Damage to Lipids, Proteins, DNA, and RNA in Mild Cognitive Impairment

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Free radical–mediated oxidative damage is thought to play a role in the pathogenesis of Alzheimer disease. Previous studies have shown oxidative damage to lipids, proteins, DNA, and RNA in multiple brain regions in late-stage Alzheimer disease. Recent studies on patients with amnestic mild cognitive impairment who have undergone autopsy have shown increased lipid peroxidation as well as protein, DNA, and RNA oxidation in multiple brain regions. These studies establish oxidative damage as an early event in the pathogenesis of Alzheimer disease that can serve as a therapeutic target to slow the progression or perhaps the onset of the disease.
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The key to Alzheimer disease (AD) is to gain an understanding of the etiologic and pathogenic factors involved in the disease. Of the pathogenic hypotheses, the oxidative stress hypothesis in AD is appealing because it involves several other hypotheses—the trace element hypothesis (iron, copper), the mitochondrial-bioenergetic decline hypothesis, and the β-amyloid peptide hypothesis. In recent years, numerous studies have strongly suggested that free radical–mediated oxidative damage plays a role in the pathogenesis of AD. Multiple articles[1-3] have demonstrated increased lipid peroxidation, protein, DNA, and RNA oxidation, and glyco-oxidation in AD. These studies have shown widespread oxidative damage throughout multiple brain regions in AD, and several immunohistochemical studies have shown that the oxidative damage is prominent in neuron cytoplasm. The problem with these studies is that they have been performed using brain specimens from patients with late-stage AD (LAD). This is a disadvantage because finding alterations in LAD does not determine whether oxidative damage is a late consequence of the disease or whether it occurs early in the disease process when it would be a potential therapeutic target.

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The present emphasis in AD clinical research has turned to early disease detection with the hope of early treatment to stop or slow the progression of the disease. The concept of mild cognitive impairment (MCI) as a phase between normal aging and early dementia and AD has served as an added stimulus for early detection.[4,5] In addition to treatment advantages, early detection could also lead to enhanced insight into pathogenetic factors in the disease.

LIPID PEROXIDATION

Recently, there have been a number of studies indicating that there is increased oxidative damage in patients with MCI or early AD (EAD). Several of these studies have shown an increase in lipid peroxidation in MCI. The brain is subject to free radical–induced lipid peroxidation because it uses one-third of the inspired oxygen, is rich in polyunsaturated fatty acid (targets for free radical attack), and is relatively high in redox transition metal ions but is relatively low in antioxidant capacity. Lipid peroxidation causes structural changes in membranes and release of bioactive α,β-unsaturated aldehydes including 4-hydroxy-2-nonenal and acrolein. It also produces isoprostanes and neuroprostanes, which are not bioactive but serve as excellent markers for arachidonic acid and docosahexaenoic acid oxidation. One of the first studies of oxidative dam-

age in the brain of patients with amnestic MCI demonstrated an increase in thiobarbituric acid–reactive substances (21%) and malondialdehyde (60%) (measurements of lipid peroxidation) in the temporal lobe compared with healthy controls.[6] Our recent study[7] showed significantly elevated mean F_2-isoprostane levels in frontal, inferior parietal lobule (IPL), and occipital regions of longitudinally followed patients with MCI compared with longitudinally followed and age-matched control subjects. Mean levels in the IPL and occipital regions were higher in patients with MCI than in patients with LAD. Mean F_4-neuroprostane levels were significantly higher in the IPL and occipital regions in patients with MCI compared with controls and higher than or equal to those in patients with LAD. Mean levels of both F_2-isoprostane and F_4-neuroprostane were higher in the hippocampus (HIP) in patients with MCI than in controls, but these did not reach statistical significance. Williams et al[8] demonstrated elevated 4-hydroxy-2-nonenal levels in the HIP, superior and middle temporal gyri, and cerebellum in longitudinally followed patients with MCI compared with healthy controls. Also in this study, acrolein levels were significantly elevated in the superior and middle temporal gyri in patients with MCI; although acrolein levels were elevated in the HIP, they were not statistically significant. Comparison of patients with EAD and control subjects showed a significant increase in 4-hydroxy-2-nonenal levels in the HIP and superior and middle temporal gyri and a significant increase in acrolein levels in all of the 3 brain regions studied. All of these studies were performed on thoroughly evaluated, longitudinally followed patients with amnestic MCI and EAD and controls who had short-postmortem-interval autopsies (mean < 4 hours). These results indicate that lipid peroxidation is an early event in AD, and they also suggest that it does not increase to a much greater level with disease progression.

Several earlier studies of cerebrospinal fluid (CSF) support the findings described earlier. In one of these, Praticò et al[9] found CSF levels of 8,12-iso-iPF_{2a}-VI significantly higher in living patients with MCI than in healthy controls. Montine et al[10,11] also found elevated levels of F_2-isoprostane in lumbar CSF of living patients with EAD (dementia < 2 years' duration).

PROTEIN OXIDATION

Keller et al[6] first showed an increase in levels of protein carbonyls (a marker of protein oxidation) in the superior and middle temporal gyri in patients with MCI compared with healthy control subjects. Using redox proteomics, Butterfield et al[12] demonstrated oxidation of several specific proteins in the HIP of patients with amnestic MCI, including Pin1, α-enolase, glutamine synthetase, and pyruvate kinase M2. Further research is required to investigate the oxidation of proteins in AD to define whether they exhibit a functional decline.

DNA OXIDATION

Oxidation of DNA causes strand breaks, DNA-DNA and DNA-protein crosslinking, and DNA base modification that could lead to alterations in transcription and protein translation. These damaged or diminished proteins can alter neuron function and eventually lead to neuron death. Mitochondrial DNA (mtDNA) may be more easily oxidized than nuclear DNA (nDNA) because of its proximity to reactive oxygen species, lack of protective histones, and limited repair capacity.[13,14] More than 20 DNA adducts have been identified, but the most thoroughly studied involves the C8 hydroxylation of guanine and formation of 8-hydroxy-2′-deoxyguanosine. Previous studies[15-18] have shown that 8-hydroxy-2′-deoxyguanosine is oxidized in nDNA and mtDNA in LAD.

Our study[19] was the first to quantify oxidized DNA base adducts in the brains from short-postmortem-interval autopsies of longitudinally followed patients with amnestic MCI using gas chromatography–mass spectrometry with selective ion monitoring and stable-labeled internal standards. 8-Hydroxy-2′-deoxyguanosine levels were significantly elevated in nDNA and mtDNA from frontal and temporal lobes of patients with MCI compared with controls. 5-Hydroxycytosine levels were significantly elevated in nDNA from 3 neocortical lobes and in frontal lobe mtDNA in patients with MCI. Levels of 8-hydroxyadenine were significantly increased in frontal, temporal, and parietal lobes in nDNA in patients with MCI compared with controls. Levels of 4,6-diamino-5-formamidopyrimidine (fapyadenine), which is formed by free radical attack of the C8 of adenine followed by ring opening under lower oxygen tension,[20-22] were significantly elevated in nDNA and mtDNA of all 3 neocortical lobes studied in patients with MCI compared with controls. Compared with our previous study of LAD,[18] this study showed that levels of 8-hydroxy-2′-deoxyguanosine, 8-hydroxyadenine, and fapyguanine were not significantly different in MCI and LAD. These results indicate that both nDNA and mtDNA are oxidized early in AD.

RNA OXIDATION

Nunomura et al[23] showed increased levels of 8-hydroxyguanine (a marker of RNA oxidation) and nitrotyrosine (a marker of protein oxidation) in EAD that decreased with disease progression in relation to increased β-amyloid peptide plaque deposition and neurofibrillary tangle formation. Their study, which did not include patients with MCI, used data from 2 patients who had AD for 3 years; the remaining patients had the disease for 6 years or longer. In support of the concept that RNA oxidation is an early event in AD, Ding et al,[24] using immunochemistry, showed significantly elevated levels of 8-hydroxyguanine in the IPL but not the cerebellum in well-documented patients with amnestic MCI compared with controls. This study also showed decreased ribosomal and transfer RNA levels and a decrease in protein synthesis capacity in IPL in patients with MCI.

A combined magnetic resonance imaging and CSF marker study of living patients with MCI showed elevated levels of CSF hyperphosphorylated tau and isoprostane and decreased hippocampal volume in patients with MCI compared with controls.[25] The combination of hippocampal volume measurements and increased CSF isoprostane measurements achieved a diagnostic accuracy of about 90%.

Although others[23,26,27] have suggested that oxidative damage is an early event in AD, they have not examined the brains of patients with MCI, the earliest detectable phase of the disease. Our studies and those of others described earlier have demonstrated oxidation of lipids, numerous proteins, DNA, and RNA in multiple brain regions in subjects with MCI, which strongly suggests that oxidative damage is involved in the pathogenesis of neuron degeneration in AD. In addition, these findings establish oxidative damage as an early therapeutic target for slowing the progression or perhaps preventing the onset of the disease.

FUTURE DIRECTIONS

Petersen et al[28] used 2000 IU of vitamin E daily for 3 years to treat patients with MCI and found no benefit from the vitamin E. However, the study did not use vitamin C in conjunction with vitamin E, which has been shown to decrease lipid peroxidation.[29] Combined effects of these 2 vitamins and diets rich in these vitamins have shown a protective effect against AD.[30-32] Because there is widespread oxidation of multiple macromolecules of lipids, proteins, DNA, and RNA in MCI, it is most likely that a single antioxidant such as vitamin E is not sufficient neuroprotection in this disorder. Better antioxidants and agents used in combination to up-regulate defense mechanisms against oxidation will be required to neutralize the oxidative component of the pathogenesis of AD. It is most likely that to optimize these neuroprotective agents, they will have to be used in the presymptomatic phase of the disease. This will involve clarifying preclinical markers and identifying genetic risk factors of AD.

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