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Targeting oxidative stress in central nervous system disorders

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Abstract

There is widespread recognition that reactive oxygen species (ROS) play key roles in normal brain function and pathology in the context of neurological disease. Oxidative stress continues to remain a key therapeutic target for neurological diseases. In developing antioxidant therapies for neurological disease, special attention should be given to the brain's unique vulnerability to oxidative insults and its architecture. Consideration of antioxidant therapy should be guided by a strong rationale for oxidative stress in the neurological disease. This review provides an overview of processes that can guide the development of antioxidant therapies in neurological diseases such as knowledge of basic redox mechanisms, unique features of brain pathophysiology, mechanisms and classes of antioxidants and desirable properties of drug candidates.

Unique Vulnerability of the Brain to Oxidative Stress

Reactive oxygen species (ROS) are recognized as important mediators of brain injury. Reactive species (RS), a broader term that include both ROS and nitrogen species contribute to, are also capable of contributing to other known mechanisms of brain injury such as mitochondrial dysfunction, proteosomal dysfunction and inflammation. Given the widespread role of oxidative stress in neuronal disorders, there has been a long quest for the development of antioxidants therapeutics. Historically, the development of natural and synthetic antioxidants as therapeutic candidates has been challenging despite promising preclinical data and enormous public interest. For example, a negative impact of antioxidant therapies has been noted on cancer metastasis and progression [1, 2]. Currently, a few drugs with antioxidant properties are approved for human use in neurological diseases (e.g. edaravone, idebenone, and dimethyl fumarate) and many more are under clinical and preclinical development. In the following sections the properties of reactive species as a therapeutic target and the challenges unique to the development of antioxidant drug candidates are discussed.

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The brain's unique vulnerability to oxidative injury is based on physiological, anatomic and functional factors. The brain comprises 2% of the body's weight in humans and yet consumes 20% of the body's oxygen (O_2) due to its high rate of metabolism. This mismatch in per weight O_2 consumption results in higher availability of O_2 , the major precursor of ROS. The bulk of the O_2 consumed is to provide an efficient electron acceptor for ATP generation which is needed for energetic consuming processes such as action potentials, synaptic machinery (exocytosis of transmitters and maintenance of energetic pumps), neurotransmission and enzymatic reactions. However, it remains a mystery as to why the brain's energy demands are so high given its closed loop of circuits [3]. The complex neuroarchitecture comprising of diverse cell types in the brain parenchyma (neurons, interneurons, stem cells, astrocytes, microglia and oligodendroglia) and function-based circuitry further dictate energetic demands. The brain's reductive environment with a relatively low partial pressure of O_2 is likely designed to retard the generation of ROS. However, endogenous factors such as modest antioxidant defenses, limited regenerative capacity, glymphatic waste disposal, dependence on excitotoxic and autooxidizable neurotransmitters, polyunsaturated fatty acids prone to peroxidation, calcium load and redox active metal burden render the brain highly vulnerable to oxidative damage (Figure 1).

Cellular Response to ROS: Redox signaling to Oxidative Damage

The modern definition of oxidative stress has been updated from the simplistic concept of "imbalance between oxidants and antioxidants" [4] to "an imbalance in prooxidants and antioxidants with associated disruption of redox circuitry and macromolecular damage" [5]. This current definition encompasses a systems biology concept with hierarchical redox circuitry based on redox nodes and potentials. The evolution of aerobic life and O_2 's obligate role as the terminal electron acceptor for ATP production has necessitated abundant and overlapping antioxidant systems. Despite its unique vulnerabilities, there is no evidence of gross insufficiencies of antioxidant or repair systems in the brain. Partial reduction of O_2 results in ROS under conditions ranging from basal brain function to pathological dysfunction. Reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide (FADH₂) provide electrons to the mitochondrial electron transport chain (ETC) via NADH ubiquinone oxidoreductase (complex I) and succinate dehydrogenase (complex II), respectively. Highly efficient passage of electrons through the ETC to the terminal cytochrome oxidase where O_2 addition ultimately makes ATP is thought to contribute to the very low levels of basal $O_2^{\cdot -}$ formation. Sequential addition of electrons to $O_2^{\cdot -}$ results in H_2O_2 , $\cdot OH$ and H_2O (Figure 2). Isolated brain mitochondria generate ROS primarily via complex I and complex III in the presence of substrates. However, inhibitor studies suggest that in comparison to complex III, complex I is a more potent source of ROS generation in brain preparations, highlighting the significance of low level complex I deficiencies observed in PD and temporal lobe epilepsy [6]. Additional notable sources of $O_2^{\cdot -}$ in many acute and chronic neuronal disorders such as stroke, traumatic brain injury, Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD) and Amyotrophic lateral sclerosis (ALS) are the NADPH oxidase (NOX) family, xanthine oxidase/xanthine dehydrogenase (XO/XDH), myeloperoxidase (MPO), cytochrome P450s, nitric oxide synthases (NOS) and phospholipase A₂ [7]. Mitochondria and NOX are

considered the major sources of $O_2^{\cdot-}$ and subsequent H_2O_2 generation under basal conditions, redox signaling and oxidative stress. The interplay between cytosolic and mitochondrial sources of ROS is emerging [8]. [6]. The fate of mitochondrial $O_2^{\cdot-}$ is governed by its rate of reaction with targets, the abundance of targets and proximity of its targets. Consequently, the three most important targets of mitochondrial $O_2^{\cdot-}$ are Sod2, labile Fe-S clusters (example aconitase) and any NO present in mitochondria. Given the 1:1 stoichiometry of $O_2^{\cdot-}$ and Fe-S center, aconitase's reaction with $O_2^{\cdot-}$ can generate twice the concentration of H_2O_2 as the dismutation reaction which uses two $O_2^{\cdot-}$ molecules to generate one H_2O_2 molecule. In brain mitochondria, H_2O_2 generated in this manner as well as other sources example alpha ketodehydrogenase (α KGDH) [9] is detoxified by the thioredoxin/peroxiredoxin (Trx/Prx) system in the presence of respiration substrates [8]. Mitochondrial H_2O_2 emission occurs when the steady-state levels of H_2O_2 exceed the capacity of the mitochondrial antioxidant systems [11]. The fate and role of mitochondria-generated H_2O_2 is not fully understood. A redox signaling role of mitochondria-generated H_2O_2 has recently been recognized [12]., however, a redox signaling role is emerging [10]. It is plausible that in respiring brain mitochondria, emission of H_2O_2 serves as a signaling event to prime NOX-derived $O_2^{\cdot-}$, thus amplifying cellular H_2O_2 levels. Secondly, H_2O_2 has been shown to initiate inflammation by activation of cytokine production by modifying redox-sensitive transcription factors resulting in redox signaling [13]. Following brain injury, aging or chronic neurodegeneration, available antioxidant and adaptive repair processes may not be sufficient to keep steady-state levels of $O_2^{\cdot-}$ and H_2O_2 in check resulting in oxidation of vulnerable macromolecules (Figure 3).

Oxidative Damage: Mediators and Targets

All cellular macromolecules can undergo oxidative damage. However, in terms of cellular organelles and macromolecules that mediate brain injury, special consideration needs to be given to mitochondria and lipids, respectively. $O_2^{\cdot-}$ is less reactive than many other RS; however, it is highly selective for certain targets and is a precursor of other RS. For example, the iron-sulfur center of mitochondrial aconitase, which mediates its toxicity in bacterial and mammalian cells [14], has been shown to exert neurotoxicity in Sod2-deficient mice, dopaminergic neurons *in vivo* and *in vitro*, and seizure-induced cell death *in vivo* [13–15]. The unique vulnerability of the acid-labile iron moiety in mitochondrial aconitase to nucleophilic $O_2^{\cdot-}$ attack and subsequent inactivation results in loss of iron and potential initiation of the Fenton reaction yielding $\cdot OH$ radicals in close vicinity to mitochondrial DNA, lipids and proteins. The importance of this mechanism in neurotoxicity has been demonstrated by the resistance of neuronal cells deficient in mitochondrial aconitase to paraquat, a redox cycling agent [15]. H_2O_2 is formed wherever $O_2^{\cdot-}$ is generated by its rapid spontaneous and SOD-catalyzed dismutation; it is also formed enzymatically as a byproduct of lipid metabolism in peroxisomes⁷. H_2O_2 has both redox signaling actions due to its relatively longer half-life and injurious effects due to its reactivity with cysteine residues in proteins and as a precursor of $\cdot OH$ and hypohalous acids such as hypochlorite (HOCl) and hypothiocyanate (HOSCN). Brain mitochondria are unique in their ability to detoxify H_2O_2 in that they consume H_2O_2 in a respiratory substrate-dependent manner via thioredoxin-peroxiredoxin (Trx-Prx) rather than in a catalase-dependent manner [8]. Modulation of

cysteine residues of proteins can mediate neurotoxicity in several neurodegenerative diseases [16, 17]. Lipid peroxidation is particularly important in brain disorders as the brain is very rich in polyunsaturated lipids. Key candidates for mediating oxidative injury are lipid oxidation byproducts malonyldialdehydimalondialdehyde, 4HNE, and acrolein. Arachidonic acid-derived oxidation products (isoprostanes, isofurans and isoketals) are produced after brain injuries [20]. Neurodegeneration can be induced by isoprostanes via receptor activation or production of toxic species such as cyclopentenone isoprostanes [21, 22]. Recent discovery of isoketals suggests their important role as mediators of protein damage due to their proclivity towards adduction [23]. Mitochondrial DNA is a notable target of oxidative damage in models of brain disorders [24]; however, the consequences of oxidative mtDNA damage have not been clearly defined. Neuronal death via apoptosis or/and necrosis is a consequence of oxidative damage in various brain disorders. It should be emphasized that antioxidant and repair processes specific to the damaged macromolecules are initiated in response to oxidative injury. For example, base excision repair processes are activated to repair oxidized DNA base adducts, thiol repair by methionine sulfoxide reductase, protein glutathionylation by glutaredoxin and oxidatively modified proteins are repaired by protein disulfide isomerases and carbonyl reductases.

Antioxidant Classes

An antioxidant [25] is defined as “a substance that, when present at a low concentration compared with that of an oxidizable substrate, inhibits oxidation of the substrate” [20]. As depicted in Figure 4, antioxidant compounds can be either direct-acting scavengers of RS or indirect-acting antioxidants.

Direct Antioxidants

Direct antioxidants can be either catalytic or stoichiometric in their mechanism. Catalytic antioxidants are often designed to mimic endogenous enzymes. SOD and catalase are endogenous metalloproteins that utilize dismutation reactions to detoxify ROS. These reactions involve one- or two-electron transfers where the electrons are accepted from one O_2^- or H_2O_2 and then donated to another and do not require reducing equivalents or ATP. Although $\cdot OH$ radicals are exceedingly promiscuous and difficult to target pharmacologically, several compounds that scavenge $\cdot OH$ radicals are included in this category including edaravone [26] and melatonin [27]. Stoichiometric natural antioxidants include vitamin E and C. The value of a catalytic mechanism is obvious in drug development as this reduces the dosage, increases efficiency and regenerates the parent compound for the duration of the drug effect. Among the antioxidant enzyme mimics, two major classes are based on endogenous enzymes that scavenge O_2^- and H_2O_2 . SOD mimics can be either selective or nonselective and most contain a redox active metal. These compounds require either fast rate of reaction with O_2^- or can accumulate in cells and tissues to high levels. The use of SOD and catalase as therapeutic agents to attenuate ROS-induced injury responses has had mixed success [28]. The main limitations of these natural products are their large size, which limits cell permeability, short circulating half-life, antigenicity and expense. An increasing number of low-molecular-weight SOD mimetics have been developed to overcome some of these limitations. For example, metalloporphyrin, salen and

macrocyclic SOD mimics are designed to mimic SOD which targets $O_2^{\cdot-}$. The manganese moiety of the manganese porphyrin SOD mimetics functions in the dismutation reaction with $O_2^{\cdot-}$ by alternate reduction and oxidation changing in its valence between Mn(III) and Mn(II), much like native SODs, whereas the catalase activity of metalloporphyrins involves additional reversible two-electron oxidations via the conjugated ring system.

Catalase mimics or glutathione peroxidase mimics target non-radicals such as H_2O_2 [29–32]. Peroxidase mimics can be selenium-based or contain a redox active metal. Selenium-based compounds need to be stable and usually require endogenous antioxidants like reduced glutathione to recycle compounds to their active state. It is highly desirable that metal-based compounds possess high affinity for the metal to avoid neurotoxicity arising from free metals that are often redox-active. Metal-based compounds can also form higher oxidation states that can be pro-oxidant under low endogenous antioxidant conditions found in the brain. Among the non-metal catalytic antioxidants, two major classes are the nitron spin traps and the fullerenes which are large C_{60} nanoparticles [33] and both can scavenge $O_2^{\cdot-}$. Both classes likely require endogenous regeneration to act catalytic. Nitroxides generated from nitrones through addition of a $RS^{\cdot}$ are stable free radicals by virtue of resonance stabilization of the unpaired electron between the nitrogen and the oxygen and can also function as chain breaking antioxidants [34]. Many of the natural antioxidant (example vitamin E and/or C, thiols, CoQ, polyphenols) and their mimics share aromatic rings substituted with hydroxyl groups [35]. They can directly scavenge peroxy and hydroxyl radicals, $ONOO^-$, and hypochlorous acid (HOCl) [36]. Major antioxidant mechanisms in this class include the ability to delocalize charge by inducing semi-quinone formation or act as a terminal adduct (i.e. chlorination or nitration product). Some of these compounds can also produce pro-oxidant effects and may induce endogenous antioxidants through Nrf2 activation [37].

Indirect Antioxidants

Indirect antioxidants either limit free radical reactions by chelating redox-active metals example desferrioxamine or inducing endogenous antioxidants example (e.g. Nrf2 pathway). Although the Nrf2 pathway includes many genes, most of which are responding to electrophilic stress, notable antioxidant genes such as glutamate cysteine ligase (GCL), thioredoxin reductase (TrxR) and heme oxygenase (HO1) are included. Compounds that induce the Nrf2 response are primarily oxidants or electrophiles example t-butyl-hydroperoxide, synthetic triterpenoids and sulforaphane. It is important to note that antioxidant action may only in part mediate efficacy of Nrf2 inducers; therefore confirmatory assays to verify antioxidant actions of Nrf2 activators are necessary [37, 38]. If the major cellular sources of oxidant production are known, it may be advantageous to target these sources directly. For example, apocynin analogs inhibit the NOXs, and various inhibitors of NOS have been used to limit the generation of nitric oxide and consequently, $ONOO^-$. AZD3241, an inhibitor of microglial myeloperoxidase, has recently undergone clinical trials for PD [39]. This study suggested that AZD3241 was well tolerated and capable of reducing microgliosis in PD patients.[32]. It is important to note that direct antioxidants, including catalytic compounds, can inhibit endogenous (indirect) antioxidant pathways. For example, a metalloporphyrin antioxidant has been shown to inhibit adaptive

Nrf2 responses by scavenging ROS that in turn activate Nrf2 [38]. Nrf2 inducers offer an advantage of activating antinflammatory and repair genes in addition to antioxidant genes which may be highly beneficial in neurological diseases characterized by inflammation and oxidative stress. This type of interaction has implications for initiation of direct antioxidant therapy. It would therefore be desirable to know the time-course of oxidant production in a disease model so that initiation of direct antioxidant therapy can be optimally timed. Precursors of endogenous antioxidants, such glutathione (GSH) can be achieved by N-acetyl cysteine (NAC) which is approved for human use in acetaminophen toxicity but shows promise in many neurological diseases such as schizophrenia, HD and PD [40, 41].

Targeted Antioxidants

Targeted antioxidants are a relatively new class of compounds developed on the rationale that non-targeted antioxidants may not achieve high concentrations at important sites of ROS production (i.e. mitochondria). Several classes of compounds have been developed to overcome this issue. Most notable are mitoquinone mesylate (MitoQ) and its analogs, which are synthesized by covalently binding ubiquinone to a triphenylphosphonium (TPP+) cation to achieve charge-based concentration in mitochondria. In fact, MitoQ has been shown to achieve over a 100-hundred-fold concentration enrichment in mitochondria and is efficacious in numerous animal models of neurological diseases and cognitive dysfunction [42–46]. Other types of targeted compounds include mitochondrial targeted vitamin E, mitochondrial targeted plastoquinone and cell permeable peptides [47–50]. A potential limitation of this targeting is its dependence on maintaining an active mitochondrial membrane potential. A common misconception of this approach is that the agent is only localized to the mitochondrial compartment, when in reality there is just a greater partitioning into the mitochondria from the cytosol than a non-targeted molecule, and one cannot rule out effects also occurring in the cytosol and other cellular compartments.

Rationale for Targeting Oxidative Stress in Brain Disorders

The growing recognition of the physiological role of ROS (notably H_2O_2) and the advent of the “(mito)hormetic” theory of ROS brings up an important question: is oxidative stress a “druggable” target for brain disorders? [51] The dual signaling vs damaging roles of ROS raises the concern that antioxidants could interfere with physiological processes. It should be emphasized that the goal of antioxidant therapy in disease states is primarily to normalize elevated ROS levels and limit oxidative damage rather than interfere with the normal physiological roles of ROS. Another argument that may explain the limited success of directly scavenging ROS is that low levels of antioxidant compounds available to target diffuse and are not available to quench high levels of compartmentalized ROS [52]. A better approach may be to utilize antioxidant drugs that target the sources of ROS such as NOX inhibitors. The use of animal models deficient in or overexpressing cellular antioxidant enzymes can provide a strong rationale for targeting oxidative stress in neurological disorders. For example, overexpression of SOD or catalase enzymes in whole animals has provided neuroprotection against the deleterious effects of a wide range of brain disorders and cognitive dysfunction [53–57]. Likewise, studies demonstrating the deficiency of compartmentalized antioxidants, such as Sod1, Sod2 and Sod3, have been useful [58–60].

Finally, an important issue that needs to be addressed before considering antioxidant therapy is to determine if ROS and oxidative damage are merely associated with the disease process or play a causative role in it. To address this question, several important criteria for assigning a causative role of ROS as well as antioxidant properties must be considered. These include determination of the identity of the specific reactive species associated with the disease process and whether the disease has a strong rationale for reactive species involvement, among other factors (Box 1).

BOX 1

Criteria That Must Be Fulfilled for Successful Antioxidant Therapy

- Determine the ROS responsible for the disease or its symptoms: does the disease have a strong rationale for ROS involvement?
- Determine the endpoints to assess target engagement
- Assess whether broad spectrum or target-specific antioxidant therapy would be most beneficial
- Determine the site of therapeutic action: is it in a specifically cellular compartment or diffuse?
- Antioxidant therapy must be sufficiently “drug-like” in stability, safety, etc.

Desirable Properties of “Drug-Like Antioxidant Compounds”

Factors that need to be considered for the development of antioxidant drugs include efficacy (high rate constant with ROS), stability, safety, favorable pharmacokinetic properties, and specificity for RS, cell and mitochondria permeable, non-antigenic properties and having non-toxic metabolites (Figure 5). Additionally, for neurological disorders, antioxidant molecules need to cross the blood-brain-barrier (BBB) and preferably show oral bioavailability. Several other important issues need consideration for the development of synthetic antioxidant drug development i.e. direct scavengers of RS. These include a) the identities of the ROS/RNS being targeted so compound screening is well aligned with the species, b) the choice of biochemical assays that predict biological activity, c) whether factors that make an ideal antioxidant are compatible with factors required for drug development (i.e. drug product stability), d) whether the end goal is to achieve symptomatic relief of primary disease outcome, disease modification and/or co-morbidities of disease such as cognitive dysfunction.

Failures and Successes in Antioxidant Development and Lessons Learned

It is often instructive to evaluate the translational process from preclinical studies to clinical trials. While idebedone was successfully translated in MS, it shows limited success in AD patients [61].[51]. However, it is instructive to consider the failure to translate two promising preclinical candidates, NXY-059 and MitoQ. NXY-059 is a nitroxide spin trap that reacts with $O_2^{\cdot -}$ *in vitro*. Its preclinical development for stroke closely adhered to the STAIR

guidelines [62] in showing efficacy: 1) in animal models of both permanent and temporary focal ischemia, 2) in more than one laboratory, 3) in more than one animal species, including nonhuman primates, 4) in both male and female animals, 5) in both behavioral and histological outcomes and 6) after administration at 1 hour after ischemia or beyond. Despite these promising preclinical efficacy results, NXY-059 was marginally positive in the first clinical trial (SAINT I) and failed the SAINT II Trial, a large randomized multicenter clinical trial (MCT) of the NXY-059, against acute ischemic stroke halting further clinical development. Similarly, MitoQ failed to show efficacy in clinical trial of Parkinson's disease (PROTECT study) in a MCT of 13 centers, placebo vs 2 doses of MitoQ [63] despite efficacy in preclinical studies. In fact, MitoQ demonstrates many favorable properties desirable in antioxidant drugs such as stability, favorable pharmacokinetic properties, BBB permeability and oral bioavailability [46]. Some reasons for the failure of NXY-059 in clinical trials may have relate to its non-ideal physiochemical properties (i.e. poor BBB penetration, non-physiological oxidation potential, and low potency) [64]. Furthermore, most clinical trials lack biomarkers assessing oxidative stress in the brain or periphery, which can verify target engagement. Recent advent of (11)C-PBR28, a microglia marker of the 18 kDa translocator protein on the outer mitochondrial membrane as a biomarker for imaging neuroinflammation may be useful in guiding clinical trials [65]. In fact, (11) C-PBR28 has been used to successfully guide a PD clinical trial for a myeloperoxidase inhibitor, AZD3241 [39]. In general, accessibility of target tissue is problematic in brain disorders. Blood-based biomarkers can also serve as important guides to antioxidant clinical trials as demonstrated by choosing the dosage of vitamin E in humans using isoprostanes as a biomarker [66]. Factors such as heterogeneity in individual human responses and responses to drug treatment are a common problem with most clinical studies. While newly established RIGOR guidelines may optimize preclinical studies [62], one cannot overlook the fact that stroke is a formidable disorder with only one approved therapy to date. In the case of neurodegenerative diseases such as PD, potential reasons for negative results may relate to delayed timing of drug administration with relation to the oxidative stress and lack of correlation with appropriate biomarker(s) of oxidative damage.

Concluding Remarks

Oxidative stress is a therapeutic target for neurological diseases due to the unique vulnerability of the brain to oxidative damage and knowledge that high levels of ROS mediate neuropathology. Clinical trials of antioxidant drugs are ongoing despite the recognition that low levels of ROS serve physiological roles in cell signaling (see Outstanding Questions Box). Antioxidant drug development should be guided by a strong rationale for oxidative stress in a given neurological disease, whether the therapeutic entity possesses desirable “drug-like” properties and its ability to decrease the oxidative burden in the affected brain region. The choice of antioxidant therapies may range from direct stoichiometric scavengers to indirect compounds that mobilize pleiotropic antioxidant gene responses. Future development of antioxidant drugs needs to take into account both the beneficial and the harmful effects of ROS by monitoring adverse effects in normal and disease populations. Failed clinical trials can provide useful information in guiding new antioxidant drug trials in the next several years. Development of brain and peripheral

biomarkers to monitor target engagement are sorely needed for CNS disorders. The lack of predictable preclinical animal studies continues to be an ongoing challenge that needs to be coupled to new RIGOR guidelines. The timing of treatment needs to be matched with the occurrence of oxidative stress rather than the disease symptoms. Selection of patients for clinical trials should consider individual variability, variability of drug responses and heterogeneity of diseases. In addition, basic science research needs to develop better drugs and interventional design as well as better targets. Understanding the mechanism(s) and sources of ROS production in disease states may allow targeting of specific enzyme(s) rather than flooding the body with antioxidants.

Outstanding Questions

- What makes the brain's architecture and function vulnerable to oxidative damage?
- Is oxidative stress a “druggable” target for brain disorders?”
- What are desirable antioxidant drug-like properties?
- When and how can reactive oxygen species be pharmacologically targeted?
- Could antioxidant therapy interfere with physiological processes?
- How can biomarkers of oxidative stress guide clinical trials?

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Trends Box

- Oxidative stress is a key a therapeutic target for many neurological diseases
- A strong rationale for oxidative stress in a given neurological disease should be the guiding factor for antioxidant therapy
- Antioxidant drug development should account for both the beneficial and the harmful effects of reactive oxygen species
- Biomarkers to monitor target engagement can guide antioxidant clinical trials for neurological diseases

Figure 1. Many unique features of the brain ranging from its function, architecture and energetics dictate its vulnerability to oxidative insults.

Figure 2.

Sequential reduction of O_2 results in formation of partially reduced species. $O_2^{\cdot -}$ is catalyzed to H_2O_2 by SODs. Catalases and peroxidases further reduce H_2O_2 to H_2O . $O_2^{\cdot -}$ can react non-enzymatically with NO^+ to form $ONOO^-$, H_2O_2 can form $HOCl$ and $HOSCN$ via MPO and $\cdot OH$ can react with PUFAs to form lipid peroxides ($L^{\cdot}$, $LO^{\cdot}$, $LOO^{\cdot}$ and $LOOH$)

Figure 3.

The redox response of cellular systems is dictated by increasing steady-state levels of ROS. ROS evoke concentration-dependent responses ranging from redox signaling, inflammation and adaptation. Oxidative stress arises when adaptive responses are overwhelmed.

Figure 4.
Classification and examples of major antioxidants based on their mechanisms.

Desirable Properties of Antioxidant Drug Candidates

Figure 5.
Desirable properties of antioxidant drug candidates.