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Chapter

The Importance of the Redox Modulation in the Prevention and Treatment of Chronic Pulmonary Diseases

Emma Borrelli

Abstract

This chapter discusses the most important mechanisms of action of oxidants in the pathogenesis of chronic pulmonary oxidative diseases and the possible use of redox modulators in the prevention and treatment of oxidant/antioxidant intracellular imbalance. Recent acquisitions on cellular physiology reported the key role, in micromolecular doses, of reactive oxygen species (ROS) as signaling molecules although excessive ROS contribute to the development and progression of a large spectrum of diseases, including chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF). Therefore, a correct understanding of the roles of redox regulation in the respiratory system during the impairment of oxidative balance and the subsequent development of chronic lung diseases appears to be important. Moreover, an interdependence between oxidant and inflammatory mediators has been shown in several experimental studies on chronic lung diseases, making more intriguing the comprehension of the pathophysiological phenomena and the therapeutic approach. This chapter discusses the role of various exogenous substances targeting oxidant/antioxidant balance in the treatment of COPD and IPF and their very limited beneficial effects due to the reduced bioavailability in the human body. Finally, the importance of novel routes of administration or a combination of redox modulators will be discussed as a promising avenue for the prevention and treatment of this common and highly disabling disease.

Keywords: redox modulator, chronic lung disease, reactive oxygen species, oxidative stress, antioxidant

1. Introduction

Clinical and experimental studies suggest that oxidative stress (OS) and cellular redox balance have been implicated in the pathobiology of two major chronic lung diseases: chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) [1]. Chronic obstructive pulmonary disease (COPD) is a pathological condition of the lung where both oxidative imbalance and inflammation play an
important role in the development and progression of the disease [2]. In support of this hypothesis, a large series of scientific studies reported in COPD an elevated oxidant generation from environmental exposures, mainly tobacco smoke and air pollutants, associated with increased amounts of reactive oxygen species (ROS) and reactive nitrogen species (RNS) released from leukocytes and macrophages during the inflammatory process in the lungs [3]. The increased levels of oxidants may have multiple consequences, such as induction of proteases, inactivation of defense mechanisms, and activation of growth factors and inflammatory pathways [4]. More interestingly, COPD is widely recognized as not simply an inflammatory/destructive lung disease but also a chronic degenerative systemic disease with extrapulmonary manifestations, such as cardiovascular disease, skeletal muscle dysfunction, osteoporosis, and neurological degeneration [5, 6]. Thus, it appears clear that, in the lung cells of COPD patients, a vicious cycle of persistent inflammation accompanied by chronic oxidative stress begins locally but rapidly becomes a chronic challenge for the organism, if the process is not properly controlled or neutralized [7]. It therefore seems obvious that, to prevent the onset and the progression of this disease, the reduction of exogenous exposition to oxidants (e.g., smoking cessation), downregulation of endogenous ROS generation and inflammation, and an increase in antioxidants could represent the best therapeutic options [8–10]. As far as IPF, it has been ascertained that many exogenous substances, such as asbestos, radiation and drugs, are involved in the pathogenesis of pulmonary fibrosis through an augmented generation of ROS and a subsequent imbalance between oxidant and antioxidant substances in the lower respiratory tract [11, 12]. In bronchoalveolar lavage fluid of patients with IPF it has been found that an increased level of 8-isoprostan (a marker of oxidative stress) respects healthy patients [13]. Mitochondrial generation of ROS in epithelial lung cells is also increased in patients with IPF, and this phenomenon could lead to an increased apoptosis of the cells. The antioxidant system is reduced as suggested by a decrease in glutathione content in the lining fluid of the epithelial cells in IPF patients [14]. Unlike COPD patients, patients affected by idiopathic fibrosis show an increased level of antioxidants and detoxification enzymes in the areas of epithelial regeneration (in fibrotic lesions the antioxidants are low). It seems that a global alteration of redox balance is more important than the trend of the single oxidant or antioxidant factors. This redox deregulation causes an activation of the epithelial TGF beta and proteases and an increase in extracellular matrix production that may ultimately contribute to the development of the end-stage fibrosis [15–17]. Despite the clear involvement of oxidant stress in chronic lung diseases, current potential treatments, such as the administration of several antioxidants, failed to protect the respiratory system against COPD and IPF. In the next paragraphs, we will briefly take into consideration the cross-talk between inflammatory and oxidative stress mediators and the possible role of the indirect stimulator of antioxidant and anti-inflammatory mediators, such as exercise, caloric reduction, or ozone therapy, in the prevention and treatment of chronic lung diseases.

1.1 Oxygen and oxidant molecules in the respiratory system: the necessary evil?

1.1.1 Source of oxidative stress in the chronic pulmonary diseases

In the lung, the main sources of oxidants are high oxygen tension, environmental factors, such as cigarette smoke and airborne particulates, and infections. If the concentrations of oxidants remain high for a long time in the pulmonary tissue,
chronic oxidative stress could begin. As far as the oxygen tension in the atmosphere, there is no doubt that it represents both challenges and opportunities for life. It is universally accepted that the energy generates through O$_2$ electron transport is a crucial mechanism for the evolution of complex multicellular organisms. However, about 2–3% of oxygen used by mitochondria, via the complex I, II, and III, during the process of oxidative phosphorylation will leak from the respiratory chain to form superoxide anion O$_2^\cdot$⁻. On the basis of recent experimental findings, the mitochondrial source of superoxide anions is much less quantitatively relevant with respect to the previous estimation. Other mitochondrial enzymes, such as dihydrolipoamide, dehydrogenase, and monoamine oxidase, are able to produce ROS [18, 19]. NAD(P)H oxidases present in cell membranes of fibroblasts, endothelial, and vascular smooth muscle cells, and particularly phagocytes, produce superoxide as a basic defensive process [20]. Other enzymes, such as nitric oxide synthase (NOS), xanthine oxidase, cytochrome P450, lipoxygenases, and even heme oxygenases (HOs), during the abnormal situation as inflammation, may be implicated in superoxide production. The reduction of superoxide anion, discovered by McCord and Fridovich in 1968, is performed by mitochondrial (SOD Mn), cytosolic (SOD Cu/Zn), and extracellular superoxide dismutases (SODs), that catalyze the dismutation to hydrogen peroxide as follows:

$$2O_2^\cdot⁻ + 2H → H_2O_2 + O_2$$

Hydrogen peroxide (H$_2$O$_2$) is not a radical molecule because it has paired electrons but it has been included among the reactive oxygen species (ROS) for its oxidant property [21]. As it is a unionized molecule, in the presence of an extracellular-cytosolic gradient, it passes throughout the cell membrane but the intracellular concentration is only about 1/10 of the extracellular one. The hydrogen peroxide has a half-life of about 1–2 seconds in plasma but less than 1 second in blood. The concentration of H$_2$O$_2$ in plasma is about 2.5 micromoles; it appears ubiquitous and it has been detected in urine and in exhaled air of patients affected by chronic lung diseases [22]. Hydrogen peroxide, due to its relatively mild reaction, can serve as the main component of intracellular signal transduction. However, when the concentration of ROS exceeds the homeostatic level, it can generate another most potent free radical, the Hydroxyl radical (OH·) via the Fenton-Jackson reaction.

$$H_2O_2 + Fe^{+++} → OH⁻ + OH⁻ + Fe^{++}$$

Hydroxyl radicals can cause covalent cross-linking of enzymes or propagates deleterious free radical reactions in a large series of molecules, such as DNA, proteins, and lipids. As far as the lipid interaction, hydroxyl radicals can eliminate a hydrogen atom from PUFA resulting in the formation of lipid radicals, which can interact further with oxygen to generate the lipid peroxyl radical. A lipid peroxidation occurs if the lipid peroxyl radical is not completely reduced by antioxidants. Lipid peroxidation products (LOP) are generally stable, can diffuse within or even escape from the cell and attack the target far from the site of the original free radical event. To avoid all these undesirable consequences, glutathione peroxidase and catalase are enzymes that play a pivotal role in preventing hydroxyl radical formation because they can definitively convert the hydrogen peroxide to water [23, 24]. For many years ROS generation was regarded as a damaging side effect of aerobic metabolism and only toxic effects were attributed to ROS. Recently, in contrast, it was recognized that ROS are part of cellular redox homeostasis and the complete elimination of these molecules would
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disrupt rather than extend the normal function of the organism [25]. In fact, the physiological production of ROS regulates many redox-dependent signaling processes that control proliferation, migration, differentiation, or cell survival by inducing specific and reversible posttranslational modifications on redox-sensitive proteins. Whether ROS will act as damaging, protective, or signaling factors depends on the delicate equilibrium between ROS production and scavenging at the proper time and side [26, 27]. In conclusion, oxygen and its derived species could be considered essential consequences and drivers for evolution and survival over Earth’s history, but an abnormal presence of ROS is associated with the development of chronic oxidative stress diseases. Only the correct understanding of the physiologic O$_2$ concentration and ROS threshold may provide novel insight into innovative strategies for treating a large number of degenerative pathologies, including chronic lung diseases [28, 29].

1.2 The antioxidant system in lung

In the human body, all organs contain a large number of antioxidant molecules to prevent inappropriate ROS production or unwanted action of ROS in cells. In the lung, the respiratory tract epithelial lining fluid (RTLF) represents a physical barrier between the external environment and the underlying respiratory tract epithelial cell layer. In this lining fluid, most of the inhaled toxicants are cleared from the lungs also thanks to the mucociliary action. A large amount of nonenzymatic low molecular weight antioxidant scavengers are contained in the RTLF, and these substances are able to directly counteract and detoxify the inhaled oxidants and thereby prevent the direct contact of inhaled toxicants with the underlying epithelium. The major antioxidant molecules in the RTLF are glutathione (GSH), ascorbic acid, uric acid, and vitamin E. Additionally, airway epithelial cells secrete certain antioxidant proteins into RTLF, which also function as antioxidant scavengers. Other ROS metabolizing enzymes in the lung tissue are superoxide dismutases (SODs), catalases, peroxiredoxins, glutathione peroxidase, thiol reductases that reverse the modifications of cysteine after oxidation, thioredoxin (TRX), glutaredoxin (GRX), phase-2 detoxifying enzymes (e.g., glutathione S-transferases (GST), metal-binding proteins (transferrin and lactoferrin). Under physiological conditions, there is an equilibrium between intracellular ROS and endogenous antioxidants. The mechanism of antioxidant defense is complex and compartmentalized allowing independent regulation of cytoplasmic, mitochondrial, and nuclear levels of ROS. Superoxide dismutase (SOD) are intracellular enzymes and represent the first line of protection against ROS. They catalyze the dismutation of O$_2^-$ in H$_2$O$_2$. There are three types of superoxide dismutase with different localization: Copper-zinc SOD (SOD1 and cytoplasmic), manganese SOD (SOD2 and mitochondrial), and zinc SOD (SOD3 and extracellular SOD) [30]. The action of SOD must be coupled with that of enzymes that degrade H$_2$O$_2$ such as catalase or glutathione peroxidase, in order to avoid increasing concentration of H$_2$O$_2$ which in presence of iron induces the formation of OH radical by the Fenton reaction. Catalase, various peroxidases, including glutathione peroxidase and glutathione S transferase, can convert H$_2$O$_2$ to H$_2$O. Other enzymes act with direct detoxification of ROS. Glutathione is a tripeptide formed by glutamic acid, cysteine, and glycine. It is represented in a simplified way by GSH (reduced form) or glutathione disulfide (GSSG) oxidized form. GSH can remove the ROS either by a direct chemical reaction or via peroxide reduction as the co-factor of GSH peroxidase inducing a cycle between the reduced and the oxidized form of glutathione [31]. The reduced form is maintained by GSH reductase. The thioredoxin/thioredoxin reductase system facilitates
the reduction of other proteins by the formation of disulfide bridges between cysteine residues. Recent experimental findings suggest that each component of this defense system has a specific function and these components are not interchangeable. This is an important concept because it means that an increased amount of one does not compensate for a deficiency of other [32].

2. Reactive oxygen species and inflammatory mediators: the dangerous crosslink

Reactive oxygen species are generally considered as proinflammatory molecules and most studies assumed that increased production of any ROS will result in cellular and organ dysfunction because of a rapid transition from one reactive oxygen intermediate to another. On the contrary, a large series of experimental findings suggest that different ROS may have distinct effects on individual cell activation. ROS have far-reaching effects on the respiratory tract and the parenchyma and increase the inflammatory response ROS activates nuclear factor-kappa beta (NF-kB), which turns on multiple inflammatory genes, resulting in enhancing the inflammatory response, oxidative stress leads to the activation of histone acetyltransferase activity, which opens the chromatin structure and is with increased transcription of multiple inflammatory genes [33]. Oxidative stress can also affect the function of antiproteases, such as alpha1-antitrypsin and secretory leukoprotease inhibitor, and thus accelerates the breakdown of elastin in the pulmonary parenchyma [34]. Patients with COPD, especially in severe disease and during exacerbations, have evidence of systemic inflammation measured either as elevated circulating cytokine, chemokine, and acute phase protein levels or as anomalies in circulating cells [35]. Persistent inflammation is associated with worse clinical results. Smoking itself can produce systemic inflammation (e.g., increased total number of leukocytes), but in patients with COPD, the degree of systemic inflammation is a sum of local and systemic inflammation [35]. The systemic inflammation in patients with COPD may contribute to its systemic manifestations and could aggravate extrapulmonary manifestations. A clinical study measured a series of plasma inflammatory markers (C-reactive protein, IL-6, CXCL8, fibrinogen, TNF-a, and leukocytes) in COPD patients and the results showed that 70% of patients with COPD had some components of systemic inflammation, and 16% had persistent inflammation [36]. Patients with persistent systemic inflammation had increased mortality and more frequent exacerbations. Systemic inflammation appears to relate to an accelerated decrease in lung function and is increased further during exacerbations. Moreover, oxidative stress drives accelerated aging through activation of phosphoinositide3-kinase (PI3K) and reduction in sirtuin-1 levels, which leads to cellular senescence and release of inflammatory proteins, which further increase oxidative stress [37, 38]. In patients affected by pulmonary fibrosis, evidence strongly suggest the important contributions of the oxidants and inflammatory mediators to the pathogenesis of IPF [39–41]. A large series of studies suggested an important contribution of ROS and RNS to the proteases/antiproteases imbalance through both activation of matrix metalloproteinases (MMP) and inactivation of protease inhibitors. It has also been suggested that ROS can both activate and inactivate MMPs, it depends on the local amount and distribution of ROS [42]. The oxidative stress and the proteolytic activation are mutually reinforcing processes that leading to tissue injury and lung fibrosis [43–45]. It is therefore clear that the prevention or the therapy of chronic lung disease must take into account the interdependence between oxidant and inflammatory systems because they are closely related.
2.1 Redox modulation in the prevention and treatment of chronic lung diseases

A large series of studies suggested a connection between impairment of the antioxidant defense system controlled by nuclear factor erythroid 2-related factor (Nrf2) and chronic lung disease development and progression [46]. Nrf2 plays a central role in controlling redox homeostasis and it is involved in the induction of several enzymes implicated in the antioxidant defense such as heme oxygenase (HO)-1, glutamate-cysteine ligase modifier subunit (GCLM) and glutamate-cysteine ligase catalytic subunit (GCLC) but also in the anti-inflammatory regulation, such as transforming growth factor (TGF)—beta and nuclear factor kappa (NF-κ)beta. In homeostatic conditions, Nrf2 is tied in the cytosol to its repressor Kelch-like ECH-associated protein 1 (Keap1) and cullin3-dependent E3 ubiquitin ligase. Two domains of Keap1 protein contain key reactive cysteine residues and the modification of these residues causes the disruption of the Nrf2-keap1 complex and the consequent activation of Nrf2. The free Nrf2 migrates in the nucleus and binds, as a heterodimer with small Maf proteins, to the antioxidant response element (ARE) in the upstream promoter region of antioxidant and phase II detoxifying enzymes genes and initiates transcription and expressions of these proteins [47]. Stimulation of Nrf2 is upregulated during short exposure to a low-intensity stressor. It has been reported a clear association between reduced Nrf2 signal pathway and the development and progression of COPD [48, 49]. For example, in an experimental setting with genetically null Nrf2 mice exposed to chronic cigarette smoke, increased alveolar destruction and inflammation were observed compared to mice with normal expression of Nrf2. In fact, in Nrf2−/− mice, the level of histone deacetylases 6 (HDAC6) was increased and this data suggests that Nrf2 could counteract the increased expression of HDAC6 by oxidative and proteolytic stress [50–52]. In fibroblast of IPF patients, it has been reported a decrease in expression and nuclear localization of Nrf2, with a reduction of related genes (HO-1, NQO1, and epoxide hydrolase). Moreover, an increase in alpha-smooth muscle actin and collagen was observed suggesting a conversion from fibroblast to myofibroblast phenotype [53]. Nrf2 is a key factor in aging and a predisposing factor for chronic lung diseases. In fact, the capacity to respond to oxidative stress through the Nrf2 activation decreases with aging. This phenomenon is probably due to many factors like the reduction of positive regulators of Nrf2 (PI3K, P62, CPB, and BRCA1) and the increase in the Nrf2 suppressors (Keap1, Bach1, and cMyc) with age [54, 55]. All together these experimental data strongly suggest that compounds able to activate Nrf2 or stabilize Keap1/DJ-1/Maf proteins could exert an important role in the prevention and therapy of chronic lung diseases, especially in situations where the endogenous antioxidant system is weakened (advanced COPD) or is less adaptable/compensatory or decreased (e.g., in IPF or aging lung) [56]. In fact, on the basis of the previously exposed mechanisms involved in the pathogenesis of COPD and IPF, it is possible to speculate that decreasing ROS overproduction or increasing antioxidants in the lung could lead to reduced pulmonary damage and a consequent systemic polymorbidity [57]. The most important Nrf2 activators are dietary and synthetic products containing sulforaphane, curcumin, and caffeic-acid phenethyl ester, which are able to induce ARE-regulated gene expression and could be also useful in chemoprevention [58, 59]. Chalcones have been tested as both redox modulators and anti-inflammatory molecules because they simultaneously inhibit the NF-κB pathway and activate Nrf2/ARE pathways with consequent induction of the expression of phase II detoxifying enzymes. Thiol compounds (N acetylcysteine and carbocysteine) have been tried in the prevention and treatment of COPD and IPF [60–62]. Unfortunately,
the clinical trials of these compounds and other molecular antioxidants, such as vitamin C and E or superoxide dismutase (SOD), showed contrasting results in the treatment of chronic lung diseases [63]. It has been suggested that the intracellular bioavailability and the heterogeneity of the patients could be the main issues in the efficacy of these therapeutic agents. Further studies on the combination of oral administration of several redox modulators were performed, but it has been reported that antioxidants, if exogenously oversupplied, can cause antioxidant stress because, as reported above, ROS are signaling molecules during the homeostatic cellular process and the full suppression of this molecule causes damage. Thus, the amount of ROS in the cellular environment can become protective or harmful depending on a fine and complex redox modulation at the proper side and time [64–67].

3. The future challenge in the prevention and treatment of chronic lung diseases

To better achieve the therapeutical effects in COPD and IPF patients, clinical trials used redox modulators at high dosages for months. The aim of this protocol is to maintain a continuous high level of Nrf2 activation in the cells. Unfortunately, prolonged and excessive Nrf2 activation may have detrimental effects, as suggested by the relationship between Nrf2 overexpression and cancer promotion or cancer cell protection from chemotherapy in lung cancer [68]. In fact, in chronic lung diseases, the association with aging lungs and COPD or IPF alterations could produce cancer development in the presence of high levels of Nrf2 activators. Other protocols of antioxidant’s administration reported that inhaled N acetylcysteine (NAC) monotherapy improves redox balance in IPF patients [69]. However, the only valid mechanism able to activate the Nrf2 pathway and the intracellular antioxidants generation is an “on and off” mechanism that could restore the oxidant/antioxidant balance and preserve the cells from the detrimental consequences of prolonged Nrf2 stimulation. For this purpose, particularly in the prevention of chronic lung diseases, it has been shown that regular exercise is one of the most successful interventions to prevent or delay chronic diseases. Aerobic exercise induces a number of biochemical signaling messages that result in changes in cell physiology, many of which are mediated by redox mechanisms. Calculated and transient redox stress caused by acute exercise increases Nrf2 activation in young animals and humans, thus improving cellular resistance to subsequent redox stressors [70, 71]. A significant increase in gene expression of Nrf2 and target SOD2 were shown in skeletal muscle of young fit males following an acute bout of cycling exercise lasting 90 min in normoxic recovery conditions. Mode, intensity, and duration of exercise could each impact the rate and amplitude of Nrf2 cycling in vivo [72]. Caloric restriction is also an endogenous Nrf2 activator, studies have shown that nutritional components may modulate the Nrf2-Keap1 system, so it may be of fundamental importance to demonstrate the beneficial effects of this system in various chronic diseases [73–75]. Other studies reported that during ozone therapy (major ozonated autohemotherapy) a transient and calculating stimulation of Nrf2 occurs [76]. In fact, ozone therapy is able to activate Nrf2 for the production of antioxidants and phase II enzymes and at the same time, it is unable to prolong this activity after about 40–60 min. This mechanism of transient Nrf2 stimulation could be very useful in the lung cells undergoing oxidative stress in chronic lung diseases for restoring a normal redox system without the risk of cellular proliferation subsequent to a continuous prolonged Nrf2 stimulation.
4. Conclusions

Experimental and clinical evidences strongly suggest that maintenance of a proper redox balance through regulation of the Nrf2/ARE pathway could be critical to the integrity and function of intracellular components in the respiratory system. It is clear that both development and progression of chronic lung diseases may be caused by an impairment of Nrf2 activation or excessive Nrf2 stimulation. The compounds that boost Nrf2 activity showed promising results but bioavailability and potential risks and benefits remain to be proved. An endogenous redox modulator like ozone, if correctly used, seems to be able to elicit a transient and calculate oxidative stress and enzymatic production of antioxidants and other detoxifying enzymes [77]. Exercise and caloric restriction are also possible indirect ways to stimulate the Nrf2/ARE pathway [78, 79]. Future studies are needed to decipher the intracellular signals involved in the activation of Nrf2/ARE- mediate gene transcription by oxidative stress and to evaluate their role in the development of chronic lung diseases.
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