Chronic Obstructive Pulmonary Disease and Oxidative Stress

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Abstract: The respiratory tract as the main entrance for various inhalative substances has great potential to generate reactive species directly or indirectly in excess. Thus, heavy smokers are at high risk for development, impairment and failed response to treatment of chronic obstructive pulmonary disease (COPD). The article is an update regarding the influence of reactive oxygen (ROS) and nitrogen (RNS) species on COPD; however, we do not intend to describe ROS and RNS actions on the entire lung tissue. Here, we focus on the airways, because in human most of the described effects of ROS and RNS species are measured on respiratory epithelial cells obtained by bronchoscopy. ROS and RNS species are physiological compounds in cells and risk factors for several respiratory diseases. In general, both kinds of species are thermodynamically stabile, but their reaction behaviors in cellular environments are very different. For example, the life times of the superoxide anion radical range from micro/milliseconds up to minutes and even hours in *in-vitro* model systems. Oxidative stress by cigarette smoke was investigated in detail by the authors of this article. In addition, original studies by the authors on the amount of fine particulate matter and trace elements in lung biopsies after defined inhalation indicate a distortion of the equilibrium between oxidants and antioxidants. We also try to present some modern views with respect to genomic medicine for future therapeutic perspectives, although this is an upcoming sector of COPD therapy.

Key Words: reactive oxygen species, oxidative stress, lung tissue, therapeutic strategies, internal medicine, respiratory medicine, genomic medicine.

INTRODUCTION

The respiratory system is well protected by enzymatic and non-enzymatic defense mechanisms [1]. But an increased burden of exogenous and endogenous reactive species creates an imbalance in the oxidant/antioxidant status in smokers and patients with chronic obstructive pulmonary disease (COPD), which is of relevance in genomic medicine. Even in respiratory diseases not associated with smoking such as pulmonary fibrosis, the balance between oxidants and antioxidants may be disturbed by such pathogenic effects (Table **1**). This review is partly based on the book "*Oxygen- and nitrogen radicals in lung disease and injury*" [2].

Radicals are chemical compounds with unpaired electron(s). Though most radicals, including oxygen and nitrogen radicals, are structurally and thermodynamically stable, they behave very differently in cells, and when they react with other compounds, their chemical lifetimes can vary from short (millisecond range) to long. A wide range of long-lived radicals is known, with life- and observation times in the range of minutes up to years (Table **2**). For example, molecular oxygen (O_2) is a stable diradical in the electronic ground state. Here, the rate constants of biomolecular reactions with respective reaction partners should be considered, which of course depend upon the exact experimental conditions (see, for example, ref. [2]). The reactivity and selecti-

vity of the superoxide anion radical ($O_2^{\cdot-}$) are modified by specific interactions and electron exchange. It is commonly accepted that the superoxide anion radical in aqueous solutions has a lifetime in the millisecond range. However, electron spin resonance spectroscopy studies in a KO_2/H_2O/iron ion system revealed for the first time a stabilization of a part of the initially added superoxide anion radicals lasting up to hours at room temperature [3]. Superoxide anion radicals adsorbed on an oxidic iron hydrate phase in aqueous systems might function as a strong oxidant similar to that species which has been suggested to be a complex between oxygen and different valence states of iron in the initiation of lipid peroxidation by ferrous iron. Further, there were serious doubts about the identity of alkoxy radicals in solution. For the first time, alkoxy radicals were directly demonstrated in solution by electron spin resonance spectroscopy [4].

Because we succeeded for the first time in finding mass-spectrometric differentiation criteria for tobacco condensate deposits in lung tissue samples [5, 6], the next step is the line of reasoning that argues for some main reaction routes of reactive oxygen (ROS) and nitrogen (RNS) species [7]. Besides iron (see Fig. **1**) *via* the Fenton reaction, many transition metals that usually occur in particulate matter, such as copper, chromium and vanadium, are known to generate hydroxyl radicals with varying efficiency. The tar component of cigarette smoke generates H_2O_2 for long periods in aqueous media [8]. Little is known about the interactions between particulate matter and ROS leading to pulmonary disease.

Physiologically, ROS are generated endogenously, for example, in mitochondria [9]. Work on oxygen sensing, and the role that reactive oxygen and nitrogen species play, has

Table. 1.   Possible Involvement of Radicals in Lung Diseases/Injuries, Modified from [2]

Disease/injury	Evidence for ROS/RNS
adult respiratory distress syndrome (ARDS)	neutrophil-mediated $O_2^{\cdot-}$, $\cdot OH$, RNS
asbestosis	$\cdot OH$ from macrophage/fiber-mediated $\cdot OH$, oxidative stress
bleomycin	lipid peroxidation/pulmonary fibrosis, $\cdot OH$
pulmonary emphysema	$\cdot OH$, $_1$-antitrypsin oxidation, $\cdot OH$
bronchial asthma	$\cdot OH$, $O_2^{\cdot-}$
silicosis	lipid peroxidation, $O_2^{\cdot-}$, $\cdot OH$, oxidative stress, $NO^{\cdot}$
idiopathic pulmonary fibrosis	$O_2^{\cdot-}$, $\cdot OH$
pulmonary hypertension	$O_2^{\cdot-}$
COPD	$O_2^{\cdot-}$, $\cdot OH$, $_1$-antitrypsin oxidation, ROS in cigarette smoke, RNS
hyperoxia	$O_2^{\cdot-}$, $\cdot OH$, oxygen toxicity antioxidant depletion
ischemia/reperfusion	ROS, $O_2^{\cdot-}$, $\cdot OH$, RNS

led to the discovery of oxidative stress and its cell-damaging effects, which can range from unremarkable dysfunctions to apoptosis. Oxidative stress indicates that the balance between oxidative and antioxidative capacities has shifted to reactive oxygen and nitrogen compounds, which can be evoked by enhanced generation of radicals or by depletion of the antioxidant pool [10]. Under severe experimental hypoxia, consequences of the cellular decrease in ATP are the formation of hypoxanthine and xanthine, which are the substrates for the massive formation of superoxide anion radicals and hydrogen peroxide *via* the oxidase activity of the

Table 2.   Some Important ROS und RNS

superoxide	$O_2^{\cdot-}$
hydrogen peroxide	H_2O_2
hydroxyl radical	$\cdot OH$
alkoxy radical	$RO^{\cdot}$
peroxy radical	$ROO^{\cdot}$
hypochlorite	OCl^{-}
singlet oxygen	$^1O_2$
ozone	O_3
nitric oxide	$NO^{\cdot}$
peroxynitrite	$ONOO^{-}$
nitrogen dioxide	NO_2

ROS and RNS also have a protective function, for instance, when microbes invade macrophages and radicals are generated to eliminate intruding pathogens. But radicals in excess can also damage cellular and molecular structures. Because the only existing method for measuring radical activity is electron-spin-resonance spectroscopy, most investigators use indirect methods, which only indicate past oxidative stress [4].
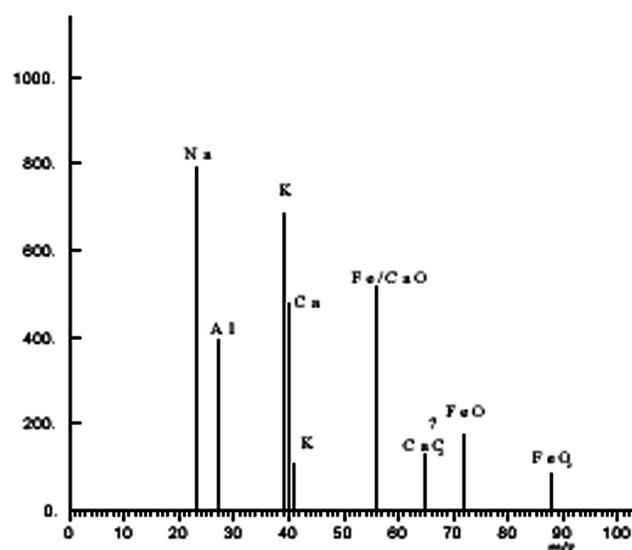
Fig. (1). Cationic mass spectrum of carbonaceous particles of a lung section from a patient suffering from COPD and graphite pneumoconiosis (*post mortem* diagnosis, see also [5]). Mean spectrum with abscissa given in m/z values (atomic mass/multiple of elementary charge) and ordinate given in arbitary units.

xanthine oxidoreductase reaction [11]. About 51% of the total consumed oxygen is used to form superoxide anion radicals in rat liver, whereas under normoxic conditions about 2% enter into this pathway [11]. The fact that oxygen affects gene expression in the adaptation process is of evident importance [7].

Enhanced generation of ROS is also induced by oxygen-enriched inspired air, hypoxic atmosphere at altitude [12], exposition to ROS/RNS generating substances such as tran-

sition metals, e.g. chrome, vanadium or copper [13], activation of ROS generating enzyme systems, or release of iron from iron pools. ROS and RNS intake may also occur through volatile substances, especially by inhalation of cigarette smoke and fine particulate matter [14]. Currently, oxidative stress is seen to be at least partly responsible for several lung diseases [15] (Table **1**), whereby both tissue damage and signal transduction on the molecular level play an important role in inflammatory lung disease. Furthermore, radicals will affect the remodeling of extracellular matrix, the inactivation of the surfactant system and of the protective antiprotease barrier.

COPD AND CIGARETTE SMOKING

The most important risk factor in the pathogenesis of COPD, chronic bronchitis and pulmonary emphysema [16], and bronchial carcinoma is cigarette smoking [17, 18]. About 90% of all COPD patients are or have been smokers [19] and 15-20% of all cigarette smokers develop clinically relevant COPD, though the etiology remains unclear.

Cigarette smoke is a major anthropogenic pollutant and contributor to the permanent load of ambient particulate matter (environmental cigarette smoke) [20, 21]. Gaseous and tar phases of cigarette smoke contain large amounts of long-lived semiquinone radical, short-lived superoxide anion and hydroxyl radical, hydrogen peroxide, NO and reactive organic compounds like carbon monoxide, ammonia, formaldehyde, N-nitrosamines, benzopyrene, benzene, isoprene, ethane, pentane, nicotine, acrolein, acetaldehyde, and other genotoxic and carcinogenic organic compounds, up to 5000 chemical compounds in total. One cigarette may deposit up to 20 mg tar in a smoker's lung. For example, in a deep drag there are 10^{17} molecules of reactive species [22], along with a NO concentration of 500-1000 parts per million [23]. People are often concomitantly exposed to fine particulate matter and exogenous radicals, such as cigarette smoke and ozone together [5]. Some of the inhaled particles themselves are able to generate radicals in the organism. Mass spectra of carbonaceous particles from cigarette condensate can detect such compounds in lung tissue biopsies from smokers [5]. Currently, there is striking evidence for oxidative stress and imbalance between oxidants and antioxidants in smokers [24]. Main molecular effects of oxidative stress are an upregulation of redox-sensitive transcription factors and proinflammatory gene expression [25].

Characteristically, the respiratory system has an abnormal response to inhaled particulate matter and gases in COPD, since neutrophils and macrophages migrate in large numbers to the lung tissue of cigarette smokers. The oxidant burden on the respiratory system imposed by inhalative toxicants, particularly cigarette smoke, is augmented by the release of radicals from neutrophils. In this context, smokers with obstructed ventilation reveal a higher number of neutrophils in the peripheral airways than smokers without bronchial obstruction. Thus, the oxidative stress in smoking COPD patients is increased [26]. Several investigations using different techniques indicated higher concentrations of indirect oxidative stress markers in the epithelial lining fluid (ELF), in respiratory condensate, in urine and in the blood of cigarette smokers and COPD patients. Cigarette smoking is also correlated with increased concentrations of myeloperoxidase (MPO) in neutrophils and is related to the degree of respiratory dysfunction [27]. It is supposed that the oxidative stress derived from MPO plays an important role in the pathogenesis of COPD.

CARBON COMPOUNDS, MAJOR ANTHROPOGENIC POLLUTANTS

According to the *USA Environmental Protection Agency*, the burden of particulate matter in ambient air caused by chemical (3%) and nonchemical (35%) industrial processes, by transportation (27%, e.g. diesel exhaust particulates, rubber losses from tires, other air pollutants generated by motor vehicles) and by fuel combustion (35%, e.g. particles from domestic fuel, fly ash, soot, tarry substances, biomass burning, combustion aerosols, power plants burning fossil fuels, and coal furnace emissions) is increasing worldwide [28]. Air pollution is also reported to be associated with increased rates of mortality and cardio-respiratory disease [20, 29-33], and mechanisms of particulate matter toxicity are under intense investigation [34]. Many types of ingested particles have the ability to generate free radicals in biological systems and to activate oxidative stress-responsive signaling pathways in cells [35]. Ambient particulate matter may also induce oxidative DNA damage in lung epithelial cells [36]. The molecular interactions between particulate matter and ROS leading to pulmonary disease are unknown.

It is very likely that combined exposure to particulate burden, exogenous ROS and ROS-generating cytokines increases particle adhesion to the epithelial wall, penetration into lung tissue, cell proliferation, inflammatory cell influx and DNA damage. In individuals chronically exposed to high levels of ambient particulate pollutants, there may be remodeling of small airways [37] and consequent chronic limitation of airway flow [38]. The chemical composition of airborne soot particles was successfully analyzed online with aerosol time-of-flight mass spectrometry [39]. Studies on carbonaceous particles from biomass burning [17, 40], volatile organic compounds [41] and pure graphite powder were used in databases to determine peak alignment of fragmentation patterns. Laser activated micromass spectrometry (LAMMA) was applied to investigate air pollutants resulting from various natural and anthropogenic sources [42, 43]. The discrimination between the composition of carbonaceous particles originating from smoking or from occupational carbon dust exposure in non-smoker's lung is of great interest and practical relevance [5, 6]. Mass spectrometry may be a valuable tool in determining the composition of inhaled organic compounds, which usually contain transition metals, deposited in the human lung. In future, in a second step, patterns of mass spectra from other lung diseases with deposition of particles might also be useful in establishing clinical diagnoses [6].

CELL-GENERATED OXIDANTS

Subpopulations of alveolar macrophages with higher density are more prevalent in smokers' lungs. Alveolar macrophages obtained by bronchoalveolar lavage from the lungs of smokers are more activated than those obtained from non-smokers, and so release increased amounts of ROS

such as $O_2^{\cdot-}$ and H_2O_2 [44, 45] (Table **2**). Airway epithelial cells are another source of ROS and type II alveolar epithelial cells can release both H_2O_2 and $O_2^{\cdot-}$ [46]. The release of ROS under concomitant presence of myeloperoxidase inactivates ₁-antitrypsin, which is the most important antiprotease enzyme in the human body, and which prevents proteolytic lung injury and development of emphysema with insufficient enzyme activity [47].

Iron is a critical element in many oxidative reactions [48]. Free iron ions will catalyze the Fenton reaction and the $O_2^{\cdot-}$ mediated Haber-Weiss reaction. The $^{\cdot}OH$ radicals react as free radicals and initiate tissue injury by lipid peroxidation. The epithelial lining fluid (ELF) as well as alveolar macrophages from smokers, especially those who develop chronic bronchitis, contain and release more iron than those of non-smokers.

ANTIOXIDATIVE CAPACITY

An antioxidant is defined as a substance that delays or inhibits the oxidation of an oxidizable substrate. Reduced glutathione (GSH) is the master non-enzymatic antioxidant. One reason that GSH is the main non-enzymatic cellular antioxidant is that the intracellular concentrations of GSH in human tissues are in the range from 0.1 – 10 mM, with the highest concentrations in liver (up to 10 mM), spleen, kidney, the lens of the eye, erythrocytes and leukocytes. However, the concentrations in the plasma are in the µM-range (about 4.5 µM). The respiratory system has several antioxidant factors that protect tissue from injury by oxidants. Non-enzymatic antioxidants in the epithelial lining fluid (ELF) gained by bronchoalveolar lavage fluid (BALF) in bronchoscopy include GSH and ascorbic acid (vitamin C) (Table **3**), which are more concentrated in ELF than in plasma. Thus, bronchial mucus and ELF have antioxidative capacity. Together, they form the most important defense against short-lived radicals [49]. Besides low molecular weight substances (see Table **3**), the antioxidant barrier of the ELF includes catalase as the major ELF antioxidant macromolecule, and plasma proteins with anti-H_2O_2 properties within a molecular weight range of 100.000 Da – 300.000 Da [50]. Other macromolecules with antioxidant properties are superoxide dismutase (36.8 ± 2.0 mU/µl ELF), glutathione reductase (0.35 ± 0.06 mU/µl ELF), glutathione peroxidase (0.05 ± 0.01 mU/µl ELF) and ceruloplasmin, which is a 134 kDa, copper-containing glycoprotein that catalyzes the oxidation of iron from ferrous to ferric. In terms of a ^{51}Cr-cytotoxicity assay for assessing hydrogen peroxide-mediated injury to lung parenchymal cells [50], catalase accounts for most of the observed anti-H_2O_2 properties of ELF [50]. These data from healthy nonsmokers are representative of lower airway fluids, but they may be not representative of alveolar surface fluids obtained from human alveolar epithelial cell line *in vitro*.

Information on the respiratory epithelial antioxidant defenses in smokers and patients with COPD is scarce. Low levels of vitamin E were demonstrated in the BALF of smokers compared to nonsmokers [51]. In contrast, slightly increased levels of ascorbic acid were found in the BALF of smokers and the alveolar macrophages showed an augmented uptake and increased concentrations of vitamin C [52]. The GSH concentration in the bronchoalveolar lavage fluid (BALF) from the lower and upper airway tract of chronic smokers is higher than in healthy nonsmoking adults. Cigarette smoke condensate can alter and influence GSH metabolism. In animal studies and in cell cultures of human alveolar epithelial cell lines, it was shown that cigarette smoke condensate produces a dose- and time-dependent depletion of intracellular GSH [53]. In consequence, activation of the glutathione redox system enzymes such as glutathione peroxidase and glucose-6-phosphatase dehydrogenase transiently decreases even in alveolar epithelial cells [54]. Changes in other antioxidants and antioxidant enzymes in response to cigarette smoke varied. Increased activity of antioxidant enzymes such as superoxide dismutase and catalase has also been reported in alveolar macrophages from young smokers [55]. It was speculated that alveolar macrophages undergo a process of adaptation to chronic oxidant exposure that may ameliorate potential damage to respiratory epithelial cells by further oxidant stress. Further, results on the antioxidant defenses in ELF of smokers are very inconsistent. The mechanisms responsible for the induction of antioxidative enzymes in alveolar macrophages by inhalation of cigarette smoke are currently unknown but very likely involve antioxidant gene induction [7].

OXIDATIVE STRESS IN LUNG TISSUE

An important link between oxidative stress and the pathogenesis of COPD is the reaction of ROS with target

Table 3.   Some Antioxidant Constituents of the Epithelial Lining Fluid (ELF) [2]

	Approximate concentration in plasma (µM)	Approximate concentration in ELF (µM)
ascorbic acid	40	100
glutathione (GSH)	4.5	100
uric acid	300	90
albumin-SH	500	70
-tocopherol (Vit. E)	25	2.5
-carotene	0.4	-

molecules of respiratory cells and the occurrence of larger amounts of modified molecules in the lungs of cigarette smokers, particularly those who develop COPD. Lipid peroxidation products were found in increased amounts in the lungs of cigarette smokers, correlating to the duration of smoking (pack years) [56]. Smoking-associated mitochondrial DNA mutations are more frequently seen in the lungs of smokers. Neutrophils have been shown to cause oxidative DNA damage in alveolar epithelial cells *in vitro* [57]. There is accumulating evidence that oxidative stress can provoke reactions with target molecules in the lung tissue of patients with COPD.

SYSTEMIC OXIDATIVE STRESS

Today, COPD is considered to have local lung and systemic effects [24]. Systemic effects are shown by decreasing peripheral muscle function and weight loss leading to reduced survival [58]. Another manifestation of a systemic effect is the presence of markers of oxidative stress in the blood of patients with COPD. This may be reflected in an increased sequestration of neutrophils in the pulmonary circulation during smoking and during exacerbations of COPD, which can be also an oxidant-mediated event [59]. Increased release of ROS from peripheral blood neutrophils has been shown during acute exacerbation of chronic bronchitis (AECB). In other studies, circulating neutrophils from patients with COPD were shown to increase O_2^- production and to upregulate their expression of adhesion molecules, which may be an oxidant-mediated effect as well. [24,60]. Lipid peroxidation products such as thiobarbituric acid reactive substances (TBARS) measured in body fluids are significantly increased in the plasma of healthy smokers and patients with AECB compared to healthy nonsmokers [60]. Secondary products of lipid peroxidation such as conjugated dienes of linoleic acid were elevated in chronic smokers [61]. An increased level of F_2-isoprostane, also a parameter of lipid peroxidation, was found as well in cigarette smokers [62]. Through oxidative stress, lipid peroxidation products also activate signaling mechanisms, which enhance airway inflammation. Measurements of antioxidant capacity in the blood can also be used as a marker of systemic oxidative stress. In smokers, the antioxidant plasma capacity is significantly decreased [63].

Within the inflammatory process, NO at low levels may induce a self-amplifying signal. In contrast, high concentrations of NO reduce the ability of cytokines to activate certain receptor genes. For example, a high concentration of NO decreases the ability of cytokines to transactivate reporter genes [64].

Thus, an important relationship between ROS/RNS and systemic inflammation is addressed by NO. Malnutrition with depletion of nutritional antioxidants is also a cause of increased ROS production [65].

THERAPEUTIC SUGGESTIONS FOR COPING WITH OXIDATIVE STRESS

There are various therapeutic approaches that may improve the oxidant-antioxidant imbalance, especially in smokers. In this context, there are several promising substances with antioxidant properties that can influence respiratory inflammation at the molecular level.

One approach is to target the inflammation by reducing the sequestration and migration of neutrophils from the pulmonary capillaries to the alveolar spaces [2]. Therapeutic options may involve drugs that can alter cell deformability, either by interfering with adhesion molecules or preventing the release of inflammatory cytokines (e.g. IL-8, leukotrien B_4). Agents that prevent the release of oxygen radicals from activated neutrophils or that may compensate for oxidants once formed by enhancing the antioxidant defense, have therapeutic impact. A recent study of a phoshodiesterase-4 inhibitor has shown a therapeutic effect in patients with COPD [66], but actually phosphodiesterase-4 inhibitors have minimal benefit on COPD patients and have not been approved in an FDA hearing for that purpose. Those drugs act by increasing cyclic adenosine monophosphate (cAMP), which decreases activation of neutrophils [67]. Another approach to enhance the lung antioxidant screen would be the use of specific spin traps, such as   -phenyl-N-tert-butylnitrone, which may react directly with oxidants in the area of inflammation [2].

Further approaches in cigarette smokers involved various antioxidants such as vitamins C and E [68], unfortunately with inconsistent results, although vitamin E has been shown to reduce oxidative stress in COPD [68]. Attempts to supplement GSH in the lung, using GSH or precursors, also were unconvincing. Nebulized glutathione increased bronchial hyperreactivity as an additional side effect [69]. Administration of the amino acid cysteine, which is rate-limiting in GSH synthesis, does not make sense, since it is oxidized to neurotoxic cystine [70]. In contrast, the cysteine-donating compound N-acetylcysteine (NAC) acts as a cellular precursor of GSH and reduces disulfide bonds. NAC has the potential to interact directly with oxidants. However, the administration of NAC to increase the plasma GSH level is dose dependent. The administration of 600 mg NAC orally in normal subjects does not significantly elevate plasma NAC within two hours. NAC 600 mg 3 times daily for 5 days significantly increased plasma GSH, but there was no associated increase in BAL or in lung tissue GSH [71]. Several European studies have shown, however, that NAC is beneficial and reduces the number of exacerbations in COPD [72]. Nacystelyn (NAL) is a lysine salt of NAC. As a thiol antioxidant compound with a neutral pH, it has mucolytic properties, in contrast to NAC. Aerosolized NAL can be applied to the lung without any significant side effects [73]. In studies comparing the effects of NAL and NAC, both were shown to increase the intracellular concentration of GSH in alveolar macrophages and inhibit hydrogen peroxide and superoxide release from circulating neutrophils of smokers and patients with COPD [74].

Molecular engineering of antioxidant genes such as glutathione peroxidase or alteration of genes involved in the synthesis of GSH itself could be a future therapeutic option [2] in genomic medicine. Another option of genomic antioxidant therapy is recombinant superoxide dismutase that prevents neutrophil influx into air spaces and IL-8 release by cigarette smoke, as shown in animal studies [75].

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