Catalytic antioxidants: a radical approach to new therapeutics

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In humans, several pathologies involve the overproduction of reactive oxygen species. Metal-containing catalytic antioxidants have emerged as a novel class of potential therapeutic agents that scavenge a wide range of reactive oxygen species. There are three structural classes of manganese-containing catalytic antioxidants that have efficacy in several oxidative stress models of human disease. The classes are divided based on their *in vitro* selectivity towards the scavenging of superoxide. The selective catalytic antioxidants include the macrocyclics, whereas the non-selective catalytic antioxidants include the salens and porphyrins. Cardiovascular, neurodegenerative and inflammatory lung disorders are all potentially important targets for catalytic antioxidant therapy.

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▼ Reactive oxygen species (ROS), such as the superoxide radical anion ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2), are formed in biological systems by the partial reduction of molecular oxygen (Figure 1). $O_2^{-\bullet}$ is produced from the one-electron reduction of molecular oxygen. Reduction of $O_2^{-\bullet}$ with a second electron generates H_2O_2, which can also be formed from a two-electron reduction of molecular oxygen. Formation of the hydroxyl radical (HO·), another ROS, is thought to occur through the one-electron reduction of H_2O_2, a reaction that is facilitated by transition metals that are in a reduced valence state (e.g. reduced copper or iron). Four electrons and two protons are required to reduce molecular oxygen to water (H_2O). Additionally, there are a large number of other reactive species that are formed from the reaction of ROS with biological molecules [e.g. polyunsaturated lipids, thiols and nitric oxide (NO)].

$O_2^{-\bullet}$ is generated from the uncoupling of the mitochondrial electron transport chain during oxidative phosphorylation and also by the catalytic action of a variety of intracellular and extracellular oxidases. The production of $O_2^{-\bullet}$, which occurs during host defense responses, is either beneficial or detrimental depending upon the site of formation, the amount generated and the prevalence of antioxidant defenses. $O_2^{-\bullet}$ is thought to act as a cell-signaling molecule and to contribute to the killing of foreign bacteria [1]. However, during acute and chronic inflammation, the overproduction of $O_2^{-\bullet}$ has been shown to contribute to tissue damage and injury [2].

H_2O_2 is generated directly from $O_2^{-\bullet}$ through a rapid dismutation reaction that can occur either enzymatically (rate of 10^9 M^{-1} s^{-1}) or non-enzymatically (rate of 10^5 M^{-1} s^{-1}). In addition, H_2O_2 is formed enzymatically as a byproduct of lipid metabolism in peroxisomes. H_2O_2 is stable at biological pH, but can participate in HO· formation in the presence of reduced transition metals. H_2O_2 readily reacts with the thiol functional group and this type of reaction is proposed to be one of the key mechanisms through which ROS participate in cell signaling. Several phosphatases contain sensitive thiol residues; the oxidization of this functionality results in inactivation of the phosphatase [3]. In addition, H_2O_2 alters cell signaling through the oxidation of thiols in transcription factors, such as reducing factor-1, activator protein-1 and nuclear factor-κB [4].

$O_2^{-\bullet}$ and H_2O_2 participate in the formation of several reactive species that have been directly implicated in tissue damage, for example, HO·. $O_2^{-\bullet}$ effects the release of iron from iron-containing proteins, thus increasing the concentration of the transition metal that is available to reduce H_2O_2 to HO·. HO· is an extremely reactive species that readily oxidizes all cellular macromolecules, including proteins, sugars, lipids and DNA. In addition, $O_2^{-\bullet}$ readily reacts with NO to form peroxynitrite ($ONOO^-$), which is unstable at physiological pH and rapidly decomposes to form potent

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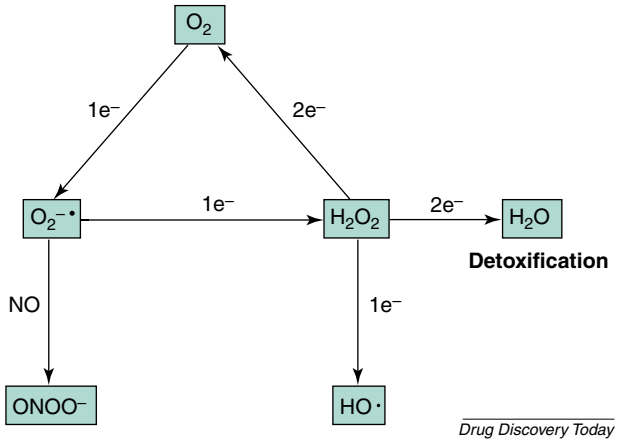

Figure 1. The generation of reactive oxygen species. Oxygen (O_2) is partially reduced by either one electron to form the superoxide radical anion ($O_2^{-\bullet}$) or two electrons to generate hydrogen peroxide (H_2O_2). In addition, further reduction of $O_2^{-\bullet}$ and H_2O_2 produces other reactive species. H_2O_2 can be reduced to form the hydroxyl radical (HO·). $O_2^{-\bullet}$ readily reacts with nitric oxide (NO) to form peroxynitrite ($ONOO^-$), which is unstable at physiologic pH and readily decomposes into potent oxidizing and nitrating species that damage cellular macromolecules such as proteins, DNA, lipids and sugars. Antioxidants detoxify these reactive species to water (H_2O).

nitrating and oxidizing species [5]. Cellular damage by oxidative modification of cellular macromolecules ensues when ROS are generated in excess of endogenous defenses. ROS are implicated in several pathological processes including tissue injury [6], inflammatory disorders [7], cardiovascular disease [8], pulmonary disease [9] and neurodegenerative diseases [10].

Catalytic antioxidants

Superoxide dismutases (SODs) and catalase are metalloproteins that catalyze 'dismutation' reactions, which detoxify ROS. A dismutation reaction is defined as a reaction in which two like-molecules react to produce two different products (i.e. A + A → B + C). SODs catalyze the formation of oxygen and H_2O_2 from two $O_2^{-\bullet}$, whereas catalase metalloproteins catalyze the reaction of two H_2O_2 to produce oxygen and water. Because these efficient reactions do not require additional reducing equivalents, no energy is taken from the cell to drive these transformations. The overall goal of cellular antioxidant defenses is to reduce ROS to water. The overexpression of these metalloproteins in cell culture and in whole animals has provided protection against the deleterious effects of a wide range of oxidative stress paradigms [11,12]. The use of SOD and catalase as therapeutic agents to attenuate ROS-induced injury responses has had mixed success [13,14]. The principal limitations of using these proteins are their large sizes, the consequences of which are low cell permeability, a short circulating half-life, antigenicity and high-manufacturing costs. To overcome many of these limitations, an increasing number of low molecular-weight catalytic antioxidants have been developed.

Development of catalytic antioxidants

The majority of catalytic antioxidants are designed with redox-active metal centers that catalyze the dismutation reaction of $O_2^{-\bullet}$ and/or H_2O_2 by a mechanism that is similar to the mode of action of the active-site metals of SOD and catalase. An ideal mimetic is stable and non-toxic. Furthermore, the size and charge of a mimetic can be exploited to target crucial cellular sites of oxidative stress.

For many years, it has been recognized that simple metal chelates react with $O_2^{-\bullet}$ and H_2O_2. However, the rates of reaction of chelates with these particular ROS are low and the complexes formed are unstable. The search for more stable and active metal chelates has resulted in the discovery of at least three classes of metal-containing catalytic antioxidants. These include the salen, macrocyclic and metalloporphyrin series (Figure 2). Within this group, the compounds are often divided into selective and non-selective classes based on the specificity of the compounds towards $O_2^{-\bullet}$ in test-tube reactions (this is why these compounds

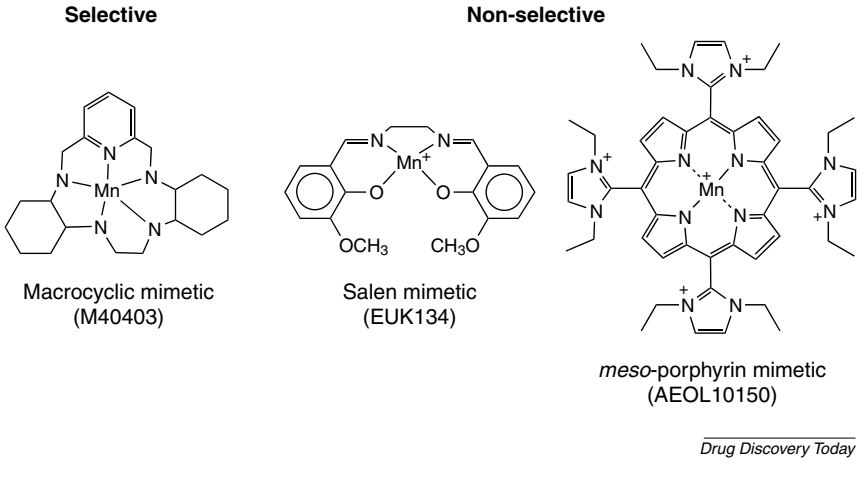

Figure 2. Structures of the different classes of catalytic antioxidants. Selectivity only refers to the *in vitro* reactivity towards superoxide versus hydrogen peroxide or peroxynitrite.

are often referred to as SOD mimetics). However, these reactions might not have relevance in more complex biological systems, where it is often difficult to demonstrate this type of selectivity. The potencies of catalytic antioxidants are often compared using their 'test tube' $O_2^{-\bullet}$ and/or H_2O_2 rate constants, which also might not be relevant in more-complex biological systems. This is particularly pertinent given the recent findings that some of these compounds accept electrons from cellular enzymes [15]. The finding that three structurally different classes of catalytic antioxidants are effective in similar oxidative stress models confirms the basic concept that small, efficient, catalytic antioxidants show promise in the treatment of ROS-mediated injuries.

Antioxidant properties of selective catalytic antioxidants

Macrocyclics

The manganese atom (Mn) at the center of the pentaaza-macrocyclic ligand-based mimetics [M series is currently under development by Metaphore Pharmaceuticals (http://www.metaphore.com)] is held by five coordination points and is only available for one-electron transfers [16]. Thus, this class of catalytic antioxidant is unique in that they are specific scavengers for $O_2^{-\bullet}$ (scavenging of H_2O_2 and $ONOO^-$ requires transfer of two electrons). During the dismutation reaction with $O_2^{-\bullet}$, the Mn(II) at the center of the macrocyclic structure undergoes alternate oxidation and reduction, which results in an interchanging valence state between Mn(II) and Mn(III). This unique feature of macrocyclic antioxidants gives them *in vitro* selectivity towards $O_2^{-\bullet}$. However, in biological systems, there are several other endogenous compounds that can also participate in one-electron reactions, including flavins and ubiquinones, and it is unclear whether or not macrocyclic antioxidants interact exclusively with $O_2^{-\bullet}$. The macrocyclics are effective in many of the same oxidative paradigms that non-selective catalytic antioxidants have been used in. The different classes of catalytic antioxidants have not been directly compared in experimental models, therefore the conditions under which one class holds an advantage over the other are currently not known.

Antioxidant properties of non-selective catalytic antioxidants

Salens

The structures of compounds in the salen class of catalytic antioxidant [EUK series is currently being developed by Eukarion (http://www.eukarion.com)] are generally aromatic, substituted ethylenediamine metal complexes. The Mn(III)-containing salen complexes have been reported to have two key antioxidant properties – the scavenging of $O_2^{-\bullet}$ and H_2O_2 [17]. However, these compounds have been shown to react with $ONOO^-$ [18] and are also thought to react with lipid peroxides. The Mn moiety of the salen compound 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 macrocyclics and porphyrins where the metal is coordinated to nitrogen atoms only. The coordination of Mn by four axial ligands results in the formation of several possible valance states, which are thought to be important in the scavenging of a wide variety of ROS and, thus, contribute to the non-selective nature of this class of antioxidant. Salens comprising Mn(III) function as $O_2^{-\bullet}$ and H_2O_2 scavengers. However, the mode of action of the salen class of compounds has yet to be elucidated, but it is thought that these molecules act by a mechanism comparable to that reported for the metalloporphyrins. The rates at which Mn(III)-containing salens scavenge H_2O_2 are similar to those reported for metalloporphyrins, but are many orders of magnitude less than those documented for endogenous catalase.

Metalloporphyrins

Metalloporphyrins [AEOL series is currently being developed by Aeolus Pharmaceuticals, which is a subsidiary of Incara Pharmaceuticals (http://www.incara.com)] are structurally different from endogenous protoporphyrins and are classed as synthetic *meso*-substituted porphyrins. Metalloporphyrins have been shown to possess at least four distinct antioxidant properties, which include scavenging $O_2^{-\bullet}$, H_2O_2, $ONOO^-$ and lipid peroxides. Analogous to the salen class of antioxidants, metalloporphyrins contain a Mn moiety that is coordinated by four axial ligands. These non-selective antioxidants participate in the dismutation reaction of $O_2^{-\bullet}$ through the alternate reduction and oxidation of the Mn moiety, which results in changes in valence between Mn(III) and Mn(II); this is similar to the reduction-oxidation reaction occurring in native SODs. The catalase activity of metalloporphyrins could be attributed to their extensive conjugated ring system that undergoes reversible one-electron oxidations, which is equivalent to the heme prosthetic groups of endogenous catalases and peroxidases [19]. In general, metalloporphyrins with higher SOD activity have greater catalase activity. It is significant that the catalase activity of most metalloporphyrins is less than 1% of native catalases. However, despite this low catalase activity, Mn-containing porphyrins are still able to protect cells from H_2O_2-mediated toxicity [20]. The mechanism by which metalloporphyrins scavenge $ONOO^-$ is thought to involve the formation of an

Table 1. Catalytic antioxidants that are effective in blocking oxidant stress in *in vitro* models

Model system	Cell type affected	Catalytic antioxidant(s)	Refs
Cytotoxicity			
Hydrogen peroxide	Fibroblast	AEOL10201	[79]
Oxygen and/or glucose deprivation	Neuronal	AEOL10201	[80]
		AEOL10113	
		AEOL10150	[69]
		AEOL10216	[32]
Neutrophil-mediated cytotoxicity	Lymphocytes	AEOL10201	[81]
MNDA	Neuronal	M40403	[82]
Paraquat	Epithelial	AEOL10216	[32]
Gentamicin	Cochlear cultures	M40403	[83]
SOD2 KO	Neuronal	AEOL10201	[84]
		AEOL10113	
Apoptosis			
T-cell receptor activation	Lymphocytes	AEOL10201	[85]
6-Hydroxydopamine	Neuronal	EUK134	[86]
Staurosporin	Neuronal	EUK134	[86]
		EUK189	
HIV	Astrocytes	M40401	[87]
Hyperoxia	Epithelial	EUK134	[88]
Diseal exhaust particles	Epithelial	EUK8	[89]
Zinc	Neuronal	EUK134	[90]
Cyclic strain	Myocytes	EUK8	[91]
		AEOL10110	
MPP+	Neuronal	AEOL10201	[92]
		EUK134	[93]

Abbreviations: KO, knockout; MNDA, *N*-methyl-D-aspartate; MPP+, 1-methyl-4-phenylpyridinium; SOD, superoxide dismutase.

oxo-Mn(IV) complex that is readily reduced to the Mn(III) oxidation state by a wide variety of endogenous antioxidants (e.g. ascorbate and glutathione), and even by $O_2^{-\bullet}$ [21]. The exact mechanism of metalloporphyrin-mediated inhibition of lipid peroxidation is not known, but it is thought to be similar to the mode of action described for metalloporphyrin-scavenging of $ONOO^-$.

Catalytic antioxidants are effective in blocking oxidant stress *in vitro*

Cytotoxicity

In vitro models of oxidative stress have proved useful in terms of confirming the antioxidant activities of catalytic antioxidants obtained from cell-free systems, and for predicting the utility of these compounds as antioxidants in more complex *in vivo* models of human disease. Given the large volume of literature on catalytic antioxidants, this review will focus on recent findings (2000 to present); earlier literature has previously been comprehensively reviewed [22–24]. Members of all classes of catalytic antioxidant have been shown to be effective in blocking oxidant stress in a wide variety of *in vitro* cytotoxicity models involving the independent or concomitant generation of $O_2^{-\bullet}$, H_2O_2 and $ONOO^-$ species (Table 1). However, there is still some debate as to the mechanism of action of these compounds that affords this defensive effect. Because the reaction of $O_2^{-\bullet}$ with different species can lead to the formation of several ROS, it is not surprising that selective and non-selective catalytic antioxidants show similar efficacy in these model systems. At μmolar levels, catalytic antioxidants appear non-toxic and protect a wide variety of different types of cultured cells against the toxicity of ROS.

Apoptosis

Apoptosis is a form of cell death that is biochemically and morphologically distinct from necrosis and that subserves physiological and pathological roles in organisms. Although the demonstration that cells undergo apoptosis when oxygen levels are low and the finding that many antioxidants fail to prevent apoptosis argue against a causal role of ROS in some apoptotic pathways, there is a large body of evidence that supports the involvement of ROS in apoptotic cell death. The release of proapoptotic factors by mitochondria, which are the major source of cellular ROS, lends further support to this argument. Furthermore, demonstrations that the delivery of SOD to neurons is protective [25,26], whereas its paucity is deleterious [27], suggest that $O_2^{-\bullet}$ is involved in neuronal apoptosis. A wide variety of apoptosis paradigms can be blocked with selective and non-selective catalytic antioxidants (Table 1). These models range from xenobiotic-induced models to physiologic models, and even to mechanical models. In addition, the cells types vary from immune cells to epithelial cells to neuronal cells. It is not clear from these studies whether the catalytic antioxidants affect a particular point in the intrinsic and/or extrinsic apoptotic pathways. However,

recent studies suggest that ROS might regulate the expression of bcl-2 and that catalytic antioxidants might indirectly block apoptosis by increasing the expression of this antiapoptotic factor [28].

Catalytic antioxidants are effective in blocking oxidant stress *ex vivo*

Catalytic antioxidants have been shown to be therapeutically effective in several *ex vivo* model systems (Table 2), including models of cardiac hypertrophy [29], vessel dysfunction [30], endotoxin [31], lipid peroxidation [15,32], compression injury [33] and oxidative paradigms in hippocampal slices [34,35]. The majority of these model systems involve the overproduction of ROS by NADPH oxidases and, in some instances, their interaction with NO.

NO is an important modulator of vessel tone and is rapidly inactivated by $O_2^{-\bullet}$. Therefore, inappropriate interplay between these reactive species can disrupt vessel function. Catalytic antioxidants have been shown to block the effects of the angiotensin II-induced elevation of $O_2^{-\bullet}$ levels in vessels, which results in hypertension [36]. Vessel dysfunction is also associated with arteriosclerosis and ROS have been implicated in the pathogenesis of this condition. Catalytic antioxidants are effective in attenuating vessel dysfunction in aorta from animal models of arteriosclerosis [30,37]. These data, together with promising studies in whole animals, suggest a potential utility of these agents in a variety of human vascular diseases, including hypertension, pulmonary hypertension, Raynaud's disease and arteriosclerosis.

Table 2. Catalytic antioxidants that are effective in blocking antioxidant stress in *ex vivo* models

Model system	Organ and/or tissue	Catalytic antioxidant(s)	Refs
Cardiac hypertrophy	Isolated heart	AEOL10110	[29]
Endotoxin	Peritoneal macrophage	AEOL10201	[31]
Vessel dysfunction	ApoE KO	AEOL10201	[30]
	Aorta	M40403	[37]
Angiotensin II-induced vasoconstriction	Aorta	EUK8	[36]
Compression injury	Cartilage	AEOL10110	[33]
Lipid peroxidation	Brain homogenate	AEOL10216	[32]
		AEOL10123	[15]
		AEOL10150	
		AEOL10158	
	LDL	AEOL10113	[94]
		AEOL10201	
		AEOL10170	
Iron	Hippocampal slices	EUK134	[34]
Kainate	Hippocampal slices	EUK134	[35]

Abbreviations: ApoE, apolipoprotein E; KO, knockout; LDL, low-density lipoprotein

Catalytic antioxidants are effective in blocking oxidant stress *in vivo*

The beneficial effects of catalytic antioxidants have been demonstrated in several *in vivo* model systems (Table 3), including models of lung fibrosis [38,39], chronic obstructive lung diseases [40], asthma [41], acute respiratory distress syndrome [42], bronchopulmonary dysplasia [43], pleurisy [44], shock [45–50], myocardial infarction [51,52], liver failure [53,54], ischemia–reperfusion [55,56], colitis [57,58], diabetes [59], transplantation [60], arthritis [61], amyotrophic lateral sclerosis [62,63], migraine [64,65], neurodegenerative diseases [66], stroke [67–70], spinal cord injury [71,72], pain [73] and dementia [74]. The efficacy of catalytic antioxidants demonstrated in an incredible diversity of model systems highlights the extensive involvement of ROS in common animal models of human disease.

Lung models

The lung functions at a higher oxygen tension than most other organs and, therefore, has a unique relationship with ROS. There is mounting evidence that indicates ROS have a key role in several lung diseases [9]. A common modality among lung diseases is an inappropriate inflammatory response. Catalytic antioxidants decrease airway hyperreactivity and inflammation in an antigen-induced mouse model of asthma [41]. Chronic obstructive pulmonary disease (COPD), which includes emphysema and bronchitis, is often associated with cigarette smoke; the combustion products of tobacco smoke have high levels of ROS. Catalytic antioxidants suppressed inflammation and protected the rat lung epithelium from cigarette smoke-induced precancerous lesions [40]. Interstitial lung disease is also associated with an increased oxidant burden and many animal models of pulmonary fibrosis use agents, such as bleomycin or ionizing radiation, that overproduce ROS [9]. Catalytic antioxidants attenuate lung fibrosis that has been induced by either bleomycin [38] or ionizing radiation [39]. Bronchopulmonary dysplasia occurs in premature infants where the lung is not fully developed and requires additional supplemental oxygen for adequate gas

Table 3. Catalytic antioxidants that are effective in blocking antioxidant stress in *in vivo* models

Model system	Species used for model system	Catalytic antioxidant(s)	Refs
Lung			
Bleomycin fibrosis	Mice	AEOL10201	[38]
Radiation fibrosis	Rats	AEOL10113	[39]
Cigarette smoke injury	Rats	AEOL10150	[40]
Antigen-induced asthma	Mice	AEOL10113	[41]
Hemorrhage-induced injury	SOD3 KO mice	AEOL10150	[42]
Bronchopulmonary dysplasia	Baboon	AEOL10113	[43]
Carrageenan-induced inflammation	Rats	M40403	[44]
Cardiovascular			
Splanchnic artery occlusion	Rats	AEOL10217	[50]
Heart ischemia–reperfusion	Rats	M40403	[51]
		EUK8	[52]
Hemorrhagic shock	Rats	EUK8	[45]
		EUK134	
Nitrate tolerance	Rats	AEOL10201	[95]
Interleukin-2-induced hypotension	Mice	M40403	[46]
Endotoxin-induced shock	Rats	M40401	[47]
		EUK8	[48]
Hypoxic pulmonary vasoconstriction	Mice	EUK8	[96]
Central nervous system			
Kainite injury	SOD2 KO mice	AEOL10201	[66]
Cerebral vasoconstriction	Amyloid Tg mice	AEOL10201	[65]
	Rats	AEOL10201	[67]
A-β cerebral vasoconstriction	Mice	AEOL10201	[64]
ALS	SOD1 Tg mice	EUK8	[63]
		EUK134	
Spinal cord injury	Rats	AEOL10201	[71]
Spongiform encephalopathy	SOD2 KO mice	EUK8	[77]
		EUK134	
Ischemia–reperfusion	Rats	AEOL10113	[68]
	Mice	AEOL10150	[69]
	Gerbils	M40401	[70]
Phencyclidine injury	Rats	M40401	[97]
Hyperalgesia	Rats	M40403	[73]
Age-induced cognitive impairment	Mice	EUK189	[74]
		EUK207	
Meningitis-induced hearing loss	Rats	AEOL10201	[98]
Liver			
Acetaminophen injury	Mice	AEOL10201	[53]
Fas-induced injury	Mice	AEOL10201	[54]
Ischemia–reperfusion	Rat	AEOL10150	[55]
Gastrointestinal			
Acetic acid-induced colitis	Rats	AEOL11201	[57]
Trinitrobenzene sulfonic acid-induced colitis	Rats	M40403	[58]
Renal			
Gentamicin injury	Rats	M40403	[99]
Endotoxin	Mice	AEOL10113	[49]
Ischemic–reperfusion	Rats	EUK134	[56]
Endocrine			
Diabetes	NOD mice	AEOL10113	[59]
Joint			
Collagen-induced arthritis	Rats	M40403	[61]

Abbreviations: A-β, amyloid-β; ALS, amyotrophic lateral sclerosis; NOD, non-obese diabetic; SOD, superoxide dismutase; Tg, transgenic.

exchange. Catalytic antioxidants suppress lung inflammation and improve lung function in a hyperoxic model of bronchopulmonary dysplasia in preterm baboons [43]. Acute respiratory distress syndrome (ARDS) is associated with sepsis and shock (conditions that are also linked to excessive ROS production) and catalytic antioxidants have shown effectiveness in several of these types of animal models [42,45,48,75].

Many of the current therapies for the treatment of lung disease are directed towards alleviating the symptoms of these diseases, but there is a medical need for therapeutics that slow or prevent the progression of lung disease. Research implicating ROS in lung disease supports the development of catalytic antioxidants for the treatment of asthma, COPD, ARDS, bronchopulmonary dysplasia and interstitial lung disease.

Cardiovascular models

A common complication of bacterial sepsis is the phenomenon referred to as 'endotoxic shock', which results in oxidative tissue damage that is partially attributed to the formation of $ONOO^-$. A serious consequence of endotoxic shock is the loss of responsiveness to vasoconstrictive agents. Selective and non-selective catalytic antioxidants are effective in endotoxin models, which is probably related to their ability to scavenge $O_2^{-\bullet}$ and/or $ONOO^-$ [45–48,50].

Another common cause of tissue injury, which is often associated with cardiac damage in myocardial infarction, arrhythmias, angina, myocardial stunning and transplantation, occurs during ischemia and subsequent reoxygenation or reperfusion. The role of excessive ROS production during ischemia–reperfusion and the protective effects of endogenous antioxidants have been well documented [76]. Selective and non-selective catalytic antioxidants are effective in a rat model of heart ischemia–reperfusion [51,52]. In addition to heart models of ischemia–reperfusion, several other organs have been shown to benefit from catalytic antioxidant treatment, including the liver [55] and the kidney [56].

Currently, there are few treatment options for either sepsis or tissue injury associated with ischemia–reperfusion. The overproduction of ROS is a key mechanism in the development of ischemia–reperfusion and the abundance of literature reporting a protective effect of antioxidants in this condition makes this a particularly attractive area for the development of catalytic antioxidant therapeutics.

Central nervous system models

As a result of the high levels of oxygen required by the brain, this organ is particularly sensitive to ROS-mediated damage. Other factors that exacerbate this sensitivity are the presence of autooxidizable neurotransmitters and a high concentration of polyunsaturated fatty acids in neuronal membranes. Furthermore, the brain has only low levels of endogenous antioxidants to protect itself from oxidative damage. Collectively, these factors make catalytic antioxidants good candidates for several acute and chronic neuronal disorders that involve the overproduction of ROS, for example, Parkinson's disease (PD), Huntington's disease (HD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), status epilepticus, stroke and trauma. Excitotoxicity (neuronal death resulting from excessive stimulation of glutamate receptors), leads to increased ROS production, which is a key pathological process in neurological disorders. Mice that lack SOD2 develop a fatal neurodegenerative phenotype that can be rescued by catalytic antioxidants [77]. Furthermore, mice that overexpress SOD2 are protected against excitotoxic injury that is induced by kainate, whereas heterozygous SOD2 knockout mice show exacerbated damage [66]. Neurons cultured from SOD2 knockout mice spontaneously die in normoxic culture conditions, but can be rescued by catalytic antioxidants [78].

Stroke is a neurodegenerative condition that often involves tissue injury that occurs during ischemia–reperfusion. These events are associated with the overproduction of ROS and can be enhanced or attenuated by modulation of endogenous SOD levels. Catalytic antioxidants are effective in attenuating cerebral vasoconstriction, which is commonly associated with hemorrhagic stroke and migraines [64,65,67]. Catalytic antioxidants are also effective in rodent vessel occlusion models of stroke [68–70].

Pain management still poses clinical challenges, particularly with the large potential of addiction to opiates. NO could play a role in inflammatory pain perception. Considering the interplay between NO and $O_2^{-\bullet}$, it could be anticipated that ROS and antioxidants modulate pain perception. A selective catalytic antioxidant (M40403) has recently been shown to block inflammation and hyperalgesia in a rat carrageenan model [73]. These data suggest that catalytic antioxidants could be developed as non-narcotic analgesic agents. Interestingly, this is also the first indication that catalytic antioxidants are being clinically tested.

These studies indicate that neuronal disorders such as ALS, trauma, pain, stroke and associated cerebral vascular disease might be amenable to catalytic antioxidant treatment. These conditions affect a large portion of the human population, but, unfortunately, there are few therapeutic options available for the treatment of many of these diseases.

Implications for the use of catalytic antioxidants in the therapy of human disease

The postulated role of ROS as final common mediators of tissue damage in diseases of diverse etiologies emphasizes the wide range of therapeutic applications of catalytic antioxidants. 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 key etiological factor that accompanies diseases involving multiple organs, comprises a major therapeutic area for antioxidant therapy. In the immune system, $O_2^{-\bullet}$ production can be triggered by host defense mechanisms such as phagocytosis, inflammatory cytokines, chemokines and immune complex formation that contribute to autoimmune diseases. Inflammatory lung, intestinal and cardiovascular disorders are all potentially important targets for catalytic antioxidant therapy.

Another major target of catalytic antioxidant therapy are diseases in which cell death occurs with or without ancillary inflammation. ROS modulate intrinsic and extrinsic apoptosis pathways and, therefore, neurodegenerative diseases that are associated with excess apoptosis are potential therapeutic targets and include PD, HD, AD, ALS, epilepsy, stroke and trauma. The detection of cell death processes occurring with and without ancillary inflammation in several of these neurodegenerative diseases provokes the speculation that the use of catalytic antioxidants that prevent some forms of apoptosis and necrosis could be particularly beneficial in the treatment of these conditions. Finally, in addition to being a key component of host defense, ROS have physiological roles in cell signaling and, consequently, the control of gene expression, which are processes that could be disrupted by antioxidant therapies. However, the carefully controlled titration of the amount of catalytic antioxidants administered to treat these disease conditions could potentially facilitate the scavenging of excess ROS, and thus restore redox balance and health to the tissue.

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