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REVIEW ARTICLE

Antioxidant Treatment in Alzheimer's Disease

Current State

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Abstract

Accumulating data from experimental and human studies indicate that oxidative stress (OS) plays a major role in the pathogenesis of Alzheimer's disease (AD). The production of reactive oxygen species (ROS), which leads to OS, can occur very early, even before the appearance of symptoms and molecular events (β-amyloid plaques and neurofibrillary tangles), leading to tissue damage via several different cellular molecular pathways. ROS can cause damage to cardinal cellular components such as lipids, proteins, and nucleic acids (e.g., RNA, DNA), causing cell death by modes of necrosis or apoptosis. The damage can become more widespread because of the weakened cellular antioxidant defense systems. Therefore, treatment with antioxidants might theoretically act to prevent propagation of tissue damage and improve both survival and neurological outcome. Indeed, several studies preformed to date examined whether dietary intake of several antioxidants, mainly vitamins, might prevent or reduce the progression of AD. Although a few of the antioxidants showed some efficacy in these trials, no answer is yet available as to whether antioxidants are truly protective against AD. Reasons for these results might include, in part, blood–brain barrier (BBB) permeability, inappropriate timing of administration, or suboptimal drug levels at the target site in the central nervous system. Thus, antioxidant cocktails or antioxidants combined with other drugs may have more successful synergistic effects. Further, well-designed intervention, as well as observational investigations based on large cohorts studied over a long period of time with several methods for assessing antioxidant exposure, including relation to BBB penetration, are needed to test this hypothesis.

Index Entries: Alzheimer's disease (AD); reactive oxygen species (ROS); oxygen stress (OS); antioxidants; blood brain barrier (BBB).

Introduction

Alzheimer's Disease

Alzheimer disease (AD) is the most common neurodegenerative disorder of the elderly, and it is considered to be the leading cause of death in the United States, after certain cancers and cardiovascular disease. Clinically, it is characterized by progressive memory loss, decline in language skills, and dementia, which is an acquired cognitive and behavioral impairment of sufficient severity to interfere significantly with social and occupational functioning. At present, the disorder afflicts ~5 million people in the United States and >30 million people worldwide. A larger number of individuals have lesser levels of cognitive impairment, which frequently evolve into a full-blown dementia, thereby increasing the number of affected persons (Clark, 2000). Prevalence of this disorder is expected to increase substantially in this century, as the disorder preferentially affects

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the elderly, who constitute the fastest growing age bracket in many countries, especially in industrialized nations. For example, statistical projections indicate that the number of persons affected by the disorder in the United States will nearly triple by the year 2050. AD is also a major public health problem when viewed from an economic perspective. In fact, the cost of caring for patients with AD was over $110 billion per year in the early 1990s in the United States, and the average annual cost per patient is about $45,000. The lifetime risk of developing AD in the United States and the industrialized nations is estimated at 1 : 4-1 : 2, and as indicated by many studies, women are at a significantly higher risk, owing to the loss of the neurotrophic effect of estrogen postmenopause. More than 14% of individuals older than 65 have AD, and the prevalence increases to at least 40% in individuals older than 80. Interestingly, some studies with nonagenarians and centenarians suggest that the risk of AD decreases in individuals older than 80 or 90. If so, age is not an unqualified risk factor for the disease, but this matter needs further study. Claims have been made that AD may affect certain ethnic and racial groups more severely than others, but this matter necessitates more study before reliable statements can be made (Clark, 2000; Maccioni, 2001).

The early-onset, familial form of Alzheimer's disease (FAD) accounts for nearly 5–10% of all cases of AD and is characterized by early manifestations of dementia, in some cases in patients ~40 years of age. It is caused by multiple mutations in the major genes: amyloid precursor protein (APP) and presenilins 1 (PS1) and 2 (PS2) (Selkoe, 2001). In contrast, late-onset AD, which is sporadic and accounts for ~90–95% of all patients, is probably attributable to the effects of genetic risk factors such as aging, apolipoprotein E (ApoE), and α2 macroglobulins (A2M), combined with different epigenetic events (Cummings and Cole, 2002).

Although the etiology of AD is unknown, it is clear that the pathophysiology is complex and evolves overlapping pathways of neuronal damage. The anatomic pathology of AD includes senile plaques (SPs), which are microscopic foci of extracellular amyloid deposition, and neurofibrillary tangles (NFTs), which are large, nonmembrane-bound bundles of abnormal fibers mainly composed of tau protein and occupy much of the perinuclear cytoplasm. AD brains exhibit a number of other abnormalities, which are cerebrocortical atrophy (predominantly at the expense of association regions), loss of synapses, gliosis, microglia activation, signs of inflammation, and oxidative damage (Cummings and Cole, 2002; Rutten et al., 2002).

In the past 3 yr, substantive advances in therapy for AD have occurred, and the treatment plan for AD includes mainly cholinesterase inhibitors. These agents are the only approved and marketed treatments currently available for AD to temporarily improve cognition or slow the rate of cognitive decline. Other components include management of comorbid conditions, treatment of behavioral symptoms and mood disorders, provision of support and resources for patient and caregiver, and compliance with state-mandated requirements to report any impairment affecting driving and abuse of the elderly. Breakthroughs in the basic science of AD have led to new insights into potential therapeutic strategies targeted at the secretases involved in the metabolism of APP. Particularly interesting were the recent studies of immune system activation, which appear to increase the clearance of the disease-associated protein. In animal models these immunologically based approaches were highly effective without demonstrating any obvious side effects. In transgenic mice with AD-related pathology, immunization has also been shown to prevent age-related cognitive impairment. However, the first clinical trial of this approach in AD patients was associated with unacceptable toxicity. These immune-based treatment approaches have great potential as rational therapies for this devastating group of disorders, but additional development is needed before they can be safely applied to humans. Other areas of investigation, such as hormone replacement therapy using estrogens, anti-inflammatory approaches, and several other therapeutic agents have had disappointing results (Knopman, 2001; Cummings et al., 2002; Wisniewski and Sigurdsson, 2002). In addition, because high cholesterol was implicated in the pathogenesis of amyloid plaques in AD, several studies examined whether the use of cholesterol-reducing agents (statins) can be protective against the disease. Indeed, recent studies indicated that patients treated with simvastatin or pravastatin have a lower prevalence of diagnosed AD (Fassbender et al., 2001; Sun et al., 2003).

Treatments that stabilize, disrupt, or reverse deposition of amyloid plaques or abnormal phosphorylation of tau protein should play a bigger role in impeding the progression of the disease than neurotransmitter-replacement therapies. Prevention or delay of the appearance of functional disabilities in

dementia will be an important and successful outcome of these initiatives.

Mild Cognitive Impairment

An increasing number of studies clearly indicate that the onset of AD is typically preceded by an interim phase known as mild cognitive impairment (MCI). This is a transitional stage between normal aging and dementia and is characterized primarily by memory deficit without clinically meaningful functional impairment. Recent data have suggested that it is associated with up to 50% probability of progressing to symptomatic AD within a 4-yr period. Thus, the rate of progression from MCI to AD is ~12% per year, supporting the concept that MCI, at least in part, represents the preceding stage of AD (Burns and Zaudig, 2002; Pratico et al., 2002).

Reactive Free Radicals and Oxidative Stress in AD

Biosynthesis of Free Radicals Leading to Oxidative Stress

A free radical is any chemical species that contains one or more unpaired electrons. Unpaired electrons alter the chemical reactivity of an atom or molecule, usually making it more reactive than the corresponding nonradical, because it acts as an electron acceptor and essentially "steals" electrons from other molecules. This loss of electrons is called oxidation, and free radicals are referred to as oxidizing agents because they tend to cause other molecules to donate their electrons. We are constantly exposed to free radicals created by electromagnetic radiation from the environment, both natural (e.g., radon, cosmic radiation) and man-made (e.g., pollutants and cigarette smoke), as well as cellular metabolisms (e.g., respiratory burst, enzyme reactions). The most common cellular free radicals are hydroxyl radical ($OH^{\bullet}$), superoxide radical ($^{\bullet}O_2^-$), and nitric oxide ($NO^{\bullet}$). Other molecules, such as hydrogen peroxide (H_2O_2) and peroxynitrite (ONO_2), are not free radicals but can lead to the generation of free radicals through various chemical reactions. Free radicals and related molecules are often classified together as reactive oxygen species (ROS) to signify their ability to lead to oxidative changes within the cell. Cells normally have a number of mechanisms to defend against damage induced by free radicals. Problems occur when production of ROS exceeds their elimination by the antioxidant defense system or when the latter is damaged. This imbalance between cellular production of ROS and the inability of cells to defend against them is called oxidative stress (OS). OS can cause cellular damage and subsequent cell death because ROS oxidizes cardinal cellular components, such as lipids, proteins, and DNA (Fig. 1) (Gilgun-Sherki et al., 2001).

OS and Excitotoxicity

Although multiple factors can precipitate OS in cells, the neurotransmitter glutamate is the major source of this process in the brain, primarily through activation of its ionotropic receptors. Glutamate and related excitatory amino acids account for most of the excitatory synaptic activity in the mammalian central nervous system (CNS) and are released by an estimated 40% of all synapses. The ionotropic receptors can be distinguished by their pharmacological and electrophysiological properties and include *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxasole-proprionic acid (AMPA), and the kainic acid (KA) receptors. Several studies provide evidence that the two phenomena are interrelated. The calcium-mediated effects of glutamate receptor activation, leading to neuronal degeneration, may involve a number of different pathways causing OS. Free radical-induced damage can occur by the stimulation of phospholipase A2 and the subsequent release of amino acids. Amino acids and oxygen radicals enhance the release of glutamate, thereby promoting a vicious circle (Gilgun-Sherki et al., 2001).

Excessive activation of glutamate receptors might be responsible for part of the neuronal damage observed in AD (Danysz et al., 1995). The role of glutamate in AD was emphasized by Greenamyre and colleagues (Greenamyre et al., 1988; Penney et al., 1990), who based their claims on the significant reduction of NMDA-type glutamate receptors in the hippocampus of AD patients. Although it is likely that a glutamate-related alteration is not the primary etiopathological factor in AD, extracellular glutamate might contribute to aggravate and accelerate neuronal degeneration. One reason for such a role is that inefficient metabolism and energy deficit occur in neurons of AD patients (Doble, 1999). These deficits lead to persistent cell depolarization and to a chronic excess of glutamate in the extracellular space, which produces persistent depolarization, will release the Mg^{2+} blockade of NMDA receptors, and produce undesired increases of intracellular Na^+ and Ca^{2+} that result in functional decline and death

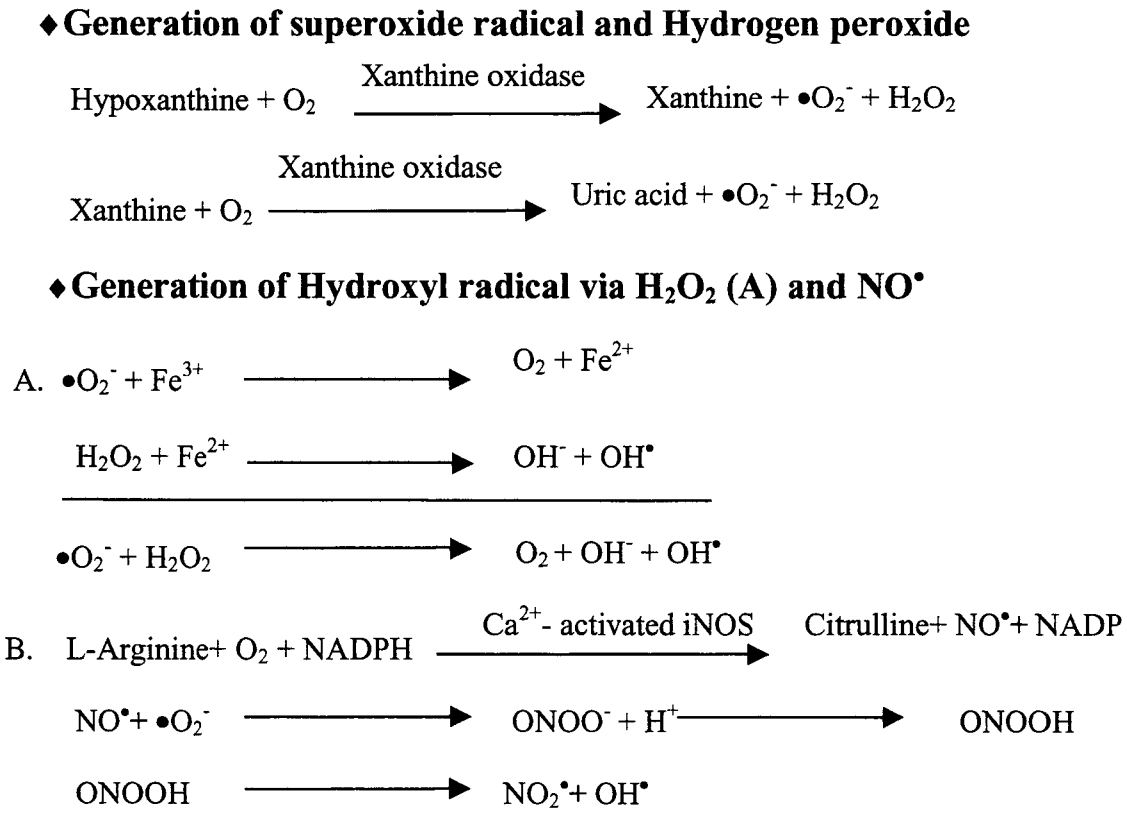

Fig. 1. Reactive oxygen species generation that may lead to cell death. NADP = nicotineamide adenine dinucleotide phosphate; NADPH = reduced form of NADP; iNOS = inducible nitric oxide synthetase.

of the neuron (Hoyer, 1996). Several studies have investigated the relationship between excitotoxicity and β-amyloid (Aβ) toxicity in vitro (Mattson et al., 1992, 1993) and demonstrated that β-amyloid peptides appears to sensate cultured neurons to excitotoxic cell death. This could provide a mechanism by which excitotoxic damage might aggravate neurodegeneration. Clinical studies that examined the efficacy of memantine (a weak and nonselective NMDA receptor channel blocker) for the treatment of AD noted some functional improvement (Gortelmeyer and Erbler, 1992; Reisberg, 2000). However, more studies are needed to confirm these data.

The excitatory amino acids and neurotransmitters, whose metabolism produces ROS, are unique in the brain as sources of OS. Other sources are generated by the high and constant use of oxygen in the mitochondria to supply the energy needs of these tissues. Free radicals are also produced by cytochrome P450 electron transport and the monoamine oxidase activity of the outer mitochondrial membrane, and attack neurons that are postmitotic cells and therefore are particularly sensitive to free radicals (Gilgun-Sherki et al., 2001).

OS and Aging

Aging in mammalian species appears to be the result of normal developmental and metabolic process responsible for graying of the hair, decrease in the rate of wound healing, and increase in the risk of diseases and death. The best-known risk factor for neurodegenerative diseases is aging. Studies have found evidence of oxidative damage to macromolecules (DNA, lipids, and proteins), especially in neurons from elderly brains, supporting the hypothesis that oxidative injury might directly cause the aging process. Additional links between OS and aging focus on mitochondria. Direct biochemical measurements of mitochondrial function demonstrate an age-dependent increase in mitochondrial deletions, point mutations, and oxidative damage to the DNA. The mitochondrial DNA in the elderly population is particularly susceptible to OS, probably because of its close proximity to the respiratory

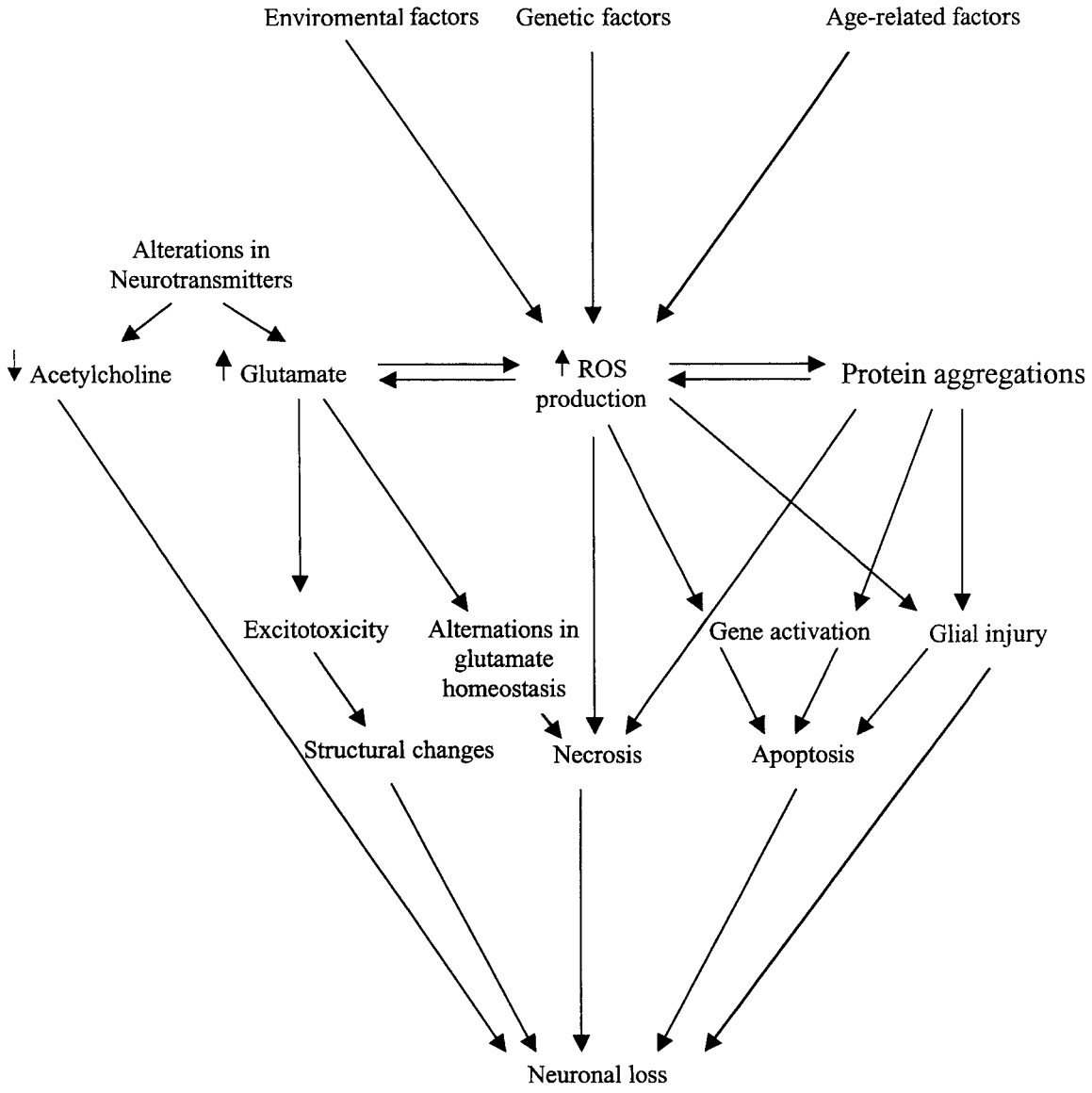

Fig. 2. The main sources of ROS and the cellular events that may lead to neuronal loss in AD.

chain, limited repair mechanisms, few noncoding sequences, and absence of histones (Cutler, 1991; Harman, 1992; Beal, 1995; Fukagawa, 1999).

OS and AD

The involvement of free radicals and OS in the pathogenesis of AD (Fig. 2) is now widely accepted (Christen, 2000; Butterfield et al., 2001; Pratico, 2002). Both NFTs and Aβ-containing plaques seems to be closely associated with markers of OS, but the exact interrelations between OS and the pathology have not been established. On one hand, Aβ was shown to be sensitive to the action of free radicals, contributing to aggregation. On the other hand, the effect of Aβ accumulation, including that arising from an Aβ-initiated inflammatory response, may include excessive generation of free radicals. The use of mice transgenic for mutated human APP has been shown to be a valuable tool for modeling several aspects of Alzheimer's pathology, including OS (Matsuoka et al., 2001). These mice have shown a strong association between fibrillar Aβ and oxidatively damaged proteins (by increased brain levels of 3-nitrotyrosine) and lipids (by increased brain levels of 4-hydroxy-2-hexenal [4-HNE]). Other studies involving transgenic mice for human APP and PS1 muta-

tions have indicated that the increase in OS in neurons precedes Aβ plaque and NFT formation (Nunomura et al., 2001; Pratico et al., 2002; Lovel et al., 2001).

In addition, examinations of the brains of AD patients in postmortem studies showed many signs of free radical attacks. These include mitochondrial and nuclear DNA damage (i.e., 8-hydroxy-2-deoxyguanosine [8-HDG], malondialdehyde [Meccoci et al., 1994; Yan et al., 1994]). Many studies have shown increased protein oxidation (i.e., carbonyls [Hensley et al., 1995]), lipid peroxidation (i.e., 4-HNE [Palmer and Burns, 1994]), isoprostanes (Montine et al., 1998), and advanced glycosylation end products (Marcus et al., 1998). Moreover, several investigators detected changes in the antioxidant enzyme activity of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and heme oxygenase-1 in several areas of the brain (Zemlan et al., 1989; Pappolla et al., 1992; Smith et al., 1994; Marcus et al., 1998).

Furthermore, aging and the ApoE4 genotype are the major risk factors for AD. As was mentioned earlier, aging is associated with increased OS in the CNS, and ApoE4 is associated with higher oxidative insults in the brains of AD patients (Ramassamy et al., 1998; Cummings, 2002). Moreover, traces of metals (iron, copper, zinc, and aluminum), capable of catalyzing reactions that produce free radicals, have been found in the brains of AD patients (Crapper et al., 1980; Deibel et al., 1997). AD is also related to mitochondrial anomalies, particularly for cytochrome-*c* oxidase, and subsequent acceleration of ROS production (Beal, 1995, 1996).

OS and MCI

A recent study showed that individuals with MCI have increased levels of lipid peroxidation in the brain before the onset of symptomatic dementia. These data suggest that measurement of lipid peroxidation may provide a reliable biomarker of brain oxidative damage that could help, together with other parameters, in identifying subjects with MCI who are at high risk for developing AD (Pratico et al., 2002).

Antioxidants in the Treatment of AD

Antioxidants

Antioxidants are exogenous (natural or synthetic) or endogenous compounds acting in several ways, including removal of O_2, scavenging ROS or their precursors, inhibiting ROS formation, and binding metal ions needed for the catalysis of ROS generation. The natural antioxidant system can be classified into two major groups: enzymatic (e.g., superoxide dismutase, catalase) and nonenzymatic or low-molecular-weight antioxidants (LMWAs). The LMWA group of molecules can be further classified into directly acting antioxidants (e.g., scavengers and chain-breaking antioxidants) and indirectly acting antioxidants (e.g., chelating agents). The former are extremely important in the defense against OS. This subgroup currently contains several hundred compounds. Most of them, including ascorbic and lipoic acids, polyphenols, and carotenoids, are derived from dietary sources. The cell itself synthesizes a minority of these molecules, such as glutathione and NADPH. Antioxidants of widely varying chemical structures have been investigated as potential therapeutic agents for neurodegenerative diseases. However, most of these agents did not show efficacy in clinical trials. This may be attributable, in part, to their limited passage through the blood–brain barrier (BBB), the major obstacle separating the brain microenvironment from the blood within the cerebrovascular tree (Gilgun-Sherki et al., 2001), and to other causes (Table 1).

Clinical Trials

The encouraging findings in the experimental models have motivated studies on humans, testing the effects of nonenzymatic/exogenous antioxidants in treating and preventing AD. Antioxidants, such as ascorbic acid and vitamin E, are free radicals scavengers that have been evaluated in the treatment of several neurological and neurodegenerative disorders. Vitamin E, a fat-soluble vitamin, has been the most widely studied antioxidant. Vitamin E analogs have shown promise as free radical scavengers in laboratory animals. Therapeutic advantages of antioxidants such as ascorbic acid and α-tocopherol (Grundman, 2000) include ready availability and low cost. Early studies on this topic carried out between 1989 and 1999 (Pitchumoni et al., 1998; Rosler et al., 1998; Flynn and Ranno, 1999) indeed provided preliminary evidence that antioxidants may have a protective effect against the development of AD. In a recent Cochrane review (Tabet et al., 2000), the outcomes of all double-blind, randomized trials carried out up to June 2000 have been analyzed, in which treatment with vitamin E at any dose was compared with placebo for patients with AD. Only one study (Sano et al., 1997) was identified as meeting the inclusion criteria of the Cochrane analysis.

Table 1
Possible Reasons for the Lesser Efficacy of Clinical Trials as Compared to Experimental Models

← Inadequate penetration into the brain due to BBB permeability.
← Current theories about the specific mode of drug action in AD brains are inappropriate.
← Current experimental models of AD are inappropriate/insufficient compared to human, because of differences in brain structure, function, and anatomy.
← Effective neuroprotection by drug in the models cannot predict success in human illness.
← Drugs that inhibit one parameter of injury might be insufficient to inactivate other parallel pathways of the pathologic cascade.
← Antioxidants should be given in very narrow range of therapeutic dosages. As with vitamins, antioxidants, given in high doses in a certain redox situation, might become pro-oxidants and toxic.
← Suboptimal dosage at the target site.

In this study (Sano et al., 1997), focusing on patients diagnosed with mild to moderate AD, a slower progression of AD in patients using vitamin E (2000 IU/d), has been shown, as compared with patients not using this antioxidant. The primary outcome used in this study of 341 participants was survival time to the first of 4 end points: death, institutionalization, loss of 2 out of 3 basic activities of daily living, or severe dementia, defined as 3 in the global Clinical Dementia Rating. The investigators reported the total numbers in each group who reached the primary end point within 2 yr for participants completing the study ("completers"). There appeared to be some benefit from vitamin E, with fewer participants reaching end point—58% (45/77) of completers compared with 74% (58/78). However, more participants taking vitamin E suffered a fall. Therefore, it was not possible to interpret the reported results for specific end points or for secondary outcomes of cognition, dependence, behavioral disturbance, and activities of daily living.

In the Cochrane review (Tabet et al., 2000) it was concluded that there is insufficient evidence of the efficacy of vitamin E in the treatment of people with AD, justifying further studies on this topic.

Another study, published a year later (Morris et al., 1998), examined the relationship between the use of vitamin E (200–800 IU/d) and vitamin C and incidence of AD in a prospective study of 633 persons aged 65 and over randomly selected from a disease-free population. After an average follow-up period of 4.3 yr, 91 participants taking vitamins met accepted criteria for the clinical diagnosis of AD. None of the 27 vitamin E supplement users had AD compared with 3.9 predicted, based on the crude observed incidence among nonusers ($p = 0.04$), and 2.5 predicted, based on age, sex, years of education, and length of follow-up interval ($p = 0.23$). None of the 23 vitamin C supplement users had AD compared with 3.3 predicted, based on the crude observed incidence among nonusers ($p = 0.1$), and 3.2 predicted, based on age, sex, years of education, and length of follow-up interval ($p = 0.04$). There was no relationship between AD and the use of multivitamins. The investigators concluded that the use of a higher dose of vitamin E and vitamin C supplements might lower the risk of AD (Morris et al., 1998). The problem with such a conclusion is that antioxidants, given in high doses, might become pro-oxidants and toxic (Table 1).

The Honolulu–Asia Aging Study ([HAAS] Japanese–American men living in Hawaii) examined whether use of vitamin E and C supplements protect against subsequent development of dementia and poor cognitive functioning. Data for this study were obtained from a subsample of the cohort interviewed in 1982 and subsequently examined by a mailed questionnaire in 1988 and the dementia prevalence survey in 1991–1993. The subjects included 3385 men, aged 71 to 93, but only 47 of them had AD. No protective effect was found for Alzheimer's dementia. Among those without dementia, use of either vitamin E or C supplements alone in 1988 was associated significantly with better cognitive test performance at the 1991–1993 examination, and use of both vitamins E and C together had borderline significance. The investigators concluded that vitamin E and C supplements might protect against vascular dementia, but not Alzheimer's dementia, and might improve cognitive function in late life (Masaki et al., 2000).

Another study (Commenges et al., 2000) investigated 1367 initially nondemented French individuals over 65 yr of age, and found, during a 5-yr follow-up period, an inverse relation between intake

of flavonoids (and not vitamin C) and the risk of incident dementia (of which AD is one possible cause). However, there were many problems regarding this study. One major error was that not all of the subjects received the same nutritional questionnaires (some of the questionnaires were more detailed). Another problem was that 16% of subjects eligible for this study dropped out or died before the 5-yr follow-up and thus were excluded from the study, and 20% dropped out or died before the 8-yr follow-up. An additional problem was that there was no direct relevance to AD.

In contrast to the HAAS study (Masaki et al., 2000), a very recent population-based study of 3734 Japanese–American men, followed up since 1965–1968 as part of the Honolulu heart program, has been published and did not find any significant association between the intake of supplemental vitamins E and C and the risk for subtypes of incident dementia (Laurin et al., 2002).

The varied results of these three studies (Masaki et al., 2000; Commenges et al., 2000; and Laurin et al., 2002), and the fact that there was no protective effect found for Alzheimer's dementia, leaves the topic unclear as to whether antioxidant supplementation might be helpful for AD.

Two studies on antioxidant intervention have recently been published. In the first (Morris et al., 2002), the relationship between AD incidence and intake of the antioxidant nutrients vitamin E, vitamin C, and β-carotene were investigated (Chicago Health and Aging Project). On an average of 1.7 yr after baseline, food frequency questionnaires were administered, and the incidence of AD cases, as evaluated in clinical examinations according to standardized criteria, were taken as the main outcome measure. The results of this prospective, longitudinal study were that persons in the highest quintile of vitamin E intake from food (>10.4 IU/d) were 70% less likely than those in the lowest quintile of intake (<7 IU/d), to be diagnosed with AD. These findings were based on new cases developing in a mean 3.9-yr interval between baseline and follow-up among 815 men and woman, black and white, aged 65 and older, free of AD at baseline.

Interestingly, the investigators observed this association only among individuals without ApoE4 genotype, suggesting that people with ApoE4 genotype may not be protected from AD by the use of vitamin E (Morris et al., 2002).

In the second study, Engelhart and coworkers (2002) determined whether dietary intake of β-carotene, flavonoids, vitamin C, and vitamin E is related to the risk of AD in a large population-based, prospective cohort study (Rotterdam Study). This study included 5396 men and women who were at least 55 years old at baseline, noninstitutionalized, dementia free, and whose dietary assessment was reliable. Incidence of AD based on standardized criteria was the main outcome measure. The results, obtained after a mean follow-up of 6 yr, indicate that persons in the highest quintile of vitamin E intake from food (>15.5 mg/d) were 43% less likely to develop AD compared with those in the lowest quintile of intake (<10.5 mg/d). This relationship was even more pronounced in current smokers, for whom intake of β-carotene and flavonoids was also preventive (Engelhart et al., 2002).

In contrast to the Chicago Health and Aging Project (Morris et al., 2002), in the Rotterdam study (Engelhart et al., 2002) the association between intake of antioxidants and AD risk did not vary between ApoE genotypes.

Surprisingly, neither study identified an association between the incidence of AD and the use of vitamin E and vitamin C supplements. This finding suggests the involvement of some other nutritional phenomena related to accurate measurement of supplement use or nutrient intake. These two latter studies are not entirely comparable because of lower incidence rates and possible differences in pathogenic phenomena between cases of AD having younger versus older ages of onset and sample sizes.

Conclusions and Future Strategies

Several findings suggest that OS might be involved in the pathogenesis of AD. These findings come from experimental and clinical studies indicating that administration of antioxidants might be useful in prevention and treatment of AD. Recent prospective studies have indicated that dietary intake of several exogenous antioxidants is associated with a lower risk for AD, suggesting that intervention with antioxidants might be beneficial in the early phases of AD or in people at risk for developing AD. However, there were many inverse results in these studies. This may be attributable, in part, to several reasons listed in Table 2. Furthermore, these studies are prone to bias, because people who use supplements may also have more health problems and more health-seeking behavior. Moreover, the necessary use of food frequency questionnaires requires patients who may have amnesic problems,

Table 2
Variations in the Clinical Studies

← **Patients:** Environmental., genetics, age, ethnic group, occupational., intelligence, habits (e.g., smoking), study scale, duration of antioxidant use, dose, dietary background, and basic morbidity status differences (including other therapies that the patients receive).
← **Drugs:** Source of supplementation: natural vs. synthetic agents (i.e., vitamin E that has eight forms).
← **Clinical criteria:** The high heterogeneity of the brain damage size and neurological and behavioral variations.
← **Design:** Population size and prospective vs. retrospective studies.

thus leading to inaccurate supplement use reports. In addition, the use of supplements in these studies is generally of short duration. Therefore, the clinical studies carried out so far did not provide the ultimate answer to whether antioxidants can truly protect or slow down the progression of AD. Generally, to achieve efficacy, a candidate antioxidant must penetrate the BBB to attain a critical therapeutic level within the CNS and must be given as early as possible, before the irreversible neuronal loss. It also should fit the precise OS physiology, for example, the type of ROS involved, the place of generation, and the severity of the damage. Thus, antioxidant cocktails or antioxidants combined with other drugs may have more successful synergistic effects. Further well-designed intervention trials, as well as observational investigations based on larger cohorts studied over a longer period of time with several methods for assessing antioxidant exposure, including relation to BBB penetration, are needed to test this hypothesis.

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