Published in final edited form as:

J Alzheimers Dis. 2010 ; 20(Suppl 2): S633–S643. doi:10.3233/JAD-2010-100507.

Mitochondria and Antioxidant Targeted Therapeutic Strategies for Alzheimer's Disease

Magali Dumont, Michael T. Lin, and M. Flint Beal*

Weill Cornell Medical College, Department of Neurology and Neuroscience, New York, NY, USA

Abstract

Oxidative stress and mitochondrial dysfunction are important features present in Alzheimer's disease (AD). They appear early and contribute to disease progression, both in human postmortem AD brains as well as in transgenic AD mouse brains. For this reason, targeting oxidative stress and mitochondria in AD may lead to the development of promising therapeutic strategies. Several exogenous antioxidant compounds have been tested and found beneficial in transgenic AD mice, such as vitamins and spices. However, their efficacy was much more modest in human trials. More recently, new strategies have been elaborated to promote endogenous antioxidant systems. Different pathways involved in oxidative stress response have been identified. Compounds able to upregulate these pathways are being generated and tested in animal models of AD and in human patients. Upregulation of antioxidant gene expression was beneficial in mice, giving hope for future avenues in the treatment of AD and other neurodegenerative disorders.

Keywords

Alzheimer's disease; antioxidants; mitochondria; oxidative stress; therapeutic strategies; transgenic mice

Oxidative Stress and Mitochondrial Dysfunction In Alzheimer's Disease

Increased oxidative stress

Alzheimer's disease (AD) pathogenesis includes elevated oxidative damage. In human AD brains, DNA, RNA, lipid and protein oxidation are increased in the cortex and the hippocampus. For example, levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) [1] and nitrotyrosine [2] are markedly augmented in AD patients compared to normal control patients. Oxidative stress markers have been considered as biomarkers for disease progression. Studies reported that levels of F₂-isoprostane in the cerebrospinal fluid (CSF) of AD patients were consistently increased compared to normal control patients [3–5]. Increased oxidative stress occurs at early stages of amyloid deposition [2]. Similarly, patients with mild cognitive impairment (MCI) have elevated DNA [6], RNA [7], and protein oxidation [8], as well as lipid peroxidation [9,10]. MCI is an intermediate state between normal aging and AD, in which there is cognitive impairment not yet severe enough to impair normal daily functioning [11]. MCI is a heterogeneous state [12], but most cases likely represent very early stages of AD, since the risk of progression to AD is increased compared to those without any cognitive impairment [13], and since the pathology

in many cases is that of mild AD [14]. Both AD and MCI data strongly suggest that oxidative stress is involved early in the disease pathogenesis.

In transgenic mouse models of AD, markers of protein oxidation and lipid peroxidation were also increased in cortex and hippocampus. This was observed prior to the appearance of amyloid plaques [15,16] and neurofibrillary tangles [17]. There is also evidence that oxidative stress can exacerbate AD pathogenesis. In human neuroblastoma cells expressing the human wild-type amyloid- β protein precursor (A β PP) transgene, administration of both peroxynitrite and FeCl₂, two potent oxidative stress inducers, augmented the secretion of A β ₁₋₄₂ and the expression of β -site A β PP-cleaving enzyme (BACE) [18].

Impaired mitochondrial functions

Mitochondria play a key role in the viability and functionality of cells by generating and scavenging free radicals. In AD, mitochondrial function is impaired [19]. Hirai and colleagues found that in AD brains, neurons with increased oxidative damage had altered mitochondrial DNA and cytochrome oxidase levels [20]. In addition, expression of several proteins involved in mitochondrial fission and fusion were affected in postmortem AD brains, leading to abnormal redistribution of mitochondria [21,22]. Wang and colleagues replicated this phenomenon in both M17 human neuroblastoma cells and primary neuronal cells by manipulation of proteins such as dynamin related protein 1 (Drp1) [22].

Mitochondria are particularly important at synapses. They actively participate in synapse formation and function. Considering that loss of synapses is the best correlate of dementia in AD [23], it is crucial to understand by which mechanisms mitochondria are involved. Li and colleagues reported that after manipulations of Drp1 and optic atrophy 1 (OPA1), reducing dendritic mitochondria content caused synaptic loss. Conversely, increasing dendritic mitochondrial content or mitochondrial activity increased the number and plasticity of spines and synapses [24].

At a young age and prior to the appearance of amyloid deposition, transgenic AD mice displayed increased levels of H₂O₂ and decreased levels of cytochrome oxidase [25]. Another group reported that in young transgenic AD mice, there was a reduction of mitochondrial membrane potential and of ATP levels [26]. In this study, mitochondrial dysfunction was associated with increased production of reactive oxygen species (ROS) [26]. Recently, Yao and colleagues confirmed that deficits in mitochondrial bioenergetics precede amyloid deposition, as evidenced by decreased mitochondrial respiration and pyruvate dehydrogenase levels and activity in transgenic mice with human A β PP, presenilin 1 (PS1), and tau mutations [27]. Taken together, these data strongly suggest that mitochondrial dysfunction occurs early in disease pathogenesis.

Several other mitochondrial key enzymes have been studied in the context of AD. In this review, we will focus our discussion on the α -ketoglutarate dehydrogenase complex (α -KGDHC) and the manganese superoxide dismutase (MnSOD). First, activity of α -KGDHC is reduced in human postmortem AD brain [28–31]. Furthermore, levels of its subunits α -ketoglutarate dehydrogenase (E1; EC 1.2.4.2) and dihydrolipoyl succinyltransferase (E2; EC 2.3.1.61 or DLST) are also diminished in brains of patients bearing the Swedish A β PP mutation KM670/671NL [32]. Recently, our group reported that both thiamine deficiency [33] and dihydrolipoyl succinyltransferase deficiency [34] exacerbated oxidative stress and increased amyloid plaque deposition in transgenic AD mice. In addition, deficiency of dihydrolipoyl succinyltransferase induced spatial memory deficits in young transgenic AD animals [34].

MnSOD is another important mitochondrial enzyme. It catalyzes the dismutation of superoxide to H₂O₂, which is then decomposed to water via catalase or glutathione

peroxidase. In transgenic AD mice, partial deficiency of MnSOD increased amyloid plaque deposition [35] and tau phosphorylation [36]. It also accelerated the onset of behavioral abnormalities in transgenic AD mice [37]. Conversely, overexpression of MnSOD is protective. We showed that MnSOD overexpression in transgenic A β PP mice reduced protein oxidation, amyloid plaque deposition, and microgliosis. It also rescued memory impairment and synaptic protein levels [38]. Our data were recently confirmed by Massaad and colleagues, who found that in another transgenic AD mouse, MnSOD overexpression decreased hippocampal superoxide levels and amyloid plaques and improved memory deficit at various ages [39].

Other studies have also provided evidence to demonstrate the importance of mitochondria in AD pathogenesis. For example, Cyclophilin D (CypD), a matrix component of the mitochondrial permeability transition pore, has been recently examined. Deficiency of CypD reduced mitochondrial, cognitive, and synaptic dysfunction but did not affect amyloid deposition in transgenic AD mice [40,41].

Exogenous Antioxidant Drugs As Therapeutic Strategies In Alzheimer's Disease

Transgenic mouse models

One well known therapeutic approach related to oxidative stress is the administration of direct antioxidant drugs. Supplementation with vitamin E was used in transgenic AD mice for its ability to reduce ROS. Authors found that vitamin E reduced lipid peroxidation in both young and aged mice, but reduced plaque burden only when the drug was administered at early ages [42]. In combination with vitamin C, vitamin E reduced memory deficits but did not affect amyloid deposition in transgenic mice with both A β PP and PS1 mutations [43]. Interestingly, genetic depletion in vitamin E in transgenic AD mice resulted in increased lipid peroxidation, and amyloid deposition and oligomerization by affecting A β clearance [44].

Melatonin is another potent antioxidant drug used in transgenic AD animal models [45]. Melatonin receptors affect mechanisms of learning and memory in mice, especially electrophysiological processes such as long-term potentiation [46]. In transgenic AD mice, administration of melatonin reduced oxidative stress and pro-apoptotic markers. It also elevated levels of SOD and glutathione, two mitochondrial enzymes involved in free radical scavenging [47]. Long term administration of melatonin improved cognitive performance and reduced amyloid deposition in transgenic AD mice [48]. Another study showed that melatonin treatment in mice injected with A β protofibrils reduced ROS and intracellular calcium levels [49].

Spices are other sources of antioxidants. Curcumin in particular has been widely used to reduce oxidative stress. In transgenic AD mice, low and high doses of curcumin decreased oxidized proteins, soluble and insoluble A β , as well as amyloid plaques [50]. *In vitro* and *in vivo*, curcumin also reduced A β aggregation [51]. As a possible mechanism for plaque clearance, curcumin can bind A β and increase A β uptake from macrophages [52]. Interestingly, short term administration of curcumin partially restored distorted neurites in transgenic AD mice [53]. Several other natural antioxidants have been studied in the context of AD therapy, such as blueberry [54] and red grape [55–57], particularly for their content of resveratrol. Fruit extracts and resveratrol itself have shown beneficial effects including lowering plaque burden and improving behavioral deficits in transgenic AD mice [58] possibly via AMP-activated protein kinase activation pathway [59].

Human patients

We turn now to a selective discussion of exogenous antioxidant drugs in human trials to treat or prevent AD or MCI. It should be noted at the outset that as yet no therapy, antioxidant or otherwise, has been shown to reverse, arrest, or even change the slope of decline in human trials.

One of the best studied antioxidants in AD is vitamin E (α -tocopherol). In 1997, the Alzheimer's Disease Cooperative Study (ADCS) published a double-blind, placebo-controlled, randomized, multicenter trial of deprenyl and vitamin E [60]. Deprenyl inhibits the free-radical generating degradation of catecholamines by monoamine oxidase B. A total of 341 patients with AD of moderate severity were assigned to placebo, deprenyl, vitamin E, or both, and followed for an average of 2 years. Unfortunately, the randomization failed, and at baseline the placebo group was significantly better cognitively than the other groups. No benefit of either deprenyl or vitamin E was seen without adjusting for this baseline difference. Beneficial delays in disease progression with vitamin E or deprenyl were seen when analyses were adjusted for baseline cognition, but the need for statistical adjustment leaves some doubt. This trial was similar to the earlier DATATOP (deprenyl and tocopherol antioxidative therapy of Parkinson's) trial, in which 800 subjects with Parkinson's disease were randomized to the same treatments, and the time to disability requiring L-dopa therapy was measured [61]. No motor benefit was seen for vitamin E, and it remains unclear whether the benefit seen with deprenyl was due to augmentation of dopamine rather than an antioxidant effect. There was no effect of either deprenyl or vitamin E on cognitive performance in early PD [62]. A subsequent ADCS trial of vitamin E in MCI showed no benefit on risk of progression to AD [63]. A total of 769 subjects randomly received vitamin E, the cholinesterase inhibitor donepezil, or placebo, and were followed for 3 years. Donepezil reduced the risk of AD for 1 year in all patients, and for all 3 years in subjects with an apolipoprotein E $\epsilon 4$ allele, with a trend toward slowing of hippocampal atrophy [64]. In contrast, there were no significant differences in rate of progression to AD between the vitamin E and placebo groups at any time point, either among all patients or among apolipoprotein E $\epsilon 4$ carriers.

It is possible that vitamin E did not have an effect in AD or MCI because of kinetic issues. For an antioxidant to be effective, it must react with oxidants faster than the oxidants react with endogenous targets. The second order rate constant for the reaction of vitamin E with most oxidants is not robustly greater than the rate constant of the oxidants with their targets, which is typically greater than $10^6 \text{ M}^{-1} \text{ s}^{-1}$. In addition, it is not known whether supplementation with vitamin E significantly increases brain levels, although levels in CSF are increased [65].

Another reason that vitamin E may not have had an effect in AD or MCI could be that oxidative stress may be most important early in disease pathogenesis. As noted above, vitamin E reduces amyloid levels and amyloid deposition in transgenic AD mice when started before plaque deposition, but not when started after plaques appear [42]. Even in clinically very mild AD (Clinical Dementia Rating 0.5), there are already sufficiently many plaques and tangles for a neuropathologic diagnosis of AD, and 50% decreases in entorhinal cortex layer II neurons [66,67]. This may be too late for antioxidants to be effective. Based on this hypothesis, antioxidants would be most efficacious in prevention.

In support of this hypothesis, several epidemiologic studies of antioxidant vitamin supplements suggest decreased risk of AD or dementia. In a prospective, population-based observational study of vitamin E and vitamin C use in 633 subjects [68], baseline use of any vitamins was determined for the preceding two weeks. After an average of 4.3 years, there were 91 incident cases of AD, none of which occurred in the 27 subjects who took vitamin E

at baseline or the 23 subjects who took vitamin C at baseline. In the Honolulu-Asia Aging Study [69], vitamin E and vitamin C use was determined in 3385 Japanese American men, and 3–10 years later cognitive status was classified as AD, vascular dementia, mixed/other dementia, low test scores without dementia, or cognitively intact. There was a significant protective effect of combined vitamin E and vitamin C for non-AD dementias but not for AD. However, among those without dementia, use of either vitamin E or vitamin C alone was associated with better cognitive performance. Similar results were found in the Canadian Study of Health and Aging [70].

In the Rotterdam study [71], dietary intake of vitamin E, vitamin C, β -carotene, and flavonoids from food sources was assessed in 5395 non-demented subjects who were then followed for an average of 6 years. The rate of incident AD was decreased by a factor of 0.82 for each 1 standard deviation increase in intake of vitamin C or vitamin E. Among current smokers, there were also significant protective effects for β -carotene and flavonoids. In the Nurses' Health Study [72], long term current users (14968 community-dwelling women) of vitamin E with vitamin C had significantly better global cognitive test scores than subjects who had never used either. In the Cache County study [73], vitamin E and vitamin C supplement use was determined in 4408 subjects, of whom 200 had prevalent AD, and in whom 104 incident AD cases were identified during follow-up. Combined use of vitamin E and vitamin C was associated with reduced AD prevalence and incidence. In follow-up from this study, increasing quartiles of vitamin C intake combined with vitamin E was associated with higher baseline Modified Mini-Mental State exam (3MS) scores, and the effect was stronger for food sources than for supplements [74].

Not all epidemiologic studies have shown positive results, though in general these studies have been smaller (< 1000 subjects). In the Monongahela Valley Independent Elders Survey of 1059 subjects [75], intake of antioxidant supplements (vitamins A, C, E, β -carotene, zinc, and selenium) was initially associated with better cognitive performance in univariate analyses. However, women and persons with higher levels of education were more likely to take antioxidants, and in multivariate analyses including age, gender, and education, there were no significant differences between antioxidant users and nonusers. In a prospective, community-based study of 815 non-demented subjects [76], vitamin E from food, but not from supplements, was associated with decreased incidence of AD in the highest quintile of vitamin E intake, a protective effect seen only among subjects not carrying an apolipoprotein E ϵ 4 allele. In the Washington Heights-Inwood Columbia Aging study of 980 non-demented subjects [77], intake of vitamin C and carotenes, or vitamin E in supplemental or dietary (nonsupplemental) forms, was not associated with decreased incidence of AD. In the Age-Related Eye Disease Study, participants were randomly assigned to receive daily antioxidants (vitamin C, vitamin E, β -carotene), zinc and copper, antioxidants plus zinc and copper, or placebo, and a cognitive battery was administered to 2166 elderly subjects after a median of 6.9 years of treatment [78]. There were no differences among the treatment groups in any of the cognitive tests. In the Women's Health Study [79], a double-blind, randomized, placebo-controlled trial of vitamin E in 39876 healthy women, 6377 women 65 years or older participated in a cognitive sub-study. There were no differences between treatment groups in global composite scores at the first or last time points or in mean cognitive change over time.

To complicate matters, there have recently been several large meta-analyses suggesting that high dose antioxidant vitamin supplementation may be associated with a slight increase in all-cause mortality. One such analysis, focused on vitamin E, combined 19 clinical trials (135967 participants) [80]. They found that in high dose vitamin E (> 400 IU/day) trials, the pooled all cause mortality risk difference was 39 per 10000 persons, whereas in low dose ($\leq$ 400 IU/day) trials, the risk difference was -16 per 10000. The Cochrane Hepato-Biliary

Group [81,82] attempted to analyze all randomized trials in adults involving β -carotene, vitamin A, vitamin C, vitamin E, and selenium. When all qualified randomized trials were included (68 trials, 232606 participants), there was no significant effect on mortality. However, in “low-bias” trials (180938 participants), antioxidant supplementation was associated with a slight (~5%) but statistically significant increase in mortality. Specifically, there were slight but statistically significant increases in all-cause mortality with β -carotene (~7% increase), vitamin A (~16% increase), and vitamin E (~4% increase).

One potential explanation for a slight increase in mortality might be that antioxidants may also act as pro-oxidants under the right circumstances. For example, vitamin C combined with ferrous iron is a standard free-radical generating system. An additional consideration is that antioxidant systems normally form a complex network. High dose supplementation with a single antioxidant vitamin in isolation could disrupt the balance of the network. The studies reviewed above, suggesting that antioxidant intake from food is superior to vitamin supplements, support the idea that the entire antioxidant network is important.

Several other antioxidant compounds have been examined in human AD trials with varying results. Idebenone is a water-soluble analog of ubiquinone. Early double-blind, placebo-controlled, randomized trials of idebenone in mild to moderate AD involved 92–450 subjects [83–85], and suggested dose-dependent, beneficial effects on cognition and slowing of disease progression for up to 2 years. Idebenone was better tolerated and associated with less deterioration than the cholinesterase inhibitor tacrine [86]. In contrast, the ADCS trial found no significant effect in 536 subjects randomized to placebo or 3 doses of idebenone [87]. There was a benefit in cognition when all 3 idebenone groups were combined, but this effect was deemed too small to be clinically significant.

Aggregation of A β and A β -induced free radicals are dependent in part on binding of Cu and Zn ions. In a pilot Phase 2 clinical trial, 36 subjects with moderate AD were randomized to placebo or clioquinol, which inhibits Cu and Zn ions from binding to A β [88]. Subjects more severely affected at baseline experienced significant worsening in the placebo group, compared with minimal deterioration in the clioquinol group. A subsequent trial of a second generation metal-binding compound, PBT2, involved 78 subjects with mild AD randomized to placebo or two doses of PBT2 [89]. Compared to placebo, the higher dose was associated with decreased CSF A β ₄₂ levels and improved performance in two tests of executive function.

The curry spice curcumin has antioxidant [50], anti-inflammatory [90], and amyloid-disaggregating properties [51] *in vitro* and in animal studies. In a small 6-month pilot trial [91], 34 subjects with AD were randomized to placebo or two doses of curcumin. There was no cognitive decline in the placebo group, and no improvement was observed with curcumin.

The Russian antihistamine latrepirdine (Dimebon) initially showed promise in a double-blind, placebo-controlled, randomized trial conducted in Russia, involving 183 subjects with mild to moderate AD [92]. At 6 months and 1 year, subjects on latrepirdine were significantly better than those in the placebo group with respect to all key outcomes – cognition, activities of daily living, behavior, and overall function. Latrepirdine also improved MMSE scores in a trial of Huntington's disease [93]. The mechanism of these effects is obscure, but have been proposed to include interaction with glutamate receptors [94], blockade of voltage-dependent calcium channels [95], and inhibition of the mitochondrial permeability transition pore [96]. However, the more recent CONNECTION trial [97], based in the US, Europe, and South America, showed no benefit for latrepirdine in any parameter [98].

In summary, antioxidant/mitochondrial-based therapies have presented a mixed picture. Many have not proved significantly effective in treatment of AD or MCI, though a few show promise. Efficacy may be greater in prevention of AD and reducing risk of cognitive decline with aging. Based on the studies reviewed here, future trials of antioxidants are likely to be more successful if focused on prevention or on very early stages of disease, and if a broad increase in the entire network of antioxidant defense systems is targeted.

Facilitation of Endogenous Antioxidant Systems As Therapeutic Strategies In Alzheimer's Disease

Another important avenue for the treatment of AD could be the facilitation of the endogenous antioxidant systems. In this review, we focus on a particular pathway involved in antioxidant and anti-inflammatory response, the nuclear factor erythroid-related factor 2/antioxidant response element (Nrf2/ARE) pathway. Nrf2 is a transcription factor, which in humans is encoded by the *NFE2L2* gene [99]. Nrf2 is a master regulator of the antioxidant response [100,101]. Its activity is regulated in part by the actin-associated protein Keap1. Keap 1 binds to Nrf2 and sequesters it in the cytoplasm. With oxidative stress, the binding is disrupted and Nrf2 is released, allowing translocation into the nucleus [100, 101]. Nrf2 can then bind to promoters with AREs, inducing the expression of genes that coordinate a cytoprotective response [102]. Many of these genes encode for mitochondrial antioxidant enzymes.

Transgenic mouse models

In transgenic AD mice, expression of Nrf2 and Nrf2/ARE regulated genes are reduced as amyloid deposition progressed [103]. The same group reported that facilitation of Nrf2 expression by both *tert*-butylhydroquinone (tBHQ) and overexpression of Nrf2 through adenovirus-mediated gene delivery protected against A β ₁₋₄₂-induced cell death in hippocampal cells *in vitro* [103]. This protection was associated with an increase of Nrf2/ARE regulated genes [103]. More recently, Kanninen and colleagues reported that intrahippocampal injection of a lentiviral vector expressing Nrf2 in A β PP/PS1 mice reduced spatial learning deficits, soluble A β , and astrogliosis. These mice also had upregulated mRNA levels of Nrf2 and heme oxygenase-1 [104]. Taken together, these data suggest that induction of endogenous antioxidant pathways could represent a promising therapeutic approach for AD [105].

Synthetic triterpenoids (TPs), derivatives of 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), have been identified as potent pharmacological inducers of the Nrf2/ARE signaling pathway *in vitro* and *in vivo* [106,107]. TPs also suppress inflammatory stress [108]. It should be noted that inflammation is an important factor as it may exacerbate oxidative stress; activated microglia may be a source of ROS in addition to neurons. Induction of the Nrf2/ARE pathway by TPs is due to disruption of the binding between Nrf2 and Keap1, allowing Nrf2 to translocate to the nucleus [107]. For these reasons, we investigated the effect of triterpenoid CDDO-methylamide (CDDO-MA) in transgenic AD mice, and found that administration of CDDO-MA improved spatial memory retention. It also reduced plaque burden, levels of A β ₄₂, inflammation, and oxidative stress [109]. Thus, the development of new drugs able to facilitate the Nrf2/ARE pathway represents a key approach for the treatment of AD.

Other endogenous pathways involved in mitochondrial function and oxidative stress have been investigated in the context of AD. In human postmortem AD brain, peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α) expression decreases as dementia progresses [110]. PGC-1 α is an important transcription cofactor that specifically

regulates genes involved in energy metabolism. Its activation is dependent on various insults including the generation of ROS. PGC-1 α interacts with the nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR- γ), which permits the interaction of this protein with multiple transcription factors. In addition, this transcription factor may influence A β PP-related gene transcription, such as BACE1 [111].

Thus, PPAR- γ agonists have been studied as potential therapeutics for AD treatment [112]. Pioglitazone and rosiglitazone, two thiazolidinediones (TZDs), selectively stimulate PPAR- γ . Rosiglitazone is also a selective ligand of PPAR- γ . Primarily used to treat diabetes, these drugs have now been administered to transgenic AD mice. After treating aged transgenic AD mice with pioglitazone, cerebrovascular functions were restored and oxidative stress was reduced [113]. Interestingly, rosiglitazone was found to stimulate neuronal mitochondrial biogenesis in an apolipoprotein E isoform-independent manner, by inducing both mitochondrial DNA and estrogen-stimulated related receptor alpha mRNA [114]. More recently, it has been shown that chronic administration of rosiglitazone reduced memory deficit in transgenic AD mice [115].

Human patients

Considering the beneficial effects of PPAR- γ agonists in mouse models of AD, several human trials have been conducted. In a pilot trial, 30 subjects with MCI or early AD were randomized to rosiglitazone ($n = 20$) or placebo ($n = 10$), and at 4 and 6 months, subjects receiving rosiglitazone performed better on tests of delayed recall [116]. However, in a larger trial with 511 subjects with mild to moderate AD, no statistically significant difference was observed between placebo and any of 3 doses of rosiglitazone [117], though exploratory subgroup analyses suggested that apolipoprotein E $\epsilon 4$ noncarriers did improve on the highest dose of rosiglitazone. This is reminiscent of the apolipoprotein E isoform-independent induction of mitochondrial biogenesis in transgenic mice [114]. In a small, open-label, controlled trial, 32 subjects with mild to moderate AD and diabetes mellitus were randomized to receive pioglitazone or not, and followed for 6 months [118]. In the pioglitazone group, there were improvements in AD Assessment Scale-Cognitive subscale scores, as well as in Wechsler Memory Scale-Revised Logical Memory performance. Pioglitazone also improved regional cerebral blood flow in the parietal lobes [119], which are typically hypometabolic in AD.

These studies clearly require replication in larger trials, but facilitators of endogenous antioxidant systems hold promise. It is also clear that other new pathways should be investigated in order to promote a more robust endogenous antioxidant response that can counteract the early increased oxidative stress in AD.

Conclusion

The literature on the importance of mitochondria in neurodegenerative disorders is abundant. In AD, oxidative stress and mitochondrial dysfunction are present early in disease pathogenesis. They also contribute actively to disease progression. Mitochondria and antioxidant targeted therapeutic strategies have been heavily studied in AD mouse models as well as human trials. However, most direct antioxidant drugs beneficial in mouse models of AD have not proved as successful in human trials, though some show promise. There may be several reasons for this. First, it should be noted that most transgenic models of AD are in fact models of amyloid deposition, and do not capture all the complexity of the human disease. Second, antioxidant and mitochondrial-based therapies are likely to be most successful when used in very early stage disease, or even for prevention. Advances in this direction will require better identification of disease at earlier stages, ideally even before clinical symptoms. Third, use of single isolated antioxidant agents may not be sufficient to

provide benefit, and could potentially even be deleterious. Recent studies suggest that facilitation of the entire endogenous antioxidant network may produce more robust effects.

Acknowledgments

This work was supported by National Institute of Health (NIH) grants AG20729 and AG14930.

References

1. Moreira PI, Nunomura A, Nakamura M, Takeda A, Shenk JC, Aliev G, Smith MA, Perry G. Nucleic acid oxidation in Alzheimer disease. *Free Radic Biol Med*. 2008; 44:1493–1505. [PubMed: 18258207]
2. 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; 60:759–767. [PubMed: 11487050]
3. de Leon MJ, Mosconi L, Li J, De Santi S, Yao Y, Tsui WH, Pirraglia E, Rich K, Javier E, Brys M, Glodzik L, Switalski R, Saint Louis LA, Pratico D. Longitudinal CSF isoprostane and MRI atrophy in the progression to AD. *J Neurol*. 2007; 254:1666–1675. [PubMed: 17994313]
4. Montine TJ, Quinn J, Kaye J, Morrow JD. F(2)-isoprostanes as biomarkers of late-onset Alzheimer's disease. *J Mol Neurosci*. 2007; 33:114–119. [PubMed: 17901555]
5. Ringman JM, Younkin SG, Pratico D, Seltzer W, Cole GM, Geschwind DH, Rodriguez-Agudelo Y, Schaffer B, Fein J, Sokolow S, Rosario ER, Gyls KH, Varpertian A, Medina LD, Cummings JL. Biochemical markers in persons with preclinical familial Alzheimer disease. *Neurology*. 2008; 71:85–92. [PubMed: 18509095]
6. Lovell MA, Markesbery WR. Oxidative DNA damage in mild cognitive impairment and late-stage Alzheimer's disease. *Nucleic Acids Res*. 2007; 35:7497–7504. [PubMed: 17947327]
7. Lovell MA, Markesbery WR. Oxidatively modified RNA in mild cognitive impairment. *Neurobiol Dis*. 2008; 29:169–175. [PubMed: 17920285]
8. Butterfield DA, Reed TT, Perluigi M, De Marco C, Coccia R, Keller JN, Markesbery WR, Sultana R. Elevated levels of 3-nitrotyrosine in brain from subjects with amnesic mild cognitive impairment: implications for the role of nitration in the progression of Alzheimer's disease. *Brain Res*. 2007; 1148:243–248. [PubMed: 17395167]
9. 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; 59:972–976. [PubMed: 12056933]
10. Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, Markesbery WR. Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology*. 2005; 64:1152–1156. [PubMed: 15824339]
11. Petersen RC, Roberts RO, Knopman DS, Boeve BF, Geda YE, Ivnik RJ, Smith GE, Jack CR Jr. Mild cognitive impairment: ten years later. *Arch Neurol*. 2009; 66:1447–1455. [PubMed: 20008648]
12. Jicha GA, Parisi JE, Dickson DW, Johnson K, Cha R, Ivnik RJ, Tangalos EG, Boeve BF, Knopman DS, Braak H, Petersen RC. Neuropathologic outcome of mild cognitive impairment following progression to clinical dementia. *Arch Neurol*. 2006; 63:674–681. [PubMed: 16682537]
13. Petersen RC, Doody R, Kurz A, Mohs RC, Morris JC, Rabins PV, Ritchie K, Rossor M, Thal L, Winblad B. Current concepts in mild cognitive impairment. *Arch Neurol*. 2001; 58:1985–1992. [PubMed: 11735772]
14. Morris JC, Storandt M, Miller JP, McKeel DW, Price JL, Rubin EH, Berg L. Mild cognitive impairment represents early-stage Alzheimer disease. *Arch Neurol*. 2001; 58:397–405. [PubMed: 11255443]
15. Pratico D, Uryu K, Leight S, Trojanowski JQ, Lee VM. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. *J Neurosci*. 2001; 21:4183–4187. [PubMed: 11404403]

16. Abdul HM, Sultana R, St Clair DK, Markesbery WR, Butterfield DA. Oxidative damage in brain from human mutant APP/PS-1 double knock-in mice as a function of age. *Free Radic Biol Med*. 2008; 45:1420–1425. [PubMed: 18762245]
17. Resende R, Moreira PI, Proenca T, Deshpande A, Busciglio J, Pereira C, Oliveira CR. Brain oxidative stress in a triple-transgenic mouse model of Alzheimer disease. *Free Radic Biol Med*. 2008; 44:2051–2057. [PubMed: 18423383]
18. Quiroz-Baez R, Rojas E, Arias C. Oxidative stress promotes JNK-dependent amyloidogenic processing of normally expressed human APP by differential modification of alpha-, beta- and gamma-secretase expression. *Neurochem Int*. 2009; 55:662–670. [PubMed: 19560504]
19. Chen X, Stern D, Yan SD. Mitochondrial dysfunction and Alzheimer's disease. *Curr Alzheimer Res*. 2006; 3:515–520. [PubMed: 17168650]
20. Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PL, Jones PK, Petersen RB, Perry G, Smith MA. Mitochondrial abnormalities in Alzheimer's disease. *J Neurosci*. 2001; 21:3017–3023. [PubMed: 11312286]
21. Cho DH, Nakamura T, Fang J, Cieplak P, Godzik A, Gu Z, Lipton SA. S-nitrosylation of Drp1 mediates beta-amyloid-related mitochondrial fission and neuronal injury. *Science*. 2009; 324:102–105. [PubMed: 19342591]
22. Wang X, Su B, Lee HG, Li X, Perry G, Smith MA, Zhu X. Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. *J Neurosci*. 2009; 29:9090–9103. [PubMed: 19605646]
23. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol*. 1991; 30:572–580. [PubMed: 1789684]
24. Li Z, Okamoto K, Hayashi Y, Sheng M. The importance of dendritic mitochondria in the morphogenesis and plasticity of spines and synapses. *Cell*. 2004; 119:873–887. [PubMed: 15607982]
25. Manczak M, Anekonda TS, Henson E, Park BS, Quinn J, Reddy PH. Mitochondria are a direct site of A beta accumulation in Alzheimer's disease neurons: implications for free radical generation and oxidative damage in disease progression. *Hum Mol Genet*. 2006; 15:1437–1449. [PubMed: 16551656]
26. Hauptmann S, Scherping I, Drose S, Brandt U, Schulz KL, Jendrach M, Leuner K, Eckert A, Muller WE. Mitochondrial dysfunction: an early event in Alzheimer pathology accumulates with age in AD transgenic mice. *Neurobiol Aging*. 2009; 30:1574–1586. [PubMed: 18295378]
27. Yao J, Irwin RW, Zhao L, Nilsen J, Hamilton RT, Brinton RD. Mitochondrial bioenergetic deficit precedes Alzheimer's pathology in female mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2009; 106:14670–14675. [PubMed: 19667196]
28. Gibson GE, Blass JP, Beal MF, Bunik V. The alpha-ketoglutarate-dehydrogenase complex: a mediator between mitochondria and oxidative stress in neurodegeneration. *Mol Neurobiol*. 2005; 31:43–63. [PubMed: 15953811]
29. Gibson GE, Zhang H, Sheu KF, Bogdanovich N, Lindsay JG, Lannfelt L, Vestling M, Cowburn RF. Alpha-ketoglutarate dehydrogenase in Alzheimer brains bearing the APP670/671 mutation. *Ann Neurol*. 1998; 44:676–681. [PubMed: 9778267]
30. Butterworth RF, Besnard AM. Thiamine-dependent enzyme changes in temporal cortex of patients with Alzheimer's disease. *Metab Brain Dis*. 1990; 5:179–184. [PubMed: 2087217]
31. Mastrogiovanni F, Bergeron C, Kish SJ. Brain alpha-ketoglutarate dehydrogenase complex activity in Alzheimer's disease. *J Neurochem*. 1993; 61:2007–2014. [PubMed: 8245957]
32. Gibson GE, Sheu KF, Blass JP, Baker A, Carlson KC, Harding B, Perrino P. Reduced activities of thiamine-dependent enzymes in the brains and peripheral tissues of patients with Alzheimer's disease. *Arch Neurol*. 1988; 45:836–840. [PubMed: 3395256]
33. Karuppagounder SS, Xu H, Shi Q, Chen LH, Pedrini S, Pechman D, Baker H, Beal MF, Gandy SE, Gibson GE. Thiamine deficiency induces oxidative stress and exacerbates the plaque pathology in Alzheimer's mouse model. *Neurobiol Aging*. 2009; 30:1587–1600. [PubMed: 18406011]
34. Dumont M, Ho DJ, Calingasan NY, Xu H, Gibson G, Beal MF. Mitochondrial dihydrolipoyl succinyltransferase deficiency accelerates amyloid pathology and memory deficit in a transgenic

- mouse model of amyloid deposition. *Free Radic Biol Med*. 2009; 47:1019–1027. [PubMed: 19596066]
35. Li F, Calingasan NY, Yu F, Mauck WM, Toidze M, Almeida CG, Takahashi RH, Carlson GA, Flint Beal M, Lin MT, Gouras GK. Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice. *J Neurochem*. 2004; 89:1308–1312. [PubMed: 15147524]
 36. Melov S, Adlard PA, Morten K, Johnson F, Golden TR, Hinerfeld D, Schilling B, Mavros C, Masters CL, Volitakis I, Li QX, Laughton K, Hubbard A, Cherny RA, Gibson B, Bush AI. Mitochondrial oxidative stress causes hyperphosphorylation of tau. *PLoS ONE*. 2007; 2:e536. [PubMed: 17579710]
 37. Esposito L, Raber J, Kekonius L, Yan F, Yu GQ, Bien-Ly N, Puolivali J, Scearce-Levie K, Masliah E, Mucke L. Reduction in mitochondrial superoxide dismutase modulates Alzheimer's disease-like pathology and accelerates the onset of behavioral changes in human amyloid precursor protein transgenic mice. *J Neurosci*. 2006; 26:5167–5179. [PubMed: 16687508]
 38. Dumont M, Wille E, Stack C, Calingasan NY, Beal MF, Lin MT. Reduction of oxidative stress, amyloid deposition, and memory deficit by manganese superoxide dismutase overexpression in a transgenic mouse model of Alzheimer's disease. *FASEB J*. 2009; 23:2459–2466. [PubMed: 19346295]
 39. Massaad CA, Washington TM, Pautler RG, Klann E. Overexpression of SOD-2 reduces hippocampal superoxide and prevents memory deficits in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2009; 106:13576–13581. [PubMed: 19666610]
 40. Du H, Guo L, Fang F, Chen D, Sosunov AA, McKhann GM, Yan Y, Wang C, Zhang H, Molkentin JD, Gunn-Moore FJ, Vonsattel JP, Arancio O, Chen JX, Yan SD. Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. *Nat Med*. 2008; 14:1097–1105. [PubMed: 18806802]
 41. Du H, Guo L, Zhang W, Rydzewska M, Yan S. Cyclophilin D deficiency improves mitochondrial function and learning/memory in aging Alzheimer disease mouse model. *Neurobiol Aging*. 2009 in press.
 42. Sung S, Yao Y, Uryu K, Yang H, Lee VM, Trojanowski JQ, Pratico D. Early vitamin E supplementation in young but not aged mice reduces Abeta levels and amyloid deposition in a transgenic model of Alzheimer's disease. *FASEB J*. 2004; 18:323–325. [PubMed: 14656990]
 43. Harrison FE, Allard J, Bixler R, Usuh C, Li L, May JM, McDonald MP. Antioxidants and cognitive training interact to affect oxidative stress and memory in APP/PSEN1 mice. *Nutr Neurosci*. 2009; 12:203–218. [PubMed: 19761651]
 44. Nishida Y, Ito S, Ohtsuki S, Yamamoto N, Takahashi T, Iwata N, Jishage K, Yamada H, Sasaguri H, Yokota S, Piao W, Tomimitsu H, Saido TC, Yanagisawa K, Terasaki T, Mizusawa H, Yokota T. Depletion of vitamin E increases amyloid beta accumulation by decreasing its clearances from brain and blood in a mouse model of Alzheimer disease. *J Biol Chem*. 2009; 284:33400–33408. [PubMed: 19679659]
 45. Hardeland R. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine*. 2005; 27:119–130. [PubMed: 16217125]
 46. Larson J, Jessen RE, Uz T, Arslan AD, Kurtuncu M, Imbesi M, Manev H. Impaired hippocampal long-term potentiation in melatonin MT2 receptor-deficient mice. *Neurosci Lett*. 2006; 393:23–26. [PubMed: 16203090]
 47. Feng Z, Qin C, Chang Y, Zhang JT. Early melatonin supplementation alleviates oxidative stress in a transgenic mouse model of Alzheimer's disease. *Free Radic Biol Med*. 2006; 40:101–109. [PubMed: 16337883]
 48. Olcese JM, Cao C, Mori T, Mamcarz MB, Maxwell A, Runfeldt MJ, Wang L, Zhang C, Lin X, Zhang G, Arendash GW. Protection against cognitive deficits and markers of neurodegeneration by long-term oral administration of melatonin in a transgenic model of Alzheimer disease. *J Pineal Res*. 2009; 47:82–96. [PubMed: 19538338]
 49. Masilamoni JG, Jesudason EP, Dhandayuthapani S, Ashok BS, Vignesh S, Jebaraj WC, Paul SF, Jayakumar R. The neuroprotective role of melatonin against amyloid beta peptide injected mice. *Free Radic Res*. 2008; 42:661–673. [PubMed: 18654881]

50. Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J Neurosci.* 2001; 21:8370–8377. [PubMed: 11606625]
51. Yang F, Lim GP, Begum AN, Ubeda OJ, Simmons MR, Ambegaokar SS, Chen PP, Kaye R, Glabe CG, Frautschy SA, Cole GM. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid *in vivo*. *J Biol Chem.* 2005; 280:5892–5901. [PubMed: 15590663]
52. Zhang L, Fiala M, Cashman J, Sayre J, Espinosa A, Mahanian M, Zaghi J, Badmaev V, Graves MC, Bernard G, Rosenthal M. Curcuminoids enhance amyloid-beta uptake by macrophages of Alzheimer's disease patients. *J Alzheimers Dis.* 2006; 10:1–7. [PubMed: 16988474]
53. Garcia-Alloza M, Borrelli LA, Rozkalne A, Hyman BT, Bacskai BJ. Curcumin labels amyloid pathology *in vivo*, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model. *J Neurochem.* 2007; 102:1095–1104. [PubMed: 17472706]
54. Joseph JA, Denisova NA, Arendash G, Gordon M, Diamond D, Shukitt-Hale B, Morgan D. Blueberry supplementation enhances signaling and prevents behavioral deficits in an Alzheimer disease model. *Nutr Neurosci.* 2003; 6:153–162. [PubMed: 12793519]
55. Ho L, Chen LH, Wang J, Zhao W, Talcott ST, Ono K, Teplow D, Humala N, Cheng A, Percival SS, Ferruzzi M, Janle E, Dickstein DL, Pasinetti GM. Heterogeneity in red wine polyphenolic contents differentially influences Alzheimer's disease-type neuropathology and cognitive deterioration. *J Alzheimers Dis.* 2009; 16:59–72. [PubMed: 19158422]
56. Ono K, Condrón MM, Ho L, Wang J, Zhao W, Pasinetti GM, Teplow DB. Effects of grape seed-derived polyphenols on amyloid beta-protein self-assembly and cytotoxicity. *J Biol Chem.* 2008; 283:32176–32187. [PubMed: 18815129]
57. Wang J, Ho L, Zhao Z, Seror I, Humala N, Dickstein DL, Thiagarajan M, Percival SS, Talcott ST, Pasinetti GM. Moderate consumption of Cabernet Sauvignon attenuates Abeta neuropathology in a mouse model of Alzheimer's disease. *FASEB J.* 2006; 20:2313–2320. [PubMed: 17077308]
58. Karuppagounder SS, Pinto JT, Xu H, Chen HL, Beal MF, Gibson GE. Dietary supplementation with resveratrol reduces plaque pathology in a transgenic model of Alzheimer's disease. *Neurochem Int.* 2009; 54:111–118. [PubMed: 19041676]
59. Vingtdoux V, Giliberto L, Zhao H, Chandakkar P, Wu Q, Simon JE, Janle EM, Lobo J, Ferruzzi MG, Davies P, Marambaud P. AMP-activated Protein Kinase Signaling Activation by Resveratrol Modulates Amyloid- β Peptide Metabolism. *J Biol Chem.* 285:9100–9113. [PubMed: 20080969]
60. Sano M, Ernesto C, Thomas RG, Klauber MR, Schafer K, Grundman M, Woodbury P, Growdon J, Cotman CW, Pfeiffer E, Schneider LS, Thal LJ. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *N Engl J Med.* 1997; 336:1216–1222. [PubMed: 9110909]
61. Shoulson I. DATATOP: a decade of neuroprotective inquiry. Parkinson Study Group. Deprenyl And Tocopherol Antioxidative Therapy Of Parkinsonism. *Ann Neurol.* 1998; 44:S160–166. [PubMed: 9749589]
62. Kieburtz K, McDermott M, Como P, Growdon J, Brady J, Carter J, Huber S, Kanigan B, Landow E, Rudolph A, et al. The effect of deprenyl and tocopherol on cognitive performance in early untreated Parkinson's disease. Parkinson Study Group. *Neurology.* 1994; 44:1756–1759. [PubMed: 7936311]
63. Parnetti L, Ambrosoli L, Abate G, Azzini C, Balestreri R, Bartorelli L, Bordin A, Crepaldi G, Cristianini G, Cucinotta D, et al. Positively for the treatment of late-onset Alzheimer's disease: a double-blind multicentre study vs citicoline and ascorbic acid. *Acta Neurol Scand.* 1995; 92:135–140. [PubMed: 7484061]
64. Jack CR Jr, Petersen RC, Grundman M, Jin S, Gamst A, Ward CP, Sencakova D, Doody RS, Thal LJ. Longitudinal MRI findings from the vitamin E and donepezil treatment study for MCI. *Neurobiol Aging.* 2008; 29:1285–1295. [PubMed: 17452062]
65. Vataassery GT, Fahn S, Kuskowski MA. Alpha tocopherol in CSF of subjects taking high-dose vitamin E in the DATATOP study. Parkinson Study Group. *Neurology.* 1998; 50:1900–1902. [PubMed: 9633757]

66. Gomez-Isla T, Price JL, McKeel DW Jr, Morris JC, Growdon JH, Hyman BT. Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *J Neurosci*. 1996; 16:4491–4500. [PubMed: 8699259]
67. Price JL, Ko AI, Wade MJ, Tsou SK, McKeel DW, Morris JC. Neuron number in the entorhinal cortex and CA1 in preclinical Alzheimer disease. *Arch Neurol*. 2001; 58:1395–1402. [PubMed: 11559310]
68. Morris MC, Beckett LA, Scherr PA, Hebert LE, Bennett DA, Field TS, Evans DA. Vitamin E and vitamin C supplement use and risk of incident Alzheimer disease. *Alzheimer Dis Assoc Disord*. 1998; 12:121–126. [PubMed: 9772012]
69. Masaki KH, Losonczy KG, Izmirlian G, Foley DJ, Ross GW, Petrovitch H, Havlik R, White LR. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. *Neurology*. 2000; 54:1265–1272. [PubMed: 10746596]
70. Maxwell CJ, Hicks MS, Hogan DB, Basran J, Ebly EM. Supplemental use of antioxidant vitamins and subsequent risk of cognitive decline and dementia. *Dement Geriatr Cogn Disord*. 2005; 20:45–51. [PubMed: 15832036]
71. Engelhart MJ, Geerlings MI, Ruitenberg A, van Swieten JC, Hofman A, Witteman JC, Breteler MM. Dietary intake of antioxidants and risk of Alzheimer disease. *JAMA*. 2002; 287:3223–3229. [PubMed: 12076218]
72. Grodstein F, Chen J, Willett WC. High-dose antioxidant supplements and cognitive function in community-dwelling elderly women. *Am J Clin Nutr*. 2003; 77:975–984. [PubMed: 12663300]
73. Zandi PP, Anthony JC, Khachaturian AS, Stone SV, Gustafson D, Tschanz JT, Norton MC, Welsh-Bohmer KA, Breitner JC. Reduced risk of Alzheimer disease in users of antioxidant vitamin supplements: the Cache County Study. *Arch Neurol*. 2004; 61:82–88. [PubMed: 14732624]
74. Wengreen HJ, Munger RG, Corcoran CD, Zandi P, Hayden KM, Fotuhi M, Skoog I, Norton MC, Tschanz J, Breitner JC, Welsh-Bohmer KA. Antioxidant intake and cognitive function of elderly men and women: the Cache County Study. *J Nutr Health Aging*. 2007; 11:230–237. [PubMed: 17508099]
75. Mendelsohn AB, Belle SH, Stoehr GP, Ganguli M. Use of antioxidant supplements and its association with cognitive function in a rural elderly cohort: the MoVIES Project. Monongahela Valley Independent Elders Survey. *Am J Epidemiol*. 1998; 148:38–44. [PubMed: 9663402]
76. 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; 287:3230–3237. [PubMed: 12076219]
77. Luchsinger JA, Tang MX, Shea S, Mayeux R. Antioxidant vitamin intake and risk of Alzheimer disease. *Arch Neurol*. 2003; 60:203–208. [PubMed: 12580704]
78. Yaffe K, Clemons TE, McBee WL, Lindblad AS. Impact of antioxidants, zinc, and copper on cognition in the elderly: a randomized, controlled trial. *Neurology*. 2004; 63:1705–1707. [PubMed: 15534261]
79. Kang JH, Cook N, Manson J, Buring JE, Grodstein F. A randomized trial of vitamin E supplementation and cognitive function in women. *Arch Intern Med*. 2006; 166:2462–2468. [PubMed: 17159011]
80. Miller ER 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med*. 2005; 142:37–46. [PubMed: 15537682]
81. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA*. 2007; 297:842–857. [PubMed: 17327526]
82. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst Rev*. 2008:CD007176. [PubMed: 18425980]
83. Bergamasco B, Scarzella L, La Commare P. Idebenone, a new drug in the treatment of cognitive impairment in patients with dementia of the Alzheimer type. *Funct Neurol*. 1994; 9:161–168. [PubMed: 7988944]

84. 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]
85. 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; 54:301–310. [PubMed: 9850939]
86. 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; 35:12–18. [PubMed: 11819153]
87. 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; 61:1498–1502. [PubMed: 14663031]
88. Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Mastwyk M, MacGregor L, Kiers L, Cherny R, Li QX, Tammer A, Carrington D, Mavros C, Volitakis I, Xilinas M, Ames D, Davis S, Beyreuther K, Tanzi RE, Masters CL. Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. *Arch Neurol*. 2003; 60:1685–1691. [PubMed: 14676042]
89. Lannfelt L, Blennow K, Zetterberg H, Batsman S, Ames D, Harrison J, Masters CL, Targum S, Bush AI, Murdoch R, Wilson J, Ritchie CW. Safety, efficacy, and biomarker findings of PBT2 in targeting Abeta as a modifying therapy for Alzheimer's disease: a phase IIa, double-blind, randomised, placebo-controlled trial. *Lancet Neurol*. 2008; 7:779–786. [PubMed: 18672400]
90. Frautschy SA, Hu W, Kim P, Miller SA, Chu T, Harris-White ME, Cole GM. Phenolic anti-inflammatory antioxidant reversal of Abeta-induced cognitive deficits and neuropathology. *Neurobiol Aging*. 2001; 22:993–1005. [PubMed: 11755008]
91. Baum L, Lam CW, Cheung SK, Kwok T, Lui V, Tsoh J, Lam L, Leung V, Hui E, Ng C, Woo J, Chiu HF, Goggins WB, Zee BC, Cheng KF, Fong CY, Wong A, Mok H, Chow MS, Ho PC, Ip SP, Ho CS, Yu XW, Lai CY, Chan MH, Szeto S, Chan IH, Mok V. Six-month randomized, placebo-controlled, double-blind, pilot clinical trial of curcumin in patients with Alzheimer disease. *J Clin Psychopharmacol*. 2008; 28:110–113. [PubMed: 18204357]
92. Doody RS, Gavrilova SI, Sano M, Thomas RG, Aisen PS, Bachurin SO, Seely L, Hung D. Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study. *Lancet*. 2008; 372:207–215. [PubMed: 18640457]
93. Kieburtz K, McDermott MP, Voss TS, Corey-Bloom J, Deuel LM, Dorsey ER, Factor S, Geschwind MD, Hodgeman K, Kayson E, Noonberg S, Pourfar M, Rabinowitz K, Ravina B, Sanchez-Ramos J, Seely L, Walker F, Feigin A. A randomized, placebo-controlled trial of latrepirdine in Huntington disease. *Arch Neurol*. 2010; 67:154–160. [PubMed: 20142523]
94. Grigorev VV, Dranyi OA, Bachurin SO. Comparative study of action mechanisms of dimebon and memantine on AMPA- and NMDA-subtypes glutamate receptors in rat cerebral neurons. *Bull Exp Biol Med*. 2003; 136:474–477. [PubMed: 14968164]
95. Lermontova NN, Redkozubov AE, Shevtsova EF, Serkova TP, Kireeva EG, Bachurin SO. Dimebon and tacrine inhibit neurotoxic action of beta-amyloid in culture and block L-type Ca(2+) channels. *Bull Exp Biol Med*. 2001; 132:1079–1083. [PubMed: 11865327]
96. Bachurin SO, Shevtsova EP, Kireeva EG, Oxenkrug GF, Sablin SO. Mitochondria as a target for neurotoxins and neuroprotective agents. *Ann N Y Acad Sci*. 2003; 993:334–344. discussion 345–339. [PubMed: 12853325]
97. ClinicalTrials.gov. A safety and efficacy study of oral Dimebon in patients with mild-to-moderate Alzheimer's disease (CONNECTION). July 14. 2009
<http://clinicaltrials.gov/ct2/show/NCT00675623>
98. Alzheimer Research Forum. Dimebon disappoints in Phase 3 trial. March 4. 2010
<http://www.alzforum.org/new/detail.asp?id=2387>
99. Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. *Proc Natl Acad Sci U S A*. 1994; 91:9926–9930. [PubMed: 7937919]

100. Li W, Kong AN. Molecular mechanisms of Nrf2-mediated antioxidant response. *Mol Carcinog*. 2009; 48:91–104. [PubMed: 18618599]
101. Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J Biol Chem*. 2009; 284:13291–13295. [PubMed: 19182219]
102. Kang KW, Lee SJ, Kim SG. Molecular mechanism of nrf2 activation by oxidative stress. *Antioxid Redox Signal*. 2005; 7:1664–1673. [PubMed: 16356128]
103. Kanninen K, Malm TM, Jyrkkanen HK, Goldsteins G, Keksa-Goldsteine V, Tanila H, Yamamoto M, Yla-Herttuala S, Levonen AL, Koistinaho J. Nuclear factor erythroid 2-related factor 2 protects against beta amyloid. *Mol Cell Neurosci*. 2008; 39:302–313. [PubMed: 18706502]
104. Kanninen K, Heikkinen R, Malm T, Rolova T, Kuhmonen S, Leinonen H, Yla-Herttuala S, Tanila H, Levonen AL, Koistinaho M, Koistinaho J. Intrahippocampal injection of a lentiviral vector expressing Nrf2 improves spatial learning in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2009; 106:16505–16510. [PubMed: 19805328]
105. van Muiswinkel FL, Kuiperij HB. The Nrf2-ARE Signalling pathway: promising drug target to combat oxidative stress in neurodegenerative disorders. *Curr Drug Targets CNS Neurol Disord*. 2005; 4:267–281. [PubMed: 15975029]
106. Liby K, Hock T, Yore MM, Suh N, Place AE, Risingsong R, Williams CR, Royce DB, Honda T, Honda Y, Gribble GW, Hill-Kapturczak N, Agarwal A, Sporn MB. The synthetic triterpenoids, CDDO and CDDO-imidazolide, are potent inducers of heme oxygenase-1 and Nrf2/ARE signaling. *Cancer Res*. 2005; 65:4789–4798. [PubMed: 15930299]
107. Liby KT, Yore MM, Sporn MB. Triterpenoids and rexinoids as multifunctional agents for the prevention and treatment of cancer. *Nat Rev Cancer*. 2007; 7:357–369. [PubMed: 17446857]
108. Dinkova-Kostova AT, Liby KT, Stephenson KK, Holtzclaw WD, Gao X, Suh N, Williams C, Risingsong R, Honda T, Gribble GW, Sporn MB, Talalay P. Extremely potent triterpenoid inducers of the phase 2 response: correlations of protection against oxidant and inflammatory stress. *Proc Natl Acad Sci U S A*. 2005; 102:4584–4589. [PubMed: 15767573]
109. Dumont M, Wille E, Calingasan NY, Tampellini D, Williams C, Gouras GK, Liby K, Sporn M, Nathan C, Flint Beal M, Lin MT. Triterpenoid CDDO-methylamide improves memory and decreases amyloid plaques in a transgenic mouse model of Alzheimer's disease. *J Neurochem*. 2009; 109:502–512. [PubMed: 19200343]
110. Qin W, Haroutunian V, Katsel P, Cardozo CP, Ho L, Buxbaum JD, Pasinetti GM. PGC-1alpha expression decreases in the Alzheimer disease brain as a function of dementia. *Arch Neurol*. 2009; 66:352–361. [PubMed: 19273754]
111. Rossner S, Sastre M, Bourne K, Lichtenthaler SF. Transcriptional and translational regulation of BACE1 expression – implications for Alzheimer's disease. *Prog Neurobiol*. 2006; 79:95–111. [PubMed: 16904810]
112. Landreth G, Jiang Q, Mandrekar S, Heneka M. PPARgamma agonists as therapeutics for the treatment of Alzheimer's disease. *Neurotherapeutics*. 2008; 5:481–489. [PubMed: 18625459]
113. Nicolakakis N, Aboukassim T, Ongali B, Lecrux C, Fernandes P, Rosa-Neto P, Tong XK, Hamel E. Complete rescue of cerebrovascular function in aged Alzheimer's disease transgenic mice by antioxidants and pioglitazone, a peroxisome proliferator-activated receptor gamma agonist. *J Neurosci*. 2008; 28:9287–9296. [PubMed: 18784309]
114. Strum JC, Shehee R, Virley D, Richardson J, Mattie M, Selley P, Ghosh S, Nock C, Saunders A, Roses A. Rosiglitazone induces mitochondrial biogenesis in mouse brain. *J Alzheimers Dis*. 2007; 11:45–51. [PubMed: 17361034]
115. Escribano L, Simon AM, Perez-Mediavilla A, Salazar-Colocho P, Del Rio J, Frechilla D. Rosiglitazone reverses memory decline and hippocampal glucocorticoid receptor down-regulation in an Alzheimer's disease mouse model. *Biochem Biophys Res Commun*. 2009; 379:406–410. [PubMed: 19109927]
116. Watson GS, Cholerton BA, Reger MA, Baker LD, Plymate SR, Asthana S, Fishel MA, Kulstad JJ, Green PS, Cook DG, Kahn SE, Keeling ML, Craft S. Preserved cognition in patients with early Alzheimer disease and amnesic mild cognitive impairment during treatment with rosiglitazone: a preliminary study. *Am J Geriatr Psychiatry*. 2005; 13:950–958. [PubMed: 16286438]

117. Risner ME, Saunders AM, Altman JF, Ormandy GC, Craft S, Foley IM, Zvartau-Hind ME, Hosford DA, Roses AD. Efficacy of rosiglitazone in a genetically defined population with mild-to-moderate Alzheimer's disease. *Pharmacogenomics J.* 2006; 6:246–254. [PubMed: 16446752]
118. Hanyu H, Sato T, Kiuchi A, Sakurai H, Iwamoto T. Pioglitazone improved cognition in a pilot study on patients with Alzheimer's disease and mild cognitive impairment with diabetes mellitus. *J Am Geriatr Soc.* 2009; 57:177–179. [PubMed: 19170800]
119. Sato T, Hanyu H, Hirao K, Kanetaka H, Sakurai H, Iwamoto T. Efficacy of PPAR-gamma agonist pioglitazone in mild Alzheimer disease. *Neurobiol Aging.* 2009 in press.