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The Role of Oxidative Stress in Alzheimer Disease

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Increasing evidence demonstrates that oxidative stress causes damage to cell function with aging and is involved in a number of age-related disorders including atherosclerosis, arthritis, and neurodegenerative disorders. In the neurodegenerative diseases, oxidative stress has been implicated in amyotrophic lateral sclerosis, Parkinson disease, Huntington disease, and Alzheimer disease (AD). The neurodegenerative disorder receiving the most attention has been AD, in which an increase occurs in oxidation of brain lipids, carbohydrates, proteins, and DNA. Some of the products of oxidation have been found in the major histopathologic alterations in AD: the neurofibrillary tangles (NFTs) and senile plaques (reviewed in Markesbery and Carney[1] and Ceballos-Picot[2]). These oxidative modifications are closely associated with a subtle inflammatory process in the brain in AD.

Oxidative stress refers to a state in which free radicals and their products are in excess of antioxidant defense mechanisms. This imbalance can occur as a result of increased free radical production or a decrease in antioxidant defenses. Free radicals are defined as any atom or molecule that has one or more unpaired electrons in its outer shell. The reduction of molecular oxygen to water is a major source of potent radicals. The initial step in this reaction yields the superoxide radical, which produces hydrogen peroxide by addition of an electron. The reduction of hydrogen peroxide yields the highly reactive hydroxyl radical. These radicals plus singlet oxygen are called reactive oxygen species (ROS). Several reactive nitrogen species, nitric oxide, and peroxynitrite also are important modulators of oxidative stress. These free radicals and others are capable of reacting with lipids, proteins, nucleic acids, and other molecules and altering their structure and function. Oxidative stress can lead to alterations in cells with an accumulation of oxidized products such as aldehydes and isoprostanes from lipid peroxidation, protein carbonyls from protein oxidation, and base adducts from DNA oxidation, all of which serve as markers of oxidation.

Because the brain is largely composed of easily oxidized lipids, has a high oxygen consumption rate, and lacks strong antioxidant defenses, it is quite vulnerable to oxidative injury. It has been demonstrated that there is an increase in oxidation in the brain with aging, which is the most consistent risk factor for AD. Another factor that makes the brain more susceptible to oxidation in AD is the presence of increased iron, a critical element in the generation of ROS. The gradual accumulation of oxidative damage over time in postmitotic neurons could account for the late-life onset and gradually progressive nature of the decline in AD.

Approximately 98% of molecular oxygen is consumed by the mitochondrial electron transport chain at the cytochrome oxidase complex. The remainder of the oxygen is reduced to hydrogen peroxide and the superoxide radical. Under stressful conditions and in aging, the electron transport system can increase ROS formation considerably. Thus, the mitochondria are both a source and a target of toxic ROS. Mitochondrial dysfunction and oxidative metabolism may play an important role in the pathogenesis of AD and other neurodegenerative diseases (see Beal[3]

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for review). Reduced cytochrome oxidase activity and messenger RNA levels have been found in autopsied brains of patients with AD. Using cybrid techniques, researchers have shown that AD cytochrome oxidase defects can be transferred into cybrid cell lines that demonstrate increased cytosolic calcium concentrations and an increase in free radical production.[4] Overall, it seems that the mechanisms by which mitochondrial dysfunction can lead to neuron degeneration in AD is through impaired production of adenosine triphosphate, altered calcium homeostasis, ROS generation, activation of the mitochondrial permeability transition, and excitotoxicity.

LIPID PEROXIDATION

Increased lipid peroxidation occurs in the brain in AD and is most prominent where degenerative changes are most pronounced.[1] Brain membrane phospholipids are composed of polyunsaturated fatty acids, which are especially vulnerable to free radical attack because their double bonds allow easy removal of hydrogen ions. Decreases in polyunsaturated fatty acids, primarily arachidonic and docosahexaenoic acids, accompany lipid peroxidation in AD. Oxidation of polyunsaturated fatty acids produces aldehydes, one of the most important of which is 4-hydroxynonenal (HNE), a highly reactive cytotoxic substance capable of inhibiting glycolysis, nucleic acid and protein synthesis, and degrading proteins. Four-hydroxynonenal levels are increased in autopsied specimens from multiple brain regions and in the cerebrospinal fluid (CSF) in subjects with AD, and HNE adducts are present in NFTs.[1] Glutathione transferases, a group of enzymes that inactivate the toxic products of oxygen metabolism including 4-hydroxyalkenals such as HNE, are markedly diminished in multiple brain regions and in the CSF in subjects with AD, suggesting a loss of protection against HNE.[5]

Four-hydroxynonenal causes degeneration and death of cultured hippocampal neurons by impairing ionmotive adenosine triphosphatase activity and disrupting calcium homeostasis.[6] Exposure of cultured hippocampal neurons to β-amyloid (βA) peptide causes a significant increase in levels of free and protein-bound HNE and increases ROS. Four-hydroxynonenal impairs glucose and glutamate transport and is capable of inducing apoptosis in cultured neurons. Administration of HNE into the basal forebrain of rats causes damage to cholinergic neurons, diminished choline acetyltransferase, and impaired visuospatial memory.[7]

The F_2-isoprostanes are prostaglandin-like compounds that are formed nonenzymatically by free radical–induced oxidation of arachidonic acid. Oxidation of docosahexaenoic acid forms F_4-neuroprostanes. F_2-isoprostanes are elevated in postmortem ventricular CSF of subjects with AD,[8] and in the lumbar CSF from living patients with probable AD, but not in the CSF from living patients with amyotrophic lateral sclerosis.[9] F_4-neuroprostane levels are elevated in postmortem ventricular CSF[8] and are more abundant in the brain than F_2-isoprostane levels. This suggests that these quantifiable markers of brain lipid peroxidation potentially could be used to assess the efficacy of therapeutic agents to decrease lipid peroxidation in AD.

PROTEIN OXIDATION

The oxidation of proteins by free radicals may also play a meaningful role in AD. Hydrazide-reactive protein carbonyl is a general assay of oxidative damage to protein. Several studies demonstrate an increase in protein carbonyls in multiple brain regions in subjects with AD and in their NFTs.[10] The oxidation of brain proteins may be at the expense of enzymes critical to neuron and glial function. Two enzymes that are especially sensitive to oxidative modification are glutamine synthetase and creatine kinase, both of which are markedly diminished in the brains of subjects with AD. Oxidative alterations in glutamine synthetase could cause alteration of glutamate concentrations and enhance excitotoxicity, whereas oxidative impairment of creatine kinase could cause diminished energy metabolism in AD.

Pathologic aggregation of proteins into fibrils is a characteristic of AD. Oxidative modifications can cause crosslinking of covalent bonds of proteins leading to fibril formation and insolubility. Neurofibrillary tangles are characterized by the aggregation and hyperphosphorylation of tau proteins into paired helical filaments. Phosphorylation is linked to oxidation through the microtubule-associated protein kinase pathway and through the activation of the transcription factor NFκB, thus potentially linking oxidation to the hyperphosphorylation of tau proteins. Oxidation of cysteine in tau protein controls the in vitro assembly of paired helical filaments. The role of oxidation damage in NFT formation is supported by the presence of protein carbonyls, nitrotyrosine (a marker of the potent radical peroxynitrite), HNE, acrolein (another highly reactive aldehyde product of lipid peroxidation), advanced glycation end products (AGE), and hemeoxygenase-1 (an antioxidant enzyme) in NFTs.[1]

DNA OXIDATION

Oxidation of DNA can produce strand breaks, sister chromatid exchange, DNA-protein crosslinking, and base modifications. The DNA damage accumulating in nondividing mammalian cells may play a major role in aging-associated changes. Several studies demonstrate an increase in oxidative DNA damage in the brains of subjects with AD (see Gabbita et al[11] for review). The most pronounced DNA adduct described is 8-hydroxy-2′-deoxyguanosine (8-OHdG), which is increased in nuclear and mitochondrial brain fractions in AD. Elevations of 5-hydroxyuracil, 8-hydroxyadenine, and 5-hydroxycytosine levels also have been found in nuclear brain fractions in subjects with AD. The pattern of damage to multiple bases is most likely due to hydroxyl radical attack on DNA. Elevations of 8-OHdG levels in intact DNA have been described in the CSF of patients with AD, along with a decrease in free 8-OHdG, representing the repair product, suggesting that there is a double insult of increased DNA damage and deficiencies in repair mechanisms responsible for removal of oxidized bases in AD.[12]

The importance of finding increased products of oxidation in the CSF of patients with in AD (HNE, F_2-isoprostanes, F_4-neuroprostanes, 8-OHdG) deserves fur-

ther study. Perhaps, coupled with the elevated tau protein levels and decreased levels of βA peptides in AD CSF,[13] they could possibly be used to improve the diagnostic accuracy of AD.

GLYCO-OXIDATION

Advanced glycation end products are posttranslational modifications of proteins that are formed when the amino group of proteins reacts nonenzymatically with monosaccharides, and may play a role in AD that is linked to oxidative modifications of βA peptides and tau.[14] Advanced glycation end products are present in senile plaques in subjects with AD, and AGE-modified βA peptides accelerate aggregation of soluble nonfibrillar βA peptides. β-Amyloid peptide binds to the receptors for AGE and generates ROS, activating NFκB, which induces expression of macrophage colony-stimulating factor, enhancing proliferation of microglia. Activated microglia are capable of producing the superoxide radical and nitric oxide. Tau and AGE antigens are localized in NFTs, and glycated tau added to neuroblastoma cells in cultures induces lipid peroxidation.

ENDOGENOUS ANTIOXIDANTS IN AD

Multiple studies of copper/zinc– and manganese-superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase activity, and gene expression in autopsied brains of subjects with AD have not demonstrated a consistent pattern of change.[1] Several studies of brains from autopsies with short postmortem interval show elevation of activity and gene expression of these antioxidants in brain regions that demonstrate an increase of lipid peroxidation in AD, possibly reflecting a compensatory rise in response to free radical generation.[1] Importantly, none of these major antioxidants is consistently diminished, indicating that this aspect of the defense mechanism against free radicals is intact. Recent evidence suggests that methionine may act as an antioxidant defense molecule in proteins by its ability to scavenge oxidants and in the process undergo oxidation to form methionine sulfoxide. The enzyme methionine sulfoxide reductase reverses methionine sulfoxide back to methionine. Our recent study shows a statistically significant decline in methionine sulfoxide reductase in postmortem brain specimens from subjects with AD,[15] which may contribute to an increase in protein oxidation in the AD brain.

CELL CULTURE AND TRANSGENIC ANIMAL EXPERIMENTS

Data from cell culture and animal experiments by Mattson[16] demonstrate that oxidative stress and dysregulation of calcium can damage neurons, which indicates a role for oxidative stress in the pathogenesis of AD. Exposure of cultured neurons to βA peptides causes an increase in oxyradical formation and radical-mediated damage to membrane lipids and proteins. β-Amyloid–induced neuron death in vitro is attenuated by antioxidants such as vitamin E and glutathione. β-Amyloid peptides are capable of spontaneously forming oxygen radicals that damage enzymes. They also generate radicals through interaction with iron and zinc, both of which are increased in the brain of subjects with AD.

Familial, early-onset, autosomal-dominant AD is associated with mutations in the presenilin genes 1 and 2 and the amyloid precursor protein. Experimental studies using cultured cells and transgenic mice expressing presenilin gene 1 mutations have yielded considerable progress in understanding the pathogenetic mechanisms of *presenilin* mutations.[17] Neurons expressing mutant presenilin gene 1 exhibit increased levels of βA peptides and altered calcium homeostasis in the endoplasmic reticulum that lead to increased ROS production, mitochondrial dysfunction, and adenosine triphosphate depletion. This causes an apoptotic death of neurons that can be prevented by vitamin E and glutathione.

Studies of transgenic mice and cultured neurons expressing the amyloid precursor protein mutations suggest that these mutations also lead to an increased production of free radicals in neurons.[16] Cultured cells expressing mutated forms of amyloid precursor protein have an increased production of βA peptides and set in motion a process of increasing oxygen free radicals, lipid peroxidation, and calcium dysregulation. Transgenic mice overexpressing the amyloid precursor protein mutation demonstrate HNE and hemeoxygenase-1 around βA peptide deposits, and iron and pentosidine (an AGE) in the center of βA deposits, indicating an association between oxidative stress and βA deposition.[18]

Meta-analysis findings from 17 epidemiologic studies suggest that nonsteroidal anti-inflammatory drugs play a protective role against AD.[19] A number of markers of inflammation are present in the brain in AD, and some are related to the morphological changes associated with AD. Although the details of the inflammatory response are beyond the scope of this review, it seems that the inflammatory cascade is important in the pathogenesis of AD and that microglia are key mediators of this response. The relationship between the inflammatory response and free radical generation is of considerable theoretical and therapeutic interest.

Although AD is probably associated with multiple etiologies and pathophysiologic mechanisms, it appears that oxidative stress is a part of the pathophysiologic process. It is not clear whether oxidative stress is a primary process in AD or the result of the disease, although emerging data indicate that oxidative damage is an early event in neurodegeneration in AD. Regardless of whether oxidative stress is a primary or secondary event, therapeutic measures to decrease the level of oxidative stress and to reduce the risk or slow the progression of the disease are appropriate. Findings of a large multicenter trial support this concept, showing that antioxidant therapy (vitamin E and/or selegiline hydrochloride) may slow the progression of AD.[20] Long-term treatment of subjects at risk for AD, using more efficacious antioxidant therapeutic agents, could potentially slow neuron degeneration and delay or prevent the onset of the disease.

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