Oxidative stress and Alzheimer disease[1,2]

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ABSTRACT Research in the field of molecular biology has helped to provide a better understanding of both the cascade of biochemical events that occurs with Alzheimer disease (AD) and the heterogeneous nature of the disease. One hypothesis that accounts for both the heterogeneous nature of AD and the fact that aging is the most obvious risk factor is that free radicals are involved. The probability of this involvement is supported by the fact that neurons are extremely sensitive to attacks by destructive free radicals. Furthermore, lesions are present in the brains of AD patients that are typically associated with attacks by free radicals (eg, damage to DNA, protein oxidation, lipid peroxidation, and advanced glycosylation end products), and metals (eg, iron, copper, zinc, and aluminum) are present that have catalytic activity that produce free radicals. β-Amyloid is aggregated and produces more free radicals in the presence of free radicals; β-amyloid toxicity is eliminated by free radical scavengers. Apolipoprotein E is subject to attacks by free radicals, and apolipoprotein E peroxidation has been correlated with AD. In contrast, apolipoprotein E can act as a free radical scavenger and this behavior is isoform dependent. AD has been linked to mitochondrial anomalies affecting cytochrome-*c* oxidase, and these anomalies may contribute to the abnormal production of free radicals. Finally, many free radical scavengers (eg, vitamin E, selegeline, and *Ginkgo biloba* extract EGb 761) have produced promising results in relation to AD, as has desferrioxamine—an iron-chelating agent—and antiinflammatory drugs and estrogens, which also have an antioxidant effect. *Am J Clin Nutr* 2000;71(suppl):621S–9S.

KEY WORDS Alzheimer disease, free radicals, antioxidants, β-amyloid, oxidative stress, metal, humans

INTRODUCTION

Alzheimer disease (AD) is no longer the condition described in texts written some 10 y ago, ie, a pathology caused by quite mysterious phenomena. Research in the fields of genetics and molecular biology has produced many findings about this common neurodegenerative disease. Many genes involved with the disease have been identified. Some of these genes are involved in early-onset forms of the disease and have a direct causal effect: the amyloid precursor protein (APP) gene located on chromosome 21 and presenilin genes 1 and 2 located on chromosomes 14 and 1, respectively. The apolipoprotein E (apo E) gene (located on chromosome 19), the α_2-macroglobulin gene located on chromosome 12, and other unidentified genes may determine susceptibility in late-onset forms and sporadic cases.

Genetic research along these lines, together with research in molecular biology investigating the main components of the 2 principal hallmarks of AD, ie, senile plaques (typically bearing β-amyloid) and neurofibrillary tangles (mainly composed of tau protein), have helped produce hypotheses about the pathogenesis of AD that no doubt come close to reflecting the real situation. In particular, it appears that a cascade of events may occur that leads to amyloidogenesis and, more specifically, to the formation and deposition of a long β-amyloid peptide (of 42 or 43 amino acids; the shorter form of 40 amino acids has no pathologic effect). Various mutations that have been identified on the *APP*, *PS1*, or *PS2* gene all feature increased production of this peptide (1). Various complementary factors, including cytokines, transforming growth factor β1, and interleukin 1, seem to be involved in triggering the process of amyloidogenesis (2, 3).

Although this amyloid hypothesis is feasible, it is still patently incomplete, particularly because there is still no proper understanding of the relations between amyloidogenesis and the development of the neurofibrillary tangles. An effort must also be made to produce a proper account of the actual neurodegenerative process itself and of neuronal death. Free radicals probably play a role in these processes. The free radical hypothesis of aging, which was proposed many years ago, posits that the age-related accumulation of reactive oxygen species (ROS) results in damage to major components of cells: nucleus, mitochondrial DNA, membranes, and cytoplasmic proteins. The imbalance between the generation of free radicals and ROS may be involved in the pathogenesis of most of the neurodegenerative disorders, including AD, as suggested by many authors for many years (4–6).

Free radicals are in fact potent deleterious agents causing cell death or other forms of irreversible damage, eg, free radicals appear to modify ≈10 000 DNA base pairs every day (7). Neurons appear to be particularly vulnerable to attack by free radicals for the following reasons: *1*) their glutathione content, an important natural antioxidant, is low (8); *2*) their membranes contain a high proportion of polyunsaturated fatty acids (9); and *3*) brain metabolism requires substantial quantities of oxygen (10). The fact that age is a key risk factor in AD provides considerable support for the free radical hypothesis because effects of the attacks by free radicals, particularly those produced by ROS, can accumulate over the years (11). Other identified risk

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factors, such as brain trauma, are also likely to be involved in the production of free radicals. The free radical hypothesis can account for the vastly heterogeneous nature of AD and the fact that both genetic and nongenetic causes are involved. Such general considerations suggest that free radicals are involved in many age-related pathologies, specifically in AD and all neurodegenerative diseases (12).

THE ROLE OF METALS IN ALZHEIMER DISEASE

Metals play a major catalytic role in the production of free radicals, and attention has centered on the role of many metals in AD, including iron, aluminum, mercury, copper, and zinc. Iron is involved in the formation of the free hydroxyl radical, which has recognized deleterious effects, as described in Fenton's and Haber-Weiss's classical reactions. Many observations provide proof that the metabolism of iron is involved in AD. The concentration of iron in the brains of AD patients is elevated. Iron, transferrin, and ferritin have been found in senile plaques (13, 14). Smith et al (15) studied the distribution of iron in the brains of AD patients using various histochemical methods and observed that the iron distribution matched the distribution of senile plaques and neurofibrillary tangles, the 2 key features of AD. The iron related to the lesions could be part of an oxidative process in situ. The characteristics of the iron-binding sites can be determined by the histidine residues and protein conformation. Indeed, when the iron chelating agent desferrioxamine is used, the histochemical labeling disappears. It is therefore reasonable to infer that an alteration in the homeostasis of iron could be involved in AD. One study showed elevated blood serum and cerebrospinal fluid concentrations of the iron-binding protein p97 in AD patients (16). The authors suggested that p97 concentrations could be used as a marker of the disease. They also suggested that p97 concentrations are useful in identifying AD patients, for following the course of the disease, and for assessing the effect of possible therapeutic approaches.

Aluminum has been suggested as a causal factor in AD, in part because of reports showing the toxicity of aluminum, the elevation of aluminum concentrations in the brains of patients with AD, and an association between aluminum concentrations in water and the prevalence of AD (17). However, some studies clearly showed that the aluminum content is not elevated in the brains regions of AD patients that are selectively vulnerable to the neuropathologic changes associated with the disease (18). The possibility of copper's involvement in AD is supported by the fact that copper can act as a catalyst in the production of ROS and by data showing that the APP molecule contains a copper-binding site (19–21). The binding of Cu(II) leads to the modification of APP via the oxidation of cysteines 144 and 158, which leads to the formation of cystine and Cu(I). In this respect, APP serves in the electron transfer to Cu(II), at least in vitro. The oxidation of the 2 cysteines to cystine results in the production of 2 electrons, yet only 1 electron is required for the reduction of Cu(II) to Cu(I). The remaining electron can be involved in the production of hydroxyl radicals (21).

The possible involvement of copper in neurodegeneration is also suggested by the fact that this metal is essential for many enzyme activities, including cytochrome-*c* oxidase and Cu/Zn superoxide dismutase (22). In a recent study, Deibel et al (23) showed lower concentrations of copper in 5 zones of the brains of AD patients, particularly in the hippocampus.

The latest metal cited as a possible factor in AD is zinc. Zinc induces a rapid tinctorial amyloid formation in humans but not in rats, which perhaps explains the scarcity of cerebral β-amyloid in these animals (24). APP binds Zn(II), and this binding modulates the functional properties of APP (eg, inhibits the cleavage of APP by α-secretase and increases binding to heparin). In addition to these data, which are directly related to AD, there is increasing evidence suggesting that zinc accumulation can mediate neuronal death associated with other brain injuries, including ischemia (25).

OXIDATIVE STRESS PRODUCTS AND ALZHEIMER DISEASE

Although data may not always be consistent, many studies have provided evidence for the deleterious consequences of oxidative stress products on certain cellular targets in AD. The oxidation of mitochondrial DNA and, to a lesser extent, of nuclear DNA has been observed in the parietal cortex of AD patients (26) as well as in elderly patients without AD. Protein oxidation has also been observed in elderly individuals with and without AD, but appears to be more marked in AD patients in the regions presenting the most severe histopathologic alterations (27). Many studies have shown increased lipid peroxidation in the brains of AD patients, particularly in the temporal lobe, where histopathologic alterations are very noticeable (28–30). These observations, however, were not corroborated by other studies (31, 32), which failed to find any difference in the basal level of peroxidation. Ramassamy et al (33) suggested that these inconsistent results are related to the apo E genotype. Those with the *E4* allele are likely to be more susceptible to peroxidation than are those without this allele.

Additionally, several studies have identified within the brains of AD patients, particularly in the neurofibrillary tangles, the end products of peroxidation: malondialdehyde (34), peroxynitrite (35, 36), carbonyls (10, 37), advanced glycosylation end products (AGEs; 34, 38), superoxide dismutase-1 (39), and heme oxygenase-1 (40, 41). Heme oxygenase-1 is a cellular enzyme that is up-regulated in the brain and in other tissues in response to an oxidative challenge or other noxious stimuli.

The lipoperoxidation phenomena could have a major, or even causal, influence on the pathogenesis of the disease. Busciglio and Yankner (42), in particular, showed that in Down syndrome, which involves a neurodegenerative component similar to or even identical to that of AD, neuronal death occurs according to a process of apoptosis that is related to an increase in lipid peroxidation and can be stopped by catalase and free radical scavengers.

The effect of ROS on membrane phospholipids could prove to be important because some alterations to membrane phospholipids may be specific to the pathogenesis of AD (43). Markesbery (14) showed that lipid peroxidation is a major cause of depletion of membrane phospholipids in AD. One of the products of lipid peroxidation, 4-hydroxynonenal, which is found in high concentrations in AD patients (44), proved to be toxic to hippocampal cells in culture (45). This highly reactive α,β-aldehyde is thought to cause neuronal death by altering the ATPases involved in ionic transfers and calcium homeostasis (14). The increased calcium concentration could itself cause a cascade of intracellular events, resulting in increased ROS and cellular death (46). Supporting evidence for this link between calcium homeostasis and free radicals derives from data showing that the

glutamate-dependent flux of calcium is associated with the production of free radicals by the mitochondria (47, 48). Nitric acid and peroxynitrite appear to play a crucial role in the excitotoxicity related to *N*-methyl-D-aspartate glutamate-receptor activation. Experiments in mice have shown that cells from neuronal nitric oxide synthase–deficient animals are resistant to toxicity induced by *N*-methyl-D-aspartate (49).

Recently, Montine et al (50) found that cerebrospinal fluid F_2-isoprostane concentrations are elevated in AD patients. These compounds are produced by free radical–catalyzed peroxidation of arachidonic acid, independent of the cyclooxygenase enzyme. This discovery is important because it confirms that lipid peroxidation is elevated in AD, but also because it suggests the possible use of the quantification of cerebrospinal fluid F_2-isoprostane concentrations as a biomarker of this disease.

PROTEIN GLYCATION AND ALZHEIMER DISEASE

Glycation phenomena have been held responsible for many age-related pathologies. Glycation of proteins starts as a nonenzymatic process with the spontaneous condensation of ketone or aldehyde groups of a sugar with a free amino acid group to form a labile Schiff base, consistent with the classical reaction first described by Maillard in 1912 (38). A cascade of reactions then results in the formation of AGEs, which are composed of irreversibly cross-linked heterogeneous protein aggregates.

Some studies showed the presence of AGEs in association with the 2 major proteins of AD, β-amyloid (51) and tau (34, 38). This observation supports the argument that AGEs are involved in the pathogenesis of AD (52, 53). Free radicals are involved in glycation processes and clearly can foster the formation of cross-linkings of β-amyloid (54). However, it has not been clearly established that these cross-linking phenomena play a causal role in AD. Mattson et al (54) propose that β-amyloid glycation is a late event in the evolution of AD and is the result of free radical generation by β-amyloid itself. On the basis of this hypothesis, glycation would not play a role in the disease. To quote Mattson et al (54), it would be "a tombstone, but not a cause."

Yan et al (55) showed that the AGE receptor, known as RAGE, is also a β-amyloid receptor. This discovery supports the idea of a relation between AGE and AD as well as between the production of free radicals and these 2 elements. The combination of AGE and RAGE can cause oxidative stress, as shown in the production of thiobarbituric acid–reactive substances, heme oxygenase-1, and the activation of nuclear transcription factor κB (NF-κB). β-Amyloid binding with RAGE also elicits the macrophage colony stimulating factor (56). This introduces an inflammatory pathway, according to a procedure linking oxidative processes and inflammation in AD. This observation could provide a partial explanation for the effect of nonsteroidal antiinflammatory drugs: their NF-κB–blocking action. However, many other studies showed that activation (not inhibition) of NF-κB can protect against neurodegeneration (57–60).

PRODUCTION OF ROS AND β-AMYLOID PROTEIN

Antioxidants providing protection from β-amyloid toxicity

Many studies have observed a direct toxic effect of β-amyloid on cultures of neurons or cell lines. If this reflects the situation in vivo, the phenomenon may offer an explanation for the role of amyloidogenesis in the pathogenesis of AD. Behl et al (61) showed that this β-amyloid toxicity on PC12 cell lines is prevented by vitamin E and by antioxidants in general. A second study by Behl et al (62) showed that hydrogen peroxide mediates this β-amyloid toxicity, which explains why catalase, which degrades hydrogen peroxide, protects the cells from β-amyloid toxicity. The same research team selected β-amyloid toxicity–resistant PC12 cell line clones and showed that they contained high concentrations of the antioxidant enzymes catalase and glutathione peroxidase (63). They also showed that the different amyloid peptides involved in various forms of amyloidosis in humans (amylin, calcitonin, and atrial natriuretic peptide) have a toxic effect similar to that of β-amyloid (64). In fact, experiments with synthetic peptides suggest that a common oxidative mechanism is found in all cases and that the amphiphilic form of the peptides rather than the structure in β layers may be the cause. The authors therefore concluded that β-amyloid toxicity could not be caused solely by an interaction through specific membrane receptors.

These pioneering studies provide clear confirmation of the involvement of the free radical process in β-amyloid toxicity. They are supported by studies of other free radical scavengers, such as *Ginkgo biloba* extract EGb 761 (65), melatonin (66), and EUK-8 (67). These scavengers also afford protection to hippocampal cells subjected to free radical stress. EGb 761 can also protect hippocampal cells from β-amyloid toxicity 4 h posttreatment (68).

β-Amyloid aggregation and production of free radicals

A dual relation exists between β-amyloid and the production of free radicals. Not only can the oxidative processes transform nonaggregated β-amyloid into aggregated β-amyloid in vitro (69), but β-amyloid itself is a source of free radicals. β-Amyloid interacts with vascular endothelial cells, producing a surfeit of free superoxide radicals that can scavenge the endothelium-derived relaxing factor and produce oxidizing agents causing lipid peroxidation (70). Even though this concerns the vascular endothelium and not the neurons, these data support the hypothesis that β-amyloid acts via the production of free radicals and plays a role in neurodegenerative processes.

Mass spectrometric and electron paramagnetic resonance spin trapping indicate that β-amyloid, in aqueous solution, fragments and generates free radical peptides (71). Some of these free radical peptides (the β-amyloid 25–35 fragment) have a potent lipoperoxidizing effect on the synaptosomal membranes in the neocortex (72). The many recent studies that focused on the involvement of the microglia as affected by β-amyloid, and therefore of inflammatory-type processes in the brains of AD patients, implicitly lead to the conclusion that free radicals are being produced because it is a well-known phenomenon associated with inflammation. McDonald et al (73) showed that the β-amyloid fibrils activate protein-tyrosine kinase–dependent signaling and superoxide production in the microglia. This research confirms the findings of other studies showing that β-amyloid stimulates ROS production in the microglia. Meda et al (74) showed that β-amyloid triggered the release of the NO_2^- radical by the microglia in rodents.

NF-κB and β-amyloid

The increase in the activity of NF-κB protects neurons against β-amyloid toxicity (75). Many new data suggest that NF-κB plays an important role in neurodegenerative disorders.

Goodman and Mattson (57) showed that activation of NF-κB may constitute a cytoprotective signaling pathway that induces expression of protective gene products such as calbindin and antioxidant enzymes. In fact, exposure of cells to oxidative stress results in activation of NF-κB (58, 76), and high constitutive NF-κB activity mediates resistance to oxidative stress in neuronal cells (77).

These data contradict those of Grilli et al (78) concerning the neuroprotective role of aspirin, which would be mediated through inhibition of NF-κB. According to Lipton (79), "it remains for future studies to determine whether we can explain the Janus faces of NF-κB activation that cause excitotoxic neurodestruction in Grilli's study but neuroprotection in the hands of Mattson." Barger et al (59) provide an explanation for these contradictory data: NF-κB activation of glial cells is probably neurotoxic but its involvement in neurons is probably neuroprotective.

It is also important to understand that antioxidants block the activation of NF-κB. However, this fact is quite logical: the activation of NF-κB is related to the importance of the free radical pool in the cells. When antioxidants decrease the quantity of free radicals, the cells no longer need to activate the protective mechanism of activation of NF-κB.

APOLIPOPROTEIN E AND OXIDATIVE STRESS

The *E4* allele of apo E has been linked to both late-onset family forms and sporadic forms of AD, whereas the *E2* allele appears to offer protection. This discovery inspired many new research projects and working hypotheses suggesting that oxidative processes play a role in AD. Apo E helps transport cholesterol through both the vascular system and the neurons. In the brain, apo E is the crucial cholesterol carrier, which explains the role it plays in neuroplasticity-related phenomena because these require changes to membrane lipids and, therefore, a cholesterol-dependent function. Poirier (80) maintains that apo E has a beneficial effect for neuronal protection but that the apo E4 isoform is less effective than are the apo E2 and E3 isoforms. (In other words, apo E4 may not be toxic, but simply capable of producing a less-favorable effect.) Miyata and Smith (81) showed that apo E has a beneficial effect against free radicals. Apo E reduced neuronal death caused by hydrogen peroxide and β-amyloid through antioxidant activity. This effect is clearly perceptible with the E2 isoform, but less so with E3 and even less so with E4.

However, Leininger-Muller et al (82) showed that apo E is sensitive to attacks by free radicals, with the responses being isoform dependent. The E4 isoform is more sensitive than is the E3 isoform, which in turn is more sensitive than is the E2 isoform. An antioxidant such as EGb 761 protects apo E from oxidation in a similarly isoform-dependent way; the effect is seen most clearly with apo E4 (82).

Ramassamy et al (33) established that another relation existed between the apo E genotype and lipoperoxidation in AD. They showed that the level of peroxidation in the brains of AD patients depended on the apo E genotype and was higher when the *E4* allele was present. This discovery is important because it explains why previous studies sometimes found an increase and sometimes found no change in lipoperoxidation in the brains of AD patients. Ramassamy et al also showed that the level of peroxidation in the brains of AD patients is inversely proportional to the concentration of apo E, which confirms the hypothesis that apo E has a beneficial effect against lipoperoxidation and that the effect is more pronounced when the patient has no *E4* allele. Ramassamy et al proved, in vitro, that EGb 761 effectively reduces the level of lipoperoxidation in the brains of AD patients.

MITOCHONDRIAL ANOMALIES AND ALZHEIMER DISEASE

Defects in the electron transport chain within the mitochondria are major factors contributing to the production of free radicals. Many studies have shown a low level of oxidative phosphorylation in AD, this being expressed both by energy deficits and the potentially toxic production of free radicals. Note that the electron transport chain that results in the formation of ATP via the reduction of oxygen to water is a complex enzymatic system consisting of 5 distinct phases: complex 1 (NADH dehydrogenase), complex 2 (succinate dehydrogenase), complex 3 (ubiquinol–cytochrome-*c* reductase), complex 4 (cytochrome-*c* oxidase), and complex 5 (ATP synthase).

Many observations have confirmed alterations in mitochondrial function in the course of aging and neurodegenerative diseases such as AD, Huntington disease, and Parkinson disease, and, for AD in particular, effects on cytochrome-*c* oxidase in complex 4 (83). Mutisaya et al (84) showed that postmortem cytochrome-*c* oxidase activity was ≈25–30% lower than normal in the cerebral cortex (frontal, parietal, temporal, and occipital) and in the platelets of AD patients. Simonian and Hyman (85) used histochemical methods to show the lower than normal cytochrome-*c* oxidase activity in the dentate gyrus and the CA4, CA3, and CA1 zones of the hippocampus. A decline in cytochrome-*c* oxidase activity is associated with decreased expression of messenger RNA (mRNA) molecules, which is lower in the midtemporal region of the brains of AD patients. Chandrasekaran et al (86) showed lower than normal mRNA concentrations in subunits 1 and 3 of cytochrome-*c* oxidase. In fact, normal amounts of cytochrome-*c* oxidase are found in the brains of AD patients; it is only the enzyme activity that is affected (87). Lakis et al (88) transferred this cytochrome-*c* oxidase defect to cybrid lines. The cybrid technique, which appears to hold great promise for the study of neurodegenerative diseases (89), uses cell lines stripped of their mitochondria and then transfers to them mitochondria from platelets (or other tissues) taken from patients with the disease to be studied. Lakis et al (88) observed increased production of free radicals by cybrids, which confirms that the cytochrome-*c* oxidase defect can produce damaging amounts of free radicals. The same reconstitution with mitochondria from AD platelets resulted in reduced cytochrome-*c* oxidase activity (90). Any possible relation between these mitochondrial anomalies and other processes involved in AD, amyloidogenesis in particular, is as yet unproved. However, Askanas et al (91), using an adenovirus as vector, showed that the transfer of the *APP* gene caused mitochondrial anomalies in a culture of muscular cells. This phenomenon could provide the explanation for inclusion-body myositis, a muscular condition similar to AD, but, on a broader scale, it could also establish a link between amyloid metabolism and mitochondrial anomalies.

Recently, the hypothesis of a link between mitochondrial function and cytochrome-*c* oxidase received considerable support with the discovery of mutations in cytochrome-*c* oxidase genes that segregate with late-onset AD (92). Cytochrome-*c* oxidase is produced by the combined effect of mitochondrial and nuclear

genes. However, these catalytic centers are encoded exclusively by 2 mitochondrial genes, *CO1* and *CO2*, which encode for subunits I and II, respectively. The gene encoding subunit III is *CO3*. Davis et al (92) described several DNA polymorphisms in *CO1* and *CO2* (but not in *CO3*) that segregate with AD. However, subsequent work by other groups concluded that these polymorphisms are not present in the mitochondrial genome but are rather nuclear pseudogenes (93, 94). Davis et al (95) recognized that the presence of these nuclear mitochondria-like DNA sequences is unlikely to cause the cytochrome-*c* oxidase defect in humans. However, they believe that an elevation in the ratio of this pseudogene relative to authentic mitochondrial DNA in the blood of AD patients still holds true.

How can mitochondrial defects have an influence on the development of AD? In theory, they can trigger 2 harmful events: the production of destructive free radicals and a reduction in energy resources. The slowing down of energy metabolism in AD has been extensively reported in pyruvate dehydrogenase (lipoamide), oxoglutarate dehydrogenase (lipoamide), and oxidative phosphorylation enzymes (96, 97). A reduction to ≈50% of normal activity of oxoglutarate dehydrogenase (lipoamide) was found in cultured AD fibroblasts (98). Observations in AD patients were also made in vivo, in which positron emission tomography was used to study glucose metabolism in patients. A reduction in the regional cerebral metabolic glucose concentration was observed at rest. This was seen throughout the neocortex and was found to correlate with the severity of dementia (99). Despite this reduced metabolism at rest, often accompanied by left-right asymmetry, some cortical regions of the brains of AD patients can still be activated during certain cognitive tasks (100). Rapoport et al (101) interpreted these findings to suggest the existence of a down-regulation process in AD that, in the early stages, is reversible. The study of gene expression supports this hypothesis. A drop in mRNA can be detected in the mitochondrial genes of the cytochrome-*c* oxidase complex and also in the mRNA of the cytochrome-*c* oxidase complex 4 subunit encoded by a nuclear gene. Rapoport et al (101, 102) maintained that, because of the lower neuronal energy requirement caused by synaptic damage in AD, there may be a down-regulation of cytochrome-*c* oxidase subunit gene expression. Reduced brain glucose utilization in the early stages of AD, as measured with positron emission tomography, would reflect this down-regulation. If this were the case, the free radicals would not be the cause of the process, but simply a consequence.

WHICH FREE RADICALS ARE INVOLVED IN ALZHEIMER DISEASE?

Many free radicals are likely involved in AD. There are excellent reasons to suspect the free hydroxyl radical as a factor in AD (15) because of its toxicity and its role in various chemical reactions, such as Fenton's, involving iron. Other suspects include the superoxide radical hydrogen peroxide, which is implicated in amyloid neurotoxicity, and peroxynitrite, which can be formed by combining superoxide and nitric oxide. Peroxynitrite can react with carbon dioxide to form unstable transmitters that act as free hydroxyl radicals. Many recent studies have confirmed the toxicity of peroxynitrite to neurons and the involvement of nitric oxide in neurologic pathologies. Smith et al (36) observed traces of oxidative modifications caused by peroxynitrite in the brains of AD patients, and this protein nitration included, among others, neurofibrillary tangles. This was not, however, observed in brains that were not affected by AD.

THERAPEUTIC USE OF ANTIOXIDANTS

All observations suggesting the involvement of ROS in the pathogenesis of AD raise the possibility of the therapeutic use of free radical scavengers and antioxidants. The hypothesis is particularly attractive because many free radical scavengers are known and many (eg, vitamins E and C, *Ginkgo biloba* extract EGb 761, melatonin, flavonoids, and carotenoids) have no major side effects. This hypothesis has been tested with a reasonable degree of success under both experimental and clinical conditions. Many experimental studies showed that free radical–scavenging substances inhibit the toxic effect of β-amyloid or hydrogen superoxide on cell cultures and organotypic hippocampal cultures (66–68).

Many preliminary clinical studies have been completed and published. Crapper McLachlan et al (103) showed that administration over a 2-y period of an iron-chelating agent, desferrioxamine, slowed the clinical development of the disease. It was hoped that the chelating effect would make it possible to inhibit iron-dependent lipid peroxidation, which, as has been seen, can play a role in the etiology of AD.

Three free radical–scavenging drugs used for therapeutic purposes in different fields were also scrutinized in clinical studies of AD and produced beneficial results: vitamin E (α-tocopherol), selegiline (also a monoamine oxidase B inhibitor), and *Ginkgo biloba* extract EGb 761(Tanakan).

The first 2 products were investigated as part of the Alzheimer's Disease Cooperative Study in 324 patients with moderately severe AD (104). The authors did not observe any significant improvement on cognitive tests (unlike observations of certain cholinesterase inhibitors, tetrahydroaminoacridine and E2020) but did observe significant delays in the time of the occurrence of the following outcomes: death, institutionalization, loss of the ability to perform basic activities of daily living, and severe dementia. These findings provided supporting evidence for the hypothesis that antioxidants may be capable of slowing the pathogenic process.

EGb 761 has been studied in clinical investigations both in Germany (105) and in the United States (106). The studies concluded that it had a positive effect on cognitive indexes, similar to the results obtained with tetrahydroaminoacridine. A formal review of >30 articles pointed to a small but significant effect of this drug on cognitive function in AD (107). Interestingly, this result differs from the findings for α-tocopherol and selegiline because neither of these 2 drugs had any significant effect on cognition. Note, however, that EGb 761 has many other effects in addition to its free radical–scavenging activity, including a protective effect on neuroreceptors in aging subjects, a monoamine oxidase–inhibiting effect (less of an effect than selegiline, but no doubt an indirect effect), and a clear effect on cognitive indexes in both animals and humans (108, 109).

Nonsteroidal antiinflammatory drugs have also been shown to have a useful effect in countering AD (110, 111). These drugs operate principally by acting on cyclooxygenases and inhibiting prostaglandin synthesis, or by decreasing glutamate-related excitotoxicity and reducing the production of ROS. Lastly, the beneficial effect of estrogen in AD may be partly attributable to its antioxidant activity. Behl et al (112) showed

that 17β-estradiol protects neuroblastoma cells against oxidative stress, and Goodman et al (113) showed that 17β-estradiol and estriol suppress membrane oxidation in hippocampal neurons induced by β-amyloid.

NUTRITION AND ALZHEIMER DISEASE

Although free radical scavengers are known to have a beneficial effect when taken in small doses as a preventive treatment, the influence of free radical scavengers in food is still difficult to prove. Many free radical scavengers are present in food, particularly in fruit and vegetables (eg, as carotenoids and flavonoids). Regular consumption of these nutritive substances may have beneficial effects. Some preliminary results have shown that diets high in antioxidant activity (strawberry extracts, spinach, and blueberry extracts) prevent the deleterious effects of oxidative stress on signal transduction and nerve growth factor in rats (114). There was also a trend toward the prevention of deficits in motor behavior. However, these experiments in animals are not directly related to AD.

Regular consumption of antioxidants in the diet may have a beneficial effect in humans. Cognitive impairment has been associated with lower vitamin C intakes (115). Fruit and vegetables could also have protective effects against stroke and vascular dementia (116). Epidemiologic studies to confirm the beneficial effects of antioxidants in AD have not yet been conducted.

CONCLUSION

The idea that free oxygen radicals might be involved in AD was originally based on general considerations focusing on free radical processes involved in aging and many medical conditions, particularly in cerebral pathologies (4, 6). This idea then inspired many research projects, which were often unable to reach any firm conclusions. Data on activities of antioxidant enzymes in AD are contradictory: some researchers have detected alterations to glutathione peroxidase and superoxide dismutase (Mn or Cu/Zn), whereas others have not (14).

Still, the involvement of free radicals in the pathogenesis of AD is, however, now widely accepted for the following reasons:

1) Neurons are particularly sensitive to free radicals.
2) Aging is the principal AD risk factor and is itself related to accumulated free radical attacks.
3) Examinations of the brains of AD patients show many signs of free radical attacks, ie, damage to mitochondrial and nuclear DNA, protein oxidation, lipid peroxidation, and AGEs.
4) Traces of substances have been found in the brains of AD patients that indicate the presence of metals (iron, copper, zinc, and aluminum) capable of catalyzing reactions that produce free radicals.
5) Free radical scavengers reduce the toxicity of β-amyloid.
6) β-Amyloid is sensitive to the action of free radicals, contributing to aggregation and itself producing peptides in free radical form.
7) Apo E is subject to free radical attacks and a correlation exists between apo E peroxidation and AD; it can also act as an isoform-dependent free radical scavenger. The oxidative status of the brain is related to the apo E genotype.
8) AD is related to mitochondrial anomalies, particularly for cytochrome-*c* oxidase, and these anomalies may explain the abnormal production of free radicals.
9) The use of many free radical scavengers (vitamin E, selegiline, and *Ginkgo biloba* extract EGb 761) has produced positive therapeutic results, as has the use of antiinflammatory drugs, estrogens, and the iron-chelating agent desferrioxamine, which inhibits the catalytic action of iron.

This evidence clearly supports the involvement of free radicals and ROS in AD. However, it is unknown whether free radicals are really one of the basic causes of the pathogenesis and neuronal damage in AD. Free radicals may act indirectly as a result of other pathologic processes. Their involvement does not seem specific to any particular disease and evidence for their role can be challenged for this reason. Metals, for example, are present in the brains of all people and not just in those with AD. It is plausible that we can describe neuropathologic disorders (eg, AD, but also Parkinson disease, Huntington disease, and amyotrophic lateral sclerosis) as a combination of 2 kinds of events: a genetic and specific defect related to a particular molecule (eg, presenilin or APP in AD, parkin in Parkinson disease, and tau in frontotemporal dementia with Parkinsonism) and the age-related abnormal production of free radicals. The development of these diseases requires these 2 events to occur. In AD, the occurrence of these 2 events is clear: recent data from rhesus monkeys show that the microinjection of β-amyloid results in profound neuronal loss, tau phosphorylation, and microglial proliferation only in aged animals (117). Even if they are not the specific cause of the disease, free radicals are likely an essential factor in the pathogenesis of AD and, consequently, are a potential target for therapy.

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