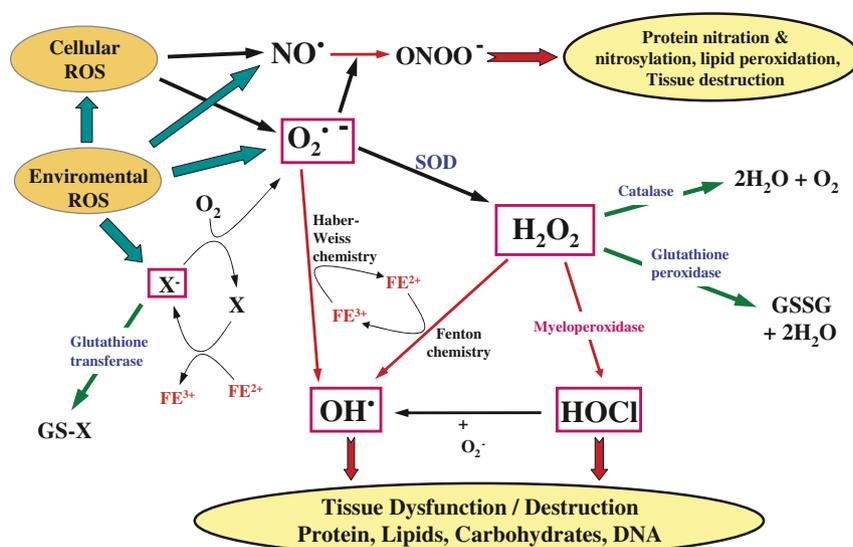
Fig. 2. Molecular consequences of oxidative stress. The relatively weak superoxide anion from both cellular and environmental sources can be transformed into more damaging and potent reactive oxygen and reactive nitrogen species, such as hyperchlorous acid, the hydroxyl radical, and peroxynitrite, through a series of enzymic and non-enzymic steps. Xenobiotic radicals from the environment can be long lived and undergo redox cycling, such as the semi-quinones and result in further superoxide anion formation as well as more powerful radical formation in the presence of free metal ions through Haber–Weiss and fenton chemistry. Endogenous antioxidant defenses glutathione transferase, glutathione peroxidase, SOD, and catalase neutralize and remove these ROS and RNS. ROS, reactive oxygen species; RNS, reactive nitrogen species; X^-, xenobiotic radical; $O_2^{\bullet-}$, superoxide anion; H_2O_2, hydrogen peroxide; $OH^{\bullet}$, hydroxide radical; HOCl, hyperchlorous acid; NO, nitric oxide; $ONOO^-$, peroxynitrite; GS-X, glutathione-xenobiotic conjugate; GSSG, oxidized glutathione dimers.

airway contraction (Kinsella et al., 1997; Okazawa et al., 1997). ROS-induced degradation of arachidonate-based phospholipids can also produce other bioactive molecules. These are 1-palmitoyl-2-(5)oxovaleroyl-sn-glycero-3-phosphorylcholine (POVPC), 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine (PGPC), and 1-palmitoyl-2-epoxyisoprostane-sn-glycero-3-phosphorylcholine (PEIPC). They are pro-inflammatory affecting both monocytes and neutrophils causing increased endothelial cell interaction (Leitinger et al., 1999) as well as increased cytokine release (Lee et al., 2000; Yeh et al., 2001), and are proposed to play an important role in various chronic inflammatory diseases (Leitinger et al., 1999). Alternatively, ROS attack of phosphatidylcholine can form a unique set of products called the PAF-like lipids. These lipids structurally mimic platelet-activating factor (PAF) and are not all as readily metabolized as PAF by PAF acetylhydrolase. Indeed, to compound matters, this enzyme is itself inactivated by ROS (Halliwell & Gutteridge, 1999). As such, these PAF-like lipids are potent agonists of monocytes and platelets through the PAF receptor (Poli et al., 2004).

Protein nitration is another product of ROS-induced tissue damage. A reaction between NO and $O_2^{\bullet-}$ results in the formation of peroxynitrite anions ($ONOO^-$), a highly reactive oxidant species. $ONOO^-$ can then in turn add a nitro group to the three position adjacent to the hydroxyl group of tyrosine to produce the stable product nitrotyrosine. Tyrosine nitration of proteins can affect enzyme activity by reducing histone deacetylase (HDAC) activity for example (Ito et al., 2004; Marwick et al., 2004). $ONOO^-$ can also induce hyperresponsiveness in airways of guinea pigs, inhibit pulmonary surfactant, induce membrane lipid peroxidation and alter tyrosine and MAP kinase activation, and damage pulmonary epithelial cells

(Dohlman et al., 1993; Groves, 1999; Beckman et al., 2000; Zhang et al., 2000). Levels of nitrotyrosine formation are elevated in both asthma (Hogg & Kalyanaraman, 1999; Kaminsky et al., 1999; Hanazawa et al., 2000) and COPD (Ichinose et al., 2000). However, levels of protein nitration have been found in induced sputum cells from COPD patients compared to those from asthmatics (Ichinose et al., 2000), possibly as a result of a higher more chronic oxidative burden. $ONOO^-$ is also capable of attacking sulphydryl groups forming nitrosothiols, a process called nitrosylation, which can also impact upon protein function. The role of denitration of modified proteins by endogenous enzymes has been proposed but it is not clear at this time whether or not this pathway is altered in patients with asthma and COPD.

Redox-sensitive molecular targets usually contain highly conserved cysteine residues, and their oxidation, nitrosylation, or the formation of disulfide links are crucial events in oxidant/redox signaling. Such molecular targets include transcription factors (NF-κB, AP-1), signaling molecules such as ras/rac or JNK, protein tyrosine phosphatases, and p21^{ras} (Lander et al., 1997). With respect to JNK, one group has shown that oxidation of a critical cysteine to a sulfenic acid residue by ROS in a phosphatase regulating JNK results in its inactivation. This leads to JNK hyperphosphorylation and activation (Kamata et al., 2005). Indeed the DNA binding ability of various transcription factors, such as Sp-1, c-Myb (Sun & Oberley, 1996), p53 (Datta et al., 2002), c-myc, EGR-1 (Huang et al., 1999), MIF-1 (Andrews, 2000), GR (Okamoto et al., 1999), and CREB (Ichiki et al., 2003), have all been shown to be redox regulated through the presence of a common cysteine residue in their DNA binding domain. The impact of ROS on such crucial intracellular mechanisms may not be confined to

abnormal disease states, but to important regulators of cell signaling under normal physiological conditions. Recent studies have shown that in response to tumour necrosis factor α (TNFα) and lipopolysaccharide (LPS), which are relevant stimuli for the inflammatory response in COPD, airway epithelial cells can concurrently produce increased amounts of intracellular ROS and RNS (Rochelle et al., 1998). Moreover, this is not confined to only a few receptors. Other receptors such as those for angiotensin-II and serotonin trigger ROS production in order to trigger downstream signaling events (Lee et al., 2001; Zimmerman et al., 2002). This intracellular production of oxidants and the subsequent changes in intracellular redox status is important in the molecular events controlling the expression of genes for inflammatory mediators (Rahman & MacNee, 1998).

The activation of redox-sensitive transcription factors, such as NF-κB and AP-1, is a necessary pre-requisite for induction of pro-inflammatory gene expression. However, at another level of gene regulation, chromatin topology also plays an important part, through which ROS can have a dramatic impact. The study of chromatin topology is itself a vast area of research with many reviews in this area which historically have been focused towards oncology (Cress & Seto, 2000; Strahl & Allis, 2000; Mai et al., 2005). In brief (see Fig. 3), DNA is tightly wrapped around a tetrameric set of core histone proteins (H2A, H2B, H3, and H4) to form chromatin. Activated transcription factors recruit co-activators that contain histone acetylase (HAT) activity. This results in histone acetylation causing the DNA to uncoil from around the histone core. As a consequence, DNA polymerases can gain access to the DNA to begin transcription. In contrast, gene transcription is shut down by histone deacetylases (HDACs) that remove acetyl groups from the histones, thereby facilitating condensation of the DNA around the histone core. The impact of ROS within this set of

events is to promote histone acetylation while at the same time inactivating certain histone deacetylases, HDAC-2, HDAC-5, and HDAC-8 (Ito et al., 2004; Marwick et al., 2004; Ito et al., 2005). Inactivation of HDAC-2 by ROS is achieved through increased nitration or carboxylation (Marwick et al., 2004). This has the net effect of promoting pro-inflammatory and antioxidant gene expression (Rahman & MacNee, 2000; Moodie et al., 2004; Rahman et al., 2004). The impact of ROS on HDAC-2 is particularly important as it has also been shown to be required for corticosteroid-mediated inhibition of the inflammatory response (Ito et al., 2000; Marwick et al., 2004).

3. Reactive oxygen species in asthma and chronic obstructive pulmonary disease

3.1. Asthma

Asthma is characterized by reversible airflow obstruction, airway hyperresponsiveness/hyperreactivity, and chronic inflammation characterized by an influx and activation of inflammatory cells (macrophages, neutrophils, eosinophils, lymphocytes, and mast cells), generation of inflammatory mediators, and epithelial cell shedding. It has been shown that inflammation driven by increased oxidative stress occurs in the airways of patients with asthma, as reviewed by Dworski (2000). Inflammatory and immune cells in the airways, such as macrophages, neutrophils, and eosinophils, release increased amounts of ROS in asthmatic patients (Sedgwick et al., 1990; Kanazawa et al., 1991; Calhoun et al., 1992; Vachier et al., 1992). ROS can result in lung injury as a result of direct oxidative damage to epithelial cells and cell shedding (Cluzel et al., 1987; Hulsmann et al., 1994). ROS have been shown to be associated with the pathogenesis of asthma by evoking
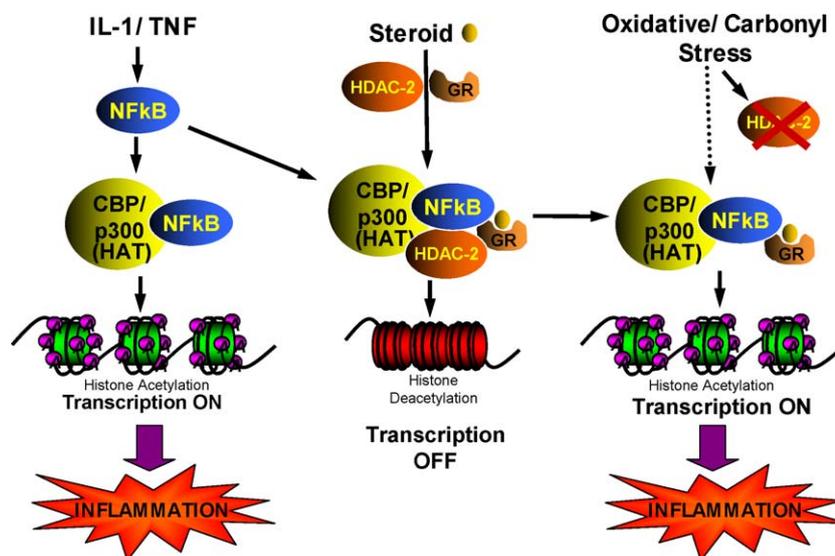

Fig. 3. Impact of oxidative stress on the regulation of chromatin structure and pro-inflammatory gene expression. Pro-inflammatory cytokines activate transcription factors, such as NF-κB, recruiting transcriptional co-activator molecules CBP/p300 containing intrinsic HAT activity resulting in histone acetylation and DNA unwinding, allowing DNA polymerases access to the DNA and pro-inflammatory gene expression. Activated corticosteroid receptors recruit HDAC into the transcriptome complex promoting histone deacetylation, chromatin condensation, and expulsion of DNA polymerases, shutting off gene expression. Oxidative stress inhibits HDAC activity as well as activating NF-κB, facilitating histone acetylation by the transcriptome complex even in the presence of activated glucocorticoid receptor.

bronchial hyperreactivity (Sadeghi-Hashjin et al., 1996; Cortijo et al., 1999) as well as directly stimulating histamine release from mast cells and mucus secretion from airway epithelial cells (Krishna et al., 1998). Furthermore, there is increased ROS during acute exacerbations of asthma (Nadeem et al., 2005). Some of the potential triggers for asthma include viral infections, air pollutants such as ozone, and cigarette smoke. Other factors that can serve as promoters of ROS production in the airways of asthmatic patients (Agosti et al., 1987; McBride et al., 1994) include lipid mediators, chemokines, adhesion molecules, and eosinophil granule proteins. All of which, through ROS generation, trigger an increased inflammatory response producing asthmatic-like symptoms. Indeed, the actions of ROS can produce many of the pathophysiological features of asthma, including enhanced arachidonic acid release, airway smooth muscle contraction, increased airway reactivity and secretions, increased vascular permeability, and increased synthesis of chemoattractants (Owen et al., 1991; Sadeghi-Hashjin et al., 1996). While much of the evidence for the involvement of ROS in the pathogenesis of asthma is indirect, numerous surrogate markers of oxidative stress have been measured. This includes markers such as exhaled H_2O_2 and NO products in breath condensate or in exhaled air (Alving et al., 1993; Antczak et al., 1997), which are elevated in asthmatics. Likewise, levels of 8-isoprostane or F_2-isoprostanes are also increased in BAL fluid of patients with asthma (Dworski et al., 1999). However, while urinary excretion of 15-F_2-Isoprostane (8-iso Prostaglandin $_2\alpha$, family of F_2-isoprostanes) was increased in mild atopic asthmatics following inhaled allergen provocation, no increase was observed after inhalation of methacholine (Dworski et al., 2001).

Neutrophils isolated from peripheral blood of asthmatic patients generate greater amounts of ROS than cells from normal subjects, and their ability to produce ROS correlates with the degree of airway hyperresponsiveness to inhaled methacholine (Seltzer et al., 1986; Hiltermann et al., 1998). Other inflammatory cells, particularly eosinophils derived from peripheral blood, also produce increased amounts of ROS and RNS after stimulation ex vivo in asthma (Cluzel et al., 1987; Sedgwick et al., 1990; Kanazawa et al., 1991; Calhoun et al., 1992; Vachier et al., 1992; Sanders et al., 1995). Moreover, in asthma, eosinophils are thought to play a critical role in the inflammatory response, as they are present in increased numbers in bronchoalveolar lavage (BAL) and blood that correlates with bronchial hyperresponsiveness (Wardlaw et al., 1988; Foreman et al., 1999). Eosinophil activation in vivo results in eosinophil peroxidase (EPO) release and oxidative damage to proteins through bromination of tyrosine residues (Mitra et al., 2000). This is evident by the increased formation of 3-bromotyrosine on proteins, in response to the specific release of EPO from eosinophils reacting with oxidants in the BAL of patients with asthma (Wu et al., 2000). In contrast, neutrophil- and monocyte-derived myeloperoxidase (MPO), which is increased in smokers and patients with COPD, produces 3-chlorotyrosine (Wu et al., 2000). BAL fluid eosinophils, alveolar macrophages, and neutrophils from

asthmatic patients produce more ROS ($O_2^{\cdot-}$, H_2O_2, hypohalites), than do those from normal subjects (Schauer et al., 1991; Teramoto et al., 1996). ROS can cause direct contraction of airway smooth muscle preparations and this effect is enhanced when the epithelium is injured or removed. Indeed, ROS-mediated injury to the airway epithelium produces hyperresponsiveness of human peripheral airways, suggesting that ROS may play a role in the pathogenesis of asthma (Hulsmann et al., 1994). This observation might provide a mechanistic link between epithelial injury arising from a variety of causes and airway hyperresponsiveness (Hulsmann et al., 1994). Moreover, these findings have been echoed in animal studies, which have shown that ROS may contribute to airway hyperresponsiveness by increasing vagal tone due to inhibition of β-adrenergic receptors and by decreasing mucociliary clearance (Owen et al., 1991; Adam et al., 1999), hence promoting airway inflammation and hyperreactivity. Recently, 2 studies by Comhair et al. have identified that impaired SOD activity has been found to be associated with airflow obstruction along with airway hyperresponsiveness and remodeling (Comhair et al., 2005a, 2005b). This inactivation of SOD in asthmatics, which has been reported in other studies (Smith et al., 1997; Comhair et al., 2000), was as a result of increased ROS causing tyrosine nitration of SOD.

It has been postulated that increased levels of HOBr production as a result of EPO release from eosinophils results in increased peroxynitrite formation by interaction of HOBr with NO. NO itself can be stored in cells as *s*-nitrosothiols (Foster et al., 2003) and this may regulate cellular apoptosis through inactivation of caspases by *s*-nitrosylation of critical cysteine residues (Liu & Stamler, 1999). Consequently, increased peroxynitrite formation could deplete intracellular stores of NO, liberating active caspases that can then induce epithelial cell apoptosis and eventual hyperresponsiveness. This is supported by evidence showing that *s*-nitrosothiol levels in asthmatics are significantly depressed (Gaston et al., 1998; Dweik et al., 2001). However, another study showed that levels of NO in exhaled breath condensate were increased in asthmatics (Alving et al., 1993; Silkoff et al., 2000). This may simply reflect a compartmentalization effect, in that different mechanisms are operating in an intracellular versus extracellular environment. The precise mechanistic role that SOD inactivation by peroxynitrite plays in this is as yet unclear. However, it is postulated that SOD inactivation by peroxynitrite could increase the overall redox state by allowing higher levels of H_2O_2 to persist thereby depleting intracellular NO stores through increased peroxynitrite formation and lowering *s*-nitrosothiol levels (Janssen-Heininger et al., 2005). Overall, this clearly shows that oxidant stress occurs in asthma, which can be reflected/detected both systemically and locally in the lungs.

3.2. Chronic obstructive pulmonary disease

COPD, as published by the British Thoracic Society Guidelines (British Thoracic Society, 1997) and the American Thoracic Society standards (American Thoracic Society, 1995),

is a slowly progressive condition characterized by airflow limitation, which is largely irreversible. Cigarette smoking is the major etiological factor in this condition. More than 90% of patients with COPD are smokers, but not all smokers develop COPD (Snider, 1989). Only, 15–20% of cigarette smokers appear to be susceptible and develop the disease, showing a faster rate of decline in forced expiratory volume in 1 sec (FEV_1) when compared to age matched non-smokers (Snider, 1989). An increased oxidant burden in smokers derives from the fact that cigarette smoke contains an estimated 10^{14} oxidants and 3000 ppm NO per puff, and many of these are relative long-lived such as tar-semiquinone, which can generate ·OH and hydrogen peroxide in the presence of free iron through the Fenton reaction (Church & Pryor, 1985; Nakayama et al., 1989; Pryor & Stone, 1993; Zang et al., 1995). Other factors that may exacerbate COPD, such as air pollutants, infections, and occupational dusts, also have the potential to produce oxidative stress (Rahman & MacNee, 1996; Repine et al., 1997).

The oxidant burden in the lungs is enhanced in smokers by the release of ROS from macrophages and neutrophils (Rahman & MacNee, 1996). Oxidants present in cigarette smoke can stimulate alveolar macrophages to produce ROS and to release a host of mediators, some of which attract neutrophils and other inflammatory cells into the lungs. Both neutrophils and macrophages, which are known to migrate in increased numbers into the lungs of COPD patients (Saetta et al., 2001; Barnes, 2004), compared with non-smokers, can generate ROS via the NADPH oxidase system (Rahman & MacNee, 1996). This increase in lung tissue inflammatory cell number in COPD becomes compartmentalized depending on cell type. Increased numbers of neutrophils are mainly located in the lumen of the lung, whereas macrophages accumulate in the tissue matrix itself (Saetta et al., 2001). Circulating neutrophils from cigarette smokers and patients with exacerbations of COPD release more $O_2^{·-}$ (Rahman et al., 1996b). Cigarette smoking is associated with increased content of MPO in neutrophils, which correlates with the degree of pulmonary dysfunction (Fiorini et al., 2000; Aaron et al., 2001). MPO activity also has a negative correlation with FEV_1 in patients with COPD, suggesting that neutrophil MPO-mediated oxidative stress may play a role in the pathogenesis of COPD (Gompertz et al., 2001).

Alveolar macrophages obtained by bronchoalveolar lavage (BAL) of smokers' lungs are also more activated compared with those obtained from non-smokers (Rahman & MacNee, 1996). One manifestation of this is the release of increased amounts of ROS such as $O_2^{·-}$ and H_2O_2 (Nakashima et al., 1987; Rahman & MacNee, 1996; Morrison et al., 1999). Exposure to cigarette smoke in vitro has also been shown to increase the oxidative metabolism of alveolar macrophages (Drath et al., 1979).

Not surprisingly, the net effect of all this ROS activity is that smokers and patients with COPD have higher levels of exhaled H_2O_2 than non-smokers (Nowak et al., 1996; Nowak et al., 1998), and levels are even higher during exacerbations of COPD (Dekhuijzen et al., 1996). This increase in H_2O_2 is

in part derived from increased release of $O_2^{·-}$ from alveolar macrophages in smokers (Dekhuijzen et al., 1996). The generation of ROS in epithelial lining fluid may also be further enhanced by the presence of increased amounts of free iron in the airspaces of smokers (Lapenna et al., 1995; Mateos et al., 1998). This is relevant to COPD because the intracellular iron content of alveolar macrophages is increased in cigarette smokers and is increased further in those who develop chronic bronchitis, compared with non-smokers (Thompson et al., 1991). In addition, macrophages obtained from smokers release more free iron in vitro than those from non-smokers (Wesselius et al., 1994). In some studies, both in stable (Jeffery, 1998) and mild exacerbations of bronchitis (Saetta, 1999), eosinophils have been shown to be prominent in the airway walls. BAL from patients with COPD has also been shown to contain increased levels of eosinophilic cationic protein (Fiorini et al., 2000). Furthermore, peripheral blood eosinophilia is also considered to be a risk factor for the development of airway obstruction in patients with chronic bronchitis and is an adverse prognostic sign (Lacoste et al., 1993; Lebowitz et al., 1995). However, despite the presence of increased number of eosinophils, specific EPO-mediated generation of 3-bromotyrosine has not been detected in COPD patients (Wu et al., 2000). This does not provide support for a role for eosinophil-mediated ROS damage in COPD. Conversely, products of oxidant-mediated protein nitration and lipid peroxidation inversely correlate with declining FEV_1 as disease severity in COPD becomes progressively worse, suggestive of a role in the pathogenesis of this disease.

As discussed earlier, cigarette smoking increases the formation of RNS and results in nitration and oxidation of plasma proteins. The levels of nitrated proteins (fibrinogen, transferrin, plasminogen, and ceruloplasmin) were higher in smokers (Pignatelli et al., 2001) compared to non-smokers. Evidence of increased $NO/ONOO^-$ activity in plasma and lung epithelial lining fluid has been shown in chronic smokers resulting in elevated formation of 3-nitrotyrosine (van et al., 1994; Petruzzelli et al., 1997; Ichinose et al., 2000). Furthermore, levels of nitrotyrosine and inducible nitric oxide synthase (iNOS) were higher in airway inflammatory cells obtained from the induced sputum of patients with COPD (Ichinose et al., 2000). With respect to lipid peroxidation products, both direct measurement through assessment of isoprostane levels and indirect measurements through the determination of levels of thiobarbituric acid reactive substances (TBARS) have shown that these products are elevated in breath condensate and in lungs of patients with stable COPD (Lapenna et al., 1995; Fahn et al., 1998; Nowak et al., 1999; Montuschi et al., 2000). Similarly, levels of plasma lipid peroxides, as assessed by TBARS, have also been shown to be elevated in COPD and negatively correlated with the FEV_1 (Montuschi et al., 2000; Tsukagoshi et al., 2000), these findings having been repeated in a larger population study (Britton et al., 1995). Likewise, increased levels of lipid peroxidation in lung tissue through detection of 4-HNE protein adducts was demonstrated. These increased 4-HNE-modified protein levels were

present in airway and alveolar epithelial cells, endothelial cells, and neutrophils in smokers with airway obstruction compared to subjects without airway obstruction (Rahman et al., 2002). More importantly, this increased level of 4-HNE-adducts in the lung inversely correlated with FEV_1, suggesting a role for 4-HNE and other carbonyl adducts in the pathogenesis of COPD.

Another more insidious aspect of ROS-driven post-translational modifications is the impact this has on corticosteroid efficacy. As highlighted earlier, ROS has a negative impact on HDAC-2 activity through increased protein nitration and carboxylation reactions. It is this level of HDAC-2 inactivation that will impact on the degree of corticosteroid responsiveness and hence the ability to resolve the inflammatory response (Fig. 3). Within mild to moderate asthma, there is an imbalance towards more HAT activity, with little or no significant change in HDAC activity (Cosio et al., 2004a; Ito et al., 2005), with the resultant outcome that corticosteroid efficacy is still intact. In contrast, COPD patients have a much greater reduction in HDAC-2 activity correlating with disease severity, as a result of chronic oxidative stress, to such an extent that corticosteroids are no longer effective in resolving the inflammatory response (Cosio et al., 2004b; Ito et al., 2005). We have also reported similar observations in an animal model of COPD (Marwick et al., 2004). Interestingly, mild asthmatics who smoke also become refractory to corticosteroid therapy as their level of HDAC-2 activity has been reduced to that seen in COPD patients (Chalmers et al., 2002). Interestingly, in COPD besides HDAC-2, the expression of other HDACs also decreases with increasing disease severity, namely, HDAC-3, HDAC-5, and HDAC-8 (Ito et al., 2005). The impact of HDAC-3 is particularly important as it has been shown to be involved in the deacetylation of nuclear NF-κB, thereby curtailing its duration of action (Chen et al., 2001).

4. Endogenous antioxidant defenses within the lung

In order to combat and neutralize the deleterious effects of ROS, various endogenous antioxidant strategies have evolved which employ both enzymatic and non-enzymatic mechanisms. The nature and composition of the antioxidant defenses will differ from tissue to tissue as well as between environments, intracellular versus extracellular. Within the lung lining fluid, several non-enzymatic antioxidant species exist, which include glutathione, ascorbic acid (vitamin C), uric acid, α-tocopherol (vitamin E), and albumin. The relative abundance of these antioxidants can differ to that observed in blood plasma (Cross et al., 1994). Nevertheless, ROS-induced lung injury can increase lung epithelial permeability (Li et al., 1994) allowing leakage of plasma constituents, which will also contain antioxidants, into the lung lining fluid providing additional antioxidant protection. Similarly, enzymatic antioxidant defenses can also differ in both anatomical and subcellular localization based on expression. This includes enzymes such as SOD, catalase, thioredoxin, glutathione peroxidase, and glutathione-S-transferase. Of these, enzymes such as SOD can

be found as different isoforms expressed either intracellularly or extracellularly. Moreover, extracellular SOD is highly expressed in the lungs mainly around blood vessels and airways (Su et al., 1997).

Table 1 highlights the level of different non-enzymatic antioxidants found in the epithelial lining fluid compared to that in plasma. While differences are clearly evident, both enzymatic and non-enzymatic antioxidant strategies are employed within the lung. An important effect of oxidative stress and inflammation is the up-regulation of protective antioxidant genes. Among antioxidants, glutathione (GSH) and its redox enzymes have an important protective role in the airspaces and intracellularly in lung epithelial cells. Indeed, GSH levels are increased in the epithelial lining fluid of both asthmatics and chronic cigarette smokers (Cantin et al., 1987; Smith et al., 1993). GSH is a tripeptide (L-γ-glutamyl-L-cysteinyl-glycine) that contains a thiol group. It functions as an antioxidant by acting as a sacrificial target for ROS and other products of lipid peroxidation, such as reactive carbonyls. In so doing, GSH becomes oxidized to its dimeric form (GSSG) or forms adducts with reactive carbonyls and other reactive xenobiotics (GS-X). Furthermore, enzymes such as glutathione peroxidase and glutathione transferase can facilitate this process (see Fig. 2). Oxidized glutathione can itself be reduced back to GSH by glutathione reductase using NADPH generated from the pentose phosphate pathway. Oxidative stress causes up-regulation of glutamate cysteine ligase, GCL (formerly known as γ-glutamylcysteine synthetase) (Rahman et al., 1996a, 1996c), an important enzyme involved in the synthesis of GSH, as an adaptive mechanism against subsequent oxidative stress. We have shown that the expression of glutamate cysteine ligase mRNA is elevated in smokers' lungs and is even more pronounced in smokers with COPD (Rahman et al., 2000). This implies that GSH synthesis is up-regulated in lungs of smokers with and without COPD. Similarly, bronchial epithelial cells of rats exposed to cigarette smoke have shown increased expression of the antioxidant genes manganese superoxide dismutase (MnSOD), metallothionein, and glutathione peroxidase (GPx). This would suggest the importance of an adaptive antioxidant gene response against the injurious effects of cigarette smoke (Gilks et al., 1998). However, Harju et al. (2002) have found that glutamate cysteine ligase immunoreactivity was decreased (possibly leading to decreased GSH levels) in the airways of smokers compared to non-smokers, suggesting that cigarette smoke predisposes lung cells to ongoing oxidant stress. In addition,

Table 1
Antioxidant constituents of plasma and lung epithelial lining fluid (ELF)

Antioxidant	Plasma, μM	ELF, μM
Ascorbic acid	40	100
Glutathione	1.5	100
Uric acid	300	90
Albumin-SH	500	70
α-Tocopherol	25	2.5
β-Carotene	0.4	–

Source: Cross et al. (1994).

Neurohr et al. (2003) recently showed that decreased GSH levels in BALF cells of chronic smokers were associated with a decreased expression of glutamate cysteine ligase-light subunit without a change in glutamate cysteine ligase-heavy subunit expression.

Important protective antioxidant genes such as genes for MnSOD, GCL, heme oxygenase-1 (HO-1), GPx, thioredoxin reductase, and metallothionein are similarly induced by various oxidative stresses including hyperoxia and inflammatory mediators such as TNF-α and lipopolysaccharide in lung cells (Lander et al., 1997; Sen, 1998; Adler et al., 1999; Thannickal & Fanburg, 2000). Indeed, the transcription factor, Nrf2, is redox sensitive (contains −SH groups) and binds to the antioxidant response element (ARE) within DNA regulating a variety of antioxidant genes. The importance of Nrf2 can be gauged from a recent report by Rangasamy et al. (2004), where they have shown that disruption of the Nrf2 gene in mice lead to an early and a more intense emphysema in response to cigarette smoke. In the same study, they have shown that the expression of nearly 50 antioxidant and cytoprotective genes in the lungs may be transcriptionally controlled by Nrf2 and all genes may work in concert to overcome the effects of cigarette smoke. Moreover, studies looking at gene polymorphisms in these protective antioxidant genes, such as glutathione transferase and HO-1, have suggested a link to onset of COPD and emphysema (Harrison et al., 1997; Exner et al., 2004). Interestingly, Rangasamy et al. have recently showed that disruption of Nrf2 also leads to susceptibility to severe allergen-induced asthma in mice. These findings indicate that although Nrf2 is important for the control of antioxidant genes, it is not specific to one inflammatory disease (Rangasamy et al., 2005). The compartmentalization and localization of various antioxidant enzymes and their functions/alterations in asthma and COPD are described in Table 2.

The major antioxidants in lung lining fluid are GSH, ascorbic acid, and uric acid (Cross et al., 1994). Like GSH, uric acid can also be found intracellularly, although at lower concentrations than that seen in plasma. It is a powerful scavenger of both ROS and RNS and can protect proteins against nitration (Simic & Jovanovic, 1989), particularly from the nitrogen dioxide radical, nitrous oxide, found in cigarette smoke (Janoff et al., 1987). Oxidation of uric acid by ROS or RNS results in the formation of allantoin, which can then be measured in various body fluids as a marker of oxidative stress (Grootveld & Halliwell, 1987). The antioxidant properties of albumin, as well as mucins, come from the presence of exposed −SH groups. Their protective effects are solely extracellular. However, albumin exists in a high concentration and acts as a sacrificial substrate able to react quickly with the very damaging and potent oxidizing peroxynitrite radicals and hypochlorous acid (Halliwell & Gutteridge, 1990b).

The remaining two protective antioxidant molecules, ascorbic acid and α-tocopherol, are derived from the diet. Both antioxidants are decreased in chronic cigarette smokers (Rahman & MacNee, 1996). Conversely, it has been shown that

Table 2
Antioxidant enzymes of the lungs, their localization, and functions in asthma and COPD

Enzyme	Lung localization	Function	Expression/activity
Cu,ZnSOD	Bronchial, alveolar epithelium, macrophages, fibroblasts, pneumocytes	Scavenges $O_2^{\bullet-}$	Decreased in COPD
EC-SOD	Bronchial epithelium, macrophages, neutrophils, vascular walls, pneumocytes	Scavenges $O_2^{\bullet-}$	Decreased in COPD
Mn,SOD	Bronchial epithelium, macrophages, neutrophils, vascular walls, pneumocytes	Scavenges $O_2^{\bullet-}$	Decreased in asthma Decreased in smoking
Catalase	Macrophages, fibroblasts, pneumocytes	Hydrogen peroxide to water	Decreased in asthma
Glutathione peroxidase	ELF cells, epithelium, macrophages, other lung cells	Organic hydroperoxides to organic hydroxides	Decreased in COPD Decreased in asthma
Heme Oxygenase-1	Alveolar, bronchial epithelium, macrophages, inflammatory cells of lungs	Heme to CO and biliverdin	Decreased in emphysema Decreased in COPD
Thioredoxin	Bronchial epithelium, macrophages	Transcriptional modulation, thiol–dithiol exchange, Prot-S-S-Prot to Prot-SH	Decreased in COPD
Glutamate cysteine ligase (catalytic subunit)	Alveolar, bronchial epithelium, macrophages	Synthesis of glutathione (first rate-limiting step)	Decreased in COPD Decreased in Smoking

there is a correlation between increased dietary antioxidant intake and improved lung function (Britton et al., 1995). Moreover, increased dietary antioxidant status, particularly vitamin E (α-tocopherol), correlated with lower levels of lipid peroxidation (Mezzetti et al., 1995). α-Tocopherol is a lipid-soluble antioxidant and as such is probably one of the most important scavengers and inhibitors of lipid peroxidation. It largely achieves this by reacting faster with the lipid peroxyl radicals than these radicals can react with other lipid molecules. Similarly, in another study, Schwartz and Weiss (1994) reported that increased dietary intake of ascorbic acid led to an improvement in lung function in both smokers and asthmatics. Ascorbic acid, otherwise known as vitamin C, is water soluble. It functions as an antioxidant by accepting free radical electrons, forming the ascorbyl radical, which is relatively unreactive. However, the ascorbyl radical will undergo a disproportionation reaction to regenerate ascorbate and dehydroascorbate, the latter rapidly breaking down to form oxalic acid and L-threonine (Halliwell & Gutteridge, 1999). Ascorbic acid has been shown to possess a whole array of antioxidant properties in vitro, from scavenging ROS and RNS, preventing lipid peroxidation to regenerating other antioxidants, such as uric acid and α-tocopherol. Like uric acid,

ascorbic acid is also a powerful scavenger of nitrous oxide which is a potent promoter of both protein nitration and lipid peroxidation (Spencer et al., 1995; Halliwell, 1996). Indeed, increases in F_2-isoprostane lipid peroxidation products are decreased in smokers who consume more ascorbate (Morrow et al., 1995). Further evidence of ascorbate's antioxidant properties in vivo is provided by the evidence highlighting its depletion in clinical conditions associated with a high oxidative stress burden (Schorah et al., 1996). Intriguingly, there is also a dark side to ascorbic acid in that in vitro it can reduce Fe (III) to Fe^{2+} thereby facilitating $OH^{\cdot}$ radical formation through Fenton chemistry. However, the relevance of this in vivo is still a subject of investigation.

5. Therapeutic intervention with antioxidants

A quick review of the literature will show that various approaches have been taken to study the benefit of antioxidants in obstructive lung disease. These range from dietary antioxidants and thiols to SOD mimetics. In view of the evidence implicating oxidative stress in the pathogenesis of chronic airways disease, one rational approach would be to consider antioxidant intervention in order to neutralize this increase in oxidative stress. This can be achieved through two approaches. Either by increasing the endogenous antioxidant enzyme defenses or by enhancing the non-enzymatic defenses through dietary or pharmacological means. For this review, the various antioxidant strategies used have been divided into six categories: dietary, thiols, spin traps, redox sensors, enzymes, and polyphenols.

5.1. Dietary

Reports of clinical benefit in asthma and COPD for increased vitamins C and E and other dietary antioxidants have been varied. Nevertheless, epidemiological studies have shown that there are decreased vitamins C and E, β-carotene, and selenium levels in cigarette smokers and that dietary antioxidant intake is an important co-factor in the development of obstructive lung disease (Anderson, 1991; Romieu & Trenga, 2001; Santos et al., 2004). Similarly, recent studies have reported a link with decreased lung function (Gilliland et al., 2003) and the presence and severity of asthma to dietary antioxidant intake, such as vitamins C and E, β-carotene, and selenium (Harik-Khan et al., 2004; Rubin et al., 2004). Interestingly, one of the studies also identified a stronger selenium–asthma association in those exposed to cigarette smoke. There was a 50% reduction in asthma prevalence when selenium levels were increased in smoke-exposed individuals as opposed to those not exposed to smoke (Rubin et al., 2004). Furthermore, one small trial has shown that there was a clinical improvement in asthmatic symptoms after being given selenium supplementation compared to placebo (Allam & Lucane, 2004). Moreover, two recent studies by the same group have shown no clinical benefit of supplemental intake of vitamin C or E when compared to current standard therapies for mild to moderate asthma (Fogarty et al., 2003; Pearson et al., 2004).

This is in contrast with earlier studies showing decreased lipid peroxidation and a corresponding improvement in lung function with increased vitamin C intake in smokers (Bucca et al., 1989; Schwartz & Weiss, 1994; Morrow et al., 1995). Supplementation with either vitamin E or β-carotene alone resulted in no clinical benefit in COPD (Rautalahti et al., 1997). Moreover, β-carotene supplementation may have a detrimental side effect, in that it may accelerate the onset of lung cancer in cigarette smokers (Repine et al., 1997). As to the impact of selenium supplementation on clinical outcome for obstructive lung diseases in general, very few studies have been done. Selenium itself is an essential dietary element of fundamental importance to human health. It is incorporated into the active site of a wide range of selenoproteins as selenocysteine and is an essential component of the antioxidant enzyme glutathione peroxidase. Currently, there are over thirty known selenoproteins, many with unknown functions (Brown & Arthur, 2001). However, what is clear is that selenium levels are decreased in COPD (Santos et al., 2004). In addition, one very early study incorporating selenium supplementation in smokers demonstrated that this resulted in reduced superoxide release from leukocytes (Clausen, 1991). Clearly, more studies are needed to evaluate the clinical benefit of selenium supplementation in chronic obstructive lung diseases.

5.2. Thiols

In the lung, thiols in the form of GSH constitute one of the main antioxidant defenses both intracellular and extracellular (see Section 4). The only other organ that has higher GSH levels is the liver. Attempts to raise GSH levels in the lung through administration of GSH itself have been tried (MacNee et al., 1991). Unfortunately, aerolization of GSH into the lung was characterized by a poor half-life (Borok et al., 1991) and the induction of bronchial hyperreactivity (Gillissen et al., 1993). Intracellular antioxidant protection after GSH administration was also limited due to its inefficient cellular uptake (MacNee & Rahman, 1999). Alternative methods of raising GSH levels have involved supplementing the GSH precursor cysteine, through the use of *N*-acetylcysteine (NAC). This approach has met with varying success in the past (MacNee et al., 1991). More recent studies using NAC have pointed to a protective benefit both in vitro and in vivo against oxidative stress (Dekhuijzen, 2004). In COPD patients, 600 mg NAC given once daily orally reduced the risk of exacerbations and improved symptom score compared to placebo (Dekhuijzen, 2004). Similarly, 2 further studies showed a clear benefit of NAC in reducing oxidant burden (De Benedetto et al., 2005; Sadowska et al., 2005). In contrast, however, the recently published BRONCUS study (Decramer et al., 2005) showed that NAC (600 mg oral, daily) was ineffective in halting the decline in lung function and prevention of exacerbations in COPD. This study followed NAC treatment (600 mg oral, daily) over a 3-year period in 523 patients with COPD. The variability in all the current studies using NAC at 600 mg, oral, daily may simply reflect the fact that the dose is not high enough. Indeed the pharmacokinetics would support this.

Given orally, 600 mg of NAC is rapidly absorbed by the gut but has a bioavailability of only 10% with a plasma half life of 6.3 hr (Dekhuijzen, 2004). This was shown to result in a transient increase in lung GSH levels after daily dosing for two weeks (Bridgeman et al., 1991). In contrast, 600 mg, oral, three times daily raised GSH levels by 50% after 5 days (Bridgeman et al., 1994). Given that 600 mg (oral) twice daily resulted in a significant 35% fall in exhaled peroxide levels from COPD patients after just 2 months (De Benedetto et al., 2005), further studies may be required at higher doses of NAC to observe any clinical benefit on lung function.

An alternative to NAC is the lysine salt of NAC, N-acystelyn (NAL). It has been used as a mucolytic for cystic fibrosis. However, it also possess antioxidant properties and can reduce both ROS levels and ROS-mediated inflammatory events in vitro (Antonicelli et al., 2002). NAL has also been shown to possess anti-inflammatory properties in vivo in reducing LPS-induced neutrophilic inflammation (Antonicelli et al., 2004). NAL has several advantages over NAC; firstly, it can enhance GSH levels twice as effectively as NAC and secondly it forms a neutral pH when in solution, unlike NAC which is acidic (Gillissen et al., 1997). This has meant that when delivered directly into the lung by aerosol in healthy volunteers, it did not cause any irritation or other side effects (App et al., 2002). Therefore, NAL may be more promising than NAC in reducing the oxidant burden in the lung in chronic airways disease.

The potential of other thiols as antioxidants are also being explored. One of these is a naturally occurring intracellular aromatic thiol found in both plants and animals, ergothioneine (Hand et al., 2005). It possess antioxidant activity, scavenging peroxynitrite thereby preventing peroxynitrite-dependent tyrosine nitration of proteins (Aruoma et al., 1997) and can block peroxide-mediated inflammatory effects in epithelial cells (Rahman et al., 2003). It remains to be seen whether this compound can be delivered effectively into the lung by aerosol and give rise to clinical benefit in COPD. However, with the recent discovery of a membrane transporter for this compound (Grundemann et al., 2005), ergothioneine could provide an alternative strategy to boosting intracellular antioxidants defenses. Erdosteine is another new thiol compound that also acts as an antioxidant, but in addition has mucoactive properties and reduces bacterial adhesiveness. In the "Equal-ife" randomized placebo controlled trial, erdosteine was dosed orally 300 mg b.i.d. for a period of 8 months (Moretti et al., 2004). Patients receiving erdosteine had significantly fewer exacerbations and spent less days in hospital than the placebo group. Moreover, patients receiving erdosteine showed no reduction in lung function over this period and showed a significant improvement in health related quality of life. A clinical trial on the combination of steroids and erdosteine in patients with COPD is awaited.

5.3. Spin traps

Spin traps were originally developed as a means of studying chemical and biological reactions encompassing free radical generation. Through the use of electron paramagnetic resonance spectroscopy, spin traps provide a very direct and specific way of measuring free radicals, such as superoxide ($O_2^{\cdot-}$). As the name suggests, spin traps effectively trap the free singlet electron forming a relatively stable product that can then be measured. Most spin traps are based around a nitrone- or nitroxide-containing molecule. As well as their in vitro use, they have been demonstrated to directly react with ROS/RNS in vivo at sites of inflammation when given therapeutically (Chabrier et al., 1999).

Early spin traps had very short half lives of less than one min, such as 5,5-dimethyl-1-pyrroline N-oxide, which would decay releasing the damaging hydroxyl radical. However, addition of various organic structures with "electron withdrawing properties" around the core pyrroline ring resulted in spin traps with greater stability and longer half lives (Shi et al., 2005). Recently, different novel classes of nitrone spin traps have been discovered, such as isoindole-based nitrones (Bottle & Micallef, 2003) and azulenyl-based nitrones (Becker et al., 2002). One such azulenyl nitrone, STANZ, may prove promising for in vivo use as it exhibits very potent antioxidant activity compared to existing nitrones. Moreover, it can rival vitamin E in its ability to inhibit lipid peroxidation in vitro. However, it remains to be seen how it will behave in vivo. Nevertheless, nitrone spin traps have been widely used as antioxidants therapeutically to counter free radical-mediated cellular damage in diseases such as stroke and the neurological conditions, Alzheimer's and Parkinson's diseases (Becker, 1999). Surprisingly, no studies have been performed in asthma and COPD, looking at the impact of spin traps on clinical endpoints, such as FEV_1. A phenyl-based nitrone spin trap developed by AstraZeneca, NXY-059, is due to enter phase III clinical trials for use in acute ischemic stroke. However, it remains to be seen whether such compounds could be developed for more long term use in chronic diseases associated with increased oxidative stress.

5.4. Redox sensor inhibitors

The intracellular redox state is not only affected by the external environment in which the cells may be exposed to, but also by the contribution that internal redox couples can make. The relative contribution that each reduced versus oxidized isoform of these couples can in turn affect the overall redox state of the cell. Conversely, the impact of external oxidative stress towards the contribution of each of the reduced/oxidized isoforms of these couples makes them particularly suited as redox sensors. Common redox sensors include the NADP/NADPH and glutathione systems. Glutathione being the most abundant redox sensor also plays an important protective role, as discussed earlier. The NADP/NADPH system can also play a protective role, particularly in the mitochondrial compartment, buffering against excessive redox shifts. Moreover, NADP/NADPH is also tightly linked to metabolism and is a necessary co-factor in many enzymatic processes. Recently, the oxidoreductase family of redox sensors, such as thioredoxin and redox effector factor-1 (ref-1), has attracted particular

interest. Thioredoxin, has been shown to play an important role in regulating redox-sensitive signaling pathways, such as NF-κB and AP-1, p38MAPK, and JNK (Filomeni et al., 2002).

Thioredoxin has been reported to associate with other proteins such as hepatopoietin (Li et al., 2005) and the apoptosis signal regulating kinase, ASK-1 (Saitoh et al., 1998), as heterodimeric complexes. Under conditions of oxidative stress, thioredoxin is released from these complexes liberating ASK-1 (Filomeni et al., 2002) and hepatopoietin (Li et al., 2005) causing ASK-1 to undergo multimerization and hepatopoietin to dimerize. The net result is that thioredoxin is now able to reduce a key thiol group within cys62 of the p65 NF-κB subunit precipitating full transcriptional activity (Qin et al., 1995). At the same time, the free ASK-1 is also activated by oxidative stress and then able to activate the pro-inflammatory p38MAPK and JNK pathways (Filomeni et al., 2002), two pathways known to be redox sensitive. By inhibiting the oxidoreductase activity of thioredoxin with the small molecular weight inhibitor MOL-294, it has been demonstrated that the both NF-κB- and AP-1-dependent transcriptional activity is blocked. This is achieved through preventing reduction of the important cys62 thiol in NF-κB allowing it to remain *s*-nitrosylated, thereby inhibiting transcriptional activity (Henderson et al., 2002). Another redox sensor inhibitor, PNRI-299, has also been demonstrated to prevent AP-1 activation. This is achieved through inhibition of another oxidoreductase family member similar to thioredoxin, namely, ref-1 (Nguyen et al., 2003). Both inhibitors have been shown in vivo to reduce airway eosinophilia, mucus hypersecretion, airway hyperresponsiveness, and cytokine release in a murine ovalbumin challenge model of asthma (Henderson et al., 2002; Nguyen et al., 2003). However, it remains to be seen whether these compounds will also prevent NF-κB/AP-1 activation and associated pro-inflammatory responses after cigarette smoke induced oxidative stress in vivo.

5.5. Enzyme mimetics

Enzyme mimetics are generally small compounds that posses catalytic activity that mimics the activity of larger enzyme-based molecules. In the case of antioxidants, this includes the SOD, catalase, and glutathione peroxidase like activities. A number of SOD mimetics based around organo-manganese complexes have been developed, which retain their antioxidant properties in vivo. These include a series of manganese-based macrocyclic ligands, such as M40401, M40403, and M40419 (Salvemini et al., 1999; Tuder et al., 2003; Muscoli et al., 2004). The second class of SOD mimetics are the manganese-metaloporphyrin-based compounds as exemplified by AEOL-10113 and AEOL-10150 (Chang & Crapo, 2002; Smith et al., 2002). The third class of manganese-based SOD mimetic are the "Salens". These are generally aromatic substituted ethylene–diamine metal complexes (Doctrow et al., 1997). An example being EUK134 (Izumi et al., 2002; Chatterjee et al., 2004). The "Salens" also posses some catalyse activity and can therefore also scavenge hydrogen peroxide, a product of SOD activity. Moreover, they are also able to decompose peroxynitrite (Sharpe et al., 2002). The mechanism of action for the non-selective nature of "Salens" against ROS and RNS is not fully known but is thought to involve the multivalent state in which this class of organo-metal complex can exist. A more detailed review of the development and properties of these catalytic antioxidants is described elsewhere (Day, 2004).

Within the various classes of SOD mimetic described here, only the metalloporphyrin-based compounds AEOL10150 and AEOL10113 have been studied in models of airway inflammation. In one study, AEOL10113 was shown to inhibit both airway inflammation and bronchial hyperreactivity in an ovalbumin challenge model of airway inflammation (Chang & Crapo, 2002). This highlighted an important mechanistic role for oxidative stress within an antigen-driven (acquired) inflammatory response. As such, a potential therapeutic benefit was postulated for use in asthma. In another study, AEOL10150 was demonstrated to inhibit cigarette smoke-induced lung inflammation (Smith et al., 2002). Again suggestive of a potential therapeutic benefit in COPD.

Another type of catalytic antioxidant is the glutathione peroxidase mimetic Ebselen. This is a selenium-based organic complex and has been shown to be a very powerful antioxidant against the highly reactive and destructive peroxynitrite radical (Jozsef & Filep, 2003). It is able to prevent both NF-κB/AP-1 activation and pro-inflammatory gene expression in human leukocytes exposed to peroxynitrite. Other studies have shown that Ebselen is also active in vivo in preventing LPS-induced airway inflammation (Haddad et al., 2002; Zhang et al., 2002). However, no studies have been reported so far on the protective effects of Ebselen in cigarette smoke induced lung inflammation. In contrast, the glutathione peroxidase mimetic BXT-51072 (Oxis, USA) and the lipid peroxidation inhibitor BO-653 (Chugai Pharma, Japan) are either currently in Phase I or pre-clinical trials in patients with COPD.

Finally, there is a class of antioxidants termed the "peroxynitrite decomposition catalysts". Although strictly neither SOD, catalase, or glutathione peroxidase mimetics, they nevertheless posses catalytic activity against peroxynitrite. Similar in structure to AEOL10150 and AEOL10113, they are iron containing porphyrin complexes, which decompose peroxynitrite into innocuous nitrate. Moreover, they have been shown to be effective in vivo in various animal models of disease associated with peroxynitrite generation (Cuzzocrea et al., 2004). It remains to be seen therefore whether this antioxidant is also effective in a smoking model for COPD, which is also associated with high levels of peroxynitrite generation.

5.6. Polyphenols

Polyphenols encompass a large group of natural antioxidants principally derived from plants. Chemically, several different classes of polyphenol exist, such as the benzoic acids, cinnamic acids, coumarins, and flavonoids. An underlying common feature of these molecules is the presence of one or more aromatic rings along with at least one hydroxyl group. Several epidemiological studies have been undertaken, which

have established a beneficial link between polyphenol intake and lower disease risk with many of the clinical benefits being attributed to both the antioxidant and anti-inflammatory properties of polyphenols (Arts & Hollman, 2005). In one Finnish study with over 10,000 participants, a significant inverse correlation was observed between polyphenol intake and the incidence of asthma (Knekt et al., 2002). Similar beneficial associations were also observed for COPD in a study encompassing over 13,000 adults. This study reported that increased polyphenol intake correlated with improved symptoms, as assessed by cough, phlegm production, and breathlessness, and improved lung function as measured by FEV_1 (Tabak et al., 2001). Two further studies appeared to corroborate these findings. The first study showed a beneficial protective effect against COPD symptoms for increased fruit intake, high in polyphenol and vitamin E content (Walda et al., 2002). In the second more recent study, a standardized polyphenol extract administered orally was shown to be effective in reducing oxidant stress and increasing PaO_2, as well as improvements in FEV_1 between enrolment and the end of the study (Santus et al., 2005).

While the above studies would appear to demonstrate an epidemiological link between polyphenol intake and clinical benefit in asthma and COPD, other studies have tried to show a direct impact of specific polyphenolic compounds on inflammation in vitro and in vivo. For example, the flavonoid resveratrol, a constituent of red wine, inhibits inflammatory cytokine release from macrophages isolated from COPD patients (Culpitt et al., 2003). Moreover, Birrell et al. (2005) have recently demonstrated that in vivo resveratrol can inhibit inflammatory cytokine expression in response to LPS challenge in rat lungs. Furthermore, in both monocytic U937 cells and airway epithelial A549 cells, resveratrol inhibits NF-κB and AP-1 activation (Manna et al., 2000; Donnelly et al., 2004). The mechanism through which this occurs is still unclear. A structural similarity between resveratrol and steroids suggested that the anti-inflammatory effects may be a result of estrogen like activity, but this has been discounted (Donnelly et al., 2004). Possible PPARα agonistic effects were also rejected (Donnelly et al., 2004). Moreover, even in the light of evidence showing that resveratrol could induce histone deacetylase activity by the sirtuins (Howitz et al., 2003), no impact on HAT/HDAC balance could be observed in A549 cells (Donnelly et al., 2004). In view of the antioxidant nature of resveratrol, it is possible that it may interact with the thioredoxin pathway, described earlier, thereby preventing redox-mediated activation of NF-κB and AP-1.

Another well-studied polyphenol is curcumin. It is the active constituent of curcuma longa, commonly known as tumeric. Like resveratrol, it has also been reported to inhibit NF-κB activation, along with IL-8 release, COX-2 expression, and neutrophil recruitment in the lungs (Biswas et al., 2005). Interestingly, one study postulates that curcumin prevents cigarette smoke-induced NF-κB activation through inhibition of IkBα kinase in human lung epithelial cells (Shishodia et al., 2003), corroborating an earlier study on the effect of curcumin on NF-κB activation (Jobin et al., 1999). However, curcumin

has also been reported to inhibit a number of other signaling pathways such as JNK, p38, Akt, JAK ERK, and PKC in a variety of different cell types (Duvoix et al., 2005). Recently, we have observed that curcumin can inhibit inflammation and restore glucocorticoid efficacy in response to oxidative stress, through up-regulation/restoration of HDAC-2 activity in U937 and Monomac6 cells. This would facilitate steroid-mediated HDAC recruitment in attenuating NF-κB-mediated chromatin acetylation and subsequent pro-inflammatory gene expression. Interestingly, it has recently been suggested that the anti-inflammatory actions of curcumin are propagated through inhibition of HAT activity, preventing NF-κB-mediated chromatin acetylation (Kang et al., 2005). However, the concentrations at which these effects were observed were at least 100 μM, a 1000-fold greater than the concentration required to restore HDAC activity. The pleiotropic nature of curcumin in targeting so many cell signaling pathways complicates the process of identifying which pathway is essential for the anti-inflammatory effects. On the other hand, it may be that the ability to prevent crosstalk between the myriad of signaling pathways is a pre-requisite for its anti-inflammatory properties. Nevertheless, as glucocorticoids are the main thrust of anti-inflammatory treatment, any therapeutic that can be used as an add-on to improve steroid responsiveness in COPD and severe asthma would be of significant clinical benefit. If that compound also possessed intrinsic anti-inflammatory properties, this would be a bonus. Clearly, clinical trials using a combination approach of a steroid with an antioxidant or polyphenol are warranted.

6. Conclusion

There is now increasing evidence that ROS generation plays a major pathophysiological role in asthma and COPD, and it is important for the severity of these conditions. ROS generation through endogenous mechanisms or exogenous cigarette smoke/environmental oxidants is critical to the inflammatory response through activation of redox-sensitive transcription factors and pro-inflammatory signaling pathways. At the same time, endogenous antioxidant mechanisms are present to attenuate this redox-mediated inflammatory response. It is when these two opposing mechanisms are out of balance that a chronic and more severe inflammatory state becomes apparent. The use of antioxidants or other pharmacological agents to boost the endogenous antioxidant system could be used to redress this imbalance. In so doing, this would provide therapeutic benefit in damping down and curtailing the severity and chronicity of the inflammatory response in asthma and COPD.

Clearly, further studies are required to understand the effect of ROS on basic cellular functions and the differential responses seen in different cell types and how this in turn impacts on the pathology of different inflammatory disease states. At the same time, endeavors into identifying new and more efficacious antioxidants as a therapeutic strategy should continue. Indeed, elucidating the mechanism of action for some of the naturally occurring antioxidants, such as the potent

enzyme mimetics and polyphenols, may lead to new therapeutic targets that can be antagonized/agonized through more conventional pharmacological approaches.

Acknowledgments

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