Published in final edited form as:

Mini Rev Med Chem. 2008 November ; 8(13): 1395–1406.

Antioxidant Therapy in Alzheimer's Disease: Theory and Practice

Gjumrakch Aliev^{1,3}, Mark E. Obrenovich⁴, V. Prakash Reddy⁷, Justin C. Shenk^{1,3}, Paula I. Moreira⁵, Akihiko Nunomura⁶, Xiongwei Zhu⁴, Mark A. Smith⁴, and George Perry^{2,*,#}

¹ Department of Biology, University of Texas at San Antonio, San Antonio, TX 78249, USA

² College of Sciences, University of Texas at San Antonio, San Antonio, TX 78249, USA

³ Electron Microscopy Research Center, University of Texas at San Antonio, San Antonio, TX 78249, USA

⁴ Department of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA

⁵ Center for Neuroscience and Cell Biology of Coimbra, University of Coimbra, Coimbra, Portugal

⁶ Department of Psychiatry and Neurology, Asahikawa Medical College, Asahikawa, Japan

⁷ Department of Chemistry, Missouri University of Science and Technology, Rolla, MO 65409, USA

Abstract

Alzheimer disease treatment has yet to yield a successful therapy that addresses the source of the damage found in brains. Of the varied proposed theories of AD etiology, reactive oxygen species (ROS) generation is cited as a common factor. Efforts to reduce the pathology associated with ROS *via* antioxidants therefore offer new hope to patients suffering from this devastating disease.

Keywords

Antioxidant; calorie restriction; estrogen; free radical; glutathione; oxidation; oxidative stress; therapy

GENERAL OVERVIEW OF ALZHEIMER DISEASE

Alzheimer disease (AD) is the leading cause of dementia. The burden of AD increases with aging of the population: an estimated 5 million people in the US suffer with over \$70 billion in expenses annually [1]. A century since its first classification by Alois Alzheimer, research has yielded neither a cure nor an effective prevention strategy. Current clinical therapies consist of cholinesterase inhibitors/NMDA receptor antagonists such as Reminyl, galantamine, Aricept (donepezil hydrochloride), Exelon (rivastigmine) and Memantine (Namenda), which offer little more than short-term palliative effects.

* Address correspondence to this author at the College of Sciences, University of Texas at San Antonio, One UTSA Circle, San Antonio, Texas, 78249, USA; Tel: +1 210 458 4450; Fax: +1 210 458 4445; george.perry@utsa.edu.

Author's Profile: George Perry is currently the Dean of the College of Sciences at The University of Texas at San Antonio. Perry's studies are focused on the mechanism of formation and physiological consequences of the cytopathology of Alzheimer disease. Dr. Perry's group has shown that oxidative damage is the initial cytopathological abnormality in Alzheimer disease.

AD is manifested as a distinguished impairment of thought, memory, and language abilities. Research over the past 20 years has yielded much information about the factors involved in the pathogenesis, but a cause has not yet been elucidated. Several major theories of AD, such as amyloid-beta ($A\beta$) toxicity [2], tauopathy [3], inflammation [4,5], oxidative stress [6–11], have all been represented broadly and argued intensely in the literature.

CLINICAL FACTS OF ALZHEIMER DISEASE

The risk of AD varies from 12% to 19% for women over the age of 65 years and 6% to 10% for men [12] and rises exponentially with age, such that up to 47% of individuals over the age of 80 develop AD [13]. On average, AD patients live about 8 years after initial diagnosis, although the disease can last for as long as 20 years. The areas of the brain that control memory and thinking skills are affected first but, as the disease progresses, neurons in other regions of the brain are also affected. Eventually, the patient with AD will need complete care, adding further emotional, physical, and financial costs to the family.

ALZHEIMER DISEASE - PATHOLOGY

Since its early classification, two distinctive hallmark lesions found in the brain of patients with AD are senile plaques and neurofibrillary tangles (NFTs) (reviewed elsewhere [14]), the latter were identified by the use of silver-staining techniques [15]. In addition, other associated markers include neuronal and dendritic loss, neuropil threads, dystrophic neurites, granulovacuolar degeneration, Hirano bodies, cerebrovascular amyloid, and atrophy of the brain [14].

Extracellular senile plaque formation remains a hallmark of AD. Bundles of $A\beta$, 10–200 μm in diameter, form the central core of the plaques and are surrounded by $A\beta$, amyloid- β protein precursor ($A\beta\text{PP}$), tau, and neurofilament proteins are reported [16].

NFTs are recognized as the major intracellular protein aggregation in AD brains, and are located primarily in the cerebral cortex, especially in the large pyramidal neurons in the hippocampal and frontotemporal regions [17]. Tau, a microtubule-associated protein found predominantly on axons, is the major component of the bundled paired helical filaments (PHF) that compose NFTs. Moreover, neurofilament proteins are also reported in NFTs [16,18]. In PHF, tau is abnormally hyperphosphorylated [3,19,20], ubiquitinated [21–23], oxidized [6,24–26], truncated [27] and aggregated into filaments [3,28,29]. The hyperphosphorylation of tau is thought to render it unable to bind to microtubules and therefore unable to promote or maintain microtubule assembly [30], although *in vivo*, while microtubules are disrupted, this has no relation to NFT [31]. The resistance to proteolytic degradation of hyperphosphorylated tau may play a key role in neurofibrillary degeneration in AD patients [32,33].

While the pathological hallmarks provide a baseline for current diagnostic standards, their role as initiators versus byproducts of disease is hotly contested [34–36].

GENETIC ETIOLOGY OF ALZHEIMER DISEASE

Only about 5% of all AD cases have an early onset and are related to genetic mutations of *presenilin 1*, *presenilin 2* [37] or the *AβPP* genes. Indeed, the majority, approximately 95%, of all AD patients are sporadic, while the late-onset cases, where the major risk factors are aging and *apolipoprotein E4 (ApoE4)* polymorphisms [38,39]. While APOE4 allele is not a determinant for AD, since a large number of individuals with APOE4 alleles never develop frank AD, in both familial and sporadic cases of disease there is accumulating evidence indicating a major role for free radicals and oxidative stress in disease pathogenesis and pathophysiology [9,10].

The greatest correlated factor for the development of AD are polymorphisms of the *ApoE* gene, such that 50% of patients with AD patients have at least one *ApoE4* allele [38,39]. Further, although familial AD only accounts for a small percentage of AD cases, many have argued that mutations in *AβPP* and *presenilin 1* and *2* are critical genetic factors in AD pathogenesis and in Aβ production [40,41].

Apolipoprotein E Gene Polymorphism

Polymorphisms of the *ApoE* gene are found to correlate with onset and risk of developing AD, such that 50% of AD patients have at least one *ApoE4* allele [38,39]. ApoE is an abundant 34-kDa glycoprotein that is synthesized and secreted mainly by astrocytes and microglia in the central nervous system (CNS). It is well established that *ApoE*, and especially the *E4* allele of *ApoE*, is a major genetic risk factor of the more common, late onset form of AD [42,43]. The influence of *ApoE* genotype on AD seems to operate *via* multiple mechanisms. For example, polymorphisms are a determinant of brain Aβ burden in individuals affected with AD [44,45]. Additionally, apolipoproteins have been suggested to act as antioxidants, with the *ApoE4* allele being less effective in this role [46] so that increased oxidative damage is found in specific brain regions of AD patients with the *ApoE E4* genotype [47].

Amyloid-β Protein Precursor

Aβ protein is the major component of senile plaque cores and is derived from the precursor protein, AβPP. AβPP is encoded on chromosome 21 (21q11–22) [48,49]. The normal function of AβPP is unknown, but it is involved in several broad physiological functions in neurons. Mutations in AβPP appear to change AβPP processing and while initially this was thought to lead to increases in Aβ, thus increasing the extracellular protein aggregation [50,51], more recent reports actually show decreases in Aβ [52]. Transgenic mice that

overexpress mutant A β PP show overproduction of A β protein, senile plaque formation and synaptic deficits without NFTs pathology, indicating a key pathological role for mutant A β PP protein [53,54]. The current data finds that A β PP mutation only accounts for a very small percentage of AD cases, 0.1–0.15% of total AD cases.

Presenilins 1 and 2

The majority (~70%) of early-onset familial AD cases are associated with mutations in two genes, presenilin 1 and presenilin 2, located on chromosomes 14 and 1, respectively [55]. Over 80 different pathogenic mutations in *presenilin 1* gene and 9 mutations in *presenilin 2* gene have been described [56]. Presenilin 1 and 2 are remarkably homologous transmembrane proteins of 463 and 448 amino acids respectively, with six and nine hydrophobic membrane-spanning domains. The physiological functions of these two proteins are unknown but may be involved in the Notch receptor pathway [57]. Other possible roles include ion channel, protein processing, or cellular trafficking functions [58]. In AD, it is thought that mutations in these proteins are associated with AD by affecting the processing of A β PP [59].

NON-GENETIC ETIOLOGY OF ALZHEIMER DISEASE

Age

Age is the single greatest risk factor for AD, and the disease rarely occurs in people under 60 years. Thereafter, AD affects 10–15% of individuals over 65 years old and up to 47% of individuals over the age of 80 [13]. This predominance of age as a major cause in AD etiology indicates that age-related events are closely involved in the development of the disease. While the processes of aging that are involved in AD pathogenesis are not fully understood, two likely candidates are altered cholinergic function and oxidative stress. Decrease in cholinergic neurons with age and disease [60] is the basis for therapy for three drugs currently on the market that stabilize acetylcholine levels in neurons. Oxidative stress factors in aging are discussed below.

Tauopathy

Hyperphosphorylation of tau makes it more resistant to proteolytic degradation, which may play a key role in neurofibrillary degeneration in AD patients [32,33]. Tau aggregation was, until quite recently, viewed as being deleterious. However, more recent evidence indicates it is a consequence of neurodegeneration. In fact, tau aggregation may be an adaptive change for the neurons to absorb oxidative stress [18,35,61,62]. Consistent with this notion, tau phosphorylation and aggregation and NFT epitopes have been shown experimentally to be consequences of both oxidative stress and post-translational oxidation of tau [26,28,63–66].

Other Risk Factors

- Vascular risk factors, including hyperlipidemia, hypertension, diabetes, and related factors of heart disease or stroke have been identified as putative antecedents to AD [67].
- With similar cardiovascular pathology, smokers have a 2–4 fold increase in risk of AD, particularly those individuals without an ApoE4 allele [68,69].
- Traumatic head injury is associated with increased risk of AD [70].
- Adults with Down syndrome develop the neuropathological changes of AD by age 40, but not all patients become demented. The risk of AD in families with a history of Down syndrome is increased 2–3 fold [71].

- Several studies show that the risk of AD among poorly educated individuals or individuals in poor living condition is significantly higher than that among well-educated persons [72,73].

Preventative Factors

- AD was found to be less frequent among postmenopausal women who used estrogen replacement therapy [74,75]. Women using hormone replacement had about a 50% reduction in disease risk with benefit only to those taking estrogen in the peri-menopausal period. While the exact mechanism for this is unclear, recent evidence points to the feedback effects of estrogen on luteinizing hormone [76–83]. While the strength in estrogen's apparent ability to reduce AD risk is interpreted from retrospective studies, to be discussed in greater detail below, nevertheless this underlies the importance of further studies and non-retrospective data to explore mechanisms of estrogen protection against AD.
- AD was also found to be less frequent among individuals who used anti-inflammatory drugs [84].
- Individuals who drink red wine in moderate amounts daily are less likely to develop AD than either heavier drinkers or abstainers [67]. The risk reduction with alcohol is possibly related to its anti-inflammatory and anti-oxidant properties or its effects on lipid metabolism by components such as resveratrol [85].

OXIDATIVE STRESS IN ALZHEIMER DISEASE

A leading theory of the cause of aging [86], free radical damage and oxidative stress are also thought to play a major role in the pathogenesis of AD. Oxidative stress is a potential source of damage to DNA, lipids, sugars and proteins within cells; any imbalance between the intracellular production of free radicals/reactive oxygen species (ROS) and antioxidant defense mechanisms results in oxidative stress [6,9,10]. Since neurons have an age-related decrease in the capacity to compensate for redox imbalance, even minor cellular stresses have the ability to lead to irreversible injury and, as such, contribute to the pathogenesis of neurodegenerative diseases. ROS are the primary mediators of oxidative injury and damage to lipids, sugars, DNA/RNA and amino acid side-chains [11,87]. Markers for oxidative damage (carbonyls, HNE, MDA and more) may increase in neurodegenerative diseases and aging, but their use as quantifiable markers for disease condition is not clear.

Free Radical Production

A molecule carrying an unpaired electron, which makes it extremely reactive and ready to acquire an electron in any way possible, is termed a free radical. In the process of acquiring an electron, the free radical will attach itself to another molecule, thereby modifying it biochemically [88]. However, as free radicals acquire an electron from the other molecules, they either convert these molecules into other free radicals, or break down or alter their chemical structure. Thus, free radicals are capable of damaging virtually any biomolecule, including proteins, sugars, fatty acids and nucleic acids [89]. Free radical damage to long-lived bio-molecules such as collagen, elastin, DNA, polysaccharides, lipids that make up the membranes of cells and organelles, blood vessel walls and lipofuscins is thought of as a major contributor to cell death [90].

The most common free radicals include superoxide, hydroxyl, alkoxyl, peroxy and nitric oxide radical. Other non-free radical molecules, such as singlet oxygen, hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl), are similar but not real free radicals. Together, the free radicals and free radical mimics are called ROS.

Free radicals have extremely short half-lives ranging from nanosecond to seconds. The shortest is only one nanosecond, (10^{-9} sec) for hydroxyl radical, and the longest half-life is 1–10 seconds, for nitric oxide radical [91]. The half-life dictates the intrinsic properties of the damaging effects of the free radicals, whether they can travel far enough to reach other cellular compartments or just attack the neighboring molecules. The further they can travel, the broader the range of molecules and organelles they can damage.

Sources of Free Radicals

There are more than six primary sources of free radicals formed endogenously within living organisms: the primary source of free radicals and oxidants is through the respiratory generation of ATP using oxygen [92–94]; peroxisomal oxidation of fatty acids, which generates H_2O_2 as a byproduct [93,94]; cytochrome P450 enzymes [94]; chronic inflammatory cells, which use a mixture of oxidants to overcome infection by phagocytosis [89,93,94]; the fifth source is from other enzymes capable of generating oxidants under normal or pathological conditions [95]; various biomolecules including thiols, hydroquinones, flavins, catecholamines, pterins and hemoglobin, which may spontaneously auto-oxidize and produce superoxide radicals [89].

Many exogenous sources, such as environmental radiation (sunlight), polluted urban air, cigarette smoke, iron and copper salts, some phenolic compounds found in many plant foods, and various drugs [89,93] could also contribute to free radical production.

Antioxidant Systems

Endogenous Antioxidants—Endogenous defense mechanisms, including enzymatic antioxidant systems and cellular molecules, protect against free radical-induced cellular damage. SOD, catalase, and glutathione peroxidase are three primary enzymes involved in direct elimination of active oxygen species (superoxide radical and H_2O_2). In addition, glutathione reductase, glucose-6-phosphate dehydrogenase, and cytosolic GST are secondary enzymes that function to decrease peroxide levels or to maintain a steady supply of metabolic intermediates like glutathione (GSH) and NADPH for optimum functioning of the primary antioxidant enzymes [96,97].

Many cellular molecules are active antioxidants in the body. For example, GSH, ascorbate (vitamin C), α -tocopherol (vitamin E) β -carotene, NADPH, uric acid, bilirubin, sodium selenite, mannitol, sodium benzoate, the iron-binding protein transferrin, dihydrolipoic acid, melatonin, plasma protein thiol, and reduced CoQ10 are all involved in protecting the body from ROS and their byproducts produced during normal cellular metabolism. Of these, GSH is the most significant component that directly quenches ROS (such as lipid peroxides like hydroxynonenal) and plays a major role in xenobiotic metabolism. Exposure to high levels of xenobiotics causes GSH to be exhausted in the process of xenobiotic neutralization and it is therefore less available to serve as an antioxidant. GSH is also important in maintaining ascorbate (vitamin C) and α -tocopherol (vitamin E) in their reduced form so they may function as antioxidants to quench free radicals [98–100].

Exogenous Antioxidants from the Diet

The most widely studied dietary antioxidants are vitamin C, vitamin E, and β -carotene. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids, as it is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is

initiated. Vitamin E is a major lipid-soluble antioxidant, and is the most effective chain-breaking antioxidant within the cell membrane, where it protects membrane fatty acids from lipid peroxidation. β -carotene and other carotenoids also provide antioxidant protection to lipid rich tissues. Fruits and vegetables are major sources of vitamin C and carotenoids. Whole grains, cereals, and high quality vegetable oils are major sources of vitamin E [101,102].

Vitamin E has been used to prevent lipid peroxidation but tocotrienol may be more effective against peroxy-radical-induced loss of cell viability [103]. Chandan and colleagues used 2,29-azobis [2-amidinopropane] hydrochloride (AAPH)-generated peroxy-radical in a model of glutamate toxicity in order to test whether vitamin E or tocotrienol protected against loss of cell viability due to glutamate toxicity after glutamate challenge in experiments where HT4 neuronal cells pre-treated with glutamate for up to 12 hours and over several concentration ranges [103]. The latter study provided evidence describing the molecular basis of tocotrienol action in protecting against glutamate toxicity at concentrations 4–10-fold lower than levels detected in plasma of supplemented humans [103]. While this may be more relevant for treating amyotrophic lateral sclerosis (ALS) than AD, in the strict sense, nevertheless, neuronal loss may be a multifactorial process in all neurodegenerative diseases and in that regard, this study demonstrated that tocotrienol was more effective than α -tocotrienol acetate in protecting neuronal cells against glutamate-induced death [103].

The Vulnerability of the Nervous System

The nervous system - including the brain, spinal cord, and peripheral nerves - is rich in both unsaturated fatty acids and iron. The double bonds in unsaturated fatty acids make them a vulnerable target for free radicals, and this, coupled with the high aerobic metabolic activity in neurons, makes the nervous system particularly susceptible to oxidative damage. The high levels of iron, while it may be essential, particularly during brain development, facilitates oxidative stress *via* iron-catalyzed formation of ROS [104,105]. In addition, those brain regions that are rich in the catecholamines, adrenaline, noradrenaline and dopamine, are exceptionally vulnerable to free radical generation. Catecholamines can induce free radicals through either spontaneous breakdown (auto-oxidation) or by being metabolized by endogenous enzymes such as monoamine oxidase. One such region of the brain is the substantia nigra, where a condition has been established between antioxidant depletion (including GSH) and tissue degeneration [9].

There is an increase in markers of oxidative stress in major neurodegenerative diseases [6,24,25,106–111] and substantial evidence that oxidative stress is a cause, or at least the initial change, in the pathogenesis of AD [112].

CLINICAL EVIDENCE OF ANTIOXIDANT AMELIORATION

Current Clinical Drugs in Use

Therapy with acetylcholinesterase inhibitors, including drugs such as Reminyl, Aricept and Exelon, which aim to stabilize acetylcholine levels in the synaptic cleft to maintain neurotransmission, is based on the hypothesis that cholinergic dysfunction in the process of aging contributes to the development of AD. Memantine, an NMDA receptor antagonist, blocks glutamate-mediated excitotoxicity. A complete review of drugs currently in clinical usage can be found elsewhere [113–115].

Antioxidant Therapy Development

The stages of free radical production may be arbitrarily divided into 1) conditions prior to their formation, 2) free radical formation and 3) adduction. The different types of antioxidant therapy are based on their intervention at different points in the stages of free radical formation. Summarized below are current strategies for developing antioxidant therapy for AD.

Prevention/Reduction of Free Radicals

Modulation of SOD, Peroxidase and Catalase or Using Their Mimics—

Transgenic mice overexpressing Cu-Zn SOD showed reduced oxidative damage in brain tissue [116] and improved cognitive function in aged rodents [117]. Overexpression of glutathione peroxidase in transgenic mice also showed anti-oxidative function and prevented homocysteine-induced endothelial dysfunction [118]. Moreover, the mimics of SOD and catalase have cytoprotective effects in AD model systems [119] and prolong life span in *C. elegans* [120]. It is reported that SOD, peroxidase and catalase activity is reduced with age and in some pathological conditions [121]. Therapy aimed at compensating for loss of activity of these enzymes is a promising approach to AD therapy.

Iron Chelators—A β PP transgenic mice treated with a Cu/Zn chelator showed improvement in general parameters and a reduction of brain A β deposition [122]. Because copper and zinc play a major role in A β toxicity and nerve cell death *via* ROS generation, chelator therapy is, in effect, antioxidant [123–125]. In one study, 48 presumed AD patients treated with desferrioxamine, a transition metal chelator, (250 mg per day), showed this class of compounds to be effective in preventing AD progression [126]. Recently, desferrioxamine and others, as FDA-preapproved drugs, were shown to limit A β protein secretion in cell culture [127]. More iron chelators are under investigation and show beneficial effect in AD treatment [128].

Caloric Restriction—Studies of caloric restriction in rodents show an attenuation of age-related deficits in learning and memory [129] and dramatically extends the life-span and reduces the incidence of age-related disease in rodents and monkeys [130,131]. The mechanism of the beneficial effect of caloric restriction is not clearly understood but is most likely *via* overall reduction in levels of oxidative stress, including in the brain [132]. In humans, a low daily caloric intake is associated with a reduced risk for AD [133]. In addition, the incidence of AD is lower in countries with low per capita food consumption compared to countries with high per capita food consumption [134,135]. Reducing caloric intake as a preventative measure in populations at high risk for AD could be combined with other AD treatments. The development of chemical mimics for caloric restriction, such as resveratrol and sirtuins, the nicotinamide adenine dinucleotide dependent protein deacetylases [136,137], may make caloric restriction for normal people more easily attainable in the future.

Free Radicals Scavengers—Various compounds have the ability to quench free radicals by reacting with them directly. These compounds include tocopherols (vitamin E) and other monophenols, ascorbate (vitamin C), carotene, flavonoids and other polyphenols, glutathione (GSH/GSSH), retinol and other polyenes, various arylamines and indole derivatives such as melatonin, ebselen and other selenium-containing compounds, mimics of catalase/SOD, etc. The major antioxidants in the group of direct antioxidants are the free-radical trapping chain-breaking antioxidants, such as phenols and carotenes.

Vitamin E has been found in rats to prevent neuronal cell death induced by hypoxia followed by oxygen reperfusion and to prevent neuronal damage from reactive nitrogen species [138]. Both vitamin E and β -carotene, by reducing oxidative stress, protect rat neurons from exposure to ethanol [139]. In an experimental model of diabetes-caused neurovascular dysfunction, β -carotene, followed by vitamins E and C, was found to effectively protect cells [140]. Vitamin E can rescue the neuronal cytotoxicity induced by aluminum in A β PP transgenic mice and reduce A β deposition in the brain by reducing isoprostane levels [141].

A significant amount of research with dietary vitamins E and C has been done in humans. In a multicenter, double blind, placebo-controlled study on 341 patients with moderately severe AD, a daily dose of approximately 1350 mg (2000 IU) vitamin E led to a slight delay of AD progression, providing the first evidence for vitamin E as a prophylaxis and treatment for AD [142]. In keeping with these results, other studies with vitamins C and E in AD patients have shown that antioxidants might have a protective effect against AD [143,144].

In one later study, 815 non-demented individuals were evaluated based on their intake of the antioxidants vitamins C and E and β -carotene. Results showed that there is a significant difference in the incidence of AD between those taking vitamin E and those who are not [145]. In another large-scale study involving 5396 non-demented individuals, it was reported that high intake of vitamins C and E significantly reduced the risk of AD [146]. Similarly, in a 5-year follow-up with 1367 non-demented individuals over 65 years of age in France, Commenges and colleagues [147] found that an intake of flavonoids significantly reduced the risk of dementia.

While promising, many studies have also reported negative results on the effectiveness of vitamin E and vitamin C intake as detailed in other reviews [148].

Estrogen—The general neuroprotective effects of estrogen (17 β -estradiol) have been the subject of much research. Generally, estrogen acts as a trophic factor in the nervous system

by altering gene transcription through interaction with its receptors ER α and ER β [149]. At the same time, in a manner more closely related to direct antioxidants, estrogen can have anti-oxidant activity independent of estrogen receptors. The structure of estrogen is similar to α -tocopherol in that both molecules contain a phenolic radical scavenging moiety and a lipophilic hydrocarbon moiety. In general, phenolic A ring estrogens have been shown to be powerful inhibitors of lipid peroxidation in various cell-free test-tube experiments [150–152]. By modifying the phenolic character of estrogen 17 β -estradiol, its antioxidant activities are lost, supporting the theory that estrogens are direct antioxidants because of their phenolic ring structure [153,154]. Furthermore, this antioxidant activity of estrogens and other phenols is strictly related to the structural prerequisites and not dependent on the interaction of these compounds with cellular estrogen receptors [151]. Meanwhile it has been shown that estrogens are strong antioxidants in different oxidative stress-induced cell degeneration models [153–156].

The optimum concentration of estrogen needed to achieve the antioxidant effect is worth consideration. Normally, estrogen is present in nanomolar concentrations *in vivo* and, in most *in vitro* studies, estrogen's antioxidant effect is achieved at a significantly higher concentration range of 1–10 μ moles. Therefore, it remains to be determined how the antioxidant effect of estrogen can be achieved therapeutically with a safe pharmacologic dose.

Hormone replacement therapy in the form of estrogen plus progestin, administered as a therapeutic agent in a Women's Health Initiative (WHI) clinical trial, was recently shown to increase the risk for probable dementia in post-menopausal women aged 65 years or older [157]. The investigators responsible for this study hypothesize that the negative effect of estrogen and progestin may be linked to the increased risk of stroke that was also reported in the estrogen/progestin treatment group, as the relationship between microinfarcts in the brain and susceptibility to AD is likely related, yet currently not well characterized. While this may indeed partially explain the results of the WHI clinical trial, only when the role of the other hormones of the hypothalamic-pituitary-gonadal axis during the climacteric years and beyond is taken into account that the results can be fully and accurately explained. It is important to recognize that the hormones of the hypothalamic-pituitary-gonadal axis have been in disequilibrium for decades in all of the women who participated in the WHI clinical trial, so if a lack of estrogen does indeed play a role in AD pathogenesis, these women have been exposed to this disease-promoting hormonal environment for years if not decades by the time the estrogen/progestin treatment was administered. This is evidenced by the fact that reports of probable dementia appeared within the first year of the study in both the

treatment and placebo groups. Therefore, it is likely predictable that the administration of estrogen/progestin in these aged women was not only unable to restore the proper functioning of the hypothalamic-pituitary-gonadal axis, but that the influx of exogenous hormones actually served to exacerbate the disease process.

Glutathione—GSH, the most abundant intracellular non-protein thiol, is the main factor which directly quenches free radicals *in vivo*. It has been shown that the level of GSH is decreased in cortical areas and in the hippocampus of patients with AD as compared with controls [158–160]. The level of GSH in red blood cells decreases with aging and in patients with AD [161]. In the healthy cell, oxidized glutathione (GSSG) rarely exceeds 10% of total cellular GSH. Therefore, the ratio of GSH/GSSG can be used as a useful indicator for oxidative status *in vivo* [162]. GSH depletion may be the ultimate factor determining vulnerability to oxidant attack. N-acetyl-cysteine (NAC), a precursor of GSH which has already been approved by the U.S. Food and Drug Administration for treatment of acetaminophen toxicity, may be an effective strategy to increase GSH and spare brain degeneration in AD patients, although this remains to be tested.

N-Acetylcysteine

Other Direct Antioxidants—Compounds such as serotonin (5-hydroxytryptamine), flavonoids, quercetin, and simple alkylphenols have been shown to prevent membrane lipid peroxidation and protect neuronal cells against oxidative cell death *in vitro* [163,164].

2,4,6-trimethylphenol (TMP) is also a potent antioxidant [151]. Additionally, being a small compound, TMP would readily cross the blood-brain barrier, thus meeting the most critical requirement for drugs used in the treatment of neurodegenerative disease. The protective potential of this compound is currently being tested experimentally in various animal models of acute neurodegeneration.

In two large clinical studies, administration of idebenone, a compound structurally similar to ubiquinone, has been reported to significantly reduce disease progression in a dose-dependent fashion [165,166]. Some *in vivo* studies in animals as well as *in vitro* studies have demonstrated a protective effect of idebenone in neuronal death [167]. More recent studies argued the effectiveness of idebenone in AD treatment [168,169], but another study showed that idebenone is better than tacrine in benefit-risk ratio in AD treatment [170]. Whether idebenone acts by modulating mitochondrial metabolic function or directly as a radical scavenger is still an open question. At the same time, while idebenone is mainly used to treat Friedreich ataxia, among other drugs, many compounds with multiple mechanisms of action may also be effective in treating a wide variety of neurodegenerative diseases.

Uric acid, an endogenous antioxidant, was also found to prevent ischemia-induced oxidative neuronal damage in rats [138]. In addition, cannabidiol is more effective than either vitamin C or E in protecting against glutamate neurotoxicity [171]. It has been demonstrated that the antioxidant activity of cannabinoid compounds is, similar to estrogens, exclusively dependent on the presence of a phenolic group and is independent of the cannabinoid receptor [172].

Other aromatic amino and imino compounds (e.g., phenothiazine, phenoxazine, iminostilbene) represent another group of direct antioxidants. Aromatic amines and imines are effective against oxidative glutamate toxicity, GSH depletion, and H_2O_2 toxicity in different cell culture systems [173]. With half-maximal effective concentrations ranging from 20–75 nM, these compounds were experimentally proven to be more effective than common standard phenolic antioxidants. These results provide a structural basis and rationale for the development of new antioxidant drugs [173,174].

Mitigating, Detoxifying or Preventing the Formation of ROS Adducts

There are yet another group of compounds that can detoxify the formed ROS adducts and repair the damage caused. These include, for example, NAC, GSH, 2-oxo-1,3-thiazolidine-4-carboxylate, carnitine, creatine, lipoic acid (thioctic acid), ubiquinone and idebenone.

The compound tenilsetam, a cognition-enhancing drug, is sometimes used to treat AD patients. Its mechanism of action is unclear but, based on *in vitro* and *in vivo* evidence, it is believed to inhibit protein glycation [175,176]. Since generation of advanced glycation endproducts is a major manifestation of oxidative stress in AD [6,108,111] glycation inhibition is thought to be neuroprotective.

Many amino acid residues of proteins are susceptible to oxidation by various forms of ROS. Oxidatively-modified proteins accumulate during aging and in a number of age-related diseases. There is an increase in oxidation of the S-containing amino acids methionine and cysteine in AD patients [177]. However, unlike oxidation of other amino acid residues, the oxidation of these two amino acids can be repaired by corresponding enzymes, methionine sulfoxide reductases (MSR), thioredoxin reductase (TrxR), thioredoxin (Trx) and NADPH. The level of MSR is decreased with aging and in AD and other neurodegenerative diseases [177]. Also mutation in the MSR gene in yeast, neuronal PC-12 cells, human T cells and *Drosophila* has been correlated with increased antioxidant capacity and the prolonged life span of these organisms [178–182].

CONCLUSIONS AND FUTURE APPLICATIONS

It has been well established that oxidative damage of cellular molecules plays an important role in neurodegenerative disorders. Oxidative damage is clearly not simply a byproduct or end product of neuronal degenerative processes but, more likely, is the direct initiation factor in neurodegeneration.

Despite the current wealth of knowledge, there is much skepticism regarding the likelihood of success with antioxidant therapy in AD. The only promising results so far are from the trial of vitamin E therapy in moderately severe AD [142,183]. Most of the antioxidants that are currently known are limited in their ability to cross the blood-brain barrier. Therefore, development or recognition of smaller antioxidant molecules that would more readily pass through the barrier and/or non-toxic or inert compounds that would carry anti-oxidant drugs from the bloodstream into the brain offers much promise. Additionally, it is imperative that future trials use combinations, rather than single antioxidants, to facilitate redox cycling, as well as maximize bioavailability to different cellular compartments.

References

1. Mayeux R. Epidemiology of neurodegeneration. *Annu Rev Neurosci* 2003;26:81–104. [PubMed: 12574495]
2. Selkoe DJ. The genetics and molecular pathology of Alzheimer's disease: roles of amyloid and the presenilins. *Neurol Clin* 2000;18:903–22. [PubMed: 11072267]
3. Lee VM, Goedert M, Trojanowski JQ. Neurodegenerative tauopathies. *Annu Rev Neurosci* 2001;24:1121–59. [PubMed: 11520930]
4. McGeer PL, McGeer EG. Inflammation, autotoxicity and Alzheimer disease. *Neurobiol Aging* 2001;22:799–809. [PubMed: 11754986]

5. Weiner HL, Selkoe DJ. Inflammation and therapeutic vaccination in CNS diseases. *Nature* 2002;420:879–84. [PubMed: 12490962]
6. Smith MA, Sayre LM, Monnier VM, Perry G. Radical AGE-ing in Alzheimer's disease. *Trends Neurosci* 1995;18:172–6. [PubMed: 7778188]
7. Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. *Free Radic Biol Med* 1997;23:134–47. [PubMed: 9165306]
8. Christen Y. Oxidative stress and Alzheimer disease. *Am J Clin Nutr* 2000;71:621S–629S. [PubMed: 10681270]
9. Perry G, Nunomura A, Hirai K, Zhu X, Perez M, Avila J, Castellani RJ, Atwood CS, Aliev G, Sayre LM, Takeda A, Smith MA. Is oxidative damage the fundamental pathogenic mechanism of Alzheimer's and other neurodegenerative diseases? *Free Radic Biol Med* 2002;33:1475–9. [PubMed: 12446204]
10. Perry G, Castellani RJ, Hirai K, Smith MA. Reactive Oxygen Species Mediate Cellular Damage in Alzheimer Disease. *J Alzheimers Dis* 1998;1:45–55. [PubMed: 12214011]
11. Picklo MJ, Montine TJ, Amarnath V, Neely MD. Carbonyl toxicology and Alzheimer's disease. *Toxicol Appl Pharmacol* 2002;184:187–97. [PubMed: 12460747]
12. Seshadri S, Wolf PA, Beiser A, Au R, McNulty K, White R, D'Agostino AB. Lifetime risk of dementia and Alzheimer's disease: The impact of mortality on risk estimates in the Framingham Study. *Neurology* 1997;49:1498–1504. [PubMed: 9409336]
13. Evans DA, Funkenstein HH, Albert MS, Scherr PA, Cook NR, Chown MJ, Hebert LE, Hennekens CH, Taylor JO. Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. *JAMA* 1989;262:2551–6. [PubMed: 2810583]
14. Alzheimer A, Stelzmann RA, Schnitzlein HN, Murtagh FR. An English translation of Alzheimer's 1907 paper, "Über eine eigenartige Erkrankung der Hirnrinde". *Clin Anat* 1995;8:429–31. [PubMed: 8713166]
15. Alzheimer A. *Zentralblatt für Vervenkrankheiten* 1906;25:1134.
16. Perry G, Lipphardt S, Mulvihill P, Kancherla M, Mijares M, Gambetti P, Sharma S, Maggiora L, Cornette J, Lobl T, Greenberg B. Amyloid precursor protein in senile plaques of Alzheimer disease. *Lancet* 1988;2:746. [PubMed: 2901596]
17. Smith MA. Alzheimer disease. *Int Rev Neurobiol* 1998;1–54. [PubMed: 9476170]
18. Liu Q, Raina AK, Smith MA, Sayre LM, Perry G. Hydroxynonenal, toxic carbonyls, and Alzheimer disease. *Mol Aspects Med* 2003;24:305–13. [PubMed: 12893008]
19. Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI. Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. *Proc Natl Acad Sci USA* 1986;83:4913–7. [PubMed: 3088567]
20. Trojanowski JQ, Lee VM. Phosphorylation of paired helical filament tau in Alzheimer's disease neurofibrillary lesions: focusing on phosphatases. *FASEB J* 1995;9:1570–6. [PubMed: 8529836]
21. Mori H, Kondo J, Ihara Y. Ubiquitin is a component of paired helical filaments in Alzheimer's disease. *Science* 1987;235:1641–4. [PubMed: 3029875]
22. Perry G, Friedman R, Shaw G, Chau V. Ubiquitin is detected in neurofibrillary tangles and senile plaque neurites of Alzheimer disease brains. *Proc Natl Acad Sci USA* 1987;84:3033–6. [PubMed: 3033674]
23. Iqbal K, Grundke-Iqbal I. Elevated levels of tau and ubiquitin in brain and cerebrospinal fluid in Alzheimer's disease. *Int Psychogeriatr* 1997;9(Suppl 1):289–96. [PubMed: 9447449]
24. 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;68:2092–7. [PubMed: 9109537]
25. Takeda A, Smith MA, Avila J, Nunomura A, Siedlak SL, Zhu X, Perry G, Sayre LM. In Alzheimer's disease, heme oxygenase is coincident with A β 50, an epitope of tau induced by 4-hydroxy-2-nonenal modification. *J Neurochem* 2000;75:1234–41. [PubMed: 10936206]
26. Liu Q, Smith MA, Avila J, DeBernardis J, Kansal M, Takeda A, Zhu X, Nunomura A, Honda K, Moreira PI, Oliveira CR, Santos MS, Shimohama S, Aliev G, de la Torre J, Ghanbari HA, Siedlak SL, Harris PL, Sayre LM, Perry G. Alzheimer-specific epitopes of tau represent lipid peroxidation-induced conformations. *Free Radic Biol Med* 2005;38:746–54. [PubMed: 15721985]

27. Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, Lu M, Fu Y, Garcia-Sierra F, LaPointe N, Miller R, Berry RW, Binder LI, Cryns VL. Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer's disease. *Proc Natl Acad Sci USA* 2003;100:10032–7. [PubMed: 12888622]
28. Perez M, Hernandez F, Gomez-Ramos A, Smith M, Perry G, Avila J. Formation of aberrant phosphotau fibrillar polymers in neural cultured cells. *Eur J Biochem* 2002;269:1484–9. [PubMed: 11874463]
29. Lovestone S, McLoughlin DM. Protein aggregates and dementia: is there a common toxicity? *J Neurol Neurosurg Psychiatry* 2002;72:152–61. [PubMed: 11796764]
30. Baudier, J.; Cole, DR. *Advances in Behavioral Biology*. Perry, G., editor. Vol. 34. Plenum Press; New York: 1987. p. 25
31. Cash AD, Aliev G, Siedlak SL, Nunomura A, Fujioka H, Zhu X, Raina AK, Vinters HV, Tabaton M, Johnson AB, Paula-Barbosa M, Avila J, Jones PK, Castellani RJ, Smith MA, Perry G. Microtubule reduction in Alzheimer's disease and aging is independent of tau filament formation. *Am J Pathol* 2003;162:1623–7. [PubMed: 12707046]
32. Eidenmuller J, Fath T, Hellwig A, Reed J, Sontag E, Brandt R. Structural and functional implications of tau hyperphosphorylation: information from phosphorylation-mimicking mutated tau proteins. *Biochemistry* 2000;39:13166–75. [PubMed: 11052669]
33. Cras P, Smith MA, Richey PL, Siedlak SL, Mulvihill P, Perry G. Extracellular neurofibrillary tangles reflect neuronal loss and provide further evidence of extensive protein cross-linking in Alzheimer disease. *Acta Neuropathol (Berl)* 1995;89:291–5. [PubMed: 7610759]
34. Smith MA, Casadesus G, Joseph JA, Perry G. Amyloid-beta and tau serve antioxidant functions in the aging and Alzheimer brain. *Free Radic Biol Med* 2002;33:1194–9. [PubMed: 12398927]
35. Lee HG, Perry G, Moreira PI, Garrett MR, Liu Q, Zhu X, Takeda A, Nunomura A, Smith MA. Tau phosphorylation in Alzheimer's disease: pathogen or protector? *Trends Mol Med* 2005;11:164–9. [PubMed: 15823754]
36. Lee HG, Zhu X, Nunomura A, Perry G, Smith MA. *Curr Alzheimer Res* 2006;3:75. [PubMed: 16472207]
37. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 2001;81:741–66. [PubMed: 11274343]
38. Ashford JW. APOE genotype effects on Alzheimer's disease onset and epidemiology. *J Mol Neurosci* 2004;23:157–65. [PubMed: 15181244]
39. Teter B. ApoE-dependent plasticity in Alzheimer's disease. *J Mol Neurosci* 2004;23:167–79. [PubMed: 15181245]
40. Hardy JA, Higgins GA. Alzheimer's disease: the amyloid cascade hypothesis. *Science* 1992;256:184–5. [PubMed: 1566067]
41. Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 2002;297:353–6. [PubMed: 12130773]
42. Saunders AM, Schmechel K, Breitner JC, Benson MD, Brown WT, Goldfarb L, Goldgaber D, Manwaring MG, Szymanski MH, McCown N, Roses A. Apolipoprotein E epsilon 4 allele distributions in late-onset Alzheimer's disease and in other amyloid-forming diseases. *Lancet* 1993;342:710–1. [PubMed: 8103823]
43. Rebeck GW, Reiter JS, Strickland DK, Hyman BT. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron* 1993;11:575–80. [PubMed: 8398148]
44. Schmechel DE, Saunders AM, Strittmatter WJ, Crain BJ, Hulette CM, Joo SH, Pericak-Vance MA, Goldgaber D, Roses AD. Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proc Natl Acad Sci U S A* 1993;90:9649–53. [PubMed: 8415756]
45. Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, Pericak-Vance M, Schmechel D, Saunders AM, Goldgaber D, Roses AD. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. *Proc Natl Acad Sci USA* 1993;90:8098–102. [PubMed: 8367470]

46. Lauderback CM, Kanski J, Hackett JM, Maeda N, Kindy MS, Butterfield DA. Apolipoprotein E modulates Alzheimer's A β (1–42)-induced oxidative damage to synaptosomes in an allele-specific manner. *Brain Res* 2002;924:90–7. [PubMed: 11743999]
47. Ramassamy C, Averill D, Beffert U, Theroux L, Lussier-Cacan S, Cohn JS, Christen Y, Schoofs A, Davignon J, Poirier J. Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. *Neurobiol Dis* 2000;7:23–37. [PubMed: 10671320]
48. Tanzi RE, Gusella JF, Watkins PC, Bruns GA, St George-Hyslop P, Van Keuren ML, Patterson D, Pagan S, Kurnit DM, Neve RL. Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* 1987;235:880–4. [PubMed: 2949367]
49. St George-Hyslop PH, Tanzi RE, Polinsky RJ, Haines JL, Nee L, Watkins PC, Myers RH, Feldman RG, Pollen D, Drachman D, Growdon J, Bruni A, Foncin JF, Salmon D, Frommelt P, Amaducci L, Sorbi S, Piacenti S, Stewart GD, Hobbs WJ, Conneally PM, Gusella JF. The genetic defect causing familial Alzheimer's disease maps on chromosome 21. *Science* 1987;235:885–90. [PubMed: 2880399]
50. Selkoe DJ. Alzheimer's disease results from the cerebral accumulation and cytotoxicity of amyloid beta-protein. *J Alzheimers Dis* 2001;3:75–80. [PubMed: 12214075]
51. Selkoe DJ. Alzheimer's disease: genotypes, phenotypes, and treatments. *Science* 1997;275:630–1. [PubMed: 9019820]
52. Bentahir M, Nyabi O, Verhamme J, Tolia A, Horre K, Wiltfang J, Esselmann H, De Strooper B. Presenilin clinical mutations can affect gamma-secretase activity by different mechanisms. *J Neurochem* 2006;96:732–42. [PubMed: 16405513]
53. Games D, Adams D, Alessandrini R, Barbour R, Berthelette P, Blackwell C, Carr T, Clemens J, Donaldson T, Gillespie F, Guido T, Hagopian S, Johnson-Wood K, Khan K, Lee M, Leibowitz P, Lieberburg I, Little S, Masliah E, McConlogue L, Montoya-Zavala M, Mucke L, Paganini L, Penniman E, Power M, Schenk D, Seubert P, Snyder B, Soriano F, Tan H, Vitale J, Wadsworth S, Wolozin B, Zhao J. Alzheimer-type neuropathology in transgenic mice overexpressing V717F β -amyloid precursor protein. *Nature* 1995;373:523–7. [PubMed: 7845465]
54. Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficits, A β elevation, and amyloid plaques in transgenic mice. *Science* 1996;274:99–102. [PubMed: 8810256]
55. Rogaev EI, Sherrington R, Rogaeva EA, Levesque G, Ikeda M, Liang Y, Chi H, Lin C, Holman K, Tsuda T, Mar L, Sorbi S, Nacmias B, Piacentini S, Chumakov I, Cohen D, Lannfelt L, Fraser PE, Rommens JM, St George-Hyslop PH. Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* 1995;376:775–8. [PubMed: 7651536]
56. Hardy J. Presenilin Mutations Directory. *Alzforum*. 2007. <http://www.alzforum.org/res/com/mut/pre/default.asp>
57. Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HAR, Haines JL, Pericak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George-Hyslop PH. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* 1995;375:754–60. [PubMed: 7596406]
58. Czech C, Tremp G, Pradier L. Presenilins and Alzheimer's disease: biological functions and pathogenic mechanisms. *Prog Neurobiol* 2000;60:363–84. [PubMed: 10670705]
59. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* 1996;2:864–70. [PubMed: 8705854]
60. Whitehouse PJ, Price DL, Clark AW, Coyle JT, DeLong MR. Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann Neurol* 1981;10:122–6. [PubMed: 7283399]

61. Liu Q, Lee HG, Honda K, Siedlak SL, Harris PL, Cash AD, Zhu X, Avila J, Nunomura A, Takeda A, Smith MA, Perry G. Tau modifiers as therapeutic targets for Alzheimer's disease. *Biochem Biophys Acta* 2005;1739:211–5. [PubMed: 15615639]
62. Takeda A, Perry G, Abraham NG, Dwyer BE, Kutty RK, Laitinen JT, Petersen RB, Smith MA. Overexpression of heme oxygenase in neuronal cells, the possible interaction with Tau. *J Biol Chem* 2000;275:5395–9. [PubMed: 10681514]
63. Perez M, Cuadros R, Smith MA, Perry G, Avila J. Phosphorylated, but not native, tau protein assembles following reaction with the lipid peroxidation product, 4-hydroxy-2-nonenal. *FEBS Lett* 2000;486:270–4. [PubMed: 11119717]
64. Zhu X, Rottkamp CA, Boux H, Takeda A, Perry G, Smith MA. Activation of p38 kinase links tau phosphorylation, oxidative stress, and cell cycle-related events in Alzheimer disease. *J Neuropathol Exp Neurol* 2000;59:880–8. [PubMed: 11079778]
65. Zhu X, Castellani RJ, Takeda A, Nunomura A, Atwood CS, Perry G, Smith MA. Differential activation of neuronal ERK, JNK/SAPK and p38 in Alzheimer disease: the 'two hit' hypothesis. *Mech Ageing Dev* 2001;123:39–46. [PubMed: 11640950]
66. Zhu X, Raina AK, Rottkamp CA, Aliev G, Perry G, Boux H, Smith MA. Activation and redistribution of c-jun N-terminal kinase/stress activated protein kinase in degenerating neurons in Alzheimer's disease. *J Neurochem* 2001;76:435–41. [PubMed: 11208906]
67. Breteler MM. Vascular risk factors for Alzheimer's disease: an epidemiologic perspective. *Neurobiol Aging* 2000;21:153–60. [PubMed: 10867200]
68. Merchant C, Tang MX, Albert S, Manly J, Stern Y, Mayeux R. The influence of smoking on the risk of Alzheimer's disease. *Neurology* 1999;52:1408–12. [PubMed: 10227626]
69. Ott A, Slioter AJ, Hofman A, van Harskamp F, Witteman JC, Van Broeckhoven C, van Duijn CM, Breteler MM. Smoking and risk of dementia and Alzheimer's disease in a population-based cohort study: the Rotterdam Study. *Lancet* 1998;351:1840–3. [PubMed: 9652667]
70. Plassman BL, Havlik RJ, Steffens DC, Helms MJ, Newman TN, Drosdick D, Phillips C, Gau BA, Welsh-Bohmer KA, Burke JR, Guralnik JM, Breitner JC. Documented head injury in early adulthood and risk of Alzheimer's disease and other dementias. *Neurology* 2000;55:1158–66. [PubMed: 11071494]
71. Nunomura A, Perry G, Pappolla MA, Friedland RP, Hirai K, Chiba S, Smith MA. Neuronal oxidative stress precedes amyloid-beta deposition in Down syndrome. *J Neuropathol Exp Neurol* 2000;59:1011–7. [PubMed: 11089579]
72. Stern Y, Gurland B, Tatemichi TK, Tang MX, Wilder D, Mayeux R. Influence of education and occupation on the incidence of Alzheimer's disease. *JAMA* 1994;271:1004–10. [PubMed: 8139057]
73. Hall KS, Gao S, Unverzagt FW, Hendrie HC. Low education and childhood rural residence: risk for Alzheimer's disease in African Americans. *Neurology* 2000;54:95–9. [PubMed: 10636132]
74. Baldereschi M, Di Carlo A, Lepore V, Bracco L, Maggi S, Grigoletto F, Scarlato G, Amaducci L. Estrogen-replacement therapy and Alzheimer's disease in the Italian Longitudinal Study on Aging. *Neurology* 1998;50:996–1002. [PubMed: 9566385]
75. Waring SC, Rocca WA, Petersen RC, O'Brien PC, Tangalos EG, Kokmen E. Postmenopausal estrogen replacement therapy and risk of AD: a population-based study. *Neurology* 1999;52:965–70. [PubMed: 10102413]
76. Casadesus G, Smith MA, Zhu X, Aliev G, Cash AD, Honda K, Petersen RB, Perry G. Alzheimer disease: evidence for a central pathogenic role of iron-mediated reactive oxygen species. *J Alzheimers Dis* 2004;6:165–9. [PubMed: 15096700]
77. Casadesus G, Zhu X, Atwood CS, Webber KM, Perry G, Bowen RL, Smith MA. Beyond estrogen: targeting gonadotropin hormones in the treatment of Alzheimer's disease. *Curr Drug Targets CNS Neurol Disord* 2004;3:281–5. [PubMed: 15379604]
78. Casadesus G, Atwood CS, Zhu X, Hartzler AW, Webber KM, Perry G, Bowen RL, Smith MA. Evidence for the role of gonadotropin hormones in the development of Alzheimer disease. *Cell Mol Life Sci* 2005;62:293–8. [PubMed: 15723165]

79. Casadesus G, Webber KM, Atwood CS, Pappolla MA, Perry G, Bowen RL, Smith MA. Luteinizing hormone modulates cognition and amyloid-beta deposition in Alzheimer APP transgenic mice. *Biochim Biophys Acta* 2006;1762:447–52. [PubMed: 16503402]
80. Webber KM, Bowen R, Casadesus G, Perry G, Atwood CS, Smith MA. Gonadotropins and Alzheimer's disease: the link between estrogen replacement therapy and neuroprotection. *Acta Neurobiol Exp (Wars)* 2004;64:113–8. [PubMed: 15190686]
81. Webber KM, Casadesus G, Perry G, Atwood CS, Bowen R, Smith MA. Gender differences in Alzheimer disease: the role of luteinizing hormone in disease pathogenesis. *Alzheimer Dis Assoc Disord* 2005;19:95–9. [PubMed: 15942328]
82. Webber KM, Casadesus G, Marlatt MW, Perry G, Hamlin CR, Atwood CS, Bowen RL, Smith MA. Estrogen bows to a new master: the role of gonadotropins in Alzheimer pathogenesis. *Ann N Y Acad Sci* 2005;1052:201–9. [PubMed: 16024763]
83. Webber KM, Casadesus G, Zhu X, Obrenovich ME, Atwood CS, Perry G, Bowen RL, Smith MA. The cell cycle and hormonal fluxes in Alzheimer disease: a novel therapeutic target. *Curr Pharm Des* 2006;12:691–7. [PubMed: 16472159]
84. Weggen S, Eriksen JL, Das P, Sagi SA, Wang R, Pietrzik CU, Findlay KA, Smith TE, Murphy MP, Bulter T, Kang DE, Marquez-Sterling N, Golde TE, Koo EH. A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature* 2001;414:212–6. [PubMed: 11700559]
85. de la Lastra CA, Villegas I. Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. *Mol Nutr Food Res* 2005;49:405–30. [PubMed: 15832402]
86. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 1956;11:298. [PubMed: 13332224]
87. Honda K, Smith MA, Zhu X, Baus D, Merrick WC, Tartakoff AM, Hattier T, Harris PL, Siedlak SL, Fujioka H, Liu Q, Moreira PI, Miller FP, Nunomura A, Shimohama S, Perry G. Ribosomal RNA in Alzheimer disease is oxidized by bound redox-active iron. *J Biol Chem* 2005;280:20978–86. [PubMed: 15767256]
88. Allen PC. Production of free radical species during *Eimeria maxima* infections in chickens. *Poult Sci* 1997;76:814–21. [PubMed: 9181613]
89. Leibovitz BE, Siegel BV. Aspects of free radical reactions in biological systems: aging. *J Gerontol* 1980;35:45–56. [PubMed: 6243146]
90. Harman LS, Mottley C, Mason RP. Free radical metabolites of L-cysteine oxidation. *J Biol Chem* 1984;259:5606–11. [PubMed: 6325443]
91. Priyadarsini KI, Khopde SM, Kumar SS, Mohan H. Free radical studies of ellagic acid, a natural phenolic antioxidant. *J Agric Food Chem* 2002;50:2200–6. [PubMed: 11902978]
92. Pierrefiche G, Laborit H. Oxygen free radicals, melatonin, and aging. *Exp Gerontol* 1995;30:213–27. [PubMed: 7556504]
93. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA* 1993;90:7915–22. [PubMed: 8367443]
94. Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev* 1998;78:547–81. [PubMed: 9562038]
95. Beal MF. Mitochondria, oxidative damage, and inflammation in Parkinson's disease. *Ann N Y Acad Sci* 2003;991:120–31. [PubMed: 12846981]
96. Vendemiale G, Grattagliano I, Altomare E. An update on the role of free radicals and antioxidant defense in human disease. *Int J Clin Lab Res* 1999;29:49–55. [PubMed: 10436261]
97. Singh M, Saini HK. Resident cardiac mast cells and ischemia-reperfusion injury. *J Cardiovasc Pharmacol Ther* 2003;8:135–48. [PubMed: 12808487]
98. Meister A. Glutathione, ascorbate, and cellular protection. *Cancer Res* 1994;54:1969s–1975s. [PubMed: 8137322]
99. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. *Am J Clin Nutr* 1995;62:1315S–1321S. [PubMed: 7495226]
100. Anderson R, Ramafi G, Theron AJ. Membrane stabilizing, anti-oxidative interactions of propranolol and dexpropranolol with neutrophils. *Biochem Pharmacol* 1996;52:341–9. [PubMed: 8694859]

101. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet* 1994;344:721–4. [PubMed: 7915779]
102. Block G. Vitamin C and cancer prevention: the epidemiologic evidence. *Am J Clin Nutr* 1991;53:270S–282S. [PubMed: 1985398]
103. Sen CK, Khanna S, Roy S, Packer L. Molecular basis of vitamin E action. Tocotrienol potentially inhibits glutamate-induced pp60(c-Src) kinase activation and death of HT4 neuronal cells. *J Biol Chem* 2000;275:13049–55. [PubMed: 10777609]
104. Bauer C, Walcher F, Holanda M, Mertzlufft F, Larsen R, Marzi I. Antioxidative resuscitation solution prevents leukocyte adhesion in the liver after hemorrhagic shock. *J Trauma* 1999;46:886–93. [PubMed: 10338408]
105. Andorn N, Kaufman JB, Clem TR, Fass R, Shiloach J. Large-scale growth of *Bordetella pertussis* for production of extracellular toxin. *Ann N Y Acad Sci* 1990;589:363–71. [PubMed: 2192661]
106. Smith MA, Richey Harris PL, Sayre LM, Beckman JS, Perry G. Widespread peroxynitrite-mediated damage in Alzheimer's disease. *J Neurosci* 1997;17:2653–7. [PubMed: 9092586]
107. Smith MA, Rudnicka-Nawrot M, Richey PL, Praprotnik D, Mulvihill P, Miller CA, Sayre LM, Perry G. Carbonyl-related posttranslational modification of neurofilament protein in the neurofibrillary pathology of Alzheimer's disease. *J Neurochem* 1995;64:2660–6. [PubMed: 7539057]
108. Smith MA, Taneda S, Richey PL, Miyata S, Yan SD, Stern D, Sayre LM, Monnier VM, Perry G. Advanced Maillard reaction end products are associated with Alzheimer disease pathology. *Proc Natl Acad Sci USA* 1994;91:5710–4. [PubMed: 8202552]
109. Smith MA, Kutty RK, Richey PL, Yan SD, Stern D, Chader GJ, Wiggert B, Petersen RB, Perry G. Heme oxygenase-1 is associated with the neurofibrillary pathology of Alzheimer's disease. *Am J Pathol* 1994;145:42–7. [PubMed: 8030754]
110. Nunomura A, Perry G, Pappolla MA, Wade R, Hirai K, Chiba S, Smith MA. RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *J Neurosci* 1999;19:1959–64. [PubMed: 10066249]
111. Castellani RJ, Harris PL, Sayre LM, Fujii J, Taniguchi N, Vitek MP, Founds H, Atwood CS, Perry G, Smith MA. Active glycation in neurofibrillary pathology of Alzheimer disease: N(epsilon)-(carboxymethyl) lysine and hexitol-lysine. *Free Radic Biol Med* 2001;31:175–80. [PubMed: 11440829]
112. Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, Jones PK, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood CS, Petersen RB, Smith MA. Oxidative damage is the earliest event in Alzheimer disease. *J Neuropathol Exp Neurol* 2001;60:759–67. [PubMed: 11487050]
113. Marlatt MW, Webber KM, Moreira PI, Lee HG, Casadesus G, Honda K, Zhu X, Perry G, Smith MA. Therapeutic opportunities in Alzheimer disease: one for all or all for one? *Curr Med Chem* 2005;12:1137–47. [PubMed: 15892629]
114. Pietrzik C, Behl C. Concepts for the treatment of Alzheimer's disease: molecular mechanisms and clinical application. *Int J Exp Pathol* 2005;86:173–85. [PubMed: 15910551]
115. Doody RS. Refining treatment guidelines in Alzheimer's disease. *Geriatrics* 2005;(Suppl):14–20. [PubMed: 16025771]
116. Cardozo-Pelaez F, Song S, Parthasarathy A, Epstein CJ, Sanchez-Ramos J. Attenuation of age-dependent oxidative damage to DNA and protein in brainstem of Tg Cu/Zn SOD mice. *Neurobiol Aging* 1998;19:311–6. [PubMed: 9733163]
117. Socci DJ, Crandall BM, Arendash GW. Chronic antioxidant treatment improves the cognitive performance of aged rats. *Brain Res* 1995;693:88–94. [PubMed: 8653426]
118. Weiss N, Zhang YY, Heydrick S, Bierl C, Loscalzo J. Over-expression of cellular glutathione peroxidase rescues homocyst(e)ine-induced endothelial dysfunction. *Proc Natl Acad Sci U S A* 2001;98:12503–8. [PubMed: 11606774]
119. Bruce AJ, Boling W, Kindy MS, Peschon J, Kraemer PJ, Carpenter MK, Holtsberg FW, Mattson MP. Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors. *Nat Med* 1996;2:788–94. [PubMed: 8673925]

120. Melov S, Ravenscroft J, Malik S, Gill MS, Walker DW, Clayton PE, Wallace DC, Malfroy B, Doctrow SR, Lithgow GJ. Extension of life-span with superoxide dismutase/catalase mimetics. *Science* 2000;289:1567–9. [PubMed: 10968795]
121. Andersen HR, Jeune B, Nybo H, Nielsen JB, Andersen-Ranberg K, Grandjean P. Low activity of superoxide dismutase and high activity of glutathione reductase in erythrocytes from centenarians. *Age Ageing* 1998;27:643–8. [PubMed: 12675104]
122. 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;30:665–76. [PubMed: 11430801]
123. Smith MA, Harris PL, Sayre LM, Perry G. Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proc Natl Acad Sci U S A* 1997;94:9866–8. [PubMed: 9275217]
124. Huang X, Cuajungco MP, Atwood CS, Hartshorn MA, Tyndall JD, Hanson GR, Stokes KC, Leopold M, Multhaup G, Goldstein LE, Scarpa RC, Saunders AJ, Lim J, Moir RD, Glabe C, Bowden EF, Masters CL, Fairlie DP, Tanzi RE, Bush AI. Cu(II) potentiation of alzheimer abeta neurotoxicity. Correlation with cell-free hydrogen peroxide production and metal reduction. *J Biol Chem* 1999;274:37111–6. [PubMed: 10601271]
125. Sayre LM, Smith MA, Perry G. Chemistry and biochemistry of oxidative stress in neurodegenerative disease. *Curr Med Chem* 2001;8:721–38. [PubMed: 11375746]
126. Crapper McLachlan DR, Dalton AJ, Kruck TP, Bell MY, Smith WL, Kalow W, Andrews DF. Intramuscular desferrioxamine in patients with Alzheimer's disease. *Lancet* 1991;337:1304–8. [PubMed: 1674295]
127. Morse LJ, Payton SM, Cuny GD, Rogers JT. FDA-preapproved drugs targeted to the translational regulation and processing of the amyloid precursor protein. *J Mol Neurosci* 2004;24:129–36. [PubMed: 15314261]
128. Liu G, Garrett MR, Men P, Zhu X, Perry G, Smith MA. Nanoparticle and other metal chelation therapeutics in Alzheimer disease. *Biochem Biophys Acta* 2005;1741:246–52. [PubMed: 16051470]
129. Ingram DK, Weindruch R, Spangler EL, Freeman JR, Walford RL. Dietary restriction benefits learning and motor performance of aged mice. *J Gerontol* 1987;42:78–81. [PubMed: 3794202]
130. Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science* 1996;273:59–63. [PubMed: 8658196]
131. Weindruch R, Lane MA, Ingram DK, Ershler WB, Roth GS. Dietary restriction in rhesus monkeys: lymphopenia and reduced mitogen-induced proliferation in peripheral blood mononuclear cells. *Aging (Milano)* 1997;9:304–8. [PubMed: 9359942]
132. Dubey A, Forster MJ, Lal H, Sohal RS. Effect of age and caloric intake on protein oxidation in different brain regions and on behavioral functions of the mouse. *Arch Biochem Biophys* 1996;333:189–97. [PubMed: 8806770]
133. Mayeux R, Sano M. Treatment of Alzheimer's disease. *N Engl J Med* 1999;341:1670–9. [PubMed: 10572156]
134. Smith MA, Petot GJ, Perry G. *J Alzheimers Dis* 1999;1:201.
135. Grant WB. *J Alzheimers Dis* 1999;1:197. [PubMed: 12214118]
136. Guarente L, Picard F. Calorie restriction--the SIR2 connection. *Cell* 2005;120:473–82. [PubMed: 15734680]
137. Delmas D, Jannin B, Latruffe N. Resveratrol: preventing properties against vascular alterations and ageing. *Mol Nutr Food Res* 2005;49:377–95. [PubMed: 15830334]
138. Yu ZF, Bruce-Keller AJ, Goodman Y, Mattson MP. Uric acid protects neurons against excitotoxic and metabolic insults in cell culture, and against focal ischemic brain injury *in vivo*. *J Neurosci Res* 1998;53:613–25. [PubMed: 9726432]
139. Copp RP, Wisniewski T, Hentati F, Larnaout A, Ben Hamida M, Kayden HJ. Localization of alpha-tocopherol transfer protein in the brains of patients with ataxia with vitamin E deficiency and other oxidative stress related neurodegenerative disorders. *Brain Res* 1999;822:80–7. [PubMed: 10082886]

140. Mitchell JH, Collins AR. Effects of a soy milk supplement on plasma cholesterol levels and oxidative DNA damage in men--a pilot study. *Eur J Nutr* 1999;38:143–8. [PubMed: 10443336]
141. Pratico D, Clark CM, Liun F, Rokach J, Lee VY, Trojanowski JQ. Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer disease. *Arch Neurol* 2002;59:972–6. [PubMed: 12056933]
142. 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;336:1216–22. [PubMed: 9110909]
143. Pitchumoni SS, Doraiswamy PM. Current status of antioxidant therapy for Alzheimer's Disease. *J Am Geriatr Soc* 1998;46:1566–72. [PubMed: 9848820]
144. Flynn BL, Ranno AE. Pharmacologic management of Alzheimer disease, Part II: Antioxidants, antihypertensives, and ergoloid derivatives. *Ann Pharmacother* 1999;33:188–97. [PubMed: 10084415]
145. Morris MC, Evans DA, Bienias JL, Tangney CC, Bennett DA, Aggarwal N, Wilson RS, Scherr PA. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. *JAMA* 2002;287:3230–7. [PubMed: 12076219]
146. Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JC, Breteler MM. Dietary intake of anti-oxidants and risk of Alzheimer disease. *JAMA* 2002;287:3223–9. [PubMed: 12076218]
147. Commenges D, Scotet V, Renaud S, Jacqmin-Gadda H, Barberger-Gateau P, Dartigues JF. Intake of flavonoids and risk of dementia. *Eur J Epidemiol* 2000;16:357–63. [PubMed: 10959944]
148. Boothby LA, Doering PL. Vitamin C and vitamin E for Alzheimer's disease. *Ann Pharmacother* 2005;39:2073–80. [PubMed: 16227450]
149. Koehler KF, Helguero LA, Haldosen LA, Warner M, Gustafsson JA. Reflections on the discovery and significance of estrogen receptor beta. *Endocr Rev* 2005;26:465–78. [PubMed: 15857973]
150. Sugioka K, Shimosegawa Y, Nakano M. Estrogens as natural antioxidants of membrane phospholipid peroxidation. *FEBS Lett* 1987;210:37–9. [PubMed: 3803578]
151. Moosmann B, Behl C. The antioxidant neuroprotective effects of estrogens and phenolic compounds are independent from their estrogenic properties. *Proc Natl Acad Sci U S A* 1999;96:8867–72. [PubMed: 10430862]
152. Behl C. Estrogens against Alzheimer disease? The female sexual hormone as a protective neurohormone. *MMW Fortschr Med* 2001;143(Suppl 2):33–5. [PubMed: 11434254]
153. Behl C, Skutella T, Lezoualc'h F, Post A, Widmann M, Newton CJ, Holsboer F. Neuroprotection against oxidative stress by estrogens: structure-activity relationship. *Mol Pharmacol* 1997;51:535–41. [PubMed: 9106616]
154. Behl C, Widmann M, Trapp T, Holsboer F. 17-beta estradiol protects neurons from oxidative stress-induced cell death *in vitro*. *Biochem Biophys Res Commun* 1995;216:473–82. [PubMed: 7488136]
155. Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, Wise PM. Estradiol protects against ischemic injury. *J Cereb Blood Flow Metab* 1998;18:1253–8. [PubMed: 9809515]
156. Goodman Y, Bruce AJ, Cheng B, Mattson MP. Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury, and amyloid beta-peptide toxicity in hippocampal neurons. *J Neurochem* 1996;66:1836–44. [PubMed: 8780008]
157. Shumaker SA, Legault C, Rapp SR, Thal L, Wallace RB, Ockene JK, Hendrix SL, Jones BN 3rd, Assaf AR, Jackson RD, Kotchen JM, Wassertheil-Smoller S, Wactawski-Wende J. Estrogen plus progestin and the incidence of dementia and mild cognitive impairment in postmenopausal women: the Women's Health Initiative Memory Study: a randomized controlled trial. *JAMA* 2003;289:2651–62. [PubMed: 12771112]
158. 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;14:213–26. [PubMed: 1958264]

159. Jenner P. Oxidative damage in neurodegenerative disease. *Lancet* 1994;344:796–8. [PubMed: 7916079]
160. Lohr JB, Browning JA. Free radical involvement in neuropsychiatric illnesses. *Psychopharmacol Bull* 1995;31:159–65. [PubMed: 7675980]
161. Liu H, Wang H, Shenvi S, Hagen TM, Liu RM. Glutathione metabolism during aging and in Alzheimer disease. *Ann N Y Acad Sci* 2004;1019:346–9. [PubMed: 15247041]
162. Asensi M, Sastre J, Pallardo FV, Lloret A, Lehner M, Garcia-de-la Asuncion J, Vina J. Ratio of reduced to oxidized glutathione as indicator of oxidative stress status and DNA damage. *Methods Enzymol* 1999;299:267–76. [PubMed: 9916205]
163. Moosmann B, Uhr M, Behl C. Neuroprotective potential of aromatic alcohols against oxidative cell death. *FEBS Lett* 1997;413:467–72. [PubMed: 9303557]
164. Skaper SD, Fabris M, Ferrari V, Dalle Carbonare M, Leon A. Quercetin protects cutaneous tissue-associated cell types including sensory neurons from oxidative stress induced by glutathione depletion: cooperative effects of ascorbic acid. *Free Radic Biol Med* 1997;22:669–78. [PubMed: 9013129]
165. Gutzmann H, Hadler D. Sustained efficacy and safety of idebenone in the treatment of Alzheimer's disease: update on a 2-year double-blind multicentre study. *J Neural Transm Suppl* 1998;54:301–10. [PubMed: 9850939]
166. Weyer G, Babej-Dolle RM, Hadler D, Hofmann S, Herrmann WM. A controlled study of 2 doses of idebenone in the treatment of Alzheimer's disease. *Neuropsychobiology* 1997;36:73–82. [PubMed: 9267856]
167. Yamada K, Tanaka T, Han D, Senzaki K, Kameyama T, Nabeshima T. Protective effects of idebenone and alpha-tocopherol on beta-amyloid-(1–42)-induced learning and memory deficits in rats: implication of oxidative stress in beta-amyloid-induced neurotoxicity *in vivo*. *Eur J Neurosci* 1999;11:83–90. [PubMed: 9987013]
168. Pluta R. No effect of anti-oxidative therapy on cerebral amyloidosis following ischemia-reperfusion brain injury. *Folia Neuropathol* 2000;38:188–90. [PubMed: 11693724]
169. Thal LJ, Grundman M, Berg J, Ernstrom K, Margolin R, Pfeiffer E, Weiner MF, Zamrini E, Thomas RG. Idebenone treatment fails to slow cognitive decline in Alzheimer's disease. *Neurology* 2003;61:1498–502. [PubMed: 14663031]
170. Gutzmann H, Kuhl KP, Hadler D, Rapp MA. Safety and efficacy of idebenone versus tacrine in patients with Alzheimer's disease: results of a randomized, double-blind, parallel-group multicenter study. *Pharmacopsychiatry* 2002;35:12–8. [PubMed: 11819153]
171. Hampson AJ, Grimaldi M, Axelrod J, Wink D. Cannabidiol and (–)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci U S A* 1998;95:8268–73. [PubMed: 9653176]
172. Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglansberger W, Di Marzo V, Lutz B. The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 2002;418:530–4. [PubMed: 12152079]
173. Moosmann B, Skutella T, Beyer K, Behl C. Protective activity of aromatic amines and imines against oxidative nerve cell death. *Biol Chem* 2001;382:1601–12. [PubMed: 11767950]
174. Moosmann B, Behl C. Cytoprotective antioxidant function of tyrosine and tryptophan residues in transmembrane proteins. *Eur J Biochem* 2000;267:5687–92. [PubMed: 10971578]
175. Munch G, Taneli Y, Schraven E, Schindler U, Schinzel R, Palm D, Riederer P. The cognition-enhancing drug tenilsetam is an inhibitor of protein crosslinking by advanced glycosylation. *J Neural Transm Park Dis Dement Sect* 1994;8:193–208. [PubMed: 7748463]
176. Shoda H, Miyata S, Liu BF, Yamada H, Ohara T, Suzuki K, Oimomi M, Kasuga M. Inhibitory effects of tenilsetam on the Maillard reaction. *Endocrinology* 1997;138:1886–92. [PubMed: 9112383]
177. Stadtman ER. Cyclic oxidation and reduction of methionine residues of proteins in antioxidant defense and cellular regulation. *Arch Biochem Biophys* 2004;423:2–5. [PubMed: 14989257]
178. Moskovitz J, Berlett BS, Poston JM, Stadtman ER. The yeast peptide-methionine sulfoxide reductase functions as an antioxidant *in vivo*. *Proc Natl Acad Sci USA* 1997;94:9585–9. [PubMed: 9275166]

179. Moskovitz J, Bar-Noy S, Williams WM, Requena J, Berlett BS, Stadtman ER. Methionine sulfoxide reductase (MsrA) is a regulator of antioxidant defense and lifespan in mammals. *Proc Natl Acad Sci USA* 2001;98:12920–5. [PubMed: 11606777]
180. Moskovitz J, Flescher E, Berlett BS, Azare J, Poston JM, Stadtman ER. Overexpression of peptide-methionine sulfoxide reductase in *Saccharomyces cerevisiae* and human T cells provides them with high resistance to oxidative stress. *Proc Natl Acad Sci USA* 1998;95:14071–5. [PubMed: 9826655]
181. Yermolaieva O, Xu R, Schinstock C, Brot N, Weissbach H, Heinemann SH, Hoshi T. Methionine sulfoxide reductase A protects neuronal cells against brief hypoxia/reoxygenation. *Proc Natl Acad Sci USA* 2004;101:1159–64. [PubMed: 14745014]
182. Ruan H, Tang XD, Chen ML, Joiner ML, Sun G, Brot N, Weissbach H, Heinemann SH, Iverson L, Wu CF, Hoshi T. High-quality life extension by the enzyme peptide methionine sulfoxide reductase. *Proc Natl Acad Sci U S A* 2002;99:2748–53. [PubMed: 11867705]
183. Grundman M. Vitamin E and Alzheimer disease: the basis for additional clinical trials. *Am J Clin Nutr* 2000;71:630S–636S. [PubMed: 10681271]

Biography