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Antioxidants as Potential Therapeutics for Lung Fibrosis

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Abstract

Interstitial lung disease encompasses a large group of chronic lung disorders associated with excessive tissue remodeling, scarring, and fibrosis. The evidence of a redox imbalance in lung fibrosis is substantial, and the rationale for testing antioxidants as potential new therapeutics for lung fibrosis is appealing. Current animal models of lung fibrosis have clear involvement of ROS in their pathogenesis. New classes of antioxidant agents divided into catalytic antioxidant mimetics and antioxidant scavengers are being developed. The catalytic antioxidant class is based on endogenous antioxidant enzymes and includes the manganese-containing macrocyclics, porphyrins, salens, and the non–metal-containing nitroxides. The antioxidant scavenging class is based on endogenous antioxidant molecules and includes the vitamin E analogues, thiols, lazaroids, and polyphenolic agents. Numerous studies have shown oxidative stress to be associated with many interstitial lung diseases and that these agents are effective in attenuating fibroproliferative responses in the lung of animals and humans.

LUNG FIBROSIS

Classifications

Human lung fibrosis has been described histopathologically as a group of interstitial pneumonias including usual interstitial pneumonia (UIP), also known as idiopathic lung fibrosis (IPF), desquamative interstitial pneumonia (DIP), respiratory bronchiolitis interstitial lung disease (RB), lymphoid interstitial pneumonia (LIP), cryptogenic organizing pneumonia (OP), diffuse alveolar damage (DAD) or acute interstitial pneumonia (AIP), and nonspecific interstitial pneumonia (NSIP) (108). A large variation is found between the prognosis of the different interstitial pneumonias. NSIP, DIP, RB, and LIP have a 5-year mortality of less than 10% and are usually responsive to corticosteroid treatment. IPF and AIP have poor prognosis with a 5-year mortality >60% and are generally unresponsive to treatment. Some evidence supports a spectrum of inflammatory and fibrotic mechanisms in interstitial pneumonias (IPs), in which the IP forms that are most responsive to corticosteroids are more inflammatory and the unresponsive IP forms are more fibrotic (199).

IPF is one of the most devastating forms of lung fibrosis and is progressive and fatal (173). IPF occurs most often in people older than 50 years, with a higher occurrence in men than in women and an overall incidence of 13 to 20/100,000 individuals (47). IPF is generally nonresponsive to conventional anti-inflammatory and immunomodulatory therapy and currently is in need of new therapeutic approaches (214). IPF patients are thought to respond poorly to antiinflammatory therapies because little inflammation exists at advanced stages of the disease and the fibrosis is driven by a dysregulated repair process with a loss of epithelial cells and accumulation of mesenchymal cells (199).

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Pathogenesis

The pathogenesis of lung fibrosis is complex and is thought to involve a number of processes that lead to an altered alveolar environment and to an abnormal repair process with accumulated fibrosis. Several factors, including age, genetic susceptibility, and environmental agents, are known to contribute to lung fibrosis (13,127,219,222). An increased interest exists in mesenchymal cells and, in particular, in the fibroblastic foci that are associated with disease progression (102). These fibroblastic foci are also associated with increased levels of active transforming growth factor beta (TGF-β) in the fibrotic lung. This cytokine is an important mediator of fibroblast differentiation into the myofibroblast phenotype (81). Overex-pression of active TGF-β produces lung fibrosis in animals (180). TGF-β is a major regulator of wound repair and a stimulant of reactive oxygen species (ROS) production in fibroblasts (212). Oxidative stress is often defined as an imbalance between ROS production and antioxidant defenses. Oxidative stress can dysregulate cell signaling (158) and is a potential target for the development of therapeutics to treat lung fibrosis (110).

OXIDATIVE STRESS AND LUNG FIBROSIS

Reactive oxygen species and antioxidant defenses

Part of the altered alveolar environment in lung fibrosis involves oxidative stress that is driven by an imbalance between oxidant production and antioxidant defenses. Reactive oxygen species (ROS) are normal byproducts of cellular metabolism and are continually produced at low levels under basal conditions. Biologically, the ROS superoxide (O_2^-) is commonly generated from the uncoupling of the cellular electron-transport systems (131). Electron-transport systems that account for a large portion of cellular O_2^- formation are the mitochondrial electron-transport system (21) and various oxidases including NADPH oxidases (35), cytochrome P450 monoxygenases (33), cyclooxygenases (115), lipoxygenases (115), nitric oxide synthases (114), and xanthine oxidoreductase (130). O_2^- can also rapidly react with nitric oxide (NO) to form the strong oxidizing and nitrating agent, peroxynitrite ($ONOO^-$). The ROS hydrogen peroxide (H_2O_2) is generated directly from O_2^- through a rapid dismutation reaction that can occur either enzymatically with superoxide dismutases (SODs, second-order rate constant of 10^9 M/sec) or spontaneously (second-order rate constant of 10^5 M/sec). This means that wherever O_2^- is generated, formation of H_2O_2 also occurs. In addition, H_2O_2 is formed enzymatically as a byproduct of lipid metabolism in peroxisomes (160). H_2O_2 is stable at biologic pH and easily crosses lipid membranes. H_2O_2 can participate in hydroxyl radical ($HO^{\cdot}$) formation in the presence of metals (78). H_2O_2 readily reacts with thiol functional groups, and this type of reaction is proposed to be a key mechanism by which ROS modulate cell-signaling events (62).

The impact of ROS may be especially important in the lung because of its large surface area and its exposure to higher oxygen levels than other tissues. The lung counters this with a formable array of antioxidant defense systems, starting with high levels of antioxidants in the epithelial lining fluid (ELF). Glutathione (GSH) is a major water-soluble antioxidant thiol in the lung ELF (27), and its levels are lower in subjects with IPF (26). In addition to GSH, the lung ELF contains other antioxidants such as ascorbate, urate, albumin, mucins, and metal-binding proteins, which are all effective at limiting oxidative damage. The lung also has a number of antioxidant enzyme systems including SODs, catalase, glutathione peroxidases (GPxs), thioredoxin, glutaredoxin, and peroxiredoxins (155). Overexpression of many of these antioxidant enzyme systems is protective against lung fibrosis (74,99,150). Many of these antioxidant systems are upregulated during lung fibrosis *via* the Nrf2 redox-sensitive transcription factor (95), that when deficient, enhances lung fibrotic responses (36). It is likely that inadequate antioxidant adaptive responses play a key role in lung fibrosis.

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Oxidative stress and disruption of cell signaling

Several phosphatases contain sensitive thiol residues that are inhibited on oxidation (31). The inactivation of phosphatases is often associated with a perceived activation of their respective kinase(s), many of which play prominent roles in inflammatory responses (154). The phosphorylation state of a protein is a steady-state level set by the relative rates of kinases and phosphatases. As steady-state levels of oxidants increase, an increase occurs in the inactivation of phosphatases and a corresponding increase in the levels and duration of phosphorylated proteins (73,189). An increase in steady-state ROS results in altering the cellular glutathione (GSH)/glutathione disulfide (GSSG) redox couple, which can in turn alter the relative oxidation status of protein thiols. In addition, H_2O_2 can alter cell signaling directly by reacting with specific thiols on transcription factors, such as reducing factor-1, activator protein-1, and inhibiting factor-κB (89,103). Many of these types of mechanisms may contribute to the often observed unregulated repair responses associated with lung fibrosis (Fig. 1).

Evidence of oxidative stress in the pathogenesis of lung fibrosis

When ROS production and antioxidant defenses are mismatched, an increase in ROS steady-state levels leads to an increase in the oxidation of cellular macromolecules. ROS are difficult to measure directly and often are assessed by measuring oxidative footprints in fluids and tissues, such as markers of protein, lipid, and DNA oxidation. Subjects with IPF have increased levels of oxidized proteins in their ELF that correlate with the percentage of neutrophils in the ELF (15,121,166). IPF subjects also have lower antioxidant capacity in their ELF than do healthy subjects (157). IPF subjects have higher levels of exhaled ethane (a marker of lipid peroxidation) than do normal subjects, and these levels are inversely correlated with PaO_2 (100). Breath condensate has been investigated as a noninvasive method to assess lung oxidative stress, and IPF subjects have higher levels of H_2O_2 and isoprostanes than do control subjects (151). Evidence also exists of increased nitrosative stress in IPF subjects (196). These data support the concept of increased oxidant formation with decreased antioxidant capacity in IPF, which is the hallmark of oxidative stress and the rationale for antioxidant therapy (Table 1).

ANTIOXIDANTS

Antioxidants have been defined many different ways, and in a very broad sense, they are agents that decrease steady-state ROS levels and protect cellular macromolecules from oxidative modification. The mechanisms by which this is achieved are many, and some are even paradoxic. For instance, some agents can produce a mild oxidative stress that results in a cellular adaptive response that increases endogenous antioxidant defenses. Some agents may inhibit cellular sources of ROS. A classic antioxidant is an agent that can rapidly react with ROS, producing less-reactive species. Catalytic antioxidants are not consumed in the reaction and are regenerated. Regardless of the mechanisms, antioxidants decrease oxidative stress and restore redox balance in biologic systems.

Catalytic antioxidant mimetics

Endogenous antioxidant enzymes are examples of catalytic antioxidants and have been used as models for the development of catalytic antioxidant mimetics. The three most prominent classes are the SOD, catalase, and GPx mimics. Most of these compounds contain a ligated transitional metal or selenium. They are generally broad-spectrum antioxidants that can scavenge O_2^-, H_2O_2, $ONOO^-$, and a variety of lipid peroxides. The SOD and catalase mimic class include macrocyclics, metallo-porphyrins, salens, and nitroxides. The GPx class includes selenium- and tellurium-based compounds.

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The potencies of these catalytic antioxidants are often compared by using their rate constants obtained under tightly defined simple chemical systems, which may or may not be relevant in more complex biologic systems. Another important note is that many of these agents can obtain electrons from cellular sources (54). These properties have two important consequences that affect the *in vivo* rate constant for the reaction with ROS and can also result in the inhibition of ROS production. The finding that many of these diverse compounds are effective in similar oxidative stress models confirms the basic concept that small, efficient, catalytic antioxidants show promise in the treatment of ROS-mediated conditions associated with injury and tissue dysfunction.

The pentaazamacrocyclic ligand-based mimetics [M40403 is currently being developed by ActivBiotics (http://www.activbiotics.com)] are unique in that they are relatively specific O_2^- scavengers, because the manganese atom (Mn) is held by five coordination points in the macrocyclic structure and is available only for one-electron transfers (Fig. 2). Mn(II) macrocylics function in the dismutation reaction with O_2^- by alternate oxidation and reduction, changing its valence between Mn(II) and Mn(III) (11). This unique aspect of these compounds gives them selectivity toward O_2^- under highly defined conditions. However, in biologic systems, it is unclear whether it is only with O_2^- that these compounds interact. A number of endogenous compounds also can partake in one-electron reactions beside O_2^-; these include flavins and ubiquinones. The macrocyclics are effective in many of the same oxidative paradigms in which nonselective catalytic antioxidant mimetics have been used. The different classes of catalytic antioxidant mimetics have not been directly compared in experimental models; therefore, the conditions under which one class holds an advantage over the others are currently not known.

Metalloporphyrins [AEOL series is currently being developed by Aeolus Pharmaceuticals (http://www.aeoluspharma.com)] are structurally different from endogenous protoporphyrins and are classified as synthetic *meso*-substituted porphyrins (Fig. 3). Metalloporphyrins have been shown to possess at least four distinct antioxidant properties, which include scavenging O_2^- (149), H_2O_2 (53), $ONOO^-$ (191), and lipid peroxides (51). Most metalloporphyrins contain either an Fe or Mn that is coordinated by four nitrogen axial ligands. The catalase-like activity of metalloporphyrins is thought to be due to their extensive conjugated ring system that can undergo reversible one-electron transfer in addition to the one-electron transfer on the metal center (70). This mechanism is similar to that proposed for the heme prosthetic groups of endogenous catalase and peroxidases. The two classes of metalloporphyrins include one group in which the SOD activities track with their catalase activities, and another group that has very little SOD activity and high catalase activity. An example of a manganese porphyrin with both high SOD and catalase-like activities is AEOL 10150 (98), whereas an example of a compound with low SOD activity and high catalase activity is AEOL 11207 (122). The compounds with high catalase-like activity still only possess a fraction of the native catalase enzyme activity under chemically defined conditions, yet they can protect cells from H_2O_2-mediated toxicity (53). This may not be a fair comparison because catalase is hard to saturate with H_2O_2 and has a relative high k_m for H_2O_2. Under biologically relevant steady-state levels of H_2O_2, the metalloporphyrins are more comparable to catalase (32). Metalloporphyrins have been shown to be effective in ameliorating oxidative stress, inflammation, and injury in a large number of animal models of human disease (50). Metalloporphyrins have plasma half-lives that range from 4 to 48 hours. Most metalloporphyrins are not extensively metabolized by the body and are largely excreted unchanged in the urine. A previous limitation of the metalloporphyrin class of compounds has been poor oral bioavailability, but several compounds in the AEOL 112 series have good oral bioavailability and longer plasma half-lives that should make them better candidates for treating chronic diseases (122).

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The salen class of catalytic antioxidant mimetics (EUK series) is currently being developed by Proteome Systems (http://www.proteomesystems.com) (Fig. 4). Generically, salens are aromatic, substituted ethylenediamine metal complexes. The Mn(III)-containing salen complexes have both O_2^- and H_2O_2 dismutation activities (63). However, like the metalloporphyrins, these compounds are not selective and can react with O_2^- and other peroxides, including $ONOO^-$ (178). The Mn moiety of the salen is coordinated by four axial ligands. One of the unique features of these compounds is that the metal center is coordinated to oxygen and nitrogen atoms, which is in contrast to the porphyrins, in which the metal is coordinated to nitrogen atoms. The coordination of Mn by four axial ligands results in the formation of several possible valance states that give these compounds their broad ROS-scavenging capabilities. The rates at which reported salens scavenge H_2O_2 are similar to those reported for metalloporphyrins, but are many orders less than those documented for catalase under similarly defined conditions (63). Salens have also been shown to protect cells against oxidative stress and are protective in a large number of animal models of human diseases (50). One of the current limitations of the salens is the stability of the parent compounds in biologic matrix, which makes it difficult to determine tissue levels and half-lives.

A number of compounds initially developed as free radical spin traps have been shown to have antioxidant properties in cell and animal systems (136). These compounds react with free radicals and form more-stable free radical products. The most frequently used compounds are the nitroxides and include *α*-phenyl-*tert*-butylnitrone (PBN) and 2,2,6,6-tetramethylpiperidine *N*-oxyl (TEMPO) (Fig. 5). These compounds have also been described as non–metal-containing SOD mimics (5). The rate of reaction with O_2^- is relatively low and thus requires large amounts (often millimolar levels) of these compounds to be present in the system to be effective (190). Fortunately, these compounds are well tolerated in animals and can achieve high tissue levels (136). These agents have a number of properties other than the reaction with ROS that could also explain some of their protective properties in models of oxidative stress. Many of these compounds can be metabolized to release NO and can inhibit enzymes that are endogenous sources of ROS (34,229).

GPx enzymes are found in every compartment within the cell and tissues and are effective scavengers of cellular peroxides. The GPx mimetic class includes mono- and diselenium–containing compounds (Fig. 6). One of the best-studied GPx-like mimics is 2-phenyl-1,2-benzisoselenazol-3(2H)-one, also known as ebselen or PZ51. Ebselen was one of the first selenium-based GPx mimics developed and catalytically scavenges peroxides in the presence of reducing equivalents such as GSH, *N*-acetylcysteine (NAC), and dihydrolipoate (DHLA) (179). The mechanism by which this occurs is still debated and may differ under different conditions. Ebselen has also been shown to stimulate the decomposition of a number of ROS, including hypochlorous acid (HOCl) (17), singlet oxygen (174), and $ONOO^-$ (128). Ebselen can readily bind cellular thiol groups on proteins, which may complicate the interpretation of biologic effects, because many cellular proteins have reactive thiols in their catalytic domains. It has been documented that ebselen can inhibit lipoxygenases (168), NADPH oxidases (46), and nitric oxide synthases (226). All of these enzymes are also potential sources of endogenous ROS. Ebselen has been shown to be protective in a number of cell-culture systems (159,194) and animal models of human disease (179). Ebselen is orally active and appears to be well tolerated in animals and humans. Newer analogues of ebselen have been developed, including BXT-51072, which has increased activity and potency in cell systems. These analogues [BXT series are being developed by Oxis International (http://www.oxis.com)] have been shown to be protective in a limited number of cell-culture systems and animal models of human disease (203).

A number of diselenide- and ditelluride-containing compounds have been reported to catalytically scavenge peroxides with higher GPx-like activity than ebselen (75,86,161,162).

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Sulfur, selenium, and tellurium belong to group IV of the periodic table and have similar chemical properties. A major difference with these types of compounds is that they usually contain a diselenide bond. Earlier compounds, such as the diphenyl diselenide (DPDS), were electrophilic agents that had cytotoxic, genotoxic, and mutagenic issues (4,163). Many previously reported diselenide compounds release free selenium during the catalytic cycle, and this may be problematic in their development as therapeutic agents. A unique aspect of a newer series of these compounds is the cyclodextrin group, which may help in directing hydrophobic peroxides toward the selenium or tellurium active site. The diselenide, 2,2′-deseleno-bis-*β*-cyclodextrin (2-SeCD), can scavenge a variety of peroxides including H_2O_2, *tert*-butyl hydroperoxide, and cumenyl hydroperoxide by using GSH as a cofactor (124). Only a limited number of cell-culture studies have been reported for these compounds (140,186), and it is still unclear whether these compounds can be successfully used in animal models of lung fibrosis.

Antioxidant scavengers

The largest categories of antioxidants are those that are reactive toward ROS, and the product of the reaction results in a less-toxic species. The naturally occurring vitamins E (*α*-tocopherol) and C (ascorbate) are such examples. Both the ascorbate and *α*-tocopherol radicals are less reactive and can be recycled by cellular reductases. Glutathione is a thiol-containing tripeptide that readily reacts with peroxides and forms a less-toxic disulfide product that is recycled by glutathione reductase. A number of synthetic compounds have been models after these endogenous antioxidants and have been shown to be protective in models of oxidative stress (Fig. 7).

A number of polyphenolic-based antioxidants are known, such as the water-soluble analogue of *α*-tocopherol, known as trolox, hindered phenols that include butylated hydroxytoluene (BHT), and various plant phenolics such as curcumin and flavonoids. These compounds are often chain-breaking antioxidants, and some have been used in the food industry as preservatives (42). In general, they require larger doses or concentrations to produce antioxidant effects in model systems because of their lower rates of reaction with ROS and their limited ability to be recycled endogenously.

Another group of compounds use a steroid nucleus substituted with antioxidant side groups and are known as lazaroids. Lazaroids are very effective at inhibiting iron-dependent lipid peroxidation (39). Lazaroids have been extensively tested as neuroprotective agents, but it is still not clear whether their neuroprotective effects are directly related to their antioxidant properties.

A large class of antioxidants is the thiol-containing compounds. The most extensively studied thiol compound is *N*-acetyl cysteine (NAC). NAC is a direct-acting antioxidant and can scavenge several ROS such as hypochlorous acid, peroxides, $^{\cdot}OH$, and $ONOO^{-}$ (45). NAC can also serve as a cellular source of cysteine for the endogenous synthesis of GSH. NAC can suppress the activation of transcription factors such as NF-*κ*B as a way to modulate cell-signaling pathways. A homocysteine derivative, erdosteine, is a prodrug that, when metabolized, produces an active thiol antioxidant metabolite (56). Erdosteine has beneficial effects in COPD patients (156). Amifostine is another prodrug that, when metabolized, produces an active thiol antioxidant used clinically as a radioprotective agent (37). Thiol-containing agents can also act as metal chelators and decrease oxidative stress by limiting the ability of transitional metal to participate in ROS formation. Paradoxically, some thiol-containing agents have the potential to create a more-reactive species when they react with ROS, which is often dependent on availability of oxygen and transitional metals.

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ANTIOXIDANTS, OXIDATIVE STRESS AND ANIMAL MODELS OF LUNG FIBROSIS

A large majority of lung fibrosis animal models involve the overproduction of oxidants, and the fibrotic effects are potentiated in antioxidant-deficient animals (Table 2). A number of drugs are known to produce lung fibrosis in humans and animals (105,228). Many of these drugs are chemotherapeutic agents that stimulate oxidative stress. Ionizing radiation is also a well-characterized method of producing lung fibrosis in animals, as well as a known adverse effect of cancer radiation treatment (41). A number of environmental exposures produce lung oxidative stress and fibrosis, including exposure to asbestos and silica (77,220). In addition, known cytokines, such as TGF-β, when overproduced, result in lung fibrosis. Most of these models have been shown to stimulate lung-injury responses and oxidative stress. A major limitation with currently available animal models of lung fibrosis is that they do not closely mimic human interstitial pneumonias, and many spontaneously resolve over time (38).

Fibrogenic drugs, antioxidants and oxidative stress

A number of chemotherapeutic agents can elevate intracellular ROS levels (141). The best-studied chemotherapeutic agent associated with oxidative stress and lung fibrosis is bleomycin. Bleomycin is thought to bind transitional metals and, in the presence of oxygen or H_2O_2, generates a strong oxidant (101,225). The lung is thought to be a target organ because of its low levels of a cysteine protease that degrades bleomycin into an inactive form (117). Bleomycin increases ROS production in lung macrophages and alveolar type II epithelial cells *in vivo* (91). Bleomycin also increases lung epithelial cell apoptosis in a ROS-dependent manner (213). Bleomycin-induced cytotoxicity and lung fibrosis can be modulated by changing the intracellular levels of endogenous antioxidants (61,84,107,112,167).

Both catalytic and scavenger antioxidants have been shown to attenuate bleomycin-induced lung fibrosis in animals (Table 3). Liposomal or lecithinized delivery of SOD with or without catalase decreases bleomycin-induced lung fibrosis in rats and mice (118,119,193,221). The catalytic antioxidant porphyrin MnTBAP attenuates bleomycin-induced lung fibrosis in mice (148). The administration of vitamin E has also been shown to have protective effects, and its deficiency potentiates bleomycin-induced lung fibrosis in animals (57,58,142,188). Thiol-containing antioxidants have been extensively studied in the bleomycin model of lung fibrosis. The best-studied thiol-containing antioxidant is NAC. Both oral and inhaled NAC decrease lung fibrosis in rats and mice (44,80,129,177). NAC also has been shown to restore lung GSH redox balance and to suppress bleomycin-induced activation of NF-κB (176). Several prodrugs, such as erdosteine and amifostine, that produce active thiol-containing metabolites have also been shown to attenuate bleomycin-induced lung fibrosis in rodents (22,88,144,145,184, 224). In addition, lazaroids are protective against bleomycin-induced lung fibrosis in rats (49,132). A number of natural products that contain polyphenolic compounds, such as *Ginko biloba* extracts and curcumin, have been found to suppress lung oxidative stress and fibrosis in rats treated with bleomycin (48,67,93,152,207).

Paraquat is a redox-active herbicide that also is known to produce fatal pulmonary fibrosis in humans (43,202) and animals (181,182). Paraquat is thought to redox cycle with cellular enzymes to produce the paraquat cation radical that rapidly reacts with oxygen to form O_2^- (3,25,76). Paraquat produces lung oxidative stress in animals (2,23,66,113,218) and humans (94,135). Both catalytic and scavenger antioxidants have been shown to attenuate paraquat-induced lung injury and fibrosis in animals (see Table 3). Administration of SOD has been shown to attenuate paraquat-induced lung injury *in vitro* (90) and *in vivo* (147,215). The manganese-containing porphyrin catalytic antioxidant MnTBAP has been shown to have protective effects against paraquat-induced injury both *in vitro* (55,97) and *in vivo* (52). In

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addition, the spin-trap PBN also has protective effects against paraquat-mediated damage (170). The administration of vitamin E has protective effects, and its deficiency potentiates paraquat-induced lung injury and fibrosis in animals (18,187). Thiol-based antioxidants also have protective effects in the paraquat model, including GSH (79,192), NAC (83,216,223), and erdosteine (92). In addition, the polyphenolic compound curcumin has been reported to have protective properties against paraquat-induced lung injury (206).

The antiarrhythmic drug amiodarone produces lung fibrosis in humans (183) and animals (28). Some data suggest a role for oxidative stress in amiodarone-induced lung fibrosis. Amiodarone inhibits mitochondrial complex I and II respiration and produces mitochondrial dysfunction in lung epithelial cells and macrophages (19). In the ventilated perfused rabbit lung system, amiodarone increases the levels of ROS and oxidized glutathione (GSSG) (106). Further studies have revealed that amiodarone is metabolized to an aryl radical that may give rise to other ROS (146,208). Both catalytic and scavenger antioxidants have been shown to attenuate amiodarone-induced lung injury and fibrosis in animals (see Table 3). PBN has been shown to directly scavenge the aryl radical produced by amiodarone (146). Vitamin E supplementation of hamsters attenuated both lung TGF-β levels and fibrosis induced by amiodarone (29,30). The thiol-based antioxidant NAC is also effective at attenuating amiodarone-induced lung fibrosis (120). In addition, the phenolic antioxidants BHA and curcumin were effective in limiting amiodarone-induced ROS and lung injury (120,152).

Radiation, antioxidants, and oxidative stress

Ionizing radiation produces fibrotic responses and generates hydrogen atom radical (H), OH, and hydrated electrons (e^-_{aq}) from the ionization of water in tissues. All three of these species are highly reactive and can generate and propagate a cascade of different ROS. Whole-body radiation decreases the levels of endogenous antioxidants and increases markers of lipid oxidation in animals and humans (9,40). Increased oxidative stress has been reported in radiation pneumonitis in humans (96) and in radiation-induced lung injury in rats (72,209). It is interesting to note that oxidative stress is still present even weeks after the radiation exposure (59). Several animal hemithoracic irradiation models of lung fibrosis have been developed and used to screen compounds for antifibrotic effects.

Both catalytic and scavenger antioxidants have been shown to attenuate radiation-induced lung injury and fibrosis in animals (see Table 3). Radiation-induced lung fibrosis is worsened in antioxidant-deficient animals (68,197) and attenuated in SOD-overexpression models (68,99, 126). Manganese-containing porphyrins and salen catalytic antioxidant mimetics have also been shown to have protective effects against radiation-induced lung fibrosis (116,153,210). The results from studies on the administration of vitamin E and its potential to limit radiation-induced lung injury and fibrosis in animals have been controversial, with some negative findings (165,217) and a recent positive result (16). Thiol-based antioxidants also have protective effects in the radiation-induced lung injury and fibrosis, including NAC (143) and amifostine (211). A number of flavonoids have also been used successfully to attenuate radiation-induced lung injury and fibrosis, including curcumin (200) and *Ginko biloba* extracts (175).

Fibrogenic cytokines and oxidative stress

A number of cytokines have been shown to stimulate fibrotic events and include TGF-β, tumor necrosis factor (TNF-α), platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), endothelin, granulocyte–macrophage colony-stimulating factor (GM-CSF), interleukin (IL-1β), IL-6, IL-10, and IL-13 (10). The best studied of these various cytokines in lung fibrosis is TGF-β. TGF-β isoforms have a number of effects on cellular responses including modulating cell growth, migration, differentiation, and apoptosis (169). TGF-β

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induces myofibroblast differentiation, extracellular matrix (ECM) synthesis, and inhibits ECM breakdown (227). TGF-β1 is abundant in BALF and present in fibroblastic foci biopsies from IPF subjects (24). Overexpression of TGF-β1 in animals induces a progressive lung fibrosis that is largely independent of inflammation (180). TGF-β1 produces oxidative stress by the induction of ROS production and a decrease in expression of cellular antioxidants (111). TGF-β1 induces ROS production by activation of NADPH oxidases (NOXs) and through mitochondrial dysfunction (185,198). TGF-β1 has been shown to decrease the expression of both catalase and mitochondrial SOD2 (82). In addition, TGF-β1 has been shown to decrease cellular GSH levels through the decreased expression and activity of γ-glutamylcysteine ligase (γ-GCL), the rate-limiting step in GSH synthesis (8). Interestingly, very few studies have been reported on the effects of antioxidants in this relatively new animal model of lung fibrosis.

Fibrogenic environmental agents and oxidative stress

A number of environmental dust and fiber exposures have been associated with the development of lung fibrosis (137). Both silica and asbestos exposures produce lung fibrosis in animals (64,104) and pneumoconiosis in humans (164). Both silica and asbestos produce injury and oxidative stress in the lungs of animals (1). In humans, a link between oxidative stress and exposure to dusts is supported by the finding of lower levels of glutathione-dependent enzymes in coal workers with pneumoconiosis (69). Numerous reports exist in the literature on the ability of both silica and asbestos exposures to increase ROS production (109,138, 172,204). In addition, beryllium exposure can produce lung granulomatous disease, and beryllium has recently been shown to stimulate increased production of ROS (171). A number of studies have reported an increased risk for developing IPF on various occupational and environmental exposures (13,87,195).

Several asbestos and silica animal models of lung fibrosis have been developed. Silica- and asbestos-induced lung fibrosis are worsened in antioxidant-deficient animals (71,125) and attenuated in catalase-overexpression models (139). Catalytic and scavenger antioxidants have been shown to attenuate asbestos- and silica-induced lung injury and fibrosis in animals (see Table 3). Catalytic antioxidants have been shown to have protective effects against silica-induced injury, including the porphyrin MnTBAP (123) and the nitroxide TEMPO (205). Thiol-based antioxidants also have protective effects in the silica-induced injuries, including NAC and GSH (12), as well as garlic extracts that are rich in thiol-containing compounds (6). The lazaroid compounds U-75412E and U-74389G are also effective against silica-induced injury (7,85).

ANTIOXIDANTS IN HUMAN IPF

Only a few antioxidants have actually been examined in humans with IPF. The thiol class has received the most attention to date. GSH (600 mg, twice daily for 3 days) has been given by inhalation to IPF subjects and found to increase ELF GSH levels and decrease ROS production in airway macrophages (20). NAC is an FDA-approved mucolytic drug that has been extensively used in cystic fibrosis subjects (65). Early studies examined the ability of oral NAC therapy (600 mg, 3 times daily for 5 days) to restore ELF GSH levels in IPF subjects (134). These studies found that this NAC regimen increased ELF GSH levels in IPF subjects 71% and was well tolerated. NAC has also been given to IPF subjects intravenously at 0.6-, 1.6-, and 4.8-g doses and found to elevate ELF GSH levels in IPF but not in normal subjects (133). None of these earlier studies looked at efficacy. NAC has been given to IPF subjects by inhalation (352 mg daily for 12 months), and some improvements were noted in exercise desaturation and high-resolution CT imaging; however, other lung-function tests and quality-of-life scores were not different from those with placebo (201). NAC has been given to a limited number of subjects with fibrosing alveolitis, in addition to immunosuppressive therapy (14). Oral NAC treatment (600 mg, 3 times daily for 12 weeks) increased lung ELF GSH levels 48%

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over baseline and was associated with improved pulmonary-function tests. A more recent multicenter randomized trial further investigated the possible benefits of oral NAC therapy in combination with azathioprine and high-dose corticosteroids in a larger cohort of IPF subjects (60). NAC treatment (600 mg, 3 times daily for 12 months) was associated with modestly improved pulmonary-function tests *versus* standard therapy alone. Interestingly, this study found that the NAC-treatment group had a lower rate of myelotoxicity from the immunosuppressive therapy. Although the initial studies with NAC in IPF showed only modest beneficial effects, it sets the stage for testing other antioxidants in IPF.

CONCLUSIONS

The evidence of a redox imbalance in lung fibrosis is substantial, and the rationale for testing antioxidants as potential new therapeutics for lung fibrosis is appealing. All the current animal models of lung fibrosis have clear involvement of ROS in their pathogenesis, and numerous examples of a wide array of different antioxidants attenuating fibroproliferative events are ample in the literature. These factors should continue to drive the investigation and the use of antioxidants to treat the progression of lung fibrosis and other fibroproliferative disorders in humans.

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ABBREVIATIONS

AIP
: acute interstitial pneumonia

α-PBN
: α-phenyl-*tert*-butylnitrone

BALF
: bronchoalveolar lavage fluid

BHA
: butylated hydroxyanisole

BHT
: butylated hydroxytoluene

CTGF
: connective tissue growth factor

COP
: cryptogenic organizing pneumonia

2-SeCD
: deseleno-bis-β-cyclodextran

DIP
: desquamative interstitial pneumonia

DAD
: diffuse alveolar damage

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DHLA
dihydrolipoate

DPDS
diphenyl diselenide

ELF
epithelial lining fluid

γ-GCL
γ-glutamylcysteine ligase

GSH
glutathione

GSSG
glutathione disulfide

GPx
gluta-thione peroxidase

GM-CSF
granulocyte–macrophage colony-stimulating factor

H_2O_2
hydrogen peroxide

$HO^{\cdot}$
hydroxyl radical

HOCl
hypochlorous acid

IPF
idiopathic pulmonary fibrosis

IL
interleukin

IP
interstitial pneumonia

LIP
lymphoid interstitial pneumonia

MnTBAP
manganese (III) tetrakis (4-benzoic acid) porphyrins

NAC
N-acetylcysteine

PDGF
platelet-derived growth factor

PMN
polymorphonuclear leukocyte

ROS
reactive oxygen species

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RB
respiratory bronchiolitis interstitial lung disease

O_2^-
superoxide

SOD
superoxide dismutase

TEMPO
tetramethylpiperidine *N*-oxyl

TGF-*β*
transforming growth factor beta

TNF-*α*
tumor necrosis factor alpha

UIP
usual interstitial pneumonia

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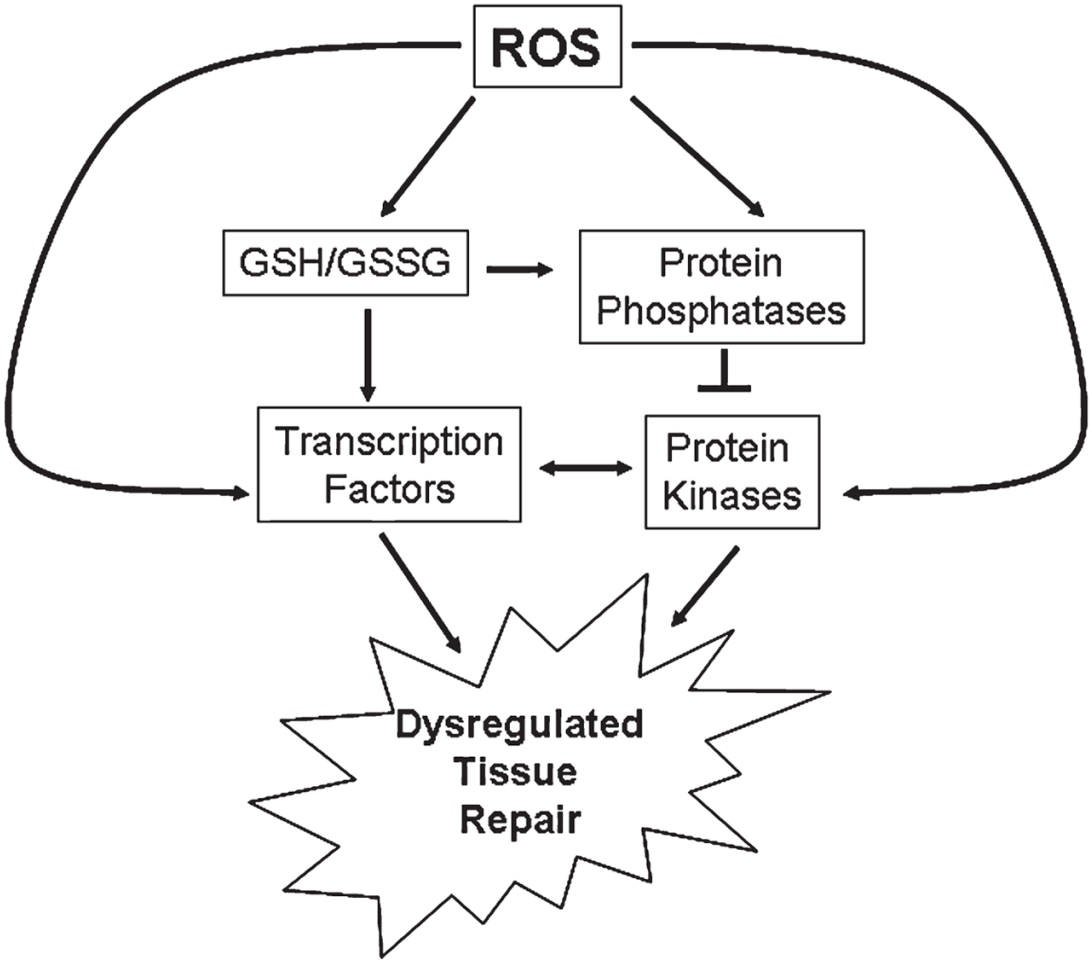

FIG. 1. Role of ROS in the dysregulation of tissue repair

Several phosphatases contain sensitive thiol residues that are inhibited on oxidation. As steady-state levels of oxidants increase, an increase in the inactivation of phosphatases and a corresponding increase in the levels and duration of phosphorylated proteins occur. An increase in steady-state ROS results in altering the cellular glutathione (GSH)/glutathione disulfide (GSSG) redox couple, which can in turn alter the relative oxidation status of protein thiols. In addition, ROS can alter cell signaling directly by reacting with specific thiols on transcription factors. Many of these types of mechanisms are thought to contribute to the often observed unregulated tissue-repair responses associated lung fibrosis.

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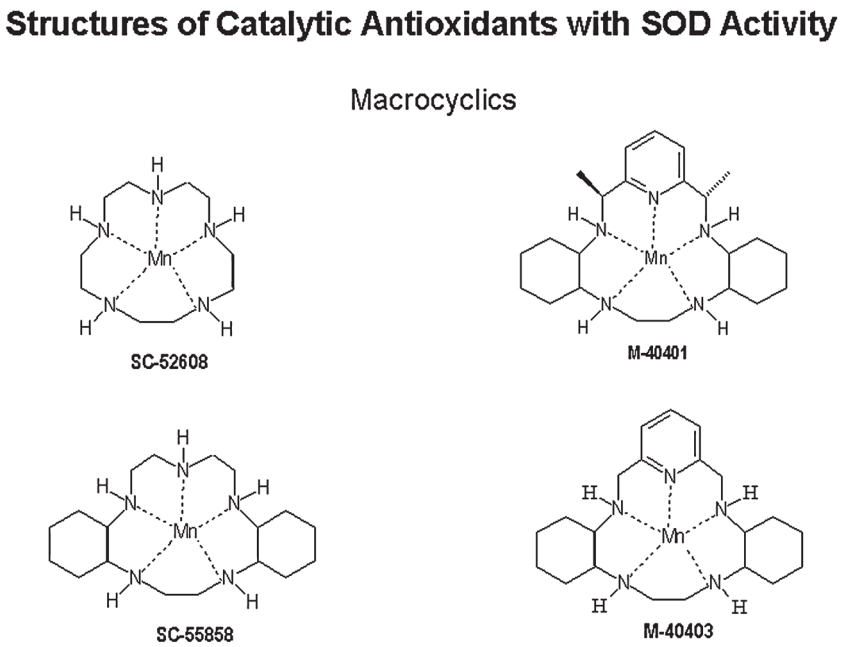

FIG. 2.
Chemical structures of macrocyclic catalytic antioxidant mimetics with SOD activity.

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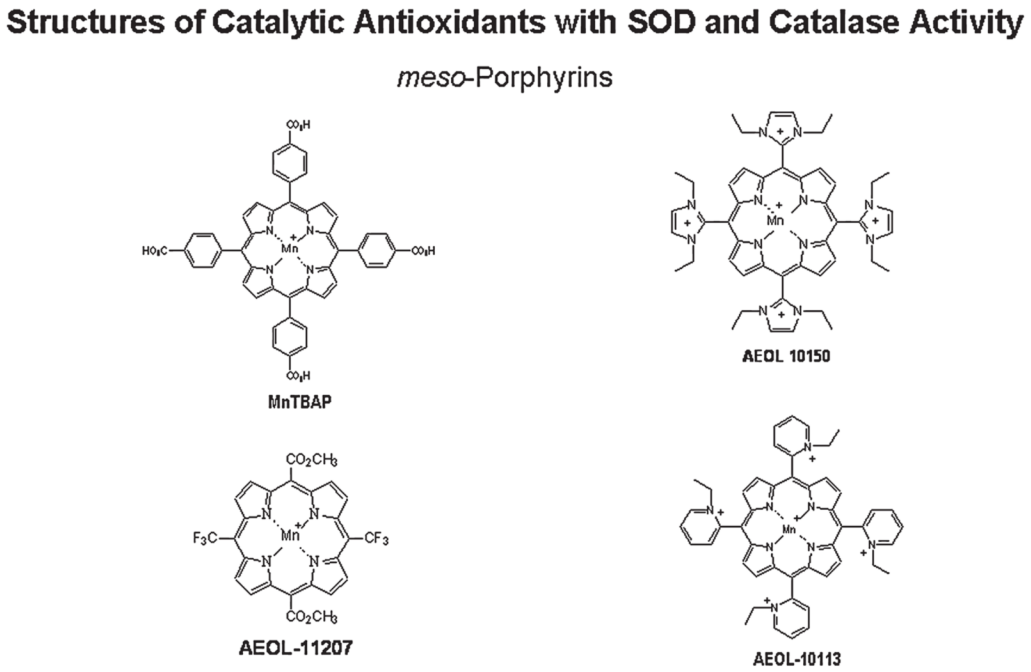

FIG. 3.
Chemical structures of metalloporphyrin catalytic antioxidant mimetics with SOD and catalase activities.

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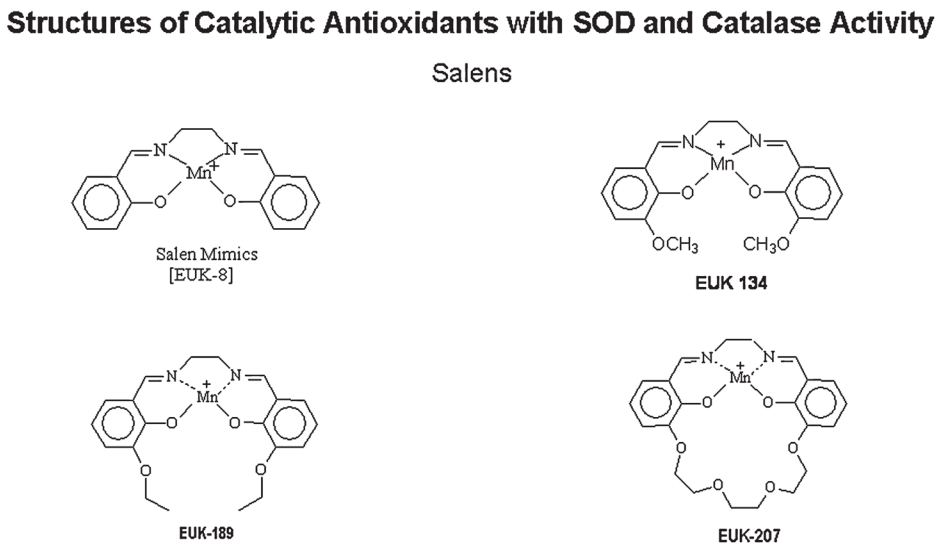

FIG. 4.
Chemical structures of salen catalytic antioxidant mimetics with SOD and catalase activities.

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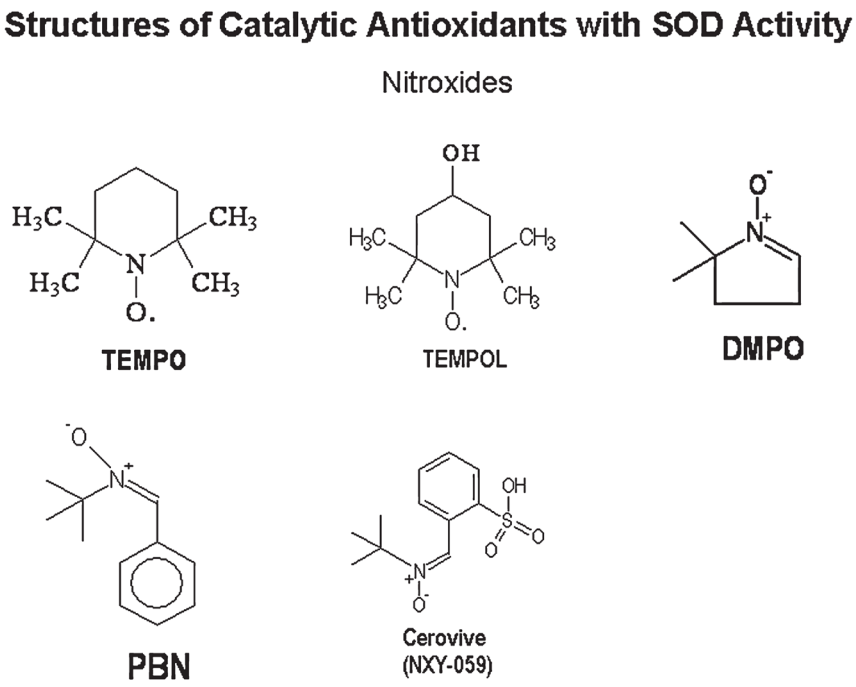

FIG. 5.
Chemical structures of nitroxide catalytic antioxidant mimetics with SOD activity.

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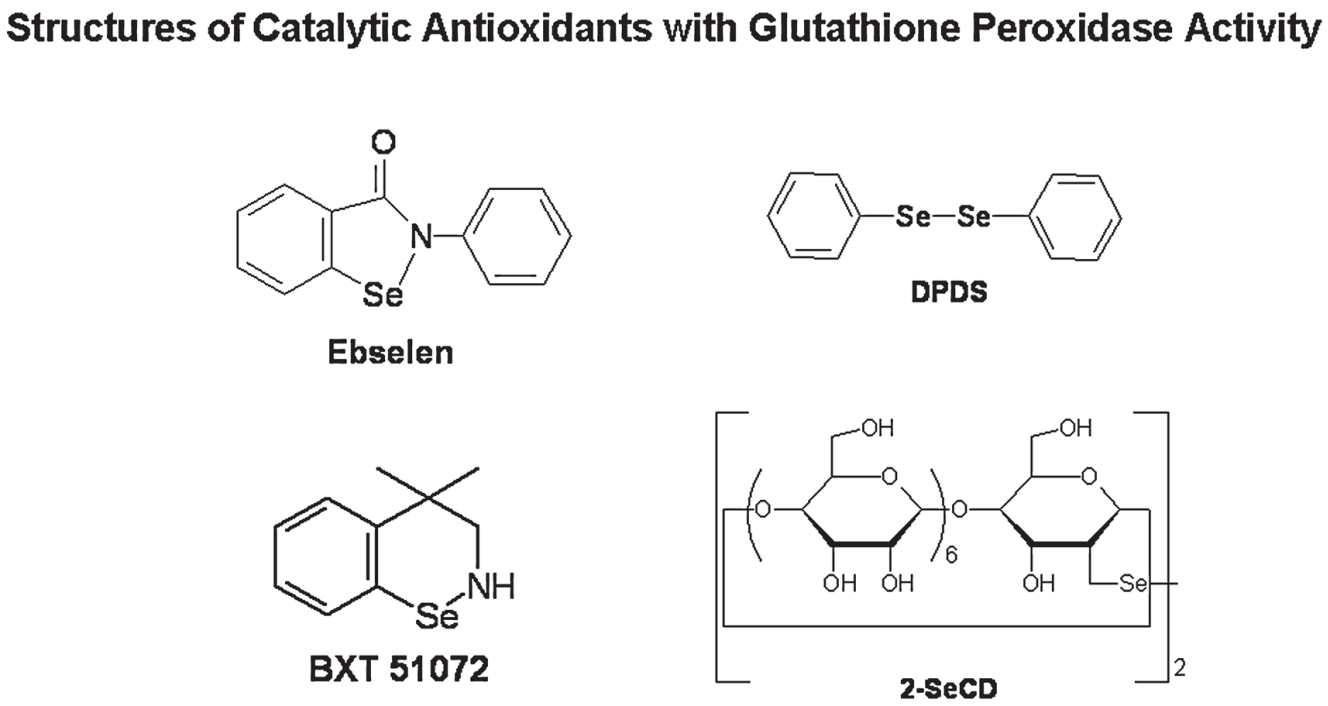

FIG. 6.
Chemical structures of selenium-containing catalytic antioxidant mimetics with glutathione peroxidase activity.

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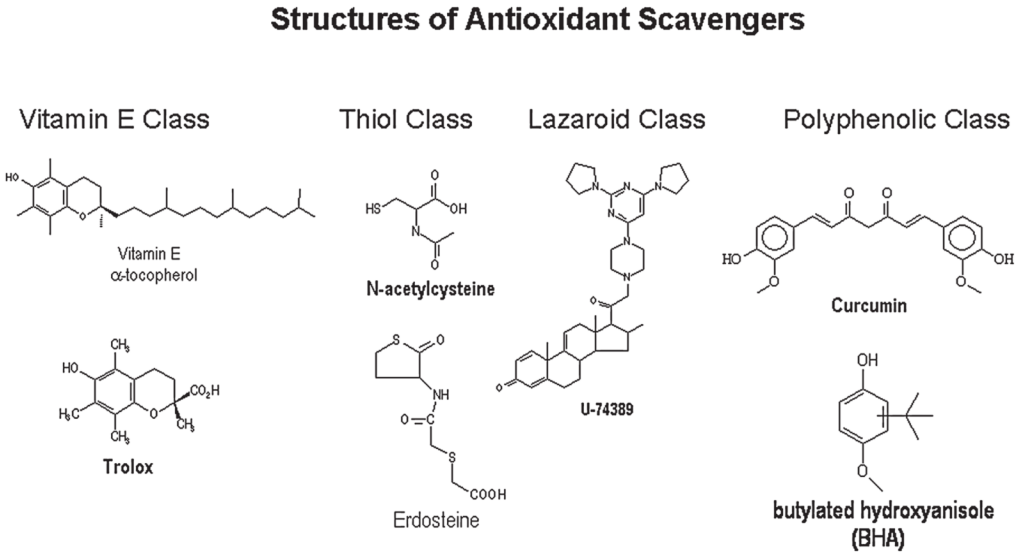

FIG. 7.
Chemical structures of antioxidant scavengers.

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Table 1

Evidence of Oxidative Stress in IPF

Blood	Sputum	BALF	BAL cells
↓ GSH	↓ GSH	↓ GSH	↑ O_2^-
↑ GSSG	↑ PMN	↑ GSSG	
↑ O_2^-	↑ IL-8	↑ PMN	
		↑ MPO	
		↑ Nitrite/nitrate	
		↑ Oxidized proteins	

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Table 2

Models of Lung Fibrosis

Fibrinogenic agents	**Species**
Bleomycin	Mice, rats, hamsters, rabbits, dogs, and primates
Paraquat	Mice, rats, hamsters, rabbits, dogs, sheep and primates
Amiodarone	Mice, rats, hamsters, rabbits, dogs, and primates
Radiation	Mice, rats, rabbits, dogs, hamsters, sheep, and primates
TGF-β	Mice, rats
Silica	Mice, rats, hamsters, rabbits, and primates
Asbestos	Mice, rats, hamsters, sheep, and primates

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Table 3

Protective Antioxidants in Animal Models of Lung Fibrosis

Fibrogenic agent	Catalytic antioxidants	Antioxidant scavengers
Bleomycin	SOD, catalase, MnTBAP	Vitamin E, NAC, erdosteine, amifostine, U-74389, curcumin, ginko biloba
Paraquat	SOD, MnTBAP, PBN, TEMPOL	Vitamin E, GSH, NAC, erdosteine, curcumin
Amiodarone	PBN	Vitamin E, NAC, BHA, curcumin
Radiation	SOD, catalase, AEOL 10113, AEOL 10150, EUK 189	Vitamin E, NAC, amifostine, curcumin, ginko biloba
TGF-β	Unknown	Unknown
Silica	Catalase, MnTBAP, TEMPO	GSH, NAC, U-75412, U-74389
Asbestos	SOD, catalase, TEMPO	NAC