Antioxidants in Central Nervous System Diseases: Preclinical Promise and Translational Challenges

Chandrashekar D. Kamat, Sunyana Gadal, Molina Mhatre, Kelly S. Williamson, Quentin N. Pye, and Kenneth Hensley*
Free Radical Biology and Aging Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA

Abstract
Oxidative damage is strongly implicated in the pathogenesis of neurodegenerative diseases including Alzheimer’s disease, amyotrophic lateral sclerosis, Huntington’s disease, Parkinson’s disease and stroke (brain ischemia/reperfusion injury). The availability of transgenic and toxin-inducible models of these conditions has facilitated the preclinical evaluation of putative antioxidant agents ranging from prototypic natural antioxidants such as vitamin E (α-tocopherol) to sophisticated synthetic free radical traps and catalytic oxidants. Literature review shows that antioxidant therapies have enjoyed general success in preclinical studies across disparate animal models, but little benefit in human intervention studies or clinical trials. Recent high-profile failures of vitamin E trials in Parkinson’s disease, and nitrone therapies in stroke, have diminished enthusiasm to pursue antioxidant neuroprotectants in the clinic. The translational disappointment of antioxidants likely arises from a combination of factors including failure to understand the drug candidate’s mechanism of action in relationship to human disease, and failure to conduct preclinical studies using concentration and time parameters relevant to the clinical setting. This review discusses the rationale for using antioxidants in the prophylaxis or mitigation of human neurodiseases, with a critical discussion regarding ways in which future preclinical studies may be adjusted to offer more predictive value in selecting agents for translation into human trials.

Keywords
Alzheimer’s disease; amyotrophic lateral sclerosis; antioxidants; Huntington’s disease; neurodegeneration; neuroinflammation; Parkinson’s disease; tocopherols

INTRODUCTION
Antioxidants are widely discussed in both the lay press and the scientific literature as health-promoting agents that may protect against various age-related diseases. There is sound rationale for hypothesizing that antioxidants could be prophylactic against central nervous system (CNS) disease. Brain protein, lipid and nucleic acid oxidation products increase at an accelerating pace with age [144,145] and further increase in cases of age-related neurodegenerative conditions such as Alzheimer’s disease (AD) [66,67,96,149,165] and Parkinson’s disease (PD) [7]. Pathogenic contributors such as amyloid-β protein (Aβ) in AD and redox-cycling hydroquinones in PD are well-established to cause or exacerbate oxidative stress in cell culture.
systems [7, 27, 95, 105]. More recent theories invoking neuroinflammatory etiologies for age-related brain disease still depend upon oxidative stress mechanisms of injury to explain the damage occurring in disparate pathologies including AD and amyotrophic lateral sclerosis (ALS) [68]. Thus, free radical scavengers or chain-terminating antioxidants ought in theory to prevent the onset or slow the progression of some, if not most, neurodegenerative conditions.

Despite the corpus of scientific evidence supporting oxidative stress as a pathogenic factor in age-related neurodisease, human clinical experience with antioxidant neuroprotectants has been generally negative. Human data from controlled trials can be divided into two categories, nutritional supplement studies and therapeutic drug trials. With respect to nutritional supplement studies most human data has been collected from vitamin E (alpha tocopherol, \( \alpha \)T) trials or trials involving mixtures of vitamin antioxidants and micronutrients (e.g., selenium, vitamin C). Although certain vitamin E trials in AD do suggest an effect on quality-of-life parameters, such as time to enter a nursing facility [131], these studies demonstrate marginal or no benefit on deterioration of measurable brain function in persons suffering mild cognitive impairment (MCI), a prelude to AD [118]. More troubling, emerging data from long-term and high-dose vitamin E supplementation studies suggest an increased risk of hemorrhagic stroke and all-cause mortality, raising concerns that vigorous antioxidant supplementation strategies may cause more harm than benefit [24, 61, 91, 103].

Currently, there have been very few human clinical trials of small-molecule drugs whose presumed mode of action is antioxidant in nature, though there are a number of such drug candidates being pressed into human trials for neurodegenerative diseases such as ALS. Such studies, if successful, could validate and perhaps vindicate decades of free radical research. Unfortunately, results thus far have been disheartening as exemplified by very recent and high-profile failure of antioxidant-based stroke therapies. In late 2006, the first human trials were completed for NXY-059 (Cerovive), a nitrone-based free radical spin trapping agent that aspired to protect neurons from stroke damage (brain ischemia/reperfusion injury) caused by free radical-induced oxidative damage. Despite abundant preclinical efficacy in rodent and primate models [64], and modestly significant benefit in the initial “SAINT-I” human clinical trial [83], the definitive “SAINT-II” trial for NXY-059 completely failed to achieve pre-set endpoints [141]. The failure of NXY-059 has massively diminished enthusiasm for pursuing neuroprotectants as drug candidates generally, and antioxidants as neuroprotectants specifically. The pharmaceutical communities’ reaction was evident in a statement by Dr. John Patterson, executive director of development at AstraZeneca PLC, which partnered with Renovis Pharmaceuticals in conducting the SAINT trials:

“The people that we’ve worked with in the outside world, the opinion leaders, think that this really has shown them that the models they use and the work that they’ve done to try and generate drugs like this, is not valid … we’re talking more generally about neuroprotection and the ability for anything, whether it’s a free radical trapping agent or other mechanism, to do something in man that’s meaningful” [58].

Such a strident statement from an entity that had been heavily invested in the concept of antioxidant therapy must be taken seriously by scientists studying redox biology and oxidative brain damage. Clearly, preclinical animal studies are essential to understand disease and to build confidence in new therapeutic approaches; however, it is becoming increasingly clear that scientists cannot extrapolate from non-human to human efficacy. At the same time, the scientific enterprise cannot accept the impossibility of mitigating CNS disease. A prudent middle ground would be to consider very judiciously the design and implementation of past animal studies that demonstrate antioxidant potential, with a goal of retesting these agents in experimental designs likely to better mimic a human clinical situation. Critical evaluation or re-evaluation would need to focus carefully on dosage, administration route and timing of drug
treatment in such a way as to recreate not only the disease process but also to model a practicable human clinical trial design [132].

The purpose of the present review, therefore, is to critically discuss preclinical studies of promising small molecules that diminish brain pathology in animal models of neurodegeneration, through mechanism(s) that likely involve diminution of oxidative damage. The review will focus on ALS, AD, PD, Huntington’s disease (HD) and stroke/brain ischemia – reperfusion injury (IRI). These pathologies are highlighted because oxidative stress components have been demonstrated convincingly for both human disease and corresponding animal models of spontaneous disease; and because the animal models have been employed in numerous published antioxidant research studies. For each of these several conditions, the review will discuss the rationale for using antioxidants; summarize principal results from preclinical studies; and evaluate results from analogous human clinical studies performed to date. An effort will be made to generalize qualities inherent to antioxidants that show benefit in rodent models of these several diseases; and to illuminate potential pitfalls that might arise in translating antioxidant therapies from animal models into human paradigms.

**AMYOTROPHIC LATERAL SCLEROSIS**

ALS (Lou Gehrig’s disease) is an age-dependent, fatal motor neuron degenerative disease affecting the motor cortex, brainstem and spinal cord. ALS may be sporadic (SALS) or familial (FALS). The molecular cause of sporadic ALS is unknown, but the disease is inexorable, with median life expectancy after diagnosis of 3–5 years although some individuals may live with the disease for much longer [38,109]. The label “ALS” is often applied loosely but technically applies only to a disease affecting anterior horn cells plus pyramidal tract involvement [38]. As such, the prevalence of ALS in the US is approximately 5 per 100,000 persons. A variety of clinically similar motor neuron diseases that display a technically different pattern of motor neuron degeneration can be collectively termed MND for “motor neuron diseases”.

Approximately 10–15% of ALS cases are familial (heritable) in nature. Of this fraction, some 20–30% is caused by mutations in the antioxidant enzyme Cu, Zn-superoxide dismutase (SOD1). More than 90 different mutations in SOD1 have been found in various kindreds afflicted with FALS [38,109]. With rare exception, hereditary ALS propagates in a dominant fashion indicating a gain-of-toxic function rather than a loss of enzyme function. The clinical features of an individual with FALS are almost indistinguishable from SALS, though some mutations such as the G93A and A4V substitutions predict a more rapid disease progression. It is not known with any certainty how the SOD1 mutations engender the clinical manifestation of FALS but the mechanism(s) likely involve some oxidative stress component (reviewed in [68]) arising directly from altered SOD1 enzymology and/or secondary to a chronic neuroinflammatory reaction (reviewed in [68]). The distinction between direct and indirect mechanisms of oxidative stress is not academic because receptor-binding drugs that interfere with immune processes, for instance microglial activation, would diminish oxidative damage and provide “antioxidant” pharmacology at much lower concentrations than might be necessary to inhibit free radical chain processes or boost cellular reducing power. The distinction between “classical antioxidant” and “pharmacological antioxidant” will be revisited at the end of this review.

Transgenic mice expressing mutant SOD1 develop paralysis within 3–6 months of age, depending on the exact mutant and background of the mouse and the SOD1 copy number. Neuron loss in these animals depends upon the expression of mutant SOD1 in both neurons and surrounding glial cells [34,62,89,107,121]. Murine neurodegeneration associated with a progressive, accelerating oxidative stress process evidenced by exponential increases in spinal cord protein carbonyl levels [11,62,65], and increased protein nitration products [90]. Evidence
from human autopsy samples corroborates increased oxidative stress in the human condition [139].

Because transgenic SOD1 mice develop a dramatic and reproducible phenotype with clearly measurable oxidative stress indices, ALS mice have become one of the preferred preclinical systems for evaluating neuroprotectant antioxidant therapies [68]. In fact, one of the first intervention studies performed on SOD1\(^{G93A}\) mice, shortly after the creation of this model, used vitamin E along with anti-excitotoxins [59]. In this early work, Gurney et al. reported that supplementation with \(\alpha\)-tocopherol from early-stage (50 d) disease significantly slowed disease progression as measured by wheel-running tests, but did not extend survival. In our laboratory we have not been able to elicit motor functional benefit in SOD1\(^{G93A}\) mice through either \(\alpha\)- or \(\gamma\)-tocopherol supplementation, as measured by repeated rotarod functional assays (data not shown). Other antioxidants have shown clear benefits in mutant SOD1 mouse models; these agents include the synthetic porphyrin and SOD-mimetic AEOL 10150 (manganese [III] tetrakis[N-N\\(^{\prime}\)-diethylimidazolium-2-yl]porphyrin) [37] and the phenolic antioxidant nordihydroguaiaretic acid (NDGA) [164].

Data from published studies of SOD1 mutant mice do need to be considered with certain caveats. First, it should be noted that a surprising variety of different treatment concepts have been shown to produce modest but statistically significant benefits in the SOD1\(^{G93A}\) mouse [21,68] yet some of the most promising agents, such as the COX-II selective inhibitor celecoxib, have failed in human clinical trials despite achieving formal efficacy in the mouse model [39,43]. Table 1 summarizes the various antioxidants studied for ALS in the important animal studies/clinical trials. Second, interventions that are effective in the SOD1 mouse model usually produce only small effects on lifespan extension. In a recent meta-analysis, Benatar estimates that drugs which produced significant benefits in SOD1 mutant mice yielded survival benefits of 13 d (weighted mean difference [21]). In the most commonly used strain of SOD1 mouse, the SOD1\(^{G93A}\) mouse which expresses high copy numbers of the mutant SOD1 gene, median lifespan is approximately 130 d [21,63,65,68] so that even the best drug treatments produce only 10% effects. Third and most troubling is the variation in survival of untreated SOD1\(^{G93A}\) mice in published drug studies. Even when one considers only studies using the fast-progressing SOD1\(^{G93A}\) animal, median control mortality can range from 100 d to 135 d. The largest relative treatment effects occur in studies in which control mortality approaches the low end of this range. For instance AEOL 10150 extended the median survival of SOD1\(^{G93A}\) mice by an impressive 27 d, from 103 d to 130 d [37], making this one of the most effective small molecule interventions ever tested in the standard ALS mouse model [21]. However, in other studies using the same mouse strain, median control survival is routinely reported in the range of 130 d [164]. Thus, one worries in these studies that the successful drug therapy may be mitigating facility-dependent stresses rather than effectively treating fundamental pathology of ALS-like disease in the experimental animals.

Care must also be taken in ascribing benefits of antioxidant therapies to the antioxidant action of the compound in question. For instance NDGA, which extends survival by 12 d in the SOD1\(^{G93A}\) mouse [164], is a potent antioxidant by virtue of its lipophilic phenolic characteristics; however, it also is a classical inhibitor of arachidonic acid 5-lipoxygenase and likely possesses other modes of pharmacological action mediated through specific protein binding targets, rather than classical antioxidant modes-of-action.

Human clinical trials have been done and continue to be pursued by using putative antioxidants against ALS. In a recent meta-analysis, Orrell, Lane and Ross searched the Cochrane Neuromuscular Disease Group Trials register, MedLine and EMBASE databases to query randomized or quasi-randomized controlled trials of antioxidant treatments for ALS [110]. The search identified 23 studies for consideration; these included trials for vitamin E (500 mg twice
daily and 1 g five times daily); N-acetylcysteine (50 mg/kg daily subcutaneous); and various mixtures of vitamin E, selenium, and methionine. No significant effect on primary outcome (survival at 12 months treatment) was observed in the meta-analysis of all antioxidants combined, and no significant differences were observed on any secondary measures [110]. These studies suggest that either there are flaws in the preclinical paradigms for selecting potential human treatments; or that the rapidly-progressing human disease is untreatable after the disease has reached a stage of diagnostic severity.

**ALZHEIMER’S DISEASE**

Oxidative stress is closely associated with the neuropathology of AD, a major neurodegenerative disorder characterized by multiple neurological events, gradual decline in cognitive functions and rapid aging of the brain tissue. Neuropathology of AD arises from numerous biochemical changes such as cholinergic deficits [53]; neuronal metabolic insult (glutamate induced excitotoxicity) [99]; and oxidative stress or damage such as lipid peroxidation and protein oxidation [108]. AD progression and memory loss involves various cellular anomalies such as: 1) accumulation of extracellular neuritic plaques of amyloid-β peptide (Aβ) [7,27,95]; 2) intracellular neurofibrillary tangles (NFTs); 3) proliferation of astrocytes, synaptic loss; and 3) progressive loss of neurons and microglial activation [66,67, 96,149,165].

**Oxidative stress in Alzheimer’s disease**

Oxidative stress in AD patients occurs due to various factors such as genetic factors (apolipoprotein E ε4 allele), germline mutations (amyloid-β protein precursor gene, presenilin-1 gene, and presenilin-2 gene), environmental causes, lifestyle-related factors (smoking) and certain health conditions such as diabetes, brain injury and hypercholesterolemia [108]. Oxidative stress is found in various in vitro (cells in culture) and in vivo models (transgenic animals) [88], as well as in tissues and fluids from patients with AD (living and postmortem brains) and cognitive diseases such as MCI and Down syndrome. Oxidative stress affects AD patients at four different levels; protein [67], nucleic acids [100], lipids [122,133,137] and enzymes [123]. Increased nitrative stress in human AD brains has been reported in the form of increased levels of protein oxidation [66], protein nitration [76], 3-nitrotyrosine, 3,3′-dityrosine in hippocampus and major regions of the brain including inferior parietal lobule (IPL), neocortical regions and ventricular cerebrospinal fluid [67]. Both nuclear and mitochondrial DNA has been modified by oxidative stress to increased levels of 8-hydroxy-2-deoxyguanosine and oxidized bases in cerebral cortex and cerebellum of AD patients as compared to age-matched control subjects [100,160]. Increased levels of malondialdehyde, a measure of lipid peroxidation, are found in human AD brains [14]. Butterfield and colleagues recently demonstrated that brain synaptosomes in AD and MCI patients had oxidative stress-mediated increased modification of phosphotidylserine, a key lipid necessary for membrane integrity [13]. Early-stage as well as late-stage AD brains expressed decreased antioxidant enzymes activities for key anti-oxidant enzymes such as: superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase [54].

To name a few examples, brain tissue from a transgenic mouse model (APPsw) of human familial AD having a “Swedish” mutant amyloid-β protein precursor (AβPP) [88] and peripheral leukocytes MCI patients have shown increased lipid peroxidation, increased oxidative damage to DNA and decreased plasma total antioxidant capacity [163]. The underlying oxidative stress in AD is mediated via various marker proteins and is supported by many preclinical investigations. Davis et al. and Meda et al. showed that Aβ/AβPP can directly induce reactive nitrogen species in cell culture models, as well as in in vivo models [7,20,27, 41,67,95,101]. Astroglial cells isolated from brains of AD patients had increased levels of heme oxygenase-1 (HO-1), a marker of oxidative stress [136]. Moreover, transgenic mouse and C.
elegans models of AD amyloidosis exhibit compromised antioxidant defense, increased protein oxidation and lipid peroxidation [44,122,137]. Similarly, the frontal, neurons, astroglial cells and blood vessels of postmortem AD brains had increased levels of nitric oxide synthase enzymes [50,93] and hydroxyl radicals [147] leading to indicative of increased production of nitrotyrosine and nitrative stress. Additionally, mitochondrial problems, energy deprivation and compromised antioxidant defense [8,14,115] are associated with increased free radical burden in AD brains. Numerous evidences for involvement of mitochondrial problems with AD came from early defects in glucose utilization and deregulation of key mitochondrial enzymes such as α-ketoglutarate dehydrogenase, pyruvate dehydrogenase and more commonly for cytochrome c oxidase (COX) [reviewed in 85].

Although these evidences suggest that diagnosis of AD has an oxidative stress component to pathology, it is not still known whether oxidative stress in AD is a cause (damage) or an effect (response to the damage). This information is crucial for designing the preclinical, as well as clinical studies for these agents to develop effective anti-AD therapies.

Antioxidant therapies for Alzheimer’s disease

Currently available anti-AD therapies can be classified as follows: 1) treating cognitive and behavioral symptoms (anti-cholinesterases, anti-oxidants); 2) treatments for sleep changes; and 3) alternative treatments such as behavioral training [108]. Adjunct therapies include pharmacological agents such as non-steroidal anti-inflammatory drugs (NSAIDs) [35,72]; metals such as copper (stabilizing the Cu/Zn SOD activity) and metal chelator, e.g., clioquinol [31]. Earlier neurotransmitter theory by Bartus in 1982 [15,53] led to the development of the very first anti-AD agents which consisted of cholinesterase inhibitors; galantamine, tacrine, donepezil, and rivastigmine [130,134,151,166] and later to the development of memantine, a NMDA-receptor antagonist [148]. Large clinical trials by the U.S. Foods and Drug Administration (FDA) in 1995 for cholinesterase inhibitors and for memantine in 2002 showed modest symptomatic benefits on cognitive, behavioral and global measures. Most of the anti-cholinesterases based therapies produce very moderate symptomatic relief and poor prognosis; therefore, the development of novel interventions which can target fundamental early changes (such as oxidative stress) has been the focus of recent anti-AD therapeutics. Antioxidant strategies are divided into three categories namely; 1) free radical scavengers, e.g., vitamins C and E, β-carotene; 2) preventive antioxidants such as metal chelators, glutathione peroxidases and SOD enzymes; and 3) de novo and repair enzymes such as lipases, proteases and DNA repair enzymes [108]. Nonspecific antioxidants include melatonin [47], omega-3 polyunsaturated fatty acid (docosahexaenoic acid) [108], curcumin [88,167], ubiquinone [16] and α-lipoic acid [124].

Various dietary supplements have been also shown to provide treatment of AD. For instance, S-adenosyl methionine (SAM) supplementation in apolipoprotein E (ApoE) deficient mice improved neuropathological features of AD [152]. Chan et al. observed neuroprotection by dietary supplementation of apple juice concentrate, rich source of SAM, in AD ApoE deficient mice [30]. Moreover, in this same mouse model, folate and vitamin E deficiency led to increased presenilin-1 expression (processes amyloid) which was later attenuated by apple juice concentrate in both juvenile and adult mice. Many other dietary components, e.g., caffeine (500 mg or 5–6 cups of coffee a day) [12], epigallocatechin-gallate esters from green tea [127] and red wine (Cabernet Sauvignon) have been shown to inhibit amyloidosis and Aβ production [159] in both cell culture and animal models. Various other factors including lifestyle factors such as calorie restriction [81,112,158], high activity in environmental enrichment [76] and voluntary exercise have been shown synergistic effects to antioxidants in mitigating AD neuropathophysiology.
Vitamin E in clinical trials of Alzheimer's disease

Vitamin E is an archetype antioxidant vitamin which has been able to reach sub-therapeutic levels in brains of AD patients and decrease lipid peroxidation susceptibility by 60% in AD patients as compared to control subjects [54,155]. Table 2 summarizes the various antioxidants studied for AD in the important animal studies/clinical trials.

Vitamin E has been frequently tested in epidemiologic and clinical studies for AD and cognitive disorders. The data from these trials are available for symptomatic treatments [29,131], as well as preventive therapies for AD [108]. Currently, clinical trials are underway for vitamin E, either alone or in combination with memantine or selenium or α-lipoic acid or with a combination of vitamin C/α-lipoic acid, coenzyme Q, the curry spice curcumin, with tryptophanmetabolite/neurotransmitter melatonin, and lutein/zeaxanthin [4]. Some of these agents have shown promising preclinical effects against amyloidopathy, behavioral decline, protein oxidation, protein carbonylation [88], and lipid oxidation [47,150] in brains of amyloid transgenic mice. Lessons from vitamin E trials have remodeled antioxidant studies suggesting a critical role of concomitant dietary and lifestyle factors [108] in improving efficacy of antioxidant therapies. Most importantly, all of these clinical trials indicated mixed results for vitamin E.

Some of the clinical trials for vitamin E, alone or in combination with vitamin C, against cognitive disorders showed positive effects for vitamin E; e.g., Honolulu-Asia Aging study (3,385 men) [98]; Chicago Health and Aging Project (815 subjects; 3.9 year follow-up study) and Nurses’ Health Study (14,986 women aged 70–79 years) [57] whereas, some studies showed contrasting effects for vitamin E [118] which include; Honolulu-Asia Aging study (2459 men; Vitamin E alone) [80,92,118], Washington Heights Study (980 subjects; 4 year follow-up study) and Cache Country Study (4740 subjects; 3 year follow-up study) [169].

Many of the above studies focused on vitamin E and C supplements alone or in combination with each other or other supplements. The Honolulu-Asia Aging Study investigated effects of 3–4 years of vitamin E or C supplements against dementia and cognitive dysfunction in Japanese-American resident men from Hawaii, aged between 71 to 93 years, for cognitive performances. Both vitamins improved cognitive performance along with protection only against non-AD dementia, suggesting that both of these antioxidants might be helpful in combating dementia and associated cognitive problems in people with late-stage AD. The Chicago Health and Aging Project undertook dose-response study of dietary (food and supplements) vitamin E (7.9–1660 IU/day), vitamin C (93–2530 mg/day), and β-carotene (1903–28788 IU/day) in 65 years and older non-AD subjects against development of AD. Vitamin E was given with or without vitamin C/β-carotene. This study indicated superiority of vitamin E over vitamin C and β-carotene. Only vitamin E alone had dose-dependent protection against risk of developing AD, although only in APOE ε4 negative subjects suggesting role of genetic status in vitamin E mediated neuroprotection.

The Nurse’s study examined effects of high-dose vitamin E (600 mg/day) with or without vitamin C (750 mg/day) on cognitive functions in women who participated in the Nurses’ Health Study from 1995 to 2000. Telephonic methods assessed cognitive function using recall of 10-word list, a short paragraph, a test of verbal fluency, and a digit span backwards test. Combination of vitamin E and C exhibited a time-dependent significant improvement in mean performance (P = 0.03) as compared to subjects with no reported vitamin intake. This study also suggested that use of specific vitamin E supplements, and not specific vitamin C supplements, is beneficial for improved cognitive function. The Honolulu-Asia Aging study (2459 men; vitamin E alone) [80,92,118] investigated dietary intake of antioxidants in middle aged versus old-aged subjects for protection against dementia. The subjects were studied over a period of 8 years from 1991 to 1999 for existence of AD and dementia. Dietary intake of vitamins E and C, β-carotene at mid-life was not associated with the risk of mid-life, as well

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as late-life, dementia. The Washington Heights Study studied the connection between intake of vitamins E and C, carotenoids in 980 elderly subjects without dementia at the start of the study. Over a 4-year period, frequency of the incidence of AD was counted. Supplemental or dietary supply of all the antioxidants failed to decrease the risk of AD. Similarly, the Cache Country Study (4740 subjects; 3-year follow-up study) found that single or combination of vitamins E and C and even multivitamin supplements have differential effects on incidences of AD [169]. Vitamin E was effective only when given with vitamin C or multivitamins in protecting against AD. Monotherapy with vitamin E could not produce any anti-AD effects. In contrast to human clinical observations, the benefit of vitamin E against AD was not observed against brain oxidative damage in transgenic APPsw mice [35]. Unconvincing data from these vitamin E trials indicate intricate physiological and pharmacological features of AD.

**Translational challenges for antioxidants in the treatment of Alzheimer’s disease**

Numerous cellular and animal models of AD have been developed and considerable efforts have been taken to identify mechanisms of redox state-mediated gene regulation in relation to AD pathology. Preclinical studies with antioxidants are very promising although their direct application to human AD is still somewhat problematic and has some caveats. These studies require a more relevant animal model to simulate human AD to help identify the exact mechanism of action for the antioxidant’s defense against AD. Some effects of these agents seem to be not mediated solely through their antioxidant functions e.g., curcumin inhibits amyloid aggregation and brain protein carbonylation in vitro and in vivo [167] in parallel to its anti-inflammatory effects on eicosanoids via inhibition of cyclooxygenase and lipoxygenase [125]. The anti-AD effects of curcumin may either arise from its direct antioxidant activity or indirectly from its anti-inflammatory functions. The relevance of brain protein carbonylation to AD pathology needs to be further addressed to support clinical applications of curcumin.

The preclinical studies of AD involving antioxidant therapeutics have similar challenges as were seen in preclinical studies of antioxidants in ALS mouse models as previously discussed. Although various preclinical models have allowed researchers to perform a quantitative target validation, they do not completely mimic the human nature of the disease especially in terms of longevity of AD in humans and qualitative similarity with human AD, especially in their profound neuropathology and underlying neurodegeneration. Since AD is associated with advanced age and its symptoms require long-term biochemical changes in the brain, it is very hard to recreate these conditions in a mouse or rat model of AD. Genetic predisposition and key biochemical changes can be reproduced in various transgenic models; however, the long-term nature of AD prohibits researchers to extrapolate the acute effects of any test drug candidate to its long-term or chronic anti-AD effects.

**Current translational challenges for Alzheimer’s disease therapeutics**

Additionally, translation of antioxidants from pre-clinical stage to clinical settings suffers from other difficulties especially pharmacokinetic (bioavailability and frequency of administration) and pharmacodynamic (therapeutic index and onset of action) constraints. For instance, even 3–8 g/day of curcumin administration in humans could not achieve therapeutic circulating levels [69]. Brain bioavailability of vitamin E in humans is very slow and may not be enough to quickly inhibit AD neuropathology unless administered as a prophylactic at very early ages [150]. The bioavailability issues can be solved by alternatively dispensing these agents using infusions, inhalations (nebulizer) or encapsulations to expedite brain and CNS levels. Amidst the pharmacodynamic constraints, use of antioxidants such as vitamin E and C in epidemiologic studies and clinical trials remains questionable.

To make matters worse, dose-correlation from the animal studies to the human studies pose many challenges. Usually the therapeutic dose or ED₅₀ [effective dose] found in animal models

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is very high and impractical to extrapolate to the human studies. Improved therapy with optimization of timing and dosing of the test agent should provide substantial benefits. In addition these intervention trials should study multiple indices and specific markers rather than measuring some non-relevant biomarkers such as thiobarbituric acid-reactive substances (TBARS). Life-style and environmental factors also vary among animal and human AD pathology thus undermining the protective effects seen in animal studies. Moreover, if AD in humans coexists with other CNS or other geriatric ailments, which are hard to mimic in animal models, this can modify drug efficacy as well as dosage regimens. This might further lead to only some symptomatic relief. Non-specific effects of the drug candidates on one or more vital systems in the human patients could pose serious threats to medical treatment. Hence, a very detailed knowledge about the exact mechanism of action for these test compounds is strongly desired. One or more of the above causes can halt the progress of a test compound from preclinical studies into the clinical settings.

Currently available antioxidants also suffer from similar drawbacks. Many antioxidants including vitamin C and E only offer symptomatic treatment without halting the underlying neurodegeneration and pathology of the disease. Since these vitamins are also available as dietary supplements and do not require a prescription, their effectiveness and safety issues are not regulated and reported to FDA. Moreover, the purity and quality of these dietary supplements remains questionable. Additionally, adverse effects of the dietary supplements are not closely monitored and reported back to the FDA which can complicate the prognosis of the disease. To add fuel to the fire, these vitamins can have serious interactions with prescribed medications or existing health conditions such as cardiovascular disorders, e.g., vitamin E [24,61,91,103], and more likely produce only a prophylactic (or preventive) effect rather than a “therapeutic” effect.

Clinical trials with antioxidants for AD need to time the start of the therapy at the right stage of the disease. Many intervention studies are started very late in the disease state, when AD pathology is already at a fulminant level. This severely modifies or even reduces therapeutic effectiveness of the test agent. Furthermore, interval change and duration of treatment can also alter therapeutic efficiency of these compounds, e.g., a 6-month trial of N-acetyl cysteine, a glutathione elevating agent [6], did not achieve formal significant differences, however, interval change from 3 to 6 months favored its treatment.

The type and composition of the test compound also affects the end result especially when certain test agents are a crude mixture of two or more constituents or extracts from an herbal source. In such case, the exact mechanism of action is hard to interpret and other factors such as caloric content, taste, and micronutrient enrichment can also have variable crosstalk with the therapy. Importantly, this makes it harder to understand what specific constituent is responsible for the observed beneficial effects. The above scenario is commonly found when antioxidant vitamins are combined with other agents, however, the observed beneficial effects require further dissection into the AD prognosis.

Although, so far, the global effects of antioxidants seen in clinical trials in alleviating AD and cognitive dysfunction are not very convincing, future success with AD therapeutics highly warrants a more relevant and appropriate animal model homologous to human AD. This will improve the screening process for discovery of novel target compounds in the future and alternatively, combining antioxidant therapies with other neurotransmitter-based therapies might produce synergistic effects against neuropathology of AD. In addition, the stringent, multi-factorial manipulations of animal models and dietary conditions along with profound epidemiologic studies and clinical trials should work as a springboard for launching an effective antioxidant campaign against human AD.
PARKINSON’S DISEASE

Rational for the use of antioxidants in PD, like ALS and AD, stems from the well-documented increase in oxidative damage to PD-affected human brain (reviewed in [60,73]) and also in the brains of animal models exposed to toxins that selectively target the nigrostriatal brain circuitry afflicted in PD [40]. Pre-clinical studies in PD benefit from the multiplicity of generally-accepted animal models. PD-like conditions can be induced in rodents or primates by various chemical manipulations including MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) intoxication [40,79]; paraquat administration [113]; 6-hydroxy-DOPA administration [142]; intrastriatal lipopolysaccharide (LPS) administration [70]; and in Lewis rats, with intravenous rotenone administration [22,23,111]. Also there are recently developed genetic models involving mutations in the PD-associated α-synuclein [49] or the PINK1 (Parkinson-induced kinase-1) gene [157]. Detailed reviews of these various animal models are available elsewhere [22,40]. It must be noted, however, that PD is unique amongst other neurodegenerative diseases because one animal model of PD, the classic MPTP model, actually originated from the accidental discovery that MPTP produces PD-like condition in human drug abusers who consume this compound [79]. Thus, at least one animal model of PD definitely mimics a specific non-heritable form of the human disease in both cause and presentation.

Antioxidant trials have achieved variable success in non-human models of PD. Most studies suggest that vitamin E (defined solely as α-tocopherol) does not protect in the most common animal models of PD including the MPTP model. Very early work by Perry’s group found that four different antioxidants (α-tocopherol, β-carotene, N-acetyl cysteine or ascorbic acid) partially protected C57-black mice against the acute neurotoxicity of MPTP [116]. Subsequent work by the same group found that neither α-tocopherol nor β-carotene in massive doses offered any protection against MPTP in a primate (marmoset) model [117]. Independent, but roughly contemporaneous, studies reported that α-tocopherol, ascorbate, dimethyl sulfoxide, cysteamine or sodium selenite offered no protection against MPTP in the mouse model [55, 97]. Thus, most studies investigating the canonical antioxidant vitamin E have been very negative with respect to observations of protective effects in the MPTP model.

Possible flaws in the early vitamin E studies may stem from low CNS bioavailability of tocopherols combined with use of the “wrong” tocopherols. In recent, more sophisticated approaches designed to compare α- or γ-tocopherol as anti-Parkinsonian agents, Itoh et al. used α-tocopherol transfer protein (TTP)-knockout mice [71]. Presumably these mice could incorporate either α- or γ-tocopherol at maximal rates dependent upon dietary content rather than the kinetics of TTP function. When TTP mice were deprived of all tocopherols, then placed on 0.1% oral α- or γ-tocopherol, then challenged with MPTP, only γ-tocopherol significantly protected against dopaminergic toxicity with almost no evident dopamine depletion [71]. These researchers measured tocopherol content in the striatum and found that γ-tocopherol incorporation into brain was substantially less than α-tocopherol incorporation, despite the apparent superiority of γ-tocopherol with respect to histological endpoints [71]. We, and others, have suggested that γ-tocopherol might be able to protect neurons differently from α-tocopherol due to the inherent ability of the former tocopherol to absorb nitration equivalents in a way that the latter cannot [63,65]. The Itoh study partially substantiates this view though nitration was not addressed explicitly. Other recent research using TTP mice seem to show that α-tocopherol depletion in non-supplemented TTP mice does not exacerbate MPTP toxicity [126], as would be expected in a situation where α-tocopherol is protective.

Although these studies appear to condemn the case for vitamin E per se as a prophylactic or treatment against PD, it is possible that vitamin E has not been tested in the right animal models and might in fact offer protection in some cases. For instance, vitamin E does significantly

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inhibit ommatidial degeneration in a drosophila model of PD wherein the drosophila PINK 1 gene was inactivated using an RNAi approach [157].

Other antioxidants besides α-tocopherol have met with greater success in treating preclinical models of PD. Table 3 summarizes the various antioxidants studied for PD in the important animal studies/clinical trials. Among antioxidants previously discussed in this review, SOD-mimicking metalloporphyrins (AEOL11207, EUK-134, EUK-189) [87,114] and epigallocatechin-gallate [33] effectively antagonize MPTP dopaminergic toxicity in mice. The synthetic nitrone-based free radical trap α-phenyl-N-tert-butyl nitrone (PBN) reproducibly protects against MPTP, though notably it does so without diminishing the level of salicylate-trappable hydroxyl radicals generated through the MPTP paradigm [48,138].

Genetic enhancement of antioxidant enzymes or direct antioxidant enzyme supplementation seems to protect against various PD models. For example, both overexpression of Cu, Zn-SOD and glutathione peroxidase (GPx) protect against paraquat + maneb-induced PD phenotype in mice [153]. Similarly lentivirus-mediated expression of GPx protects against 6-hydroxydopa [128]. Choi et al. report that SOD protein can be engineered with a 21-peptide transduction sequence that facilitates protein delivery across cell membranes and into brain tissue [32,46]. Remarkably, this PEP-1-SOD completely protected against paraquat-mediated striatal damage in mice when the engineered protein was injected intraperitoneally [32]. From these latter pieces of work, it seems clear that specific antioxidant intervention strategies can prove highly successful against multiple preclinical models of neurodegeneration. Taken together these several studies provide proof-of-concept for antioxidant therapy, at least in non-human PD. The failure of other treatments and especially of α-tocopherol in preclinical models warns that not all purported antioxidants are equivalent and that antioxidant interventions are not generalizable and may be therapy- and/or PD model-specific.

A number of well-conducted human clinical trials have explored antioxidant therapies and particularly vitamin E supplementation in PD. Epidemiology studies utilizing large sample sizes in the Nurses’ Health Study (76,890 women followed for 14 years) and the Health Professionals Follow-Up Study (47,331 men followed for 12 years) suggest that dietary intake (from food only, rather than supplements) of vitamin E diminishes risk of PD among both men and women whereas multivitamin supplement usage and total vitamin E intake did not correlate with PD risk [1,170]. It may be noteworthy that amongst dietary habits, consumption of nuts was significantly associated with reduced PD risk (pooled RR = 0.57) [170]. Nuts are known to be very rich in γ-tocopherol [84]. We have previously argued that α-tocopherol and γ-tocopherol are correlated in healthy subjects so that epidemiological studies associating dietary vitamin E or plasma vitamin E with health benefits may have indexed an unanticipated auto-correlation between the two tocopherol variants [63]. PD brain, plasma and CNS are not depleted in α-tocopherol [42,104] but γ-tocopherol has not been investigated. In light of recent findings described above that γ-tocopherol uniquely protects against an animal model of PD, more epidemiological studies are justified to explore non-α-tocopherol correlations with PD risk.

Intervention studies of antioxidants have been performed in human PD. The now famous DATATOP (Deprenyl and Tocopherol Antioxidant Therapy of Parkinsonism) provided placebo, vitamin E (2000 IU/d), deprenyl, or vitamin E plus deprenyl to 8900 patients with early PD. After 14 months of controlled observation and more than a decade of follow-up, there appeared to be no benefit of the vitamin E supplementation strategy [140]. Weber and Ernst provide a recent metanalysis of three vitamin E clinical trials (2 observational, 1 prospective randomized); four trials of coenzyme Q10 (CoQ10) and 1 study of glutathione [161]. Of these trials only the CoQ10 trials demonstrate some “minor treatment benefits” that...
probably map to partial correction of mitochondrial electron transport chain deficiencies in PD rather than antioxidant effects per se [161]. A second recent meta-analysis of vitamin E clinical trials in AD, PD, tardive dyskinesia and cataract reaches essentially the same conclusion and “Discourages individual vitamin E supplements that usually contain 400 IU of α-tocopherol” [120].

HUNTINGTON’S DISEASE

HD is an inherited triplet repeat disease wherein a polyglutamine tract of the huntingtin (Htt) protein is expanded from less than 30 to perhaps 200 tandem glutamine residues [156]. HD causes selective loss of mostly the medium spiny neurons of the caudate nucleus. The mechanism(s) of mutant Htt-induced degeneration are much debated but likely involve both loss of neurotrophic functions [82] and gains of toxic functions [156]. In the latter category oxidative stress indices are widely reported to increase in human and animal models of HD, perhaps secondary to mitochondrial dysfunction, excitotoxin-mediated oxidative stress and neuroinflammation [26].

The earliest preclinical models of HD, still employed today, were intrastriatal injection of the neurotoxin quinolinic acid into rats or systemic chronic intoxication with the mitochondrial complex II-inhibitor 3-nitropropionic acid (3NP) [18,25]. Table 4 summarizes the various antioxidants studied for HD in the important animal studies/clinical trials. These strategies have been employed in rodents and even primates with considerable success and reproducibility [17,25,51,129]. After the discovery of the mutant Htt gene product as the genetic cause of human HD, transgenic mice were developed that express the first exon of mHtt with an approximately 150-count polyglutamine expansion. The most commonly employed of these animals is the so-called R6 mouse model [86]. These animals develop basal ganglial degeneration but also have other symptoms including diabetes with motor functional decline beginning at approximately 30 d and death occurring by 100–120 d ([86]; personal observations, KH). More sophisticated animal transgenic models employing full-length mHtt expression from yeast artificial chromosomes (YAC) are becoming popular [154], as are “knock-in” models that express mHtt forms with regional-specificity [102]. These mice are likely to more realistically model the human pathology, but they develop disease much more slowly so that intervention testing in these animals requires many months of experimentation.

Relative to the other diseases previously discussed, fewer antioxidant trials have been conducted in HD preclinical models. Flint Beal’s group, who pioneered the quinolinic acid model of HD, found that none of the antioxidants vitamin E, β-carotene, or ascorbic acid provided protection against quinolinate-induced striatal neurotoxicity when administered systemically for several days prior to toxin challenge [19]. As a positive therapeutic control, NMDA receptor antagonists did provide some benefit in this study. Latter investigations into antioxidants by the Beal group found that the thiol antioxidant and mitochondrial enzyme cofactor lipoic acid improved survival in both the R6/2 and the N171-82Q transgenic mouse models of HD, whereas the antioxidant and free radical scavenger 2-sulfo-tert-butyl nitronate (S-PBN) had no effect [10]. More recent work suggests that vitamin E plus coenzyme Q10 provide some mitigation of 3NP-induced striatal energy deficits in aged rats [75]. Ehrnhoef er et al. find that epigallocatechin-gallate significantly reduced mHtt toxicity in a yeast model and slowed motor function decline in a transgenic mouse model [45]. High dose coenzyme Q10 extended the life of R6/2 mice, in a manner that was dose and source-dependent [146]; this study was noteworthy in measuring 8-hydroxyguanosine, which was reduced by the CoQ10 treatment, as a marker of oxidative damage.

Human clinical trials in HD are ongoing. Peyser et al. performed a prospective, double-blind, placebo-controlled study of high-dose α-tocopherol on a cohort of 73 HD patients. Vitamin E
had no effect on neurologic or psychiatric symptoms but post hoc analysis revealed a significant effect of intervention on neurological symptoms in early stage patients [119]. Coenzyme Q10 has received the most intense human clinical scrutiny in HD. A multicenter, blinded, randomized study employing 347 early HD patients receiving 300 mg CoQ10 twice daily failed to produce a significant change in the primary measure of total functional capacity (TFC) between baseline and 30 months [5], though there was a non-significant trend toward slowing the TFC decline and beneficial trends in secondary measures [5].

STROKE – ISCHEMIA/REPERFUSION INJURY

Animal models of stroke differ from the neurodegeneration models previously discussed in that most stroke models require a physical injury or surgical modification to an otherwise genetically and toxicologically intact animal. In principle, this accelerates the rate at which drug treatments can be tested, because an ischemia/reperfusion experiment requires days to weeks rather than weeks to months. On the other hand this introduces significant challenges in modeling human stroke which is a result of both genetic predisposition and prolonged environmental factors that are difficult to reproduce in preclinical studies. The lack of specific gene mutations causative for adult stroke have precluded development of robust murine genetic models for IRI, though there is a stroke-prone hypertensive rat model that mimics certain types of cerebrovascular disease entailing lacunar infarction and intracerebrovascular hemorrhage [106]. Thus less than ten rodent models of focal stroke have been utilized and most of these produce large focal lesions more closely resembling fatal human infarctions than smaller, more potentially treatable human cerebrovascular events [28]. Most of the antioxidant preclinical trials have utilized carotid artery occlusion in the Mongolian gerbil (chosen because most gerbils lack a complete circle of Willis thus allowing efficient unilateral restriction of cerebral blood flow); middle cerebral artery occlusion (MCAO) in the rat; and vertebral artery/carotid artery occlusion in rodents [28]. Table 5 summarizes the various antioxidants studied for stroke/IRI in the important animal studies/clinical trials.

Stroke is widely considered to have an oxidative stress component originating directly from the physical biochemistry of the ischemia/reperfusion event, and later from secondary events such as excitotoxicity and neuroinflammation [9]. In the early stages of ischemia, oxygen tension drops leading to an effective blockade of mitochondrial electron transport and an accumulation of reducing equivalents. Upon reperfusion these can rapidly promote incomplete reduction of molecular oxygen yielding free radicals and peroxides [78]. Conversion of xanthine dehydrogenase to the superoxide-generating xanthine oxidase (XO) has also been implicated as an early source of free radicals during reperfusion [94]. Mice engineered to over-express SOD1 or GPx1 have some resistance to transient brain ischemia/reperfusion injury [74,77,162,168] whereas GPx1 knockout animals suffer exacerbated stroke damage [36]. Thus, there is a strong basis to expect that antioxidant agents might be valuable in mitigating neurodegeneration resulting from a transient ischemic event. From the practical standpoint, however, the early events in ischemia/reperfusion injury are unlikely to be treatable because one cannot predict the occurrence of stroke or initiate treatment immediately after a cerebrovascular event; however, later stage events including neuroinflammatory cascades and secondary oxidative stress events can be mitigated, at least in theory [9,135].

Numerous efforts have been made to do so using rodent and primate models and many therapeutic candidates with presumptive antioxidant modes of action (reviewed in [94]). These therapies have been divided into classes consisting of agents that scavenge radicals (chain breakers or classical antioxidants such as vitamin E, sulfhydryl compounds and nitroto spin traps);agents that accelerate reactive oxygen detoxification (superoxide dismutase, catalase or peroxidase conjugates and mimetics); and agents that decrease rates of radical generation (e.g., XO inhibitors like allopurinol, nitric oxide synthase inhibitors or nonsteroidal anti-
inflammatory agents and cyclooxygenase-inhibitors) [94]. A surprising number of antioxidant manipulations reportedly reduce IRI lesion volume in non-human experiments. An incomplete list of these agents would include allopurinol, oxypurinol, and selective cyclooxygenase-II inhibitors NS-398 and nimesulide; PBN, sulfated PBN and azulanyl nitrones; lipoic acid, N-acetyl cysteine, glutathione monoethyl ester, uric acid, vitamin E, resveratrol, PEGylated or otherwise derivatized SOD, AEOL- and EUK-series SOD1 inhibitors; the GPx mimetic ebselen; and aminosterols or “lazaroids” that combine radical scavenging actions with iron chelating functions ([143] and thoroughly reviewed in [94]).

Despite the impressive number of antioxidants that benefit various preclinical models of stroke, only four candidate drugs have advanced to human trials [56,94]. The lazaroid Tirilazad and the nitrone NXY-059 (Cerovive) both failed in thorough multi-phase human clinical trials with Tirilazad demonstrating possible toxic effects [2,56,94]. NXY-059 did produce significant benefits ($p = 0.038$) in the modified Rankin functional scale in the Stroke-Acute Ischemic NXY Treatment-I (SAINT-I) trial employing 1722 patients, though no significance was detected by the more rigorous National Institutes of Health Stroke Scale (NIHSS) or the Barthel Index [83]. Expansion of the study to 3206 subjects in the SAINT-II trial failed to produce any significant result of NXY-059 ($p = 0.33$; [141]). The SAINT I/II trial disappointment was intense because NXY-059 and its PBN predecessor had shown consistent benefits in multiple stroke models including gerbil, rat and primate models; the drug had shown preclinical efficacy even when administered up to six hours after the ischemic event; and because the SAINT I/II development program had been conducted using many of the STAIR (Stroke Therapy Academic Industry Round table) guidelines outlining “best industry” practices for this sort of translational endeavor [52]. Moreover, the very large population size in the SAINT I/II study and the pronounced statistical discrepancy in functional recovery scores between the smaller and larger trial, greatly dismayed the research and clinical communities.

Ebselen and the radical scavenger edaravone (MCI-186, Radicut) have been tested in relatively small Japanese studies. The ebselen trials employed small populations (< 200 ebselen-treated patients) and did not show clinical improvement at three months post-treatment (reviewed in [56]). In 2001, edavarone was introduced into Japanese clinics for treatment of acute ischemic stroke [94]. In a study published in 2003 employing 252 acute ischemic stroke patients with a three month follow-up, edaravone produced a significant ($p = 0.0382$) benefit according to the modified Rankin scale [3]. It should be noted, however, that NXY-059 produced a similar benefit in the SAINT-I trial that was not at all reproduced when the number of patients were increased in the SAINT-II trial [141]. Thus extreme caution is warranted in the interpretation of small clinical trial results in human ischemia/reperfusion injury.

**SUMMARY AND CONCLUSIONS**

A review of studies conducted over the past twenty years, that tested agents claimed to be “antioxidant” in animal models of five different neurological conditions with strongly-implicated oxidative stress components, revealed a generally favorable series of outcomes, with the antioxidant therapies proving largely efficacious across the models. In contrast, a review of the literature regarding antioxidant efficacy in human supplementation trials or clinical drug trials revealed little to no effect of commonly-employed antioxidant substances; and no benefit from the most heavily-studied synthetic antioxidant drug candidates tested against stroke. Even more disconcerting, there is emerging data that suggests very high-dose or long-term supplementation with vitamin E may pose certain health risks.

The reasons for the disjunction between the animal model studies and the human clinical experience may be due either to a flaw in the theory concerning oxidative stress as a pathological contributor to neurodisease; or a flaw in the implementation of that theory. Theory
would be flawed if oxidative stress plays no role in neuron dysfunction or death; which is to say, if oxidative stress is merely an epiphenomenon. In this case, antioxidant interdiction would likely decrease biomarkers of oxidative stress in animal models (or humans) without imparting an observable benefit on neuron viability, histological indicators or behavioral outcomes. Evidence from animal models suggests this is not the case; generally speaking, purported antioxidant therapies both diminish oxidative damage (e.g., measured by protein carbonylation in the case of curcumin in murine models of AD) and also slow disease progression. The caveat in this last statement is that most studies of putative antioxidants do not simultaneously report oxidative stress measures AND pathological endpoints so it is difficult to correlate the diminution of oxidative stress biomarkers with the overall phenotypic/outcomes benefit of the test agents. This is an area that can be improved in future studies, especially with the advent of increasing numbers of technically facile assays for monitoring oxidative stress in tissue lysates.

Likewise, there is abundant evidence that oxidative stress occurs in human neurodegenerative disease; however, there is little experimental data from human studies to discern whether this exacerbation of oxidative stress is contributive to the severity of the disease, because human studies necessarily must be observational in nature. Nonetheless, it would appear that many animal models do recapitulate the oxidative stress component of their corresponding human disease counterpart.

If the antioxidant theory is valid, then the implementation of antioxidant strategies must be flawed. There is abundant reason to suspect this is the case. Academic studies generally are performed in such a way as to bias in favor of a treatment effect, by administering large concentrations of test agent early in disease or before experimental disease or injury occurs. This may be appropriate in early studies to prove a concept, but such studies do not in any way mimic the human clinical situation. In order for a preclinical study to engender confidence that a test agent might work in a human clinical situation, the test agent would need to impart benefit to the non-human model at dosages and times that might be achieved safely and practically in a human. Of course accomplishing such objective would be very difficult as it would require routine pharmacokinetic assessments of drug disposition in the animal model, and ideally in comparison with known human pharmacological parameters. This level of detail may not be practical in academic studies that are limited by time and money that can be applied to a project; and by lack of interest amongst academic scientific reviewers in such tedious pharmacological details. Nonetheless, animal studies will continue to fail in their prediction of human clinical efficacy unless more attention is devoted to “humanizing” the animal research. Additionally, the identification and extrapolation of the key biomarkers from animal research to humans is critical and crucial to the early diagnosis and clinical success of the antioxidant therapeutics.

Finally the oxidative stress and antioxidant research community needs to attend to the meaning of the term “antioxidant”. The term is applied loosely to mean any agent that decreases oxidant concentration by either scavenging oxidants catalytically (e.g., the metalloporphyrins) or stoichiometrically (as in the case of nitrone-based free radical traps). In reality, however, many substances can be antioxidant in vitro under conditions that are not relevant in vivo. More to the point, many antioxidants actually contain inherent pharmacological activity by virtue of binding to and reacting with specific receptors or enzyme targets. For instance, curcumin and nordihydroguaiaretic acid both act as chain-breaking antioxidants in vitro but also bind and antagonize cyclooxygenase and lipoxygenase, at nM concentrations. The pharmacological activity of such compounds may result in the diminution of oxidant production and hence be a very potent and true “antioxidant” mode of action in vivo; however, studies of such multifunctional compounds should consider the extent to which benefits in animal models arise from drug-induced changes in paracrine signaling dynamics, such as circuits driven by eicosanoids and prostacyclins.

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One might argue that the mechanism of action of an antioxidant is not germane to estimating the likelihood that the efficacy of the agent will extrapolate from pre-clinical studies to human clinical trials. On the contrary, an understanding of the mechanism of action of a drug is crucial to the design and implementation of human trials, for example, to avoid undesirable activity of the drug at target sites outside the diseased organ or to avoid dosage at inappropriate times when the target-of-action is temporally pleiotropic (e.g., beneficial early in the course of disease but detrimental later in the disease).

The success of multitudinous preclinical antioxidant studies overwhelmingly obligates the scientific community to continue researching oxidative stress with long-term goals of manipulating oxidative stress processes for clinical benefit. Despite the recent clinical disappointments of antioxidant therapies, sound rationale remains to develop improved antioxidant pharmacophores or formulations for the prophylaxis and mitigation of human neurodegenerative disease.

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**References**


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Table 1

Summary of the various antioxidants studied for amyotrophic lateral sclerosis

<table>
<thead>
<tr>
<th>Disease</th>
<th>Test agent</th>
<th>Primary endpoint</th>
<th>Result/Outcome</th>
<th>Model</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS</td>
<td>Vitamin E, Synthetic porphyrins</td>
<td>Motor neuron architecture, 3-NT and malondialdehyde in spinal cord</td>
<td>Therapeutic benefit with improved survival</td>
<td>Mouse</td>
<td>37</td>
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<td></td>
<td>AEOL 10150, riluzole</td>
<td>Onset, progression of disease and overall lifespan</td>
<td>Delayed disease onset and progression and did not prolong survival</td>
<td>Mouse</td>
<td>59</td>
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<td>NDGA</td>
<td>Nitrite release, gliosis</td>
<td></td>
<td>Improved life-span and motor dysfunction</td>
<td>Mouse</td>
<td>164</td>
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<tr>
<td>Celecoxib</td>
<td>PGE-2 release, rate of change in upper extremity motor function</td>
<td>Mouse studies: Inhibition of PGE-2 production in the spinal cord with prolonged survival. Human studies: No improvements in motor dysfunction, muscle strength and no adverse effects</td>
<td>Mouse</td>
<td>39,43</td>
<td></td>
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<tr>
<td>Single or mixture of antioxidants</td>
<td>Meta-analysis from nine studies: Post-12 month treatment survival</td>
<td>Lack of any significant beneficial effects for antioxidants used alone or in combination</td>
<td>Human</td>
<td>109</td>
<td></td>
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</table>

3-NT: 3-nitrotyrosine; NDGA: nordihydroguaiaretic acid; PGE-2: prostaglandin E-2.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Test agent</th>
<th>Primary endpoint</th>
<th>Result/Outcome</th>
<th>Model</th>
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<tr>
<td>AD</td>
<td>SAM</td>
<td>GST inhibition, presenilin-1 expression</td>
<td>Improvement in neuropathological features</td>
<td>Mouse</td>
<td>152</td>
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<tr>
<td></td>
<td>Apple juice concentrate</td>
<td>PS-1 expression in ApoE−/− mice</td>
<td>Improved neuroprotection via inhibition of PS-1 expression</td>
<td>Mouse</td>
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<td></td>
<td>Curcumin</td>
<td>Binding to Aβ species and brain oxidative damage and plaque formation</td>
<td>Facilitates disaggregation of Aβ and reduction in AD associated neuropathology</td>
<td>Mouse</td>
<td>88,167</td>
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<td></td>
<td>Vitamin E and C</td>
<td>Behavioral performance, lipid peroxidation and glutathione in plasma samples</td>
<td>Decreased TBARS levels and decreased lipid peroxidation susceptibility</td>
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<td>54,155</td>
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<td></td>
<td>Vitamin E alone or with Vitamin C</td>
<td>Honolulu-Asia Aging Study (dementia and cognitive function)</td>
<td>Protection against vascular dementia and not against AD dementia</td>
<td>Human</td>
<td>98</td>
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<tr>
<td></td>
<td></td>
<td>Chicago Health and Aging Project (telephone tests of cognitive function)</td>
<td>Vitamin E, and NOT vitamin C, offered modest cognitive benefits in older women</td>
<td>Human</td>
<td>57</td>
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<tr>
<td></td>
<td></td>
<td>Nurse’s Health Study</td>
<td>Vitamin E, and NOT vitamin C, offered modest cognitive benefits in older women</td>
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<td>Vitamin E alone</td>
<td>Honolulu-Asia Aging Study (dementia)</td>
<td>Failure to lower the AD risk</td>
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<td>Washington Heights Study</td>
<td>Lack of decreased risk of AD by neither dietary, supplemental, nor total intake of vitamin E</td>
<td>Human</td>
<td>92</td>
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<td>Cache County Study</td>
<td>Lack of decreased risk of AD by vitamin E alone</td>
<td>Human</td>
<td>61,143</td>
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<td></td>
<td>Prophylactic protection in young versus aged mice</td>
<td>Decreased amyloid deposition and lipid peroxidation in only in mice receiving vitamin E at younger ages and not in later ages</td>
<td>Mouse</td>
<td>150</td>
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</table>

SAM: S-adenosyl methionine; GST: Glutathione-s-transferase; PS-1: presenilin-1; TBARS: thioarbituric acid reactive substances; CoQ10: coenzyme Q10.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Test agent</th>
<th>Primary endpoint</th>
<th>Result/Outcome</th>
<th>Model</th>
<th>Ref</th>
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<tr>
<td>PD</td>
<td>Vitamins E and C, β-carotene, NAC</td>
<td>Striatal dopamine content and loss of dopaminergic neurons</td>
<td>Protection against MPTP-neurotoxicity and prevention of neuronal loss</td>
<td>Mouse</td>
<td>116</td>
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<tr>
<td>PD</td>
<td>Vitamin E, β-carotene, ascorbate, sodium selenite, γ-tocopherol, AEOL11207, Cu-Zn SOD, GPx</td>
<td>MPTP-induced toxicity</td>
<td>Lack of protection against neurotoxicity in dopaminergic nigrostriatal neurons</td>
<td>Mice Marmoset</td>
<td>55, 97, 117</td>
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<tr>
<td>PD</td>
<td>Vitamin E</td>
<td>Protection against PD in drosophila</td>
<td>Inhibition of PD-associated ommatidial degeneration</td>
<td>Drosophila</td>
<td>157</td>
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<tr>
<td>PD</td>
<td>Vitamin E (dietary), multivitamin supplement</td>
<td>Risk of PD in men and women</td>
<td>Lowered risk of PD only for high dose of dietary vitamin E</td>
<td>Human</td>
<td>170</td>
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<td>PD</td>
<td>Deprenyl and vitamin E</td>
<td>Risk of PD</td>
<td>Lack of any anti-PD benefits</td>
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<tr>
<td>PD</td>
<td>Vitamin E, CoQ10, glutathione</td>
<td>Meta-analysis of 8 clinical trials for risk of PD</td>
<td>Minor protection only by CoQ10 via improved mitochondrial function</td>
<td>Human</td>
<td>161</td>
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</table>

NAC: N-acetyl cysteine; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; GPX: glutathione peroxidases; CoQ10: coenzyme Q10.
### Table 4
Summary of the various antioxidants studied for Huntington’s disease

<table>
<thead>
<tr>
<th>Disease</th>
<th>Test agent</th>
<th>Primary endpoint</th>
<th>Result/Outcome</th>
<th>Model</th>
<th>Ref</th>
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<tr>
<td>HD</td>
<td>Vitamin E, β-carotene, Vitamin C</td>
<td>Striatal quinolinic acid toxicity, striatal lesions</td>
<td>Lack of anti-quinolineate protection</td>
<td>Rat</td>
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<tr>
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<td>Thiol, lipoic acid, S-PBN</td>
<td>Total life-span in mouse model of HD</td>
<td>Improved survival only by thiol and lipoic acid</td>
<td>Mouse</td>
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<tr>
<td></td>
<td>Vitamin E with CoQ10</td>
<td>3-NP induced toxicity; activity of creatine kinase and functions of mitochondrial respiratory chain</td>
<td>Partial protection against 3-NP toxicity</td>
<td>Rat</td>
<td>75</td>
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<td></td>
<td>Epigallocatechin-gallate</td>
<td>mHtt cytotoxicity, aggregation of mutant htt exon 1 protein</td>
<td>Protection against mHtt toxicity and motor function decline</td>
<td>Yeast Mouse</td>
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<tr>
<td></td>
<td>Vitamin E (high doses)</td>
<td>HD-associated neurologic and neuropsychological symptoms</td>
<td>Partial beneficial effects against HD-associated motor decline</td>
<td>Human</td>
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<td>CoQ10</td>
<td>Risk of early HD</td>
<td>Lack of slowing of functional decline</td>
<td>Human</td>
<td>5</td>
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</tbody>
</table>

S-PBN: 2-sulpho-tert-phenylbutyrimitone; 3-NP: 3-nitropropionic acid; mHtt: mutant polyglutamine (polyQ)-containing protein huntingtin.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Test agent</th>
<th>Primary endpoint</th>
<th>Result/Outcome</th>
<th>Model</th>
<th>Ref</th>
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</thead>
<tbody>
<tr>
<td>Stroke/IRI</td>
<td>NXY-059 (Cerovive), Tirilazoid</td>
<td>Post-stroke Neurological and functional outcome</td>
<td>Failure as a neuroprotectant, lack of functional outcome</td>
<td>Human</td>
<td>2,83,94</td>
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<td>NXY-059 (Cerovive)</td>
<td>Disability at 90-days</td>
<td>Improvement only in the primary outcome and not in neurological outcome</td>
<td>Human</td>
<td>83,141</td>
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<td>Ebselen</td>
<td>MCA occlusion</td>
<td>Modest neuroprotection when given as a prophylactic dose</td>
<td>Rat</td>
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<td>Edaravone</td>
<td>Post-stroke functional outcome based on modified Rankin scale</td>
<td>Significant improvements in the functional outcome</td>
<td>Human</td>
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<td>Resveratrol</td>
<td>Inhibition of focal ischemia by MCA occlusion</td>
<td>Prevention of oxidative damage and motor impairment</td>
<td>Rat</td>
<td>94,143</td>
</tr>
</tbody>
</table>

MCA: middle cerebral artery.