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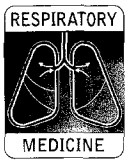

Topical Reviews

Characterization of *N*-acetylcysteine and ambroxol in anti-oxidant therapy

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Reactive free oxygen radicals are known to play an important role in the pathogenesis of various lung diseases such as idiopathic pulmonary fibrosis (IPF), adult respiratory distress syndrome (ARDS) or cystic fibrosis (CF). They can originate from endogenous processes or can be part of exogenous exposures (e.g. ozone, cigarette smoke, asbestos fibres). Consequently, therapeutic enhancement of anti-oxidant defence mechanisms in these lung disorders seems a rational approach. In this regard, *N*-acetyl-L-cysteine (NAC) and ambroxol have both been frequently investigated.

Because of its SH group, NAC scavenges H_2O_2 (hydrogen peroxide), ·OH (hydroxol radical), and HOCl (hypochlorous acid). Furthermore, NAC can easily be deacetylated to cysteine, an important precursor of cellular glutathione synthesis, and thus stimulate the cellular glutathione system. This is most evident in pulmonary diseases characterized by low glutathione levels and high oxidant production by inflammatory cells (e.g. in IPF and ARDS). NAC is an effective drug in the treatment of paracetamol intoxication and may even be protective against side-effects of mutagenic agents. In addition NAC reduces cellular production of pro-inflammatory mediators (e.g. TNF-*α*, IL-1).

Also, ambroxol [*trans*-4-(2-amino-3,5-dibromobenzylamino)-cyclohexane hydrochloride] scavenges oxidants (e.g. ·OH, HOCl). Moreover, ambroxol reduces bronchial hyperreactivity, and it is known to stimulate cellular surfactant production. In addition, ambroxol has anti-inflammatory properties owing to its inhibitory effect on the production of cellular cytokines and arachidonic acid metabolites. For both substances effective anti-oxidant and anti-inflammatory function has been validated when used in micromolar concentrations. These levels are attainable *in vivo* in humans. This paper gives an up-to-date overview about the current knowledge of the hypothesis that oxidant-induced cellular damage underlies the pathogenesis of many human pulmonary diseases, and it discusses the feasibility of anti-oxidant augmentation therapy to the lung by using NAC or ambroxol.

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Introduction

Reactive oxygen species (ROS) are constantly formed in the lung and serve a physiological role. Once radicals are formed they can (a) destroy micro-organisms, normal or neoplastic mammalian cells and (b) modulate the inflammatory response (e.g. mediator release of inflammatory cells). However, when generated in excess, or in inappropriate environments, and when anti-oxidant defence is insufficient, radicals can damage DNA, lipids, proteins and carbohydrates [for review see 1–3]. In the lung in particular, various diseases are known where oxygen metabolites play a predominant role in the pathogenesis (Table 1).

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Based on this knowledge, anti-oxidant augmentation therapy seems to be a rational approach in pulmonary diseases characterized by an overload of radicals or lack of anti-oxidants. In this regard, *N*-acetyl-L-cysteine (NAC) and ambroxol, both widely used in Europe as mucolytic agents, have been investigated thoroughly in the recent past. The purpose of this article is to summarize these findings in order to assess clinical feasibility of anti-oxidant therapy with these drugs.

The Role of Oxidants in Living Systems

FREE RADICALS

A free radical is any species capable of independent existence that contains one or more unpaired electrons, where an unpaired electron is one that occupies an atomic or

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TABLE 1. Reactive oxygen intermediates are regarded to play a considerable role (a) in the pathogenesis of various pulmonary inflammatory disorders and (b) after inhalation of various substances [summarized from (59,155–158]

Disease or exposure	Source of oxidant
Adult respiratory distress syndrome (ARDS)	Stimulated PMN cells
Cystic fibrosis	Stimulated PMN cells, lack of fat-soluble anti-oxidants (caused by exocrine pancreatic insufficiency)
Idiopathic pulmonary fibrosis (IPF)	Stimulated PMN cells, and alveolar macrophages, pulmonary GSH deficiency
Asthma	Stimulated PMN cells and eosinophils
Hyperoxia	O_2^- formation from transport chains
Ozone	O_3: formation of O_2^-, ·OH and H_2O_2, release of cyclo-oxygenase metabolites of arachidonic acid
Asbestosis	Fe^{2+}, stimulated macrophages
Silicosis	Fe^{2+} after formation of silicato-iron complexes
Paraquat toxicity	Redox cycling
Bleomycin toxicity	Formation of O_2^- and ·OH from Fe^{2+}–bleomycin complex
Cigarette smoking	H_2O_2, NO_x peroxyl compounds, quinones, stimulated PMN cells and macrophages
Ionizing radiation	O_2^- formation by ionization
HIV infection	Systemic deficiency of acid-soluble thiols

TABLE 2. Examples of oxygen species [the terms oxidants and ROS includes oxygen radicals and other derivates of O_2 (non-radicals) (4)]

Formula	Description	Comments
Radicals		
O_2^-	Superoxide anion	Produced by a number of enzymes, auto-oxidation reactions and non-enzymatic electron transfers. Conversion to H_2O_2 [by superoxide dismutase (SOD) and O_2] or ·OH (Fenton reaction).
·OH	Hydroxy radical	Highly reactive
$RO_2^{\cdot}$, $RO^{\cdot}$	Peroxyl, alkoxyl radicals	Occur as organic radicals during the breakdown of peroxides of lipids (peroxidation)
NO·, $NO_2^{\cdot}$	Oxides of nitrogen	Nitric oxide and nitrogen dioxide can be found *in vivo* (NO·) and in polluted air (NO_2).
Non-radicals		
H_2O_2	Hydrogen peroxide	H_2O_2 occurs from O_2^- by many enzymatic reactions. From H_2O_2 ·OH can be formed.
HOCl	Hypochlorous acid	It is formed by action of myeloperoxidase in inflammatory reactions
O_3	Ozone	Formed in the environment.

molecular orbital by itself (4). ROS is a collective term that includes not only free oxygen radicals but also some derivatives of O_2 that do not contain unpaired electrons such as H_2O_2 (hydrogen peroxide), $^1O_2$ (singlet oxygen), O_3 (ozone) and HOCl (hypochlorous acid) (Table 2). The reactivity of the most frequently occurring ROS is as follows (5):

$$O_2 < O_2^- < H_2O_2 < \cdot OH$$

Oxidants are generated by various chemical processes (e.g. certain drugs, metals) and enzymatic reactions, or they can directly be inhaled (e.g. smoking, ozone, hyperoxia) [for review see (6–8)].

Myeloperoxidase, present in polymorphonuclear (PMN) cells and released during respiratory burst, converts H_2O_2 into HOCl, another highly toxic oxidant which can further react to form more long-lived chloramines (9). Triggered by SOD, O_2^- (superoxide anion) quickly dismutates to H_2O_2 from which highly reactive ·OH (hydroxyl radical) can be formed in the presence of transition metals such as iron. Iron- (or copper-) catalysed reactions are often referred to as either the Haber–Weiss reaction (formation of ·OH from H_2O_2 and O_2^-) or

the Fenton reaction (formation of ·OH from H_2O_2) (10,11). Since H_2O_2 easily diffuses through cell membranes ·OH formation occurs extra- as well as intracellularly. ·OH always causes site-directed cell damage where it occurs.

Generation of oxygen radicals by inflammatory cells is known to play an important part in the killing of several bacterial and fungal strains. H_2O_2 is used in a thyroid peroxidase mediated reaction to produce thyroid hormones or it can induce gene expression (e.g. cytoplasmatic gene transcription factor) (5,12). Because tumour necrosis factor (TNF), hyperthermia, and anti-cancer drugs such as adriamycin produce oxidants that cause tumour cell lysis, ROS seem to be meaningful in the natural defence against the development of cancer (13). Apart from the natural role oxygen radicals play in the host defence system, in inflammatory processes they notably can contribute to the severity of various pulmonary diseases. Since oxygen radicals are able to induce DNA-strand breaks, which activate prooncogenes such as K-*ras*, C-*RaF*-I and c-*fos*, oxygen radicals are also associated with an increased incidence of malignancies as proposed in asbestos exposure related tumours (14,15).

Activated leukocytes (both PMN cells and macrophages) release highly reactive reduced oxygen species such as O_2^-, H_2O_2 and others (·OH, HOCl, NO_x and HO_2). The local concentrations of oxidants formed by PMN cells, which occur during normal cellular oxidative phosphorylation, can be extremely high: 2×10^6 PMN cells stimulated with 10^{-8}M f-Met–Leu–Phe produce 10^{-8}M O_2^- within 1 min (9). Secretion of toxic oxygen metabolites into the extracelluar milieu is known as respiratory burst.

Also, inhalation of inorganic as well as organic particles generates oxidant stress via activation of inflammatory cells. Asbestos fibres (e.g. crocidolite) have a high redox potential through their iron content (up to 28%), forming ·OH in the Fenton reaction (16). Asbestos fibres and silica in addition trigger cytokine (e.g. TNF) release which stimulates inflammatory cells to produce high amounts of oxidants. In rat lungs inhaled crocidolite enhances antioxidant activity, and alveolar macrophages have been seen to undergo lipid peroxidation that can be blocked by anti-oxidants (17,18).

Interestingly, various cellular mediators released by stimulated inflammatory cells may further contribute to the oxidant load. Inflammatory mediators such as TNF, IL-1, IL-6, IL-8, various lipid mediators such as arachidonic acid metabolites prostaglandins, leukotrienes (e.g. B_4), the phospholipid platelet-activating factor and others are involved in the autocrine or paracrine activation of macrophages, neutrophils, fibroblasts, epithelial cells and endothelial cells (19–22). Both activated blood monocytes and neutrophils are attracted into the alveolar space by eicosanoid and cytokines released from these alveolar macrophages and thus enhance oxidant levels in the epithelial lining. Arachidonic acid metabolites can initiate oxidant release of activated inflammatory cells and enhance anti-oxidant enzyme expression in bronchoepithelial cells (23–27). Phospholipase A_2 releases arachidonic acid, which is involved directly or as a co-factor in the activation of the neutrophil respiratory burst (1,28). In contrast, mediators such as TNF, lipopolysaccharide (LPS) and IL-1 induce in epithelial cell types expression of anti-oxidants (e.g. MnSOD) (26,29–31).

THE PATHOGENESIS OF OXIDANT-RELATED INJURY

The term lung injury incorporates a wide range of phenomena characterized by widely differing manifestations, including, among others, functional alterations in gas exchange, excess of lung water, alterations of pulmonary haemodynamics, increased vascular permeability and histological evidence of structural damage. An impressive variety of insults may cause acute and chronic lung injury. One group of these insults affects the lung parenchyma directly, whereas others affect the lung via systemic mechanisms (32).

Lung injury is clearly marked by measurable changes as result of an inflammatory response where circulating PMN cells accumulate in the local vasculature and migrate through the vessel wall, the interstitium, and the epithelial barriers to gain access to the alveolar lumen.

Hyperoxia leads to a significant increase of intracellular O_2^- formation which may be one of the reasons why patients ventilated under hyperoxic conditions more easily develop ARDS (33). Recent experiments done in healthy volunteers exposed to 100% O_2 for 12–18 h by a full-face mask not only demonstrated that oxygen caused tracheobronchitis in most of the volunteers but also revealed that epithelial cells are not able to protect themselves from free radicals by triggering anti-oxidant (SOD and catalase) gene expression during this time period (34). Thus, oxidant-related cell injuries such as tracheobronchitis or ARDS in humans or low survival rates in animal experiments are typical consequences (33,35).

Bleomycin and the herbicide paraquat cause oxidant formation as a result of redox cycling which is Fe dependent (36,37). Because of its high affinity to DNA, bleomycin induces site-directed ·OH formation which causes crucial DNA damage. Certain xenobiotics, activated by intracellular reductases and thereby undergoing oxidative redox cycling, exert cell damage via intracellular generation of O_2^-. Lung injuries caused by paraquat, bleomycin and nitrofurantoin are considered a chemical form of oxygen toxicity. As a consequence paraquat toxicity, characterized by alveolitis, oedema and bleomycin, is used as a model for acute lung injury and pulmonary fibrosis (1,38).

Cigarette smoke, which has been estimated to contain 10^{14} oxidants per puff, and ionizing radiation induce inflammatory cells to release oxidants, but both may also cause cell injury through direct oxidant formation (39,40). Ozone, which contains unpaired electrons, degrades to highly reactive ·OH.

Oxidants indirectly contribute to pulmonary injury by inactivating anti-proteolytic proteins such as α_1-antitrypsin (α_1-AT) and secretory leukoprotease inhibitor (SLPI). ROS cause methionine sulphoxide formation at the active site and thus inactivate these anti-proteases. High methionine

sulphoxide levels in bronchoalveolar lavage (BAL) fluid of patients with ARDS and in BAL fluid of cigarette smokers indicate that oxidative processes inactivate anti-proteases in their airways more frequently than in normal volunteers (41). Because α_1-AT is the most important anti-protease to inactivate neutrophil elastase specifically, part of the oxidant-induced injury may also be due to secondary proteolytic damage caused by inactivation of α_1-AT. Malfunction of α_1-AT and therefore high activity levels of elastases are known to promote the development of lung emphysema (41). Like anti-proteolytic agents, anti-oxidants such as SOD can also be inactivated by oxidants (e.g. H_2O_2) (1,42).

There is multiple evidence that oxidative events associated with acute lung injury may also induce lipid peroxidation and consequently affect the lung surfactant system (43). Disturbance of the surfactant systems leads to acceleration of further lung injury (44).

Anti-oxidants

PRINCIPLES OF ANTI-OXIDANT DEFENCE

Normally, in the lung exists an oxygen-rich environment sensitively balanced between the toxicity of oxidants and protective measures of various intracellular and extracelluar anti-oxidants. Overlapping activities of anti-oxidants (e.g glutathione and catalase are both scavenging H_2O_2) indicates tight control of redox balance specifically to protect the cells without blocking the physiological role of cellular oxidants. To encounter the damaging effect of oxygen metabolites, the cells in the lung have a sophisticated network of anti-oxidant defence mechanisms. If exposed to oxidants, pulmonary cells (e.g. bronchoepithelial cells) are even able to enhance their anti-oxidant defence. For example, in smokers glutathione levels in BAL fluid are generally higher than in non-smokers; asbestos fibres cause an increase in MnSOD mRNA levels (45–48). However, the interrelationship between ROS and anti-oxidants in humans is very complex, and thus just the basic mechanism should be mentioned here [for further reviews see (7,49–51)].

Oxidant-scavenging systems can be categorized into the following groups: (a) enzyme systems, (b) fat-soluble compounds, (c) water-soluble compounds and (d) high molecular weight anti-oxidants (49). According to this classification, these agents are present in varying degrees in the intracellular or extracelluar spaces: (a) SOD, catalase, glutathione redox cycle compounds and glutathione peroxidase, glutathione reductase, glucose-6-phosphodihydrogenase; (b) vitamin E, β-carotene, bilirubin; (c) vitamin C, uric acid, glucose, cysteine, cystamine, reduced glutathione (GSH), taurine; (d) tracheobronchial mucus, albumin. SOD, catalase and the compounds of the glutathione redox cycle are the primary intercellular anti-oxidant defence mechanism to cope with ROS.

The most important H_2O_2-removing system in human cells is the glutathione redox cycle including the glutathione peroxidases (GSHPX). GSH is oxidized to GSSG and thus removes H_2O_2. Glutathione reductase regenerates GSH from GSSG, with NADPH as a source of reducing power (Fig. 1) (52). Located in the peroxisomes, catalase catalyses like GSH the dismutation of H_2O_2 into water and O_2:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Because of their conversion into ·OH and into reactive radical metal complexes, the conversion of O_2^- and H_2O_2 into less toxic products is from major importance in living cells (53). Therefore, sufficient anti-oxidant augmentation therapy can only be expected when these oxidants are scavenged or cellular metabolism leading to increased production of oxidants is attenuated.

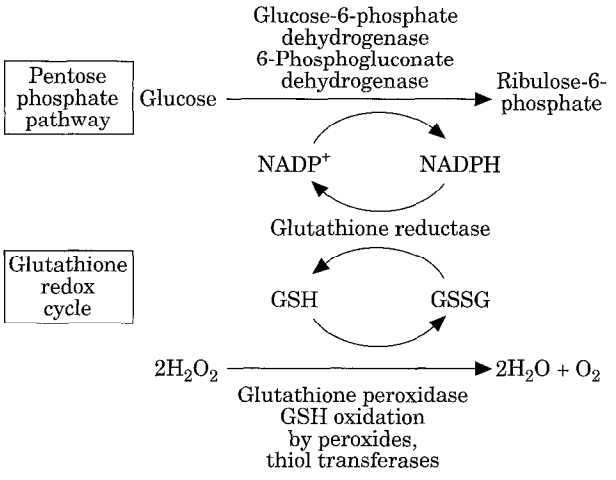

FIG. 1. Glutathione redox cycle. Reduced glutathione (GSH) is oxidized to GSSG and thus removes H_2O_2. Although GSH alone can react with a variety of peroxides, the reaction is more efficiently catalysed by glutathione peroxidase or thiol transferases. Glutathione reductase regenerates GSH from GSSG, with NADPH as a source of reducing power (52).

PHARMACOLOGICAL INTERVENTIONS TO ENHANCE CELLULAR ANTI-OXIDANT DEFENCE

SOD, catalase and GSH in particular, but also a whole variety of other substances with direct and indirect anti-oxidant capabilities (see previous section), prevent oxidative cell injury in cell culture as well as *in vivo* in animal models and in humans (38,49,54–56). However, the effectiveness of anti-oxidant treatment *in vivo* faces a dilemma: the administration of SOD (although available in recombinant form) and catalase is limited by the large molecular mass of both proteins. Liposome encapsulation (or conjugations with polyethylene glycol) has been successfully used to enhance SOD, catalase and GSH efficiency, but absence of phagocytes was required (57,58). Aerosolization of pure GSH was characterized by a low half-life (55). Vitamin E protects just vitamin-E-deficient animals. Although vitamin C can scavenge oxygen species and thus reduce the occurrence of malignancies, it is generally not considered as a major anti-oxidant because it has additional pro-oxidant

properties (38,49). Another approach would be to stimulate cells to produce anti-oxidants. NAC has such a potential, because when deacetylated it provides cysteine for cellular glutathione synthesis (49,59). Other substances such as ambroxol also have direct anti-oxidant properties and like NAC a whole variety of indirect anti-oxidant functions. Ambroxol is able to reduce oxidant-related cell damage through inhibition of phospholipases, stimulation of the lung surfactant system (e.g. stimulation of alveolar phospholipid secretion), inhibition of cytokines such as IL-1 and TNF, and inhibition of chemotaxis response of neutrophils to formyl-methionyl-leucyl-phenylalanine (fMLP) (60–62).

N-acetylcysteine

DIRECT ANTI-OXIDANT ACTION OF *N*-ACETYLCYSTEINE

NAC, which was developed in the 1960s, is the *N*-acetyl derivative of the naturally occurring amino acid L-cysteine (63). Because of its ability to reduce disulphide bonds it is widely used to reduce viscosity and elasticity of mucus and is virtually non-toxic (64). The molecular weight is 163·2 g mol^{-1} and the molecular formula is $C_5H_9NO_3S$. The oxidant scavenger function of NAC can easily be explained by the chemistry of this molecule. Because of its SH group [Fig. 2(a)] NAC has the potential to interact directly with oxidants such as H_2O_2 to form H_2O_2 and O_2 (64,65). In this reaction two NAC molecules are oxidized to a disulphide bond [Fig. 2(a)]. The rate constant with O_2^- is significantly low (64,66). NAC, like many thiols such as GSH, is an excellent scavenger of ·OH radical (rate constant: $1{\cdot}36 \times 10^{10}$ M^{-1} s^{-1}). In addition, NAC also scavenges HOCl (64).

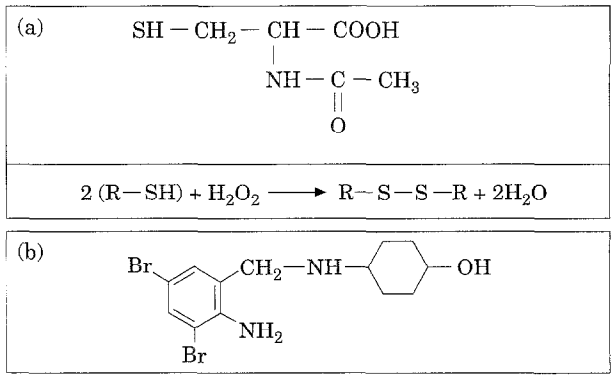

FIG. 2. (a)The molecule of *N*-acetylcysteine (*N*-acetyl-L-cystein) [modified according to (63)]. NAC is the *N*-acetyl derivative of the naturally occurring amino acid L-cysteine. Through its SH group it has mucolytic and, like glutathione, subsidiary anti-oxidative capabilities. In addition, the SH group can be oxidized with oxidants such as H_2O_2 to form H_2O_2 and O_2 (64,65). In this reaction two NAC molecules are oxidized to a disulfide bond (R=residue). (b) The molecule of ambroxol [*trans*-4-(2-amino-3,5-dibromobenzylamino)-cyclohexane hydrochloride]. Ambroxol has a high affinity for lung tissue resulting, in approximately 20 times higher concentrations in the lung than in plasma after oral or i.v. administration (143). It has been used for more than 20 yr for its mucolytic capabilities (154). Furthermore, ambroxol has been proven to scavenge oxidants directly and reduces peroxidative cellular metabolites in *in vitro* and animal studies (60,133).

GLUTATHIONE PRECURSOR FUNCTION OF *N*-ACETYLCYSTEINE *IN VITRO* AND IN ANIMAL EXPERIMENTS

In addition to oxidant scavenger function, there is plenty of evidence showing that NAC promotes cellular glutathione production, and thus NAC reduced or even prevented oxidant mediated damage to cell culture or animals.

Administration of $\geq 10^{-5}$ M NAC to A549 cells (ATCC), a human-derived cell line with epithelial characteristics, resulted in an approximately ten-fold increase of intracellular glutathione levels (65). Because bovine pulmonary artery endothelial cells and lung type II epithelial cells transported cysteine more efficiently than cystine it seems likely that cysteine is the principal substrate for cellular glutathione production (67). Cysteine is derived from *N*-deacetylation processes of NAC and/or from the reduction of cystine to cysteine (68). Although additional mechanisms have been discussed, it seems likely that *N*-deacetylation of NAC represents the key mechanism for the cellular glutathione precursor effect (67,69,70).

Wagner *et al.* investigated in 12 dogs the preventive effect of intravenous NAC against O_2 toxicity (71). Concomitantly, NAC treatment resulted in a significant decrease of pulmonary vascular resistance, and arterial carbon dioxide tension, delayed development of abnormal ventilation–perfusion relationships and reduced alveolar and interstitial oedema (71). In endotoxin-exposed rats NAC treatment improved ARDS-like injury by ameliorating structural lung damage and reducing lung wet weight and lung albumin leakage and it resulted in a reduction of thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ (prostaglandin) in BAL fluid. The authors related these results mainly to the GSH precursor effect of NAC (72).

NAC also protects against pulmonary O_2 toxicity as well as against ischaemic and reperfusion damage. Various physiological evaluation measures in dogs ventilated with 100% O_2 for 54 h were improved in the NAC group (71). When only given on reperfusion after artificial ischaemia in isolated rabbit hearts, NAC had no altering effect on cellular glutathione levels. However, when NAC was administered prior to coronary ischaemia (60 min), tissue GSH was increased by almost 40%, and ischaemia-induced decrease of GSH and protein SH, as well as leakage of creatine kinase, was limited. Interestingly, mitochondria, isolated after ischaemia, maintained their natural oxidative phosphorylating capacities (73).

In addition to this evidence of its anti-oxidant functions, NAC has also anti-inflammatory capabilities. Oral administration of NAC reduces humoral markers in BAL

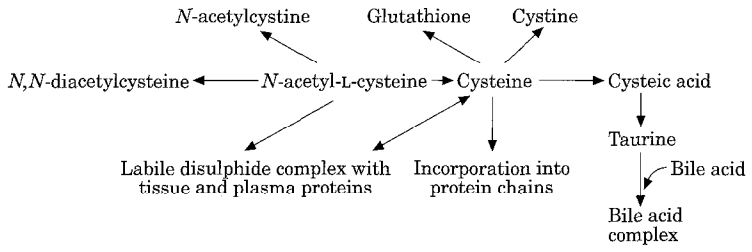

FIG. 3. Metabolic pathway of NAC [modified according to (63)].

fluid (NAC in healthy individuals) (74). NAC increases leukotriene B_4 (LTB_4) secretion, which is usually found in low levels in alveolar macrophages of smokers (75).

Because NAC is acidic in solution, inhalation of this substance may be related to coughing. A new approach is the development of nacystelyn (NAL), a lysine salt of NAC. NAL can easily be administered into the lung by aerosolization without causing significant side-effects (76). The mucolytic activity of NAL may be more potent than the activity of NAC at equimolar concentrations (77). Surprisingly, NAL seems to have an additional inhibitory effect on human neutrophil elastase activity *in vitro* (76,77). This dual function would make this drug particularly useful in inflammatory airway diseases in which an overload of proteases play a major role in the pathogenesis (e.g. cystic fibrosis, α_1-AT deficiency) (78,79). Future analyses have to show whether NAL can hold its promise also *in vivo*. Moreover, it has to be demonstrated whether NAL has inhibitory functions on free radicals and whether it has GSH precursor function like NAC. In comparison with NAC, NAL revealed similar H_2O_2 scavenger capabilities as well as cellular GSH precursor function when used in micromolar concentrations, which was recently demonstrated *in vitro* (65).

PULMONARY METABOLISM OF *N*-ACETYLCYSTEINE

A schematic representation of the proposed metabolism of NAC is given in Fig. 3. After intravenous or oral application NAC is quickly absorbed and undergoes rapid and extensive metabolism in gut wall and liver, resulting in a bioavailability of about 10% (80,81). Deacetylation of NAC arises through metabolic deposition which is catalysed by *N*-deacetylase located in or on the cells. At 1 h after administration plasma total NAC is present in a covalently protein-bound form. After i.v. administration the terminal half-life of total acetylcysteine is 5·6 h (82). At 10–12 h after a single dose of NAC only traces could be found (83). After i.v. administration, the renal clearance accounts for about 30% of the total clearance, and approximately 30% is secreted in urine during the first 12 h (84).

As a main condition for anti-oxidant function in the lung, concentrations of NAC or its metabolites in the lung interstitium, bronchoepithelial cells and/or in the epithelial lining have to be sufficiently elevated (micromolar levels). In patients ($n=10$; among these $n=5$ underwent pneumonectomy or lobectomy) with respiratory disorders total radioactivity in plasma, lung tissue and bronchial secretions was determined after oral demonstration of 100 mg [^{35}S]NAC. Lung tissue radioactivity (after 5 h) was comparable with plasma levels (85). Just small amounts of radioactivity were found in the bronchial sections (85). After administration of 200 mg t.i.d. for 2 weeks to healthy volunteers neither NAC nor its metabolites were detected in the BAL fluid (86). Also 600–1800 mg NAC [given daily by mouth to patients with chronic obstructive pulmonary disease (COPD)] failed to increase levels of NAC, cysteine and glutathione in BAL fluid, ELF and lung tissue (87). It can be concluded from these findings, and in contrast to the increase of plasma levels, that after oral or i.v. administration NAC can only be expected on the epithelial lining or in bronchoepithelial wall in traces at best. However, NAC frequently has to be used to balance the oxidant–anti-oxidant system in human pulmonary diseases.

ANTI-OXIDANT PROPERTIES OF *N*-ACETYLCYSTEINE IN HUMANS

To investigate anti-oxidant properties of NAC, in most studies doses between 200 mg and 600 mg daily (oral administration), and up to 150 mg kg^{-1} (i.v. as a loading dose) in patients with ARDS, were administered (75,87–90). Overall, it is difficult to relate successful treatment exclusively to the anti-oxidant effect of NAC since it also has mucolytic properties. Most studies indicate that the therapeutic potential of NAC in the treatment of inflammatory disorders of the human lung is associated with its direct scavenger effect and/or its ability to increase cysteine, the main cellular glutathione precursor. Some authors, however, failed either to increase anti-oxidant function in human lung or to reduce the activation state of inflammatory cells by oral NAC administration (91–93).

NAC in Healthy Individuals

The effect of NAC in enhancing anti-oxidant defence in the lungs of healthy volunteers is marginal. In 11 normal, non-smoking volunteers (aged 24–37 years) O_2^- as well as H_2O_2 release of freshly isolated neutrophils from whole blood remained unchanged after oral administration of 600 mg NAC daily for 5 days (93). In normal individuals i.v. administration of various doses of NAC had no significant effect on glutathione concentrations in BAL fluid. Compared with baseline values ($0·79 \pm 0·17\ \mu$M),

administration of 600 mg NAC ($0{\cdot}92 \pm 0{\cdot}33\ \mu$M), 1·8 g NAC ($1{\cdot}4 \pm 0{\cdot}4\ \mu$M) and 4·8 g NAC ($1{\cdot}33 \pm 0{\cdot}46\ \mu$M) did not significantly increase total glutathione levels (3 h after i.v. application) (94). Meyer *et al.* hypothesized that lack of glutathione increase in BAL fluid after NAC administration might be due to various reasons. First, NAC may support glutathione synthesis only in situations of increased demand on the cellular glutathione pool following oxidative stress (e.g. after paracetamol intoxication or in patients suffering from IPF) (94). Second, the rapid clearance of most of the NAC and its products could limit its effect and, third, increasing glutathione levels in BAL fluid might suppress local glutathione synthesis by feed-back inhibition (94,95).

In addition to the failure of NAC to increase glutathione or cysteine levels in BAL fluid (or ELF) in healthy individuals, NAC (200 mg t.i.d.) significantly ($P<0{\cdot}001$) reduced various inflammatory parameters in BAL fluid in 11 healthy smokers: lactoferrin, eosinophil cationic protein, anti-chymotrypsin and chemotactic activity of neutrophils. In serum–plasma the concentrations of myeloperoxidase and elastase were also reduced by NAC (74).

Kleinfeld *et al.* (92) found in six healthy normolipidaemic adult volunteers that NAC (2 × 600 mg for 4 weeks, followed by 2 × 1200 mg for 2 weeks) had no effect on the susceptibility of LDL (low-density lipoprotein) oxidizability. Unexpectedly and in contrast to other investigations, NAC administration even resulted in reduction of plasma GSH and increase of GSSG (92).

NAC in Respiratory Disorders

Among others, patients with ARDS, IPF or COPD have been the primary targets for clinical studies to evaluate the efficacy of NAC in anti-oxidant therapy.

ARDS is of multifactorial origin, and at a certain stage of its development strong oxidative stress through massive PMN activation becomes a predominant condition. Concomitant glutathione-mediated anti-oxidant protection is low in the alveolar fluid of these patients (96). Mechanical ventilation with high concentrations of oxygen further enforces the release of reactive oxygen metabolites (33,97). To balance the oxidant–anti-oxidant system, patients with ARDS ($n=30$) were treated i.v. with high doses of NAC. After an initial dose of 150 mg kg^{-1} followed by 24 mg kg^{-1} every 4 h, initially low plasma and red cell glutathione levels increases. In addition, improvements of chest radiograph oedema, vascular resistance, oxygen delivery and oxygen consumption were achieved (98). Spies *et al.* (89) confirmed this result in a placebo-controlled study: in the NAC-responder group (50% of all patients) 65% of patients with a septic shock who initially received 150 mg kg^{-1} in 15 min of NAC, followed by 12·5 mg kg^{-1} h^{-1}, had a better survival rate (89). With i.v. NAC (40 mg kg^{-1} day^{-1}) Suter and coworkers improved systemic oxygenation and reduced the need for ventilatory support in patients presenting with mild-to-moderate acute lung injury. However, the development of ARDS and patients' survival (mortality) was not significantly reduced by this therapy: the 1 month mortality rate was 22% for the NAC group ($n=32$) and 35% for the placebo group ($n=29$) (99).

IPF is characterized by alveolar inflammation, exaggerated release of reactive oxygen metabolites and subnormal concentrations of glutathione in the ELF (100,101). Direct glutathione administration via the aerosol route but also NAC given i.v. or by mouth increased significantly BAL fluid (or ELF) glutathione levels (55,90,94). In 17 non-smoking patients with biopsy-proven IPF receiving 3 × 600 mg NAC day^{-1} for 5 days BAL fluid total glutathione levels increased significantly ($P<0{\cdot}006$) from $0{\cdot}99 \pm 0{\cdot}12$ to $1{\cdot}54 \pm 0{\cdot}24\ \mu$M (90). Also, i.v. administration of different NAC concentrations to patients with IPF increased total glutathione in BAL fluid significantly ($P<0{\cdot}03$). BAL fluid total glutathione levels rose from $0{\cdot}99 \pm 0{\cdot}25\ \mu$M to $1{\cdot}79 \pm 0{\cdot}37\ \mu$M (after 1·8 g NAC). A further increase of BAL fluid total glutathione levels after higher doses (4·8 g NAC) was not observed ($1{\cdot}47 \pm 0{\cdot}34\ \mu$M) (94). High concentrations of total glutathione were almost entirely due to GSH (about 94–97%) (94). However, these results do not mandate clinical efficacy of NAC treatment because just biochemical alterations have been demonstrated in these studies. This has still to be demonstrated in long-term studies.

Also, in COPD patients NAC treatment was tested to improve anti-oxidant levels although this disease, with the possible exception of severe cases, is not characterized by glutathione deficiency. NAC has been used for over two decades in the treatment of COPD and some studies demonstrated beneficial effect, but others have not (102–104). Oral administration of NAC to patients with COPD (two groups, one received 600 mg once daily, the second 600 mg thrice daily) resulted in a significant ($P<0{\cdot}05$) increase of NAC in blood 1·5 h after the drug was given (after 5 days treatment). Sustained elevation of plasma glutathione and cysteine levels was only observed in the group treated with the higher dosage. In contrast, neither GSH nor cysteine levels rose in BAL fluid, ELF or lung tissue (87).

These results, a negative effect in healthy volunteers or patients with COPD (normal glutathione levels in BAL fluid) and beneficial effect of NAC treatment in patients with IPF or ARDS (low glutathione levels in BAL fluid, high oxidant load), support the hypothesis of negative feedback inhibition (see previous section) (95). In agreement with this concept, patients with a hereditary deficiency of glucose -6-phosphodehydrogenase, who are more sensitive to oxidative stress, were also successfully treated with NAC. In these patients NAC treatment augmented not only glucose-6-phosphodehydrogenase activity but also reduced effective lipid peroxidation and made these erythrocytes more resistant to incubation with acetylphenylhydracine, an agent causing oxidative stress (105,106).

NAC is virtually non-toxic. When used in humans NAC rarely causes anaphylaxis, tachycardia or hypotension (107,108). Only very high concentrations in *in vivo* and *in vitro* experiments revealed that $>10^{-3}$ M reduces chemotaxis and >0·1 M increases cytotoxicity of PMN cells (107). Because of the low bioavailability, it is not feasible to achieve these concentrations in humans (84,109).

N-ACETYLCYSTEINE AS AN ANTIDOTE

From 5% to 10% of paracetamol is metabolized to *N*-acetylbenzochionimine. This metabolite reacts with thiols and thus can be detoxified. However, toxic amounts of paracetamol (intake about ≥ 6 g) overwhelm cellular glutathione supplies, eventually causing liver necrosis (110). Because approximately 70% of NAC is metabolized in the liver, NAC has been proven to be a useful antidote in paracetamol intoxication, which conjugates with reduced glutathione (63,111). In the liver, such antidote action is related to NAC as an effective precursor to supply intracellular cysteine, which is intracellularly transformed into GSH. Thus, the NAC-mediated GSH increase not only conjugates paracetamol to less toxic metabolites but it also makes GSH available for anti-oxidant defence purposes intracellularly as well as extracelluarly (63).

NAC given orally also has a cardioprotective effect in patients treated with doxorubicin, it reduces cyclophosphamide-induced haemorrhagic cystitis and it attenuates bleomycin-related changes in lung function (112,113).

Patients successfully treated for lung cancer remain at high risk to develop a secondary primary tumour. In Euroscan [the European Chemoprevention Study under the auspices of the European Organization for Research and Treatment of Cancer (EORTC)] patients after successful treatment of lung or head and neck cancer are randomized as follows: NAC (600 mg day^{-1} for 2 yr), retinol palmitate, the combination of both or no drugs. To date, more than 2600 patients have been included. Only 8% suffered from side-effects (mild gastrointestinal symptoms) (114,115). With NAC, the authors hope to reduce the risk of second tumours. Reduction of the development of second tumours is based on the knowledge that thiols inhibit the mutagenic response to potent carcinogens such as benzo[a]pyrene, 2-aminofluorene and aflatoxin β_1, reduces oxidant-mediated DNA damage and stimulates DNA repair mechanisms (115).

Ambroxol

PHYSIOLOGICAL EFFECTS OF AMBROXOL

Ambroxol [*trans*-4-(2)amino-3,5-dibromobenzylamino)-cyclohexane hydrochloride; Fig. 2(b)] stimulates synthesis and secretion of surfactant by type II pneumocytes (116,117) and inhibits sodium absorption by airway epithelial cells (118). It can also accelerate the maturation of the foetal lungs by increasing the lecithin: sphingomyelin ratio (119). Ambroxol is used as a mucolytic drug and lower sputum viscosity by normalizing secretions from bronchial glands. In this context, ambroxol, has been widely tested to prevent chronic bronchitis exacerbations, to improve the clearance mechanism of the epithelial surface of the airways, to decrease bronchial hyperreactivity and even to promote secretion mechanisms in patients with otitis media and to reduce the occurrence of neonatal respiratory distress syndrome by induction of foetal lung maturation (120–125).

ANTI-OXIDANT ACTIVITY OF AMBROXOL *IN VITRO*

At concentrations of 10^{-6}–10^{-5} M and $2{\cdot}5$–7×10^{-5} M respectively ambroxol revealed a capacity to inhibit ·OH- and HOCl-mediated reactions (126). As a scavenger of these two ROS ambroxol was almost equipotent to dimethylsulphoxide and methionine (126). The inhibition of O_2^--dependent auto-oxidant of pyrogallol and decomposition of H_2O_2 by ambroxol was low over a large concentration range. The capacity of ambroxol to scavenge HOCl and ·OH can explain the above-mentioned inhibitory effect of this drug on chloramine-T- and cigarette-smoke-induced decrease of serum elastase inhibitory capacity (127,128). Both of these factors inactivate a_1-AT via generation of HOCl and ·OH.

Ambroxol was shown to inhibit lipid peroxidation in liver homogenates exposed to the Fe^{2+}–ascorbate system. At a concentration of $2{\cdot}1 \times 10^{-4}$ M it decreased two-fold the conversion of liver lipids into thiobarbituric acid reactive substances (129). In another study $2{\cdot}5 \times 10^{-4}$ M ambroxol protected linoleic acid from peroxidative damage induced by the H_2O_2–Fe^{2+}–EDTA system *in vitro* (126). At very high concentrations (millimolar range), which, however, are irrelevant for therapeutic use in humans, ambroxol completely inhibited ·OH-induced peroxidation of linoleic acid as expressed by malondialdehyde (MDA) generation. The authors speculate that two mechanisms are responsible for these effects, first direct ·OH scavenging effect of ambroxol and second the ability of the two nitrogen atoms of the drug molecule to chelate iron (130).

AMBROXOL AS LIPID ANTI-OXIDANT IN ANIMAL STUDIES

Ambroxol was demonstrated to reveal protective efficacy for lung and heart lipids that was comparable with and even higher than those of NAC and methionine (131–133). Ambroxol injected once per day (70 mg kg^{-1}) for three consecutive days decreased the LPS (17 mg kg^{-1}) induced lipid peroxidation in murine organs (133). The lung and heart levels of conjugated dienes (CDs) in animals pretreated with ambroxol were 3·3 and 1·7 times lower than those observed in the control group which received buffer and subsequently LPS (132). NAC did not inhibit the LPS-induced (IL-1- and TNF-dependent) hypothermic reaction in mice, while ambroxol inhibited the loss of body temperature from $4{\cdot}1 \pm 1{\cdot}3$ to $2{\cdot}2 \pm 0{\cdot}9$°C (132,133). These protective effects of ambroxol may result from its ability to scavenge ·OH and the inhibition of IL-1 and TNF release from LPS-activated monocytes (126,134).

In another study mice were injected i.p. with equimolar doses ($1{\cdot}7 \times 10^{-4}$ M kg^{-1}) of ambroxol, NAC, methionine or solvent once per day for three consecutive days. Lipid peroxidation was induced in organ homogenates by means of heat (50°C) or 10^{-5} M H_2O_2 (131). Ambroxol inhibited the thermal generation of CDs (one of the first lipid peroxidation products) in lung homogenates. This inhibition ($59{\cdot}9 \pm 33{\cdot}1$%) was similar to that with NAC ($61{\cdot}1 \pm 26{\cdot}1$%) but was 1·4-fold higher than that observed

TABLE 3. Effects of ambroxol *in vitro* and *ex vivo*

Action	Concentration or dose	Conditions
(1) Direct anti-oxidant activity		
• ·OH scavenger	10^{-3}–10^{-1} M	*In vitro*
• HOCl scavenger	$2{\cdot}5$–$7{\cdot}5 \times 10^{-5}$ M	*In vitro*
(2) Lipid anti-oxidant: inhibition of lipid peroxidation induced by		
• ·OH	$2{\cdot}5 \times 10^{-4}$–5×10^{-3} M	*In vitro*
• H_2O_2	i.p. 3×70 mg kg^{-1}	*Ex vivo*
• temperature, 50°C	i.p. 3×70 mg kg^{-1}	*Ex vivo*
• endotoxin (LPS)	i.p. 3×70 mg kg^{-1}	*In vitro*
• doxorubicin	i.v. 1×70 mg kg^{-1}	*In vivo*
(3) Inhibition of human phagocytes		
• chemotaxis	10^{-5} M	*In vitro*
• spontaneous migration	$9{\cdot}6$–$3{\cdot}8 \times 10^{-5}$ M	*In vitro*
• chemiluminescence	$1{\cdot}9 \times 10^{-4}$ M	*In vitro*
• IL-1 and TNF release	$2{\cdot}4 \times 10^{-5}$–$2{\cdot}4 \times 10^{-4}$ M	*In vitro*
• modulation of intracellular Ca^+	10^{-4} M	*In vitro*

The results obtained in the millimolar range are interesting from a scientific point of view but irrelevant in humans; because of low bioavailability it is not feasible to achieve concentrations of this order of magnitude by therapeutic administration.

for methionine. In addition, H_2O_2-induced generation of CDs in lungs from ambroxol-treated mice was 3·1-fold lower than that found in lung homogenates from the control group. In contrast, NAC and methionine failed to protect the lung lipids from H_2O_2-provoked peroxidative damage (131). This finding indicates that injection of ambroxol increases the lung anti-oxidant defence against heat and H_2O_2.

When applied first, a single intravenous injection of ambroxol (70 mg kg^{-1}) completely protected heart lipids from peroxidative damage induced by doxorubicin in mice (135). The heart content of CD and MDA in the animals that received ambroxol and doxorubicin was about three-fold lower than that found in the doxorubicin-treated mice. Ambroxol revealed also a protective action against other anti-cancer drugs and xenobiotics (e.g. bleomycin, paraquat) that are known to induce oxidative stress in tissues (136,137).

ANTI-INFLAMMATORY PROPERTIES OF AMBROXOL

The inhibition of phospholipases A present in lung and alveolar lysosomes is an important mechanism by which ambroxol enhances the content of surfactant in the bronchoalveolar lining fluid (61). Ambroxol decreased the total activity of phospholipases A in rabbit alveolar macrophages, peritoneal cells and PMN cells (138,139). Furthermore, ambroxol at physiological concentrations (micromolar range) inhibited the spontaneous migration and chemotactic response of human PMN cells to purulent sputum, zymosan-activated serum and fMLP (60,140). Human monocytes cultured in medium containing ambroxol released less IL-1 and TNF after stimulation with LPS (134). The significant inhibition of biosynthesis of these two pro-inflammatory–pro-oxidant cytokines was observed just at an ambroxol concentration of 10 μM. Ambroxol ($2{\cdot}4$–$9{\cdot}6 \times 10^{-5}$ M) attenuated the spontaneous and zymosan-induced lucigenin-dependent chemiluminescence of human PMN cells (6). This inhibition of phagocyte chemiluminescence may be a result of phospholipase inhibition with subsequent suppression of the respiratory burst and a direct scavenging effect of ROS. However, pre-incubation of human PMN cells and monocytes with ambroxol had no influence on phagocytosis and killing of *Candida albicans* (141). In dogs exposed to ozone aerolsolized ambroxol inhibited the development of airway hyperresponsiveness to acetylcholine but did not decrease the PMN cell influx into the lower airways (142).

Table 3 summarizes the main effects of ambroxol indicating anti-oxidant and anti-inflammatory properties. Most of these effects came from *in vitro* or animal experiments using concentrations which are feasible to achieve (micromolar range) in human lung tissue (143).

ANTI-OXIDANT FUNCTION OF AMBROXOL IN HUMANS

Despite substantial evidence of the apparent anti-oxidative effect of ambroxol *in vitro* or in animals, only few studies in humans have been published. One describes that additional administration of ambroxol in cancer patients prevented deterioration of the lung function related to anti-cancer treatment with bleomycin and nitrosourea (144). Red blood cells of pre-term infants of mothers pre-treated i.v. with ambroxol revealed a decreased content of lipid

peroxidation products and enhanced anti-oxidant defence (145). While NAC cannot be detected in BAL fluid after i.v. or oral administration, ambroxol has a very high affinity for lung tissue, resulting in approximately 20 times higher levels in the lung than in serum. Thus, micromolar concentrations can be achieved in lung tissue after the administration of 1 g ambroxol (143). In a large double-blind multicentre trial 252 patients were enrolled receiving 1 g day^{-1} of ambroxol i.v. for six consecutive days before upper abdominal surgery. In this trial Feigz *et al.* demonstrated that there was a significant reduction in atelectasis between the ambroxol and the placebo groups ($p<0{\cdot}05$; ambroxol 10·6%, placebo 23·9%) (146). Furthermore, high doses of ambroxol (1 g day^{-1}) may prevent postoperative ARDS and bronchopulmonary complications after chest surgery (147). I.v. administered ambroxol (10 mg kg^{-1}, twice daily for 1 week) was shown to increase survival of 28 neonates (birth weight <2000 g) with idiopathic respiratory distress syndrome which is related to its stimulatory effect on cellular surfactant production (148). To understand the anti-inflammatory effect of surfactant, it is important to know that it contains anti-oxidants such as SOD and catalase and that it inhibits cytokine release and thus reduces the respiratory burst of inflammatory cells (149–151). By stimulating surfactant production in the airways, ambroxol may indirectly reduce the oxidant burden in the lung. Bronchial reactivity to metacholine was reduced two-fold (comparison *vs.* placebo) when ambroxol was administered orally to asthmatic patients (90 mg day^{-1}, for a 14 day period) (122). To summarize, numerous parameters (physiological and biochemical) for both substances indicate the potential usefulness of this therapeutic approach.

References

1. Weiss, SJ. Oxygen, ischemia and inflammation. *Acta Physiol Scand* 1986; **548:** 9–37.
2. Schraufstätter IU, Cochrane CG. Oxidants: types, sources, and mechanisms of injury. In: Crystal RG, West JB, eds. *The lung, Scientific foundations*. New York: Raven Press, 1991, pp. 1803–1810.
3. Schraufstätter IU, Hyslop PA, Jackson J *et al.* Oxidant and protease injury of the lung. *Bull Eur Physiopathol Respir* 1987; **23:** 297–302.
4. Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*, 2nd edition. Oxford: Clarendon Press, 1989.
5. Halliwell B, Cross CE. Oxygen-derived species: their relation to human disease and environmental stress. *Environ Health Perspect* 1994; **102:** 5–12.
6. Winsel K, Becher G. Effects of ambroxol on chemiluminescence of phagocytic cells and Na-arachidonate induced bronchoconstriction in guinea pigs. *Eur Respir J* 1992; **5:** 289s.
7. Hoidal JR, Kennedy TP. Oxidative damage and respiratory disease. In: Chow CK, ed. *Cellular Antioxidant Defense Mechanisms*. New York: CRC Press Inc. 1988, 53–70.
8. Jenkinson SG. Free radical effects on lung metabolism. *Clin Chest Med* 1989; **10:** 37–47.
9. Weiss SJ, Lambert MB, Test ST. Long-lived oxidants generated by human neutrophils: characterization and bioactivity. *Science* 1983; **222:** 625–628.
10. Haber F, Weiss JJ. The catalytic decomposition of hydrogen peroxide by iron salts. *Proc R Soc London* 1934; **147:** 332–352.
11. Fenton HJH. Oxidation of tartaric acid in the presence of iron. *J Chem Soc Trans* 1894; **65:** 899–910.
12. Ward PA. Oxygen radicals, cytokines, adhesion molecules, and injury. *Environ Health Perspect* 1994; **102:** 13–16.
13. Meeting report on oxy radicals and antioxidative response in cancer: 12th Sapporo cancer seminar. *Cancer Res* 1993; **53:** 3207–3210.
14. Jackson IH. Potential molecular mechanisms of oxidant-induced carcinogenesis. *Environ Health Perspect* 1994; **102:** 155–158.
15. Duthie SJ, Ma M, Ross MA *et al.* Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes. *Cancer Res* 1996; **56:** 1291–1295.
16. Hansen K, Mossman BT. Generation of superoxide (O_2^-) from alveolar macrophages exposed to asbestiform and nonfibrous particles. *Cancer Res* 1987; **47:** 1681–1686.
17. Goodglick LA, Pietras LA, Kane AG. Dissociation of lipid peroxidation from asbestos toxicity. *Am Rev Respir Dis* 1988; **137:** A402.
18. Mossman BT, Marsh JP, Shatos MA. Alteration of superoxide dismutase activity in tracheal epithelial cells by asbestos and inhibition of cytotoxicity by antioxidants. *Lab Invest* 1986; **54:** 204–212.
19. Adler KB, Fischer BM, Wright DT, Cohn LA *et al.* Interactions between respiratory epithelial cells and cytokines: relationships to lung inflammation. In: Boland BM, Cullinan J, Rogers T, eds. *Cells and Cytokines in Lung Inflammation*, New York: New York Academy of Sciences, 1994, pp. 128–145.
20. Kelley J. Cytokines of the lung. *Am Rev Respir Dis* 1990; **141:** 765–788.
21. Fitzpatrick FA, Murphy RC. Cytochrome P-450 metabolism of arachidonic acid: formation and biological actions of "epoxygenase"-derived eicosanoids. *Pharmacol Rev* 1989; **40:** 229–241.
22. Thivierge M, Rola-Pleszczynski M. Involvement of both cyclooxygenase and lipoxygenase pathways in platelet-activating factor-induced interleukin-6 production by alveolar macrophages. In: Boland BM, Cullinan J, Rogers T, eds. *Cells and Cytokines in Lung Inflammation*. New York: New York Academy of Sciences, 1994, pp. 213–222.
23. Wong GHW, Goeddel DV. Induction of manganous superoxide dismutase by tumour necrosis factor: possible protective mechanism. *Science* 1988; **242:** 941–943.
24. Visner GA, Dougall WC, Wilson JM *et al.* Regulation of manganese superoxide dismutase by lipoplysaccharide, interleukin-1, and tumor necrosis factor. *J Biol Chem* 1990; **265:** 2856–2864.

25. Jacobs RF, Tabor DR, Burks AW *et al.* Elevated Interleukin-1 release by human alveolar macrophages during the adult respiratory distress syndrome. *Am Rev Respir Dis* 1989; **140:** 1686–1692.
26. Tsan M-F, White JE, Treanor C *et al.* Molecular basis for tumor necrosis factor-induced increase in pulmonary superoxide dismutase activities. *Am J Physiol (Lung Cell Mol Physiol)* 1990; **259:** L506–L512.
27. Needleman P, Turk J, Jakschik BA *et al.* Arachidonic acid metabolism. *Ann Rev Biochem* 1986; **55:** 69–102.
28. Abramson SL, Malech HL, Galin JI. Neutrophils. In: Crystal RG, West JB, eds. *The Lung Scientific Foundation.* New York: Raven Press, 1991, pp. 553–563.
29. White JE, Tsan M-F. Induction of pulmonary Mn superoxide dismutase mRNA by interleukin 1. *Am J Physiol (Lung Cell Mol Physiol)* 1994; **266:** L664–L671.
30. Harris CA, Derbin KS, Hunte-McDonough B *et al.* Manganese superoxide dismutase is induced by IFN-gamma in multiple cell types. Synergistic induction by IFN-gamma and tumor necrosis factor or IL-1. *J Immunol* 1991; **147:** 149–154.
31. Hirose K, Longo DL, Oppenheim JJ *et al.* Overexpression of mitochondrial manganese superoxide dismutase promotes the survival of tumor cells exposed to interleukin-1, tumor necrosis factor, selected anticancer drugs, and ionizing radiation. *FASEB J* 1993; **7:** 361–368.
32. Davis WB, Rennard SI, Bitterman PB *et al.* Pulmonary oxygen toxicity: early reversible changes in human alveolar structures induced by hyperoxia. *N Engl J Med* 1983; **309:** 878–883.
33. Tagan M-C, Markert M, Schaller M-D *et al.* Oxidative metabolism of circulating granulocytes in adult respiratory distress syndrome. *Am J Med* 1991; **91**(Suppl. 3C): 3C-72S–3C-78S.
34. Erzurum SC, Danel C, Gillissen A *et al.* In vivo antioxidant gene expression in human airway epithelium of normal individuals exposed to 100% O_2. *J Appl Physiol* 1993; **75**(3): 1256–1262.
35. Tsan M-F, White JE. Kinetics of pulmonary superoxide dismutase in interleukin-1-induced oxygen-tolerant rats. *Am J Physiol (Lung Cell Mol Physiol)* 1992; **263:** L342–L347.
36. Burger RM, Kent TA, Howitz SB *et al.* Mossbauer study of iron bleomycin and its activation intermediates. *J Biol Chem* 1983; **86:** 139–142.
37. Cunningham ML, Ringrose PS, Lokesh BR. Bleomycin cytotoxicity is prevented by superoxide dismutase. *Cancer Lett* 1983; **21:** 149–153.
38. Cantin AM, Crystal RG. Oxidants, antioxidants, and the pathogenesis of emphysema. *Eur J Respir Dis* 1985; **66**(Suppl. 139): 7–17.
39. Church T, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect* 1985; **64:** 111–126.
40. Huninghake GW, Crystal RG. Cigarette smoking and lung destruction: accumulation of neutrophils in the lungs of cigarette smokers. *Am Rev Respir Dis* 1983; **128:** 833–838.
41. Ogushi F, Hubbard RC, Fells GA *et al.* Risk factors for emphysema: cigarette smoking is associated with a reduction in the association rate constant of lung α_1-antitrypsin for neutrophil elastase. *J Clin Invest* 1991; **87:** 1060–1065.
42. Rigo A, Viglino P, Scarpa M, Rotilio G. Reduction and oxidation of bovine superoxide dismutase by H_2O_2. In: Rotilio G, ed. *Superoxide and Superoxide in Chemistry* Amsterdam: Elsevier Science, 1985, pp. 185–188.
43. Takahashi H, Abe M, Hashimoto S *et al.* In vivo effect of lipopolysaccharide on alveolar and peritoneal macrophages of rats: superoxide anion generation and 5-lipoxygenase metabolism of arachidonic acid. *Am J Respir Cell Mol Biol* 1993; **8:** 291–298.
44. Schraufstätter IU, Hyslop PA, Hinshaw DB *et al.* Hydrogen peroxide-induced injury of cells and its prevention by inhibitors of poly(ADH-ribose) polymerase. *Proc Natl Acad Sci USA* 1986; **83:** 4908–4912.
45. Janssen YM, Marsh JP, Driscoll KE *et al.* Increased expression of manganese-containing superoxide dismutase in rat lungs after inhalation of inflammatory and fibrogenic minerals. *Free Radic Biol Med* 1994; **16:** 315–322.
46. Marks-Konczalik J, Gillissen A, Schärling B *et al.* Regulation of manganese superoxide dismutase gene expression in human bronchoepithelial cells after exposure to crocidolite, silica and rockwool in vitro. *Eur Respir J* 1995; **8:** 351s.
47. Gillissen A, Jaworska M, Wickenburg D *et al.* Superoxide dismutase, glutathione and catalase levels in bronchoepithelial cells after exposure to crocidolite, silica and basaltwool in vitro. *Eur Respir J* 1995; **8:** 464s.
48. Linden M, Hakansson L, Ohlsson K *et al.* Glutathione in bronchoalveolar lavage fluid from smokers in related to humoral markers of inflammatory cell activity. *Inflammation* 1989; **13**(6): 651–658.
49. Heffner JE, Repine JE. Pulmonary strategies of antioxidant defense. *Am Rev Respir Dis* 1989; **140:** 531–554.
50. Aruoma OI. Characterization of drugs as antioxidant prophylactics. *Free Radic Biol Med* 1996; **20:** 675–705.
51. Harris ED. Regulation of antioxidant enzymes. *FASEB J* 1992; **6:** 2675–2683.
52. Meister A. Glutathione metabolism and its elective modification. *J Biol Chem* 1988; **262:** 17205–17208.
53. Meister A, Larsson A. Glutathione synthetase deficiency and other disorders of the gamma-glutamyl cycle. In: Schriver CR, Beyuet AL, Sly WS *et al.*, eds. *The Metabolic Basis of Inherited Disease.* New York: McGraw-Hill, 1989, pp. 855–868.
54. Gillissen A, Roum JH, Hoyt RF *et al.* Augmentation of respiratory epithelial lining fluid antioxidant screen by aerosolization of recombinant human Cu^{++}/Zn^{++} superoxide dismutase. *Chest* 1993; **104:** 811–815.
55. Borok Z, Buhl R, Grimes GJ *et al.* Effect of glutathione aerosol on oxidant–antioxidant imbalance in

idiopathic pulmonary fibrosis. *Lancet* 1991; **338:** 215–216.
56. Gillissen A, Birrer P, Buhl R *et al.* Recombinant secretory leukoprotease inhibitor augments glutathione levels in respiratory epithelial lining fluid. *J Appl Physiol* 1993; **75:** 825–832.
57. Jurima-Romet M, Barber RF, Demeester J *et al.* Distribution studies of liposome-encapsulated glutathione administered to the lung. *Int J Pharm* 1990; **63:** 227–235.
58. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am J Med* 1991; **91:** 3C-14S–3C-22S.
59. Cantin AM, Béin R. Glutathione and inflammatory disorders of the lung. *Lung* 1991; **169:** 123–138.
60. Stockley RA, Shaw J, Burnett D. Effect of ambroxol on neutrophil chemotaxis in vitro. *Agents Actions* 1988; **24:** 292–296.
61. Heath MF, Jacobson W. The inhibition of lysosomal phospholipase A from rabbit lung by ambroxol and its consequences for pulmonary surfactant. *Lung* 1985; **163:** 337–334.
62. Marcatili S, Guarino C, Giannattasio A *et al.* Alterations of the endoalveolar surfactant after surgery with extracorporeal circulation. *Respiration* 1990; **57:** 233–238.
63. Ventresca GP, Cicchetti V, Ferrari V. Acetylcysteine. In: Braga PC, Allegra L, eds. *Drugs in Bronchial Mucology*. New York: Raven Press, 1989, pp. 77–102.
64. Aruoma OI, Halliwell B, Hoey BM *et al.* The antioxidant action of *N*-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Radic Biol Med* 1989; **6:** 593–597.
65. Gillissen A, Jaworska M, Orth M *et al.* Nacystelyn and *N*-acetylcysteine augment cellular antioxidant defense in two distinctive ways. *Respir Med* 1997; **91:** 159–168.
66. Vanderbist F, Maes P, Nève J. In vitro comparative assessment of the antioxidant activity of nacystelyn against three reactive oxygen species. *Arzneimitteltherapie* 1996; **8:** 783–788.
67. Phelps DT, Deneke SM, Daley DL *et al.* Elevation of glutathione levels in bovine pulmonary artery endothelial cells by *N*-acetylcysteine. *Am J Respir Cell Mol Biol* 1992; **7:** 293–299.
68. Deneke SM, Susanto I, Vogel KA *et al.* Mechanisms of use of extracelluar glutathione by lung epithelial cells and pulmonary artery endothelial cells. *Am J Respir Cell Mol Biol* 1995; **12:** 662–668.
69. Cotgreave I, Moldéus P, Schuppe I. The metabolism of *N*-acetylcysteine by human endothelial cells. *Biochem Pharmacol* 1991; **42:** 13–16.
70. Deneke SM. Induction of cysteine transport in bovine pulmonary artery endothelial cells by sodium arsenite. *Biochim Biophys Acta* 1992; **1109:** 127–131.
71. Wagner PD, Mathieu-Costello O, Bebaut DE *et al.* Protection against pulmonary O_2 toxicity by *N*-acetylcysteine. *Eur Respir J* 1989; **2:** 116–126.
72. Feddersen CO, Barth P, Püchner A *et al.* *N*-acetylcysteine vermindert funktionelle und strukturelle, ARDS-typische Lungenschädigungen bei endotoxinbehandelten Ratten. *Med Klin* 1993; **88**(4): 198–206.
73. Ceconi C, Curello S, Cargnoni A *et al.* The role of glutathione status in the protection against ischaemic and reperfusion damage: effects of *N*-acetylcysteine. *Mol Cell Cardiol* 1988; **20:** 5–13.
74. Eklund A, Eriksson Ö, Haklansson L *et al.* 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* 1988; **1:** 832–838.
75. Linden M, Wieslander E, Eklund A *et al.* Effects of oral *N*-acetylcysteine on cell content and macrophage function in bronchoalveolar lavage from healthy smokers. *Eur Respir J* 1988; **1:** 645–650.
76. Hardy JG, Everard ML, Coffiner M *et al.* Lung deposition of a nacystelyn metered dose inhaler formulation. *J Aerosol Med* 1993; **6:** 37–44.
77. Marriott C, Ingham S, Coffiner M *et al.* Determination of the mode of action of a novel mucolytic agent, nacystelyn. *Eur Respir J* 1993; **6:** 438s.
78. Gillissen A, Schmidt EW, Rasche B *et al.* The biochemical behaviour of alpha 1-antitrypsin under substitution therapy at homozygote (PI-ZZ) patients. *Klin Wochenschr* 1998; **67:** 328–335.
79. Snider GL. Emphysema: the first two centuries – and beyond. Part II. *Am Rev Respir Dis* 1992; **146:** 1615–1622.
80. Ruffman R, Wendel A. GSH rescue by *N*-acetylcysteine. *Klin Wochenschr* 1991; **6:** 279–283.
81. Issels RD, Nagele A, Eckert K-G *et al.* Promotion of cystine uptake and it utilization for glutathione biosynthesis induced by cysteamine and *N*-acetylcysteine. *Biochem Pharmacol* 1988; **37:** 881–888.
82. Henderson AH hayes P. Acetylcysteine as a cytoprotective antioxidant inpatients with severe sepsis: potential new use for an old drug. *Crit Care* 1994; **28:** 1086–1088.
83. De Caro L, Chizzi A, Costa R. Pharmacokinetics and bioavailability of oral acetylcysteine in healthy volunteers. *Arzneim-Forsch/Drug Res* 1989; **39:** 382–386.
84. Borgstrom L, Kagedal B, Paulsen P. Pharmacokinetics on *N*-acetylcysteine in man. *Eur J Clin Pharmacol* 1986; **31:** 217–222.
85. Rodenstein D, De Coster A, Gazzaniga A. Pharmacokinetics of oral acetylcysteine absorption, binding and metabolism in patients with respiratory disorders. *Clin Pharmacokinet* 1978; **3:** 247–254.
86. Cotgreave I, Eklund A, Larsson A *et al.* No penetration of orally administered *N*-acetylcysteine into bronchoalveolar lavage fluid. *Eur J Respir Dis* 1987; 73–77.
87. Bridgeman MME, Marsden M, Selby C *et al.* Effect of *N*-acetylcysteine on the concentrations of thiols in plasma, bronchoalveolar lavage fluid, and lung tissue. *Thorax* 1994; **49:** 670–675.
88. Jepsen S, Herlevsen P, Knudsen P *et al.* Antioxidant treatment with *N*-acetylcysteine during adult

respiratory distress syndrome: a prospective, randomized, placebo-controlled study. *Crit Care Med* 1992; **20**(7): 918–923.
89. Spies CD, Reinhart K, Witt I *et al.* Influence of *N*-acetylcysteine on indirect indicators of tissue oxygenation in septic shock patients: results from a prospective, randomized, double-blind study. *Crit Care Med* 1994; **22:** 1738–1746.
90. Meyer A, Buhl R, Magnussen H. The effect of oral *N*-acetylcysteine on lung glutathione levels in idiopathic pulmonary fibrosis. *Eur Respir J* 1994; **7:** 431–436.
91. Vecchiarelli A, Dottorini M, Pietrella D *et al.* Macrophage activation by *N*-acetylcysteine in COPD patients. *Chest* 1994; **105:** 806–811.
92. Kleinveld HA, Demacker PNM, Stalenhoef AFH. Failure of *N*-acetylcysteine to reduce low-density lipoprotein oxidizability in healthy subjects. *Eur J Clin Pharmacol* 1992; **43:** 639–642.
93. Drost E, Lannan S, Bridgeman MME *et al.* Lack of effect of *N*-acetylcysteine on the release of oxygen radicals from neutrophils and alveolar macrophages. *Eur Respir J* 1991; **4:** 723–729.
94. Meyer A, Buhl R, Kampf S *et al.* Intravenous *N*-acetylcysteine and lung glutathione of patients with pulmonary fibrosis and normals. *Am J Respir Crit Care Med* 1995; **152:** 1055–1060.
95. Meister A. Glutathione deficiency produced by inhibition of its synthesis, and its reversal: applications in research and therapy. *Pharmacol Ther* 1991; **51:** 155–194.
96. Pacht ER, Timerman AP, Lykens MG *et al.* Deficiency of alveolar fluid of patients with adult respiratory distress syndrome. *Chest* 1991; **100:** 1397–1403.
97. Cochrane CG, Spragg R, Revak SD. Pathogenesis of the adult respiratory distress syndrome. Evidence of oxidant activity in bronchoalveolar lavage fluid. *J Clin Invest* 1983; **71:** 754–761.
98. Bernard GR. *N*-acetylcysteine in experimental and clinical acute lung injury. *Am J Med* 1991; **91**(Suppl. 3C): 3C-54S–3C-59S.
99. Suter PM, Domenighetti G, Schaller M-D *et al.* *N*-acetylcysteine enhances recovery from acute lung injury in man. *Chest* 1994; **105:** 190–194.
100. Cantin A, Hubbard RC, Crystal RG. Glutathione deficiency in the epithelial lining fluid of the lower respiratory tract of patients with idiopathic pulmonary fibrosis. *Am Rev Respir Dis* 1989; **139:** 370–372.
101. Strausz J, Müller-Quernheim J, Steppling H *et al.* Oxygen radical production by alveolar inflammatory cells in idiopathic pulmonary fibrosis. *Am Rev Respir Dis* 1990; **141:** 124–128.
102. Clarke SW. Chronic bronchitis in the 1990s: up-to-date treatment. *Respiration* 1991; **58:** 43–46.
103. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1995; **152:** S77–S120.
104. British Thoracic Society Research Committee. Oral *N*-acetyl cysteine and exacerbation rates in patients with chronic bronchitis and severe airways obstruction. *Thorax* 1985; **40:** 832–835.
105. Clemens MR, Schmid B. Freie Radikale und Antioxidantien in Hämatologie und Onkologie. *Atemw-Lungenkrkh* 1991; **17:** S26–S34.
106. Pérez-Trallero E, Garcia-Arenzana JM, Montes M *et al.* Lipoprotein(A) reduction by N-acetylcysteine. *Lancet* 1991; **337:** 672–673.
107. Kharazmi A, Nielsen H, Schiotz PO. N-acetylcysteine inhibits human neutrophil and monocyte chemotaxis and oxidative metabolism. *Int J Immunopharmac* 1988; **10**(1): 39–46.
108. Flanagan RJ, Meredith TJ. Use of N-acetylcysteine in clinical toxicology. *Am J Med* 1991; **3C:** 131S–139S.
109. Burgunder JM, Varriale A, Lauterburg BH. Effect of N-acetyl cysteine on plasma cysteine and glutathione following paracetamol administration. *Eur J Clin Pharmacol* 1989; **36:** 127–131.
110. Prescott LF, Donovan JW, Jarvie DR *et al.* The disposition and kinetics of intravenous N-acetylcysteine in patients with paracetamol overdosage. *Eur J Clin Pharmacol* 1989; **37:** 501–506.
111. Bonanomi L, Gazzaniga A. Toxocological, pharmacokinetic and metabolic studies of acetylcysteine. *Eur J Respir Dis* 1980; **61:** 45–51.
112. Myers C, Bonow R, Palmeri S *et al.* A randomized controlled trial assessing the prevention of doxorubicin cardiomyopathy by N-acetylcysteine. *Seminars in Oncology* 1983; **10:** 53–55.
113. Levy L, Vredevoe DL. The effect of N-acetylcysteine on cyclophosphamide immunoregulation and antitumor activity. *Seminars in Oncology* 1983; **10:** 7–16.
114. De Vries N, Zandwijk van N, Pastorino U. The EUROSCAN study: progress report. *Am J Otolaryngol* 1993; **14:** 62–66.
115. Zandwijk van N. N-acetylcysteine for lung cancer prevention. *Chest* 1995; **107:** 1437–1441.
116. Cerutti P, Kapanci Y. Effects of metabolite VIII of bromhexine (Na 872) on type II epithelium of the lung. An experimental and morphological study with reference to surfactant secretion. *Respiration* 1979; **37:** 241–251.
117. Elmer G, Kapanci Y. Effect of ambroxol on pneumocyte type II cell. A morphological and biochemical study. *Curr Probl Clin Biochem* 1985; **13:** 47–55.
118. Tamaoki J, Chiotani A, Yamauchi F *et al.* Ambroxol inhibits Na^+ absorption by canine airway epithelial cells in culture. *J Pharm Pharmacol* 1991; **43:** 841–843.
119. Petten GR, Mears GJ, Taylor PJ. The effects of NA 872 on pulmonary maturation in the fetal lamb and rabbit. *Am J Obstet Gynecol* 1978; **130:** 35–40.
120. Anonymous. Prevention of chronic exacerbations with ambroxol (mucosolvan retard) – an open, long-term study in 5,635 patients. *Respiration* 1989; **55:** 84–96.
121. Olivieri D, Zavattini G, Tomasini G *et al.* Ambroxol for the prevention of chronic bronchitis exacerbations: long-term multicenter trial. Protective effect of

ambroxol against winter semester exacerbations: a double-blind study versus placebo. *Respiration* 1987; **51:** 42–51.
122. Mellillo G, Cocco G. Ambroxol decreases bronchial hyperreactivity. *Eur J Respir Dis* 1986; **69:** 316–320.
123. Ericsson CH, Juhasz J, Jonsson E *et al.* Ambroxol and simple chronic bronchitis. *Respiration* 1987; **51:** 33–36.
124. Germouty J, Jirou-Najou JL. Clinical efficacy of ambroxol in the treatment of bronchial stasis. *Respiration* 1987; **51:** 37–41.
125. Passali D, Zavattini G. Multicenter study on the treatment of secretory otitis media with ambroxol. *Respiration* 1987; **51:** 52–59.
126. Nowak D, Antczak A, Krol M *et al.* Antioxidant properties of ambroxol. *Free Radic Biol Med* 1994; **16:** 517–522.
127. Rozniecki J, Nowak D. Effect of ambroxol on the chloramine-T-induced decrease of serum elastase inhibitory capacity in vitro. *Lung Respir* 1987; **4:** 14–15.
128. Rozniecki J, Nowak D. Protective effect of ambroxol on serum elastase inhibitory capacity against chloramine-T and cigarette smoke extract. *Bull Eur Physiopathol Respir* 1987; **12:** 366s.
129. Zimmermann RG, Weber G. Inhibition of lipid peroxidation by bromhexin, ambroxol, and *N*-acetylcysteine. *Doc. No.: U961-0608 edn.*, Biberach an der Riss: Dr K Thomae GmbH, 1991.
130. Ledwozyw A, Jablonka S. Tusinska R. The effect of ambroxol on peroxidative processes in dog lung mitochondria. *Pol Arch Weter* 1991; **31:** 105–113.
131. Nowak D, Antczak A, Pietras T *et al.* Protective effect of ambroxol against heat- and hydrogen peroxide-induced damage to lung lipids in mice. *Eur Respir J* 1994; **7:** 1629–1634.
132. Nowak D, Pietras T, Antczak A *et al.* Inhibition of endotoxin-induced lipid peroxidation by ambroxol in mice. *Lung Respir* 1993; **10:** 14–15.
133. Nowak D, Pietras T, Antczak A *et al.* Effect of bacterial lipopolysaccharide on the content of lipid peroxidation products in lungs and other organs of mice. *Anton Leeuwenhoek Int J Gen M* 1993; **63:** 77–83.
134. Bianchi M, Mantovani A, Erroi A *et al.* Ambroxol inhibits interleukin 1 and tumor necrosis factor production in human mononuclear cells. *Agents Actions* 1990; **31:** 275–279.
135. Nowak D, Pierscinski G, Drzewoski J. Ambroxol inhibits doxorubicin-induced lipidperoxidation in heart of mice. *Free Radic Biol Med* 1995; **19:** 659–663.
136. Nowak D, Antczak A, Bialasiewicz P *et al.* Effect of ambroxol on cytosolic calcium rise in human polymorphonuclear leukocytes after stimulation with n-formyl-methionyl-leucyl-phenylalanine and concanavalin A. *Int J Immunopathol Pharmacol* 1994; **7:** 47–56.
137. Donnini M, Luisetti M, Diomede L. Ambroxol reduces paraquat toxicity in the rat. In: Wichert von P, Müller B, eds. *Basic Research on Lung Surfactant. Progress in Respiratory Research.* Basel: Karger, 1990, pp. 329–332.
138. Grabner R. Influence of cationic amphiphilic drugs on the phosphatidylcholine hydrolysis by phospholipase A_2. *Biochem Pharmacol* 1987; **36:** 1063–1067.
139. Peers SH. Flower RJ. Effects of ambroxol upon macrophage and neutrophil phospholipases. *6th Int Conf of Prostaglandins, Florence*, 1986 p. 245s.
140. Rozniecki J, Nowak D. Ambroxol inhibits spontaneous migration of human neutrophils. *Clin Exp Allergol* 1990; **20:** 27s.
141. Capsoni F, Ongari AM, Minonzio F *et al.* Effect of ambroxol on human phagocytic cell function. *Boll Ist Sieroter Milan* 1985; **64:** 236–239.
142. Chitano P, di Stefano A, Finotto S *et al.* Ambroxol inhibits airway hyperresponsiveness induced by ozone in dogs. *Respiration* 1989; **55:** 74–78.
143. Mezzetti M, Colombo L, Marini MG *et al.* A pharmacokinetic study on pulmonary tropism of ambroxol on patients under thoracic surgery. *Surg Intensive Care* 1990; **13:** 179–185.
144. Moseley PL, Shasby DM, Brady M *et al.* Lung parenchymal injury induced by bleomycin. *Am Rev Respir Dis* 1984; **130:** 1082–1086.
145. Novak Z, Varga SI, Kovacs L *et al.* The effects of oradexone and ambroxol pretreatment on the oxidative sensitivity of the red blood cells in preterm infants. *Clin Chim Acta* 1989; **182:** 241–246.
146. Fegiz G, Volpino P, Piat G *et al.* Prevention by ambroxol of bronchopulmonary complications after upper abdominal surgery: double-blind Italian multicenter clinical study. *Lung* 1991; **169:** 69–76.
147. Romanini BM, Sandri MG, Tosi M *et al.* Ambroxol for prophylaxis and treatment of broncho-pulmonary complications after chest surgery. *Int J Clin Res* 1986; **2:** 123–127.
148. Marini A, Franzetti M, Gios G *et al.* Ambroxol in the treatment of idiopathic respiratory distress syndrome. An interim report on a controlled double-blind multicenter study versus placebo. *Respiration* 1987; **51:** 60–67.
149. Thomassen MJ, Antal JM, Connors M *et al.* Characterization of Exosurf (surfactant)-mediated suppression of stimulated human alveolar macrophage cytokine release. *Am J Respir Cell Mol Biol* 1994; **10:** 399–404.
150. Matalon S, Holm BA, Baker RR *et al.* Characterization of antioxidant activities of pulmonary surfactant mixtures. *Biochim Biophys Acta* 1990; **1035:** 121–127.
151. Ahuja A, Oh N, Chao W *et al.* Inhibition of the human neutrophil respiratory burst by native and synthetic surfactant. *Am J Respir Cell Mol Biol* 1996; **14:** 496–503.
152. Guyatt GH, Townsend M, Kazim F *et al.* A controlled trial of ambroxol in chronic bronchitis. *Chest* 1987; **92:** 618–621.
153. Ericsson CH, Juhasz J, Mossberg B *et al.* Influence of ambroxol on tracheobronchial clearance in simple chronic bronchitis. *Eur J Respir Dis* 1987; **70:** 163–170.

154. Dirksen H, Hermansen F, Groth S *et al.* Mucociliary clearance in early simple chronic bronchitis. *Eur J Respir Dis Suppl* 1987; **153:** 145–149.
155. Davis WB, Pacht ER. Extracelluar antioxidant defenses. In: Crystal RG, West JB, Barnes PJ *et al.*, eds. *The Lung, Scientific Foundations*, New York: Raven Press, 1991, pp. 1821–1828.
156. Eck HP, Gmünder H, Hartmann M *et al.* Low concentrations of acid-soluble thiol (cysteine) in the blood plasma of HIV-1 infected patients. *Biol Chem Hoppe-Seyler* 1989; **370:** 101–108.
157. Jackson RM, Frank L. Ozone induced tolerance of hyperoxia in rats. *Am Rev Respir Dis* 1984; **129:** 425–429.
158. Smith LJ, Houston M, Anderson J. Increased levels of glutathione in bronchoalveolar lavage fluid from patients with asthma. *Am Rev Respir Dis* 1993; **147:** 1461–1464.