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REVIEW

Oxidative Stress and Respiratory System: Pharmacological and Clinical Reappraisal of N-Acetylcysteine

Pierachille Santus,[1] Angelo Corsico,[2] Paolo Solidoro,[3] Fulvio Braido,[4] Fabiano Di Marco,[5] and Nicola Scichilone[6]

1 Università degli Studi di Milano, Dipartimento di Scienze della Salute. Pneumologia Riabilitativa Fondazione Salvatore Maugeri-Istituto Scientifico di Milano-IRCCS, Milano, Italy

2 Respiratory Disease Unit, Fondazione IRCCS Policlinico San Matteo, University of Pavia, DMM, Pavia, Italy

3 SCDO Pneumologia, Dipartimento Cardiovascolare e Toracico, Città della Salute e della Scienza di Torino, Presidio Molinette, Torino, Italy

4 Clinica Malattie Respiratorie e Allergologia Dipartimento di Medicina Interna (DiMI) Azienda Ospedaliera Universitaria IRCCS San Martino di Genova, Genova, Italy

5 Università degli Studi di Milano, Dipartimento di Scienze della Salute, Pneumologia, Ospedale San Paolo, Milano, Italy

6 Dipartimento Biomedico di Medicina Interna e Specialistica (Di.Bi.M.I.S.), Sezione di Pneumologia, University of Palermo, Palermo, Italy

Abstract

The large surface area for gas exchange makes the respiratory system particularly susceptible to oxidative stress-mediated injury. Both endogenous and exogenous pro-oxidants (e.g. cigarette smoke) trigger activation of leukocytes and host defenses. These mechanisms interact in a "multilevel cycle" responsible for the control of the oxidant/antioxidant homeostasis. Several studies have demonstrated the presence of increased oxidative stress and decreased antioxidants (e.g. reduced glutathione [GSH]) in subjects with chronic obstructive pulmonary disease (COPD), but the contribution of oxidative stress to the pathophysiology of COPD is generally only minimally discussed. The aim of this review was to provide a comprehensive overview of the role of oxidative stress in the pathogenesis of respiratory diseases, particularly COPD, and to examine the available clinical and experimental evidence on the use of the antioxidant N-acetylcysteine (NAC), a precursor of GSH, as an adjunct to standard therapy for the treatment of COPD. The proposed concept of "multilevel cycle" helps understand the relationship between respiratory diseases and oxidative stress, thus clarifying the rationale for using NAC in COPD. Until recently, antioxidant drugs such as NAC have been regarded only as mucolytic agents. Nevertheless, several clinical trials indicate that NAC may reduce the rate of COPD exacerbations and improve small airways function. The most plausible explanation for the beneficial effects observed in patients with COPD treated with NAC lies in the mucolytic and antioxidant effects of this drug. Modulation of bronchial inflammation by NAC may further account for these favorable clinical results.

Introduction

The primary role of the respiratory system is to provide an adequate supply of oxygen (O_2) to the tissues, in order to ensure normal oxygenation and organ functions. The large surface area for gas exchange makes the respiratory system particularly susceptible to oxidative stress-mediated injury: both the high environmental O_2 concentration in the lung and exogenous pollutants inhaled through the breath may play a role in the production of pro-oxidant reactive oxygen species (ROS) and reactive nitrogen species (RNS). Endogenous and exogenous pro-oxidants trigger cellular activation, with the release of proinflammatory mediators and proteases, and host defenses such as antioxidant enzymatic and non-enzymatic systems. All these mechanisms interact in a "multilevel cycle" responsible for the control of the oxidant/antioxidant homeostasis. Oxidative stress resulting from an oxidant/antioxidant imbalance in favor of oxidants causes biological modifications that are implicated in several pathological conditions, including respiratory diseases that

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Correspondence to: Dr. Nicola Scichilone, Dipartimento Biomedico di Medicina Interna e Specialistica (DIBIMIS), Sezione di Pneumologia, University of Palermo, Palermo, Italy, via Trabucco 180, 90146 Palermo, Italy, phone: +39 091 6802655, fax: +39 091 6882842, email: nicola.scichilone@unipa.it

may be caused and/or aggravated by the exposure to air pollution and cigarette smoke.

In fact, inhaled oxidants initiate a number of pathologic processes, including inflammation of the airways, that may contribute to the pathogenesis and/or exacerbation of respiratory diseases (1). Recent evidence indicates that exposure to environmental pollution, even at low levels, can increase the risk of emergency admissions for acute respiratory diseases and exacerbation of obstructive lung diseases in the general population (2), likely due to an increase in lung inflammation (3). Cigarette smoke contains a very high number of chemical compounds, and both the tar and gas phases contain numerous free radicals and other oxidants (4) that may induce an oxidant/antioxidant imbalance within the lung (5).

Chronic obstructive pulmonary disease (COPD) is a pathological lung condition directly related to cigarette smoke (6). There is now a large body of evidence that an increase in oxidative stress plays an important role in the pathogenesis of COPD (7). Markers of oxidative stress have been demonstrated in the airways, breath condensate, sputum, blood, and urine of smokers and patients with COPD (4, 6). There is also evidence that endogenous antioxidants such as reduced glutathione (GSH) and lung GSH biosynthesizing enzymes are significantly decreased in these patients (8). Furthermore, oxidative stress has been reported to increase further, and GSH levels to be depleted, during severe COPD exacerbations (9). Inhaled bronchodilators and inhaled corticosteroids are the mainstay of treatment for COPD (6, 10). However, given their key role in the pathogenesis of COPD, oxidative stress and inflammation could represent promising therapeutic targets for the treatment of this disease (11, 12).

N-acetylcysteine (NAC) is a precursor of L-cysteine and GSH that has been used for decades as a mucolytic agent in the treatment of different respiratory diseases. Being a precursor of GSH synthesis, NAC may provide additional benefits in conditions such as COPD, where the endogenous GSH pool is depleted. Several studies have investigated the role of NAC in the treatment of COPD. The aim of this review was to provide a comprehensive overview of the role of ROS and oxidative stress in the pathogenesis of respiratory diseases, particularly COPD, and to review the available clinical and experimental evidence on the use of NAC as an adjunct to standard treatment for COPD.

Lung and oxidative stress: Pathophysiology

Oxidative stress occurs in response to the production of cellular ROS and RNS during endogenous metabolic reactions. Inhaled oxidants in the ambient air, including ozone, nitrogen dioxide, diesel exhaust and cigarette smoke, are also well-established causes of oxidative stress. Under normal conditions, the production of endogenous ROS is tightly regulated, and endogenous antioxidants protect tissues against the exposure to free radicals (13).

Although low-to-moderate concentrations of ROS and RNS are necessary for physiological functions such as defense against infectious agents and cellular signaling pathways (13), uncontrolled activation of ROS production leading to an imbalance between oxidants and antioxidants such as GSH can have detrimental consequences (Figure 1). The most common ROS, i.e. the superoxide anion ($O_2^{-}\bullet$) and the hydroxyl radical

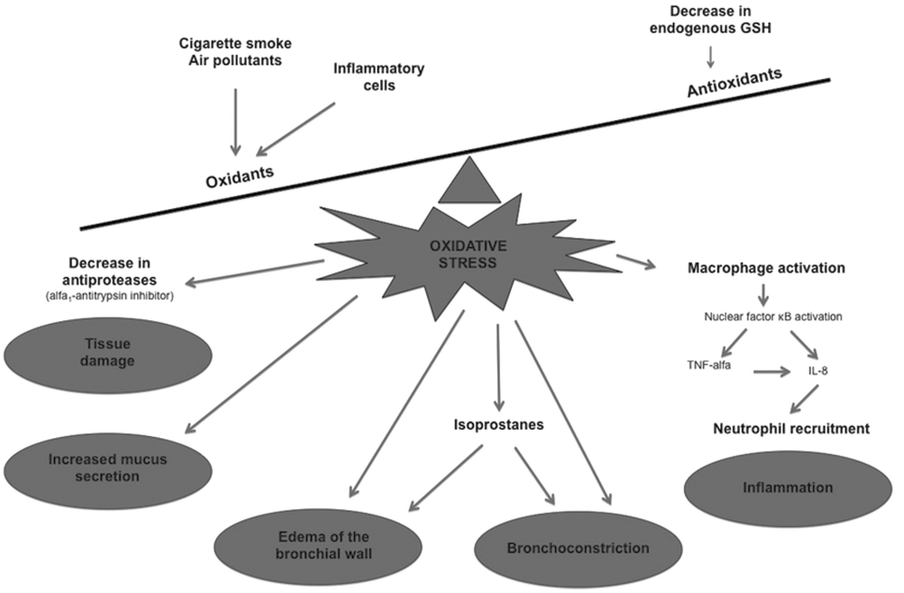

Figure 1. Etiology and pathogenic role of oxidative stress in COPD. An excess in pro-oxidants (e.g. cigarette smoke) may overwhelm the antioxidant defense system of the body, causing an oxidant/antioxidant imbalance and therefore oxidative stress. Oxidative stress is involved many of the pathogenic processes underlying COPD, such as direct tissue damage, inactivation of antiproteases, mucus hypersecretion, vascular barrier dysfunction leading to edema of the bronchial wall, bronchoconstriction (both via direct action and through the production of isoprostanes from lipid peroxidation) and enhanced lung inflammation through activation of redox-sensitive transcription factors in leukocytes.

(•OH), are directly associated with oxidative modifications of biochemical systems such as proteins, lipids and carbohydrates. Oxidative stress is involved many of the pathogenic processes underlying COPD, such as direct tissue damage, inactivation of antiproteases, mucus hypersecretion, vascular barrier dysfunction leading to edema of the bronchial wall, bronchoconstriction and enhanced lung inflammation through activation of redox-sensitive transcription factors in leukocytes (7, 14, 15) (Figure 1).

The multilevel cycle

Figure 2 illustrates the multilevel interpretation of the relationship between oxidative stress and lung damage. The interaction of exogenous pro-oxidants such as cigarette smoke or air pollutants with the bronchial epithelium and the alveolar-capillary membrane represents the first step of the cycle. In addition to exogenous pro-oxidants, respiratory tissues are also exposed to endogenous ROS. Therefore, both exogenous and endogenous stimuli may lead to an increase in ROS and RNS, causing lipid peroxidation and oxidative damage to proteins and DNA and sustaining oxidative stress (5).

In Figure 2 this cycle is identified by continuous black arrows. The second level of the process is represented by cellular activation, both directly by endogenous oxidants and indirectly via production of molecular mediators such as tumor necrosis factor α (TNFα) by the bronchial epithelium. Finally, the third level is represented by the host defenses against oxidative stress. These are very important, since the physiological defense system acts as the arbitrator between healthy and pathological conditions via different endogenous pathways.

First level: outside and inside

Cigarette smoke contains large amounts of free radicals in both the gas and the tar phases, and is considered as an important risk factor for the development of various types of lung diseases and cancers (16). The gas phase free radicals of tobacco smoke include both inorganic and organic substances such as ROS, epoxides, peroxides, nitric oxide (NO•), nitrogen dioxide, peroxynitrite ($ONOO^-$), peroxynitrates and many other molecules (17, 18). Twenty years ago Pryor and Stone reported that gas phase cigarette smoke contains approximately 1015 organic radicals per puff, which are primarily of the alkyl, alkoxy, and peroxyl type (19).

NO• is present in cigarette smoke in concentrations of 500–1000 ppm, i.e. one of the major exogenous sources of NO• to which smokers are exposed. NO• reacts swiftly with the superoxide anion (O_2^-•) to form peroxynitrite ($ONOO^-$) and with organic peroxyl radicals to give alkyl peroxynitrites (ROONO) (20). In addition to cigarette smoke, air pollutants may also be considered as important inductors of lung oxidative stress. Indeed, lung inflammation, as particularly demonstrated in COPD, is

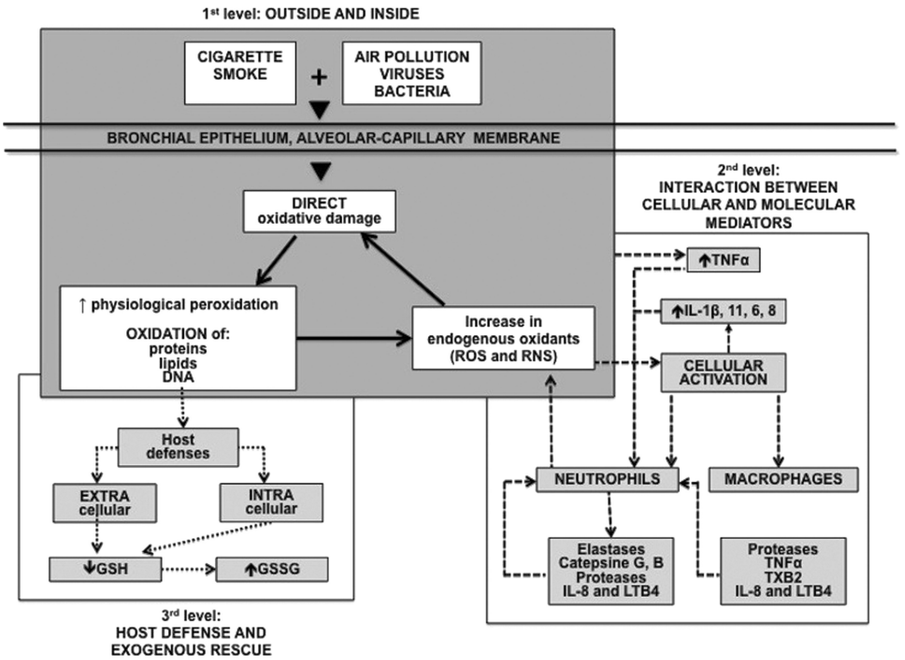

Figure 2. The concept of "multilevel cycle". The interaction of exogenous pro-oxidants such as cigarette smoke or air pollutants with the bronchial epithelium and the alveolar-capillary membrane represents the first level, "outside and inside" (continuous black arrows). In the second level, "interaction between cellular and molecular mediators", (broken arrows) cellular activation due to both exogenous and endogenous ROS and RNS triggers a vicious oxidative cycle sustained by the release of inflammatory mediators and proteases activation. The second level of the process is represented by cellular activation, both directly by endogenous oxidants and indirectly via production of molecular mediators such as tumor necrosis factor α (TNFα) by the bronchial epithelium. Activated leukocytes release proinflammatory cytokines, chemokines and proteases that perpetrate oxidative stress. The third level, "host defense and exogenous rescue", (dotted arrows) is represented by the host defenses against oxidative stress, such as GSH. O_2^-•, superoxide anion; •OH, hydroxyl radical; TNFα, tumor necrosis factor alpha; IL, Interleukin; RNS, reactive nitrogen species; ROS, reactive oxygen species; LTB4, leukotriene B4; TXB2, tromboxane B2; GSH, reduced glutathione; GSSG, glutathione oxidized form.

related to lipid cell membrane peroxidation (21, 22), and it is likely that sulphur dioxide (SO_2), particulate matter of less than 10 microns in diameter (PM10), cold air and a variety of hydrated metal ions contribute to airways cytotoxicity, inflammation and mucociliary clearance dysfunction (21, 23).

Free radicals attack cell constituents by damaging protein structure, lipids and DNA sequences (Figure 2), thus increasing the risk of developing various lung diseases. Inhaled radicals produce adducts that contribute to many of the negative effects of tobacco smoke in the lung (18). Finally, the role of microorganisms implicated in respiratory infections should also be considered. Viral and bacterial infections are associated with the vast majority of severe COPD exacerbations requiring hospitalization, and the presence of infection itself is related to exacerbation severity (24). The human respiratory syncytial virus (RSV) is causative agent of severe infections in people of all ages (25). Increased sputum production and cough as a consequence of changes in ciliary beat of the bronchial epithelium and in mucin production are the main clinical manifestations of COPD exacerbations; both processes may be affected by RSV, which induces the destruction of ciliated epithelial cells as well as an increase in mucus production (26, 27).

RSV-induced epithelial damage involves an oxidative reaction with ROS production, either by leukocytes or epithelial cells themselves, as mediators of the epithelial cell damage (28, 29). ROS intermediates are necessary for RSV infection and are involved in the inflammatory response of host cells (30). In addition, Sethi *et al.* (31) recently demonstrated that exacerbations associated with acquisition of new strains of bacteria, namely *H. influenzae*, *S. pneumoniae*, *M. catarrhalis,* and *P. aeruginosa*, were clearly associated with a more intense neutrophilic inflammatory response in the airways, and with more intense systemic inflammation, compared with exacerbations not associated with such strain acquisition. It has even been suggested that suppression of inflammation might be more important than the removal of bacteria for the improvement of symptoms and functional abnormalities associated with COPD exacerbations (24).

Second level: interaction between cellular and molecular mediators

It is a natural consequence that the first level results in cellular activation, triggering a vicious oxidative cycle sustained by the release of inflammatory mediators and proteases activation (Figure 2). Neutrophils and macrophages are the main cell types involved in this process within the lung. It is well known that neutrophils represent an important defense against bacterial and viral infections in the lung. On the other hand, these cells may promote an inflammatory response that can contribute to the pathogenesis of several lung diseases, including COPD and severe asthma (32).

Neutrophil activation may have several deleterious effects in the lungs. These cells release proinflammatory ROS and serine proteases, including neutrophil elastase and proteinase-3, which may cause emphysema via degradation of elastin fibers and stimulation of mucus secretion, and matrix metalloproteinase-8 (MMP-8) and MMP-9, which break down elastin and collagen. Furthermore, a dysregulation of MMP-3, possibly caused by the –1171 5A/6A polymorphism or other linked variants, may affect the progression and severity of COPD (33). Neutrophils themselves release factors, such as leukotriene B_4 (LTB4) and interleukin 8 (IL-8), that attract other neutrophils, thereby perpetuating the chronic inflammation that underlies COPD (34).

Oxidants, either inhaled such as cigarette smoke or generated by leukocytes, can inactivate the α1 protease inhibitor, i.e. α1-antitrypsin, the inhibitor of elastase, by oxidation of its active site. *In vitro* studies indicate that oxidants present in cigarette smoke, as well as those spontaneously released by alveolar macrophages obtained from smokers, may induce functional inactivation of the α1 protease inhibitor, which results in the reduction of its inhibitory effect on neutrophil elastase (35, 36).

The key point is that neutrophils, when activated, release proteases and neutrophil elastases from their granules. Hence, in cigarette smokers there is a potential for a greater protease burden. Moreover, neutrophils release ROS able to inactivate anti-proteases in the immediate peri-cellular region (5). Saetta (37) demonstrated that also macrophages have an important role in airway inflammation. In fact, these Authors found an increased number of macrophages and T lymphocytes in bronchial biopsies obtained from the central airways of smokers, as compared with non-smokers. Cigarette smoke is known to stimulate alveolar macrophages to release chemotactic factors for neutrophils, which amplifies the oxidative vicious cycle in the lung (38).

Consistently, a recent study demonstrated that lung inflammation is generally characterized by local recruitment of pro-inflammatory cells such as neutrophils and macrophages, which are involved in the up-regulation of various signaling molecules, e.g. cytokines (TNF-α, IL-6), chemokines (IL-8) and adhesion molecules (Figure 2). These mediators, along with an increased ROS production, play a key role in the development of several inflammatory respiratory diseases, including COPD (39).

Third level: host defense and exogenous rescue

The human respiratory system has evolved biological mechanisms to counteract pathogenic *noxae* and reduce disease risks. With regard to oxidative stress, the close relationship between lung and oxygen has driven the respiratory system to develop a series of defense mechanisms capable of reducing the potentially detrimental consequences of the increase in ROS and RNS. To protect themselves against these oxidant species, the lungs

are endowed with efficient antioxidant defense systems such as antioxidant enzymes and non-enzymatic antioxidants, including GSH, albumin, uric acid, vitamins C and E, and other low molecular weight organic molecules (40). Vitamin E is an interesting antioxidant system that may also have an effect on lipid peroxidation. In particular, vitamin E has been demonstrated to dose-dependently inhibit the receptor-mediated activation of neutrophils that results in the synthesis of leukotrienes in individuals with asthma (41).

Among the endogenous antioxidant systems, GSH represents one of the most important defenses. However, GSH does not have an unlimited potential, even though it appears to be very active and effective. GSH is a tripeptide composed of glutamic acid, cysteine, and glycine. Thanks to its sulfhydryl group, it functions as an antioxidant, protecting against free radicals and other oxidants. GSH has also been implicated in immune modulation and inflammatory responses (42). GSH is the predominant non-protein sulfhydryl in cells, and plays a key role in maintaining the cellular redox status, defined as the ratio between the concentration of oxidizing and reducing equivalents (43). The *de novo* synthesis of GSH from its amino-acid constituents is essential to face the increased GSH demand that occurs in response to oxidative stress. GSH synthesis involves two enzymatic steps catalyzed by γ-glutamylcysteine synthetase and glutathione synthetase (44).

The GSH redox system

The GSH redox system is crucial in maintaining intracellular GSH/Glutathione oxidized (GSSG) homeostasis, which is critical to normal cellular physiological processes and represents one of the most important antioxidant defense systems in lung cells (44). This system uses GSH as a substrate in the detoxification of peroxides such as H_2O_2 and lipid peroxides, a reaction that involves glutathione peroxidase. This reaction generates GSSG, which is then reduced to GSH by glutathione reductase in a reaction requiring the hexose monophosphate shunt pathway involving the reduced nicotinamide adenine dinucleotide phosphate (NADPH).

Maintenance of a high intracellular GSH/GSSG ratio (>90%) minimizes accumulation of disulphides and provides a reducing environment within the cell. However, when oxidants or other environmental stress alter this ratio, the shift in the GSH/GSSG redox buffer may influence a variety of cellular signaling processes, such as activation of transcription factors and cellular activation (45). Knowledge of the mechanisms of GSH regulation and balance between the release and expression of pro- and anti-inflammatory mediators could lead to the development of novel therapies based on the pharmacological manipulation of the production or gene transfer of this important antioxidant in lung inflammation and injury (45).

It should also be noted that, besides inhibiting the inflammatory response, GSH exerts other functions. GSH is essential for some functions of the immune system, both innate and adaptive, which could explain the association between low levels of GSH and increased susceptibility to infections in several diseases, including COPD (46). Given the important role of oxidative stress in the pathogenesis of respiratory diseases, it has been proposed that antioxidant drugs such as NAC be used in combination with conventional therapy in the management of these conditions.

N-Acetylcysteine (NAC)

NAC is a precursor of GSH. Both agents act as free oxygen radical scavengers. Messier *et al.* (47) recently demonstrated that NAC provides protection against injury induced by cigarette smoke in alveolar type II (ATII) cells and lung tissue obtained from knockout mice lacking the nuclear factor erythroid 2-related factor-2 (Nrf2, a redox-sensitive transcription factor that is a key regulator of the antioxidant defense system) both *in vivo* and *in vitro*. This finding supports the protective role of NAC, which acts as a direct scavenger of free radicals like OH•, H_2O_2 and O_2^-• in an Nrf2-independent manner (47, 48).

This new evidence is of considerable importance, since a reduction in Nrf2-dependent endogenous antioxidants has been described in patients with COPD (8). Another interesting *in vitro* study (30) demonstrated that, after influenza virus and RSV infection, there is an increase in intracellular levels of H_2O_2 and a decrease in intracellular thiols. NAC restored this imbalance by decreasing the H_2O_2 concentration and restoring thiol levels. As a consequence, virus titers decreased and viral proliferation was inhibited. Finally, the same authors recently showed that NAC may restore epithelial functions after RSV infection via inhibition of the expression of adhesion molecules and RSV replication, and by restoring antioxidant capacity, intracellular H_2O_2 levels and glutathione content in normal human bronchial epithelial cells (49). In recent years, clinical trials on NAC and COPD have been conducted, which allows more specific and interesting considerations.

Mechanism of action of NAC

NAC is a thiol and mucolytic agent, a precursor of L-cysteine and reduced glutathione (GSH). The characteristic of NAC of being a precursor of GSH represents the most important pharmacological property of this drug. NAC can be administered orally, via aerosol or intravenously. The drug is rapidly absorbed following oral administration (50), and is quickly metabolized to cysteine, which is a direct precursor in the synthesis of intracellular GSH. As such, NAC acts as an antioxidant by restoring the pool of intracellular reduced GSH, which is often depleted in conditions associated with increased oxidative stress and inflammation (8, 9, 50). It has been shown that oral administration of NAC 600 mg/day for 5 days significantly increases GSH concentrations in the bronchoalveolar lavage fluid of treated individuals, as

compared with those who did not receive NAC ($p < 0.05$ 1–3 hours after the last dose of NAC) (51).

Due to its free sulfhydryl group, which confers NAC with the ability to reduce disulphide bonds, the drug is widely used to reduce viscosity and elasticity of the mucus (52). As mentioned, NAC can also act as direct scavenger of free radicals such as OH, H_2O_2 and $O_2^{-}\bullet$ (48). In a randomized, placebo-controlled trial, treatment with NAC significantly reduced H_2O_2 concentration in the expired breath condensate of patients with stable COPD (53). In particular, patients treated with NAC 600 mg/day for up to 12 months showed a progressive decrease from baseline in H_2O_2 concentration, which achieved statistical significance after 6 months of treatment ($p < 0.03$). After 9 and 12 months of treatment, the H_2O_2 concentration in the expired breath condensate was 2.3- and 2.6-fold lower in patents treated with NAC as compared with placebo-treated patients ($p < 0.04$ and $p < 0.05$, respectively) (53).

The antioxidant effect of NAC has been assessed in a similar study, which confirmed NAC's ability to significantly reduce exhaled H_2O_2 levels in patients with COPD (54). In this randomized, placebo-controlled trial, 55 patients with stable COPD were randomized to receive NAC 600 mg b.i.d. ($n = 32$) or placebo ($n = 23$) for 2 months. In patients treated with NAC, exhaled H_2O_2 levels were significantly reduced after 1 month of treatment (from 1.28 ± 0.61 μM to 0.91 ± 0.44 μM; $p = 0.007$ *versus* baseline), with a further decrease at the end of the second month of treatment (0.83 ± 0.41 μM; $p = 0.0001$ *versus* baseline) (54). Conversely, no significant changes were observed in H_2O_2 levels in the placebo group at any time point.

The antioxidant properties of NAC make this drug a promising therapeutic agent for the modulation of cellular and molecular mechanisms occurring in biological systems (Figure 2). In particular, being NAC able to modulate the cellular redox status, it can interfere with several pathways and regulate the activity of nuclear factors such as nuclear transcription factor-κB (NF-κB) (55). Specifically, NAC may affect thioredoxin and glutaredoxin expression, which are part of ubiquitously expressed thiol-reducing systems next to glutathione.

Thioredoxin regulates the DNA binding of NF-κB by reducing a cysteine of the p50 subunit, while glutaredoxin, by sensing the changes in the redox state of GSH/GSSG, may alter the signal transduction pathways involved in the modulation of biological responses such as the inflammatory response (56). The different mechanisms of action of NAC and its possible beneficial effects are summarized in Figure 3.

N-Acetylcysteine in the treatment of chronic bronchitis and COPD

The clinical efficacy of N-acetylcysteine for the treatment of patients with chronic bronchitis and COPD has been documented in several clinical trials and metanalyses (Table 1).

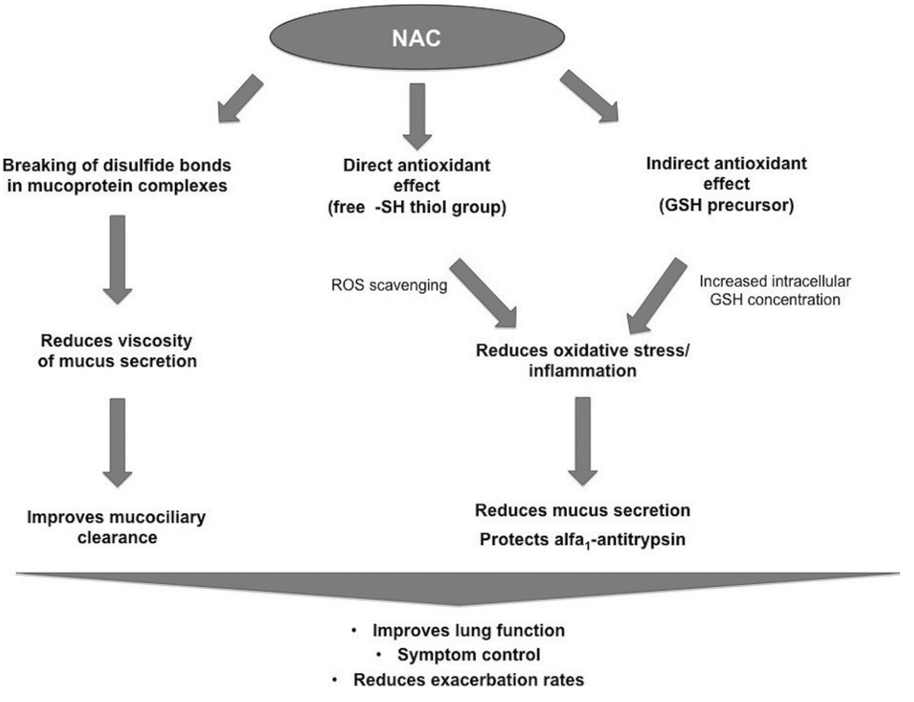

Figure 3. Mechanisms of action of N-acetylcysteine (NAC). NAC acts as a mucolytic, antioxidant and antinflammatory agent. The free sulfhydryl group confers NAC with the ability to reduce disulphide bonds, thus decreasing mucus viscosity and facilitating mucociliary clearance. The antioxidant activity of NAC may be both direct (the free sulfhydryl group may serve as a ready source of reducing equivalents) and indirect (through replenishment of intracellular GSH levels) antioxidant effects. Thus, NAC may break the vicious oxidative cycle by reducing oxidative stress and, subsequently, inflammation. Overall, these effects may result in improvements in symptoms, lung function and reduced exacerbation rates.

Table 1. Overview of the main clinical trials on the antioxidant activity and clinical efficacy of NAC in the treatment of chronic bronchitis, COPD and COPD exacerbations

Author	Condition	N. Patients	Study design	Treatment(s)	Duration	Main results
Bridgeman *et al.* 1991 [51]	Patients undergoing bronchoscopy and bronchoalveolar lavage	34	Open, randomised trial; three groups of patients	– Not treated ($n = 10$) – NAC 600 mg/d, bronchoalveolar lavage – 1–3 hours ($n = 12$) – 16–20 hours ($n = 12$) After the last dose	5 days	Greater GSH concentrations in the lavage fluid of group 2 (lavage performed 1–3 hours after the last dose), as compared with controls ($p < 0.05$)
Pela *et al.* 1999 [57]	Moderate to severe COPD	169	Multicentre, randomised, controlled, open label trial	– Standard therapy ($n = 84$) – Standard therapy + NAC 600 mg/d ($n = 85$)	6 months	41% reduction in the number of exacerbations in patients treated with NAC vs controls ($p < 0.05$)
Kasielski and Nowak 2001 [53]	COPD	44	Double-blind, placebo controlled, randomised trial	– Placebo ($n = 22$) – NAC 600 mg/d ($n = 22$)	12 months	Greater reduction in H2O2 concentration in patients treated with NAC vs controls ($p < 0.04$ at 9 months and $p < 0.05$ at 12 months)
Gerrits *et al.* 2003 [65]	COPD	1219	Retrospective analysis of patients hospitalised for COPD, who had received N-acetylcysteine following discharge from their first admission or had not	– Not treated with NAC ($n = 754$) – Treated with NAC 200 to > 600 mg/d ($n = 465$)	Up to 1 year	Reduction in the risk of rehospitalisation for COPD by about 30% in patients treated with NAC (RR = 0.67)
De Benedetto *et al.* 2005 [54]	Stable COPD	55	Randomised placebo controlled trial	– Placebo ($n = 23$) – NAC 600 mg/bid ($n = 32$)	2 months	Significant reduction in H_2O_2 concentration in exhaled air condensate vs baseline after 1 month ($p < 0.007$) and after 2 months ($p = 0.0001$) of treatment. Significant reductions vs placebo at 1 and 2 months.
Decramer *et al.* 2005 [59]	Moderate COPD	523	Multicentre randomised, placebo-controlled trial	– Placebo ($n = 267$) – NAC 600 mg/d ($n = 256$)	3 years	21% reduction in exacerbations in the subgroup of patients treated with NAC and no inhaled corticosteroids vs placebo ($p = 0.04$)
Zuin *et al.* 2005 [61]	COPD exacerbations	123	Double-blind, randomised placebo-controlled trial	– Placebo ($n = 42$) – NAC 600 mg/d ($n = 41$) – NAC 1.200 mg/d ($n = 39$)	10 days	Significantly higher proportion of patients achieving normalised CRP levels compared with placebo – NAC 600 mg/d (52% of patients); – NAC 1200 mg/d (90% of patients); – placebo (19% of patients); ($p < 0.01$ NAC vs placebo)
Sutherland *et al.* 2006 [58]	COPD	2214 (1820 included in the analysis)	Metanalysis of 8 randomised controlled trials	– Placebo ($n = 920$) – NAC 400 mg/d up to 1200 mg/d ($n = 900$)	≥ 3 months	≈50% reduction in the risk of COPD exacerbations in patients treated with NAC ($p = 0.001$)
Stav *et al.* 2009 [63]	Moderate to severe COPD	24	Randomised, double blind, placebo-controlled, crossover study	– Placebo ($n = 24$) – NAC 600 mg/bid ($n = 24$)	6 weeks	Reduction in air trapping • increase in IC ($p < 0.0033$ vs placebo) • increase in FVC after exercise ($p < 0.0029$ vs placebo) • reduction in the RV/TLC ratio after exercise ($p < 0.001$ vs placebo)
Stey *et al.* 2000 [62]	Chronic bronchitis	2011	Systematic review of 11 randomised, placebo-controlled trials	– Placebo ($n = 733$) – NAC 400-600 mg/d ($n = 723$)	3–6 months	Greater percentage of patients with no exacerbations during the treatment period (NAC= 48.5%, placebo = 31.2% ($p < 0.05$)
Tse *et al.* 2013 [60]	Stable COPD	120	Randomised, double blind, placebo-controlled trial	– Placebo ($n = 62$) – NAC 600 mg/bid ($n = 58$)	1 year	Decrease in exacerbation frequency – NAC = 0.96 episodes/year – Placebo = 1.71 episodes/year ($p = 0.019$)

COPD exacerbations

The efficacy of NAC in reducing the frequency of exacerbations was assessed in a clinical trial involving 169 patients with moderate to severe COPD (57). Patients were randomized to standard therapy alone (β_2-agonists, anticholinergics, theophylline and inhaled and/or oral corticosteroids) or standard therapy plus NAC 600 mg once daily for 6 months. This multicenter, open, randomized controlled trial demonstrated a statistically significant ($p < 0.05$) 41% reduction in the number of exacerbations in the group treated with NAC, as compared with the control group (standard therapy). Furthermore, a small but significant ($p < 0.001$) difference in the severity of exacerbations (as measured with

an exacerbation severity score) was observed in favor of the NAC group over the control group.

A more recent meta-analysis (58) of data from 8 trials including a total of 2214 randomized patients showed that NAC significantly reduced the odds of experiencing exacerbations over the treatment period by about 50% (odds ratio = 0.49, 95% CI: 0.32–0.74; $p = 0.001$). This effect appeared to be attenuated by the use of inhaled corticosteroids, but was not affected by smoking. Overall, the results of this meta-analysis indicate that NAC, by reducing the rate of exacerbations, may help modify the natural history of moderate-to-severe COPD.

The BRONCUS study (Bronchitis Randomized on NAC Cost-Utility Study) is a pivotal study on the effect of NAC in a large number of patients with COPD (59). In this double-blind, placebo-controlled, randomized study, 523 patients with COPD were followed for 3 years to examine the effects of NAC 600 mg/day *versus* placebo (both on top of standard therapy) on lung function and rate of exacerbations. To be eligible for the study, patients with moderate COPD had to report at least two exacerbations during the two years prior to the enrolment. Overall, NAC failed to demonstrate an effect on the frequency of exacerbations (hazard ratio 0.99, 95% CI: 0.89–1.10; $p = 0.85$); however, a significant 21% reduction in the rate of exacerbations was found in the subgroup of patients not taking inhaled corticosteroids (hazard ratio 0.79, 95% CI: 0.63–0.99; $p = 0.04$), as compared with the placebo group.

In the HIACE trial, a double blind, randomized, placebo-controlled trial in 120 patients with stable COPD treated with NAC 600 mg b.i.d. or placebo for 1 year, the frequency of exacerbations was significantly reduced by 44% in the NAC group as compared to the placebo group (0.96 *versus* 1.71 times/year, $p = 0.019$) (60) (Figure 6). Furthermore, at the end of the study more patients in the NAC group were free of exacerbations, as compared with those treated with placebo (53.8% *versus* 37.5%), although this result did not achieve statistical significance ($p = 0.088$). The Authors concluded that the reduction in the rate of COPD exacerbations might be related to the antioxidant and antiinflammatory effects of NAC, which may lead to a significant improvement in small airways function and therefore in air trapping (60).

The efficacy of a short-term treatment with NAC (600 and 1200 mg/day for 10 days) on acute COPD exacerbations was assessed in a clinical randomized, double blind, placebo-controlled trial (61). The main finding of this study was a significant reduction in inflammatory markers, particularly C-reactive protein (PCR), associated with NAC treatment. At baseline, 81 out of 123 patients in the three treatment arms had CRP levels >0.5 mg/dL. At the end of the treatment period, the proportion of patients whose CRP levels normalized was significantly higher ($p < 0.01$) both in the NAC 600 mg group (52% of patients) and the NAC 1200 mg group (90% of patients), as compared with the placebo group (19%) (61). Furthermore, a statistically significant reduction in interleukin 8 (IL-8) levels was observed in the group treated with NAC 1200 mg/day ($p < 0.001$ *versus* baseline). Normalization of inflammatory markers was associated with clinical improvements, with reductions in difficulty of expectoration ($p = 0.042$ and $p < 0.001$ in the NAC 600 mg and 1200 mg groups, respectively) and other symptoms (e.g. cough frequency and intensity) (61).

A systematic review of randomized controlled trials (RCTs) comparing NAC with placebo was undertaken to analyze the effects of NAC treatment on COPD exacerbations (62). This analysis, which considered 11 studies including 2011 patients, revealed that a greater number of patients were free of exacerbations during treatment with NAC 400-600 mg/day for 3–6 months, as compared to patients treated with placebo. In particular, 351 out of 723 patients treated with NAC (48.5%) did not experience exacerbations during the treatment period, as compared with 229 out of 733 (31.2%) patients who received placebo (Figure 4). This difference was statistically significant ($p < 0.05$).

Lung hyperinflation

A reduction in oxidative stress and inflammation by NAC in the lower respiratory tract might have a favorable effect on the phenomenon of air trapping. Chronic airflow obstruction in COPD is caused by a combination of small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema) (6). Chronic inflammation leads to structural changes that result in narrowing of the small airways, whereas destruction of the lung parenchyma, also due to inflammation, leads to loss of alveolar attachments to the small airways and reduces the lung elastic recoil (6). As a consequence, the ability of the airways to remain patent during expiration decreases, causing air trapping and lung hyperinflation.

Stav *et al*. assessed the effect of NAC on lung hyperinflation in a double blind, randomized, placebo-controlled crossover trial in 24 patients with moderate to severe COPD and lung hyperinflation (age >40 years,

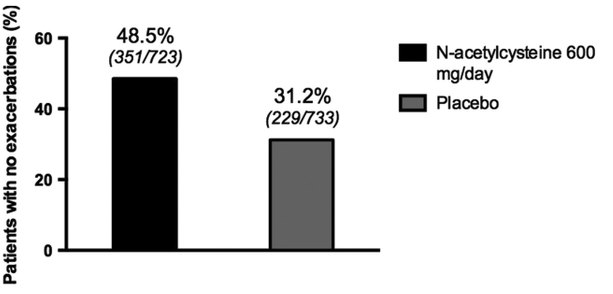

Figure 4. Percent of patients with no COPD exacerbations during treatment with NAC 600 mg/day or placebo for 12–24 weeks. Meta-analysis of 11 clinical trials involving 2011 patients with chronic bronchitis/COPD. Difference between groups was statistically significant ($p < 0.05$) (62).

FEV_1 58% of predicted, mean residual volume [RV] to total lung capacity [TLC] ratio: 137% of predicted, inspiratory capacity [IC] 2.2 L) (63). Treatment with NAC 1200 mg/day for 6 weeks significantly increased IC ($p < 0.0033$ *versus* placebo) and forced vital capacity (FVC) measured after exercise ($p < 0.0029$), and significantly reduced the RV/TLC ratio after exercise ($p < 0.01$ *versus* placebo).

Of note, this functional improvement was associated with increased physical performance. These findings demonstrate that NAC may significantly reduce air trapping caused by dynamic hyperinflation, even after a relatively short treatment period. The effects of NAC on airway resistance and lung hyperinflation have also been explored in the HIACE trial (60). HIACE showed that the antioxidant/antinflammatory activity of NAC may reduce the resistance of the lower airways, as indicated by a significant ($p = 0.037$) improvement in forced expiratory flow 25% to 75% ($FEF_{25-75\%}$) and forced oscillation technique (FOT) parameters ($p < 0.05$), which are used to specifically assess small airways function.

Lung function

The rate of decline in FEV_1 was a co-primary endpoint of the BRONCUS study. In this study, the rate of decline in FEV_1 was unaffected by treatment with NAC (54 mL/year *versus* 47 mL/year in the NAC and placebo group, respectively) (59). However, it should be noted that the dropout rate was significantly lower in the NAC group than in the placebo group (27% and 37%, respectively; $p = 0.018$). This difference could explain the lack of effect on primary variables.

Furthermore, the results of a secondary analysis of patients who completed the study suggest that NAC may have favorable effects on lung function in patients with COPD. A statistically significant reduction from baseline in functional residual capacity (FRC) was found at the end of the study in patients treated with NAC (mean difference: –0.374 ± 1.03; $p < 0.0001$), but not in those assigned to placebo (Figure 5). Furthermore, another study showed a small but significant improvement in FEV_1 in COPD patients treated with NAC 600 mg/day plus standard therapy for 6 months (57).

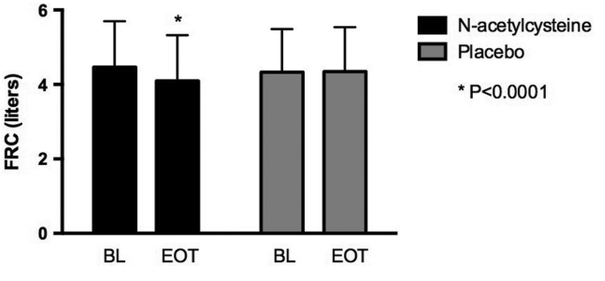

Figure 5. Change from baseline in functional residual capacity (FRC) in patients who completed the treatment period in the BRONCUS study. FRC was significantly reduced from baseline (BL) to the end of treatment (EOT) in patients treated with NAC 600 mg/day (n = 120), as compared with those treated with placebo (n = 107) (59).

COPD symptoms

The aforementioned systematic review of RCTs also addressed the effect of NAC on COPD symptoms (62). For each trial, the percentage of patients reporting improvements in symptoms was higher in NAC-treated individuals than in those in the placebo arm. The difference in favor of NAC reached statistical significance in the largest clinical trial, consisting of 611 patients (66% of the total number of patients in all trials considered) (62, 64). The authors of the meta-analysis concluded that of 100 treated patients, 26 (number-needed-to-treat, 3.8) would report that the NAC treatment led to improvement of their bronchitis-related symptoms, who would not have done so had they all received placebo (62).

Hospitalizations for COPD

In the HIACE trial, a trend toward reduction in the frequency of hospital admissions for COPD (0.5 times/year vs 0.80 times/year) and average number of hospitalization days (1.8 days/year vs 4.2 days/year) was observed with NAC 600 mg b.i.d. as compared with placebo (60). This trend did not reach statistical significance, likely due to the relatively small number of patients enrolled in the study.

A retrospective analysis of 1219 patients with COPD aged ≥55 years and hospitalized for a COPD exacerbation (65) evaluated the efficacy of NAC treatment following discharge on prevention of re-hospitalizations for COPD. To this aim, the population considered was divided into two groups, i.e. patients treated and not treated with NAC. All patients were studied starting from the first discharge after a COPD exacerbation to their first re-hospitalization, death or up to a maximum follow-up of one year.

This study showed that NAC treatment reduced the risk of re-hospitalization for COPD by approximately 30%, as illustrated by the Kaplan–Meier curve in Figure 6 (relative risk = 0.67; 95% Confidence Interval [CI]:

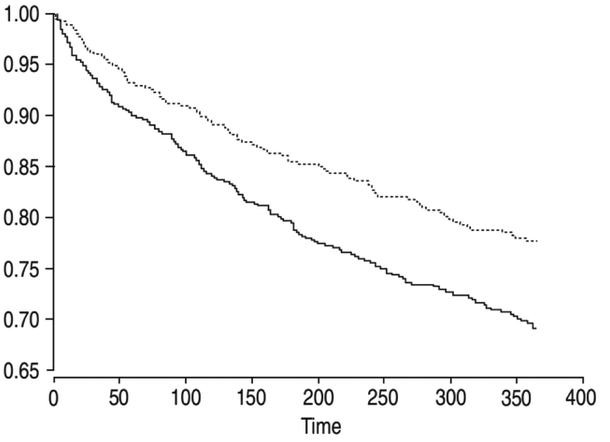

Figure 6. Kaplan-Meier curve showing a 30% reduction in the risk of readmission to hospital for COPD in patients treated (dotted line) and not treated (solid line) with NAC after discharge for COPD exacerbation. Risk reduction was dose-dependent. Reproduced with permission from (65).

0.53-0.85). Stratification of patients based on average daily dose of NAC during the follow-up period showed that the risk of re-hospitalization was significantly lower in patients treated with high daily doses of NAC, with a statistically significant dose/response trend ($p < 0.0001$).

Systemic manifestation of COPD

Although primarily viewed as a respiratory disease, COPD has both pulmonary and systemic effects. Oxidative stress is believed to play an important role in the systemic manifestations of COPD (66), which include osteoporosis and muscle wasting. Of note, besides being associated with reduced exercise capacity and health status, low fat free mass is also an independent predictor of mortality in COPD patients (67). The effects of NAC on exercise performance have been assessed in both healthy subjects and COPD patients.

NAC was shown to reduce respiratory muscle fatigue and delay time to fatigue in healthy volunteers (68–70). A randomized, double-blind, placebo-controlled crossover study in which nine severe COPD patients performed localized dynamic quadriceps endurance tests at 40% of maximal strength after treatment with NAC (1800 mg/day for 4 days and a last 600-mg dose on the day of the test) showed a significant 25% improvement in endurance time after NAC treatment compared with placebo (71). In the same study, treatment with NAC prevented the increase in exercise-induced oxidative stress observed in patients treated with placebo after the test (71).

In the study by Stav *et al*., treatment with NAC 1200 mg/day for 6 weeks significantly increased exercise time compared with placebo, with treatment differences of 22 s, which was highly significant (63). Conversely, in the HIACE trial NAC failed to improve exercise capacity (60). To the best of our knowledge, no data are available on the effects of NAC on bone resorption in COPD patients. However, the results of a randomized, double-blind, placebo-controlled pilot study in early-postmenopausal women suggest that NAC may slow bone resorption (72).

Tolerability

The efficacy of high-dose NAC should be balanced against the potential risk of side effects. No serious adverse events have been reported in clinical trials involving NAC. In the HIACE study (60), long-term treatment with NAC (600 mg b.i.d. for 1 year) was safe and well tolerated, with no significant differences in adverse effects *versus* placebo (5.2% *versus* 8%, respectively). Chronic use of an even higher dose of NAC (1800 mg daily), such as that employed in the treatment of interstitial pulmonary fibrosis in the multicenter, randomized, placebo-controlled IFIGENIA study (73), was well tolerated.

Conclusions

The antioxidant/antinflammatory activity of NAC, documented in both experimental and clinical pharmacological studies, may be attributed to NAC's ability to act on the oxidative stress that characterizes COPD, both indirectly by restoring the endogenous pool of reduced glutathione (NAC provides cysteine for GSH biosynthesis) and via a direct antioxidant effect (NAC contains a free thiol –SH group that is highly protective against ROS). The rationale for using NAC in the treatment of COPD is further supported by the definition of COPD provided by the recent Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines, which describe COPD as a disease characterized by persistent airflow limitation that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung (6).

Indeed, the GOLD guidelines highlight the pathogenic role of oxidative stress resulting from inhalation of pro-oxidant agents and/or a reduction in endogenous antioxidants as an important amplifying mechanism in COPD (6). Similarly, the American Thoracic Society and the European Respiratory Society acknowledge that the systemic manifestations of COPD are likely to be, at least in part, the result of shared mechanisms that also contribute to the structural and functional changes within the lungs, including systemic inflammation, altered apoptosis, and oxidative stress (74). NAC targets mucus hypersecretion, oxidative stress and inflammation, which are all involved in the pathogenesis of COPD.

These properties account for the favorable clinical outcomes achieved with short- and long-term NAC treatment in patients with COPD. In particular, NAC appears to be a valuable adjunct to standard therapy for preventing COPD exacerbations. Although the BRONCUS study failed to demonstrate a significant reduction in exacerbation rates with NAC, several other studies indicate that this drug, particularly when given at high doses (1200 mg/day), may have beneficial effects on the frequency and severity of COPD exacerbations. Preliminary results from the randomized, placebo-controlled PANTHEON study (75), which enrolled more than 1000 patients with moderate-to-severe COPD, suggest that high-dose NAC (1200 mg/daily for 1 year) may reduce exacerbations in a significant proportion of patients, with a better improvement in the subgroup with moderate COPD (76).

Lung hyperinflation and exercise capacity also seem to be favorably affected by NAC treatment. Of note, these parameters are important predictors of health status. Due to the increasing interest in drugs targeting oxidative stress and inflammation, and despite being an "old" drug, NAC has recently been the subject of several investigations in the field of respiratory medicine. Both a strong rationale and the results of several clinical trials that demonstrated the efficacy and tolerability of the drug support the use of NAC as an adjunct to standard treatment in patients with COPD. Evidence from recent studies will help define which patients may benefit more from NAC treatment.

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Declaration of Interest Statement

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