Antioxidant Therapy in Alzheimer's Disease: Theory and Practice

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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 devastative disease.

Key Words: Antioxidant, calorie restriction, estrogen, free radical, glutathione, oxidation, oxidative stress, therapy.

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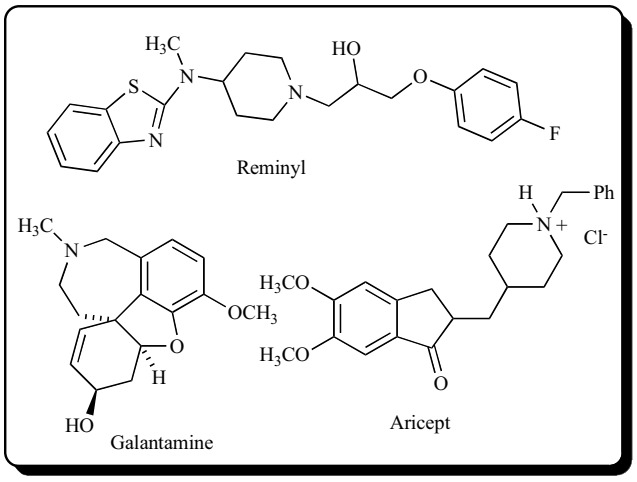
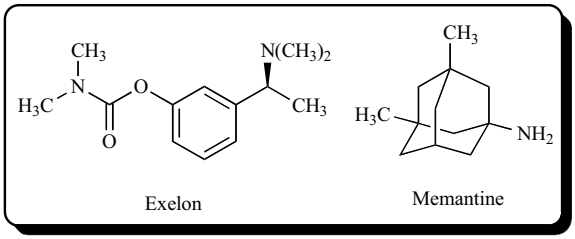
Perry's studies are focused on the mechanism of formation and physiological consequences of the cytopathology of Alzheimer disease. Dr. Perry's group has shown that oxidative damage is the initial cytopathological abnormality in Alzheimer disease.

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.





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 (Aβ) 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

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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 Aβ, 10-200 μm in diameter, form the central core of the plaques and are surrounded by Aβ, amyloid-β protein precursor (AβPP), 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], ubiquinated [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 AβPP show overproduction of Aβ protein, senile plaque formation and synaptic deficits without NFTs pathology, indicating a key pathological role for mutant AβPP protein [53,54]. The current data finds that AβPP 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 AβPP [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 antioxidant 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 biomolecules 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, peroxyl 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 in-

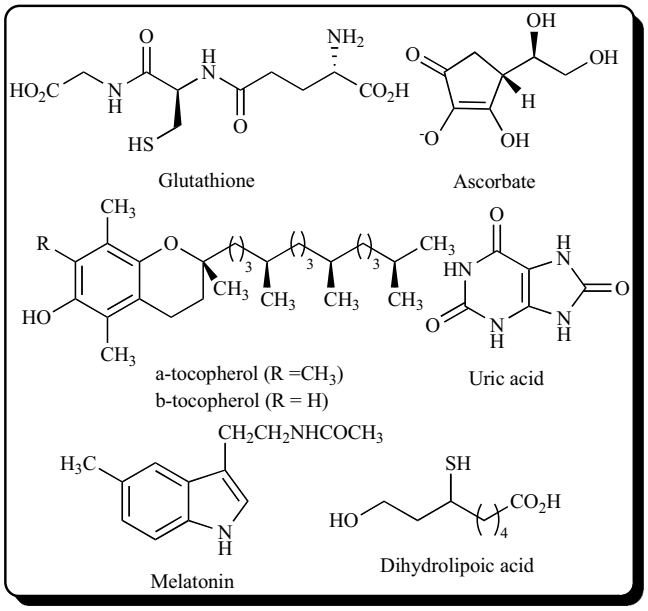
fection by phagocytosis [89,93,94]; the fifth source is from other enzymes capable of generating oxidants under normal or pathological conditions [95]; various biomolecules including thiols, hydroquinones, flavins, catecholamines, pterins and hemoglobin, which may spontaneously auto-oxidize and produce 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 peroxyl-radical-induced loss of cell viability [103]. Chandan and colleagues used 2,29-azobis [2-amidinopropane] hydrochloride (AAPH)-generated peroxyl-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 αtocotrienol 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 sub-

stantial 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

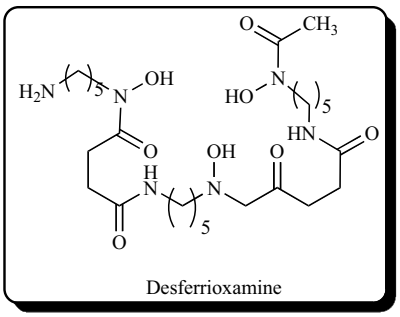
Transgenic mice overexpressing Cu-Zn SOD showed reduced oxidative damage in brain tissue [116] and improved cognitive function in aged rodents [117]. Overexpression of glutathione peroxidase in transgenic mice also showed antioxidative function and prevented homocysteine-induced endothelial dysfunction [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

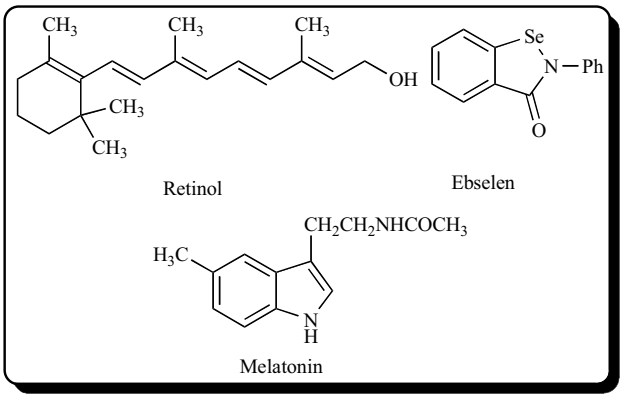
AβPP transgenic mice treated with a Cu/Zn chelator showed improvement in general parameters and a reduction of brain Aβ deposition [122]. Because copper and zinc play a major role in Aβ toxicity and nerve cell death *via* ROS generation, chelator therapy is, in effect, antioxidant [123-125]. In one study, 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 Aβ 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

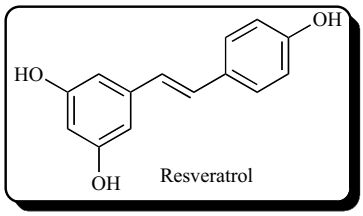


of age-related disease in rodents and monkeys [130,131]. The mechanism of the beneficial effect of caloric restriction is not clearly understood but is most likely *via* overall reduction in levels of oxidative stress, including in the brain [132]. In humans, a low daily caloric intake is associated 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, B-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 AβPP transgenic mice and reduce Aβ deposition in the brain by reducing isoprostane levels [141].

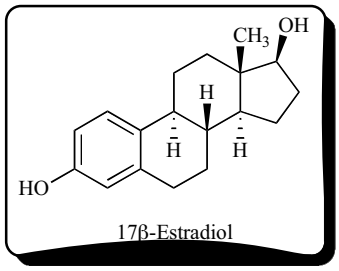
A significant amount of research with dietary vitamins E and C has been done in humans. In a multicenter, double blind, placebo-controlled study on 341 patients with moderately severe AD, a daily dose of approximately 1350 mg (2000 IU) vitamin E led to a slight delay of AD progression, providing the 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 ERα and ERβ [149]. At the same time, in a manner more closely related to direct antioxidants, estrogen can have antioxidant activity independent of estrogen receptors. The structure of estrogen is similar to β-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].



17β-Estradiol

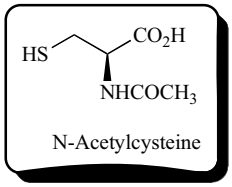
The optimum concentration of estrogen needed to achieve the antioxidant effect is worth consideration. Normally, estrogen is present in nanomolar concentrations *in vivo* and, in most *in vitro* studies, estrogen's antioxidant effect is achieved at a significantly higher concentration range of 1-10 μmoles. 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 postmenopausal 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

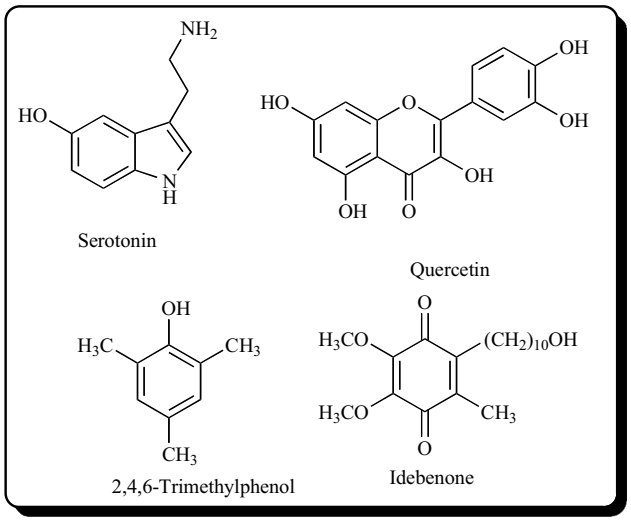
GSH, the most abundant intracellular non-protein thiol, is the main factor which directly quenches free radicals *in vivo*. It has been shown that the level of GSH is decreased in cortical areas and in the hippocampus of patients with AD as compared with controls [158-160]. The level of GSH in red blood 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.



Other Direct Antioxidants

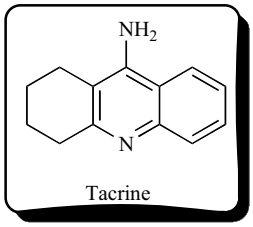
Compounds such as serotonin (5-hydroxytryptamine), flavonoids, quercetin, and simple alkylphenols have been shown to prevent membrane lipid peroxidation and protect neuronal cells against oxidative cell death *in vitro* [163,164].



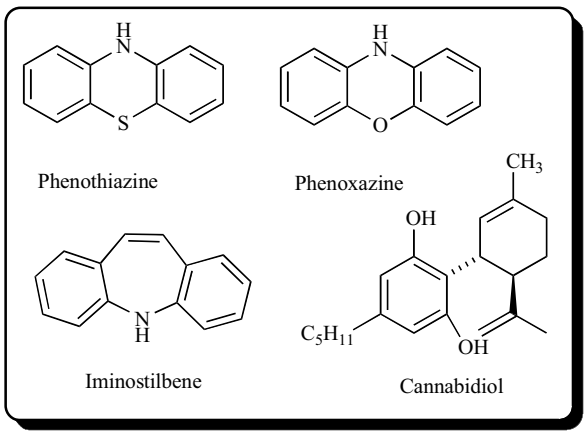
2,4,6-trimethylphenol (TMP) is also a potent antioxidant [151]. Additionally, being a small compound, TMP would readily cross the blood-brain barrier, thus meeting the most critical requirement for drugs used in the treatment of neurodegenerative disease. The protective potential of this compound is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



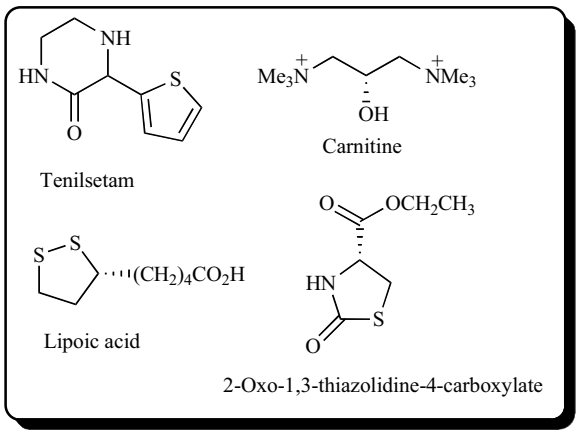
Other aromatic amino and imino compounds (e.g., phenothiazine, phenoxazine, iminostilbene) represent another group of direct antioxidants. Aromatic amines and imines are effective against oxidative glutamate toxicity, GSH depletion, and H_2O_2 toxicity in different cell culture systems [173]. With half-maximal 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, methioninine 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 by-product 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 antioxidant 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 bioavailablility to different cellular compartments.

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