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REVIEW

Oxidative stress in the pathogenesis of diffuse lung diseases: A review

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Summary
Oxidative stress is an imbalance between oxidants (reactive oxygen and nitrogen species) and antioxidants that may affect lipids, DNA, carbohydrates and proteins. The lung is continuously exposed to endogenous and exogenous oxidants (cigarette smoke, mineral dust, ozone, radiation). Reactive oxygen and nitrogen species are mainly produced by phagocytes as well as by polymorphonuclear, alveolar, bronchial and endothelial cells. A potential role of oxidative stress in the pathogenesis of diffuse lung diseases (particularly idiopathic pulmonary fibrosis) has been demonstrated. Increased oxidant levels and decreased antioxidant defences can contribute to the progression of idiopathic pulmonary fibrosis and other diffuse lung diseases.

The growing number of papers on the different aspects of oxidant/antioxidant imbalance in diffuse lung diseases in the last decade reflects increasing interest in this topic and suggests that specific DLDs may be characterized by specific patterns of oxidation and antioxidant responses. The study of oxidative stress can provide insights into etiopathogenesis and favour the discovery of new treatments. In this review of the literature on oxidants and antioxidants

Abbreviations: ROS, reactive oxygen species; RNS, reactive nitrogen species; BAL, bronchoalveolar lavage; Ssc, pulmonary fibrosis associated with systemic sclerosis; IPF, idiopathic pulmonary fibrosis; DLD, diffuse lung diseases; UIP, usual interstitial pneumonia; DIP, desquamative interstitial pneumonia; EAA, extrinsic allergic alveolitis; COPD, chronic obstructive pulmonary disease; DNPH, dinitrophenylhydrazine; NO, nitric oxide; CO_2, carbon dioxide; H_2O_2, hydrogen peroxide; FVC, forced vital capacity; MMPs, matrix metalloproteinases; EC-SOD, extracellular superoxide dismutase; t-PA, tissue-type plasminogen activator; u-PA, urokinase-type plasminogen activator; HO, heme oxygenase; NAC, *N*-acetylcysteine; PAI-1, plasminogen activator inhibitor 1; MAPK, mitogen-activated protein kinase; NRF2, nuclear factor-erythroid 2 p45 subunit-related factor 2; NF-kB, nuclear factor-kB; Keap1, Kelch-like enoyl-CoA hydratase-associated protein 1; TGF-beta, transforming growth factor beta.

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in diffuse lung diseases, the focus is on idiopathic pulmonary fibrosis, sarcoidosis, pneumoconiosis and pulmonary fibrosis associated with systemic sclerosis.

Contents

Introduction

Definition of diffuse lung diseases and oxidative stress

Diffuse lung diseases (DLD) are a heterogeneous group of lung disorders with different aetiologies that evolve towards pulmonary fibrosis of variable severity.[1–3] They are characterized by various pathogenetic mechanisms: sarcoidosis and lung diseases involving connective tissue are systemic immunoinflammatory diseases, whereas idiopathic pulmonary fibrosis (IPF) is a severe epithelial/fibroblastic disorder limited to the lung.[4,5] IPF is characterized by rapid progression to diffuse fibrosis which destroys lung parenchyma. The aetiology of this disease is still unknown, although there is evidence that cellular redox status and oxidative stress contribute to progression.[6–15] Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are free radicals generated physiologically during oxidative phosphorylation.[9,12,16] They have various physiological roles and are removed rapidly from the body: their persistence can cause cell dysfunction and cell death.[9] Defence mechanisms against oxidants involve enzyme and non-enzyme antioxidant systems. An imbalance between generation of ROS/RNS and antioxidant defences leads to a negative condition known as oxidative/nitrosative stress[7,9] in which cell antioxidants are insufficient to keep ROS/RNS below a toxic threshold due to excessive production of ROS/RNS and/or loss of cell antioxidant defences (Fig. 1).[7] Oxidative/nitrosative stress can affect proteins, lipids, carbohydrates and nucleic acids that are the main components of cells.[17–26]

Oxidizing agents can be produced endogenously by metabolic reactions (including activation of phagocytes or mitochondrial electron transport during respiration) or exogenously by cigarette smoking, toxins, pollution, radiation, drugs and other causes (Table 1).[20,25] During inflammatory processes, activated macrophages and neutrophils can release a great amount of hydrogen peroxide and superoxide via the phagocytic isoform of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The massive production of antimicrobial and tumoricidal ROS in an inflammatory environment plays an important role in bodily defences but when inflammation becomes chronic it induces persistent activation of macrophages and neutrophils that become a persistent source of oxidative damage of DNA and cell components.[25,26] Chronic inflammation-induced production of ROS/RNS in the lung may predispose individuals to lung diseases.[20,21] Activated macrophages and neutrophils dominate inflammatory responses in DLD and are particularly elevated in bronchoalveolar lavage (BAL) of patients with IPF, sarcoidosis and pulmonary fibrosis associated with systemic sclerosis (Ssc).[16,17,22]

In IPF, the pulmonary redox imbalance is due to an increase in oxidants associated with extracellular glutathione deficiency[7–9,13] which has been associated with disease progression (smokers with IPF are reported to have worse survival and severity-adjusted survival than non-smokers with IPF).[14] Very recently Daniil et al. determined oxidative burden in serum of IPF patients by a simple reproducible method based on analysis of total hydroperoxides.[15] They concluded that systemic oxidative stress levels were significantly higher in IPF patients than in controls and negatively correlated with lung function parameters (forced vital capacity and diffuse lung capacity for carbon monoxide) and dyspnoea severity.[15]

Imbalance between oxidants and antioxidants is a pathogenetic mechanism also recognized in other DLD, such as pulmonary fibrosis associated with systemic sclerosis (Ssc), sarcoidosis and pneumoconiosis. Oxidative stress is thought to induce progression in Ssc by interaction of oxygen radicals with vascular endothelium components and fibroblasts.[22–24] The description of oxidant/antioxidant balance in the lungs and evidence of its involvement in the pathogenesis of different DLDs is the main topic of the present review.

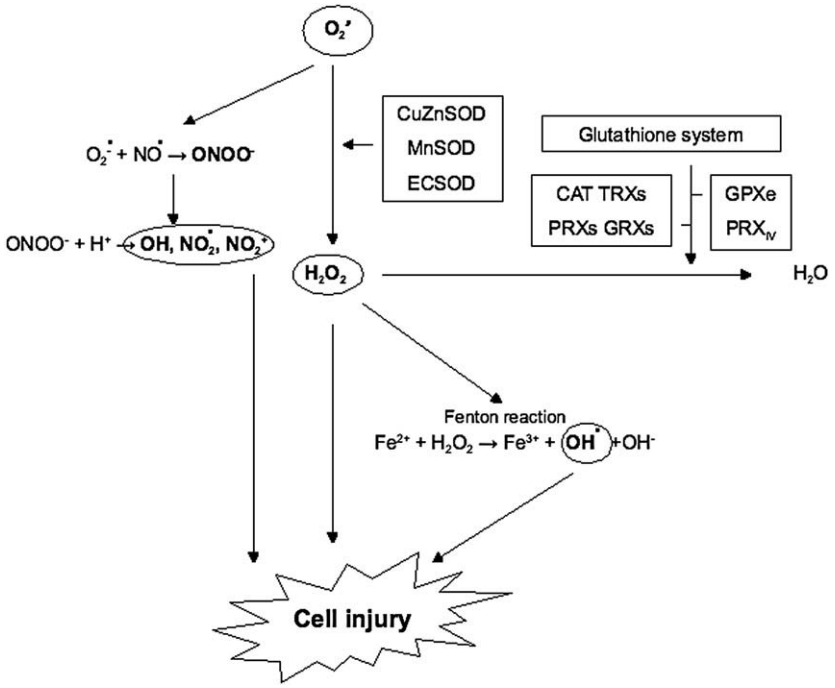


Figure 1 Reactions of superoxide ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), and nitric oxide ($NO^{\bullet}$) and cell injury. The activation of inflammatory cells results in generation of $NO^{\bullet}$ and other RNS by nitric oxide synthase. The major antioxidant enzymes scavenging superoxide and hydrogen peroxide are: superoxide dismutases (copper, zinc-SOD, manganese-SOD, extracellular-SOD); catalase (CAT), thioredoxins (TRXs), peroxyredoxins (PRXs), glutaredoxins (GRXs) [intracellular origin]; glutathione peroxidase (GPXe), peroxyredoxin IV (PRXIV) [extracellular origin], and glutathione antioxidant system [intra- and extracellular origins].

Oxidants and diffuse lung diseases

- Pathophysiological role of ROS and RNS
- Effects of ROS/RNS in the pathogenesis of DLD and BAL findings

Because of their anatomy, location and function, the lungs are highly susceptible to oxidative damage. The main ROS/RNS implicated in the oxidative-induced damage are listed in Table 2.[9] In non-phagocytic cells, mitochondria are the main source of ROS, but smooth endoplasmic reticulum and microsomes may also produce free radicals.[25] Moderate physiological levels of ROS are important to modulate cell functions such as apoptosis, gene expression, signal transduction and defence against pathogens.[26] Up to 1% of the mitochondrial electron flow leads to formation of superoxide anion under normal conditions. Any interference with electron transport can increase superoxide production.[9,25,26] Under physiological conditions reactive oxygen species induce secretion of mucus, remodel extracellular matrix and blood vessels, regulate expression of antiproteases and trigger alveolar repair responses, apoptosis and proliferation, modulating immunological responses in the lung.[9]

Reactive nitrogen species are oxidant molecules that may regulate cell survival. Nitric oxide (NO) is an endothelial vasorelaxant with many biochemical effects produced through activation of NO synthases.[19] Most NO-derived species interact with glutathione and other thiols, oxidizing them to nitrosated thiols. Peroxynitrite-mediated reactions are enhanced by carbon dioxide (CO_2).[19]

Table 1 Exogenous and endogenous sources of oxidants (ROS/RNS).

Exogenous sources	Endogenous sources
• Cigarette smoke	• Inflammatory cells
• Exogenous toxins	• Fibroblast
• Pollution	• Epithelial cells
• Hyperoxia	• Endothelial cells
• Radiation	• Respiratory chain
• Carcinogens	• Xantine and NADPH oxidase
• Drugs	

Table 2 Main reactive oxygen and nitrogen species.

Reactive oxygen species (ROS)	Reactive nitrogen species (RNS)
• Superoxide ($O_2^{-\bullet}$)	• Nitric oxide ($^{\bullet}NO$)
• Hydrogen peroxide (H_2O_2)	• Nitrogen dioxide ($^{\bullet}NO_2$)
• Hydroxyl radical ($HO^{\bullet}$)	• Nitrous acid (HNO_2)
• Peroxyl radical ($RO_2^{\bullet}$)	• Dinitrogen tetroxide (N_2O_4)
• Alkoxyl radical ($RO^{\bullet}$)	• Dinitrogen trioxide (N_2O_3)
• Hydroperoxyl radical ($HO_2^{\bullet}$)	• Peroxynitrite ($ONOO^-$)
• Singlet oxigen ($^1O_2$)	• Peroxynitrous acid (ONOOH)
• Ozone (O_3)	• Alkyl peroxynitrites (ROONO)
	• Nitronium cation (NO_2^+)
	• Nitryl chloride (NO_2Cl)

ROS/RNS released by phagocytes are involved in lung tissue damage in interstitial lung diseases.[9] Increased activation and abundance of inflammatory cells in smoking-related lung diseases, IPF and pneumoconiosis may explain the high levels of hydroxyl radical and superoxide anion concentrations in these diseases.[19,27] In 1987 it was demonstrated that neutrophils, macrophages and eosinophils from bronchoalveolar lavage of IPF patients overproduce ROS causing epithelial injury.[11] The crucial function of nitrosyl-induced stress in DLD and enhanced expression of inducible nitric oxide synthase in lung tissue of patients with silicosis, granulomatous lung diseases and histiocytosis was reported by several authors.[28,29] Oxidative-mediated lung and vascular injury has also been documented in patients with Ssc, who also showed elevated levels of oxidants: hydroperoxides are abundant in serum of Ssc patients and their concentrations are correlated with clinical parameters and microvascular impairment.[30] Rahman and MacNee demonstrated that increased ROS/RNS levels play a major role in inflammation through activation of transcription factors (i.e. nuclear factor-kB, NF-kB), signal transduction and gene expression of proinflammatory mediators.[31] ROS/RNS also regulate many proinflammatory and profibrotic cytokines involved in lung fibrogenesis and in tissue damage occurring in DLD (see the section ''Interactions of ROS/RNS with mediators of diffuse lung diseases'').[9]

The most widely studied oxidative stress-induced modification of proteins is the formation of carbonyl groups on amino acid residues. Carbonyls are considered a biomarker of oxidative stress and used to quantify oxidative damage in polypeptide chains.[17–21] Analysis of oxidative stress products in bronchoalveolar lavage demonstrated significantly higher carbonylated protein content in patients with diffuse lung diseases than in controls, with an imbalance between oxidants and antioxidants (Fig. 2).[22,32] Proteomic analysis of BAL protein targets of carbonylation in DLD revealed that IPF patients had more carbonylated proteins than sarcoidosis, Ssc patients and controls.[22] Carbonylation was a selective process involving certain proteins crucial in fibrogenesis that were oxidized in IPF but not in sarcoidosis or systemic sclerosis patients (Fig. 3).[22] Albumin, immunoglobulins and complement C3 were carbonylated in BAL of Ssc. In IPF patients, complement C3, transferrin, immunoglobulin light chains, immunoglobulin A and a group of six plasma proteins were the protein targets of carbonylation. In sarcoidosis patients, albumin, immunoglobulins, alpha1-antitrypsin, complement C3 were found to be oxidized.[22] These results and other evidence suggested different patterns of oxidation and antioxidant responses in different DLD. The specific pathogenetic mechanisms are associated with different inflammatory cells recruited in each DLD, resulting in carbonylation of different targets of oxidation that enables identification of oxidation profiles characteristic of the various diseases.[17,22] Some antioxidant enzymes and numerous proteolytic fragments of apolipoprotein A1, haptoglobin β, serotransferrin and α-1-antitrypsin have been observed specifically in BAL of IPF patients and not in other DLDs, indicating the high proteolytic activity typical of IPF.[17,18]

Elevated oxidative and nitrosative stress in idiopathic pulmonary fibrosis has also been demonstrated by increased levels of H_2O_2 in expired breath condensate,[33] increased eosinophilic mediators and myeloperoxidase concentrations in BAL,[34] enhanced levels of lipid peroxidation products (such as 8-isoprostane) in BAL and condensate[35] and elevated nitric oxide concentrations in exhaled breath.[34–39]

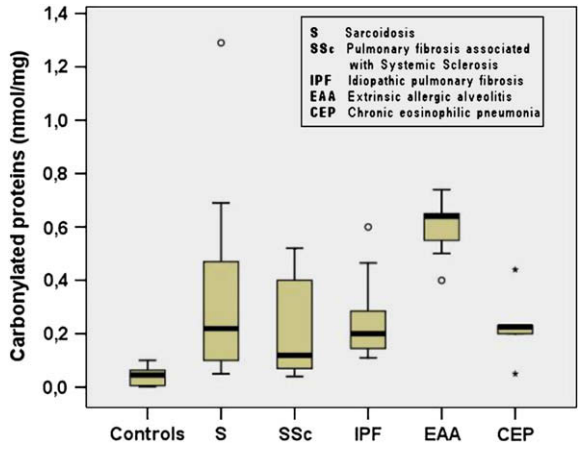


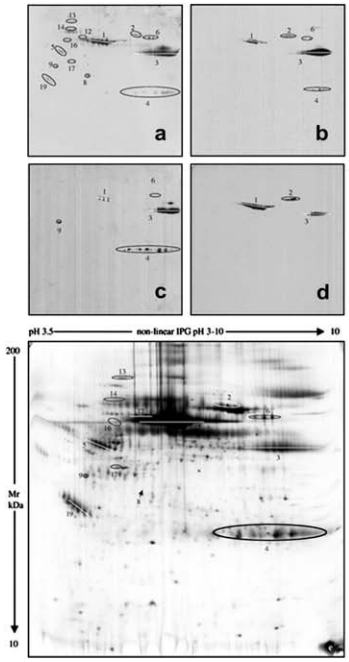
Figure 2 Carbonylated protein concentrations in bronchoalveolar lavage of patients with different interstitial lung diseases (S: sarcoidosis; SSc: pulmonary fibrosis associated with systemic sclerosis; IPF: idiopathic pulmonary fibrosis; EAA: extrinsic allergic alveolitis; CEP: chronic eosinophilic pneumonia) (see Ref. 39).

Interactions of ROS/RNS with the protease/antiprotease system

Extracellular matrix degradation mediated by ROS/RNS and protease/antiprotease system. Role of matrix metalloproteinases and plasminogen activator inhibitor 1

Altered mechanisms of extracellular matrix degradation in patients with DLD may lead to irreversible fibrotic lung damage. It has been demonstrated that the structural remodelling occurring in these diseases is mediated by matrix metalloproteinases (MMPs) and their specific inhibitors (tissue inhibitors of metalloproteases (TIMPs).[40] Protease and antiprotease expression in tissue and BAL of IPF patients suggests increases in different proteases, particularly matrix metalloproteinase-7 (or matrilysin) that contributes to lung epithelial damage in IPF.[23,40,41] Oxidant radicals participate in protease/antiprotease imbalance by activating MMPs through modification of their cysteine domain and inactivating TIMPs.[40,41] ROS can also directly induce MMP gene transcription.[40] ROS/RNS may also inactivate the protease system. MMP activation or inactivation is determined on the basis of local concentrations.[7,9,40] The main antioxidant enzymes inhibiting oxidative activation of MMPs are glutathione peroxidase and extracellular superoxide dismutase (EC-SOD).[42,43]

Degradation of the extracellular matrix is also triggered by plasmin. Activation of tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) is normally regulated by plasminogen activator



Spot Number	Carbonylated proteins	Sarcoidosis	Pulmonary fibrosis in SSc	Idiopathic pulmonary fibrosis	Controls
1	Albumin	3/3	3/3	3/3	3/3
2	Transferrin	-	3/3	3/3	3/3
3	Ig G heavy chian α	3/3	3/3	3/3	3/3
4	Ig light chian κ, λ	1/3	2/3	3/3	3/3
5	α1-antitrypsin	2/3	-	2/3	3/3
6	Complement C3 β	2/3	1/3	2/3	2/3
7	Haptoglobulin α	-	-	1/3	-
8	Haptoglobulin β (frag)	-	-	2/3	-
9	Complement C3	3/3	-	3/3	-
10	Superoxide dismutase	-	-	1/3	-
11	Transthyretin	-	-	1/3	-
12	Hemopexin	-	-	2/3	-
13	Ceruloplasmin	-	-	2/3	-
14	Immunoglobulin A S-chian	-	-	3/3	-
15	Immunoglobulin A heavy chian	-	-	-	1/3
16	α2-antiplasmin	-	-	2/3	-
17	Apolipoprotein A-IV	-	-	1/3	2/3
18	α1-antichymotrypsin	-	-	-	1/3
19	Pulmonary surfactant-associated protein A	-	-	1/3	1/3

Figure 3 Proteomic analysis of bronchoalveolar lavage from patients with idiopathic pulmonary fibrosis, sarcoidosis and pulmonary fibrosis associated with systemic sclerosis. On the right: list of carbonylated proteins observed by immunoblot analysis and their corresponding frequencies in healthy subjects and patients with sarcoidosis, Ssc and IPF. On the left: carbonylated proteins are indicated with arrows (see Ref. 22).

inhibitor 1 (PAI-1) that is crucial in controlling degradation of the extracellular matrix.[7] In IPF, abnormal repair of lung tissue may be the consequence of altered activation of PAI-1 activity, as recently reported by Liu.[44] Reactive oxygen species upregulate the transcription rate of the PAI-1 gene by activating mitogen-activated protein kinase pathways and/or the NF-kB pathway. PAI-1 plays a pivotal role in the development of lung fibrosis and ROS/RNS mediate induction of PAI-1 through different growth factors, cytokines, and other agents.[44] ROS/RNS are modulators of protease/antiprotease balance which contribute to extracellular matrix degradation, a key event in the pulmonary structural remodelling occurring in diffuse lung diseases.[7,9,23,40–44]

ROS/RNS regulation of apoptosis

- Apoptosis mediators
- Apoptosis in IPF
- Apoptosis induced by ROS/RNS in DLD

Apoptosis (programmed cell death) manifests through a highly conserved signalling pathway. It plays an important role in normal lung homeostasis, participating in the pathogenesis of a variety of lung diseases, including fibrotic lung disorders.[45–47]

Caspase family protein is crucial in apoptosis. Pro-caspases (inactive cytoplasmic forms) activated through extrinsic or intrinsic pathways can migrate to the nucleus, causing the ultimate breakdown of the cell.[45] The extrinsic pathway depends on activation of members of the TNF receptor superfamily (including Fas/CD95) that bind ligands (e.g. FasL) in the extracellular space, creating Fas-associated death domains (FADD) and activating caspases 8 and 10 and subsequently caspases 3 and 7 that promote apoptosis directly (Fig. 4).[45] The intrinsic pathway is due to internal cell stressors (such as DNA damage) that activate proapoptotic Bcl-2 proteins (e.g. Bax, Bac) and/or inactivate antiapoptotic Bcl-2 proteins (e.g. Bcl-2 itself) leading to mitochondrial release of cytochrome c, activation of caspase 9 and consequently caspase 3 and 7 that cause apoptosis (Fig. 4).[45] Activation of caspases may be inhibited by various molecules, including inhibitors of apoptosis IAP.[48]

Alveolar epithelial cell apoptosis by the Fas—FasL pathway has been documented in pulmonary fibrosis[46,47,49] in which proapoptotic mediators (p53, p21, Bax) and caspase 3 were found to be upregulated while Bcl-2 and other antiapoptotic molecules were downregulated.[50] TGF-beta 1 is an inductor of alveolar epithelial cell apoptosis through caspase activation, upregulation of p21 and downregulation of antiapoptotic Bcl-2[51]; this cytokine also inhibits fibroblast apoptotic phenomena in animal models of lung fibrosis and induces ROS/RNS production by fibroblasts.[52] In IPF, fibroblasts have been found resistant to Fas-mediated apoptosis due to an increase in Fas-activated FADD-like IL-1-converting enzyme-like inhibitory of apoptosis protein (FLIP).[53,54]

Kuwano et al. demonstrated an association between increased mitochondrial generation of reactive oxygen species and apoptosis of alveolar epithelial cells in IPF patients.[49] Loss of epithelial cells leads to destruction of alveolar basement membrane and recruitment of fibroblasts in situ to repair the damage by deposition of extracellular matrix. The resistance of fibroblasts to apoptosis

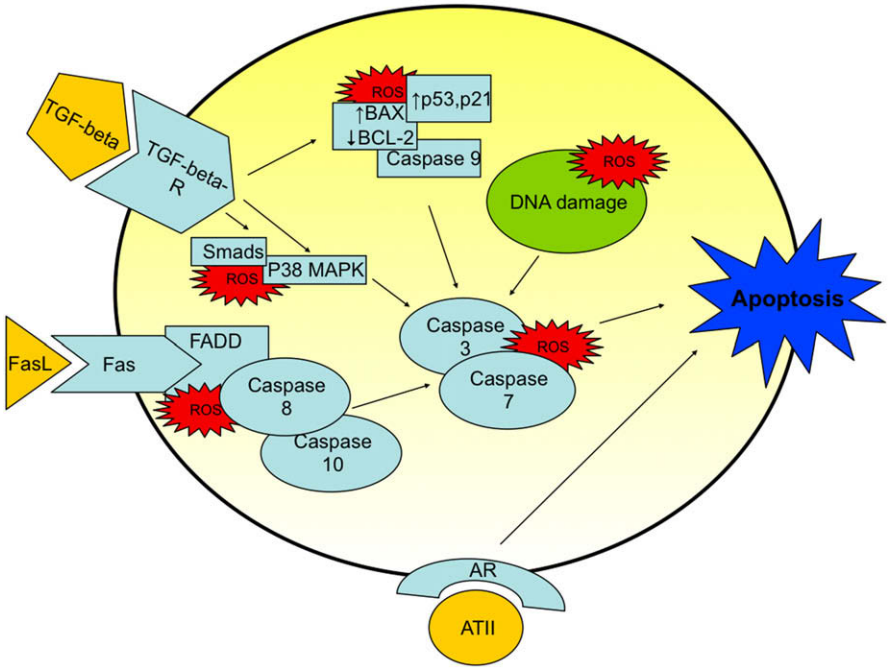


Figure 4 Apoptosis in alveolar epithelial cells of patients with idiopathic pulmonary fibrosis (IPF): intrinsic and extrinsic pathways and ROS involvement in apoptotic mechanisms. ROS = reactive oxygen species; p38 MAPK = p38 mitogen-activated protein kinase; Smads = mothers against decapentaplegia in *Drosophila* (*Mad*) gene and the related *Sma* gene in *Caenorlabditis elegans*; FasL = Fas ligand; Fas = apoptosis-mediating surface antigen (aka Apo-1 and CD95); FADD = Fas-associated death domain; BAX = BCL-2-associated protein; BCL-2 = B-cell lymphoma 2; ATII = angiotensin II; AR = angiotensin receptor (subtype I).

may determine persistent matrix deposition with consequent destruction of normal lung architecture.[45] ROS/RNS may upregulate cell death by interacting with caspase 3, inducing cytochrome c release from mitochondria, inducing DNA fragmentation and activation of mitogen-activated protein kinase (MAPK) pathway with subsequent activation of ERK, JNK and p38 MAPK (reviewed in Ref. 55). Stimulation of receptor tyrosine kinases or activation of protein kinase C-mediated pathways has also been associated with oxidant-induced apoptosis.[48] All these molecular alterations have been documented by enhanced apoptosis in lungs of IPF patients at bronchiolar and alveolar levels.[48]

Many intracellular signalling pathways implicated in ROS-mediated apoptosis are active in IPF.[47,48] Thioredoxin 1 (see the section ''Antioxidant protection and diffuse lung diseases'') is a radical scavenger, reported to protect the lungs of transgenic mice against hyperoxia-induced oxidative damage and to prevent apoptosis of alveolar epithelial cells by inducing upregulation of the antiapoptotic mediator Bcl-2.[56] ROS/RNS regulation of apoptosis in idiopathic pulmonary fibrosis is reported in Fig. 4.

Interactions of ROS/RNS with mediators of diffuse lung diseases

- TGF-β interactions with ROS/RNS in IPF
- Other cytokines associated with oxidative stress in DLD

Transforming growth factor-β (TGF-β) is an important profibrotic growth factor with several immunological functions, involved in the aberrant lung repair mechanism characteristic of many fibrotic lung disorders.[57–60] Transforming growth factor-β is synthesized as inactive precursor and secreted as part of a latent complex: L-TGF-β. Immediately after lung injury, this cytokine exerts its proinflammatory and chemotactic activities by recruiting myofibroblasts.[61] In advanced stages of injury (i.e. lung fibrosis) it participates in lung tissue repair, mediating abnormal remodelling.[7] This profibrotic growth factor is over-expressed in IPF tissues by type II epithelial cells, fibroblasts and alveolar macrophages.[57] The persistence of this cytokine enhances activation of immunoinflammatory cells, alters synthesis, deposition and turnover of matrix components and stimulates over-production of type I and type III collagen by different types of cells.[48,53] Myofibroblast activation and production of extracellular matrix in the lung may be the consequence of persistently elevated TGF-β levels.[7]
TGF-β may induce over-production of reactive oxygen species in IPF by activation of NADPH oxidase; at the same time ROS can activate latent TGF-beta 1, upregulating TGF-β expression.[62,63] Since TGF-beta is a potent inhibitor of glutathione synthesis, it also promotes the antioxidant deficit typical of IPF.[64] On the other hand, extracellular superoxide dismutase prevents lung injury and stabilizes extracellular matrix components, inactivating TGF-β.[65]

Oxidants/antioxidants can interact with different mediators of DLD; for example ROS generated by silica particles and by silica-activated cells can induce expression of several inflammatory cytokines, such as TNF-α, TGF-β and IL-1β, responsible for lung damage and progression of fibrotic disease.[66,67] Some authors also report correlations

between oxidative burst and concentrations of certain cytokines in different DLD. Lenz and coworkers reported an inverse correlation between BAL carbonyl levels and INF-γ concentrations in sarcoidosis and a low IL-10/IL-8 mRNA ratio in BAL cells of IPF patient.[23] IL12 is a Th1 proinflammatory cytokine that is elevated in Sjogren Syndrome. Overexpression of IL12 in lung tissues of mice has been associated with increased oxidative stress (evaluated by nitrotyrosine staining).[68]

Antioxidant protection and diffuse lung diseases

- Main non-enzymic antioxidants of the lungs and DLD
- Main antioxidant enzymes in health and DLD and the regulatory function of NRF2

1. Superoxide dismutases (SODs):
   - Extracellular superoxide dismutase (EC-SOD)
   - Copper/zinc superoxide dismutase (Cu/Zn-SOD)
   - Manganese superoxide dismutase (Mn-SOD)
2. Catalase
3. Glutathione and glutathione peroxidase
4. Peroxyredoxins
5. Thioredoxins
6. Glutaredoxins
7. Heme oxygenases

Certain low molecular weight compounds exert antioxidant functions under physiological conditions. Non-enzymic molecules involved in defence against oxidants include vitamins, such as vitamin C and vitamin E, beta carotene, retinol, uric acid and glutathione (see below).[9] The antioxidant properties of vitamin E on bleomycin-induced lung fibrosis have been documented. Infusion of high doses of vitamin E considerably reduces the fibrotic effects of bleomycin on mouse lung tissue by reducing the hydroxyproline/soluble protein ratio.[69] A more recent research showed an inverse correlation between mortality risk and plasma concentrations of retinol and vitamin E in a population of patients with asbestosis.[70]

The lungs are protected from the negative effects of oxidant persistence in tissues by endogenous agents named antioxidants. Antioxidants may be non-enzyme or enzyme proteins. The former include glutathione, vitamins (alpha-tocopherol and ascorbic acid), beta carotene and uric acid; the latter, superoxide dismutases, catalases and peroxidases.[9,71] Proteins crucial in cellular antioxidant defences are: peroxyredoxins, thioredoxins, glutaredoxins and heme oxygenases.[9,17,18] Here we describe the principal antioxidant enzymes in the lungs and discuss their potential involvement in diffuse lung diseases.

Superoxide dismutases (SODs)

Extracellular-SOD (EC-SOD), copper/zinc-SOD (Cu/Zn-SOD) and manganese-SOD (Mn-SOD) are different superoxide dismutases that protect the lungs against oxidative stress by reducing superoxide anion to hydrogen peroxide, which is then converted to water by catalase and glutathione peroxidase.[9] The role of SODs in the pathogenesis of chronic inflammatory lung disorders has been widely described in the recent literature.[65,72,73]

EC-SOD is a slightly hydrophobic glycoprotein that binds cell surfaces and matrix components. Expression of EC-SOD is cell- and tissue-specific and is found predominantly in lungs, heart, blood vessels, placenta and kidneys. In normal lung tissue, EC-SOD is expressed by alveolar macrophages, bronchial epithelium, vascular endothelium, extracellular matrix and epithelial cells.[9]

EC-SOD normally protects the lungs against fibrosis by preventing oxidative degradation of the matrix and by binding type I and type IV collagen via its heparin/matrix binding domain.[65] Its expression has been evaluated in lung tissue samples from patients with extrinsic allergic alveolitis (EAA), sarcoidosis, IPF, desquamative interstitial pneumonia (DIP) and chronic obstructive pulmonary disease (COPD).[9,74] The authors demonstrated lower immunoreactivity for EC-SOD in DLD patients than controls, suggesting the possibility of its involvement in the pathogenesis of DLD.[74] In IPF, EC-SOD is practically absent in fibrotic areas and fibroblast foci, suggesting that it is depleted where the oxidative burst is particularly strong.[74] The prospect that EC-SOD is downregulated in IPF while its copper/zinc and manganese forms are upregulated in lung diseases is an intriguing topic.[74,75] Its decrease in IPF could be due to the extremely high concentrations of oxidants in this severe DLD and also to EC-SOD localisation in the extracellular matrix and cell surface, trigger points for oxidative-mediated lung damage in IPF.[39,65,75] In IPF, many matrix components are sensitive to oxidative modification/degradation and a significantly elevated turnover of extracellular matrix has been reported; EC-SOD reduction is therefore clearly associated with increased risk of oxidative stress-mediated matrix degradation in this disease.[65]

Copper/zinc superoxide dismutase is located in epithelial cells, fibroblasts and alveolar macrophages.[9] Its main function is to scavenge O_2^-, inhibiting redox generation of ROS and RNS.[9] Positive immunoreactivity of this enzyme has been documented in bronchial epithelium of sarcoidosis and extrinsic allergic alveolitis patients.[76] Although the exact pathogenetic role of this enzyme in oxidative-mediated lung injury is still unclear, its concentrations in serum from idiopathic pulmonary fibrosis patients have been found elevated with respect to controls.[72]

Manganese superoxide dismutase is an 88-kDa protein expressed by polymorphonuclear cells, bronchial epithelial cells, endothelial cells and pneumocytes.[77] Gene expression of this protein is stimulated by increased exogenous and endogenous oxidants in the airways. Increased concentrations of Mn-SOD were reported by Harju and colleagues in healthy smokers.[75] Chang et al. evaluated expression of Mn-SOD in lung tissue of rats exposed to hyperoxia.[76]

Mn-SOD is over-expressed by alveolar macrophages in sarcoidosis and extrinsic allergic alveolitis granulomas through cytokine-mediated induction during granuloma formation.[78,79] In lung tissue of IPF patients, increased expression of Mn-SOD is reported in type II pneumocytes and alveolar macrophages.[79] Mild expression of Mn-SOD is described in alveolar type II epithelial cells and macrophages from DIP samples.[72,79]

Catalase

Catalase is a 240-kDa protein mainly found in macrophages, pneumocytes and lung fibroblasts. It exerts its antioxidant

function by reducing hydrogen peroxide (produced by SODs) to water. The real involvement of this enzyme in oxidative lung damage is still unclear and controversial data is available on oxidant-mediated catalase production.[9]

In 2000 Lakari et al. evaluated catalase and Mn-SOD expression by immunohistochemistry in lung tissue from patients with IPF, DIP, EAA and sarcoidosis.[79] Catalase and Mn-SOD were expressed in alveolar regions of DIP and usual interstitial pneumonia (UIP) and in granulomas of sarcoidosis and extrinsic allergic alveolitis, suggesting a protective role against progression of diffuse lung diseases.[79] The role of catalase, SODs and antioxidant enzymes in the pathogenesis of Ssc was recently reviewed by Gabrielli et al.[80] A significant reduction in catalase and SOD levels in blood of Ssc patients has been reported.[16,80]

Glutathione and glutathione peroxidase

The physiological antioxidant role of this tetrameric protein is much better described than that of catalase.[9] In normal conditions, bronchial epithelial cells, alveolar macrophages and other cell lines synthesize glutathione, that it is generally abundant in epithelial lining fluid of normal lungs. Its main function is to convert hydroperoxides to less dangerous hydroxides, protecting cells against oxidative damage (Fig. 5).[9] Glutathione peroxidases are a family of enzymes including three selenium-dependent enzymes and one selenium-independent antioxidant peroxidase. The glutathione peroxidases are divided into cellular and extracellular forms.[9] The extracellular form of selenium-dependent glutathione peroxidase is mainly located in epithelial lining fluid together with glutathione and is produced by bronchial epithelial cells and alveolar macrophages.[9] Its main function is to protect alveolar epithelial cells against oxidative stress, such as hyperoxia-induced lung damage.[9] The activity of the extracellular glutathione peroxidase appears to be enhanced in sputum from patients with cystic fibrosis.[81]

Glutathione and glutathione peroxidase levels are elevated in various granulomatous lung diseases, including chronic beryllium disease.[82] Glutathione metabolism is altered in IPF: in this disease BAL glutathione levels are lower than in controls and administration of *N*-acetylcysteine (NAC) stimulates glutathione synthesis, partially restoring glutathione levels at alveolar level.[83–86] In vivo experiments in bleomycin-induced lung fibrosis have confirmed that *N*-acetylcysteine reduces the primary inflammatory events, preventing cell damage and development of IPF.[87]

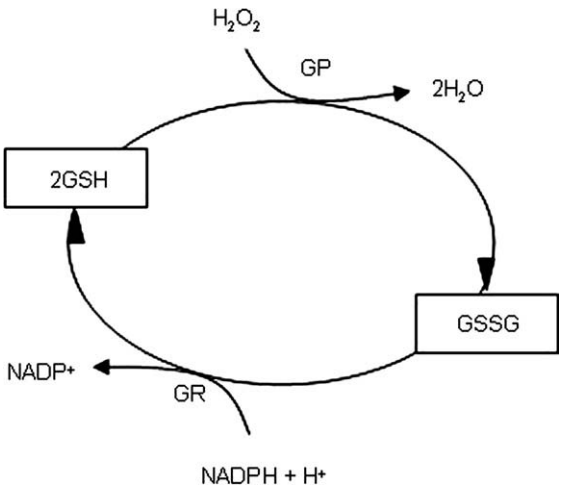


Figure 5 Glutathione cycle. Glutathione peroxidase (GP) catalyzes oxidation of glutathione (GSH) to its oxidized form (GSSG), scavenging hydrogen peroxide (H_2O_2). GSSG is reconverted to GSH by glutathione reductase (GR).

Peroxyredoxins

Peroxyredoxins are a group of antioxidant enzymes involved in the breakdown of hydrogen peroxide. Six different types of peroxyredoxins (I–VI) have been characterized in human lungs. They protect lungs against oxidative stress by reducing a wide spectrum of peroxides.[8] Peroxyredoxin 1 is mainly expressed by bronchial epithelial cells and alveolar macrophages. Peroxyredoxin 2 is expressed by alveolar type II cells and is associated with platelet-derived growth factor (PDGF) signalling and cell proliferation.[56] The peroxyredoxins mainly expressed in the lungs are peroxyredoxins 3, 5 and 7, typically found in bronchial and alveolar epithelial cells and macrophages.[8,9] Increased expression of different peroxyredoxins has been reported in lung cancer, alveolitis and hypoxic conditions.[9] The potential role of peroxyredoxins in the pathogenesis of diffuse lung diseases is still unclear. Recently Vuorinen and colleagues observed that peroxyredoxin 2 was mainly localised in lung epithelium and alveolar macrophages of IPF patients with very low expression by cells of fibroblastic foci.[88]

Thioredoxins

Bronchial epithelium and alveolar macrophages are the main sources of thioredoxins in the lungs. This group of oxido-reductases (derived from the flavoprotein family) is composed of thioredoxin and thioredoxin reductase components.[9,89] The major functions of these enzymes are regulation of cell proliferation in the presence of NADP–NADPH, chemotactic activity, suppression of leukocyte infiltration into sites of inflammation, NF-kB activation and protection of tissues against exogenous oxidants.[89] Thioredoxin has been observed to reduce the viscosity of sputum in cystic fibrosis patients and airway inflammation and hyper-reactivity in asthmatics.[90,91] Expression of these enzymes in lung tissue of patients with DLD was analysed by immunohistochemistry by Tiitto et al.[92] Thioredoxin and thioredoxin reductase were highly concentrated in areas of metaplastic epithelium from IPF patients (but almost absent in fibrotic areas), in alveolar macrophages from DIP patients and in granulomas of sarcoidosis patients, suggesting involvement of thioredoxins in ongoing cell regeneration and inflammation.[92] Thioredoxin-transgenic mice and mice receiving thioredoxin treatment showed decreased bleomycin-induced cellular infiltrates and fibrotic changes in lung tissue in the bleomycin-induced lung fibrosis model.[9,89] Kinnula et al. reported that thioredoxins and thioredoxin reductase were expressed in metaplastic epithelium from UIP tissue samples but were absent in areas of active fibrosis, suggesting elevated oxidant generation in IPF.[8]

Glutaredoxins

Glutaredoxins (Grx1 cytosolic, Grx2 mitochondrial) preserve the ideal redox state of cells by reducing protein

disulphides to sulphydryls. They are expressed abundantly by alveolar cells, bronchial epithelial cells and lung macrophages.[8,93] The large quantity of Grx1 in alveolar macrophages of healthy lung tissue suggests a fundamental role of this protein in the physiological regulation of oxidant/antioxidant balance. Several cytokines activate Grx1 expression and TGF-beta downregulates its expression, as demonstrated in vivo in experiments with interstitial lung diseases.[93] In pulmonary sarcoidosis, allergic alveolitis and lung fibrosis, expression of glutaredoxins is much reduced with consequent loss of antioxidant defences.[94]

Heme oxygenases

Heme oxygenase-1 (HO-1) is a 30-kDa protein that exerts antiapoptotic, anti-inflammatory and antioxidant activities by converting heme to carbon monoxide and biliverdin. Alveolar macrophages, bronchial epithelial cells and inflammatory cells release HO-1 under different stimuli.[95–97] Heme oxygenase knockout mice are very sensitive to oxidative stress.[96] HO-1 is an inducible isoform of heme oxygenase thought to be an oxidative stress-responsive protein. It has been found upregulated in smokers and patients with asthma, acute lung injury, cystic fibrosis or chronic rejection of lung transplant, and downregulated in severe chronic obstructive pulmonary disease.[9,95–97] Protective effects of HO-1 have also been proposed in asbestosis and silicosis.[97] Sato et al. reported that it attenuates chronic silicosis in mice and humans by suppressing ROS activities; its expression was found enhanced in this disease and the enzyme may therefore be regarded as a potential marker of disease severity.[97] There is evidence that HO is a heat-shock antioxidant protein mainly expressed in the inflammatory phases of DLD.[9] An interesting study by Ye et al. showed that HO-1 was depleted in BAL cells of IPF patients, supporting the hypothesis that an oxidative imbalance is responsible for progression of IPF.[95] Protective functions of HO-1 in the respiratory system were reviewed by Ryter et al. in 2007.[96] The anti-inflammatory cytokine IL-10, hyperoxia, exogenous nitric oxide, diesel exhaust particles and quinones are stimuli that induce HO-1 expression.[98] Molecules inhibiting HO-1 expression and activity include proinflammatory cytokines (IL-1, IL-17, TNF-α) and zinc-deuteroporphyrin.[99] The expression of HO-1, thioredoxins and other antioxidant enzymes is regulated by nuclear factor-erythroid 2 p45 subunit-related factor 2 (NRF2), a transcriptional factor activating antioxidant responses. Its function is essential for defence against ROS/RNS damage.[100] NRF2 may be suppressed by the enzyme Kelch-like enoyl-CoA hydratase-associated protein 1 (Keap1) that sequesters NRF2 in cytoplasm by creating an inactive Keap1/NRF2 complex. In response to oxidative stress, NRF2 (dissociated from Keap1) binds specific antioxidant elements in antioxidant enzyme promoter and defence protein genes and regulates their expression in many tissues.[100,101] In this way NRF2 protects against oxidant-mediated lung injury and influences susceptibility to IPF.[43] Studies on animal models have shown that NRF2 protects against fibrogenesis: mouse models, knockout for NRF2, showed significantly reduced antioxidant activities and enhanced susceptibility to bleomycin-induced lung fibrosis.[43]

Conclusion

- Oxidative stress in the pathogenesis of DLD
- Future therapeutic approach

Oxygen-derived free radicals produced by phagocytes are believed to contribute to lung tissue damage occurring in DLD. A lung defence mechanism against oxidative stress produces antioxidant molecules. Bronchoalveolar lavage protein composition shows different protein profiles with different levels of antioxidant proteins in sarcoidosis, IPF and Ssc.[17,102] Different pathways counteracting oxidative-mediated tissue damage have therefore been hypothesised for different lung pathogenesis.[22] Detailed analysis of oxidant and antioxidant molecules in DLD corroborates the hypothesis that different DLD have specific patterns of oxidant-mediated lung injury and antioxidant defences.[22] However, oxidant/antioxidant imbalance has been thoroughly investigated for some DLD (such as IPF) while very little data is available for others. Better knowledge of the antioxidant enzymes involved in the regulation of cell redox status in DLD could enable potential new biomarkers of lung disease severity and activity to be identified, just as heme oxygenase-1 was found to be a biomarker of silicosis.[96] The study of oxidative stress in the pathogenesis of DLD could open new therapeutic horizons. Recent attempts to correct oxidant/antioxidant imbalance in DLD[83–86,94,103] showed that high doses of antioxidants (which can prevent epithelial cell damage by oxygen radicals) could reverse extracellular glutathione deficiency and oxidative damage in patients with IPF. As oxidative stress has been associated with the pathogenesis of DLD such as sarcoidosis, pulmonary fibrosis associated with systemic sclerosis and pneumoconiosis, novel clinical trials to verify the effectiveness of antioxidants in these diseases are urgently required.

In conclusion, research into oxidant/antioxidant balance in diffuse lung diseases is a promising field that can provide insights into pathogenetic mechanisms and open new therapeutic perspectives.

Conflict of interest

The authors declare that they have no conflicts of interest related to the present article.

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