Journal of Neuropathology and Experimental Neurology (2006) 65 (7):631-641.

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Running Title: Oxidative Stress in Alzheimer's disease

Number of text pages: 50 Figures: 2 Tables: 5

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ACKNOWLEDGMENT

Work in the authors' laboratories is supported by funding from Japan Society for the Promotion of Science.

ABSTRACT

Genetic and lifestyle-related risk factors for Alzheimer disease (AD) are associated with an increase in oxidative stress, suggesting that oxidative stress is involved at an early stage of the pathologic cascade. Moreover, oxidative stress is mechanistically and chronologically associated with other key features of AD, namely, metabolic, mitochondrial, metal, and cell-cycle abnormalities. Contrary to the commonly held notion that pathological hallmarks of AD signify etiology, several lines of evidence now indicate that aggregation of amyloid-β and tau is a compensatory response to underlying oxidative stress. Therefore, removal of proteinaceous accumulations may treat the epiphenomenon rather than the disease and may actually enhance oxidative damage. Although some antioxidants have been shown to reduce the incidence of AD, the magnitude of the effect may be modified by individual factors such as genetic predisposition (e.g. apolipoprotein E genotype) and habitual behaviors. Because calorie restriction, exercise, and intellectual activity have been experimentally shown to promote neuronal survival through an enhancement of endogenous antioxidant defenses, a combination of dietary regimen of low total calorie and rich antioxidant nutrients and maintaining physical and intellectual activities may ultimately prove to be one of the most efficacious strategies for AD prevention.

Key Words: Alzheimer disease, antioxidant, mild cognitive impairment, oxidative stress, prevention, risk factor, therapy

INTRODUCTION

The process of neurodegeneration in Alzheimer disease (AD) is a dynamic, multifaceted biochemical phenomenon and is essentially lifelong. Symptomatic disease, on the other hand, although more easily recognized and more easily diagnosed pathologically, represents the end stage. The simple fact that senile plaques and neurofibrillary tangles (NFTs) can be seen with a light microscope should not imply etiology, yet the primacy of these lesions in terms of disease pathogenic theories, and the *de facto* reliance on them to assess animal models and response to therapy, suggests otherwise. Too little attention is paid to early, presymptomatic biochemistry, both in terms of furthering knowledge of pathogenesis and targeting therapy. Targeting measurable but manifestly end-stage lesions such as senile plaques and NFTs may not only be ineffective, but may accelerate the disease.

Oxidative stress, on the other hand, has been shown in numerous studies to precede the cardinal neuropathologic manifestations of AD. Oxidative modifications are further seen in cerebral tissue in early stages of AD, whereas oxidation of nucleic acids, proteins, and lipids are all prominent and early changes in AD, and oxidatively modified macromolecules are present in biologic fluids from the patients with AD as well as cellular and animal AD models (reviewed in Ref. 1).

Accompanying oxidative insult are attempts at apoptosis and regeneration, both as early phenomena before the formation of senile plaques and NFTs. The attempt at apoptosis is incomplete or "abortive" (2), and this makes mechanistic sense given the evanescent time interval over which apoptosis occurs in general. Similarly, attempts at regeneration by diseased neurons, or cell-cycle re-entry, which is a pervasive and early change in the AD brain (3, 4), is ineffective and likely predisposes diseased neurons to further degeneration.

Evidence is accumulating that the end-stage lesions in AD, namely senile plaques and NFTs, are a fundamental adaptive, rather than deleterious, response. The adaptive nature is evidenced by sequestration of redox-active metals by senile plaques and intraneuronal inclusions (5-9), and the increasing recognition that inclusion-bearing neurons are generally protected from immediate cell death (10-12). In the context of chronic neurodegeneration in AD, therefore, the idea of removal of end-stage lesions as a viable treatment option remains dubious in our view, whereas intervention that targets fundamental early changes such as oxidative stress and aberrant cell-cycle reentry is manifestly more logical.

GENES AND RISK FACTORS ASSOCIATED WITH ALZHEIMER DISEASE ARE INTIMATELY IMPLICATED IN OXIDATIVE STRESS

The prevalence of AD increases exponentially throughout aging with approximately half of

the population afflicted by the age of 95 (13), suggesting that advanced age is a major risk factor for AD. Like in other organ systems, cells in the brain encounter a cumulative burden of oxidative and metabolic stress that may be a universal feature of the aging process as well as a major causal factor of senescence. Moreover, the brain is especially vulnerable to free radical damage because of its high content of easily peroxidizable unsaturated fatty acids, high oxygen consumption rate (accounting for 20-25% of the total body oxygen consumption, but for less than 2% of the total body weight), and relative paucity of antioxidant enzymes compared with other organs (e.g. the content of catalase in brain is only 10-20% of liver and heart) (14-16).

Germline mutations leading to familial, early-onset AD are also oxidative stress-causing mutations. Elevated vulnerability to oxidative stress-induced cell death and /or reduced antioxidant defenses have been demonstrated in: (i) cell lines expressing mutant human amyloid β protein precursor (AβPP), presenilin-1 (PS-1), or presenilin-2 (PS-2) (17-20); (ii) transgenic mice expressing mutant human AβPP and/or PS-1 as well as knock in mice expressing mutant human PS-1 (21-28); (iii) fibroblasts and lymphoblasts from patients with familial AD with AβPP or PS-1 gene mutations (29); and (iv) cerebral cortex of autopsied brain samples from patients with AβPP or PS-1 gene mutations (30, 31). Moreover, the possession of one or both apolipoprotein E (*APOE*) ε4 alleles, a major genetic risk factor for late-onset familial and sporadic AD (32), is associated with oxidative stress. *In vitro*, *APOE* shows allele specific

antioxidant activity, with *APOE* ε2 the most effective and *APOE* ε4 being the least effective (33). Oxidative damage in *APOE* genotype-dependent manner has been further demonstrated in autopsy brain samples of AD (34-36).

Medical risk factors for AD include traumatic brain injury, stroke, hypertension, diabetes mellitus, hypercholesterolemia, hyperhomocysteinemia (37-41). Environmental and lifestyle-related risk factors for AD include aluminum exposure, smoking, high calorie intake, lack of exercise, and lack of intellectual activities (42-45). As it turns out, all these risk factors are associated with an increase in production and propagation of reactive oxygen species and/or a decrease in endogenous antioxidant capacity (14, 46-51). Similarly, nutrients or agents inhibiting free radical formation reduce the incidence of AD. This includes not only the free radical scavenging vitamins C and E (52), but also estrogen, non-steroidal anti-inflammatory drugs, statins, omega-3 polyunsaturated fatty acids, and red wine (53-57), all of which show antioxidant activities (58-62). Furthermore, calorie restriction, exercise, and intellectual activity have been shown to promote neuronal survival through an enhancement of endogenous antioxidant-defenses in experimental animals (14, 63).

Germline mutations and risk factors for AD that cause or promote oxidative damage as well as agents, nutrients, and behavior that prevent or attenuate oxidative damage are summarized in Table 1. Overall, the data indicates that oxidative stress is intimately associated with the

pathogenesis of AD and acts "upstream" of the pathologic process.

OXIDATIVE STRESS IS AN EARLY AND PROMINENT FEATURE OF ALZHEIMER DISEASE

The early involvement of oxidative stress in AD is demonstrated by recent studies on cell culture models, transgenic animal models, post-mortem brains, and biological fluids from subjects with AD, mild cognitive impairment (MCI), and Down syndrome (Tables 2 and 3). Using an *in situ* approach to identify markers of nucleic acid and protein oxidation in human brain, we found that oxidative damage is more pronounced in AD subjects with lesser amounts of Aβ deposition or AD subjects with shorter disease duration (64). Furthermore, we found that oxidative damage precedes Aβ deposition in brain tissue from Down syndrome, a putative model of AD with predictable neuropathologic progression over time (65). These findings are consistent with the increased nucleic acid oxidation in cerebrospinal fluid from AD subjects, in which the shorter the disease duration, the greater the oxidative damage (66). Moreover, individuals with MCI or very mild AD show: (i) increased levels of lipid peroxidation and nucleic acid oxidation in postmortem brain tissue (67, 68) (Fig. 1); (ii) increased levels of lipid peroxidation and nucleic acid oxidation in cerebrospinal fluid, plasma, urine and peripheral leukocytes (69, 70); and (iii) decreased levels of plasma antioxidants and total plasma antioxidant capacity (71, 72).

Up-regulation of heme-oxygenase-1, a sensitive marker of oxidative stress, is observed in astroglial cells in postmortem brains of AD and MCI (73).

The human data is supported by the experimental studies using cell culture and transgenic animal models of AD. Increased lipid peroxidation and protein oxidation and decreased copper/zinc superoxide dismutase (SOD) activity precede Aβ plaque deposition or Aβ fibril formation in transgenic mouse and *C.elegans* models of AD amyloidosis (23, 74, 75). Oxidative stress induces intracellular Aβ accumulation and tau phosphorylation in cell cultures (76-78), and vitamin E reduces Aβ and tau lesions in transgenic animals (79, 80). Furthermore, dietary copper stabilizes brain copper/zinc SOD activity and reduces Aβ production in AβPP transgenic mice (81). AβPP mutant mice crossed with manganese SOD heterozygous knockout mice show increased Aβ plaque deposition in brain (82). The early involvement of oxidative stress in the pathological cascade of AD is likely to be closely associated with other key features of AD such as metabolic dysfunction, mitochondrial dysfunction, metal dysregulation, and cell-cycle dysreguration (reviewed in Ref. 83). In fact, all these features are observed as early-stage events of AD, which are shown in human (3, 84-86) and transgenic animal models (4, 87-89). In short, oxidative stress not only is an upstream event but also has been shown to play a fundamental, direct role in the pathogenesis of AD by a variety of mechanisms.

THE PATHOLOGY OF ALZHEIMER DISEASE MAY REPRESENT A RESPONSE TO OXIDATIVE STRESS

Neuropathologic assessment is a means of definitive diagnosis of patients with dementia, and establishes benchmarks by which models of neurodegenerative diseases are validated. Nevertheless, because the diagnosis of AD rests in the semi-quantitative *association* of lesions with a known clinical phenotype (rather than the *presence or absence* of lesions), it remains an open question whether the basic pathology of AD represents effect rather than cause, or response to injury rather than injury *per se*. Likewise, evidence that neurodegenerative inclusions are epiphenomenal, or even protective, is accumulating. In a cellular model of Huntington disease, a neurodegenerative disorder caused by an abnormal polyglutamine expansion, the formation of neuronal intranuclear inclusions was associated with improved neuronal survival and decreased levels of mutant *huntingtin* protein throughout the neuron (12). Similarly, recent studies indicate that toxic oligomers or protofibrils of Aβ are responsible for cell death, and that the fibrillar Aβ in typical amyloid plaques may actually be neuroprotective (reviewed in Ref. 90, 91). These findings further support our previous studies demonstrating the sequestration of heavy metals by pathological inclusions (5, 7-9), and the general concept that neurodegenerative disease pathology fundamentally represents the response to the disease process as is the case for other amyloids.

It is noteworthy that senile plaques and NFTs are present in a considerable percentage of brains of cognitively normal elderly subjects, often in large numbers. A study investigating autopsied subjects aged between 69 and 100 who were cognitively intact revealed that 49% of those normal subjects met the Khachaturian criteria for AD based on senile plaque density, 25% met the CERAD criteria for "definite" AD based on senile plaque density, and 24% were Braak stages IV-VI (92). Although uncommon, the presence of Braak stage VI AD in occasional cognitively intact elderly individuals should highlight the limited role AD pathology plays in etiology (92). Furthermore, the poor correlation between neuronal loss and senile plaque density as well as between disease severity and senile plaque density in AD is well known (93). Accordingly, AβPP transgenic mice showing massive deposition of Aβ plaques in brains lack consistent, widespread neuronal loss (94). By contrast, neuronal loss and clinical severity correlate with NFT density, with the amount of neuronal loss exceeding the amount of NFTs (95, 96). In a tau transgenic mouse, in which the overexpression of mutant human tau can be regulated by tetracycline, turning off tau expression halts neuronal loss and reverses of memory defects. However, surprisingly, in this model, NFTs continue to accumulate (97). This is consistent with a report on transgenic mice expressing nonmutant human tau, in which neuronal death occurs independently of NFT formation (4). In an ultrastructural study of human tissue, a reduction in number and total length of microtubules in pyramidal neurons in AD was unrelated

to the presence of NFTs (98). In contrast, neurons with NFTs are estimated to be able to survive for decades (10), indicating that NFTs themselves are not obligatory for neuronal death in AD. Given these data, and that oxidative stress precedes structural (pathologic) alterations, it appears more and more that the pathology is not only nonetiologic, but a compensatory phenomenon to the fundamental oxidative injury that drives neuronal death in AD (Fig. 2).

Although high concentration of Aβ in a micromolar range can lead to oxidative stress in various biologic systems (reviewed in Ref. 99), it is apparent from cell (76, 77), animal (23, 74, 75, 80-82), and human (65) studies that oxidative stress chronologically precedes Aβ deposition. Moreover, increased density of Aβ plaque deposition is associated with decreased levels of nucleic acid oxidation in neurons in postmortem brains from patients with sporadic and familial AD and Down syndrome (30, 64, 65). These findings indicate that the process of Aβ plaque formation is linked to neuronal protection against oxidative stress. Recently, *in vitro* and *in vivo* studies have demonstrated an antioxidant activity of Aβ. Monomeric $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ has been shown to protect cultured neurons from iron and copper induced toxicity (100). In concordance with this, coinjection of iron and $A\beta_{1\text{-}42}$ into rat cerebral cortex is significantly less toxic than injection of iron alone (101). Furthermore, addition of physiological concentrations (in a low nanomolar range) of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ has been shown to protect lipoproteins from oxidation in cerebrospinal fluid and plasma (99). These Aβ peptides fail to prevent metal-independent

oxidation and $A\beta_{25-35}$ lacking the metal binding site located in N-terminal domain (histidine at positions 6, 13, and 14, and tyrosine at position 10) is less effective at inhibiting oxidation. Therefore, it is likely that the mechanism by which Aβ inhibits oxidation is through chelating metal ions (99). Indeed, copper, iron, and zinc are elevated in the rims and cores of Aβ plaques in postmortem brains of AD (6, 9). We propose that chelation of redox-active copper and iron is a most important mechanism of the protective function of Aβ, and that elevation of zinc, a redox-inert antioxidant, may be a homeostatic response to oxidative stress, which subsequently accelerates the formation of Aβ plaques (102).

The findings that Aβ actually plays an important role in antioxidant defenses may equally apply to tau. Cellular (78), animal (79), and human (65) studies suggest that oxidative stress chronologically precedes NFT formation. Oxidative stress activates several kinases including glycogen synthase kinase-3 and mitogen-activated protein kinases, which are activated in AD and are capable of phosphorylating tau. Once phosphorylated, tau becomes particularly vulnerable to oxidative modification and consequently aggregates into fibrils (reviewed in Ref. 103-105). Therefore, NFT formation is likely to be a result of neuronal oxidation, which is accompanied by an induction of an antioxidant enzyme heme oxygenese-1 (105). In fact, in postmortem brains of AD, neuronal oxidative damage to nucleic acid is actually decreased by the presence of NFTs when neurons with NFTs are compared to neurons free of NFTs (64). Although

the exact mechanism how the NFT formation opposes to oxidative stress is unknown, redox-active iron accumulation is strikingly associated with NFTs (5) and tau is found capable of binding to iron and copper by which it may exert antioxidant activities (8).

As we have reviewed here, the disease-specific proteins, Aβ and tau, potentially play a protective role against oxidative stress. However, the efficiency of the protective function may be dependent on aggregation state of the protein (100). Recently, an increasing body of evidence has been collected to support the hypothesis that oligomers, not monomers or fibrils, are the toxic fraction of the protein (reviewed in Ref. 90, 91). Therefore, further study is required to adequately assess the relationship between oxidative stress and oligomer formation, which may provide an important clue to successful early therapeutic intervention in AD.

ANTIOXIDANT STRATEGY FOR ALZHEIMER DISEASE: LESSONS FROM VITAMIN E STUDIES

Approaches to treatment during different stages of AD can be divided as primary prevention for asymptomatic population, secondary prevention for subjects with MCI, and symptomatic treatment for patients with AD (106). Antioxidant defenses are classified as three groups, namely, (i) the preventive antioxidants such as SOD, glutathione peroxidase, and metal chelating proteins, (ii) the radical-scavenging antioxidants such as vitamins C and E, and (iii) the

repair and de novo enzymes such as lipase, protease, and DNA repair enzymes (107). A metal chelating agent, clioquinol (108) and radical-scavenging antioxidants (52, 109-111) have been proposed for reducing the risk of AD and/or slowing the progression of AD. In particular, vitamin E has been frequently tested by prospective epidemiologic studies and clinical trials, and the data for vitamin E are presently available through primary and secondary prevention (52, 110-114) and symptomatic treatment for AD (109).

Vitamin E is the most important lipid-soluble chain-breaking natural antioxidant in mammalian cells and is able to cross the blood-brain barrier and accumulate at therapeutic levels in the brain, where it reduces lipid peroxidation (115). In a cross-sectional study of 4,809 elderly, decreasing serum levels of vitamin E per unit of cholesterol were consistently associated with increasing levels of poor memory, whereas serum levels of vitamins A and C, β-carotene, and selenium were not associated with poor memory performance (116). An association of vitamin E intake with better cognitive function in the elderly has been found in large cohort studies. In the Honolulu-Asia Aging Study of 3,385 men aged 71 to 93 years, supplementary intake of either vitamin C or E was associated with better cognitive function (117). The Chicago Health and Aging Project with samples of 2,889 community residents aged 65 to 102 years found that supplementary or dietary intake of vitamin E, but not vitamin C or carotenes, was inversely related to cognitive decline (118). In the Nurses' Health Study of 14,968 women aged 70 to 79

years, long-term and current users of supplements containing vitamins C and E had better cognitive function (119).

However, data from prospective studies relating intake of vitamin E and risk of AD are conflicting. Among recent prospective cohort studies summarized in Table 4, the Rotterdam study was performed with the largest sample size and found that dietary intake of vitamin E was associated with a low risk of AD (52). This association was more prominent among current smokers and did not vary by *APOE* genotype, excluding supplement users, or controlling for supplement use. The Chicago Health and Aging Project found that dietary, but not supplementary, intake of vitamin E was associated with a low risk of AD only among non-carriers for *APOE* ε4 allele (110). In the Washington Heights-Inwood Columbia Aging Project, however, no association was found between dietary or supplementary intake of vitamin E and a decreased risk of AD (112). Although the Cache Country Study also demonstrated no beneficial effect of supplementary intake of vitamin E alone on reduction in risk of AD, it found an association between a combined supplementary intake of vitamins C and E and a reduced risk of AD (111). The Honolulu-Asia Aging Study of elderly men with higher age range (71-93 years) found an association between a combined supplementary intake of vitamins C and E and a reduced risk of vascular dementia but not AD (117). The same study group also examined the midlife dietary intake of antioxidants and risk of late-life dementia, in which men aged 45 to 68 years at

enrollment were followed up for approximately 30 years. Dietary intake of vitamin C, vitamin E, β-carotene, or flavonoids was not associated with a reduced risk of dementia or its subtype (113). Only the Rotterdam study found a beneficial effect of dietary intake of vitamin C alone on reduction in risk of AD, which was more prominent among current smokers. In this study, β-carotene or flavonoids showed a similar effect only among current smokers (52). In summary, the results to date are really inconsistent. However, they may suggest the importance of having a balanced combination of several antioxidant nutrients to exert a significant effect on the prevention of AD. Intake of the nutrients from foods is preferable to that from supplements in some studies, indicating that a sufficient intake of a certain nutrient from food may reflect well-balanced intake of other nutrients that are important for its absorption and/or biological activity (120). Even if antioxidant nutrients are taken in an appropriate way, their effects may be modified by individual factors such as genetic predisposition (e.g. *APOE* genotype) and habitual behavior such as smoking. Recently, in a double blind, 3-year follow up study, high doses of vitamin E (2,000 IU per day) was reported to have no benefit in subjects with MCI (114). The lack of efficacy of vitamin E to prevent the progression of MCI to AD indicates that a single supplementary vitamin has no significant effect in the secondary prevention of AD, which is consistent with the previous cohort studies on the progression of the cognitively normal elderly to AD.

In contrast to the MCI trial, in a double-blind, placebo-controlled, randomized, multi-center 2-year study, 2,000 IU per day of vitamin E has been shown to slow progression in patients with moderate AD (109). Vitamin E significantly delayed the time to the primary outcome (death, institutionalization, loss of the ability to perform basic activities of daily living, or severe dementia), whereas it did not slow the rate of decline on cognitive functioning assessed by the Alzheimer Disease Assessment Scale or the Mini-Mental State Examination. Although not only vitamin E but also a selective monoamine oxidase B inhibitor, selegiline, or a combination of vitamin E and selegiline was found to have a similar beneficial effect in this study, vitamin E has been widely used for patients with AD because of its low cost and perceived safety, as current practice guidelines recommend (121, 122). However, the safety of high-dosage or long-term supplementation of vitamin E has recently been called into question. Indeed, a meta-analysis with vitamin E dosage varied between 16.5 and 2,000 IU per day and average follow-up period ranged from 1.4 to 8.2 years suggested that high-dosage (daily dose of 400 IU and over) of vitamin E supplements may increase all-cause mortality (123). Moreover, long-term supplementation (median follow-up period of 7.0 years) of vitamin E with daily dose of 400 IU may increases the risk for heart failure in patients with vascular disease or diabetes mellitus (124). Together with the increased all-cause mortality associated with high-dosages of β-carotene (125) and the increased risk of cardiovascular disease mortality with high-dosages of vitamin C

in postmenopausal women with diabetes (126), use of any high-dosage vitamin supplements should be discouraged until evidence of efficacy is documented from appropriately designed clinical trials. Of note, vitamins E and C, carotenoids, and flavonoids may loose their effectiveness as antioxidants or even act as prooxidants under certain circumstances *in vitro*, for example, at high concentration or high partial pressures of oxygen, in the presence of metal ions such as copper or iron, under mild oxidative condition without co-antioxidants, and at high concentration of carotenoid itself (127-130).

ANTIOXIDANT STRATEGY FOR ALZHEIMER DISEASE: TARGETING MODIFIABLE RISK FACTORS

When we consider the highly complex system for the fine regulation of cellular redox balance in human body, it is no wonder that extrinsic *in vitro* antioxidants may show only limited effects on reduction of oxidative damage in biologic systems. Therefore, we should survey ways to activate our intrinsic system to reduce oxidative damage, which might be effective in the retardation of disease progression, at least in subclinical and early-stage AD. If the process of Aβ deposition is closely associated with antioxidant function, as we have discussed in this review, this process will be activated during times when oxidative stress is high and the endogenous antioxidant defenses are compromised. On the other hand, if this system is efficient and/or is

supported by exogenous antioxidant supplementation, the antioxidant effects of Aβ may not be necessary. Indeed, several substances such as vitamin E, nonsteroidal antiinflammatory drugs, the metal chelator clioquinol, copper (stabilizing copper/zinc SOD), melatonin, omega-3 polyunsaturated fatty acid (docosahexaenoic acid), and the curry spice curcumin reduce the levels of Aβ and/or Aβ deposition in brains of transgenic animal model of AD (80, 81, 131-137) (Table 5). Because all these substances are antioxidants (59, 60, 81, 131-133), they constitute potential candidates in antioxidative strategy for treatment of AD. However, as we have learned from the vitamin E studies, each of them may only show limited clinical benefit despite their dramatic effect under experimental conditions.

It is well established that experimental animals on caloric restriction that lowers steady-state levels of oxidative stress show several signs of retarded aging (138, 139). Recently, the association between caloric intake and the risk of AD was analyzed (44, 140). Compared with individuals in the lowest quartile of total caloric intake or fat intake, those in the highest quartile had an increased risk of AD. This increased risk was significant only among individuals carrying *APOE* ε4 allele. The hazard ratios of AD for total caloric intake and fats intake are 2.27 and 2.31, respectively (44). Of note, caloric restriction attenuates Aβ deposition in AβPP transgenic mice, which is achieved by feeding 60% or 70% of the calories consumed by the pair-controlled ad libitum diet (141, 142). Indeed, it has been suggested that a daily calorie intake

in range of 1,800-2,200 for moderately active adults may dramatically reduce the risk of AD, Parkinson disease, and stroke (63).

A life style-related factor other than diet (i.e. inactivity) is considered to be a risk factor of AD. When activities were evaluated by using a scale in terms of "diversity" (total numbers of activities) and "intensity" (hours per month), the scores were significantly lower in passive diversity, intellectual diversity, and physical diversity as well as intellectual intensity in early and middle adulthood (from ages 20 to 60 years) of the patients with AD (45). In the Canadian Study of Health and Aging, a large-scale prospective cohort of 9,008 subjects aged 65 years or older, physical activity was associated with lower risks of cognitive impairment, AD and dementia of any types (143). Participation in leisure activities, including intellectual and physical activities, was associated with a reduced risk of development of both AD and vascular dementia in a prospective cohort of 469 subjects aged 75 years or older (144). In another large-scale prospective cohort of 3,375 subjects aged 65 years or older, an inverse association between physical activity and risk for AD or vascular dementia was identified among *APOE* ε4 noncarriers but not *APOE* ε4 carriers (145). This study also suggested that participating in a number of different activities may be as or more important than frequency, intensity, and duration of physical activity with respect to dementia risk. Exercise is effective not only in the stages of primary and secondary prevention, but also in symptomatic treatment for AD. A

randomized, controlled trial of 153 patients with AD demonstrated that exercise combined with teaching caregivers behavioral management techniques improved physical health and depression of patients with AD (146). In AβPP/PS-1 double transgenic mice, environmental enrichment reduced Aβ deposition, which was striking in enriched mice exhibiting high activity levels (percent time running) (147). Another transgenic model with double-mutant form of AβPP also showed reduced Aβ deposition by 5 months of voluntary exercise (148). Importantly, experimental studies indicate that an activation of an intrinsic antioxidant defense system is involved in the mechanisms by which caloric restriction, intellectual activity, and exercise promote neuronal survival. Each of them induces a mild cellular stress response, and consequently neurons respond to these stresses by activating signaling pathways that produce growth factors and protein chaperones, which is followed by the production of antioxidant enzymes such as copper/zinc SOD, manganese SOD, gluthathione peroxidase, and catalase (14).

In summary, daily diet and activities may be important targets in the antioxidative strategy for the prevention and treatment of AD. Clearly, further studies are required to establish the regimens of the practical intervention at each stage of AD.

CONCLUSION

Most of the known genetic, medical, environmental, and lifestyle-related factors for AD are

associated with increased oxidative stress. In concord, human cases at the preclinical stage of AD (e.g. subjects with MCI, young adults with Down syndrome) as well as cellular and animal models of AD provide consistent evidence that oxidative insult is a significant early event in the pathologic cascade of AD. Contrary to the general accepted role of the pathologic hallmarks, the process of aggregation of Aβ and tau proteins may be involved in a compensatory, protective response to oxidative insult. Focusing on recently reported modifiable risk factors for AD, we suspect that maintaining a low-calorie diet as well as intellectual and physical activities represents an important antioxidative strategy for the prevention of AD.

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Figure Legends

Fig. 1 Oxidized nucleoside, 8-hydroxyguanosine (8OHG), is abundant in vulnerable neurons in very mild Alzheimer disease (AD). Neuronal 8OHG immunoreactivity showing cytoplasmic predominace is prominent in the temporal cortex from a 92-year-old case of pathologically definite AD with premortem Clinical Dementia Rating (CDR) score of 0.5 (**A**). In the same case, however, 8OHG is virtually undetectable in the cerebellar Purkinje cells (*arrows*) (**B**). In a 90-year-old cognitively normal case with no AD pathology and premortem CDR score of 0, the neuronal 8OHG immunoreactivity is faint in the temporal cortex (**C**). Scale bar = 100 μm.

Fig. 2 Chronologic changes in the pathology and level of oxidative stress in Alzheimer disease (AD). **A.** *A classic model* of neuronal death with the progression of AD pathology. It is hypothesized that formation of senile plaque (SP) and/or neurofibrillary tangle (NFT) is closely associated with neuronal death. In this model, an increased level of oxidative stress is accompanied with the formation of abundant pathology and synergistically contributes to the neuronal death. Indeed, this model is *not* compatible with the recent findings accumulated in this review. **B.** *An alternative model* of neuronal death in AD where oxidative stress has a primary and central role. In this model, level of oxidative stress is manifest before the formation of SP

and NFT. It is hypothesized that increased oxidative stress causes rapid neuronal death without formation of SP and NFT. Intraneuronal accumulation of Aβ and tau may be involved in the process of neuronal death, whereas formation of SP and NFT may be associated with neuronal survival response against oxidative stress.

Table 1. Germline mutations, risk factors and protective factors for Alzheimer disease (AD) are associated with oxidative stress.

Germline mutations and risk factors for AD that cause or promote oxidative damage
Germline mutations
Amyloid β protein precursor gene, Presenilin-1 gene, Presenilin-2 gene
Risk factors for AD
Advanced age
Genetic risk factor
Apolipoprotein E ε4 Allele
Medical risk factors
Traumatic brain injury, Stroke, Hypertension, Diabetes mellitus
Hypercholesterolemia, Hyperhomocysteinemia
Environmental and lifestyle-related factors
Aluminum exposure, Smoking
Protective factors for AD that prevent or attenuate oxidative damage
Nutrients and agents
Vitamins C and E
Estrogen, Nonsteroidal antiinflammatory drugs, Statins
Omega-3 polyunsaturated fatty acids
Lifestyle-related factors
Low calorie intake, Exercise, Intellectual activity
Fish intake, Wine consumption

Table 2. Temporal primacy of oxidative stress in the pathologic cascade of Alzheimer disease: Animal models

Animal models	**Findings**
AβPP transgenic mice and C.elegans	1) Increased lipid peroxidation, protein oxidation, and decreased Cu/Zn SOD activity precedes Aβ plaque deposition or Aβ fibril formation (23, 74, 75). 2) Vitamin E supplementation reduces Aβ levels and Aβ plaque deposition (80). 3) Dietary copper stabilizes brain Cu/Zn SOD activity and reduces Aβ production (81).
AβPP mutant mice crossed with Mn SOD heterozygous knockout mice	Aβ plaque deposition is accelerated in brains of AβPP mutant mice with Mn SOD (+/-) compared to AβPP mutant mice with Mn SOD (+/+) (82).
Human tau transgenic mice	Vitamin E supplementation suppresses the development of tau pathology (79).

AβPP, amyloid β protein precursor; Cu/Zn SOD, copper/zinc superoxide dismutase; Mn SOD, manganese superoxide dismutase

Table 3. Temporal primacy of oxidative stress in the pathologic cascade of Alzheimer disease (AD): Human samples

Materials/Subjects	**Findings**
Post-mortem brains from subjects with Down syndrome	Oxidative damages to nucleic acid and protein precede Aβ plaque deposition in a series of aging brains (65).
Post-mortem brains from subjects with AD	1) Oxidative damages to nucleic acid and protein are more prominent in AD subjects with lesser amounts of Aβ plaque deposition or AD subjects with shorter disease duration (64). 2) Oxidative damage to nucleic acid is more prominent in hippocampal neurons free of NFTs compared to neurons with NFTs (64). 3) Oxidative damage to nucleic acid is increased in a presymptomatic case with presenilin-1 gene mutation (30).
Post-mortem brains from subjects with MCI	1) Oxidative damage to nucleic acid, protein and lipid are increased (67, 68). 2) Heme-oxygenase-1, a sensitive marker of oxidative stress, is up-regulated in astroglia (73).
CSF from subjects with AD	Oxidative damage to nucleic acid is more prominent in AD subjects with shorter disease duration or AD subjects with higher scores in Mini-Mental State Examination (66).
CSF, plasma, urine and peripheral leukocytes from subjects with MCI	1) Lipid peroxidation in CSF, plasma, and urine is increased (69). 2) Oxidative damage to DNA in peripheral leukocytes is increased (70). 3) Plasma antioxidants (vitamins A, C, E, carotenoids, SOD, etc) and plasma total antioxidant capacity are decreased (71, 72).

CSF, Cerebrospinal fluid; MCI, mild cognitive impairment; NFTs, neurofibrillary tangles; SOD, superoxide dismutase

Table 4. Summary of epidemiologic prospective cohort studies of vitamin E intake and risk of Alzheimer disease (AD)

Authors	*Setting*	Subjects	Follow-up period	Results
Masaki et al (117)	*Honolulu-Asia Aging Study*	n = 3,385 (only men), age 71-93 years		Combination of supplementary vitamins E and C was effective for vascular dementia but not for AD.
Engelhart et al (52)	*Rotterdam Study*	n = 5,395, age ≥ 55 years, mean 67.7 years	mean 6.0 years	**Dietary vitamin E was effective**, which was more prominent among current smokers and was not influenced by *APOE* genotype or controlling for supplement use.
Morris et al (119)	*Chicago Health and Aging Project*	n = 815, age ≥ 65 years, mean 73.3 years	mean 3.9 years	**Dietary vitamin E was effective** among non-carriers for *APOE*ε4 allele, whereas supplementary vitamin E was not effective.
Luchsinger et al (112)	*Washington Heights-Inwood Columbia Aging Project*	n = 980, age ≥ 65 years, mean 75.3 years	mean 4.0 years	Neither dietary nor supplementary vitamin E was effective.
Zandi et al (111)	*Cache Country Study*	n = 4,740, age ≥ 65 years, mean 75.3 years	mean 3.0 years	Supplementary vitamin E alone was not effective, whereas **combination of supplementary vitamins E and C was effective.**
Laurin et al (113)	*Honolulu-Asia Aging Study*	n = 2,459 (only men), age 45-68 years, mean 52.4 years	mean 30.2 years	Dietary intake of vitamin E was not effective.

Table 5. Substances and behaviors that reduce the levels of Aβ and/or Aβ deposition in brains of transgenic animal model of Alzheimer disease

Substance/Behavior	**References**
Vitamin E	(80)
Nonsteroidal antiinflammatory drugs	(135, 136)
Metal chelator (clioquinol)	(132)
Copper (stabilizing the Cu/Zn SOD activity)	(81)
Melatonin	(133)
Omega-3 polyunsaturated fatty acid (docosahexaenoic acid)	(134)
Curcumin	(131, 137)
Calorie restriction	(141, 142)
High activity in environmental enrichment	(147)
Voluntary exercise	(148)

Of note, these substances and behaviors are associated with a decrease in oxidative stress.

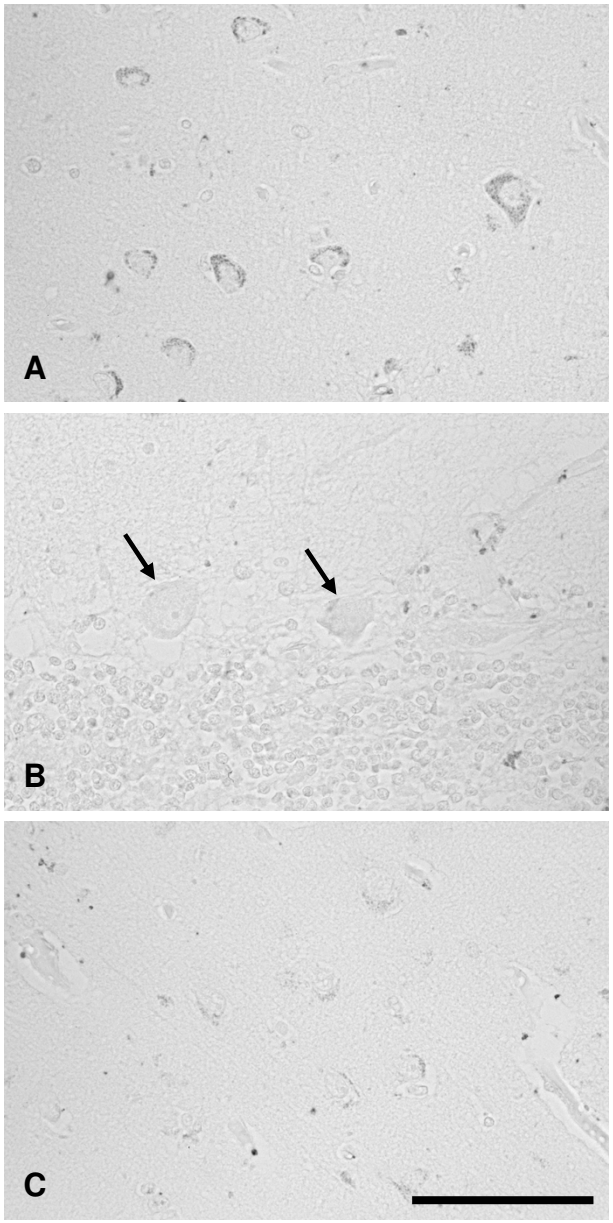

Fig. 1
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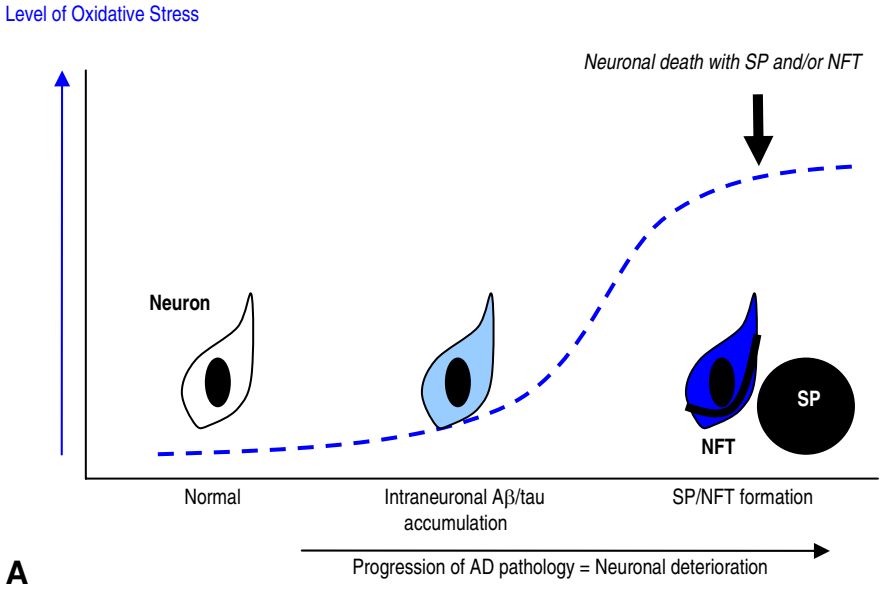

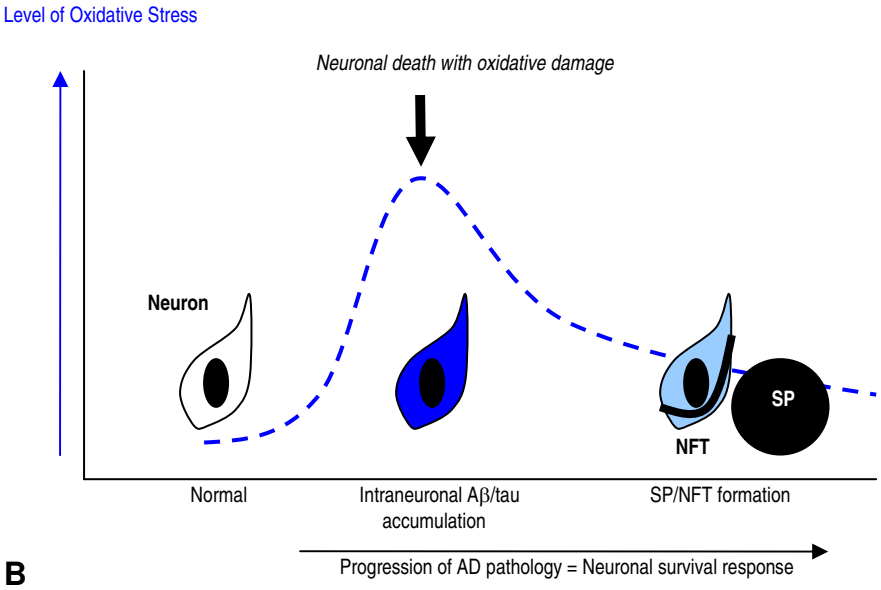

Fig. 2
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