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

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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

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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, αT) 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 neurodegenerationis 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 α-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 α- or γ-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′-diethylimidazolium-2-yl]porphyrin) [37] and the phenolic antioxidant nordihydroguairetic 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 life-style 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 life style 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

as late-life, dementia. The Washington Heights Study studied connection between intake of vitamins E and C, carotenes 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 ED_{50} [effective dose] found in animal models

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

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 trails 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-butylnitrone (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]. Ehrnhoefer 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 nitrone 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, sulfinated PBN and azulenyl 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, edarovone 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.

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

1. The Parkinson Study Group. Effects of tocopherol and deprenyl on the progression of disability in early Parkinson's disease. N Engl J Med 1993;328:176–183. [PubMed: 8417384]
2. The RANTTAS Investigators. A randomized trial of tirilazad mesylate in patients with acute stroke (RANTTAS). Stroke 1996;27:1453–1458. [PubMed: 8784112]
3. Effect of a novel free radical scavenger, edaravone (MCI-186) on acute brain infarction. Randomized, placebo-controlled, double-blind study at multicenters. Cerebrovasc Dis 2003;15:222–229. [PubMed: 12715790]
4. ClinicalTrials.gov, Accessed June 12, 2008.
5. A randomized, placebo-controlled trial of coenzyme Q10 and remacemide in Huntington's disease. Neurology 2001;57:397–404. [PubMed: 11502903]
6. Adair JC, Knoefel JE, Morgan N. Controlled trial of N-acetylcysteine for patients with probable Alzheimer's disease. Neurology 2001;23:1515–1517. [PubMed: 11673605]
7. Adams JD Jr, Chang ML, Klaidman L. Parkinson's disease – redox mechanisms. Curr Med Chem 2001;8:809–814. [PubMed: 11375751]
8. Adams JD Jr, Klaidman LK, Odunze IN, Shen HC, Miller CA. Alzheimer's and Parkinson's disease. Brain levels of glutathione, glutathione disulfide, and vitamin E. Mol Chem Neuropathol 1991;14:213–226. [PubMed: 1958264]
9. Alexandrova ML, Bochev PG, Markova VI, Bechev BG, Popova MA, Danovska MP, Simeonova VK. Oxidative stress in the chronic phase after stroke. Redox Rep 2003;8:169–176. [PubMed: 12935315]
10. Andreassen OA, Ferrante RJ, Dedeoglu A, Beal MF. Lipoic acid improves survival in transgenic mouse models of Huntington's disease. Neuroreport 2001;12:3371–3373. [PubMed: 11711888]
11. Andrus PK, Fleck TJ, Gurney ME, Hall ED. Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. J Neurochem 1998;71:2041–2048. [PubMed: 9798929]
12. Arendash GW, Schleif W, Rezai-Zadeh K, Jackson EK, Zacharia LC, Cracchiolo JR, Shippy D, Tan J. Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain beta-amyloid production. Neuroscience 2006;142:941–952. [PubMed: 16938404]
13. Bader-Lange ML, Cenini G, Piroddi M, Abdul HM, Sultana R, Galli F, Memo M, Butterfield DA. Loss of phospholipid asymmetry and elevated brain apoptotic protein levels in subjects with amnestic mild cognitive impairment and Alzheimer disease. Neurobiol Dis 2008;29:456–464. [PubMed: 18077176]

14. Balazs L, Leon M. Evidence of an oxidative challenge in the Alzheimer's brain. Neurochem Res 1994;19:1131–1137. [PubMed: 7824065]
15. Bartus RT, Dean RL III, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science 1982;217:408–414. [PubMed: 7046051]
16. Beal MF. Mitochondrial dysfunction and oxidative damage in Alzheimer's and Parkinson's diseases and coenzyme Q10 as a potential treatment. J Bioenerg Biomembr 2004;36:381–386. [PubMed: 15377876]
17. Beal MF, Ferrante RJ. Experimental therapeutics in transgenic mouse models of Huntington's disease. Nat Rev Neurosci 2004;5:373–384. [PubMed: 15100720]
18. Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KJ, Martin JB. Replication of the neurochemical characteristics of Huntington's disease by quinolinic acid. Nature 1986;321:168–171. [PubMed: 2422561]
19. Beal MF, Kowall NW, Swartz KJ, Ferrante RJ, Martin JB. Systemic approaches to modifying quinolinic acid striatal lesions in rats. J Neurosci 1988;8:3901–3908. [PubMed: 2461437]
20. Behl C, Davis JB, Lesley R, Schubert D. Hydrogen peroxide mediates amyloid beta protein toxicity. Cell 1994;77:817–827. [PubMed: 8004671]
21. Benatar M. Lost in translation: treatment trials in the SOD1 mouse and in human ALS. Neurobiol Dis 2007;26:1–13. [PubMed: 17300945]
22. Betarbet R, Sherer TB, Greenamyre JT. Animal models of Parkinson's disease. Bioessays 2002;24:308–318. [PubMed: 11948617]
23. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. Nat Neurosci 2000;3:1301–1306. [PubMed: 11100151]
24. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 2007;297:842–857. [PubMed: 17327526]
25. Brouillet E, Jacquard C, Bizat N, Blum D. 3-Nitropropionic acid: a mitochondrial toxin to uncover physiopathological mechanisms underlying striatal degeneration in Huntington's disease. J Neurochem 2005;95:1521–1540. [PubMed: 16300642]
26. Browne SE, Beal MF. Oxidative damage in Huntington's disease pathogenesis. Antioxid Redox Signal 2006;8:2061–2073. [PubMed: 17034350]
27. Butterfield DA, Martin L, Carney JM, Hensley K. A beta (25–35) peptide displays H2O2-like reactivity towards aqueous Fe2+, nitroxide spin probes, and synaptosomal membrane proteins. Life Sci 1996;58:217–228. [PubMed: 9499162]
28. Carmichael ST. Rodent models of focal stroke: size, mechanism, and purpose. Neuro Rx 2005;2:396–409. [PubMed: 16389304]
29. Cash AD, Perry G, Smith MA. Therapeutic potential in Alzheimer disease. Curr Med Chem 2002;9:1605–1610. [PubMed: 12171555]
30. Chan A, Shea TB. Supplementation with apple juice attenuates presenilin-1 overexpression during dietary and genetically-induced oxidative stress. J Alzheimers Dis 2006;10:353–358. [PubMed: 17183144]
31. Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA, Barnham KJ, Volitakis I, Fraser FW, Kim Y, Huang X, Goldstein LE, Moir RD, Lim JT, Beyreuther K, Zheng H, Tanzi RE, Masters CL, Bush AI. Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. Neuron 2001;30:665–676. [PubMed: 11430801]
32. Choi HS, An JJ, Kim SY, Lee SH, Kim DW, Yoo KY, Won MH, Kang TC, Kwon HJ, Kang JH, Cho SW, Kwon OS, Park J, Eum WS, Choi SY. PEP-1-SOD fusion protein efficiently protects against paraquat-induced dopaminergic neuron damage in a Parkinson disease mouse model. Free Radic Biol Med 2006;41:1058–1068. [PubMed: 16962931]
33. Choi JY, Park CS, Kim DJ, Cho MH, Jin BK, Pie JE, Chung WG. Prevention of nitric oxide-mediated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease in mice by tea phenolic epigallocatechin 3-gallate. Neurotoxicology 2002;23:367–374. [PubMed: 12387363]

34. Clement AM, Nguyen MD, Roberts EA, Garcia ML, Boillee S, Rule M, McMahon AP, Doucette W, Siwek D, Ferrante RJ, Brown RH Jr, Julien JP, Goldstein LS, Cleveland DW. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. Science 2003;302:113–117. [PubMed: 14526083]
35. Cole GM, Morihara T, Lim GP, Yang F, Begum A, Frautschy SA. NSAID and antioxidant prevention of Alzheimer's disease: lessons from in vitro and animal models. Ann N Y Acad Sci 2004;1035:68–84. [PubMed: 15681801]
36. Crack PJ, Taylor JM, Flentjar NJ, de Haan J, Hertzog P, Iannello RC, Kola I. Increased infarct size and exacerbated apoptosis in the glutathione peroxidase-1 (Gpx-1) knockout mouse brain in response to ischemia/reperfusion injury. J Neurochem 2001;78:1389–1399. [PubMed: 11579147]
37. Crow JP, Calingasan NY, Chen J, Hill JL, Beal MF. Manganese porphyrin given at symptom onset markedly extends survival of ALS mice. Ann Neurol 2005;58:258–265. [PubMed: 16049935]
38. Cudkowicz ME, Kenna-Yasek D, Sapp PE, Chin W, Geller B, Hayden DL, Schoenfeld DA, Hosler BA, Horvitz HR, Brown RH. Epidemiology of mutations in superoxide dismutase in amyotrophic lateral sclerosis. Ann Neurol 1997;41:210–221. [PubMed: 9029070]
39. Cudkowicz ME, Shefner JM, Schoenfeld DA, Zhang H, Andreasson KI, Rothstein JD, Drachman DB. Trial of celecoxib in amyotrophic lateral sclerosis. Ann Neurol 2006;60:22–31. [PubMed: 16802291]
40. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003;39:889–909. [PubMed: 12971891]
41. Davis RE, Miller S, Herrnstadt C, Ghosh SS, Fahy E, Shinobu LA, Galasko D, Thal LJ, Beal MF, Howell N, Parker WD Jr. Mutations in mitochondrial cytochrome c oxidase genes segregate with late-onset Alzheimer disease. Proc Natl Acad Sci USA 1997;94:4526–4531. [PubMed: 9114023]
42. Dexter DT, Ward RJ, Wells FR, Daniel SE, Lees AJ, Peters TJ, Jenner P, Marsden CD. Alpha-tocopherol levels in brain are not altered in Parkinson's disease. Ann Neurol 1992;32:591–593. [PubMed: 1456747]
43. Drachman DB, Frank K, Dykes-Hoberg M, Teismann P, Almer G, Przedborski S, Rothstein JD. Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. Ann Neurol 2002;52:771–778. [PubMed: 12447931]
44. Drake J, Link CD, Butterfield DA. Oxidative stress precedes fibrillar deposition of Alzheimer's disease amyloid beta-peptide (1–42) in a transgenic Caenorhabditis elegans model. Neurobiol Aging 2003;24:415–420. [PubMed: 12600717]
45. Ehrnhoefer DE, Duennwald M, Markovic P, Wacker JL, Engemann S, Roark M, Legleiter J, Marsh JL, Thompson LM, Lindquist S, Muchowski PJ, Wanker EE. Green tea (−)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. Hum Mol Genet 2006;15:2743–2751. [PubMed: 16893904]
46. Eum WS, Kim DW, Hwang IK, Yoo KY, Kang TC, Jang SH, Choi HS, Choi SH, Kim YH, Kim SY, Kwon HY, Kang JH, Kwon OS, Cho SW, Lee KS, Park J, Won MH, Choi SY. In vivo protein transduction: biologically active intact pep-1-superoxide dismutase fusion protein efficiently protects against ischemic insult. Free Radic Biol Med 2004;37:1656–1669. [PubMed: 15477017]
47. Feng Z, Qin C, Chang Y, Zhang JT. Early melatonin supplementation alleviates oxidative stress in a transgenic mouse model of Alzheimer's disease. Free Radic Biol Med 2006;40:101–109. [PubMed: 16337883]
48. Ferger B, Teismann P, Earl CD, Kuschinsky K, Oertel WH. The protective effects of PBN against MPTP toxicity are independent of hydroxyl radical trapping. Pharmacol Biochem Behav 2000;65:425–431. [PubMed: 10683482]
49. Fernagut PO, Chesselet MF. Alpha-synuclein and transgenic mouse models. Neurobiol Dis 2004;17:123–130. [PubMed: 15474350]
50. Fernandez-Vizarra P, Fernandez AP, Castro-Blanco S, Encinas JM, Serrano J, Bentura ML, Munoz P, Martinez-Murillo R, Rodrigo J. Expression of nitric oxide system in clinically evaluated cases of Alzheimer's disease. Neurobiol Dis 2004;15:287–305. [PubMed: 15006699]
51. Ferrante RJ, Kowall NW, Cipolloni PB, Storey E, Beal MF. Excitotoxin lesions in primates as a model for Huntington's disease: histopathologic and neurochemical characterization. Exp Neurol 1993;119:46–71. [PubMed: 8432351]

52. Feuerstein GZ, Zaleska MM, Krams M, Wang X, Day M, Rutkowski JL, Finklestein SP, Pangalos MN, Poole M, Stiles GL, Ruffolo RR, Walsh FL. Missing steps in the STAIR case: a Translational Medicine perspective on the development of NXY-059 for treatment of acute ischemic stroke. J Cereb Blood Flow Metab 2008;28:217–219. [PubMed: 17579658]
53. Francis PT, Palmer AM, Snape M, Wilcock GK. The cholinergic hypothesis of Alzheimer's disease: a review of progress. J Neurol Neurosurg Psychiatry 1999;66:137–147. [PubMed: 10071091]
54. Galbusera C, Facheris M, Magni F, Galimberti G, Sala G, Tremolada L, Isella V, Guerini FR, Appollonio I, Galli-Kienle M, Ferrarese C. Increased susceptibility to plasma lipid peroxidation in Alzheimer disease patients. Curr Alzheimer Res 2004;1:103–109. [PubMed: 15975074]
55. Gong L, Daigneault EA, Acuff RV, Kostrzewa RM. Vitamin E supplements fail to protect mice from acute MPTP neurotoxicity. Neuroreport 1991;2:544–546. [PubMed: 1751810]
56. Green AR, Ashwood T. Free radical trapping as a therapeutic approach to neuroprotection in stroke: experimental and clinical studies with NXY-059 and free radical scavengers. Curr Drug Targets CNS Neurol Disord 2005;4:109–118. [PubMed: 15857295]
57. Grodstein F, Chen J, Willett WC. High-dose antioxidant supplements and cognitive function in community-dwelling elderly women. Am J Clin Nutr 2003;77:975–984. [PubMed: 12663300]
58. Gryta T. Neuroprotectant therapy faces uphill battle after failure. Dow Jones Newswires. Nov 16;2006
59. Gurney ME, Cutting FB, Zhai P, Doble A, Taylor CP, Andrus PK, Hall ED. Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. Ann Neurol 1996;39:147–157. [PubMed: 8967745]
60. Hald A, Lotharius J. Oxidative stress and inflammation in Parkinson's disease: is there a causal link? Exp Neurol 2005;193:279–290. [PubMed: 15869932]
61. Hayden KM, Welsh-Bohmer KA, Wengreen HJ, Zandi PP, Lyketsos CG, Breitner JC. Risk of mortality with vitamin E supplements: the Cache County study. Am J Med 2007;120:180–184. [PubMed: 17275460]
62. Hensley K, Abdel-Moaty H, Hunter J, Mhatre M, Mou S, Nguyen K, Potapova T, Pye QN, Qi M, Rice H, Stewart C, Stroukoff K, West M. Primary glia expressing the G93A-SOD1 mutation present a neuroinflammatory phenotype and provide a cellular system for studies of glial inflammation. J Neuroinflammation 2006;3:2. [PubMed: 16436205]
63. Hensley K, Benaksas EJ, Bolli R, Comp P, Grammas P, Hamdheydari L, Mou S, Pye QN, Stoddard MF, Wallis G, Williamson KS, West M, Wechter WJ, Floyd RA. New perspectives on vitamin E: gamma-tocopherol and carboxyelthylhydroxychroman metabolites in biology and medicine. Free Radic Biol Med 2004;36:1–15. [PubMed: 14732286]
64. Hensley, K.; Carney, JM.; Stewart, CA.; Tabatabaie, T.; Pye, Q.; Floyd, RA. Nitrone-based free radical traps as neuroprotective agents in cerebral ischaemia and other pathologies. In: Green, RC.; Cross, AJ., editors. Neuroprotective Agents and Cerebral Ischaemia. Academic Press Limited; San Diego, CA: 1997. p. 299-317.
65. Hensley K, Floyd RA, Gordon B, Mou S, Pye QN, Stewart C, West M, Williamson K. Temporal patterns of cytokine and apoptosis-related gene expression in spinal cords of the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. J Neurochem 2002;82:365–374. [PubMed: 12124437]
66. Hensley K, Hall N, Subramaniam R, Cole P, Harris M, Aksenov M, Aksenova M, Gabbita SP, Wu JF, Carney JM, Lovell M, Markesbery WR, Butterfield DA. Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. J Neurochem 1995;65:2146–2156. [PubMed: 7595501]
67. Hensley K, Maidt ML, Yu Z, Sang H, Markesbery WR, Floyd RA. Electrochemical analysis of protein nitrotyrosine and dityrosine in the Alzheimer brain indicates region-specific accumulation. J Neurosci 1998;18:8126–8132. [PubMed: 9763459]
68. Hensley K, Mhatre M, Mou S, Pye QN, Stewart C, West M, Williamson KS. On the relation of oxidative stress to neuroinflammation: lessons learned from the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. Antioxid Redox Signal 2006;8:2075–2087. [PubMed: 17034351]
69. Hsu CH, Cheng AL. Clinical studies with curcumin. Adv Exp Med Biol 2007;595:471–480. [PubMed: 17569225]

70. Iravani MM, Kashefi K, Mander P, Rose S, Jenner P. Involvement of inducible nitric oxide synthase in inflammation-induced dopaminergic neurodegeneration. Neuroscience 2002;110:49–58. [PubMed: 11882372]
71. Itoh N, Masuo Y, Yoshida Y, Cynshi O, Jishage K, Niki E. gamma-Tocopherol attenuates MPTP-induced dopamine loss more efficiently than alpha-tocopherol in mouse brain. Neurosci Lett 2006;403:136–140. [PubMed: 16716512]
72. Jantzen PT, Connor KE, DiCarlo G, Wenk GL, Wallace JL, Rojiani AM, Coppola D, Morgan D, Gordon MN. Microglial activation and beta-amyloid deposit reduction caused by a nitric oxide-releasing nonsteroidal anti-inflammatory drug in amyloid precursor protein plus presenilin-1 transgenic mice. J Neurosci 2002;22:2246–2254. [PubMed: 11896164]
73. Jenner P. Oxidative stress in Parkinson's disease. Ann Neurol 2003;53(Suppl 3):S26–S36. [PubMed: 12666096]
74. Kamii H, Mikawa S, Murakami K, Kinouchi H, Yoshimoto T, Reola L, Carlson E, Epstein CJ, Chan PH. Effects of nitric oxide synthase inhibition on brain infarction in SOD-1-transgenic mice following transient focal cerebral ischemia. J Cereb Blood Flow Metab 1996;16:1153–1157. [PubMed: 8898687]
75. Kasparova S, Sumbalova Z, Bystricky P, Kucharska J, Liptaj T, Mlynarik V, Gvozdjakova A. Effect of coenzyme Q10 and vitamin E on brain energy metabolism in the animal model of Huntington's disease. Neurochem Int 2006;48:93–99. [PubMed: 16290265]
76. Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, Markesbery WR. Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurology 2005;64:1152–1156. [PubMed: 15824339]
77. Kinouchi H, Epstein CJ, Mizui T, Carlson E, Chen SF, Chan PH. Attenuation of focal cerebral ischemic injury in transgenic mice overexpressing CuZn superoxide dismutase. Proc Natl Acad Sci USA 1991;88:11158–11162. [PubMed: 1763030]
78. Kutala VK, Khan M, Angelos MG, Kuppusamy P. Role of oxygen in postischemic myocardial injury. Antioxid Redox Signal 2007;9:1193–1206. [PubMed: 17571958]
79. Langston JW, Ballard P, Tetrud JW, Irwin I. Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 1983;219:979–980. [PubMed: 6823561]
80. Laurin D, Masaki KH, Foley DJ, White LR, Launer LJ. Midlife dietary intake of antioxidants and risk of late-life incident dementia: the Honolulu-Asia Aging Study. Am J Epidemiol 2004;159:959–967. [PubMed: 15128608]
81. Lazarov O, Robinson J, Tang YP, Hairston IS, Korade-Mirnics Z, Lee VM, Hersh LB, Sapolsky RM, Mirnics K, Sisodia SS. Environmental enrichment reduces Abeta levels and amyloid deposition in transgenic mice. Cell 2005;120:701–713. [PubMed: 15766532]
82. Leavitt BR, van Raamsdonk JM, Shehadeh J, Fernandes H, Murphy Z, Graham RK, Wellington CL, Raymond LA, Hayden MR. Wild-type huntingtin protects neurons from excitotoxicity. J Neurochem 2006;96:1121–1129. [PubMed: 16417581]
83. Lees KR, Zivin JA, Ashwood T, Davalos A, Davis SM, Diener HC, Grotta J, Lyden P, Shuaib A, Hardemark HG, Wasiewski WW. NXY-059 for acute ischemic stroke. N Engl J Med 2006;354:588–600. [PubMed: 16467546]
84. Lehmann J, Martin HL, Lashley EL, Marshall MW, Judd JT. Vitamin E in foods from high and low linoleic acid diets. J Am Diet Assoc 1986;86:1208–1216. [PubMed: 3745745]
85. Leuner K, Hauptmann S, Abdel-Kader R, Scherping I, Keil U, Strosznajder JB, Eckert A, Muller WE. Mitochondrial dysfunction: the first domino in brain aging and Alzheimer's disease? Antioxid Redox Signal 2007;9:1659–1675. [PubMed: 17867931]
86. Li JY, Popovic N, Brundin P. The use of the R6 transgenic mouse models of Huntington's disease in attempts to develop novel therapeutic strategies. NeuroRx 2005;2:447–464. [PubMed: 16389308]
87. Liang LP, Huang J, Fulton R, Day BJ, Patel M. An orally active catalytic metalloporphyrin protects against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity in vivo. J Neurosci 2007;27:4326–4333. [PubMed: 17442816]
88. Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 2001;21:8370–8377. [PubMed: 11606625]

89. Lino MM, Schneider C, Caroni P. Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. J Neurosci 2002;22:4825–4832. [PubMed: 12077179]

90. Liu D, Bao F, Wen J, Liu J. Mutation of superoxide dismutase elevates reactive species: comparison of nitration and oxidation of proteins in different brain regions of transgenic mice with amyotrophic lateral sclerosis. Neuroscience 2007;146:255–264. [PubMed: 17368952]

91. Lonn E, Bosch J, Yusuf S, Sheridan P, Pogue J, Arnold JM, Ross C, Arnold A, Sleight P, Probstfield J, Dagenais GR. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. JAMA 2005;293:1338–1347. [PubMed: 15769967]

92. Luchsinger JA, Tang MX, Shea S, Mayeux R. Antioxidant vitamin intake and risk of Alzheimer disease. Arch Neurol 2003;60:203–208. [PubMed: 12580704]

93. Luth HJ, Munch G, Arendt T. Aberrant expression of NOS isoforms in Alzheimer's disease is structurally related to nitrotyrosine formation. Brain Res 2002;953:135–143. [PubMed: 12384247]

94. Margaill I, Plotkine M, Lerouet D. Antioxidant strategies in the treatment of stroke. Free Radic Biol Med 2005;39:429–443. [PubMed: 16043015]

95. Mark RJ, Hensley K, Butterfield DA, Mattson MP. Amyloid beta-peptide impairsion-motive ATPase activities: evidence for a role in loss of neuronal Ca2+ homeostasis and cell death. J Neurosci 1995;15:6239–6249. [PubMed: 7666206]

96. Markesbery WR, Lovell MA. Damage to lipids, proteins, DNA, and RNA in mild cognitive impairment. Arch Neurol 2007;64:954–956. [PubMed: 17620484]

97. Martinovits G, Melamed E, Cohen O, Rosenthal J, Uzzan A. Systemic administration of antioxidants does not protect mice against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP). Neurosci Lett 1986;69:192–197. [PubMed: 3489911]

98. Masaki KH, Losonczy KG, Izmirlian G, Foley DJ, Ross GW, Petrovitch H, Havlik R, White LR. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. Neurology 2000;54:1265–1272. [PubMed: 10746596]

99. Masliah E, Alford M, DeTeresa R, Mallory M, Hansen L. Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. Ann Neurol 1996;40:759–766. [PubMed: 8957017]

100. Mecocci P, MacGarvey U, Beal MF. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann Neurol 1994;36:747–751. [PubMed: 7979220]

101. Meda L, Cassatella MA, Szendrei GI, Otvos L Jr, Baron P, Villalba M, Ferrari D, Rossi F. Activation of microglial cells by beta-amyloid protein and interferon-gamma. Nature 1995;374:647–650. [PubMed: 7715705]

102. Menalled LB, Sison JD, Dragatsis I, Zeitlin S, Chesselet MF. Time course of early motor and neuropathological anomalies in a knock-in mouse model of Huntington's disease with 140 CAG repeats. J Comp Neurol 2003;465:11–26. [PubMed: 12926013]

103. Miller ER III, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. Ann Intern Med 2005;142:37–46. [PubMed: 15537682]

104. Molina JA, de Bustos F, Jimenez-Jimenez FJ, Benito-Leon J, Orti-Pareja M, Gasalla T, Tallon-Barranco A, Navarro JA, Arenas J, Enriquezde-Salamanca R. Cerebrospinal fluid levels of alpha-tocopherol (vitamin E) in Parkinson's disease. J Neural Transm 1997;104:1287–1293. [PubMed: 9503274]

105. Murray IV, Liu L, Komatsu H, Uryu K, Xiao G, Lawson JA, Axelsen PH. Membrane-mediated amyloidogenesis and the promotion of oxidative lipid damage by amyloid beta proteins. J Biol Chem 2007;282:9335–9345. [PubMed: 17255094]

106. Nabika T, Cui Z, Masuda J. The stroke-prone spontaneously hypertensive rat: how good is it as a model for cerebrovascular diseases? Cell Mol Neurobiol 2004;24:639–646. [PubMed: 15485135]

107. Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, Przedborski S. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. Nat Neurosci 2007;10:615–622. [PubMed: 17435755]

108. Nunomura A, Castellani RJ, Zhu X, Moreira PI, Perry G, Smith MA. Involvement of oxidative stress in Alzheimer disease. J Neuropathol Exp Neurol 2006;65:631–641. [PubMed: 16825950]

109. Orrell RW. Amyotrophic lateral sclerosis: copper/zinc superoxide dismutase (SOD1) gene mutations. Neuromuscul Disord 2000;10:63–68. [PubMed: 10677867]
110. Orrell RW, Lane RJ, Ross M. Antioxidant treatment for amyotrophic lateral sclerosis/motor neuron disease. Cochrane Database Syst Rev 2007:CD002829. [PubMed: 17253482]
111. Panov A, Dikalov S, Shalbuyeva N, Taylor G, Sherer T, Greenamyre JT. Rotenone model of Parkinson disease: multiple brain mitochondria dysfunctions after short term systemic rotenone intoxication. J Biol Chem 2005;280:42026–42035. [PubMed: 16243845]
112. Patel NV, Gordon MN, Connor KE, Good RA, Engelman RW, Mason J, Morgan DG, Morgan TE, Finch CE. Caloric restriction attenuates Abeta-deposition in Alzheimer transgenic models. Neurobiol Aging 2005;26:995–1000. [PubMed: 15748777]
113. Peng J, Peng L, Stevenson FF, Doctrow SR, Andersen JK. Iron and paraquat as synergistic environmental risk factors in sporadic Parkinson's disease accelerate age-related neurodegeneration. J Neurosci 2007;27:6914–6922. [PubMed: 17596439]
114. Peng J, Stevenson FF, Doctrow SR, Andersen JK. Superoxide dismutase/catalase mimetics are neuroprotective against selective paraquat-mediated dopaminergic neuron death in the substantial nigra: implications for Parkinson disease. J Biol Chem 2005;280:29194–29198. [PubMed: 15946937]
115. Perier C, Tieu K, Guegan C, Caspersen C, Jackson-Lewis V, Carelli V, Martinuzzi A, Hirano M, Przedborski S, Vila M. Complex I deficiency primes Bax-dependent neuronal apoptosis through mitochondrial oxidative damage. Proc Natl Acad Sci USA 2005;102:19126–19131. [PubMed: 16365298]
116. Perry TL, Yong VW, Clavier RM, Jones K, Wright JM, Foulks JG, Wall RA. Partial protection from the dopaminergic neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by four different antioxidants in the mouse. Neurosci Lett 1985;60:109–114. [PubMed: 3877260]
117. Perry TL, Yong VW, Hansen S, Jones K, Bergeron C, Foulks JG, Wright JM. Alpha-tocopherol and beta-carotene do not protect marmosets against the dopaminergic neurotoxicity of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. J Neurol Sci 1987;81:321–331. [PubMed: 3121800]
118. Petersen RC, Thomas RG, Grundman M, Bennett D, Doody R, Ferris S, Galasko D, Jin S, Kaye J, Levey A, Pfeiffer E, Sano M, van Dyck CH, Thal LJ. Vitamin E and donepezil for the treatment of mild cognitive impairment. N Engl J Med 2005;352:2379–2388. [PubMed: 15829527]
119. Peyser CE, Folstein M, Chase GA, Starkstein S, Brandt J, Cockrell JR, Bylsma F, Coyle JT, McHugh PR, Folstein SE. Trial of d-alpha-tocopherol in Huntington's disease. Am J Psychiatry 1995;152:1771–1775. [PubMed: 8526244]
120. Pham DQ, Plakogiannis R. Vitamin E supplementation in Alzheimer's disease, Parkinson's disease, tardive dyskinesia, and cataract: Part 2. Ann Pharmacother 2005;39:2065–2072. [PubMed: 16288072]
121. Pramatarova A, Laganiere J, Roussel J, Brisebois K, Rouleau GA. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. J Neurosci 2001;21:3369–3374. [PubMed: 11331366]
122. Pratico D, Uryu K, Leight S, Trojanoswki JQ, Lee VM. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. J Neurosci 2001;21:4183–4187. [PubMed: 11404403]
123. Premkumar DR, Smith MA, Richey PL, Petersen RB, Castellani R, Kutty RK, Wiggert B, Perry G, Kalaria RN. Induction of heme oxygenase-1 mRNA and protein in neocortex and cerebral vessels in Alzheimer's disease. J Neurochem 1995;65:1399–1402. [PubMed: 7543935]
124. Quinn JF, Bussiere JR, Hammond RS, Montine TJ, Henson E, Jones RE, Stackman RW Jr. Chronic dietary alpha-lipoic acid reduces deficits in hippocampal memory of aged Tg2576 mice. Neurobiol Aging 2007;28:213–225. [PubMed: 16448723]
125. Rao CV. Regulation of COX and LOX by curcumin. Adv Exp Med Biol 2007;595:213–226. [PubMed: 17569213]
126. Ren YR, Nishida Y, Yoshimi K, Yasuda T, Jishage K, Uchihara T, Yokota T, Mizuno Y, Mochizuki H. Genetic vitamin E deficiency does not affect MPTP susceptibility in the mouse brain. J Neurochem 2006;98:1810–1816. [PubMed: 16787402]

127. Rezai-Zadeh K, Shytle D, Sun N, Mori T, Hou H, Jeanniton D, Ehrhart J, Townsend K, Zeng J, Morgan D, Hardy J, Town T, Tan J. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. J Neurosci 2005;25:8807–8814. [PubMed: 16177050]
128. Ridet JL, Bensadoun JC, Deglon N, Aebischer P, Zurn AD. Lentivirus-mediated expression of glutathione peroxidase: neuroprotection in murine models of Parkinson's disease. Neurobiol Dis 2006;21:29–34. [PubMed: 16023352]
129. Roitberg BZ, Emborg ME, Sramek JG, Palfi S, Kordower JH. Behavioral and morphological comparison of two nonhuman primate models of Huntington's disease. Neurosurgery 2002;50:137–145. [PubMed: 11844244]
130. Rosler M, Anand R, Cicin-Sain A, Gauthier S, Agid Y, Dal-Bianco P, Stahelin HB, Hartman R, Gharabawi M. Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. BMJ 1999;318:633–638. [PubMed: 10066203]
131. Sano M, Ernesto C, Thomas RG, Klauber MR, Schafer K, Grundman M, Woodbury P, Growdon J, Cotman CW, Pfeiffer E, Schneider LS, Thal LJ. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. N Engl J Med 1997;336:1216–1222. [PubMed: 9110909]
132. Savitz SI, Fisher M. Future of neuroprotection for acute stroke: in the aftermath of the SAINT trials. Ann Neurol 2007;61:396–402. [PubMed: 17420989]
133. Sayre LM, Zelasko DA, Harris PL, Perry G, Salomon RG, Smith MA. 4-Hydroxynonenal-derived advanced lipid peroxidation end products are increased in Alzheimer's disease. J Neurochem 1997;68:2092–2097. [PubMed: 9109537]
134. Scarpini E, Scheltens P, Feldman H. Treatment of Alzheimer's disease: current status and new perspectives. Lancet Neurol 2003;2:539–547. [PubMed: 12941576]
135. Schaller B. Prospects for the future: the role of free radicals in the treatment of stroke. Free Radic Biol Med 2005;38:411–425. [PubMed: 15649644]
136. Schipper HM, Bennett DA, Liberman A, Bienias JL, Schneider JA, Kelly J, Arvanitakis Z. Glial heme oxygenase-1 expression in Alzheimer disease and mild cognitive impairment. Neurobiol Aging 2006;27:252–261. [PubMed: 16399210]
137. Schuessel K, Schafer S, Bayer TA, Czech C, Pradier L, Muller-Spahn F, Muller WE, Eckert A. Impaired Cu/Zn- SOD activity contributes to increased oxidative damage in APP transgenic mice. Neurobiol Dis 2005;18:89–99. [PubMed: 15649699]
138. Schulz JB, Henshaw DR, Matthews RT, Beal MF. Coenzyme Q10 and nicotinamide and a free radical spin trap protect against MPTP neurotoxicity. Exp Neurol 1995;132:279–283. [PubMed: 7789466]
139. Shibata N, Nagai R, Uchida K, Horiuchi S, Yamada S, Hirano A, Kawaguchi M, Yamamoto T, Sasaki S, Kobayashi M. Morphological evidence for lipid peroxidation and protein glycoxidation in spinal cords from sporadic amyotrophic lateral sclerosis patients. Brain Res 2001;917:97–104. [PubMed: 11602233]
140. Shoulson I. DATATOP: a decade of neuroprotective inquiry. Parkinson Study Group. Deprenyl And Tocopherol Antioxidative Therapy Of Parkinsonism. Ann Neurol 1998;44:S160–S166. [PubMed: 9749589]
141. Shuaib A, Lees KR, Lyden P, Grotta J, Davalos A, Davis SM, Diener HC, Ashwood T, Wasiewski WW, Emeribe U. NXY-059 for the treatment of acute ischemic stroke. N Engl J Med 2007;357:562–571. [PubMed: 17687131]
142. Simola N, Morelli M, Carta AR. The 6-hydroxydopamine model of Parkinson's disease. Neurotox Res 2007;11:151–167. [PubMed: 17449457]
143. Sinha K, Chaudhary G, Gupta YK. Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. Life Sci 2002;71:655–665. [PubMed: 12072154]
144. Smith CD, Carney JM, Starke-Reed PE, Oliver CN, Stadtman ER, Floyd RA, Markesbery WR. Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease. Proc Natl Acad Sci USA 1991;88:10540–10543. [PubMed: 1683703]
145. Smith CD, Carney JM, Tatsumo T, Stadtman ER, Floyd RA, Markesbery WR. Protein oxidation in aging brain. Ann N Y Acad Sci 1992;663:110–119. [PubMed: 1362341]

146. Smith KM, Matson S, Matson WR, Cormier K, Del Signore SJ, Hagerty SW, Stack EC, Ryu H, Ferrante RJ. Dose ranging and efficacy study of high-dose coenzyme Q10 formulations in Huntington's disease mice. Biochim Biophys Acta 2006;1762:616–626. [PubMed: 16647250]

147. Sofic E, Sapcanin A, Tahirovic I, Gavrankapetanovic I, Jellinger K, Reynolds GP, Tatschner T, Riederer P. Antioxidant capacity in postmortem brain tissues of Parkinson's and Alzheimer's diseases. J Neural Transm Suppl 2006:39–43. [PubMed: 17447414]

148. Sonkusare SK, Kaul CL, Ramarao P. Dementia of Alzheimer's disease and other neurodegenerative disorders –memantine, a new hope. Pharmacol Res 2005;51:1–17. [PubMed: 15519530]

149. Sultana R, Perluigi M, Butterfield DA. Protein oxidation and lipid peroxidation in brain of subjects with Alzheimer's disease: insights into mechanism of neurode-generation from redox proteomics. Antioxid Redox Signal 2006;8:2021–2037. [PubMed: 17034347]

150. Sung S, Yao Y, Uryu K, Yang H, Lee VM, Trojanowski JQ, Pratico D. Early vitamin E supplementation in young but not aged mice reduces Abeta levels and amyloid deposition in a transgenic model of Alzheimer's disease. FASEB J 2004;18:323–325. [PubMed: 14656990]

151. Tariot PN, Solomon PR, Morris JC, Kershaw P, Lilienfeld S, Ding C. A 5-month, randomized, placebo-controlled trial of galantamine in AD. The Galantamine USA-10 Study Group. Neurology 2000;54:2269–2276. [PubMed: 10881251]

152. Tchantchou F, Graves M, Ortiz D, Chan A, Rogers E, Shea TB. S-adenosyl methionine: A connection between nutritional and genetic risk factors for neurodegeneration in Alzheimer's disease. J Nutr Health Aging 2006;10:541–544. [PubMed: 17183426]

153. Thiruchelvam M, Prokopenko O, Cory-Slechta DA, Richfield EK, Buckley B, Mirochnitchenko O. Overexpression of superoxide dismutase or glutathione peroxidase protects against the paraquat + maneb-induced Parkinson disease phenotype. J Biol Chem 2005;280:22530–22539. [PubMed: 15824117]

154. van Raamsdonk JM, Warby SC, Hayden MR. Selective degeneration in YAC mouse models of Huntington disease. Brain Res Bull 2007;72:124–131. [PubMed: 17352936]

155. Veinbergs I, Mallory M, Sagara Y, Masliah E. Vitamin E supplementation prevents spatial learning deficits and dendritic alterations in aged apolipoprotein E-deficient mice. Eur J Neurosci 2000;12:4541–4546. [PubMed: 11122365]

156. Walker FO. Huntington's disease. Lancet 2007;369:218–228. [PubMed: 17240289]

157. Wang D, Qian L, Xiong H, Liu J, Neckameyer WS, Oldham S, Xia K, Wang J, Bodmer R, Zhang Z. Antioxidants protect PINK1-dependent dopaminergic neurons in Drosophila. Proc Natl Acad Sci USA 2006;103:13520–13525. [PubMed: 16938835]

158. Wang J, Ho L, Qin W, Rocher AB, Seror I, Humala N, Maniar K, Dolios G, Wang R, Hof PR, Pasinetti GM. Caloric restriction attenuates beta-amyloid neuropathology in a mouse model of Alzheimer's disease. FASEB J 2005;19:659–661. [PubMed: 15650008]

159. Wang J, Ho L, Zhao Z, Seror I, Humala N, Dickstein DL, Thiyagarajan M, Percival SS, Talcott ST, Pasinetti GM. Moderate consumption of Cabernet Sauvignon attenuates Abeta neuropathology in a mouse model of Alzheimer's disease. FASEB J 2006;20:2313–2320. [PubMed: 17077308]

160. Wang J, Xiong S, Xie C, Markesbery WR, Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. J Neurochem 2005;93:953–962. [PubMed: 15857398]

161. Weber CA, Ernst ME. Antioxidants, supplements, and Parkinson's disease. Ann Pharmacother 2006;40:935–938. [PubMed: 16622156]

162. Weisbrot-Lefkowitz M, Reuhl K, Perry B, Chan PH, Inouye M, Mirochnitchenko O. Overexpression of human glutathione peroxidase protects transgenic mice against focal cerebral ischemia/reperfusion damage. Brain Res Mol Brain Res 1998;53:333–338. [PubMed: 9473716]

163. Weissman L, Jo DG, Sorensen MM, de Souza-Pinto NC, Markesbery WR, Mattson MP, Bohr VA. Defective DNA base excision repair in brain from individuals with Alzheimer's disease and amnestic mild cognitive impairment. Nucleic Acids Res 2007;35:5545–5555. [PubMed: 17704129]

164. West M, Mhatre M, Ceballos A, Floyd RA, Grammas P, Gabbita SP, Hamdheydari L, Mai T, Mou S, Pye QN, Stewart C, West S, Williamson KS, Zemlan F, Hensley K. The arachidonic acid 5-lipoxygenase inhibitor nordihydroguaiaretic acid inhibits tumor necrosis factor alpha activation of microglia and extends survival of G93A-SOD1 transgenic mice. J Neurochem 2004;91:133–143. [PubMed: 15379894]

165. Williamson KS, Gabbita SP, Mou S, West M, Pye QN, Markesbery WR, Cooney RV, Grammas P, Reimann-Philipp U, Floyd RA, Hensley K. The nitration product 5-nitro-gamma-tocopherol is increased in the Alzheimer brain. Nitric Oxide 2002;6:221–227. [PubMed: 11890747]

166. Winblad B, Engedal K, Soininen H, Verhey F, Waldemar G, Wimo A, Wetterholm AL, Zhang R, Haglund A, Subbiah P. A 1-year, randomized, placebo-controlled study of donepezil in patients with mild to moderate AD. Neurology 2001;57:489–495. [PubMed: 11502918]

167. Yang F, Lim GP, Begum AN, Ubeda OJ, Simmons MR, Ambegaokar SS, Chen PP, Kayed R, Glabe CG, Frautschy SA, Cole GM. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. J Biol Chem 2005;280:5892–5901. [PubMed: 15590663]

168. Yang G, Chan PH, Chen J, Carlson E, Chen SF, Weinstein P, Epstein CJ, Kamii H. Human copper-zinc superoxide dismutase transgenic mice are highly resistant to reperfusion injury after focal cerebral ischemia. Stroke 1994;25:165–170. [PubMed: 8266365]

169. Zandi PP, Anthony JC, Khachaturian AS, Stone SV, Gustafson D, Tschanz JT, Norton MC, Welsh-Bohmer KA, Breitner JC. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the Cache County Study. Arch Neurol 2004;61:82–88. [PubMed: 14732624]

170. Zhang SM, Hernan MA, Chen H, Spiegelman D, Willett WC, Ascherio A. Intakes of vitamins E and C, carotenoids, vitamin supplements, and PD risk. Neurology 2002;59:1161–1169. [PubMed: 12391343]

Table 1

Summary of the various antioxidants studied for amyotrophic lateral sclerosis

Disease	Test agent	Primary endpoint	Result/Outcome	Model	Ref
ALS	Vitamin E, Synthetic porphyrins	Motor neuron architecture, 3-NT and malondialdehyde in spinal cord	Therapeutic benefit with improved survival	Mouse	37
	AEOL 10150, riluzole	Onset, progression of disease and overall lifespan	Delayed disease onset and progression and did not prolong survival	Mouse	59
	NDGA	Nitrite release, gliosis	Improved life-span and motor dysfunction	Mouse	164
	Celecoxib	PGE-2 release, rate of change in upper extremity motor function	*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	Mouse Human	39,43
	Single or mixture of antioxidants	Meta-analysis from nine studies: Post-12 month treatment survival	Lack of any significant beneficial effects for antioxidants used alone or in combination	Human	109

3-NT: 3-nitrotyrosine; NDGA: nordihydroguairetic acid; PGE-2: prostaglandin E-2.

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Table 2

Summary of the various antioxidants studied for Alzheimer's disease

Disease	Test agent	Primary endpoint	Result/Outcome	Model	Ref
AD	SAM	GST inhibition, presenilin-1 expression	Improvement in neuropathological features	Mouse	152
	Apple juice concentrate	PS-1 expression in ApoE−/− mice	Improved neuroprotection via inhibition of PS-1 expression	Mouse	30
	Curcumin	Binding to Aβ species and brain oxidative damage and plaque formation	Facilitates disaggregation of Aβ and reduction in AD associated neuropathology	Mouse Human	88,167
	Vitamin E and C	Behavioral performance, lipid peroxidation and glutathione in plasma samples	Decreased TBARS levels and decreased lipid peroxidation susceptibility	Mouse Human	54,155
	Vitamin E alone or with Vitamin C	Honolulu-Asia Aging Study (dementia and cognitive function)	Protection against vascular dementia and not against AD dementia	Human	98
		Chicago Health and Aging Project (telephone tests of cognitive function)	Vitamin E, and NOT vitamin C, offered modest cognitive benefits in older women	Human	57
		Nurse's Health Study	Vitamin E, and NOT vitamin C, offered modest cognitive benefits in older women	Human	80,118
	Vitamin E alone	Honolulu-Asia Aging Study (dementia)	Failure to lower the AD risk	Human	80,118
		Washington Heights Study	Lack of decreased risk of AD by neither dietary, supplemental, nor total intake of vitamin E	Human	92
		Cache County Study	Lack of decreased risk of AD by vitamin E alone	Human	61,143
		Prophylactic protection in young versus aged mice	Decreased amyloid deposition and lipid peroxidation in only in mice receiving vitamin E at younger ages and not in later ages	Mouse	150

SAM: S-adenosyl methionine; GST: Glutathione-s-transferase; PS-1: presenilin-1; TBARS: thiobarbituric acid reactive substances; CoQ10: coenzyme Q10.

Table 3

Summary of the various antioxidants studied for Parkinson's disease

Disease	Test agent	Primary endpoint	Result/Outcome	Model	Ref
PD	Vitamins E and C, β-carotene, NAC	Striatal dopamine content and loss of dopaminergic neurons	Protection against MPTP-neurotoxicity and prevention of neuronal loss	Mouse	116
	Vitamin E, β-carotene, ascorbate, sodium selenite	MPTP-induced toxicity	Lack of protection against neurotoxicity in dopaminergic nigrostriatal neurons	Mice Marmoset	55,97,117
	γ-tocopherol, AEOL11207, Cu-Zn SOD, GPx	MPTP- and paraquat-induced neurotoxicity	Protection against dopaminergic toxicity and decreased lipid peroxides in the midbrain and striatum	Mouse	71,153
	Vitamin E	Protection against PD in drosophila	Inhibition of PD-associated ommatidial degeneration	Drosophila	157
	Vitamin E (dietary), multivitamin supplement	Risk of PD in men and women	Lowered risk of PD only for high dose of dietary vitamin E	Human	170
	Deprenyl and vitamin E	Risk of PD	Lack of any anti-PD benefits	Human	140
	Vitamin E, CoQ10, glutathione	Meta-analysis of 8 clinical trials for risk of PD	Minor protection only by CoQ10 via improved mitochondrial function	Human	161

NAC: N-acetyl cysteine; MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; GPX: glutathione peroxidases; CoQ10: coenzyme Q10.

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Table 4

Summary of the various antioxidants studied for Huntington's disease

Disease	Test agent	Primary endpoint	Result/Outcome	Model	Ref
HD	Vitamin E, β-carotene, Vitamin C	Striatal quinolinic acid toxicity, striatal lesions	Lack of anti-quinolineate protection	Rat	19
	Thiol, lipoic acid, S-PBN	Total life-span in mouse model of HD	Improved survival only by thiol and lipoic acid	Mouse	10
	Vitamin E with CoQ10	3-NP induced toxicity; activity of creatine kinase and functions of mitochondrial respiratory chain	Partial protection against 3-NP toxicity	Rat	75
	Epigallocatechin-gallate	mHtt cytotoxicity, aggregation of mutant htt exon 1 protein	Protection against mHtt toxicity and motor function decline	Yeast Mouse	45
	Vitamin E (high doses)	HD-associated neurologic and neuropsychological symptoms	Partial beneficial effects against HD-associated motor decline	Human	119
	CoQ10	Risk of early HD	Lack of slowing of functional decline	Human	5

S-PBN: 2-sulpho-tert-phenyibutyinitrone; 3-NP: 3-nitropropionic acid; mhtt: mutant polyglutamine (polyQ)-containing protein huntingtin.

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Table 5

Summary of the various antioxidants studied for stroke/IRI

Disease	Test agent	Primary endpoint	Result/Outcome	Model	Ref
Stroke/IRI	NXY-059 (Cerovive), Tirilazoid	Post-stroke Neurological and functional outcome	Failure as a neuroprotectant, lack of functional outcome	Human	2,83,94
	NXY-059 (Cerovive)	Disability at 90-days	Improvement only in the primary outcome and not in neurological outcome	Human	83,141
	Ebselen	MCA occlusion	Modest neuroprotection when given as a prophylactic dose	Rat	56
	Edaravone	Post-stroke functional outcome based on modified Rankin scale	Significant improvements in the functional outcome	Human	3
	Resveratrol	Inhibition of focal ischemia by MCA occlusion	Prevention of oxidative damage and motor impairment	Rat	94,143

MCA: middle cerebral artery.

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