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Elevated 4-hydroxyhexenal in Alzheimer's disease (AD) progression

Melissa A. Bradley[a], Shuling Xiong-Fister[b], William R. Markesbery[b,c], Mark A. Lovell[a,b,*]

[a] Department of Chemistry, University of Kentucky, Lexington, KY, USA
[b] Sanders-Brown Center on Aging and Alzheimer's Disease Center, University of Kentucky, Lexington, KY, USA
[c] Departments of Neurology and Pathology, University of Kentucky, Lexington, KY, USA

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Abstract

Multiple studies have demonstrated elevations of α, β-unsaturated aldehydes including 4-hydroxynonenal (HNE) and acrolein, in vulnerable regions of mild cognitive impairment (MCI), preclinical Alzheimer's disease (PCAD), and late stage Alzheimer's disease (LAD) brain. However, there has been limited study of a third member, 4-hydroxyhexenal (HHE), a diffusible lipid peroxidation product of the ω-3 polyunstataturated fatty acids (PUFAs). In the present study levels of extractable and protein-bound HHE were quantified in the hippocampus/parahippocampal gyrus (HPG), superior and middle temporal gyri (SMTG), and cerebellum (CER) of MCI, PCAD, LAD, and normal control (NC) subjects. Levels of extractable and protein-bound HHE were increased in multiple regions in the progression of Alzheimer's disease (AD). Extractable HHE was significantly elevated in the hippocampus/parahippocampal gyrus (HPG) of PCAD and LAD subjects and protein-bound HHE was significantly higher in MCI, PCAD, and LAD HPG. A time- and concentration-dependent decrease in survival and a concentration-dependent decrease in glucose uptake were observed in primary cortical cultures treated with HHE. Together these data support a role for lipid peroxidation in the progression of Alzheimer's disease.
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1. Introduction

Oxidative damage to cellular macromolecules, including nucleic acids, proteins, and lipids, is a feature of aging as well as many neurodegenerative diseases including Alzheimer's disease (AD). Multiple studies have shown oxidative damage, including nucleic acid oxidation (Gabbita et al., 1998; Lovell et al., 1999; Mecocci et al., 1994; Nunomura et al., 2001; Shan et al., 2003), protein modifications (Ding et al., 2006; Drake et al., 2004; Lovell et al., 1998, 2000b; Pocernich and Butterfield, 2003; Shao et al., 2008; Sultana et al., 2006), and generation of by-products of lipid peroxidation (Lovell et al., 2001; Markesbery and Lovell, 1998; McGarth et al., 2001) are significantly increased in late-stage Alzheimer's disease (LAD) compared with age-matched normal control (NC) subjects. In addition, markers of oxidative damage have been observed in mild cognitive impairment (MCI), the earliest clinical manifestation of AD (Butterfield et al., 2006; Ding et al., 2005; Markesbery et al., 2005; Shao et al., 2008; Wang et al., 2006; Williams et al., 2006). These observations suggest oxidative damage may play a potential role in the pathogenesis of AD.

Lipids represent a class of biomacromolecules whose proper function is vital to cellular homeostasis, but are also vulnerable to oxidative damage by reactive oxygen species (ROS). Peroxidation of lipids results in compromised integrity of cellular membranes and the generation of diffusible aldehydic by-products including the α, β-unsaturated aldehydes acrolein, 4-hydroxyhexenal (HHE), and 4-hydroxynonenal (HNE). The toxicity of α, β-unsaturated aldehydes is attributed to their soft electrophilic nature that is highly reactive with cysteine, histidine, and lysine amino

* Corresponding author at: Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY 40536, USA. Tel.: +1 859 257 1412 × 251; fax: +1 859 323 2866.
E-mail address: malove2@uky.edu (M.A. Lovell).

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acid residues (LoPachin et al., 2009). HNE and acrolein are elevated in MCI and LAD brain (Butterfield et al., 2006; Lovell et al., 2001; Markesbery and Lovell, 1998; Reed et al., 2008; Williams et al., 2006) and have been shown to be toxic in neuron cultures (Lovell et al., 2000a, 2001; Pocernich and Butterfield, 2003). HHE is a by-product of oxidative damage to ω-3 polyunsaturated fatty acids (PUFAs) including docosahexaenoic acid, the predominate ω-3 PUFA in gray matter (Long et al., 2008; Van Kuijk et al., 1990). Concentrations of docosahexaenoic acid (DHA) are approximately 30–50 times higher than the predominate ω-6 PUFA, arachidonic acid (Lim et al., 2005; Pawlosky et al., 2001; Salem et al., 2001) making it an abundant target for oxidative attack.

In the current studies levels of extractable and protein-bound HHE were quantified in vulnerable brain regions, the hippocampus/parahippocampal gyrus (HPG) and superior and middle temporal gyri (SMTG), and the cerebellum (CER), a nonvulnerable brain region from preclinical Alzheimer's disease (PCAD), MCI, LAD, and NC subjects. Levels of extractable HHE were quantified using gas chromatography mass spectrometry with negative chemical ionization (GC/MS/NCI) and protein-bound levels of HHE were determined by dot blot immunochemistry and an HHE specific antibody. In addition, levels of protein carbonyls were quantified by immunochemistry. To determine the rate of HHE generation in the presence of AD physiologically relevant oxidative insults, DHA was treated with amyloid β peptide ($A\beta_{1-40}$, $A\beta_{1-42}$), as well as iron (II)/ascorbic acid. Levels of extractable HHE generated from oxidized DHA were determined by gas chromatography mass spectrometry with negative chemical ionization. To investigate the effect of HHE on primary cortical neurons, cell viability was assessed at 3, 6, 12, and 24 hours in cultures treated with increasing HHE concentrations (1–100 μM). Additionally, the effect of HHE (1 to 100 μM) on glucose uptake was assessed at 6 hours.

2. Methods

2.1. Neuron cultures

Experimental protocols were in compliance with federal guidelines for animal experimentation and were approved by the University of Kentucky Institutional Animal Care and Use Committee (IACUC). Dissociated primary cortical neuron cultures were established from E-18 rat embryos as previously described (Lovell et al., 2000a, 2001). Studies were carried out on cells 7 days in culture by switching to Locke's solution consisting of 1 mL of 154 mM NaCl, 5.6 mM KCl, 2.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 3.6 mM $NaHCO_3$, 10 mM glucose, 5 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) (pH 7.2) with 10 mg/L gentamicin sulfate and treated with HHE (Cayman Chemical, Ann Arbor, MI, USA) at 1, 10, 25, 50, and 100 μM for increasing periods of time.

2.2. Cell viability studies

Cell viability was assessed by quantifying reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazodium bromide (MTT) as a measure of mitochondrial viability as previously described (Mosmann, 1983) or by counting the number of undamaged cortical neurons in premarked microscopic fields before treatment and at each time point (0, 3, 6, 12, and 24 hours) as previously described (Xie et al., 2000). Cells were identified as damaged by the presence of dendritic beading and vacuolization of the cell body. A significant positive correlation ($r = 0.97$) was observed between levels of reduced MTT and cell counts.

2.3. Glucose transport assays

The effect of HHE on glucose uptake was assessed by treating cortical neurons 7 days in culture with 1, 10, 25, 50, and 100 μM HHE for 6 hours and assessing glucose transport as described by Mark et al. (1997). Briefly, cultures were switched to Locke's with glucose and treated with HHE for 6 hours. Following treatment cultures were switched to Locke's without glucose and 1 μCi of ^{14}C labeled glucose added for 5 minutes. Cultures were washed 3 times with phosphate-buffered saline (PBS) and immediately lysed by the addition of 200 μL of 0.5% sodium dodecyl sulfate (SDS)/0.5M NaOH. Blanks for glucose uptake assays included cultures treated with 10 μM phloretin before the addition of radiolabeled glucose. Protein content was determined in 10 μL aliquots of the lysate using the Pierce bicinchonic acid (BCA) method and counts per minute (CPM) were normalized to the total protein content for each individual sample. Results are expressed as mean ± standard error of the mean (SEM) % of control and represent the mean of 5–6 dishes per each concentration per experiment and 3 separate experiments.

2.4. Brain specimen sampling

Tissue specimens from the HPG, SMTG, and CER of short postmortem interval (PMI) autopsies of 7 LAD subjects (4 men [M]:3 women [W]), 8 MCI subjects (3 M:5 W), 10 PCAD subjects (1 M:9 W) and 10 age-matched NC subjects (2 M:8 W) were obtained through the Neuropathology Core of the University of Kentucky Alzheimer's Disease Center (UK-ADC). Samples were flash frozen in liquid nitrogen and maintained at -80 °C until used for analysis.

PCAD and NC subjects were followed longitudinally in the UK-ADC Normal Control Clinic and underwent neuropsychological testing, and physical and neurological examinations annually. All NC subjects had neuropsychological test scores in the normal range and showed no evidence of memory decline. Although there are not well defined criteria for the identification of PCAD subjects, the UK-ADC tentatively describes PCAD subjects as those with sufficient AD pathologic alterations at autopsy to meet intermediate or high National Institute on Aging - Reagan Institute (NIA-RI) criteria, moderate or frequent neuritic plaque scores according to

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