Trends in Pharmacological Sciences

CellPress
REVIEWS

Opinion

Thiol-Based Drugs in Pulmonary Medicine: Much More than Mucolytics

Mario Cazzola,[1,*] Luigino Calzetta,[1] Clive Page,[2] Paola Rogliani,[1] and Maria Gabriella Matera[3]

Thiol-based drugs are considered as mucolytics because they decrease the viscosity and mostly decrease the elasticity of bronchial secretions by reducing disulfide bonds in proteins. However, they can also act as antioxidant drugs directly through free sulfhydryl groups that serve as a source of reducing equivalents, as well as indirectly through the replenishment of intracellular glutathione (GSH) levels. Modulation of neurokinin A levels may also be related to the effect of thiol drugs on oxidative stress. Moreover, thiol-based drugs interfere with inflammatory pathways and modulate human bronchial tone. They might also be considered as therapeutic agents against some types of infection because they reduce bacterial adhesion to the respiratory epithelial cell surface and inhibit biofilm formation, causing biofilm disruption and thereby improving the efficacy of antibiotic therapy.

Thiol-Containing Compounds

Drugs containing the thiol moiety (or those metabolized to thiol-containing species) are often used in pulmonary medicine as **mucolytics** (see Glossary) to manage the thick mucus secretions associated with several lung diseases. There are several thiol-based drugs, but *N*-acetyl L-cysteine (NAC), *S*-carboxymethyl-L-cysteine [3-(*S*-carboxymethylthio)alanine] (S-CMC), and *N*-(carboxymethylthioacetyl)-homocysteine thiolactone (erdosteine) have been most extensively studied in the treatment of pulmonary diseases. The documented activities of these thiol-containing compounds are summarized in Table 1, and the correlations between their specific effects are shown in Figure 1.

Thiol-Based Drugs as Mucolytics

Although some data do not support the use of thiol-based drugs in pulmonary medicine [1–3], there is also evidence that these three thiol-containing compounds are effective and safe mucolytic agents [4,5].

NAC, a thiol-based drug with a free sulfhydryl group (-SH), disrupts the structure of the mucus. It breaks the disulfide bonds (S–S) that connect mucin proteins by donating electrons to the thiol groups of mucin monomer cysteine (Cys) residues [6,7]. This pharmacological effect results in depolymerization of mucin oligomers, changes in the rheology of mucin-rich secretions, and consequent reduction in the elasticity and viscosity of the mucus [8].

S-CMC, which is also available as its lysine salt (S-CMC-lys), does not have a free SH group and therefore does not appear to break mucin S–S bonds, and instead acts via alternative mechanisms. Essentially, S-CMC replaces fucomucins by sialomucins probably via intracellular stimulation of sialyl transferase activity, modulates active ion transport across the airway epithelium, and increases mucociliary clearance velocity [9,10]. Erdosteine contains two S atoms, one of which is a thioether in the aliphatic side chain and the other is enclosed in the heterocyclic ring (thiolactone) [11]. It is a **prodrug** that is metabolized to the ring-opening compound, metab-

Highlights

Thiol groups are common functional groups in protein structure. They stabilize the tertiary and quaternary structures of proteins by forming intra- and interchain disulfide bonds (S–S), play many roles in metabolism and homeostasis, and are important in several physiological and pathological processes.

Drugs containing the thiol moiety (-SH) impact on a variety of biological systems. *N*-acetyl L-cysteine, *S*-carboxymethyl-L-cysteine, and erdosteine have been studied in the treatment of pulmonary diseases such as chronic obstructive pulmonary disease, bronchiectasis, and idiopathic pulmonary fibrosis. However, the implementation of these drugs in pulmonary medicine remains limited.

Thiol-based drugs act as mucolytics and antioxidants. They also interfere with inflammatory pathways, modulate human bronchial tone, and should be considered in some infections because thiols reduce bacterial adhesion to the respiratory epithelial cell surface and inhibit biofilm formation, causing biofilm disruption and improving the efficacy of antibiotic therapy.

[1]Unit of Respiratory Medicine, Department of Experimental Medicine, University of Rome 'Tor Vergata', Rome, Italy
[2]Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London, London, UK
[3]Unit of Pharmacology, Department of Experimental Medicine, University of Campania 'Luigi Vanvitelli', Naples, Italy

*Correspondence:
mario.cazzola@uniroma2.it (M. Cazzola).

Table 1. Documented Activities of the Main Thiol-Containing Compounds[a]

		Erdosteine	NAC	S-CMC
Mucolytic activity	Reduced viscosity of mucoprotein solutions	+	+	+
	Reduced bronchial secretions		+	+
	Increased mucociliary clearance	+	+	+
Antioxidant activity	Reduced pro-oxidant profile	+	+	+
	Increased antioxidant profile	+	+	+
Anti-inflammatory activity	Reduced neurogenic inflammation		+	
	Reduced cytokine release	+	+	+
	Reduced proteinase synthesis	+	+	
	Reduced levels of proinflammatory proteins and activation of transcription factors	+	+	+
Direct and indirect antibacterial activity	Increased neutrophil killing activity		+	
	Bacteriostatic effect		+	
	Reduced bacterial adhesion on epithelium	+	+	+
	Reduced biofilm formation		+	
	Improved antibiotic activity	+	±	+
	Viral replication and infectivity		+	+
Impact on bronchial contractile tone	Reduced bronchial desensitization		+	
	Improved effect of bronchodilator agents	+	±	

[a]Symbols and abbreviations: +, demonstrated activity; ±, inconsistent activity; empty cells indicate that the activity has not been investigated; NAC, *N*-acetyl L-cysteine; S-CMC, *S*-carboxymethyl-L-cysteine [3-(*S*-carboxymethylthio)alanine].

olite M1 or (±)-*N*-(2-carboxymethylthioacetyl)homocysteine, which contains a pharmacologically active -SH group [11]. Hence, the metabolite M1 has mucolytic properties.

Thiol-Based Drugs Exhibit Pharmacological Actions beyond Mucolytic Activity

Accumulating evidence suggests that thiol drugs also possess potent antioxidant and anti-inflammatory properties [8,12]. In addition, thiol-based drugs have been reported to exhibit antibacterial activity against a variety of medically important bacteria [13,14] and are also able to influence bronchial tone [8], activities that are likely related to their antioxidant and anti-inflammatory properties. These multifaceted actions of thiol-based drugs beyond mucolytic activity may be of relevance to the ability of these drugs to reduce exacerbations in patients with chronic obstructive pulmonary disease (COPD), and it is therefore timely to reconsider whether this drug class should only be referred to as mucolytics.

Thiol-Based Drugs as Antioxidants

The potential ability to influence oxidative stress is the most intriguing among the non-mucolytic activities of thiol-based drugs, although it is not yet clear if these drugs are really effective in controlling oxidative stress. Oxidative stress is an excess production of reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), and hydrogen peroxide (H_2O_2), as well as reactive nitrogen species (RNS), relative to the levels of antioxidants (Box 1). ROS generation initiates the activation of various signaling pathways including c-Src, protein kinase C (PKC), epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), phosphoinositide 3-kinase (PI3K)/Akt, and mitogen-activated protein kinases (MAPKs), as well as of transcription factors such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1), and hypoxia-inducible factor (HIF)-1α, and ultimately induces the expression of inflammatory target proteins [15]. Overproduction of these proteins may contribute to pulmonary diseases such as

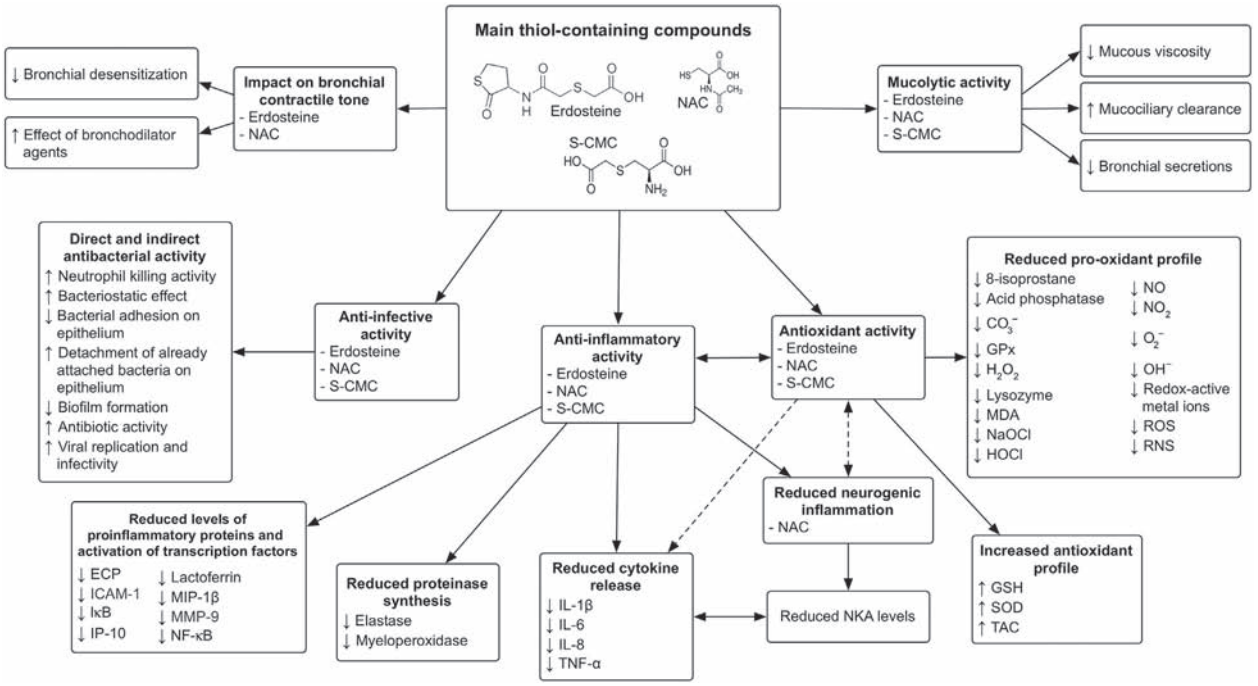

Figure 1. Graphical Representation of the Documented Activities of the Main Thiol-Containing Compounds in Pulmonary Medicine and Correlations between Specific Effects. Broken arrows represent indirect correlations. Abbreviations: ECP, eosinophil cationic protein; GPx, glutathione peroxidase; GSH, glutathione; IκB, nuclear factor of κ light polypeptide gene enhancer in B cells inhibitor; ICAM-1, intercellular cell adhesion molecule-1; IL, interleukin; IP-10, interferon-γ-inducible protein 10; MDA, malondialdehyde; MIP-1β, macrophage inflammatory protein 1β; MMP-9, metalloproteinase-9; NAC, *N*-acetyl L-cysteine; NO, nitric oxide; NF-κB, transcription factor nuclear factor-κB; NKA, neurokinin A; ROS, reactive oxygen species; RNS, reactive nitrogen species; S-CMC, *S*-carboxymethyl-L-cysteine [3-(*S*-carboxymethylthio)alanine]; SOD, superoxide dismutase; TAC, total antioxidant capacity.

Box 1. Oxidative Stress and GSH

Oxidative stress has been defined as a disturbance in the balance between the production of free radicals or ROS and antioxidant defenses, which may lead to tissue injury [83]. In a healthy organism in which mediators of oxidative stress and inflammation are in balance with the counteracting detoxifying and anti-inflammatory molecules, free radicals are normal and necessary to some degree. In addition to causing some damage, they also stimulate repair [84].

Free radicals or ROS become an issue when their production is excessive, and they overwhelm the repair processes because of a shift towards oxidative stress that can cause DNA and protein damage, inflammation, and finally cell death [84]. The ratio of GSH to oxidized glutathione (GSSG) may be used as a marker of oxidative stress. However, the calculation and quantification of this ratio is not easy and depends on the detection method, the tissue of interest, and the abundance of recycling enzymes such as GPx1. At present there is no quantitative means to characterize individuals with oxidative stress, and none of the so-called biomarkers of oxidative stress allows us to assess accurately and definitively oxidative stress in a manner that can be directly applied in the clinic [83].

Oxidative stress derived from several sources, such as cigarette smoke, inhaled oxidants, and endogenous ROS can lead to inflammation and an imbalance of oxidants and antioxidants in favor of oxidants. This in turn results in various cellular and molecular alterations leading to COPD/emphysema [84].

Antioxidant capacity in the lung is substantially reduced as a result of cigarette smoking and other exacerbations, and oxidative stress persists long after the cessation of cigarette smoking or exacerbation owing to continued production of ROS from endogenous sources [16].

GSH has been referred to as the 'master antioxidant'. It neutralizes free radicals and removes toxins from the body, including heavy metals. GSH is also crucial for the proper function of the immune system. In addition, it reduces inflammation in the body. Diminished GSH levels elevate cellular vulnerability to oxidative stress [85].

Box 2. Chronic Lung Diseases

Asthma: according to the World Health Organization (WHO) definition, asthma is a chronic disease characterized by recurrent attacks of breathlessness and wheezing which vary in severity and frequency from person to person.[ii] Symptoms may occur several times per day or week in affected individuals, and for some people become worse during physical activity or at night. The strongest risk factors for developing asthma are a combination of genetic predisposition with environmental exposure to inhaled substances and particles that may provoke allergic reactions or irritate the airways.

Chronic obstructive pulmonary disease (COPD): according to the WHO definition, COPD is a lung disease characterized by chronic obstruction of lung airflow that interferes with normal breathing and is not fully reversible.[iii] The more familiar terms 'chronic bronchitis' and 'emphysema' are no longer used but are now included within the COPD diagnosis. The primary cause of COPD is tobacco smoke (including second-hand or passive exposure). However, COPD is not simply a 'smoker's cough' but an underdiagnosed, life-threatening lung disease. In addition, exposure to indoor air pollution such as the use of biomass fuels for cooking and heating contributes to the burden of COPD.

Idiopathic pulmonary fibrosis (IPF): according to the definitions adopted by the American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association, IPF is a specific form of chronic, progressively fibrosing interstitial pneumonia of unknown cause, occurring primarily in older adults, and limited to the lungs [86]. It is characterized by progressive worsening of dyspnea and lung function, and is associated with a poor prognosis.

Bronchiectasis: according to the WHO definition, bronchiectasis is an abnormal widening of one or more airways.[iv] Small glands in the lining of the airways normally make a small amount of mucus. Mucus keeps the airways moist, and traps any dust and dirt in the inhaled air. Because bronchiectasis creates abnormal widening of the airways, additional mucus tends to form, and this pools in parts of the widened airways. Widened airways with extra mucus are prone to infection.

Cystic fibrosis: an autosomal recessive disorder associated with mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene (chromosome 7q31.2) that encodes a protein that functions as an apical epithelial chloride channel. Cystic fibrosis is a complex disorder caused by dysfunctional chloride ion transport across epithelial surfaces, leading to thickening of secretions (e.g., mucus, digestive fluids, sweat), and is characterized by chronic and progressive pulmonary, pancreatic, gastrointestinal, and hepatobiliary involvement [87].

acute respiratory distress syndrome (ARDS), asthma, cystic fibrosis (CF), idiopathic pulmonary fibrosis (IPF), and COPD (Box 2) [16,17].

Loss of antioxidant capacity is mainly due to decreased levels of GSH and/or its precursor, Cys [16]. As a carrier of an active thiol group, GSH acts as an antioxidant either directly by interacting with ROS/RNS and electrophiles or by operating as a reductant substrate for other enzymatic (e.g., glutathione peroxidase, GPx) and non-enzymatic (e.g., vitamin C) antioxidants [5]. GSH has important roles in cellular defense against cellular oxidant aggression, redox regulation of protein thiols, and in maintaining redox homeostasis that is crucial for proper functioning of cellular processes including apoptosis [16,17].

Both extracellular and intracellular levels of GSH are often abnormal in many chronic lung diseases such as asthma, COPD, IPF, bronchiectasis, and CF [15]. Bacterial infection, inflammation, or smoking can elevate GSH levels in the **bronchoalveolar lavage fluid** (BALF) to avoid further damage to the lung, but the ability of a compromised lung to maintain a normal GSH basal level may contribute to the progression of these lung diseases [16,17].

Increased levels of circulating GSH might be useful [18], but the low absorption of GSH, mainly due to the action of an intestinal enzyme, γ-glutamyl transpeptidase, which recycles GSH precursors and may prevent significant intact absorption of GSH *per se* from oral supplementation [19], the short apparent half-life of GSH, and the dependence of GSH availability on transporter expression for uptake and transport into the BALF [20] make oral administration of GSH problematic. Moreover, GSH induces toxicity at the high doses needed to adequately restore GSH levels.

Synthesis of GSH occurs via a two-step ATP-requiring enzymatic process in which Cys is the fundamental substrate [17]. Cys undergoes rapid oxidation in solution, generating inactive Cys–Cys

Glossary

Bacterial adhesion: a complex but essential step in the colonization of environments that contain surfaces exposed to fluid flow. It is now well established that, to initiate infection at a particular site, bacteria must adhere to host cells or to extracellular material covering these cells.
Biofilms: a community of microorganisms attached to an inert or living surface by a self-produced polymeric matrix, or an assemblage of microbial cells that are associated with a surface and enclosed in a matrix of primarily polysaccharide material.
Bronchoalveolar lavage fluid (BALF): the fluid that is flushed into a small part of the lung and then recollected for examination using a bronchoscope that is passed through the mouth or nose into the lungs. BAL is the most common manner to sample the components of the epithelial lining fluid and to determine the protein composition of the pulmonary airways.
Free radicals: highly reactive and unstable molecules, usually but not always oxygen molecules. They are atoms or groups of atoms with an odd (unpaired) number of electrons that can be formed when oxygen interacts with specific molecules.
8-Isoprostane: a prostanoid resembling prostaglandin F2α but that has inverted stereochemistry at the 8-position. Expired breath condensate levels of 8-isoprostane are a marker of increased lipid peroxidation in patients with pulmonary disease.
Lipopolysaccharide (LPS): also called endotoxin, LPS is a component of the outer membrane of Gram-negative bacteria and is released from the bacterial surface via outer membrane vesicles (blebs), which may be released from the bacterial cell surface, or by lysis and disintegration of the organism.
Mucolytics: medications that change the biophysical properties of secretions by degrading mucin polymers, DNA, fibrin, or F-actin in airway secretions, generally decreasing viscosity.
Neurokinin A (NKA): an endogenous peptide of the tachykinin family that acts as a potent agonist of the NK-2 receptor, the most important tachykinin receptor involved in smooth muscle contraction. In addition, NKA is a potent agonist of the NK-1 receptor and could therefore mediate some of the effects traditionally attributed to substance P.

[17]. NAC has advantages over Cys because it is relatively resistant to oxidation to Cys–Cys, although the rates of cellular uptake of NAC and its deacetylation may not be adequate to maintain sufficient Cys levels for endogenous GSH biosynthesis [16].

NAC is able to deliver -SH moieties and provides a source for Cys required in the biosynthesis of GSH, but a direct effect on oxidative stress is unlikely because the rate constants for its reaction with relevant physiological oxidants such as H_2O_2, and $O_2^{\bullet-}$ (0.16 and 68 $M^{-1}s^{-1}$, respectively, at pH 7.4 and 37°C) [21] are too low to make a significant contribution to oxidant scavenging, considering that the rate constants for the reaction of GSH with H_2O_2, and $O_2^{\bullet-}$ are 15 $M^{-1}s^{-1}$ [22] and ~200 $M^{-1}s^{-1}$ [23], respectively. An indirect effect leading to replenishment of intracellular GSH levels may be a more likely possibility, but it is has also been suggested that increased Cys is converted to hydrogen sulfide (H_2S) through enzymatic catabolism, and subsequent mitochondrial H_2S oxidation increases sulfane sulfur levels, leading to the observed antioxidant effects of NAC [21].

Nevertheless, in an *ex vivo* model of COPD exacerbation, NAC increased the total antioxidant capacity by ~90% because of a ~50% increase in GSH levels and a ~150% increase in superoxide dismutase activity compared to **lipopolysaccharide** (LPS)-incubated bronchi, and reduced the levels of pro-oxidant factors activated by LPS by ~30% [12]. Furthermore, NAC decreased the release of **neurokinin A** (NKA) induced by LPS stimulation, an effect that correlated with a reduction in GPx activity and in the levels of H_2O_2, malondialdehyde (MDA), and nitric oxide (NO) [24]. In patients with COPD, NAC affected the redox balance of the body by increasing GSH levels in plasma and BALF [25], and reduced both ROS production by alveolar macrophages and exhaled H_2O_2 [26].

Erdosteine inhibits the effects of **free radicals** produced by cigarette smoke and reduces the production of $O_2^{\bullet-}$, H_2O_2, and NO, as well as the release of acid phosphatase and lysozyme from LPS-activated macrophages [11]. The erdosteine derivative, M1, regulates ROS produced by rat neutrophils, guinea pig eosinophils, and human neutrophils and is effective in preventing H_2O_2-induced oxidative stress and DNA damage in A549 human lung adenocarcinoma cells through the scavenging of intracellular ROS [27]. Presumably, cells exposed to erdosteine are affected by M1, which is related to homocysteine. Experimentally, erdosteine prevented or reduced tissue damage induced by oxidative stress of various causes [28,29].

In COPD patients, erdosteine affected ROS levels in current smokers, reduced exercise-induced oxidative stress by improving the oxidant/antioxidant imbalance, and decreased ROS and **8-isoprostane** plasma levels in those undergoing acute exacerbations of COPD (AECOPD) [11]. S-CMC reacts with and reduces ROS, but its scavenger effects, which result from the reducing function of the thioether group, are weaker than those of NAC [30]. COPD patients treated with S-CMC show a marked reduction of exhaled 8-isoprostane [31].

Thiol-Based Drugs as Anti-Inflammatory Drugs

ROS generation initiates the activation of various signaling pathways and ultimately induces the expression of inflammatory target proteins [15]. Therefore, targeting oxidizing molecules in conjunction with inflammatory mediators and transcription factors, while maintaining intracellular GSH, may be a novel therapeutic strategy for treating inflammatory lung diseases. However, GSH forms mixed disulfides with proteins when it reacts with ROS [32]. This process, called glutathionylation, can be either anti- or proinflammatory depending on the protein targeted [32].

There is evidence that NAC inhibits NF-κB activation, which is central to the regulation and expression of stress response genes under oxidative challenge, in human bronchial epithelial cells

pKa: the acid dissociation constant.
Planktonic: bacteria that are freely floating. They can become sessile bacteria by adhering to a surface.
Prodrug: a pharmacologically inactive substance that, after administration, is metabolized into a pharmacologically active drug.
TOLLIP: also known as Toll-interacting protein, this is an inhibitory adaptor protein that in humans is encoded by the *TOLLIP* gene.

[33] *in vitro* through inhibition of IκB kinases (IKKs) [34], and attenuates endotoxin-induced NF-κB activation in rat lung tissue *in vivo* [33]. Nevertheless, NAC can also affect other signal transduction pathways and the expression of various genes, and directly modulates the activity of several common transcription factors both *in vitro* and *in vivo* [35].

NAC reduced TNF-α/IL-1β-stimulated intercellular cell adhesion molecule-1 (ICAM-1) expression and IL-8 release in endothelial and epithelial cells [36], plasma concentrations of myeloperoxidase and elastase, and the levels of lactoferrin and eosinophil cationic protein in BALF [37]. Furthermore, NAC attenuated the chemoattractant properties of neutrophils both in BALF [36] and sputum [38] of COPD patients, inhibited IL-8 and matrix metalloproteinase-9 release and ICAM-1 expression from cells harvested in BALF also from IPF patients [39], and decreased neutrophil burden, the number of airway neutrophils actively releasing elastase-rich granules, and IL-8 in sputum samples from patients with CF [40]. However, 4 months of oral NAC did not alter markers of inflammation including IL-8 and TNF-α in BALF obtained from asymptomatic asbestos-exposed individuals in a double-blind, placebo controlled trial [41]. Intravenous NAC also failed to attenuate an increase in IL-6 in patients undergoing lung resection [42].

However, it must be mentioned that experimental data indicate that higher concentrations of NAC than those needed to modulate any oxidant/antioxidant imbalance would be necessary to elicit anti-inflammatory effects, whereas a lower concentration, corresponding to oral administration of NAC at 200 mg/day, is adequate to reduce IL-6 levels, which are inversely correlated with NKA release [12]. This finding suggests that NAC at low concentrations may fine-tune neurogenic inflammation, a deleterious condition that may support the vicious circle between oxidative stress and inflammation in human bronchi [24].

Erdosteine also elicits anti-inflammatory effects, and inhibits LPS-induced NF-κB activation and IL-6 and IL-1β production in mouse macrophages, and attenuates the increase of inflammatory cells in the BALF of rats following instillation of LPS [11]. Concentrations of M1 achievable in the clinical setting inhibit neutrophil elastase release induced by the synthetic chemotactic peptide *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) in a concentration-dependent manner [43]. Furthermore, erdosteine drastically decreased the level of proinflammatory IL-8 in the peripheral blood of COPD patients who smoked [11].

S-CMC also reduces pulmonary inflammation and mucus overproduction in mice exposed to cigarette smoke after infection with influenza virus via activation of NF-E2-related factor (Nrf) 2 [44]. It also attenuates H_2O_2- and TNF-α-induced inflammation in human lung alveolar type II epithelial cells *in vitro* through suppressing NF-κB and extracellular regulated kinase 1/2 (ERK1/2)/MAPK signaling pathways [45,46].

Antibacterial and Antiviral Effects of Thiol-Based Drugs

Some bacterial pathogens have evolved not only to survive the oxidative stress encountered during infection but also in some cases to utilize them to thrive in the face of redox challenges during infection [47]. For this reason, thiol-based drugs may be useful against these pathogens.

NAC and GSH dramatically increase neutrophil killing of *Staphylococcus aureus in vitro*, an effect that is not necessarily only due to protection of the cells from self-induced oxidative stress [48]. A possible explanation is that they reduce the concentration of extracellular NO.

NAC also has bacteriostatic properties against several pathogens, including *Pseudomonas aeruginosa*, *S. aureus*, and *Klebsiella pneumoniae*, although it is apparently without significant direct antibacterial activity [49]. Furthermore, it induces the release of proinflammatory membrane vesicles by non-typeable *Haemophilus influenzae* (NTHI), *Moraxella catarrhalis*, and

P. aeruginosa, but not by *Streptococcus pneumoniae* [50]. NAC also exhibits potent antimycobacterial effects and may limit *Mycobacterium tuberculosis* infection both through suppression of the host oxidative response and direct antimicrobial activity [51]. These bacteriostatic effects of NAC could be mediated by the inhibition of Cys utilization by the bacteria, but the Cys requirements differ among bacteria and can even vary between strains, which could explain the differences in bacterial sensitivity to NAC [52]. Even more important is the ability of thiol-based drugs to reduce **bacterial adhesion** to the respiratory epithelial cell surface, which is the first step in the development of respiratory tract infections, particularly when mucociliary function is impaired.

There is evidence that NAC, S-CMC, and erdosteine inhibit bacterial adhesion to oropharyngeal epithelial cells and can increase the detachment of bacteria without affecting their virulence [13,14,53]. These effects vary according to hosts and strains. Equally notable is the ability of the thiol-based drugs to also affect **biofilms** [13]. This observation is important because bacteria in biofilms can be two- to 1000-fold more resistant to antimicrobial agents than the corresponding **planktonic** forms [54].

NAC plays a role in the various steps of biofilm formation: adhesion to inert and living surfaces, matrix production and organization, and dispersal of preformed biofilms [13]. It also reduces the production of extracellular polysaccharides (EPSs), the major structural components of the biofilm of most bacteria [55]. NAC at pH<**pKa** can penetrate the matrix and eventually kill 100% of bacteria embedded in the biofilm [56]. The microcolonies of killed bacteria swell in size and passively shed them. However, NAC used intravenously or in the presence of blood increases bacterial biofilm formation rather than inhibiting it [57].

The mucolytic activity of thiol-based drugs allows better penetration of antibiotics into the sites of infection, thus influencing their antimicrobial effects. There is evidence that erdosteine significantly increases antibiotic concentrations in sputum, but not in serum [58], and S-CMC administered with amoxicillin causes an increase of the quantitative levels of antibiotic even in purulent bronchial secretions [58].

The addition of M1 to clarithromycin potentiates the inhibition of *S. aureus* adhesion to human mucosal cells relative to clarithromycin alone [59]. Furthermore, NAC increases the activity of penicillins [60] and slightly increases the activities of amoxicillin, erythromycin, and levofloxacin in some biofilm-associated *S. pneumoniae* strains that otherwise are not very sensitive to these antibiotics [61]. However, it decreases ceftriaxone and aminoglycoside activity, and can even make carbapenems chemically unstable at high concentrations [60].

Thiol-based drugs are an effective strategy against influenza virus infection because virus-induced oxidative stress is important in the regulation of the host immune system [62]. Supplementation with GSH inhibits viral replication, infectivity, and influenza virus-induced apoptosis and generation of virus particles, and also depresses viral matrix protein expression, virus-induced caspase activation, and Fas upregulation [62]. It may also antagonize the major pathogenic processes of influenza virus H5N1 [62].

NAC inhibits H5N1 replication and H5N1-induced production of proinflammatory molecules, which are associated with oxidant sensitive pathways, and alleviates lung damage by inhibiting TLR4 protein levels [8]. The protective effect of NAC against respiratory virus infection includes inhibition of NF-κB translocation to the nucleus, phosphorylation of p38 MAPK, inhibition of ICAM-1, and restoration of the normal ciliary activity [8]. The inhibitory activity of NAC against influenza A viruses appears to be strain-dependent [8]. Combination of NAC with ribavirin or oseltamivir enhanced the anti-influenza effects of these drugs [8].

S-CMC reduces pulmonary inflammation and mucus overproduction in mice exposed to cigarette smoke after infection with influenza virus via activation of Nrf2 [44]. Furthermore, it reduces the expression of sialic acids, which are receptors for influenza virus, and increases the pH in endosomes of human airway epithelial cells [63].

Thiol-Based Drugs as Modulators of Bronchial Tone

Oxidants can influence the function of airway smooth muscle (ASM) because contraction produces free radicals, and GSH decreases ASM contraction induced by different stimuli [8]. These observations suggest that thiol-based drugs may be a possible therapeutic option to modulate bronchial tone.

NAC is effective in restoring the physiologic contractility of ASM depressed by chronic stimulation with LPS, likely because it is able to prevent neurogenic inflammation and an increase in NKA [24]. Furthermore, NAC enhances the bronchodilator effects of muscarinic receptor antagonists, but not of β_2-agonists [64]. By contrast, erdosteine enhances airway responses to salbutamol in patients with mild-to-moderate COPD, an effect related to the peculiar protection against lipid peroxidation rather than to its scavenging activity because lipid peroxidation of β_2-adrenoceptors does not reverse with NAC treatment [11].

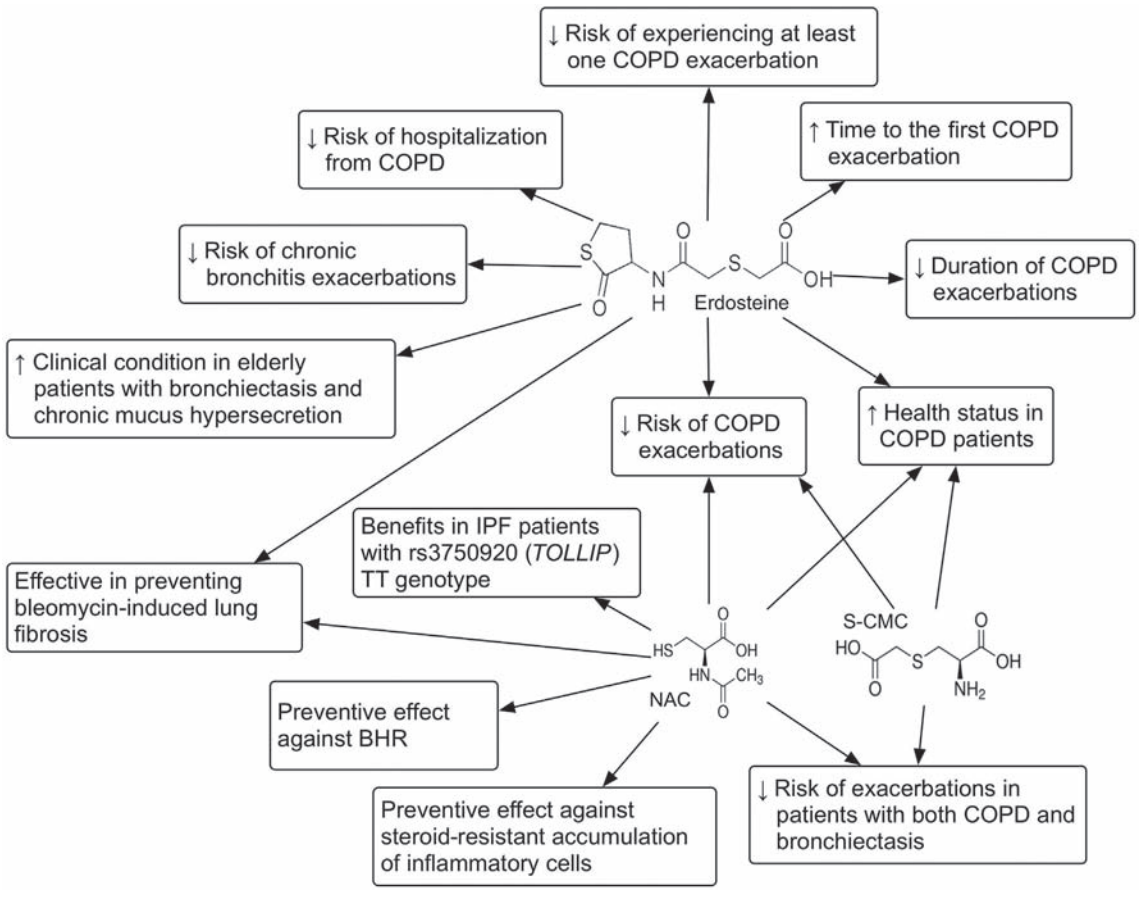

Figure 2. Graphical Representation of the Clinical Effects of the Main Thiol-Containing Compounds in Pulmonary Medicine. Abbreviations: BHR, bronchial hyper-responsiveness; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis; NAC, *N*-acetyl L-cysteine; S-CMC, *S*-carboxymethyl-L-cysteine [3-(*S*-carboxymethylthio) alanine]; TOLLIP, Toll-interacting protein.

Importance of the Route of Administration

Thiol-based drugs are usually given orally, but they may be more effective when administered by inhalation because they can directly act on the airway, a possibility that has several advantages such as reaching the correct anatomical target at high concentrations, thus avoiding drug metabolism by the liver and intestine, and also fast onset, a lower drug dose, and reduced systemic side effects [13]. However, there is little literature on inhaled thiol-based drugs, which is limited in any case to NAC. In fact, no information is available on the effects of erdosteine or S-CMC administered by this route.

However, clinical data in CF and COPD do not support the efficacy of inhaled NAC with respect to improvement of lung function or reduction in pulmonary exacerbations [2,3,65,66]. It has been suggested that the low intrinsic reducing activity and short half-life of NAC on airway surfaces are responsible for its ineffectiveness [7], and there is also evidence that a temporary and short-lived H_2O_2 increase is related to redox interactions in the airways of COPD patients [67]. Nevertheless, inhaled NAC monotherapy was associated with improved redox imbalance in patients with early IPF [68].

Clinical Effectiveness of Thiol-Based Drugs in Pulmonary Medicine

The potential clinical impact of thiol-based drugs in pulmonary medicine is summarized in Figure 2. The recent Global Initiative for Chronic Obstructive Lung Diseases (GOLD 2019) report[i] has listed NAC, S-CMC, and erdosteine as additional therapies to consider in the treatment of COPD because recent data have shown that some of these drugs (erdosteine [69] and NAC [4]) can reduce exacerbations in patients with COPD, even in patients taking inhaled corticosteroids. However, no distinction is made between these drugs because they have only been investigated by indirect comparisons [4]. Nevertheless, given recent clinical data with NAC and erdosteine, wider use of these drugs in the treatment of COPD needs to be considered [69,70].

With regard to other lung diseases, it has been hypothesized that thiol-based drugs may be useful for treating IPF because of the involvement of oxygen radicals and reduced GSH levels in IPF pathogenesis and progression [71]. In effect, NAC and erdosteine have been shown to prevent bleomycin-induced fibrosis in laboratory animals [72]. The antifibrotic activity probably results from their -SH groups which have potent free radical scavenging activities. However, clinical trials with NAC have yielded disappointing results [71,73], although inhaled NAC alone or in combination with pirfenidone may be a reasonable option in a minority of IPF patients [72], likely those with the rs3750920 (***TOLLIP***) TT genotype [74].

The addition of NAC to standard-of-care asthma medication had no significant effect on asthma exacerbations [75], although there is evidence that this thiol-based drug prevents airway hyperresponsiveness and the accumulation of steroid-resistant inflammatory cells in an animal model of exacerbation of asthma [76], and also restores oxidant–antioxidant balance through a decrease in NADPH oxidase expression/ROS generation/lipid peroxide formation and increased total antioxidant capacity [8].

Although NAC and S-CMC seem to be ineffective in treating non-CH bronchiectasis [77,78], they are recommended by the recent Spanish guidelines for the treatment of non-CF bronchiectasis in adults because they can reduce exacerbations [79]. Interestingly, erdosteine added to routine chest physiotherapy provided some physiological and clinical benefits in the treatment of elderly patients with bronchiectasis and chronic mucus hypersecretion [80]. NAC and S-CMC have also been used to 'treat' CF, but there is no evidence that they are beneficial [81].

Concluding Remarks and Future Perspectives

Thiol-based drugs clearly exhibit multiple pharmacological actions of relevance to the treatment of a range of respiratory diseases including COPD, asthma, and possibly IPF. However, some

Outstanding Questions

Do we really understand the detailed biochemistry and biological roles of Cys and GSH in living systems?

Is current knowledge about free radicals or ROS sufficient to establish clinical therapeutic strategies regarding the use of an antioxidant regimen in pulmonary medicine, or is there a need to enlarge our knowledge?

Is the ability of thiol-based drugs to reduce the risk of AECOPD related to their mucolytic, antioxidant, anti-inflammatory, or antimicrobial activities, or to all of these?

Are the NAC doses approved for daily treatment able to induce a true antioxidant or even anti-inflammatory effect?

Are thiol-based drugs in conjunction with other antifibrotic agents useful in the treatment of at least some specific groups of patients suffering from IPF?

Can thiol-based drugs actually modulate bronchial tone in humans and also be used for treating patients with asthma?

Can local administration of thiol-based drugs provide an effective way to increase their concentration in the airways?

crucial questions remain to be addressed (see Outstanding Questions) to better understand which of these pharmacological effects contribute to the clinical benefits observed with this drug class. Nonetheless, recent clinical data with NAC and erdosteine suggest that, at least in patients with mild to moderate COPD, wider use of these drugs should be considered as a safe and inexpensive way to reduce exacerbations.

However, we must also highlight that a recent analysis from the *Cochrane Database of Systematic Reviews* concludes that NAC likely has a modest beneficial effect on respiratory exacerbations and quality of life in patients with COPD, an effect that tends to be smaller in recent larger studies [66]. In fact, one basic study explained the possible lack of efficacy of NAC by its weak potency [7], and another basic study highlighted the dual (anti- and pro-oxidant) effects of thiol-based drugs under oxidative stress conditions [82], raising doubts about the utility of thiol drugs, at least those currently available.

Thinking more positively based on what was highlighted above, we agree that the implementation of these drugs in pulmonary medicine remains limited because of the heterogeneity of clinical studies, drug dosing, and formulations, as well as difficulty in identifying the appropriate target population among those with COPD [70] and likely also those with IPF.

Disclaimer Statement

M.C., L.C., and C.P. are or have been consultants to Zambon (Italy), a company that manufactures and markets NAC, and to Recipharm (Sweden), a company that manufactures and markets erdosteine. M.G.M. has received a research grant partially funded by Zambon (Italy). P.R. has no competing interests.

Resources

[i] https://goldcopd.org/wp-content/uploads/2018/11/GOLD-2019-v1.5-FINAL-04Nov2018_WMS.pdf

[ii] www.who.int/respiratory/asthma/definition/en/

[iii] www.who.int/respiratory/copd/en/

[iv] www.who.int/respiratory/other/bronchiectasis/en/

References

1. Poole, P.J. and Black, P.N. (2006) Mucolytic agents for chronic bronchitis or chronic obstructive pulmonary disease. *Cochrane Database Syst. Rev.*, CD001287
2. Tam, J. *et al.* (2013) Nebulized and oral thiol derivatives for pulmonary disease in cystic fibrosis. *Cochrane Database Syst. Rev.*, CD007168
3. Nash, E.F. *et al.* (2009) Nebulized and oral thiol derivatives for pulmonary disease in cystic fibrosis. *Cochrane Database Syst. Rev.*, CD007168
4. Cazzola, M. *et al.* (2017) Impact of mucolytic agents on COPD exacerbations: a pair-wise and network meta-analysis. *COPD* 14, 552–563
5. Cazzola, M. *et al.* (2018) Impact of erdosteine on chronic bronchitis and COPD: a meta-analysis. *Pulm. Pharmacol. Ther.* 48, 185–194
6. Aldini, G. *et al.* (2018) N-acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. *Free Radic. Res.* 52, 751–762
7. Ehre, C. *et al.* (2019) An improved inhaled mucolytic to treat airway muco-obstructive diseases. *Am. J. Respir. Crit. Care Med.* 199, 171–180
8. Calzetta, L. *et al.* (2018) Multifaceted activity of N-acetyl-l-cysteine in chronic obstructive pulmonary disease. *Expert Rev. Respir. Med.* 12, 693–708
9. Colombo, B. *et al.* (1994) Stimulation of Cl^- secretion by the mucoactive drug S-carboxymethylcysteine-lysine salt in the isolated rabbit trachea. *Eur. Respir. J.* 7, 1622–1628
10. Hooper, C. and Calvert, J. (2008) The role for S-carboxymethylcysteine (carbocisteine) in the management of chronic obstructive pulmonary disease. *Int. J. Chron. Obstruct. Pulmon. Dis.* 3, 659–669
11. Dal Negro, R. *et al.* (2018) Erdosteine: drug exhibiting polypharmacy for the treatment of respiratory diseases. *Pulm. Pharmacol. Ther.* 53, 80–85
12. Cazzola, M. *et al.* (2017) Pharmacological investigation on the anti-oxidant and anti-inflammatory activity of N-acetylcysteine in an ex vivo model of COPD exacerbation. *Respir. Res.* 18, 26
13. Blasi, F. *et al.* (2016) The effect of N-acetylcysteine on biofilms: implications for the treatment of respiratory tract infections. *Respir. Med.* 117, 190–197
14. Braga, P.C. *et al.* (1999) Effects of erdosteine and its metabolites on bacterial adhesiveness. *Arzneimittelforschung* 49, 344–350
15. Lee, I.T. and Yang, C.M. (2012) Role of NADPH oxidase/ROS in pro-inflammatory mediators-induced airway and pulmonary diseases. *Biochem. Pharmacol.* 84, 581–590
16. Matera, M.G. *et al.* (2016) Oxidation pathway and exacerbations in COPD: the role of NAC. *Expert Rev. Respir. Med.* 10, 89–97
17. Rushworth, G.F. and Megson, I.L. (2014) Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits. *Pharmacol. Ther.* 141, 150–159
18. Rogers, L.K. and Cismowski, M.J. (2018) Oxidative stress in the lung – the essential paradox. *Curr. Opin. Toxicol.* 7, 37–43
19. Zhang, H. *et al.* (2005) Gamma-glutamyl transpeptidase in glutathione biosynthesis. *Methods Enzymol.* 401, 468–483
20. Gould, N.S. and Day, B.J. (2011) Targeting maladaptive glutathione responses in lung disease. *Biochem. Pharmacol.* 81, 187–193
21. Ezeriņa, D. *et al.* (2018) N-acetyl cysteine functions as a fast-acting antioxidant by triggering intracellular H_2S and sulfane sulfur production. *Cell Chem. Biol.* 25, 447–459

22. Abedinzadeh, Z. *et al.* (1989) Kinetic study of the oxidation mechanism of glutathione by hydrogen peroxide in neutral aqueous medium. *Can. J. Chem.* 67, 1247–1255
23. Jones, C.M. *et al.* (2002) Electron paramagnetic resonance spin trapping investigation into the kinetics of glutathione oxidation by the superoxide radical: re-evaluation of the rate constant. *Free Radic. Biol. Med.* 32, 982–990
24. Calzetta, L. *et al.* (2018) N-acetylcysteine protects human bronchi by modulating the release of neurokinin A in an ex vivo model of COPD exacerbation. *Biomed. Pharmacother.* 103, 1–8
25. Bridgeman, M.M. *et al.* (1991) Cysteine and glutathione concentrations in plasma and bronchoalveolar lavage fluid after treatment with N-acetylcysteine. *Thorax* 46, 39–42
26. Kasielskim, M. and Nowak, D. (2001) Long-term administration of N-acetylcysteine decreases hydrogen peroxide exhalation in subjects with chronic obstructive pulmonary disease. *Respir. Med.* 95, 448–456
27. Marabini, L. *et al.* (2011) Protective effect of erdosteine metabolite I against hydrogen peroxide-induced oxidative DNA-damage in lung epithelial cells. *Arzneimittelforschung* 61, 700–706
28. Boyaci, H. *et al.* (2006) Effects of erdosteine on bleomycin-induced lung fibrosis in rats. *Mol. Cell. Biochem.* 281, 129–137
29. Demiralay, R. *et al.* (2006) Comparison of the effects of erdosteine and N-acetylcysteine on apoptosis regulation in endotoxin-induced acute lung injury. *J. Appl. Toxicol.* 26, 301–308
30. Nogawa, H. *et al.* (2009) Carbocisteine can scavenge reactive oxygen species in vitro. *Respirology* 14, 53–59
31. Carpagnano, G.E. *et al.* (2004) Exhaled interleukine-6 and 8-isoprostane in chronic obstructive pulmonary disease: effect of carbocysteine lysine salt monohydrate (SCMC-Lys). *Eur. J. Pharmacol.* 505, 169–175
32. Coppo, L. and Ghezzi, P. (2011) Thiol regulation of pro-inflammatory cytokines and innate immunity: protein S-thiolation as a novel molecular mechanism. *Biochem. Soc. Trans.* 39, 1268–1272
33. Sadowska, A.M. *et al.* (2007) Antioxidant and anti-inflammatory efficacy of NAC in the treatment of COPD: discordant in vitro and in vivo dose-effects: a review. *Pulm. Pharmacol. Ther.* 20, 9–22
34. Oka, S. *et al.* (2000) N-acetylcysteine suppresses TNF-induced NF-κB activation through inhibition of IκB kinases. *FEBS Lett.* 472, 196–202
35. Samuni, Y. *et al.* (2013) The chemistry and biological activities of N-acetylcysteine. *Biochim. Biophys. Acta* 1830, 4117–4129
36. Radomska-Leśniewska, D.M. *et al.* (2006) Influence of N-acetylcysteine on ICAM-1 expression and IL-8 release from endothelial and epithelial cells. *J. Physiol. Pharmacol.* 57, 325–334
37. Eklund, A. *et al.* (1988) Oral N-acetylcysteine reduces selected humoral markers of inflammatory cell activity in BAL fluid from healthy smokers: correlation to effects on cellular variables. *Eur. Respir. J.* 1, 832–838
38. van Overveld, F.J. *et al.* (2000) Induced sputum of patients with chronic obstructive pulmonary disease (COPD) contains adhesion-promoting, therapy-sensitive factors. *Inflamm. Res.* 49, 8–13
39. Radomska-Leśniewska, D.M. *et al.* (2010) N-acetylcysteine inhibits IL-8 and MMP-9 release and ICAM-1 expression by bronchoalveolar cells from interstitial lung disease patients. *Pharmacol. Rep.* 62, 131–138
40. Tirouvanziam, R. *et al.* (2006) High-dose oral N-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis. *Proc. Natl. Acad. Sci. U. S. A.* 103, 4628–4633
41. Alfonso, H. *et al.* (2015) Effect of N-acetylcysteine supplementation on oxidative stress status and alveolar inflammation in people exposed to asbestos: a double-blind, randomized clinical trial. *Respirology* 20, 1102–1107
42. Bastin, A.J. *et al.* (2016) Systemic inflammation and oxidative stress post-lung resection: effect of pretreatment with N-acetylcysteine. *Respirology* 21, 180–187
43. Braga, P.C. *et al.* (2006) Effect of metabolite I of erdosteine on the release of human neutrophil elastase. *Pharmacology* 77, 150–154
44. Yageta, Y. *et al.* (2014) Carbocisteine reduces virus-induced pulmonary inflammation in mice exposed to cigarette smoke. *Am. J. Respir. Cell Mol. Biol.* 50, 963–973
45. Wang, W. *et al.* (2015) Carbocisteine attenuates hydrogen peroxide-induced inflammatory injury in A549 cells via NF-κB and ERK1/2 MAPK pathways. *Int. Immunopharmacol.* 24, 306–313
46. Wang, W. *et al.* (2016) Carbocisteine attenuates TNF-α-induced inflammation in human alveolar epithelial cells in vitro through suppressing NF-κB and ERK1/2 MAPK signaling pathways. *Acta Pharmacol. Sin.* 37, 629–636
47. Reniere, M.L. (2018) Reduce, induce, thrive: bacterial redox sensing during pathogenesis. *J. Bacteriol.* 200 e00128-18
48. Nygren, H. *et al.* (2004) Development of a wound dressing targeting neutrophil function. *World J. Surg.* 28, 337–342
49. Parry, M.F. and Neu, H.C. (1977) Effect of N-acetylcysteine on antibiotic activity and bacterial growth *in vitro*. *J. Clin. Microbiol.* 5, 58–61
50. Volgers, C. *et al.* (2017) Effects of N-acetyl-L-cysteine on the membrane vesicle release and growth of respiratory pathogens. *FEMS Microbiol. Lett.* 364, fnx087
51. Amaral, E.P. *et al.* (2016) N-acetyl-cysteine exhibits potent anti-mycobacterial activity in addition to its known anti-oxidative functions. *BMC Microbiol.* 16, 251
52. Guédon, E. and Martin-Verstraete, I. (2006) Cysteine metabolism and its regulation in bacteria. *Microbiol. Monogr.* 5, 195–218
53. Mitchell, S.C. and Steventon, G.B. (2012) S-carboxymethyl-L-cysteine. *Drug Metab. Rev.* 44, 129–147
54. Svensäter, G. and Bergenholtz, G. (2004) Biofilms in endodontic infections. *Endod. Top.* 9, 27–36
55. Olofsson, A.C. *et al.* (2003) N-acetyl-L-cysteine affects growth, extracellular polysaccharide production, and bacterial biofilm formation on solid surfaces. *Appl. Environ. Microbiol.* 69, 4814–4822
56. Kundukad, B. *et al.* (2017) Mechanistic action of weak acid drugs on biofilms. *Sci. Rep.* 7, 4783
57. Yin, S. (2018) The interaction of N-acetylcysteine and serum transferrin promotes bacterial biofilm formation. *Cell. Physiol. Biochem.* 45, 1399–1409
58. Ricevuti, G. *et al.* (1988) Influence of erdosteine, a mucolytic agent, on amoxycillin penetration into sputum in patients with an infective exacerbation of chronic bronchitis. *Thorax* 43, 585–590
59. Braga, P.C. *et al.* (2001) Bacterial adhesiveness: effects of the SH metabolite of erdosteine (mucoactive drug) plus clarithromycin versus clarithromycin alone. *Chemotherapy* 47, 208–214
60. Landini, G. *et al.* (2016) Effect of high N-acetylcysteine concentrations on antibiotic activity against a large collection of respiratory pathogens. *Antimicrob. Agents Chemother.* 60, 7513–7517
61. del Prado, G. *et al.* (2010) Biofilm formation by *Streptococcus pneumoniae* strains and effects of human serum albumin, ibuprofen, N-acetylcysteine, amoxicillin, erythromycin, and levofloxacin. *Diagn. Microbiol. Infect. Dis.* 67, 311–318
62. Liu, M. *et al.* (2017) The role of oxidative stress in influenza virus infection. *Microbes Infect.* 19, 580–586
63. Yamaya, M. *et al.* (2010) Inhibitory effects of carbocisteine on type A seasonal influenza virus infection in human airway epithelial cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 299, 160–168
64. Sinojia, R. *et al.* (2014) Priming of beta-2 agonist and antimuscarinic induced physiological responses induced by 1200mg/day NAC in moderate to severe COPD patients: a pilot study. *Respir. Physiol. Neurobiol.* 191, 52–59
65. Rogers, D.F. (2002) Mucoactive drugs for asthma and COPD: any place in therapy? *Expert Opin. Investig. Drugs* 11, 15–35
66. Poole, P. *et al.* (2015) Mucolytic agents versus placebo for chronic bronchitis or chronic obstructive pulmonary disease. *Cochrane Database Syst. Rev.*, CD001287
67. Rysz, J. *et al.* (2007) Increased hydrogen peroxide concentration in the exhaled breath condensate of stable COPD patients after nebulized N-acetylcysteine. *Pulm. Pharmacol. Ther.* 20, 281–289
68. Muramatsu, Y. *et al.* (2016) Effect of inhaled N-acetylcysteine monotherapy on lung function and redox balance in idiopathic pulmonary fibrosis. *Respir. Investig.* 54, 170–178
69. Dal Negro, R.W. *et al.* (2017) Effect of erdosteine on the rate and duration of COPD exacerbations: the RESTORE study. *Eur. Respir. J.* 50, 1700711

70. Vézina, F.A. and Cantin, A.M. (2018) Antioxidants and chronic obstructive pulmonary disease. *Chronic. Obstr. Pulm. Dis.* 5, 277–288
71. Martinez, F.J. *et al.* (2014) Randomized trial of acetylcysteine in idiopathic pulmonary fibrosis. *N. Engl. J. Med.* 370, 2093–2101
72. Yildirim, Z. *et al.* (2005) Attenuation of bleomycin-induced lung fibrosis by oral sulfhydryl containing antioxidants in rats: erdosteine and N-acetylcysteine. *Pulm. Pharmacol. Ther.* 18, 367–373
73. Rogliani, P. *et al.* (2016) Pirfenidone, nintedanib and N-acetylcysteine for the treatment of idiopathic pulmonary fibrosis: a systematic review and meta-analysis. *Pulm. Pharmacol. Ther.* 40, 95–103
74. Oldham, J.M. *et al.* (2015) TOLLIP, MUC5B, and the response to N-acetylcysteine among Individuals with idiopathic pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* 192, 1475–1482
75. Aliyali, M. *et al.* (2010) Effects of N-acetylcysteine on asthma exacerbation. *Iran. J. Allergy Asthma Immunol.* 9, 103–109
76. Eftekhari, P. *et al.* (2013) Preventive effect of N-acetylcysteine in a mouse model of steroid resistant acute exacerbation of asthma. *EXCLI J.* 12, 184–192
77. Hill, A.T. *et al.* (2011) Primary care summary of the British Thoracic Society guideline on the management of non-cystic fibrosis bronchiectasis. *Prim. Care Respir. J.* 20, 135–140
78. Snijders, D. *et al.* (2013) Inhaled mucoactive drugs for treating non-cystic fibrosis bronchiectasis in children. *Int. J. Immunopathol. Pharmacol.* 26, 529–534
79. Martínez-García, M.Á. *et al.* (2018) Spanish guidelines on treatment of bronchiectasis in adults. *Arch. Bronconeumol.* 54, 88–98
80. Crisafulli, E. *et al.* (2007) Effectiveness of erdosteine in elderly patients with bronchiectasis and hypersecretion: a 15-day, prospective, parallel, open-label, pilot study. *Clin. Ther.* 29, 2001–2009
81. Rubin, B.K. (2014) Cystic fibrosis: myths, mistakes, and dogma. *Paediatr. Respir. Rev.* 15, 113–116
82. Sajewicz, W. (2010) Effect of thiol drugs on tert-butyl hydroperoxide induced luminol chemiluminescence in human erythrocytes, erythrocyte lysate, and erythrocyte membranes. *Chem. Biol. Interact.* 186, 144–151
83. Tsalamandris, S. *et al.* (2018) The role of oxidative stress. In *Coronary Artery Disease* (Tousoulis, D., ed.), pp. 95–100, Elsevier
84. Finkel, T. and Holbrook, N.J. (2000) Oxidants, oxidative stress and the biology of ageing. *Nature* 408, 239–247
85. Teskey, G. *et al.* (2018) Glutathione as a marker for human disease. (Vol. 87), Advances in Clinical Chemistry (Makowski, G.S., ed.), pp. 141–159, Elsevier
86. Raghu, G. *et al.* (2018) Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. *Am. J. Respir. Crit. Care Med.* 198, e44–e68
87. Michl, R.K. *et al.* (2016) Clinical approach to the diagnosis and treatment of cystic fibrosis and CFTR-related disorders. *Expert Rev. Respir. Med.* 10, 1177–1186