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REVIEW

Antioxidant properties of *N*-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease

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Antioxidant properties of N-*acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease. P.N.R. Dekhuijzen. ©ERS Journals Ltd 2004.*

ABSTRACT: Oxidative stress has been implicated in the pathogenesis and progression of chronic obstructive pulmonary disease.

Both reactive oxidant species from inhaled cigarette smoke and those endogenously formed by inflammatory cells constitute an increased intrapulmonary oxidant burden. Structural changes to essential components of the lung are caused by oxidative stress, contributing to irreversible damage of both parenchyma and airway walls. In addition, oxidative stress results in alterations in the local immune response, increasing the risk of infections and exacerbations, which, in turn, may accelerate lung function decline.

The antioxidant *N*-acetylcysteine, a glutathione precursor, has been applied in these patients in order to reduce symptoms, exacerbations and the accelerated lung function decline. This article reviews the presently available experimental and clinical data on the antioxidative effects of *N*-acetylcysteine in chronic obstructive pulmonary disease.
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Oxidative stress has been implicated in the pathogenesis and progression of chronic obstructive pulmonary disease (COPD) [1–5]. Both reactive oxidant species (ROS) from inhaled cigarette smoke and those endogenously formed by inflammatory cells constitute an increased intrapulmonary oxidant burden. Data from *in vitro*, *ex vivo* and *in vivo* studies indicate two sorts of effect of oxidative stress relevant to COPD. Structural changes to essential components of the lung are caused by oxidative stress, contributing to irreversible damage of both parenchyma and airway walls [6, 7]. In addition, oxidative stress may result in alterations in the local immune response [7]. Theoretically, this might increase the risk of infections and exacerbations, which, in turn, may accelerate lung function decline [8], although there are no data to support this contention.

Oxidative stress is closely linked to inflammation. The inflammatory process in patients with stable COPD is dominated by macrophages, CD8+ T-lymphocytes and neutrophils, and to a lesser extent mast cells, in the bronchial submucosa and alveoli [5]. Increased production of mediators, such as interleukin (IL)-8, tumour necrosis factor-α (TNF-α) and leukotriene B_4, which both attract inflammatory cells and increase oxidant production by these cells, has been found.

Attenuation of oxidative stress would be expected to result in reduced pulmonary damage and a decrease in local infections, contributing to attenuation of the progression of COPD. At present the only antioxidant widely available for the treatment of patients with COPD is *N*-acetylcysteine (NAC). The present article reviews the available experimental and clinical data on the antioxidative effects of NAC in relation to COPD.

Oxidants and antioxidants in the lungs of patients with COPD

Several reviews have summarised the available data on the presence (table 1) and consequences (table 2) of oxidative stress in the lungs of "healthy" smokers and smokers with COPD [1–5, 9]. Cigarette smoke is a major source of oxidants, *e.g.* free radicals, including semiquinone and hydroxyl radicals, nitric oxide and hydrogen peroxide, in the lungs [3]. Furthermore, cigarette smoke promotes the influx and activation of neutrophils and macrophages. Leukocytes from smokers release more oxidants, such as the superoxide anion and H_2O_2, than leukocytes from nonsmokers [10]. The alveolar macrophages of smokers contain increased amounts of iron and release more free iron than those of nonsmokers [11]. The presence of free iron facilitates the generation of very reactive hydroxyl radicals.

An important part of the pulmonary antioxidant defence is located in the epithelial lining fluid (ELF). Vitamin C and E levels in ELF are depleted in smokers, but glutathione (GSH) levels are increased [6, 10, 12]. These effects are dependent on the time course of exposure to tobacco smoke. Acute exposure causes marked depletion of antioxidants in plasma [13, 14], intracellular GSH in erythrocytes [15] and GSH in ELF [10, 12].

Alterations in the lung caused by oxidative stress

Both *in vitro* and *in vivo* experiments have demonstrated that oxidative stress may cause alterations in essential

Table 1. – Indices (biochemical markers) of increased oxidative stress in chronic obstructive pulmonary disease

Elevated breath hydrogen peroxide and 8-isoprostane levels
Decreased plasma antioxidant capacity
Elevated plasma lipid peroxide (TBARS) levels
Plasma protein sulphydryl oxidation
Increased exhaled carbon monoxide
Release of ROS from peripheral blood neutrophils and AMs
Increased urinary isoprostane $F_{2\alpha}$-III levels

TBARS: thiobarbituric acid reactive substance; ROS: reactive oxidant species; AM: alveolar macrophage. Modified from [2].

Table 2. – Alterations in components of the lung caused by oxidative stress

Airway wall
Contraction of airway smooth muscle
Impairment of β-adrenoceptor function
Stimulation of airway secretion
Pulmonary vascular smooth muscle relaxation or contraction
Activation of mast cells
Alveolar epithelial cell layer
Increased permeability by detachment
Decreased adherence
Increased cell lysis
Lung matrix
Decreased elastin synthesis and fragmentation
Decreased collagen synthesis and fragmentation
Depolymerisation of proteoglycans
Antiproteases
Inactivation of α_1-proteinase inhibitor
Inactivation of secretory leukoprotease inhibitor
Pulmonary microcirculation
Increased permeability
PMN sequestration
Increased PMN adhesion to endothelium of arterioles and venules
Transcription factors
Switch-on of TNF-α, IL-8 and other inflammatory protein genes

PMN: polymorphonuclear neutrophil; TNF-α: tumour necrosis factor-α; IL: interleukin. Modified from [6, 7].

components of the lung, contributing to pathological abnormalities and functional changes (table 2) [6, 7].

Increased amounts of ROS have been shown to reduce the synthesis of elastin and collagen [16, 17]. Fragmentation of these major constituents of the lung skeleton may also occur. In addition, ROS may affect the structure of components of the extracellular matrix, such as hyaluronate [7]. Depolymerisation of the proteoglycans in the lung reduces the viscosity of the extracellular matrix.

Oxidative stress may also initiate or amplify alterations in the airway wall. Lipid peroxidation may initiate the release of arachidonic acid from membrane phospholipids, leading to release of prostaglandins and leukotrienes. Increased levels of ROS may also increase IL-1 and -8 production in several cell systems [18, 19].

Other changes include changes in protein structure, leading to altered antigenicity and thus immune responses, contraction of smooth muscle, impairment of β-adrenoceptor function, stimulation of airway secretion, pulmonary vascular smooth muscle relaxation or contraction, and activation of mast cells [7]. Antiproteases such as α_1-proteinase inhibitor (α_1-PI) and secretory leukoprotease inhibitor may be inactivated by ROS [20]. In particular, oxidation of the active site of α_1-PI, the so-called methionine residue, reduces the ability of α_1-PI to inactivate neutrophil elastase [21].

Changes in the alveolar epithelial cell layer occur both as a direct result of inhaled ROS and through the above-mentioned alterations [22]. The permeability of this part of the lung is increased by detachment of the cellular layer, reduced adherence of cells and increased cell lysis [7].

Sequestration of neutrophils, initiated by inhaled tobacco smoke, may occur in the lung microcirculation [23]. Both a reduction in the deformability of neutrophils and an increase in neutrophil adhesion to the vascular endothelium, due to increased levels of adhesion molecules, are involved in this pulmonary sequestration. The increased numbers and prolonged presence of these inflammatory cells contributes to the cycle of locally increased ROS production, attraction of new inflammatory cells, *etc*.

Finally, oxidative stress activates the transcription factor nuclear factor-κB (NF-κB), which switches on the genes for TNF-α, IL-8 and other inflammatory proteins [5, 24], enhancing inflammation.

Taken together, these data strongly suggest that oxidative stress is an important pathogenetic factor in the alterations in the lungs of patients with COPD.

Pharmacotherapy of COPD

Pharmacotherapy in patients with COPD is primarily focused on maximal bronchodilatation by inhaled anticholinergics and β_2-agonists [25]. The role of anti-inflammatory treatment is the subject of many mechanistic and clinical long-term interventional trials. Neither inhaled corticosteroids (ICS) nor high dosages of oral corticosteroids affect the number of inflammatory cells or concentrations of cytokines and proteases in induced sputum from COPD patients [26, 27]. Dexamethasone does not inhibit basal or stimulated release of IL-8 by alveolar macrophages in COPD patients compared to healthy smokers [28]. Corticosteroids inhibit apoptosis and thus stimulate survival of neutrophils [29]. ICS do not affect activation markers of neutrophils, such as myeloperoxidase and human neutrophil lipocalin [27]. They do, however, reduce serum IL-8 levels, which may result in a reduction in the influx of neutrophils [30]. This effect may possibly be mediated by the effects of ICS on airway epithelial cells [31]. Biopsy studies showed a reduction in the number of mast cells by ICS [32]. Treatment with ICS reduces the concentration of exhaled NO [33] and H_2O_2 [34] in exhaled air. Recent placebo-controlled trials show no effect of prolonged treatment on the course of forced expiratory volume in one second (FEV1) in mild COPD [35–38]. A reduction in the number and severity of exacerbations was observed in the Inhaled Steroids in Obstructive Lung Disease study in patients with an FEV1 of ~50% of the predicted value [37]. In severe COPD patients, recent studies using a combination of ICS and long-acting β_2-agonists showed an additive effect on the number of exacerbations compared to ICS or long-acting β_2-agonists alone [39–42]. It is difficult, however, to attribute these effects specifically to an antioxidative effect, given the strong anti-inflammatory potency of ICS. The potential role and positioning of antioxidant therapy with NAC is discussed below.

Pharmacology of *N*-acetylcysteine

Antioxidant properties

NAC exhibits direct and indirect antioxidant properties. Its free thiol group is capable of interacting with the electrophilic groups of ROS [43, 44]. This interaction with ROS leads to intermediate formation of NAC thiol, with NAC disulphide as a major end product [45]. In addition, NAC exerts an indirect antioxidant effect related to its role as a GSH precursor. GSH is a tripeptide made up of glutamic acid, cysteine and glycine. It serves as a central factor in protecting against internal toxic agents (such as cellular aerobic respiration and metabolism of phagocytes) and external agents (such as NO, sulphur oxide and other components of cigarette smoke, and pollution). The sulphydryl group of cysteine neutralises these agents. Maintaining adequate intracellular levels of GSH is essential to overcoming the harmful effects of toxic agents. GSH synthesis takes place mainly in the liver (which acts as a reservoir) and the lungs. Synthesis takes place in the cellular cytoplasm in two separate

enzymatic stages. In the first, the amino acids glutamic acid and cysteine are combined by γ-glutamylcysteine synthetase, and, in the second, GSH synthetase adds glycine to the dipeptide γ-glutamylcysteine to form GSH. *In vitro*, NAC acts as a precursor of GSH as it can penetrate cells easily and is subsequently deacylated to form cysteine [43].

The availability of amino acids for GSH synthesis is a fundamental factor in its regulation. Cellular levels of glutamic acid and glycine, but not cysteine, are plentiful. Consequently, GSH synthesis depends on the availability of cysteine. In the case of (relative) depletion of GSH levels or increased demand, GSH levels may be increased by delivering additional cysteine *via* NAC. However, it is impossible to administer the active form of cysteine, L-cysteine, because of low intestinal absorption, poor water solubility and rapid hepatic metabolism. NAC, with the acetyl radical linked to amine function, eliminates these disadvantages. The required quantity of cysteine may thus be administrated to maintain adequate levels of GSH in the lungs. Other cysteine derivatives, in which the sulphydryl group is blocked (carboxymethylcysteine), do not have this precursor action.

Clinical pharmacology

NAC is rapidly absorbed after oral administration in both animals and humans [46–48]. The maximum plasma concentration is reached 2–3 h after administration [30] and the plasma half-life is 6.3 h. NAC undergoes extensive hepatic metabolism, resulting in a low bioavailability of ~10% for the unchanged molecule.

As expected, NAC cannot be detected in plasma or bronchoalveolar lavage fluid (BALF) following oral administration for 5–14 days [49, 50]. In contrast, cysteine and GSH levels were increased transiently in plasma [49, 50] and lung [50] after oral administration of NAC 600 mg once daily. In patients with COPD, however, plasma concentrations of GSH were unchanged after this dose of NAC, whereas 600 mg three times daily increased plasma GSH levels [51]. With this higher dose, administered for 5 days to patients who underwent lung resection surgery (n=11), cysteine and GSH levels were increased by ~50% compared to untreated patients (n=11). This difference was, however, not significant, which was probably due to the high variation in concentrations of cysteine and GSH. Nevertheless, these data suggest that there is a transient dose-dependent effect of NAC on lung cysteine and GSH levels.

Antioxidant and anti-inflammatory effects

The efficacy of NAC as a precursor in GSH synthesis has been studied in isolated mouse lungs [43]. Cigarette smoke administered directly to the lung through the trachea caused a dose-dependent reduction in total pulmonary GSH. Administering NAC together with cigarette smoke prevented the loss of pulmonary GSH and abolished the effects of cigarette smoke. NAC reduced H_2O_2-induced damage to epithelial cells *in vitro* [22] and NF-κB activation in some cells [52]. In addition, NAC treatment reduced cigarette smoke-induced abnormalities in polymorphonuclear neutrophils (PMNs) [53], alveolar macrophages, fibroblasts and epithelial cells *in vitro* [54–57]. Treatment with NAC also attenuated rat secretory cell hyperplasia induced by tobacco smoke [58] and prevented hypochlorous acid-mediated inactivation of α_1-PI *in vitro* [59]. In a rat model of cigarette smoke-induced alterations in small airways, NAC prevented thickening of the airway wall and improved distribution of ventilation [60].

In addition to its effects on PMNs, NAC also influences the morphology and markers of oxidative stress in red blood cells (RBCs). An increased percentage of RBCs in COPD patients is morphologically damaged, with high concentrations of H_2O_2 and lowered levels of thiols [61]. Such alterations are correlated with reduced oxygen exchange [62]. Treatment of COPD patients with 1.2 or 1.8 mg·day^{-1} NAC for 2 months improved RBC shape, reduced H_2O_2 concentrations by 38–54% and increased thiol levels by 50–68% [63].

Treatment with NAC may alter lung oxidant/antioxidant imbalance. NAC (600 mg·day^{-1}) given orally increased lung lavage GSH levels [50], reduced $O_2^{\cdot -}$ production by alveolar macrophages [55] and decreased BALF PMN chemiluminescence *in vitro* [64]. In addition, 600 mg·day^{-1} NAC in COPD patients reduced sputum eosinophil cationic protein concentrations and the adhesion of PMNs [65]. *In vitro*, NAC reduced adhesion of *Haemophilus influenzae* and *Streptococcus pneumoniae* to oropharyngeal epithelial cells [66].

Effects on cigarette smoke-induced changes

Three studies have investigated the effects of 600 mg·day^{-1} NAC given orally on parameters of inflammation in the BALF of "healthy" smokers [55, 67, 68]. NAC resulted in a tendency towards normalisation of the cell composition, with an increase in lymphocyte concentration ($p<0.05$) [55]. In addition, improvements were observed in the phagocytic activity of alveolar macrophages [55], and an increase in secretion of leukotriene B_4 ($p<0.05$), which shows a chemotactic activity that represents an important defence mechanism against aggressive agents [55]. In addition, NAC reduced the stimulated production of $O_2^{\cdot -}$ (from $p<0.01$ to $p<0.05$, depending on the type of stimulus) [67]. Finally, a reduction in the levels of various markers of inflammatory activity, such as eosinophil cationic protein, lactoferrin and antichymotrypsin ($p<0.05$), was found after administration of NAC [68].

Effects on elastase activity

Treatment with NAC resulted in a considerable reduction in elastase activity, in both the bronchoalveolar cavity and plasma, related to its property of scavenging HOCl [44].

Modulatory effect on genes

Redox signalling forms part of the fundamental mechanisms of inflammation, such as cytokine induction, proliferation, apoptosis and gene regulation for cell protection. Oxidants act as mediators of signal transduction, *e.g.* activation of NF-κB and activation protein 1. NAC has been shown to inhibit activation of NF-κB, which controls the cellular genes for intracellular adhesion molecules in intact cells [52]. In addition, NAC has been shown to inhibit the expression of vascular cell adhesion molecule-1 in human endothelial cells [69].

Effects on oxidative stress induced by viruses

Oxidant production in respiratory cells rises when they become infected with pathogenic viruses, and the oxidative stress is accompanied by increased production of a variety of inflammatory mediators. NAC has been shown to play a protective role in increasing the resistance of mice to influenza virus [70]. Influenza virus increased the production of ROS in

epithelial cells and activated NF-κB transcription factor [71]. Pretreatment with NAC attenuated virus-induced NF-κB and IL-8 release. Mice infected intranasally with influenza virus APR/8 showed high BALF levels of xanthine oxidase, TNF-α and IL-6 as early as 3 days after infection [72]. Xanthine oxidase levels were also elevated in serum and lung tissue. Administration orally of 1 g·kg body weight^{-1}·day^{-1} NAC significantly reduced the mortality rate of the infected mice ($p<0.005$). Rhinoviruses also stimulated increased production of H_2O_2 and oxidative stress of human respiratory epithelial cells [73]. Oxidative stress, in turn, caused activation of NF-κB and release of IL-8, and this effect was blocked by NAC in a dose-dependent manner.

Effect on exhaled biomarkers of oxidative stress

Increased levels of H_2O_2 in exhaled breath condensate (EBC) have been shown in stable COPD patients, with a further increase during exacerbations [74]. Treatment with NAC 600 mg once daily for 12 months reduced the concentration of H_2O_2 in EBC compared to placebo in stable COPD patients (FEV1 ~60–70% pred) [75]. This effect was observed in the second 6 months of the treatment period. A higher dose of NAC (1.2 g once daily) reduced the concentration of H_2O_2 in EBC within a period of 30 days, suggesting that there is a dose-dependent effect on this marker of oxidative stress [76].

Mucolytic effects

In addition to these antioxidant actions, NAC exhibits mucolytic properties by destroying the disulphide bridges of mucoprotein macromolecules after inhalation. This pharmacological action is due to the presence of a free sulphydryl group in the NAC molecule [77, 78]. Mucus viscosity is reduced *in vitro* in human tracheobronchial secretions [79]. NAC also decreased the viscosity of canine tracheal mucus, leading to improved mucociliary transport [80]. In an animal model of chronic bronchitis, oral NAC inhibited smoke-induced goblet cell hyperplasia [81] and associated mucus hypersecretion [82]. In addition, NAC reduced the time to recovery of goblet cell numbers after smoking cessation [83].

Clinical efficacy of *N*-acetylcysteine in COPD

The clinical efficacy of NAC has been investigated in a number of both open and double-blind studies of patients with chronic bronchitis, with and without COPD. The effects on symptoms, viral and bacterial infections, number and severity of exacerbations, and lung function decline are discussed separately.

Clinical symptoms

An open clinical trial including 1,392 patients demonstrated the efficacy of NAC at a dose of 600 mg·day^{-1} in reducing the viscosity of expectorations, promoting expectoration and reducing the severity of cough [84]. After 2 months of treatment with NAC, the viscosity of expectorations improved in 80% of cases, the nature of the expectorations improved in 59%, difficulty in expectorating improved in 74% and the severity of cough improved in 71%.

Improvement in clinical symptoms as a result of treatment with NAC has been shown in a long-term double-blind trial with parallel groups conducted in several centres to which 744 patients with chronic bronchitis were recruited [85]. Patients were randomly divided into two groups, one treated with NAC and the other with placebo. The results confirmed the efficacy of NAC regarding the parameters related to bronchial hypersecretion.

Bronchial bacterial colonisation

In an open cross-sectional study performed in 22 smokers with no chronic bronchitis, 19 smokers with chronic bronchitis, with or without airway obstruction, and 14 healthy non-smokers, the bacterial flora and effect of NAC on bacterial numbers were investigated [86]. The number of bacterial colonies was highest in smokers with chronic bronchitis. In addition, the number of intrabronchial bacteria was significantly lower in patients treated with NAC compared to other patients. This effect was more obvious in patients with chronic obstructive bronchitis.

N-*acetylcysteine and viruses*

The effects of NAC on influenza and influenza-like episodes have been studied in 262 patients suffering from nonrespiratory chronic degenerative diseases [87]. Compared to placebo, NAC, 600 mg twice daily for 6 months, resulted in a significant decrease in both the frequency and severity of influenza-like episodes. Local and systemic symptoms were also significantly reduced in the group receiving NAC. Although seroconversion towards influenza virus was similar in the two groups, only 25% of virus-infected subjects treated with NAC developed the symptomatic form of the condition compared with 79% of the placebo group.

Lung function decline

In an open observational survey in Sweden, the decline in FEV1 in COPD patients who took NAC for 2 yrs was less than that in a reference group receiving usual care [88]. This favourable effect was particularly apparent in COPD patients aged >50 yrs (annual decline in FEV1 of 30 mL) compared to the reference group (annual decline in FEV1 of 54 mL). After 5 yrs, the reduction in FEV1 in the NAC group was less than that in the reference group (B. Lundbäck, Unit for Lung and Allergy Research, National Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden, personal communication). Clearly, it should be noted that the nature of the study design precludes firm conclusions regarding the effect of NAC on lung function decline in COPD.

Exacerbations

In a recent systematic review by STEY *et al.* [89], data on prevention of exacerbation, improvement of symptoms and adverse effects were extracted from original reports (fig. 1). The relative benefit and number needed to treat were calculated for both individual trials and the combined data. Of the 39 trials retrieved, 11 (2,011 patients analysed), published 1976–1994, were regarded as relevant and valid according to preset criteria [85, 90–99]. Except for one study [97], these were placebo-controlled randomised trials. In nine of the studies, 351 of 723 (48.5%) patients receiving NAC showed no exacerbation compared with 229 of 733 (31.2%) patients receiving placebo (relative benefit 1.56 (95%

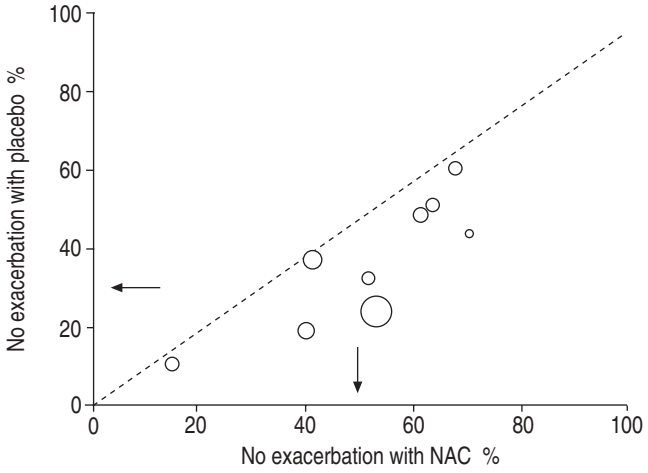

Fig. 1. – Correlation of absence of exacerbation with oral *N*-acetylcysteine (NAC) and placebo in patients with chronic obstructive pulmonary disease and/or chronic bronchitis. ○: trial (symbol size proportional to trial size); -----: line of equality. Arrows represent weighted means. Reproduced with permission from [89].

confidence interval (CI) 1.37–1.77), number needed to treat 5.8 (95% CI 4.5–8.1)). There was no evidence of any effect of study period (12–24 weeks) or cumulative dose of NAC on efficacy. In five of the trials, 286 of 466 (61.4%) patients receiving NAC reported improvement of their symptoms compared with 160 of 462 (34.6%) patients receiving placebo (relative benefit 1.78 (95% CI 1.54–2.05), number needed to treat 3.7 (95% CI 3.0–4.9)). These findings are in line with the outcomes of two previous meta-analyses using less-precise selection of these studies [100, 101], and confirm that NAC has a clinically significant effect on the number and impact of exacerbations. Again, it should be stressed that the patients included in these studies were not characterised in as detailed a fashion as would currently be demanded according to, for example, the Global Initiative for Chronic Obstructive Lung Disease guidelines [25].

With NAC, 68 of 666 (10.2%) patients reported gastrointestinal adverse effects compared to 73 of 671 (10.9%) taking placebo. With NAC, 79 of 1,207 (6.5%) patients withdrew from the study due to adverse effects, compared to 87 of 1,234 (7.1%) receiving placebo.

Conclusions

Oxidative stress is considered to be an important part of the inflammatory response to both environmental and internal signals. Transcription factors such as NF-κB and activation protein 1 are activated by oxidative stress, and, in turn, amplify the inflammatory response to noxious stimuli. In this way, both oxidative stress and inflammation are involved in the complex pathophysiology of COPD, in terms of both pathogenesis and the progression of the disease. The benefits of ICS in severe COPD are limited, and no effects have been found in mild and moderate COPD.

In vitro and *in vivo* data show that *N*-acetylcysteine protects the lungs against toxic agents by increasing pulmonary defence mechanisms through its direct antioxidant properties and indirect role as a precursor in glutathioine synthesis. In patients with chronic obstructive pulmonary disease, treatment with *N*-acetylcysteine at a dose of 600 mg once daily reduces the risk of exacerbations and improves symptoms compared to placebo. Whether this benefit is sufficient to justify the routine and long-term use of *N*-acetylcysteine in all patients with chronic bronchitis has been addressed recently in the Bronchitis Randomized On NAC Cost-Utility Study [102]. This phase III randomised double-blind placebo-controlled parallel-group multicentric study was designed to assess the effectiveness of *N*-acetylcysteine 600 mg daily for 3 yrs in altering the decline in forced expiratory volume in one second, exacerbation rate and quality of life in patients with moderate-to-severe chronic obstructive pulmonary disease. In addition, the cost/utility of the treatment was estimated. This study has recently been completed and will provide further data for establishing the application of *N*-acetylcysteine as an antioxidant in patients with chronic obstructive pulmonary disease.

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