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Catalase and glutathione peroxidase mimics

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Abstract

Overproduction of the reactive oxygen species (ROS) superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are increasingly implicated in human disease and aging. ROS are also being explored as important modulating agents in a number of cell signaling pathways. Earlier work has focused on development of small catalytic scavengers of O_2^-, commonly referred to as superoxide dismutase (SOD) mimetics. Many of these compounds also have substantial abilities to catalytically scavenge H_2O_2 and peroxynitrite ($ONOO^-$). Peroxides have been increasingly shown to disrupt cell signaling cascades associated with excessive inflammation associated with a wide variety of human diseases. Early studies with enzymatic scavengers like SOD frequently reported little or no beneficial effect in biologic models unless SOD was combined with catalase or a peroxidase. Increasing attention has been devoted to developing catalase or peroxidase mimetics as a way to treat overt inflammation associated with the pathophysiology of many human disorders. This review will focus on recent development of catalytic scavengers of peroxides and their potential use as therapeutic agents for pulmonary, cardiovascular, neurodegenerative and inflammatory disorders.

Keywords

Catalytic antioxidants; Cell signaling; Drug development; Hydrogen peroxide; Inflammation; Oxidative stress

1. Endogenous catalytic hydrogen peroxide scavengers

Hydrogen peroxide (H_2O_2) is generated directly from superoxide (O_2^-) through a rapid dismutation reaction that can occur either enzymatically with superoxide dismutases (SOD) or spontaneously. This means that wherever O_2^- is generated there is also formation of H_2O_2. In addition, H_2O_2 is formed enzymatically as a by-product of lipid metabolism in peroxisomes. H_2O_2 is stable at biological pH and easily crosses lipid membranes. H_2O_2 can participate in hydroxyl radical ($HO^\bullet$) formation in the presence of reduced transition metals. Oxidative stress is traditionally defined as an imbalance between reactive oxygen species (ROS) production and antioxidant defense against these ROS. A consequence of oxidative stress is an increase in the formation of oxidized cellular macromolecules. Critical cysteinethiol groupson proteins are a common site ofoxidation and many of these cysteines are important in maintaining

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proteins in a proper conformation for catalytic function. This is a common mechanism proposed for how oxidative stress can disrupt cell signaling pathways leading to unregulated inflammatory responses [1]

SODs and catalase are metalloproteins that catalyze "dismutation" reactions, which detoxify O_2^- and H_2O_2, respectively. SODs catalyze the formation of oxygen and H_2O_2 from two O_2^-, whereas catalase catalyzes the formation of oxygen and water from two H_2O_2 molecules.

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

Because these efficient reactions do not require additional reducing equivalents, no energy is required from the cell. The overall goal of cellular antioxidant defenses is to reduce ROS to water. Mammalian catalase is a tetramer in which each monomer contains an iron heme (porphyrin) group bound to the catalytic site [2]. The heme groups are protected, being buried in a non-polar pocket with narrow hydrophobic channels to aid in H_2O_2 selectivity. It should be noted that catalase also possesses peroxidase activity and is known to oxidize short chain alcohols to their corresponding aldehydes [3]. Overexpression of SOD and catalase in cultured cells and whole animals has provided protection against the deleterious effects of a wide range of oxidative stress paradigms [4].

Another class of endogenous catalytic H_2O_2 scavengers is the selenium-containing peroxidases [5]. This is a broad group of enzymes that utilize H_2O_2 as a substrate along with an endogenous source of reducing equivalence. One of the best studied families of peroxidases are the glutathione peroxidases (GPx). GPxs are tetrameric proteins where each monomer contains one atom of selenium at the catalytic site. The active site of GPx contains a selenocysteine where the sulfur in cysteine has been replaced by selenium (R-SeH). During the catalytic cycle, a selenol (protein-Se^-) reacts with peroxide (H_2O_2 or lipid peroxide, LOOH) resulting in a selenenic acid (protein-SeOH). The selenenic acid group is reduced back to a selenol by two glutathiones (GSH) which are in turn oxidized to disulfide (GSSG) and LOOH is reduced to its corresponding alcohol (LOH).

$$H_2O_2+2GSH \rightarrow GSSG+2H_2O$$

$$LOOH+2GSH \rightarrow GSSG+H_2O+LOH$$

The GSSG is converted back to two GSH by glutathione reductase that uses reducing equivalents derived from β-nicotinamide adenine dinucleotide phosphate (NADPH). Overexpression of GPx has been shown to be protective against oxidative stress in cultured cells and whole animals [6,7]. Not all of the peroxidases detoxify H_2O_2. A number of non-specific peroxidases, such as myleoperoxidase, eosinophil peroxidase and lactoperoxidase, actually form more reactive produces such as hypochlorite, hypothiocyanite, and hypobromous acid [8].

A new family of proteins have been discovered that are known as the thioredoxin-dependent peroxidases or peroxiredoxins (Prx). These proteins can directly reduce peroxides and the oxidized protein is regenerated indirectly by thioredoxin (Trx) reductase [9,10]. There are at least 13 mammalian Prxs and they are widely abundant in mammalian cells. Prx have been found to be induced in response to oxidative stress [11] and are protective against oxidative

stress when over-expressed in animals [12]. The Prxs and Trxs work in tandom with the GPxs to maintain cellular peroxide steady-state levels and also maintain cellular protein cysteines in a reduced state (Fig. 1). During oxidative stress, where cellular peroxides are elevated, there is an increased level of oxidized protein cysteines that leads to inactivation of phosphatases and transcription factors that are thought to drive dysregulated inflammatory reactions [13]. This is the drug target and rationale for the development of catalase and GPx mimics.

2. Development of catalytic hydrogen peroxide scavengers

There are two main design strategies to detoxify peroxides. One approach models after the catalase dismutation reaction and focuses on compounds with redox-active metal centers that often containing either manganese (Mn) or iron (Fe). Another strategy models after the GPx enzymes using either selenium or tellurium active sites. An ideal mimetic is stable and non-toxic at therapeutically efficacious concentrations. The size and charge of the mimetic is often exploited to target cellular sites of oxidant production, such as the mitochondria, and to improve their pharmacodynamic properties.

Many simple metal chelates readily react with H_2O_2. However, the rates of reaction with these chelates are generally low and the complexes formed are relatively unstable. Recent developments in the field have yielded more stable and active metal chelates (Fig. 2). These include the salens, metalloporphyrins, and other metal complexes that can dismutate H_2O_2 under highly defined conditions. Likewise, a number of selenium-containing compounds have been developed around the GPx mechanism of H_2O_2 decomposition (Fig. 3).

All of the reported catalytic H_2O_2 scavengers react with a wide range of ROS and are not specific for H_2O_2. The potencies of these peroxide scavengers are based on rate constants derived under very defined and often non-biologically relevant conditions. For example, most compounds are routinely screened in buffers using very high H_2O_2 (1–10 mM) levels that are two orders of magnitude higher than the 1–100 nM levels that occur physiologically [14]. The findings 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.

3. Antioxidant properties of catalase-like hydrogen peroxide scavengers

3.1. Metalloporphyrins

Metalloporphyrins [AEOL series is currently being developed by Aeolus Pharmaceuticals, Laguna Niguel, CA (http://www.aeoluspharma.com)] are structurally different from endogenous protoporphyrins and are classified as synthetic *meso*-substituted porphyrins. Metalloporphyrins have been shown to possess at least four distinct antioxidant properties, which include scavenging O_2^- [15], H_2O_2 [16], $ONOO^-$ [17], and LOOH [18]. Most metalloporphyrins contain either a Fe or Mn moiety 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 transfers in addition to the one-electron transfers on the metal center. This mechanism is similar to that proposed for the heme prosthetic groups of endogenous catalase and peroxidases. There are two classes of metalloporphyrins wherein one group the SOD activities track with their catalase activities and another group that has very little SOD activity with high catalase activity. Examples of a manganese porphyrins with both high SOD and catalase-like activities are the pyridinium and imidazolium-substituted *meso*-porphyrins such, as AEOL 10113 and 10150 [19], whereas examples of compounds with low SOD activity and high catalase activity are MnTBAP [16] and AEOL 11207 [20]. It is still unknown which antioxidant activities are the most important in mediating the protective effects of metalloporphyrins in models of oxidative stress.

Metalloporphyrins have been shown to be effective in ameliorating oxidative stress, inflammation and injury in a large number of *in vitro* [21] and animal models of human disease (Table 1). Metalloporphyrins have plasma half-lives that range from 4 to 48 h. 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 their poor oral bioavailability, but several compounds in the AEOL 112-series have been shown to have good oral bioavailability and longer plasma half-lives which should make them better candidates for treating chronic diseases [22].

3.2. Salens

The salen class of catalytic antioxidants (EUK series) is currently being developed by Proteome Systems, North Ryde, Australia (http://www.proteomesystems.com). Generically, salens are aromatic, substituted ethylenediamine metal complexes. The Mn(III)-containing salen complexes have both O_2^- and H_2O_2 dismutation activities [23]. However, like all the small molecular weight scavengers, these compounds are not selective and can react with O_2^- and other peroxides and $ONOO^-$. 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 where the metal is only coordinated to nitrogen atoms. The coordination of Mn by four axial ligands results in the formation of several possible valence 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 [23]. Salens have also been shown to protect cells against H_2O_2-mediated injury [24]. Salens have been shown to be efficacious in a large number of animal models of human diseases (Table 1). One of the current limitations of the salens is the stability of the parent compounds in biological matrix which makes it difficult to determine tissue levels and half-lives.

3.3. Other metal complexes

There are a number of other metal containing macrocyclic compounds that have been described as catalase mimics. Iron complexes of 14-membered macrocycles have been shown to catalytically scavenge H_2O_2 to oxygen and water [25]. These compounds have been shown to be effective only in cell culture systems [26]. It is unclear how stable the complexes will be in more complex biological systems. Another group of compounds with H_2O_2-scavenging activity are the dimanganese complexes. A number of bacteria have catalase enzymes that use a dimanganese center to dismutate H_2O_2 and a number of investigators have tried to emulate this strategy with small molecules and peptides. Some examples of the small molecules are the nitro and chloro-substituted dimanganese complexes of 1,5-bis(5-salicylidenamino) pentan-3-ol (5-NO_2-salpent & 5-Cl-sal-pent), 1,5-bis(2-hydroxybenzophylideneamino)pentan-3-ol (2-OH-benzpent), and 1,5-bis(2-hydroxynaphtylideneamino)pentan-3-ol (2-OH-Napthpent) [27]. Very limited data is currently available whether they have protective properties in biological systems. It should be noted that free manganese is an efficient scavenger of H_2O_2 and so stability is an important feature to establish for any claim of catalytic H_2O_2 scavenging by a metal complex.

4. Antioxidant properties of glutathione peroxidase-like hydrogen peroxide scavengers

4.1. Ebselen

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.

It catalytically scavenges peroxides in the presence of reducing equivalents such as GSH, N-acetylcysteine (NAC), and dihydrolipoate (DHLA) [28]. 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) [29], singlet oxygen [30], and $ONOO^-$ [31]. Ebselen can readily bind cellular thiol groups on proteins which may complicate the interpretation of biological effects since many cellular proteins have reactive thiols in their catalytic domains. In fact, it has been documented that ebselen can inhibit lipoxygenases [32], NADPH oxidases [33], and nitric oxide synthases [34]. 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 [28] and animal models of human disease (Table 1). Ebselen is orally active and appears to be well tolerated in animals and humans.

Newer analogs of ebselen have been developed including BXT-51072, which has increased activity and potency in cell systems. These analogs [BXT-series are being developed by Oxis International, Foster City, CA (http://www.oxis.com)] have been shown to be protective in a limited number of cell culture systems [35] and animal models of human disease (Table 1).

4.2. Diselenide and ditelluride compounds

A number of diselenide and ditelluride containing compounds have been reported to catalytically scavenge peroxides with higher GPx-like activity than ebselen [36]. Sulfur, selenium, and tellurium belong to group VI of the periodic table and have similar chemical properties. Early compounds, such as the diphenyl diselenide (DPDS), were electrophilic agents that have cytotoxic, genotoxic, and mutagenic effects [37,38]. Many previously reported diselenide compounds release free selenium during the catalytic cycle, which may be problematic in their development as therapeutic agents. A unique aspect of a newer series of these compounds is the cyclodextrin (CD) group which may help in directing hydrophobic peroxides towards 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 using GSH as a cofactor [39]. Only a limited number of cell culture studies have been reported for these compounds [40], and it is still unclear whether these compounds can be successfully used in animal models of human disease.

4.3. Peptide compounds

Many proteins and peptides contain cysteine residues that can be readily modified to contain selenium and a number of investigators have examined whether these types of compounds have GPx-like activities. Examples of this include seleno-subtilisin which was produced by chemical modification of the serine protease, substilisin. This compound was found to have GPx-like activity [41]. Another strategy used a phage library of random 15-mer selenopeptides that was screened for GPx activity and generated some active GPx mimics (15SeP and $15SeP_1$). The peptides were found to increase the GPx-like activity of treated cultured cells and protected them against H_2O_2-mediated lipid peroxidation and cytotoxicity [42]. In order to increase the selectivity of the selenoprotein toward GSH, a number of selenium-containing monoclonal antibodies (i.e. Se-4A4 and Se-scFv2F3) were raised against GSH-S-2,4-dinitrophenyl t-butyl ester and found to have GPx-like activity [43]. The selenium-containing antibody Se-4A4 has been shown to protect isolated cardiac mitochondria against xanthine oxidase-induced oxidative modification [44]. Very limited data exist on whether any of these approaches have produced compounds with biological activity in more complex biological systems.

5. Catalytic antioxidants are effective in ameliorating oxidative stress *in vitro*

5.1. Cytotoxicity

In vitro models of oxidative stress have proved useful in verifying utility of catalytic antioxidants under more complex biological conditions [21]. Members of all classes of catalytic antioxidants with H_2O_2-scavenging activities have been shown to be effective in blocking oxidative stress in a variety of *in vitro* cytotoxicity models involving oxidant production [21, 24,28]. The mechanism(s) by which these catalytic antioxidants produce their protective effects are still highly debated and largely unknown. As discussed earlier, many of the compounds are capable of scavenging a number of different ROS and many may also decrease ROS by inhibiting endogenous ROS production [19]. These issues cloud the utility of H_2O_2-scavenging screens to select potentially biologically potent compounds. Overall, many of the catalytic antioxidant H_2O_2 scavengers at μmolar levels appear non-toxic and show similar efficacy in protecting a wide variety of different types of cultured cells against the toxicity of ROS.

5.2. Apoptosis

Apoptosis is a form of cell death that is biochemically and morphologically distinct from necrosis and has physiological and pathological roles in biological systems. There is an increasing body of literature that supports the involvement of ROS in some apoptotic pathways. The release of pro-apoptotic factors by mitochondria, which are a major source of ROS, lends further credence to this argument. Delivery of catalase and GPx to cells is protective, whereas paucity of either is deleterious, also supporting H_2O_2 involvement in cellular apoptosis [45]. A wide assortment of apoptosis paradigms can be ameliorated by catalytic antioxidants with H_2O_2-scavenging activity [21,24,28]. Apoptosis can be limited by catalytic antioxidants with catalase or GPx-like activities in a number of different cell types. It is not clear from these studies whether the catalytic antioxidants affect a particular point in the intrinsic and/or extrinsic apoptotic pathways. Some studies suggest that ROS might regulate the expression of pro-apoptotic factors, and antioxidants may directly block apoptosis by increasing the expression of anti-apoptotic factors, such as bcl-2 [46].

5.3. Inflammation

A number of recent studies have suggested that catalytic antioxidants with the ability to scavenge H_2O_2 can attenuate markers of inflammation such as cytokines, chemokines and adhesion molecules. H_2O_2 has been shown to activate a number of transcription factors including NFkB, AP-1 and Nrf2 [47]. In addition, peroxides and other oxidants can inactivate kinase signaling pathways through the inhibition of protein phosphatases [48]. These pathways likely contribute to the protective effects of many catalytic H_2O_2 scavengers in a variety of cell models of inflammation [21,24,28].

6. Catalytic antioxidants are effective in ameliorating oxidative stress *in vivo*

The beneficial effects of catalytic antioxidants with H_2O_2-scavenging capabilities have been demonstrated in numerous *in vivo* model systems (Table 1). These model systems cover diseases associated with the pulmonary system (such as fibrosis, asthma, acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), bronchopulmonary dysplasia (BPD), and pleurisy), cardiovascular system (including sepsis, hypertension, and myocardial infarction), neurologic system (including amyotrophic lateral sclerosis (ALS), migraine, spinal cord injury, stroke, Parkinson's disease (PD), and dementia), digestive system (including liver injury, transplantation, hepatitis, and colitis), endocrine system (diabetes), and the renal system (injury, sepsis, and transplantation). The diversity of the various models systems by which these catalytic antioxidants have shown efficacy speaks

to the important role ROS/reactive nitrogen species (RNS) play in animal models of human disease.

6.1. Pulmonary models

The lung functions at higher oxygen tensions than most other organs creating a unique relationship with ROS. There is increasing evidence that ROS have an important role in several lung diseases [49]. A common feature of many lung diseases is an inappropriate or dysfunctional inflammatory response. Catalytic antioxidants decrease airway hyperreactivity and inflammation in antigen-induced mouse models of asthma [50]. This is in contrast to a recent finding which reported high overexpression of catalase (8-fold) in the lung actually increased airway reactivity in a mouse model of asthma [51]. A potential explanation for this paradox is that many catalytic antioxidants have bell-shaped dose-responses and that very high levels can actually give the opposite response. An important goal of antioxidant therapy is to restore redox balance. Given the role of H_2O_2 in cell signaling pathways, it is conceivable that excessive scavenging of H_2O_2 may be detrimental under certain conditions.

COPD, which includes emphysema and bronchitis, is strongly associated with cigarette smoke that is a rich source of ROS [52]. In fact, cigarette smoke has been shown to inhibit catalase activity [53]. The diselenide GPx-like mimic diphenyl diselenide has been shown to protect rat pup lungs from oxidative changes associated with exposure to cigarette smoke [54]. Likewise, the metalloporphyrin AEOL 10150, which has both high SOD and catalase-like activities, attenuated inflammation and protected rat lung epithelium from cigarette smoke-induced precancerous lesions [55]. These data support the development of catalytic antioxidants for the treatment of COPD.

Interstitial lung disease is also associated with oxidative stress and many of the animal models of lung fibrosis use agents that overproduce or stimulate the production of ROS. Bleomycin is a redox-cycling chemotherapeutic agent that produces lung fibrosis in rodents and humans. Bleomycin-induced lung fibrosis can be attenuated by a liposomal mixture of SOD and catalase [56]. The metalloporphyrin AEOL 10201, also known as MnTBAP, has low SOD activity and moderate catalase activities and attenuated bleomycin-induced lung fibrosis in mice [57]. Ionizing radiation is also known to produce lung fibrosis in animals and man. Administration of a mixture of polyethylene glycol-tagged SOD and catalase has been shown to attenuate radiation-induced lung fibrosis in mice without affecting radiation-induced tumor killing [58]. Similarly, a couple of different metalloporphyrins (AEOL 10113 & 10150) have been shown to decrease radiation-induced lung fibrosis in rats [59,60]. The manganese-containing salen EUK-189 also was found to ameliorate early DNA damage in a rat model of lung irradiation [61]. These studies illustrate the potential utility of catalytic antioxidants in the treatment of interstitial lung disease.

ARDS is associated with sepsis and shock and both of these conditions involve the over production of ROS and reactive nitrogen species. A related lung disorder is BPD that occurs in premature infants where the lung is not fully developed and requires supplemental oxygen for adequate gas exchange. Both ARDS and BPD animals models commonly use either hyperoxia or endotoxin exposures, both of which are known to elevate lung ROS production. Both hyperoxia and endotoxin models have been shown to be responsive to modulation of endogenous SOD and catalase lung levels [62,63]. The metalloporphyrin AEOL 10113 was found to be beneficial in a preterm baboon model of BPD [64] and AEOL 10150 was found to attenuate hemorrhage-induced acute lung injury in SOD3 KO mice [65]. The manganese containing salen EUK 8 was found to be protective in an endotoxin-mediated swine model of ARDS [66]. These finding support the further development of catalytic antioxidants for the treatment of ARDS and BPD.

6.2. Cardiovascular models

Cardiovascular disease is a major cause of death in humans. A common cause of tissue injury directly related to the cardiovascular system is ischemia-reperfusion (IR). IR is associated with hemorrhage [67], myocardial infarction, arrhythmias, angina, myocardial stunning and transplantation. The role of excessive ROS production during IR and the protective effects of endogenous antioxidants have been well documented. Catalytic antioxidants with H_2O_2-scavenging activities are effective in animal models of heart IR [68,69]. In addition, several other organ systems have been shown to benefit from catalytic antioxidant treatments in IR, including the liver [70,71], lung [65], brain [72–75] and kidney [76].

Hypertension is a well characterized risk factor for cardiovascular disease. A number of systems are involved in the complex regulation of blood pressure and include cardiac output, fluid balance, vasodilatation and renal function. An important system in blood pressure regulation is the renin/angiotensin system which has also been shown to regulate vascular ROS production [77]. A well known anti-hypertensive agent captopril is both an angiotensin-converting enzyme inhibitor and antioxidant [78]. Catalytic antioxidants with H_2O_2-scavenging activities are effective in animal models of hypertension [79,80]. These studies suggest that catalytic antioxidants may be useful antihypertensive agents.

Systemic infection or sepsis frequently results in the overproduction of ROS and RNS that has devastating consequences for the cardiovascular system. A serious consequence of sepsis is the loss of vascular tone and its responsiveness to vasoconstrictive agents in a condition referred to as shock. A number of catalytic antioxidants with H_2O_2-scavenging activities are effective in animal models of sepsis induced by bacteria or endotoxin [66,81–86]. The well-established role of ROS and RNS in these conditions and the abundance of literature supporting a protective role of the endogenous antioxidant defenses make this a very attractive arena for development of catalytic antioxidants.

6.3. Nervous system models

The brain consumes a large amount of oxygen and is particularly sensitive to ROS-mediated damage. Factors that are thought to contribute to this phenomena are the presence of autooxidizable neurotransmitters, the high levels of polyunsaturated fatty acids in neuronal membranes, and modest levels of endogenous antioxidants. Collectively, these factors make catalytic antioxidants with H_2O_2-scavenging activities good candidates for several acute and chronic neuronal disorders that involve the overproduction of ROS such as PD [87], Huntington's disease (HD) [88], Alzheimer's disease (AD) [89], ALS [90], stroke [91], and trauma [92].

PD is a chronic neurodegenerative disorder where a large body of literature supports a role for ROS and oxidative stress and neuronal loss in the substantia nigra. Much of this data is linked to the mitochondrial dysfunction of complex I associated with this disease [93]. There have been numerous reports of increases in lipid, DNA and protein oxidative changes in PD and animal models of PD [94]. In addition, there are reports of decreases in antioxidants such as GSH, increased iron content and activation of NFkB [95]. Toxins that increase ROS in the substantia nigra such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) also produce PD-like symptoms and pathology in man and animals [96]. Catalytic antioxidants with H_2O_2-scavenging activities are effective in animal models of PD [22,97]. Recent studies with the orally active metalloporphyrin AEOL 11207 demonstrated neuroprotection in a mouse MPTP model that was also associated with decreased oxidative stress [22]. Currently available PD therapies are largely limited to palliative treatments with unpleasant side effects and opens PD as an attractive target for the development of catalytic antioxidants.

A number of dementias, such as AD and HD, are memory disorders that have been associated with protein aggregation and increased oxidative stress in the cortex and hippocampus [98]. Some investigators have suggested that amyloid may increase H_2O_2 production through metal-catalyzed reactions [99]. A few studies have shown that catalytic antioxidants can protect neurons from amyloid-induced cytotoxicity [100]. Increased production of ROS and RNS may also be an indirect consequence of amyloid protein deposits stimulating an inflammatory response from microglial cells [101]. In addition, there is some evidence that NO plays a role in memory and that catalytic antioxidants can be protective [102]. Aging is also a factor in the loss of cognitive function and catalytic antioxidants have also shown to be effective at slowing this process [103]. These data suggest that dementias may be a fruitful area for the further development of catalytic anti-oxidants.

ALS is a motor neuron disorder that leads to progressive loss of motor function, muscle atrophy and death within a few years. Most cases of ALS are sporadic, but about 10% are familial and some of these are associated with genetic mutations. Some familial forms of ALS are associated with mutations in SOD1, and transgenic mice overexpressing one of the SOD1 mutations develop a progressive degenerative disease of motor neurons [104]. It is thought that there is a gain of function associated with the mutant SOD1 that leads to the motor neuron loss in these transgenic mice, and the mechanisms by which this occurs are highly debated. The importance of oxidative stress in ALS is also highly debated even though ample evidence exists that it occurs in this disease [90]. A few studies have shown that catalytic antioxidants with H_2O_2-scavenging activities can prolong survival in the SOD1 mutant transgenic mice [105,106]. AEOL 10150 has completed phase I safety testing in ALS patients.

Stroke is a leading cause of death in humans with few treatment options. Stroke is an acute neurodegenerative condition that often involves tissue injury due to IR events. Stroke is associated with increased ROS production, and injury can be enhanced or attenuated by modulation of endogenous antioxidants [107]. A number of catalytic antioxidants with H_2O_2-scavenging activities are effective in animal vessel occlusion models of stroke [72–74]. Catalytic antioxidants are also effective in attenuating cerebral vasoconstriction that is commonly associated with hemorrhagic stroke and migraines [108,109]. Ebselen has had some success in human phase II clinical trials [75] and is currently being evaluated in human phase III clinical trials for stroke in Japan sponsored by Daiichi Sankyo Pharmaceuticals. These studies suggest that stroke may be a potential target for catalytic antioxidants.

7. Implications for the use of catalytic antioxidants as modulators of human disease

The postulated role of ROS as a terminal mediator of tissue injury and dysfunction in diseases of diverse etiologies emphasizes the wide range of therapeutic opportunities for catalytic antioxidant development. Pathologies that are most likely to benefit from catalytic antioxidant therapy include conditions in which a clear role for ROS has been established. Inflammation, which is a pivotal etiological factor in many human pathophysiologic processes involving multiple organ systems, is a major therapeutic opportunity for catalytic antioxidant development given the increasingly important role ROS are being given in modulating cell signaling pathways. The role of ROS has been well documented in host defense mechanisms involving phagocytosis, cytokines, chemokines and immune complex formation, all of which can contribute to auto-immune disorders. Inflammatory lung, intestinal, and cardiovascular diseases all are potentially important targets for catalytic antioxidant therapy.

An important unaddressed issue in the development of catalytic antioxidants is the mechanism by which these agents diminish oxidative stress and injury in animals and humans. To date, catalytic antioxidants are defined by their chemistry under highly defined and largely non-

biological conditions. The relevance of using this classification system is still yet to be verified. In fact, over time the potential number of mechanisms these agents possess in biological systems has increased substantially. A more important fact is that these compounds have potent biological effects that often track with their ability to suppress oxidative stress in a large variety of animal models of human disease. However, definitive proof that these compounds are effective in human disease is still needed.

8. Conclusion

Emerging research is strengthening the role of ROS in redox-mediated cell signaling pathways that have well-established roles in human disease and aging. A novel class of compounds that efficiently scavenge cellular peroxides are being developed to modulate cellular ROS and alter some of the aberrant cell signaling associated with many human disorders. There is promising data in the literature that these compounds may have potential therapeutic use in pulmonary, cardiovascular, neurodegenerative and inflammatory disorders.

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Abbreviations

AP-1
: activator protein 1

AD
: Alzheimer’s disease

ALS
: amyotrophic lateral sclerosis

ARDS
: acute respiratory distress syndrome

BPD
: bronchopulmonary dysplasia

COPD
: chronic obstructive pulmonary disease

CD
: cyclodextran

DHLA
: dihydrolipoate

DPDS
: diphenyl diselenide

GSH
: glutathione

GSSG
: glutathione disulfide

GPx
glutathione peroxidase

HD
Huntington's disease

H_2O_2
hydrogen peroxide

$HO^{\bullet}$
hydroxyl radical

HOCl
hypochlorous acid

IR
ischemia-reperfusion

LOOH
lipid peroxide

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

MPTP
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

NAC
N-acetylcysteine

NADPH
β-nicotinamide adenine dinucleotide phosphate

NO
nitric oxide

O_2
oxygen

PD
Parkinson's disease

ROOH
peroxides

Prx
peroxiredoxin

$ONOO^-$
peroxynitrite

RNS
reactive nitrogen species

ROS
reactive oxygen species

O_2^-
superoxide

SOD
: superoxide dismutase

TAA
: tetraaza macrocycle

Trx
: thioredoxin

H_2O
: water

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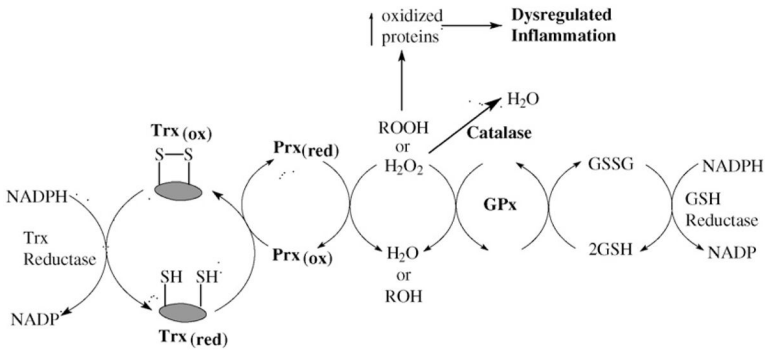

Fig. 1.

Endogenous scavenging of cellular peroxide. The cell's steady-state peroxide (ROOH) levels are largely maintained by the activities of catalase, glutathione peroxidases (GPx) and the thioredoxin-assisted peroxidases (peroxiredoxins, Prx). This system also maintains cellular protein cysteines in a reduced (red) state. During oxidative stress, where cellular peroxides are elevated, there is an increased level of oxidized (ox) protein cysteines that leads to inactivation of phosphatases and transcription factors and dysregulated inflammatory reactions. This is the drug target and rationale for the development of catalase and GPx mimics.

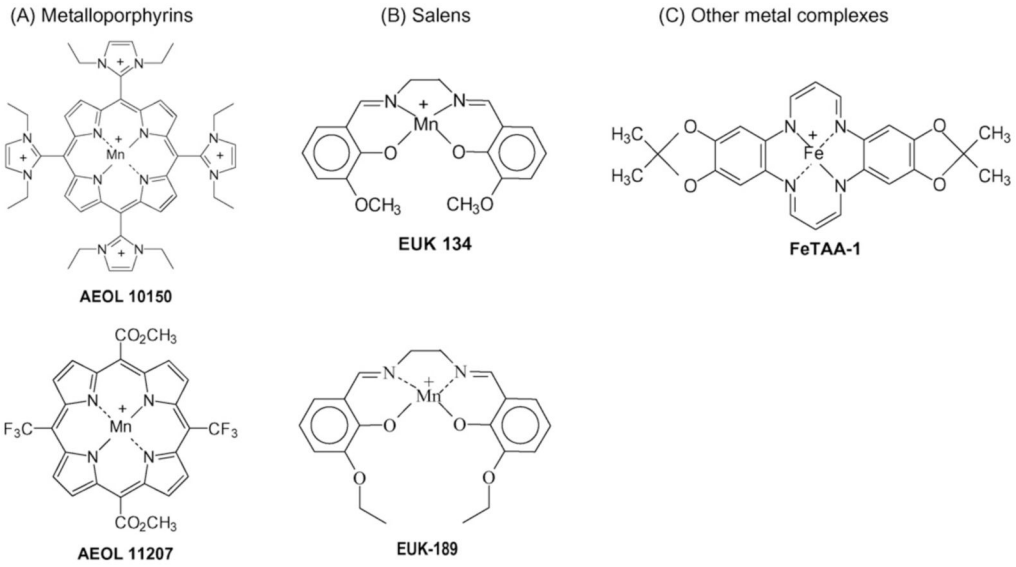

Fig. 2.
Examples of catalase-like mimics chemical structures: (A) metalloporphyrins; (B) salens; and (C) other metal complexes.

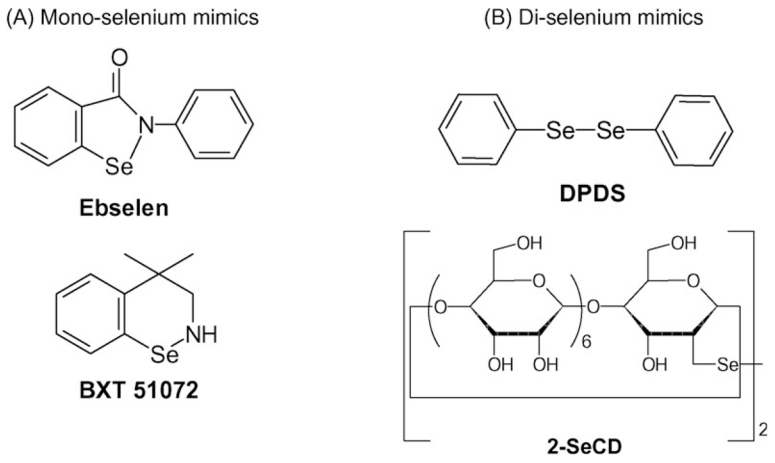

Fig. 3.
Examples of glutathione peroxidase-like mimics chemical structures: (A) mono-selenium mimics; and (B) di-selenium mimics.

Table 1

Examples of catalytic antioxidants with H_2O_2-scavenging activity that are effective in attenuating oxidative stress in *in vivo* models..

Model system	Species	Active site	Compound(s)	Reference
Pulmonary				
Bleomycin fibrosis	Mice	Mn	AEOL 10201	[57]
Radiation fibrosis	Rats	Mn	AEOL 10113	[59]
		Mn	AEOL 10150	[60]
		Mn	EUK 189	[61]
Cigarette smoke	Rats	Se	DPDS	[54]
		Mn	AEOL 10150	[55]
Endotoxin	Pigs	Mn	EUK 8	[66]
Bronchopulmonary dysplasia	Preterm baboons	Mn	AEOL 10113	[64]
Antigen-induced asthma	Mice	Mn	AEOL 10113	[50]
Hemorrhage	SOD3 KO mice	Mn	AEOL 10150	[65]
Cardiovascular				
Heart ischemia/reperfusion	Mice	Se	BXT 51072	[68]
	Aged rats	Mn	EUK 8	[110]
	Rats	Mn	AEOL 10113	[69]
Dilated cardiomyopathy	SOD2 KO mice	Mn	AEOL 10201	[111]
	Harlequin mutant mice	Mn	EUK 8	[83]
Sepsis	Rats	Mn	AEOL 10113	[84]
		Mn	EUK 8	[81]
Hemorrhage	Rats	Mn	EUK 8	[67]
		Mn	EUK 134	
Nervous system				
Neurofibromatosis	Flies	Mn	AEOL 10201	[112]
		Mn	AEOL 10150	
MPTP	Monkeys	Se	Ebselen	[97]
	Mice	Mn	AEOL 11207	[22]
ALS	Mutant SOD1	Mn	AEOL 10150	[105]
	Tg mice	Mn	EUK 8	[106]
		Mn	EUK 134	
Stroke	Rats	Se	Ebselen	[74]
		Mn	EUK 134	[73]
	Mice	Mn	AEOL 10113	[72]
		Mn	AEOL 10150	
	Humans	Se	Ebselen	[75]
Spinal cord trauma	Rats	Mn	AEOL 10201	[113]
		Se	Ebselen	[114]
	Mice	Mn	AEOL 10150	[115]
Cerebral vasoconstriction	Amyloid Tg mice	Mn	AEOL 10201	[108]
	Rats	Mn	AEOL 10201	[109]
Cognitive function	Aged mice	Mn	EUK 189	[103]

Model system	Species	Active site	Compound(s)	Reference
		Mn	EUK 207	
Hepatic/gastrointestinal/renal				
Ischemia/reperfusion	Rat	Mn	AEOL 10150	[70]
		Mn	EUK 134	[76]
		Se	Ebselen	[71]
Endotoxin	Mice	Se	Ebselen	[85]
		Mn	AEOL 10113	[82]
	Rats	Mn	EUK 134	[86]
Fas	Mice	Mn	AEOL 10201	[116]
Ethanol	Rats	Se	Ebselen	[117]
Carbon tetrachloride	Rats	Se	Ebselen	[118]
Hyperthermia	Aged rats	Mn	EUK 189	[119]
Colitis	Rats	Mn	AEOL 11201	[120]