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Redox proteomic identification of 4-Hydroxy-2-nonenal-modified brain proteins in amnestic mild cognitive impairment: Insight into the role of lipid peroxidation in the progression and pathogenesis of Alzheimer's disease

Tanea Reed,[a] Marzia Perluigi,[a,b] Rukhsana Sultana,[a,c] William M. Pierce,[d] Jon B. Klein,[e] Delano M. Turner,[d] Raffaella Coccia,[b] William R. Markesbery,[f,g,h] and D. Allan Butterfield[a,c,f,*]

[a]*Department of Chemistry, University of Kentucky, Lexington, KY 40506-0055, USA*
[b]*Department of Biochemical Sciences, University of Rome "La Sapienza", Rome 00185, Italy*
[c]*Center of Membrane Sciences, University of Kentucky, Lexington, KY 40506-0059, USA*
[d]*Department of Pharmacology, University of Louisville School of Medicine and VAMC, Louisville, KY, USA*
[e]*Kidney Disease Program and Core Proteomics Laboratory, University of Louisville School of Medicine and VAMC, Louisville, KY, USA*
[f]*Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536, USA*
[g]*Department of Neurology, University of Kentucky, Lexington, KY 40536-0230, USA*
[h]*Department of Pathology, University of Kentucky, Lexington, KY 40536-0230, USA*

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Numerous investigations point to the importance of oxidative imbalance in mediating AD pathogenesis. Accumulated evidence indicates that lipid peroxidation is an early event during the evolution of the disease and occurs in patients with mild cognitive impairment (MCI). Because MCI represents a condition of increased risk for Alzheimer's disease (AD), early detection of disease markers is under investigation. Previously we showed that HNE-modified proteins, markers of lipid peroxidation, are elevated in MCI hippocampus and inferior parietal lobule compared to controls. Using a redox proteomic approach, we now report the identity of 11 HNE-modified proteins that had significantly elevated HNE levels in MCI patients compared with controls that span both brain regions: Neuropolypeptide h3, carbonyl reductase (NADPH), α-enolase, lactate dehydrogenase B, phosphoglycerate kinase, heat shock protein 70, ATP synthase α chain, pyruvate kinase, actin, elongation factor Tu, and translation initiation factor α. The enzyme activities of lactate dehydrogenase, ATP synthase, and pyruvate kinase were decreased in MCI subjects compared with controls, suggesting a direct correlation between oxidative damage and impaired enzyme activity.

We suggest that impairment of target proteins through the production of HNE adducts leads to protein dysfunction and eventually neuronal death, thus contributing to the biological events that may lead MCI patients to progress to AD.

Keywords: Mild cognitive impairment; Lipid peroxidation; HNE; 4-hydroxynonenal; Alzheimer's disease; Redox proteomics

Introduction

Amnestic mild cognitive impairment (MCI) is currently described as the transitional phase between normal aging and early Alzheimer's disease (AD), with a subtle but measurable memory disorder not associated with dementia. Individuals with MCI are at an increased risk of developing AD, or another form of dementia with a rate of progression between 10% and 15% per year (Petersen, 2003), although there have been cases where patients have reverted to normal (Apostolova et al., 2006). Consequently, it is important to identify subjects with MCI. Accumulating evidence indicates that reactive oxygen species-mediated reactions, particularly of neuronal lipids, are extensive in AD brain areas involved in the disease processes (Butterfield and Lauderback, 2002; Montine et al., 2002). In recent years, investigations have pointed to the functional importance of oxidative imbalance as a crucial event in mediating AD pathogenesis (Butterfield and Lauderback, 2002; Markesbery, 1997; Zhu et al., 2005).

Brain, with its high oxygen consumption, copious amounts of redox transition metals, and enrichment in polyunsaturated fatty acids (O'Brien and Sampson, 1965; Skinner et al., 1993) is

* Corresponding author. Department of Chemistry, Center of Membrane Sciences, and Sanders-Brown Center on Aging, University of Kentucky, Lexington KY 40506-0055, USA. Fax: +1 859 257 5876.
E-mail address: dabcns@uky.edu (D.A. Butterfield).
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particularly vulnerable to lipid peroxidation (Pamplona et al., 2005). Lipid peroxidation is a complex process involving the interaction of oxygen-derived free radicals with polyunsaturated fatty acids, resulting in a variety of highly reactive electrophilic aldehydes that are capable of easily attaching covalently to proteins by forming adducts with cysteine, lysine, or histidine residues (Esterbauer et al., 1991) through Michael addition (Butterfield and Stadtman, 1997). Among the aldehydes formed, malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE) represent the major products of lipid peroxidation (Esterbauer et al., 1991).

HNE is an alpha, beta-unsaturated alkenal product of omega-6 polyunsaturated fatty acids and is a major cytotoxic end product of lipid peroxidation that mediates oxidative stress-induced death in many cell types (Tamagno et al., 2003; Uchida, 2003). HNE accumulates in membranes at concentrations of 10 μM to 5 mM in response to oxidative insults (Esterbauer et al., 1991) and invokes a wide range of biological activities, including inhibition of protein and DNA synthesis (Ding et al., 2005; Drake et al., 2004; Poot et al., 1988), disruption of Ca^{2+} homeostasis, membrane damage, cell death (Esterbauer et al., 1991), and activation of stress signaling pathways (Okada et al., 1999; Tamagno et al., 2003).

Glutathione, a major antioxidant in the human brain, has been shown to detoxify HNE in cells by free radical capture (Bains and Shaw, 1997; Subramaniam et al., 1997). Glutathione *S*-transferase and the multidrug resistant protein, MRP1, which exports the GSH conjugate of HNE, out of neurons become functionally impaired (Boyd-Kimball et al., 2006). Glutathione *S*-transferases have a high catalytic activity against HNE and are oxidatively modified and downregulated in AD brain (Sultana and Butterfield, 2004). As a corollary, overexpression of glutathione *S*-transferase can combat the effects of HNE toxicity in culture (Xie et al., 2001).

Several publications report that the brain of MCI patients is under oxidative stress. Increased levels of thiobarbituric acid reactive substance (TBARS), malondialdehyde (MDA), F_2 isoprostanes and F_4 neuroprostanes, soluble protein-bound HNE, specific markers of *in vivo* lipid peroxidation (Butterfield and Stadtman, 1997), were significantly elevated in cerebrospinal fluid (CSF), plasma, urine, and brain of MCI patients compared with controls (Butterfield et al., 2006b; Keller et al., 2005; Pratico et al., 2002; Williams et al., 2006), suggesting that lipid peroxidation may be an early event in the pathogenesis of the disease.

To gain insight into the role of lipid peroxidation in the progression from MCI to AD, we used a redox proteomic approach to identify specific HNE-bound proteins in hippocampal and inferior parietal lobule (IPL). Specimens were obtained from short post-mortem interval (PMI) autopsies from subjects with MCI and normal control subjects. Based on the observation that generally the oxidative modification of a protein leads to its dysfunction, redox proteomic represents an efficient tool to gain insight into the role of oxidative stress in the pathogenesis and progression of AD (Butterfield et al., 2006a, 2007a,b; Sultana et al., 2006a).

Materials and methods

Control and MCI brains

Hippocampal and IPL samples were obtained at autopsy from nine MCI patients and nine age-and sex-matched controls. Autopsy samples were obtained via the Rapid Autopsy Program of the University of Kentucky Alzheimer's Disease Center (UK ADC) with an average PMI of 3 h for MCI patients and control subjects (Table 1). The normal control subjects in this study were seven women and two men, and the average age was 82±2.1 years. The amnestic MCI patients were five women and four men, and the average age was 89±1.3 years (Table 1). All subjects came from our longitudinally followed normal control group that has annual neuropsychological testing, and neurological and physical examinations. Control subjects had no cognitive complaints, normal neuropsychological test scores, intact activities of daily living (ADLs), and normal neurologic examinations. Amnestic MCI patients met the criteria described by Petersen (Petersen, 2003) which included: a memory complaint supported by an informant, objective memory test impairment (age- and education-adjusted), general normal global intellectual function, intact ADLs, Clinical Dementia Rating score of 0.0 to 0.5, no dementia, and a clinical evaluation that revealed no other cause for memory decline. The control subjects showed no significant histopathological alterations and the average Braak score was 1.1. The MCI patients had an average Braak score of 4.0.

Chemicals

All chemicals were of the highest purity and were obtained from Sigma-Aldrich (St. Louis, MO, USA). The anti-HNE antibodies and anti-rabbit IgG (secondary antibody) were obtained from Chemicon (Temecula, CA, USA).

Sample preparation

Brain samples were sonicated and suspended in 10 mM HEPES buffer (pH 7.4) containing 137 mM NaCl, 4.6 mM KCl, 1.1 mM KH_2PO_4, 0.1 mM EDTA, and 0.6 mM $MgSO_4$ as well as proteinase inhibitors: leupeptin (0.5 mg/mL), pepstatin (0.7 μg/mL), type II S soybean trypsin inhibitor (0.5 μg/mL), and PMSF (40 μg/mL). Homogenates were centrifuged at 14,000 ×*g* for 10 min to remove debris. Protein concentration in the supernatant was determined by the BCA method (Pierce, Rockford, IL, USA).

Table 1
Profile of subjects used in this study

	Age (years)	Gender	Brain weight (g)	PMI (h)	Braak
Control 1	86	Female	1300	3.75	I
Control 2	74	Male	1400	4	I
Control 3	86	Female	1150	1.75	I
Control 4	90	Female	1110	4	II
Control 5	76	Female	1315	2	I
Control 6	79	Male	1240	1.75	II
Control 7	75	Female	1330	3.50	I
Control 8	86	Female	1200	3.50	0
Control 9	84	Female	900	3.00	I
Average	82±2.1		1216±36	3.02±0.4	1.1
MCI 1	91	Female	1155	5	III
MCI 2	93	Female	1050	2.75	III
MCI 3	87	Male	1200	3.5	VI
MCI 4	87	Male	1170	2.25	III
MCI 5	88	Female	1080	2.25	V
MCI 6	82	Female	1075	3	III
MCI 7	87	Male	1530	2.75	IV
MCI 8	99	Female	930	2.00	V
MCI 9	84	Male	1350	3.50	IV
Average	89±1.3		1171±20	3.00±0.3	4.0