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Antioxidant therapies in COPD

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Abstract: Oxidative stress is an important feature in the pathogenesis of COPD. Targeting oxidative stress with antioxidants or boosting the endogenous levels of antioxidants is likely to be beneficial in the treatment of COPD. Antioxidant agents such as thiol molecules (glutathione and mucolytic drugs, such as N-acetyl-L-cysteine and N-acetylcysteine), dietary polyphenols (curcumin, resveratrol, green tea, catechins/querceetin), erdosteine, and carbocysteine lysine salt, all have been reported to control nuclear factor-kappaB (NF-κB) activation, regulation of glutathione biosynthesis genes, chromatin remodeling, and hence inflammatory gene expression. Specific spin traps such as α-phenyl-N-tert-butyl nitron, a catalytic antioxidant (ECSOD mimetic), porphyrins (AEOL 10150 and AEOL 10113), and a superoxide dismutase mimetic M40419 have also been reported to inhibit cigarette smoke-induced inflammatory responses in vivo. Since a variety of oxidants, free radicals, and aldehydes are implicated in the pathogenesis of COPD, it is possible that therapeutic administration of multiple antioxidants will be effective in the treatment of COPD. Various approaches to enhance lung antioxidant capacity and clinical trials of antioxidant compounds in COPD are discussed.

Keywords: reactive oxygen species, lipid peroxides, polyphenols, glutathione, antioxidants, NF-κB, corticosteroids, chronic obstructive pulmonary disease, lungs

Introduction

Reactive oxygen species (ROS), such as superoxide anion ($O_2^{\cdot-}$) and the hydroxyl radical ($\cdot OH$), are highly unstable species with unpaired electrons capable of initiating oxidation. Lungs are continuously exposed to oxidants, generated either endogenously by metabolic reactions (eg, from mitochondrial electron transport during respiration or during activation of phagocytes) or exogenously, such as from air pollutants or cigarette smoke. Production of ROS has been directly linked to oxidation of proteins, DNA, and lipids, which may cause direct lung injury or induce a variety of cellular responses through the generation of secondary metabolic reactive species. ROS may alter remodeling of extracellular matrix and blood vessels, stimulate mucus secretion, inactivate antiproteases, cause apoptosis, and regulate cell proliferation (Rahman and MacNee 1996a, 1999). Furthermore, increased levels of ROS have been implicated in initiating inflammatory responses in the lungs through the activation of transcription factors such as nuclear factor-kappaB (NF-κB) and activator protein-1 (AP-1), signal transduction, chromatin remodeling, and gene expression of proinflammatory mediators (Rahman and MacNee 1998; Rahman 2003) (Figure 1). Since a variety of oxidants, free radicals, and aldehydes are implicated in the pathogenesis of COPD, it is possible that therapeutic administration of a variety of antioxidants will be effective in the treatment of COPD.

This paper discusses the rationale for antioxidant therapeutic intervention in the light of oxidative stress in the management of COPD.

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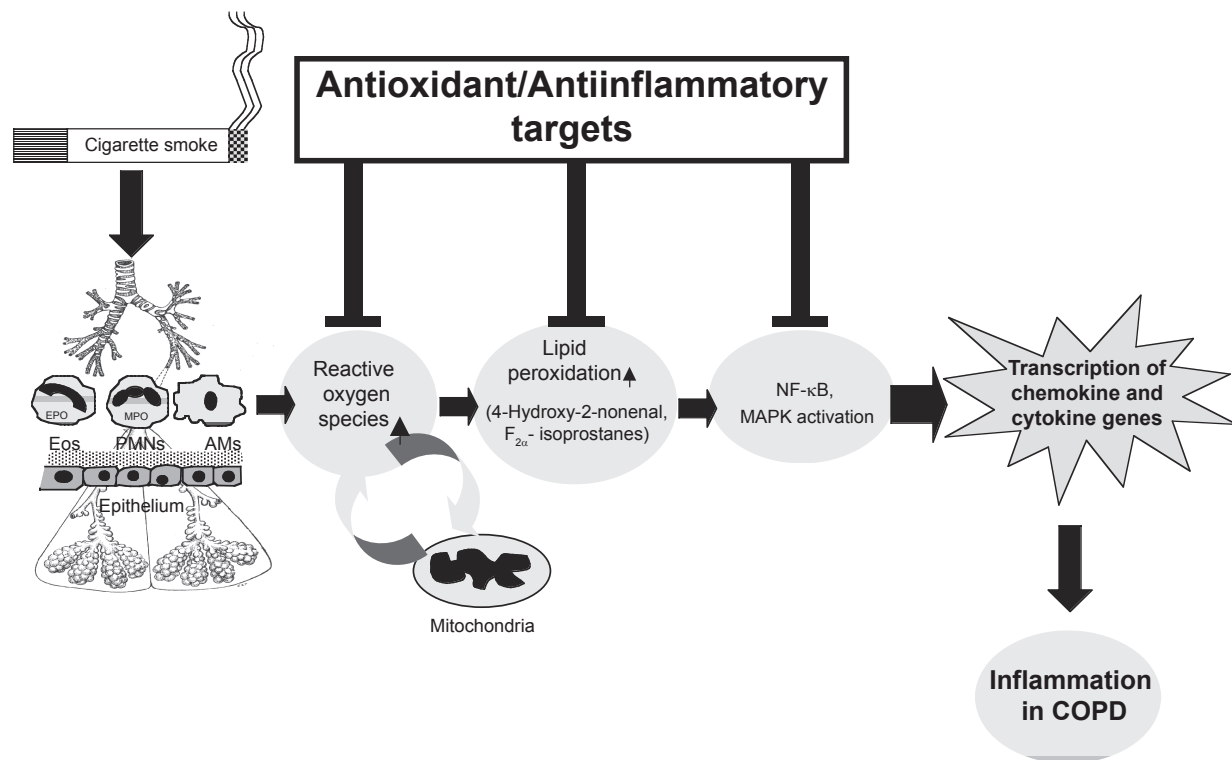


Figure 1 Mechanism of reactive oxygen species (ROS)-mediated lung inflammation. Inflammatory response is mediated by oxidants inhaled and/or released by the activated neutrophils, alveolar macrophages, eosinophils, and epithelial cells, leading to production of ROS and membrane lipid peroxidation. Activation of transcription of the proinflammatory cytokine and chemokine genes, up-regulation of adhesion molecules, and increased release of proinflammatory mediators are involved in the inflammatory responses in patients with COPD.

Inhaled oxidants and cigarette smoke

COPD is a slow, progressive condition characterized by airflow limitation, which is largely irreversible (ATS 1995; Pauwels et al 2001). Cigarette smoking is the major etiological factor in this condition. More than 90% of patients with COPD are smokers, but only 15%–20% of cigarette smokers show a rapid decline FEV₁ and develop the disease (Snider 1989). An increased oxidant burden in smokers derives from the fact that cigarette smoke contains more than 10¹⁷ oxidant/free radical molecules per puff and more than 4700 chemicals (Church and Pryor 1985).

Cell-derived ROS

The common feature of COPD is the development of an inflammatory response, characterized by activation of epithelial cells and resident macrophages and the recruitment and activation of neutrophils, eosinophils, monocytes, and lymphocytes (Rahman and MacNee 1996a). The activation of macrophages, neutrophils, and eosinophils generates O₂^{•-}, which is rapidly converted to H₂O₂ under the influence of superoxide dismutase (SOD), and •OH is formed

non-enzymatically in the presence of Fe²⁺ as a secondary reaction. ROS can also be generated intracellularly from several sources. The primary ROS-generating enzyme is NADPH oxidase, a complex enzyme system that is present in phagocytes and epithelial cells. In addition to NADPH oxidase, phagocytes employ other enzymes to produce ROS, which involves the activity of heme peroxidases (myeloperoxidase, MPO) or eosinophil peroxidase (EPO). Superoxide anion and H₂O₂ can also be generated by mitochondria and the xanthine/xanthine oxidase (XO) reaction. XO activity has been shown to be increased in cell-free bronchoalveolar lavage (BAL) fluid and plasma from COPD patients, compared with normal individuals. This has been associated with increased O₂^{•-} and lipid peroxide levels (Pinamonti et al 1998).

Oxidative stress biomarkers in exhaled breath condensate

Identification of non-invasive biomarkers of oxidative stress is important because antioxidant clinical trials or supplementation studies could potentially be monitored by measuring markers of oxidative stress. Recent studies have focused on applying non-invasive techniques, such as biomarkers

Table 1 Antioxidant therapeutic interventions in COPD**Antioxidant compounds**

Thiol compounds: N-acetyl-L-cysteine, N-acetylcystein, glutathione esters, thioredoxin, procysteine, erdosteine, N-isobutyrylcysteine
 Inducers of glutathione biosynthesis
 Antioxidant vitamins (vitamin A, E, C), β -carotene, CoQ₁₀
 Polyphenols (curcumin, resveratrol, quercetin, and green tea catechins)
 Nitron spin traps
 Superoxide dismutase and glutathione peroxidase mimetics
 Ebselen
 Porphyrins

in exhaled breath condensate to evaluate oxidative stress in COPD (Rahman and Kelly 2003; Rahman and Biswas 2004). Smokers and patients with COPD have higher levels of exhaled H₂O₂ than non-smokers, and levels are even higher during exacerbations of COPD (Kharitonov and Barnes 2001; Montuschi and Barnes 2002). The levels of 8-iso-prostaglandin F₂ α (8-isoprostane) in exhaled breath condensate are elevated in healthy smokers and more markedly in patients with COPD, reflecting the degree of oxidative stress (Rahman and MacNee 1998; Montuschi et al 2000). Non-specific lipid peroxidation products, such as thiobarbituric acid reactive substances (TBARS), have also been shown to be elevated in breath condensate and in lungs of patients with stable COPD (Pratico et al 1998; Nowak et al 1999). Other specific products of lipid peroxidation such as malondialdehyde and 4-HNE have also been shown to be increased in exhaled breath condensate of COPD patients (Rahman et al 2000; Rahman, van Schadewijk, et al 2002; Aoshiba et al 2003).

Antioxidant therapeutic interventions

Systemic antioxidant capacity and antioxidant vitamins

Smoking and exacerbations of COPD result in decreased antioxidant capacity in plasma in association with depleted protein sulfhydryls in the plasma (Rahman et al 1996, 1997; Corradi et al 2003). The decrease in antioxidant capacity in smokers occurs transiently during smoking and resolves rapidly after smoking cessation. In exacerbations of COPD, however, antioxidant capacity remains low for several days after the onset of the exacerbation, tending to return towards normal values at the time of recovery from the exacerbation (Rahman et al 1997). The depletion of antioxidant capacity could in part be explained by the increased release of ROS from peripheral blood neutrophils, as shown by a significant negative correlation between neutrophil superoxide anion release and plasma antioxidant capacity (Rahman et al 1996).

Thus, there is clear evidence that oxidants in cigarette smoke markedly decrease plasma antioxidants, which may play an important role in the pathogenesis of COPD. Furthermore, it is possible that interindividual differences in antioxidant capacity may contribute to the differences in susceptibility to cigarette smoke-induced COPD. Therefore, it is imperative to propose the rationale for antioxidant therapy ameliorating the increased oxidative stress and consequently the inflammatory response in COPD. There are various options to enhance the lung antioxidant screen (Table 1).

Depletion of total antioxidant capacity in smokers is associated with decreased levels of major plasma antioxidants in smokers (Pelletier 1970; Petruzzelli et al 1990; Duthie et al 1991; Antwerpen et al 1993; Bridges et al 1993; Mezzetti et al 1995; Rahman and MacNee 1996a). These studies show depletion of vitamin C, vitamin E, β -carotene, and selenium in the serum of chronic smokers and in patients with COPD (Pelletier 1970; Chow et al 1986; Duthie et al 1991; Antwerpen et al 1993; Bridges et al 1993; Mezzetti et al 1995; Tug et al 2004). Moreover, decreased vitamin E and vitamin C levels were reported in leukocytes and BAL fluids from smokers (Barton and Roath 1976; Bridges et al 1990; Theron et al 1990). Ascorbate appears to be a particularly important antioxidant in the plasma (Pacht et al 1988). Cigarette smoke-induced lipid peroxidation of plasma *in vitro* is decreased by ascorbate (Cross et al 1994). Reduced levels of vitamin E and a marginal increase in vitamin C have been shown in the BAL fluid of smokers, compared with non-smokers (Rahman and MacNee 1996b). Similarly, alveolar macrophages from smokers have both increased levels of vitamin C and augmented uptake of ascorbate, suggesting that these cells are trying to redress their antioxidant balance (Rahman and MacNee 1996b).

Dietary antioxidant supplementation is one of the simplest approaches to boost antioxidant defense systems. Supplementation of vitamin C, vitamin E, and β -carotene has been attempted in cigarette smokers and patients with COPD (Cross et al 1993; Allard et al 1994; Rautalahti

Table 2 Clinical trials conducted for the efficacy of antioxidants in smokers and in COPD

Trial	Antioxidant used	Aim of study	Disease/ Condition	Outcome	Reference
BRONCUS	NAC	Effect of NAC on FEV ₁	COPD	30% reduction in COPD hospitalization obtained without change on decline in FEV ₁	Decramer et al 2001, 2005
Systematic Cochrane review of 23 randomized, controlled trails	NAC (2 months of oral therapy)	Effect of NAC and antibiotics on number of days of disability	COPD	Significant reduction in days of disability (0.65 day per patient per month) and 29% reduction in exacerbations. No difference in lung function	Poole and Black 2001, 2003
Systematic Cochrane review of randomized, controlled trials; 11 of 39 retrieved trials	NAC	Use of validated score to evaluate the quality of each study	COPD	9 trials showed prevention of exacerbation; 5 addressed improvement of symptoms compared with 34.6% of patients receiving placebo	Stey et al 2000
Meta-analysis of published trials	NAC	Assess possible prophylactic benefit of prolonged treatment	COPD	23% decrease in number of acute exacerbations	Grandjean et al 2000
–	NAC (600mg once daily for 12 months)	Effect of NAC on H ₂ O ₂ and TBARS in exhaled breath condensate	COPD	No change in TBARS; reduced H ₂ O ₂ levels	Kasielski and Nowak 2001
–	β-Carotene (20 mg/day) and vitamin E (50 mg/day)	Effect on symptoms (chronic cough, phlegm, or dyspnea)	COPD	No benefit on symptoms	Habib et al 1999
–	β-Carotene (20 mg daily for 4 weeks)	Effect on lipid peroxide levels in exhaled breath	Smokers	Reduced lipid peroxidation (pentane levels) in exhaled breath	Allard et al 1994; Rautalahti et al 1997; Steinberg and Chait 1998
The MORGEN study	Diet rich in polyphenols and bioflavonoids (catechin, flavonol, and flavone) (58 mg/day)	Effect on FEV ₁ , chronic cough, breathlessness, and chronic phlegm	COPD	Positively associated with decline in FEV ₁ and inversely associated with chronic cough and breathlessness, but not chronic phlegm	Tabak et al 2001
European countries (Finnish, Italian, and Dutch cohorts)	Diet rich in fruits, vegetables, and fish intake	Effect on 20-year COPD mortality	COPD	24% lower COPD mortality risk	Walda et al 2002
–	Vitamin C (500 mg daily for 4 weeks)	Effect on lipid peroxide levels in breath and plasma	Smokers	No change in lung function No change in lipid peroxidation (breath pentane and plasma MDA levels)	Allard et al 1994
–	Vitamin C (600 mg), vitamin E (400 IU), β-carotene (30 mg)	Effect on lipid peroxidation	Smokers	Reduced lipid peroxidation	Aghdassi et al 1999; Lykkesfeldt et al 2000
–	Vitamin E (400 IU twice daily for 3 weeks)	Effect on breath ethane levels	Smokers	No effect on breath ethane levels	Habib et al 1999

Abbreviations: BRONCUS, Bronchitis Randomized on NAC Cost-Utility Study; MDA, malondialdehyde; NAC, N-acetyl-L-cysteine; TBARS, thiobarbituric acid reactive substances.

et al 1997; Steinberg and Chait 1998; Aghdassi et al 1999; Habib et al 1999; Lykkesfeldt et al 2000) (Table 2). In the general population there is a positive association between dietary intake of antioxidant vitamins and lung function. Epidemiological studies have demonstrated negative associations of dietary antioxidant intake with pulmonary function and with obstructive airway disease (Grievink et al 1998). Britton and co-workers (1995) showed a positive association between dietary intake of the antioxidant vitamin E and lung function in a population of 2633 subjects, supporting the hypothesis that this antioxidant may have a role in protecting against the development of COPD. Another study has suggested that antioxidant levels in the diet could be a possible explanation for differences in COPD mortality in different populations (Sargeant et al 2000). Dietary polyunsaturated fatty acids may also protect cigarette smokers against the development of COPD (Shahar et al 1999). These studies support the concept that dietary antioxidant supplementation including polyphenols may be a possible therapy to prevent or inhibit oxidative stress and inflammatory responses, which are key features in the development of COPD.

Antioxidant vitamin supplementation reduces oxidant stress in smokers, measured as a decrease in pentane levels in breath as an indication of lipid peroxides in the airways (Euler et al 1996). Dietrich and colleagues (2002) have recently shown that vitamin C or an antioxidant mixture containing vitamin C, α -lipoic acid, and vitamin E decreases plasma F_2 -isoprostane levels in smokers with high body mass index, suggesting modulation of lung oxidative stress with these dietary supplements. This study suggested that cigarette smoking depletes a variety of multiple antioxidants needed to quench an array of free radicals present in cigarette smoke and inhibit inflammatory response induced by cigarette smoking. However, robust clinical trials using dietary antioxidant vitamins and polyphenols are urgently needed to address the beneficial effects of these antioxidants in COPD.

Directly increasing lung antioxidant capacity

The development and progress of COPD are associated with increased oxidative stress or decreased antioxidant resources (Boots et al 2003). The most direct way to redress the oxidant imbalance in COPD would be to increase the lung's capacity to produce antioxidants. A variety of means by which to do

this have been attempted with varying success.

Lung thiols: glutathione and its biosynthesis

The thiol antioxidant glutathione (GSH) is concentrated in epithelial lining fluid compared with plasma (Cantin et al 1987; Pacht et al 1988) and has an important protective role in the airspaces and intracellularly in epithelial cells. Several studies have suggested that GSH homeostasis may play a central role in the maintenance of the integrity of the lung airspace epithelial barrier. Decreasing the levels of GSH in epithelial cells leads to loss of barrier function and increased permeability (Li et al 1996; Morrison et al 1999). Human studies have shown elevated levels of GSH in epithelial lining fluid in chronic cigarette smokers compared with non-smokers (Cantin et al 1987; Morrison et al 1999). However, this increase is not present immediately after acute cigarette smoking (Morrison et al 1999). The twofold increase in BAL fluid GSH in chronic smokers may not be sufficient to deal with the excessive oxidant burden during smoking, when acute depletion of GSH may occur (Harju et al 2002). Harju and colleagues (2002) found that the immunoreactivity of glutamate cysteine ligase (GCL), the rate-limiting enzyme in GSH synthesis, was decreased in the airways of smokers compared with non-smokers, suggesting that cigarette smoke predisposes lung cells to ongoing oxidant stress. Neurohr and colleagues (2003) recently showed that decreased GSH levels in BAL fluid cells of chronic smokers were associated with a decreased expression of GCL light subunit without a change in GCL heavy subunit expression. Increasing the activity of GCL would be expected to increase cellular GSH levels. The induction of GCL by molecular means to increase cellular GSH levels or GCL gene therapy also holds great promise in protection against chronic inflammation and oxidant-mediated injury in COPD.

Direct increase of lung cellular levels of GSH would be a logical approach to enhance the antioxidant potential in the treatment of COPD. In fact, extracellular augmentation of GSH has been tried through intravenous administration of GSH, oral ingestion of GSH, and aerosol inhalation of nebulized GSH in an attempt to reduce inflammation in various lung diseases (Rahman and MacNee 1999, 2000). However, all these routes of administration lead to undesirable effects, which suggests that direct GSH therapy may not be an appropriate way of increasing GSH levels in lung epithelial lining fluid and cells in COPD. The bioavailability of GSH, pH, and osmolality in the inflammatory microenvironment, and resultant formation of toxic products

(GSSG and GSH-adducts) are further challenges for direct GSH administration. Alternative formulations may address bioavailability, such as liposomal delivery, but at present it seems that direct administration of GSH will not be successful in treating COPD.

N-acetyl-L-cysteine (NAC)

NAC, a cysteine-donating reducing compound, acts as a cellular precursor of GSH and becomes deacetylated in the gut to cysteine following oral administration (Cotgreave 1997). NAC may also reduce cystine to cysteine, which is an important mechanism for intracellular GSH elevation *in vivo* in lungs. It reduces disulfide bonds (a property of a good reducing agent), but also has the potential to interact directly with oxidants. NAC is also used as a mucolytic agent, to reduce mucus viscosity and to improve mucociliary clearance. A Cochrane systematic review evaluated the effects of treatment with mucolytic agents in patients with COPD. Mucolytic treatment was associated with a significant reduction of 0.79 exacerbations per patient per year compared with placebo, a 29% decrease. How mucolytic agents work is unknown, although they may reduce exacerbations by altering mucus production, by breakdown of sulfhydryl groups, or through antibacterial or immunostimulatory effects (Poole and Black 2001, 2003). Although small-scale trials failed to demonstrate any clear clinical benefits, a few meta-analyses have shown a small but significant clinical benefit in COPD (Grandjean et al 2000; Dekhuijzen 2004).

Pharmacological administration of NAC has been used, with varying success, in an attempt to enhance lung GSH in patients with COPD (Rasmussen and Glennow 1988; Bridgeman et al 1994). Van Schooten et al (2002) have reported that in a randomized, double-blind, placebo-controlled phase II trial, a 6-month oral dose of 600 mg twice daily reduced various plasma and BAL fluid oxidative biomarkers in smokers. Similarly, it has been shown that treatment with NAC 600 mg once daily for 12 months also reduced the concentration of H_2O_2 in exhaled breath condensate compared with placebo in stable COPD patients (Kasielski and Nowak 2001). A more recent clinical trial also proves that oral administration of NAC 600 mg twice daily for 2 months rapidly reduces the oxidant burden in airways of stable COPD patients (De Benedetto et al 2005). This reduction in oxidative biomarkers results in clinical benefit such as reduction in bronchial hypersecretion (Aylward et al 1980) in addition

to a decline in FEV_1 and in exacerbations (Stey et al 2000). Orally administered NAC has been shown to increase phagocytic activity of BAL macrophages from healthy smokers (Linden et al 1993); similar results were not seen in COPD patients, possibly owing to active concentrations of NAC not reaching the lung, as *in vitro* analysis of cells supports an induction of phagocytosis by NAC (Vecchiarelli et al 1994). It has also been reported recently that orally administered NAC increased the quadriceps endurance time of severe COPD patients (Koechlin et al 2004), thus suggesting that NAC administration may have beneficial effects on the systemic oxidative stress associated with COPD. However, a multicenter study using NAC delivered by metered-dose inhalers in patients with chronic cough failed to show a positive effect on wellbeing, sensation of dyspnea, cough, or lung function (Dueholm et al 1992).

Although there is a body of evidence that the administration of NAC provides benefit for COPD patients, it is not clear whether this represents a maintenance therapy (Decramer et al 2001). A phase III multicenter trial, Bronchitis Randomized on NAC Cost-Utility Study (BRONCUS), has recently been completed, with the aim of addressing this question and determining whether the effectiveness of NAC as an "antioxidant" results in an alteration in the rate of decline in FEV_1 , exacerbation rate, and quality of life in patients with moderate to severe COPD and hence supporting NAC administration as a maintenance therapy for COPD (Gerrits et al 2003) (Table 2). The results of the phase III BRONCUS trial showed no effect on decline in FEV_1 but a reduction in overinflation in patients with severe COPD and exacerbation rate in patients not treated with inhaled glucocorticoids (Decramer et al 2005).

N-acystelyn (NAL)

NAL, a lysine salt of NAC, is a mucolytic and antioxidant (reducing) thiol compound. The advantage of NAL over NAC is that it has a neutral pH in solution, whereas NAC is acidic. NAL can be aerosolized into the lung without causing significant side effects (Gillissen et al 1997). Gillissen and co-workers compared the effect of NAL and NAC and found that both drugs enhanced intracellular GSH in alveolar epithelial cells and inhibited hydrogen peroxide and $O_2^{\cdot-}$ released from human blood-derived polymorphonuclear leukocytes from smokers with COPD. NAL also inhibited ROS generation induced by serum-opsonized

zymosan by human polymorphonuclear neutrophils. This inhibitory response was comparable to the effects of NAC (Gillissen et al 1997). Recently, Antonicelli and colleagues (2002) have shown that NAL inhibited oxidant-mediated interleukin (IL)-8 release in alveolar epithelial A549 cells, suggesting an antiinflammatory effect of NAL. Therefore, NAL may represent an interesting alternative approach to augment the antioxidant screen, thereby inhibiting inflammatory responses in the lungs, and it has the advantage over other antioxidant agents in that it may be administered by inhalation. A clinical trial using NAL in the treatment of COPD is in progress.

N-isobutyrylcysteine (NIC)

Because NAC becomes hydrolyzed in biological systems, the measured bioavailability of the drug is low. Thus, it was speculated that a drug might be synthesized that had greater bioavailability than NAC and could be used as a more effective treatment for chronic bronchitis. NIC is a NAC-like thiol compound that does not undergo effective first-pass hydrolysis and hence has higher oral bioavailability than NAC (Ekberg-Jansson et al 1999). The oral bioavailability can be as high as 80%, depending on food intake. However, when evaluated as a therapy for exacerbations of chronic bronchitis, NIC performed no better than placebo and not as well as NAC (Gillissen et al 1997). Furthermore, a study of NIC also failed to reduce exacerbation rates in patients with COPD (Ekberg-Jansson et al 1999).

Erdosteine

Erdosteine is a new thiol compound that acts as an antioxidant, but in addition has mucoactive properties and reduces bacterial adhesiveness. In the "Equalife" randomized, placebo-controlled clinical study, erdosteine was administered orally 300mg twice daily for 8 months (Moretti et al 2004). Patients receiving erdosteine had significantly fewer exacerbations and spent fewer 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. It is not clear whether this clinical benefit is due to antioxidant effects of erdosteine. The mucolytic effect of erdosteine is perhaps due to the presence of a sulfhydryl group. It may be possible that erdosteine reduces bacterial colonization through a direct effect on adhesion.

Procysteine

Procysteine (L-2-oxothiazolidine-4-carboxylate) is a cysteine-donating compound that increases the cysteine levels of the cells and has greater bioavailability than NAC. This thiol compound is well tolerated and has been shown to increase mitochondrial levels of GSH in alveolar type II cells (Guidot and Brown 2000). Glutathione esters, particularly GSH monoethyl esters, can increase the GSH levels of the cells by cleavage of ester bond (an ethyl group esterified to glycine). GSH esters have been shown to increase GSH levels in the lungs of rats; however, this compound can be cytotoxic, and variation in the uptake levels of GSH has been shown in various cellular models (Butterworth et al 1993).

Antioxidant enzyme mimetics and spin traps

Increased activity of antioxidant enzymes (SOD and superoxide catalase) in alveolar macrophages from young smokers has been reported (McCusker and Hoidal 1990). However, Kondo and co-workers (1994) found that the increased superoxide generation by alveolar macrophages in elderly smokers was associated with decreased antioxidant enzyme activity when compared with that of non-smokers. The activities of CuZn superoxide dismutase (CuZnSOD), glutathione-S-transferase, and glutathione peroxidase are all decreased in alveolar macrophages from elderly smokers (Gilks et al 1998).

The activities of SOD and glutathione peroxidase have been shown to be higher in the lungs of rats exposed to cigarette smoke (York et al 1976). McCusker and Hoidal (1990) have also demonstrated enhanced antioxidant enzyme activity in alveolar macrophages from hamsters after cigarette smoke exposure, which resulted in reduced mortality when the animals were subsequently exposed to >95% oxygen. They speculated that alveolar macrophages undergo an adaptive response to chronic oxidant exposure that ameliorates potential damage to lung cells from further oxidant stress. The mechanisms for the induction of antioxidant enzymes in erythrocytes (Toth et al 1986), alveolar macrophages (McCusker and Hoidal 1990), and lungs (York et al 1976) by cigarette smoke exposure are currently unknown.

Spin traps such as α -phenyl-N-tert-butyl nitron react directly with reactive oxygen and reactive nitrogen species at the site of inflammation (Chabrier et al 1999). Smith and colleagues (2002) have shown that intratracheal instillation of a catalytic antioxidant, manganese(III) meso-tetrakis (N,N'-diethyl-1,3-imidazolium-2-yl) porphyrin

(AEOL 10150 and AEOL 10113), inhibited the cigarette smoke-induced inflammatory response (decreased number of neutrophils and macrophages) in rats after 2 days or 8 weeks (6 hours/day, 3 days/week) exposure. These compounds also mimic extracellular SOD and superoxide catalase, scavenging both lipid peroxides and peroxy-nitrite, and have been shown to be effective in a number of animal models of lung disease.

SOD mimetic M40419 has been shown to block the development of emphysema and significantly reduce lung markers of oxidative stress in an animal model (Tuder et al 2003). Animal studies have shown that recombinant SOD treatment can prevent the neutrophil influx to the airspaces and IL-8 release induced by cigarette smoking through a mechanism involving down-regulation of NF- κ B (Nishikawa et al 1999). This further substantiates the idea that compounds with antioxidant enzyme properties may be able to act as novel antiinflammatory drugs by regulating the molecular events in COPD.

Glutathione peroxidase mimic

Small molecules with enzymatic activity similar to glutathione peroxidase, such as the seleno-organic compound ebselen, have been developed. Ebselen increases the efficiency of GSH as an antioxidant and can thus be used as therapy against oxidative stress and inflammation. Recent studies have shown that ebselen inhibits airway inflammation (neutrophil recruitment and chemokine expression) in response to lipopolysaccharide in various animal models (Haddad et al 2002; Zhang et al 2002). It would be interesting to see whether similar results can be obtained with ebselen in response to smoking *in vivo*.

Redox sensor molecules

There is a range of small redox molecules such as β -strand mimetic templates MOL-294 and PNRI-299 that have been shown to inhibit NF- κ B and AP-1-mediated transcription and block allergic airway inflammation in a mouse asthma model (Henderson et al 2002). The mechanism of inhibition is based on the reversible inhibition of redox sensor proteins (similar to redox effector factor-1). These redox compounds are novel and have been shown to reduce airway eosinophil infiltration, mucus hypersecretion, edema, and cytokine release in a mouse asthma model. However, the activity of these compounds in cigarette smoke-induced oxidative stress and proinflammatory mediator release assays *in vitro* or *in vivo* has not been reported.

Inhibition of superoxide production from inflammatory neutrophils: phosphodiesterase 4 inhibitor

Various mechanisms inhibit superoxide production *in vitro* through increasing intracellular levels of cyclic adenosine monophosphate (cAMP); the most advanced of these being the use of phosphodiesterase 4 (PDE4) inhibitors. These inhibitors act by increasing intracellular concentrations of cAMP, which has a broad range of antiinflammatory effects on various key effector cells involved in asthma and COPD (Lipworth 2005). Raising cAMP in neutrophils blocks the assembly of NADPH oxidase and hence inhibits superoxide production. These compounds also potently inhibit expression of a variety of cytokines, such as tumor necrosis factor (TNF)- α and monocyte inflammatory protein (MIP)-1 β , and therefore may have a broad antiinflammatory profile. Clinical benefit of PDE4 inhibitors has been demonstrated in COPD (Compton et al 1999; Soto and Hanania 2005), although these compounds are dose limited by emetic and cardiac effects (Sturton and Fitzgerald 2002). This is perhaps due to inhibition of a wrong isoform of PDE4 isoenzyme (PDE4D), instead of PDE4B (antiinflammatory). Currently the PDE4 inhibitor daxos and roflumilast (Altana) is in phase III trials and may represent the most likely new mechanism to enter the market for the treatment of COPD in the short term.

Modulation of redox-sensitive transcription factors and inflammatory pathways

A number of transcription factors involved in the regulation of a variety of inflammatory genes, such as NF- κ B and AP-1, are activated by oxidative stress (Rahman 2002). NF- κ B exists as a heterodimeric complex usually of p50 and p65/RelA subunits. Di Stefano and colleagues (2002) demonstrated increased expression of the p65 protein of NF- κ B in bronchial epithelium of smokers and patients with COPD. The increased expression of p65 in epithelial cells was correlated with the degree of airflow limitation in patients with COPD. Similarly, Caramori and co-workers (2003) have shown that the p65 subunit of NF- κ B was increased in sputum macrophages but not in sputum neutrophils during exacerbations of COPD, suggesting cell-specific activation of this factor. The activation of NF- κ B in monocytes/macrophages can then trigger the release of proinflammatory mediators in lung epithelial fluid, which would then amplify the inflammatory cascade

by activation of epithelial cells as well as recruitment of neutrophils to the airways. Activation of NF- κ B by oxidative stress is inhibited by co-incubation with NAC in vitro, providing evidence for activation of this transcription factor in these patients being due to oxidative stress (Schreck et al 1992). Small molecule inhibition of this pathway is currently of great interest as a potential means to down-regulate the inflammation characteristic of COPD. I- κ B kinase-2 (IKK)-mediated phosphorylation of I- κ B is required for its subsequent ubiquitination and degradation; therefore small molecule inhibitors of this enzyme would be expected to block the nuclear translocation of NF- κ B. A variety of compounds are in preclinical development for this target from GlaxoSmithKline, AstraZeneca, Altana, and Tularik, but as yet no compound suitable for clinical studies has been reported in the literature. Alternative means to inhibit NF- κ B activation, such as inhibitors

of ubiquitin ligase (Wertz et al 2004), peptide inhibitors of NEMO (a protein that forms a complex with IKK 2) (Choi et al 2003), PPAR α agonists, and direct inhibition of NF- κ B transport through nuclear pores, are being investigated in preclinical studies.

A recent study reported on the potential therapeutic effect of the small molecule antagonist siRNA against NF- κ B subunit p65 in airway epithelial cell lines (McIntyre et al 2003). In this in vitro study, cells treated with TNF α showed reduced NF- κ B p65 expression and concomitant IL-6 and CXCL8 expression. An antisense antagonist for NF- κ B p65 may be beneficial in inhibiting the NF- κ B-mediated inflammatory genes in patients with COPD.

NF- κ B-targeted therapies, inhibitors of IKK, may prove to be useful in antiinflammatory therapies (Table 3). The potential disadvantage of using inhibitors of cell signaling molecules is that mitogen-activating protein kinase (MAPK), NF- κ B, and

Table 3 Direct and indirect antioxidant drugs under development for the treatment of COPD

Drug name	Drug type	Company	Indication	Stage of development
Ariflo-S207499	PDE4 inhibitor	SmithKline Beecham	COPD	Phase III
Roflumilast	PDE4 inhibitor	Altana	COPD and asthma	Phase III in EuropePhase II/III in US
D-4418	PDE4 inhibitor	CellTech in UK in collaboration with Merck	COPD and asthma	Phase II
SCH351591	PDE4 inhibitor	Schering-Plough in collaboration with CellTech of UK	COPD and asthma	Phase I
Daxos	PDE4 inhibitor	Altana	COPD	Phase III
IC485	PDE4 inhibitor	Icos Corp, WA, USA	COPD and rheumatoid arthritis	Preclinical
BAY019-8004 (a benzofuran derivative)	PDE4 inhibitor	Bayer	COPD and asthma	Phase I
ATRA (all trans retinoic acid)	Derivative of vitamin A	Roche	Emphysema	Phase II; approved as Vesanoïd for acute promyelocytic leukemia
Mucolytic	N-acetyl-L-cysteine and its derivatives, carbocysteine and N-isobutylcysteine,	Zambon	COPD	Phase III No effects on lung function but frequency of exacerbations reduced
NAL (CAS 89344-48-9)	N-acetylcystein	SMB Pharma, Belgium	COPD	Phase I
BO-653	Lipid peroxidation inhibitor	Chugai Pharma, Japan	COPD	Preclinical
BXT-51072	Glutathione peroxidase mimetic	Oxis, USA	COPD	Phase I
ZD4407	5-lipoxygenase inhibitor	AstraZeneca	COPD	Preclinical
NF- κ B inhibitors	IPL-576092 (an analog of contignasterol, a natural compound)	Aventis Pharma	COPD	Phase II
NF- κ B inhibitors	BMS345541 and SPC600839	BristolMyersSquibb and Celgene/Serono	COPD	Clinical trials awaited

IKK are general signal transduction proteins involved in multiple processes and therefore inhibitors may have unacceptable side effects. In addition, inhibition of these molecules may also impair defense mechanisms against infections.

Oxidative stress and steroid efficacy in COPD

It has been suggested that oxidative stress may have a role in the poor efficacy of corticosteroids in COPD. Ito and co-workers (2001) showed a role for histone acetylation and deacetylation in IL-1 β -induced TNF α release in alveolar macrophages derived from cigarette smokers. They also suggested that oxidants may play an important role in the modulation of histone deacetylase (HDAC) and inflammatory cytokine gene transcription. Furthermore, we have shown that both cigarette smoke/H₂O₂ and TNF α caused an increase in histone acetylation (HAT activity) leading to IL-8 expression in monocytes and alveolar epithelial cells both in vitro and in vivo in rat lungs (Rahman, Gilmour, et al 2002; Marwick et al 2004; Moodie et al 2004).

Glucocorticoid suppression of inflammatory genes requires recruitment of HDAC2 to the transcription

activation complex by the glucocorticoid receptor (Ito et al 2001; Rahman et al 2004). This results in deacetylation of histones and a decrease in inflammatory gene transcription. A reduced level of HDAC2 was associated with increased proinflammatory response and reduced responsiveness to glucocorticoids in alveolar macrophages obtained from smokers (Ito et al 2001; Rahman, Gilmour, et al 2002; Marwick et al 2004; Moodie et al 2004; Rahman et al 2004). Culpitt and co-workers have shown that cigarette smoke solution stimulated release of IL-8 and granulocyte-macrophage colony-stimulating factor, which was not inhibited by dexamethasone, in alveolar macrophages obtained from patients with COPD compared with those of smokers (Culpitt, Rogers, Shah, et al 2003). They suggested that the lack of efficacy of corticosteroids in COPD might be due to steroid insensitivity of macrophages in the respiratory tract. Thus, the cigarette smoke/oxidant-mediated reduction in HDAC2 levels in alveolar epithelial cells and macrophages will not only increase inflammatory gene expression but also cause a decrease in glucocorticoid function in patients with COPD (Figure 2). HDAC activity has also been measured in bronchial biopsies and alveolar macrophages

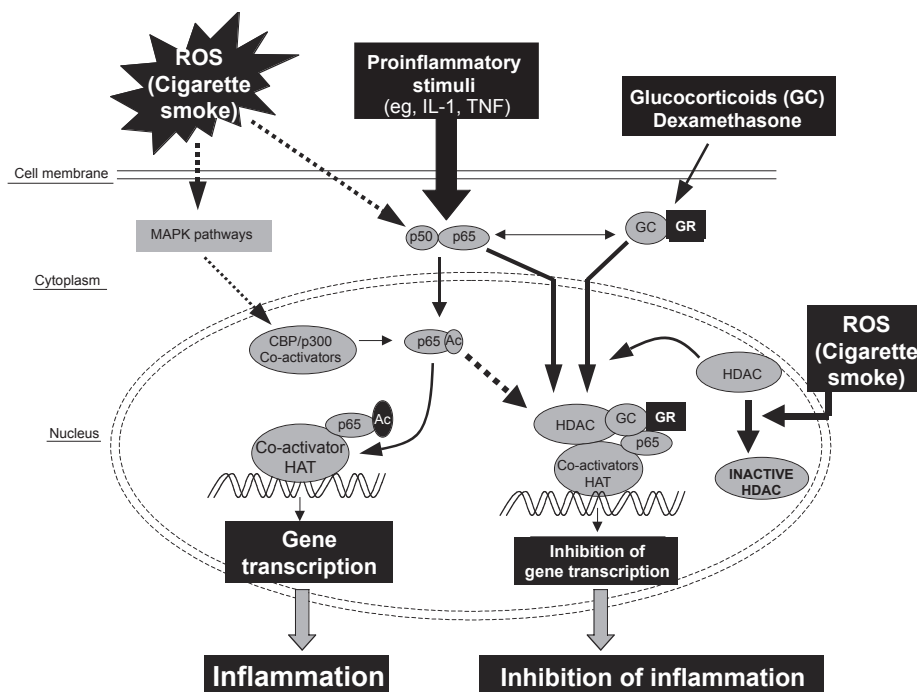


Figure 2 Model showing the possible mechanism of histone acetylation by oxidative stress and its repression by corticosteroids (GCs), leading to inhibition of gene transcription. Mitogen-activating protein kinase (MAPK) signaling pathways may be activated by oxidative stress, leading to histone acetylation. Direct interaction between co-activators (HAT), histone deacetylase (HDAC), and the glucocorticoid receptor (GR) may result in repression of the expression of proinflammatory genes. HDAC forms a bridge with HAT to inhibit gene transcription. However, when the HDAC is inhibited by oxidants or the NF- κ B subunit p65 is acetylated, steroids may not be able to recruit HDACs into the transcriptional complex to inhibit proinflammatory gene expression.

from COPD patients and smoking controls, demonstrating a significant decrease in HDAC activity, the magnitude of which increased with severity of disease (Ito et al 2005). Moreover, protein expression of HDAC2 was decreased in a similar manner in COPD patients.

Consequently, a potential means by which to treat COPD would be to increase HDAC2 expression and activity such that steroids regain their antiinflammatory activity. We have shown that co-incubation of cells with NAC and H₂O₂ protects HDAC2 from down-regulation and reduction of specific activity (Moodie et al 2004). In addition, it has been reported that theophylline has a similar effect in lung macrophage cells, increasing HDAC2 expression and re-sensitizing the cells to steroids (Borja et al 2004). Alternative means of up-regulating HDAC2 activity would be of great interest for potential combination therapies of the future.

Polyphenols

Dietary polyphenols have antioxidant and antiinflammatory properties that may explain their beneficial effects (Arts and Hollman 2005). Curcumin is an active principle of the perennial herb *Curcuma longa* (commonly known as turmeric). Turmeric has a long traditional use in the Orient for many ailments, particularly as an antiinflammatory agent. Recent studies have reported that curcumin inhibits NF- κ B expression/activation, IL-8 release, cyclooxygenase (COX)-2, heme oxygenase-1, cytokines, and neutrophil recruitment in the lungs (Shishodia et al 2003; Biswas et al 2005). Curcumin has multiple properties to protect against cigarette smoke-mediated oxidative stress (Shishodia et al 2003). It acts as oxygen radical and hydroxyl radical scavenger, increases antioxidant glutathione levels by induction of GCL, and acts as an antiinflammatory agent through inhibition of NF- κ B and IL-8 release in lung cells. Resveratrol, a flavonoid found in red wine, is an effective inhibitor of inflammatory cytokine release from macrophages in COPD patients (Culpitt, Rogers, Fenwick, et al 2003). This antiinflammatory property of resveratrol may be due to its ability to induce sirtuins and HDAC activity (Howitz et al 2003). A recent in vivo study has shown that resveratrol inhibits inflammatory cytokine expression in response to lipopolysaccharide in rat lungs (Birrell et al 2005). The molecular mechanisms of antiinflammatory properties of dietary polyphenols against cigarette smoke/oxidative stress have not yet been studied. This compound

may induce phase II detoxifying genes by Nrf-2-dependent mechanisms.

Recent studies from our laboratory show that these dietary polyphenols restore glucocorticoid functions in response to oxidative stress imposed by cigarette smoke by up-regulation of HDAC activity in the monocyte/macrophage (U937) and MonoMac6 cell lines (Rahman et al 2005). This was associated with restoration of HDAC1, HDAC2, and HDAC3 levels, suggesting that dietary polyphenol-mediated inhibition of proinflammatory cytokines increases formation of HDAC-p65 complex with glucocorticoid receptor, hence rendering NF- κ B ineffective. The other possible mechanism of polyphenol-mediated inhibition of inflammatory response is by quenching oxidants and aldehydes and inhibiting histone deacetyltransferase activity. Catechins present in green tea (epigallocatechin-3-gallate) in addition to theophylline may be effective in cigarette smoke-mediated oxidative stress and inflammatory response (Schwartz et al 2005). However, this compound has never been tested in in vitro or in vivo smoking models. Overall, these dietary polyphenols and flavonols may not only act as antioxidant/antiinflammatory agents, but also possibly increase the efficacy of glucocorticoids in COPD.

Bioflavonoids possess both antioxidant and antiinflammatory properties and hence may influence chronic inflammatory diseases such as COPD. Tabak and colleagues (2001) studied the intake of catechins, flavonols, and flavones in relation to pulmonary function and COPD symptoms in 13 651 adults from three Dutch cities. Dietary intake of catechin (eg, green tea polyphenols, epigallocatechin gallate), flavonol (eg, quercetin and kaempferol), and flavone (eg, apigenin and luteolin) was positively associated with FEV₁ and inversely associated with chronic cough and breathlessness, but not chronic phlegm. More importantly, single-component (such as catechin) intake was independently associated with FEV₁ and all three COPD symptoms, whereas flavonol and flavone intake was independently associated with chronic cough only. The importance of this study was further substantiated by a study by Walda and colleagues (2002), who showed the beneficial protective effect of fruit containing polyphenols and vitamin E against COPD symptoms in 20-year COPD mortality from three European countries, in Finnish, Italian, and Dutch cohorts. These important studies certainly encourage further multinational studies to demonstrate the beneficial effects of a high intake of nutraceuticals (polyphenols/bioflavonoids) against COPD symptoms.

Conclusions

There is now considerable evidence for the increased generation of ROS in COPD, which may be important in the pathogenesis of this condition. There are several small molecule compounds in clinical trials that target oxidant signaling or quench oxidants derived from cigarette smoke. Antioxidant and/or antiinflammatory agents such as thiol molecules, spin traps, dietary polyphenols, antioxidant mimetics, and inhibitors of oxidative stress-induced signaling pathways present potential means by which to treat this element of COPD. Antioxidant compounds may also enhance the efficacy of glucocorticoids by quenching oxidants and aldehydes, increasing histone deacetylase activity in COPD patients. Dietary polyphenols, such as resveratrol and curcumin, inhibit cigarette smoke/oxidant-induced NF- κ B activation, histone acetylation, and proinflammatory cytokine release and restore glucocorticoid functions via a mechanism involving up-regulation of HDAC activity. Thus, dietary polyphenols regulate inflammatory response at the molecular level, and possibly this is a way forward to restore glucocorticoid efficacy in the treatment of smoking-induced chronic inflammatory diseases. An effective wide-spectrum antioxidant therapy that has good bioavailability and potency is urgently needed to control the localized oxidative and inflammatory processes that occur in the pathogenesis of COPD. Although thiol antioxidant treatments have shown promising effects in targeting ROS and oxidant-mediated cellular alterations, in vivo cigarette smoke studies and human clinical trials of other small molecule antioxidants with dual activities (antioxidant and antiinflammatory) are urgently needed to validate these compounds as clinical therapies.

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