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Review Article

Metalloporphyrin catalytic antioxidants for the potential treatment of neurodegenerative diseases

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CONTENTS

Abstract

Oxidative injury has been implicated in the etiology of a number of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and Huntington's disease. Antioxidants, in particular catalytic small-molecular-weight antioxidant compounds that detoxify reactive species, are novel therapeutic agents and therefore of great interest as potential therapies for chronic neurodegenerative diseases. Manganese-containing *meso*-porphyrin catalytic antioxidants that mimic the activities of superoxide dismutase (SOD) and catalase and decompose peroxynitrite ($ONOO^-$) have demonstrated efficacy in cellular and animal models of neurodegenerative diseases, offering hope that metalloporphyrin antioxidants may be promising drugs of the future in human neurodegenerative diseases.

Reactive oxygen species and reactive nitrogen species as potential drug targets

Superoxide

Reactive oxygen species (ROS) are normal byproducts of cellular metabolism and inflammation that are continually produced at low levels under normal conditions. The formation of superoxide (O_2^-) occurs by the one-electron reduction of molecular oxygen (O_2). Biologically, O_2^- is commonly generated from the uncoupling of the cellular electron transport systems (1). Cellular electron transport systems that account for a large portion of cellular O_2^- formation are the mitochondrial electron transport system (2) and various oxidases, including NADPH oxidases (3), cytochrome P-450 monoxygenases (4), cyclooxygenases (5), lipoxygenases (5), nitric oxide synthases (6) and xanthine oxidoreductase (7). O_2^- also rapidly reacts with nitric oxide (NO) to form the strong oxidizing and nitrating agent peroxynitrite ($ONOO^-$). Another role for O_2^- is its ability to liberate iron from iron-containing proteins (8, 9).

Hydrogen peroxide

Further reduction of O_2^- leads to the formation of hydrogen peroxide (H_2O_2), which means that wherever O_2^- is generated there is also formation of H_2O_2. H_2O_2 can also be formed enzymatically as a byproduct of lipid metabolism in peroxisomes (10) and as inactivation of iron-containing proteins such as aconitase. H_2O_2 is stable at biological pH and easily crosses lipid membranes. H_2O_2 can participate in hydroxyl radical ($HO^\cdot$) formation in the presence of reduced transition metals (11). 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 (12).

Several phosphatases contain reactive thiol residues that are inhibited upon oxidation (13). As steady-state levels of oxidants rise, there is an increase in the inactivation of phosphatases and a corresponding increase in the levels and duration of phosphorylated proteins, many of which play prominent roles in inflammatory responses (14-16). An increase in steady-state ROS results in alteration of 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 directly alter cell signaling by reacting with specific thiols on transcription factors, such as activator protein 1 (AP-1) and inhibitor of kappaB (IkB) (17, 18). Many of

these types of mechanisms are thought to contribute to the often observed unregulated inflammatory responses associated with human diseases.

Reactive nitrogen species

Reactive nitrogen species (RNS) also play a role in oxidative stress. Nitric oxide (NO) acts as a signal transduction molecule in vasodilatation (19, 20) and neuronal signaling (21), and nitrosylation is proposed to be a redox-sensitive protein modification involved in signal transduction (reviewed in 22, 23). NO also reacts with O_2^- in a reaction that is diffusion-limited, forming $ONOO^-$ (Fig. 1), a powerful oxidant. This reaction can serve as a mechanism to control levels of O_2^- and/or NO, affecting cell signaling by NO. Additionally, $ONOO^-$ and its breakdown products can react with proteins, resulting in nitration of tyrosine residues (24). Both O_2^- and NO are produced in the course of the inflammatory response, leading to the formation of nitrotyrosine, which can be used as a molecular footprint of nitrosative stress in inflammation (25). Nitrotyrosine has been considered a hallmark of oxidative stress and is increased in many disease states, including Parkinson's disease (26) and Alzheimer's disease (27).

Endogenous antioxidant defenses

The production of ROS in various cellular compartments is a consequence of normal metabolism and is tightly regulated by endogenous antioxidant defenses, which include small-molecular-weight compounds such as glutathione and urate. Many of these compounds either provide reducing equivalents or their reaction products result in a less reactive species and often require regeneration by cellular enzymes. An advantage of these types of compounds is that they tend to be nonspecific and can react with a wide range of reactive species, including electrophilic compounds in addition to the more classic ROS. The cell also relies on detoxifying enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase and peroxiredoxins to limit the steady-state levels of endogenous reactive species. These enzyme systems tend to be more specific for the ROS they detoxify and can turn over many more ROS than stoichiometric antioxidants. The production of ROS is either beneficial or detrimental, depending on the site of formation, the amount generated and the prevalence of antioxidant defenses. 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 are often assessed by measuring oxidative footprints in fluids and tissues, such as markers of protein, lipid and DNA oxidation. The mitochondria are a prominent cellular source of endogenous ROS in both health and disease. Mitochondrial O_2^- is formed as a result of excessive electrons arising from inhibition of normal electron transport or a "leak", which allows for the partial reduction of molecular oxygen. O_2^- radicals formed under physiological conditions have been estimated to account for 0.1-0.4% of electron flow (28, 29).

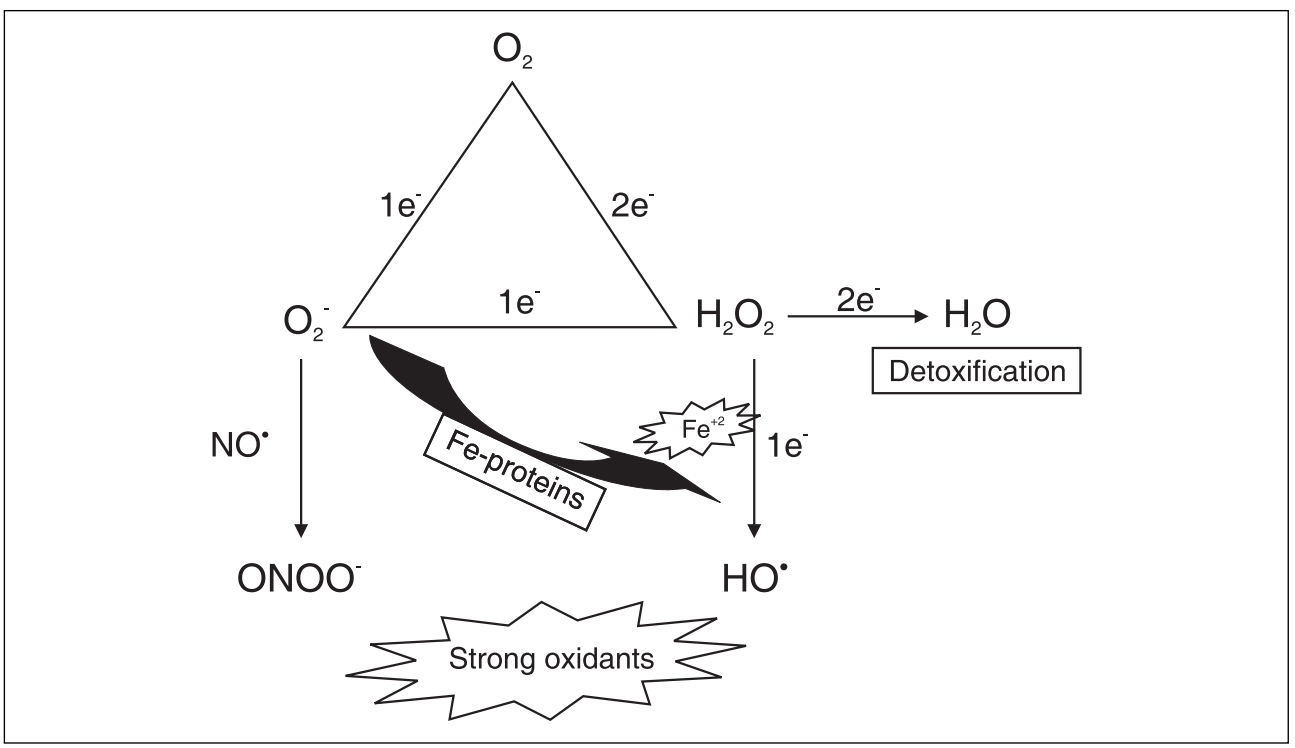

Fig. 1. A schematic representation of the interconversion and formation of major reactive oxygen/nitrogen species.

The major detoxification pathway for O_2^- is by the mitochondrial SOD MnSOD, which dismutes O_2^- to H_2O_2 and oxygen. The significance of mitochondrial O_2^- toxicity is evident by the lethal phenotype and severe pathologies present in the MnSOD-null mouse (30, 31). Mice lacking MnSOD are either embryonic or neonatal lethal, depending on the genetic background (32). MnSOD-null mice on the CD1 background survive about 8 days but develop fatty liver, metabolic acidosis and cardiomyopathy (33). Treatment with AEOL-10201 (Fig. 2) results in prolongation of the lifespan of the mice and improvement of the liver and heart pathology, but a neurodegenerative phenotype is uncovered, indicating the importance of detoxifying endogenous mitochondrial O_2^- in the brain (34).

The significance of enhanced O_2^- production is of particular interest in chronic neurodegenerative diseases such as Parkinson's disease, as patients exhibit a brain and systemic decrement in the activity of complex I (35). This is because O_2^- production increases dramatically when electron flow is inhibited at complex I. Once excessive mitochondrial O_2^- is generated, it can readily inactivate aconitase, resulting in release of iron (36), as well as H_2O_2, due to oxidation of the enzyme's labile Fe-S cluster (8), which can ultimately form toxic hydroxyl radicals (37).

Metalloporphyrin catalytic antioxidants

Catalytic antioxidants, which are small-molecule mimics of SOD and/or catalase and are also potent detoxifiers of lipid peroxides and peroxynitrite (reviewed in 38), hold particular promise. Because they are catalytic and not merely free radical scavengers, these compounds are much more potent antioxidants than dietary additives such as vitamin E that act stoichiometrically. The manganese *meso*-porphyrin catalytic antioxidants combine the broad spectrum of reactivity towards reactive species of the stoichiometric antioxidants with the catalytic efficiency of the endogenous antioxidant enzymes. Additionally, these synthetic compounds can be chemically modified to increase their ability to cross the

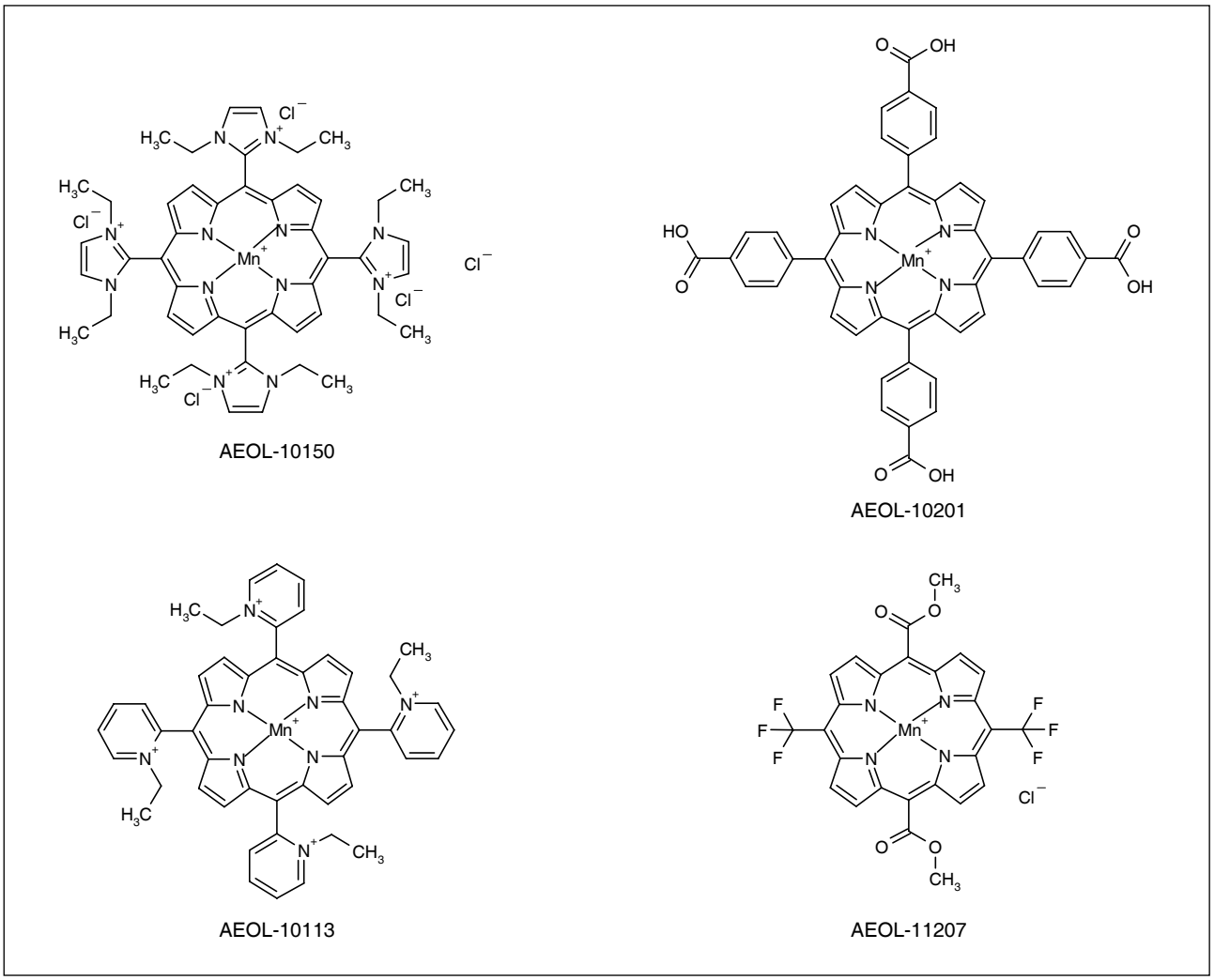

Fig. 2. Three classes of metal-containing catalytic antioxidants. Metalloporphyrins AEOL-10150, AEOL-10201 (MnTBAP), AEOL-10113 and AEOL-11207.

blood–brain barrier, as well as their availability to various subcellular compartments, such as mitochondria (39). This review will focus on the manganese *meso*-porphyrin class of catalytic antioxidants and their application in neurodegenerative disease.

Metalloporphyrins (AEOL series, developed by Aeolus Pharmaceuticals, http://www.aeoluspharma.com) are structurally different from endogenous protoporphyrins and are classified as synthetic *meso*-substituted porphyrins. Most metalloporphyrins contain an Mn moiety that is coordinated by four nitrogen axial ligands (Fig. 2). These compounds have been demonstrated to scavenge O_2^-, H_2O_2, lipid peroxyl radicals and $ONOO^-$ (reviewed in 40). In the dismutation of O_2^-, the manganese changes its valence state between Mn(III) and Mn(II) in a series of alternate reduction and oxidation steps, similar to the alternating reduction and oxidation that occurs in native SODs. 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. Initially, two development paths were followed, where one path used electrochemistry profiles to develop compounds that were active in the dismutation of O_2^- and the other was a general combinatory synthesis approach. From these approaches, two classes of metalloporphyrins emerged wherein one group had very high SOD and catalase activities and another group had very little SOD activity but high catalase activity (41). The groups were also found to scavenge lipid peroxyl radicals and $ONOO^-$, which may involve formation of an oxo-Mn(IV) complex that is reduced by endogenous antioxidants such as ascorbate and glutathione (42).

The antioxidant properties of metalloporphyrins have been studied both in vitro and in vivo, where several compounds have been demonstrated to have biological activity, as will be discussed below. The potencies of these compounds are often based on rate constants derived under very defined and often nonbiologically relevant conditions. A question remains, however, regarding which antioxidant activity is most important to protection in a given system. Compound activities in vivo do not always correlate with the activities measured in vitro, making it difficult to predict activity in a disease model.

Metalloporphyrins have been shown to be effective in ameliorating oxidative stress, inflammation and injury in a large number of animal models of human disease (38). 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 was 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 (43).

An important unaddressed issue in the development of metalloporphyrin catalytic antioxidants is the mechanism by which this type of agent reduces oxidative stress and injury in animals and humans. To date, metalloporphyrin catalytic antioxidants are defined by their chemistry in highly defined and largely nonbiological systems. The relevance of using this classification system has yet to be verified. In fact, over time the potential number of mechanisms these agents may 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 work in human disease is still needed.

Pharmacological studies using metalloporphyrins in acute and chronic neuronal diseases (Table I)

Acute neuronal disorders

Stroke is the major acute neuronal disorder in which damage due to ROS is well established, resulting in multiple clinical trials of free radical scavengers. Increased formation of ROS in the context of ischemic stroke usually occurs during the subsequent reperfusion of the occluded brain area when blood flow is resumed. Reactive oxygen and nitrogen species have been implicated in the death of cells in the ischemic penumbra, the area containing slowly dying cells that surrounds a core of necrotic tissue. The rationale for testing metalloporphyrins in stroke came primarily from the observation that overexpression of Cu/Zn SOD protects against focal ischemic injury in mice (44, 45) and global ischemia and reperfusion injury or transient focal ischemia in rats (46), suggesting that O_2^- is a toxic species. Accordingly, AEOL-10113 (Fig. 2) reduced infarct size when administered up to 6 h after focal ischemia (47). AEOL-10150 (Fig. 2) also reduced infarct size in a transient ischemia model (48), and attenuated the increase in inflammatory gene expression in response to transient middle cerebral artery occlusion (49). These results have led to optimism that catalytic antioxidants might have therapeutic value in stroke.

Epilepsy is another major acute neuronal disorder in which the role of ROS is beginning to be recognized (50). Temporal lobe epilepsy, a major type of acquired epilepsy, occurs to a greater extent in older individuals and seizures often develop in stroke and Alzheimer's disease patients. Prolonged seizure activity causes a depletion of glutathione, particularly mitochondrial glutathione (51). Epileptic neuronal death is at least in part dependent on mitochondrial O_2^- production (52, 53), as well as oxidative damage from activation of inflammatory responses via the plasma membrane NADPH oxidase (54). The metalloporphyrin AEOL-10201, injected directly into the brain, has been shown to decrease oxidative DNA damage, aconitase inactivation and neuronal death following acute seizures from kainate injection, without interrupting seizure duration or latency (55).

Table I: Summary of major in vivo studies of metalloporphyrins in acute and chronic neurodegenerative diseases.

Disease	Compound	Treatment protocol	Results
Stroke/ischemia	AEOL-10150 AEOL-10113	90-Min mouse middle cerebral artery occlusion. Compounds administered intracerebroventricularly (i.c.v.) 90 min or 6 h after initiation of reperfusion.	Both compounds decreased infarct size at 90 min. AEOL-10150 reduced infarct size when administered 6 h after reperfusion (47, 48).
Epilepsy/excitotoxicity	AEOL-10201	Kainic acid-treated rats. Compound administered via osmotic minipump 48 h before treatment.	Pretreatment with AEOL-10201 improved neuronal survival, prevented aconitase inactivation and oxidative DNA damage, but did not affect behavioral seizures (53).
Parkinson's disease	AEOL-11207	MPTP mouse model of parkinsonism. Compound administered subcutaneously (s.c.) and orally (p.o.).	Compound protected against MPTP-induced nigral cell death, dopamine depletion, glutathione depletion and 3-nitrotyrosine formation when administered s.c. or p.o. (43).
Amyotrophic lateral sclerosis (ALS)	AEOL-10150	G93A mouse model of ALS. Compound administered at the onset of symptoms.	Compound extended survival from onset up to 3-fold, increased motor neuron survival and reduced astrogliosis and markers of oxidative stress (70).
	AEOL-10150 + phenylbutyrate (PBA)	G93A mouse model of ALS. Compounds administered alone or in combination at time of disease onset.	AEOL-10150 increased survival by 11%, PBA by 13% and combined treatment by 19%. Treatment reduced markers of oxidative stress and increased expression of protective genes (71).

Chronic neurodegenerative diseases

Parkinson's disease and amyotrophic lateral sclerosis (ALS) (56) are two chronic neurodegenerative diseases in which metalloporphyrins have shown promise in preclinical studies. Parkinson's disease is an age-related progressive disorder characterized by the selective death of dopaminergic neurons in the substantia nigra (57). Parkinson's disease represents one of the best examples of a disease in which a role for ROS appears critical. The vast majority of cases are sporadic. Decreased complex I activity in the substantia nigra of Parkinson's disease patients relative to age-matched controls is considered a prominent source of $O_2^{\cdot}$ (35). The importance of complex I is further supported by the discovery that MPTP, a compound that induces a parkinsonian-like syndrome in humans and other species including mice, is an inhibitor of complex I (58, 59).

In addition to increased ROS production, inhibition of complex I primarily results in reduced ATP production. Evidence of oxidative stress in the brains of Parkinson's disease patients includes oxidative modification of DNA (60), proteins (61) and membrane lipids (62, 63).

Metalloporphyrin catalytic antioxidants have been found to be neuroprotective in several animal models of Parkinson's disease. AEOL-10201 protects against 6-hydroxydopamine toxicity (64). A major advancement in the field of catalytic antioxidants was the demonstration that AEOL-11207 (Fig. 2), a lipophilic metalloporphyrin, protected against MPTP toxicity in vivo following oral administration (43). This compound belongs to a new class of metalloporphyrins, the AEOL-112 series of glyoxylate metalloporphyrins (41), which were designed to have greater lipid solubility, oral bioavailability and to cross the blood–brain barrier.

In vitro assays were used to rank compounds from the AEOL-112 series for their ability to scavenge mitochondrially generated H_2O_2 (65). The IC_{50} for AEOL-11207 in this in vitro assay (~100 nM) correlates well with the brain concentrations achieved in vivo (approximately 200 nM) at which neuroprotective effects were observed (43). Oral administration of AEOL-11207 inhibited MPTP-induced death of dopaminergic neurons in the substantia nigra, as well as loss of dopamine levels in the striatum, and oxidative damage including 3-nitrotyrosine levels, 4-hydroxy-2-nonenol levels and a decrease in the ratio of oxidized to reduced glutathione (43, 66). The oral efficacy of AEOL-11207 in a well-established model of Parkinson's disease suggests the potential clinical utility of this class of compounds.

ALS, or Lou Gehrig's disease, involves a relentless and progressive loss of motor neurons resulting in muscle atrophy. The majority of cases are sporadic, but a small percentage of familial cases are associated with gain-of-function mutations in SOD1, which has led to the development of mutant mouse models in which therapies and mechanisms can be tested. Although SOD1 mutations do not directly result in increased superoxide levels, there is substantial evidence for increased oxidative stress in ALS. Mice carrying a mutant human form of SOD1 accumulate damaged DNA, proteins and lipids (66, 67), and neural tissue from ALS patients demonstrates oxidative damage (68, 69). Expression of mutant SOD1 also results in mitochondrial dysfunction and increased oxidative and nitrative damage in astrocytes, which could be rescued by mitochondrially targeted antioxidants (69). Prolongation of lifespan in the mouse model of ALS has been a major approach for testing potential therapies. Metalloporphyrins have shown promise in animal models

of ALS. AEOL-10150 extended survival from symptom onset up to threefold when it was administered to each mouse on the first day of muscle weakness in the mouse G93A model of ALS (70). In this study, AEOL-10150 was combined with dietary creatine and rofecoxib, with no additional increase in survival. In another study, AEOL-10150 was administered alone or in combination with the histone deacetylase inhibitor phenylbutyrate (PBA) at symptom onset in the G93A mouse model (71). All three treatments were found to extend survival, but the combination therapy was the most effective, extending survival by ~20%.

Therapeutic considerations for the treatment of neurodegenerative diseases

The development of therapeutic agents for the treatment of both acute and chronic neurodegenerative diseases requires that the agents have efficacy in animal models, safety, stability, blood–brain barrier permeability, oral bioavailability and a favorable plasma and brain half-life. Additional considerations for catalytic antioxidants include cell permeability, a high rate constant for ROS and low antigenicity. Collectively, the metalloporphyrins (Fig. 2) satisfy the majority of these requirements. Notably, the glyoxylate metalloporphyrin AEOL-11207 shows particular promise as a therapeutic agent in Parkinson's disease due to its efficacy in the MPTP mouse model, oral bioavailability and long plasma and brain half-life (43). Future applicability of catalytic antioxidants to chronic neurodegenerative diseases such as Parkinson's disease and ALS is promising. One caveat with respect to potential application in Parkinson's disease: in the animal model, metalloporphyrins have been administered concurrently with exposure to the parkinsonian toxin in young adult mice. The translation of the efficacy of the compound in older individuals already presenting parkinsonian symptoms at the time of intervention remains a challenge.

Conclusions

Oxidative stress is a component of and plays a complex role in many neurodegenerative diseases. In some cases, oxidative stress is secondary to other pathology, e.g., oxidative damage occurring as the result of an immune response initiated by cell death. In other cases, oxidative damage due to mitochondrial dysfunction is a primary cause of cell death and neuronal loss. Metalloporphyrins can intervene at each of these steps, preventing cell death in response to mitochondrial dysfunction or excitotoxicity, and preventing the immune response-driven vicious cycle of increased oxidative and nitrative damage, which can contribute to subsequent cell death and disease progression. Therefore, there are multiple ways in which metalloporphyrins can exert the protective effects observed in in vitro and in vivo models of neurodegeneration, and much promise that these compounds may represent useful therapies in human disease.

Disclosure

Dr. Day is a consultant for and holds equity in Aeolus Pharmaceuticals, which is commercially developing metalloporphyrins as human therapeutic agents.

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