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Biomarkers of lipid peroxidation in Alzheimer disease (AD): an update

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Abstract

Increasing evidence suggests free radical mediated oxidation of biological substrates is a key feature of Alzheimer's disease (AD) pathogenesis. While it has long been established that biomarkers of lipid peroxidation (LPO) are elevated in AD brain as well as ventricular CSF postmortem, more recent studies have demonstrated increased LPO biomarkers in postmortem brain from subjects with mild cognitive impairment (MCI), the earliest clinically detectable phase of dementia and preclinical AD (PCAD), the earliest detectable pathological phase. Furthermore, multiple LPO biomarkers are elevated in readily accessible biological fluids throughout disease progression. Collectively these studies demonstrate that LPO is an early feature during disease progression and may be considered a key pathway for targeted therapeutics as well as an enhancer of diagnostic accuracy for early detection of subjects during the prodromal phase.

Keywords

Mild cognitive impairment; Preclinical Alzheimer's disease; Alzheimer's disease; Oxidative stress; Lipid peroxidation

Introduction

Clinically, Alzheimer's disease (AD) is characterized by an insidious onset and progressive cognitive decline. Currently 5.1 million Americans aged 65 years or older are estimated to suffer from AD, a figure that is projected to increase to 13.8 million in 2050 (Hebert et al. 2013), consistent with an aging American population. It has been proposed that the development of effective interventional therapies has the potential to significantly reduce prevalence and associated cost of care. However, to date, no therapies exist to halt or alter the course of AD progression due largely to the lack of a clearly defined disease etiology.

An extended prodromal phase during disease progression is associated with the insidious nature of AD onset and is considered key to understanding the underlying disease etiology, but to date antemortem clinical diagnostic accuracy of patients along the AD continuum has

proved challenging. However, two distinct sub-populations have been identified along this continuum on the basis of either clinical diagnoses antemortem or pathological burden postmortem. Pioneered by Petersen et al. (Petersen et al. 1999), mild cognitive impairment (MCI) is considered the earliest detectable clinical phase of AD representing the initial deviation from normal aging. Subsequent cognitive decline (Petersen et al. 1999), ventricular expansion, and global brain atrophy are accelerated in MCI subjects compared to age-matched cognitively normal control (NC) subjects (Jack et al. 2008). The second sub-population, preclinical Alzheimer's disease (PCAD), can only be identified postmortem. Antemortem, PCAD subjects are indistinguishable from aged-matched NC subjects but are differentiated based the presence of significant AD-associated pathology in the transentorhinal region (Schmitt et al. 2000) at autopsy. The identification of these two distinct phases along the disease continuum has the potential to provide invaluable insight into disease etiology.

Despite intensive study in both the early prodromal stages and late-stage AD (LAD), defining the etiology has remained elusive. Unlike familial AD, which is associated with genetic mutations in key proteins and enzymes associated with amyloid precursor protein processing, the initiating event responsible for onset of sporadic AD, particularly early in the prodromal phase remains masked by secondary events. To date, no single hypothesis has been proposed that fully encompasses both the clinical and pathological features of AD. However a precipitating event eliciting multiple secondary cascades may help to explain the multifaceted nature of AD pathogenesis.

One particular hypothesis of this nature that has received considerable interest is the oxidative damage hypothesis, an extension of the free radical theory of aging (Harman 1956). While the free radical theory of aging focuses on elevated oxidative damage during aging throughout the body, the oxidative damage hypothesis emphasizes the potential role of oxidative damage in the brain during the pathogenesis of AD (reviewed in (Chang et al. 2014; Zhao and Zhao 2013). High metabolic energy demands, elevated transition metal content, and limited antioxidant defenses relative to other organs make the brain a prime target for oxidative damage, mediated by reactive nitrogen (RNS) and oxygen species (ROS) (reviewed in (Kohen and Nyska 2002)). Multiple lines of research suggest that oxidative damage of cellular substrates including nucleic acids (reviewed in (Lovell and Markesbery 2007; Markesbery and Lovell 2006; Moreira et al. 2008)), proteins (reviewed in (Butterfield and Kanski 2001; Sultana et al. 2006)), and lipids (reviewed in (Montine et al. 2002; Sultana et al. 2013)) occur during the progression of AD.

Lipid peroxidation: biochemistry

In contrast to controlled enzymatic lipid metabolism, lipid peroxidation (LPO) is a non-enzymatic process that proceeds in an uncontrolled manner characterized by three distinct phases: initiation, propagation, and termination (reviewed in (Yin et al. 2011)) (Fig 1A). Initiated by hydroxyl, alkoxyl, or peroxyl radicals, LPO precedes via hydrogen abstraction at the methylene group adjacent to the carbon-carbon double bond of unsaturated fatty acids. Oxygenation of the carbon-centered lipid radical generates a lipid peroxyl radical that subsequently abstracts a hydrogen from adjacent unsaturated fatty acids, initiating a self-

perpetuating chain reaction, leading to amplification of the initial oxidative event. Termination occurs either as a result of radical-radical neutralization or radical interaction with chain-breaking antioxidants such as, α-tocopherol, i.e. vitamin E (as reviewed in (Halliwell and Chirico 1993; Yin et al. 2011)).

Secondary by-products of LPO can be categorized as either products of oxygenated lipid rearrangement or decomposed hydroperoxide by-products (Fig 1B and 1C). The products of oxygenated lipid rearrangement include Isoprostane (IsoP) isomers, which are derived from the polyunsaturated fatty acid (PUFA) arachidonic acid (ARA), and neuroprostane (NeuroP) isomers derived from the PUFA docosahexaenoic acid (DHA). Unlike enzymatically-produced prostaglandins derived from free PUFAs, IsoPs and NeuroPs are formed *in situ* and are stored within the membrane but may be released following hydrolysis. Although, ARA- and DHA-derived hydroperoxide decomposition produces a variety of secondary by-products (reviewed in (Shichiri 2014)) the most commonly studied hydroperoxide decomposition by-products include malondialdehyde (MDA), acrolein (ACR), 4-hydroxy-2-nonenal (HNE), and 4-hydroxy-2-hexenal (HHE) (Fig 1B and 1C) due to their associated toxicity (reviewed in (Del Rio et al. 2005; Long and Picklo 2010; LoPachin et al. 2009). Although, hydroperoxide decomposition products are considered to be end products of LPO, they are anything but.

LPO in the AD brain

Important factors to consider when evaluating cross-sectional studies of LPO in AD brain include detection methods, types of sample analyzed, and whether subjects had confirmed diagnoses of AD. Early quantification of LPO secondary by-products focused on the spectrophotometrically detectable heat mediated-condensation products of aldehydes with thiobarbituric acid (TBA) in the classic thiobarbituric acid reactive substances (TBARS) assay. Although generally attributed to MDA, TBA is reactive with a wide range of aldehydes and is sensitive to *in vitro* LPO during sample preparation (Halliwell and Whiteman 2004). Development of direct quantification methodologies including high pressure liquid chromatography (HPLC) coupled with electrochemical detection (HPLC-EC) or fluorescence, HPLC tandem mass spectrometry (LC-MS/MS), or gas chromatography mass spectrometry (GC-MS) allowed superior selectivity and sensitivity for LPO secondary by-products and TBA-adducts formed in complex matrices. However, because these methodologies utilize bulk tissue samples the levels of LPO, secondary by-products quantified reflect CNS LPO as a whole, rather than a single cell type. In contrast, indirect quantification of secondary by-products by immunochemical techniques also allows quantification of a distinct pool of LPO of secondary by-products, protein adducts. Furthermore, immunohistochemical techniques offer the distinct advantage of antigen localization.

IsoPs and NeuroPs: Postmortem

Endoperoxide intermediates of ARA and DHA LPO either reduce to F_2-IsoPs and F_4-NeuroPs or undergo molecular rearrangement to E_2-, D_2-, A_2- or J_2- IsoPs and E_4- or D_4-NeuroPs (Fam et al. 2002; Morrow et al. 1990; Reich et al. 2000; Roberts et al. 1998; Yin et

al. 2002). F_2-IsoPs and F_4-NeuroPs, the most commonly studied, are biological stable, making them ideal metabolites for monitoring LPO during AD progression.

While mean levels of the F_2-IsoPs, $iPF_{2\alpha}$-III and $iPF_{2\alpha}$-IV, quantified by GC-MS, were elevated in the frontal (FP) and temporal pole (TP) of late-stage AD (LAD) subjects compared to age-matched NC subjects, these levels did not correlate with the duration of AD (Pratico et al. 1998). In contrast, mean levels of IsoP isomers in neocortical regions quantified by GC-MS were comparable to levels in LAD compared to age-matched NC subjects for a smaller cohort (Reich et al. 2001). However, F_2-IsoP isomers were detectable and elevated in the ventricular cerebrospinal fluid (vCSF) of LAD subjects, suggesting that biological fluids in direct contact with the brain reflect ongoing LPO (Pratico et al. 1998). Furthermore, elevated mean levels of total F_2-IsoP isomers in vCSF of LAD subjects were associated with cortical atrophy as rated postmortem by a skilled neuropathologist, Braak staging scores, and apolipoprotein E 4 (*APOE*) genotype (Montine et al. 1999) as well as mutations in the hereditary hemochromatosis gene HFE (Pulliam et al. 2003). Similarly, levels of NeuroP isomers quantified by GC-MS in the same subject cohort were significantly elevated in neocortical brain specimens, specifically the superior and middle temporal gyrus (SMTG) and inferior parietal lobule (IPL) of LAD subjects (Reich et al. 2001), as well as the TP (Musiek et al. 2004) and occipital area 17 and 18 (OCC) (Nourooz-Zadeh et al. 1999) compared to age-matched NC subjects. However, total levels of IsoP and NeuroP isomers were not significantly correlated with Braak staging scores or dependent on *APOE* genotype (Reich et al. 2001). Higher detectable levels of NeuroP isomers compared to IsoP isomers within a single cohort (Nourooz-Zadeh et al. 1999; Reich et al. 2001) suggest that DHA is preferentially oxidized. Mean levels of F_2-IsoP and F_4-NeuroP isomers were both significantly higher in the IPL and OCC of MCI subjects compared to age-matched NC subjects (Markesbery et al. 2005), suggesting that ARA and DHA LPO occurs early during disease progression. Comparable levels of both F_2-IsoP and F_4-NeuroP isomers in neocortical brain regions of MCI and LAD (Markesbery et al. 2005) suggest that ARA and DHA LPO oxidation is initiated early and continues throughout the progression of AD.

Hydroperoxide Decomposition: Postmortem

The frequency and intensity of immunohistochemical staining of the physiologically favored cyclic hemiacetal HNE histidine adduct (Fig 2A) were significantly increased in the hippocampus (HIP) of LAD subjects compared to age-matched NC subjects in both pyramidal and to a lesser extent non-pyramidal neurons (Fukuda et al. 2009). In addition, the HNE lysine-derived pyrrole adducts (Fig 2B) were strongly, but not exclusively, associated with neurofibrillary tangle (NFT)-bearing neurons in LAD subjects (Montine et al. 1997a; Montine et al. 1997b; Sayre et al. 1997). More recently, fluorophoric HNE modifications localized to neuronal cytoplasm, corresponding to grandovacular degeneration, were significantly elevated in the HIP of LAD subjects compared to age-matched NC subjects (Zhu et al. 2012). HNE adduct colocalization with non-perivascular senile plaques was relatively weak (Sayre et al. 1997) and reported in only a fraction of AD subjects (Ando et al. 1998). A similar pattern was observed for the acrolein lysine adduct, N^{ε}-(3-formyl-3, 4-dehydropiperidino)-lysine (FDP-lysine) (Fig 2C) with abundant staining in NFT- bearing neurons with minimal presence in NFT-free neurons (Calingasan et al. 1999a). In contrast to

HNE adducts, acrolein adducts were not specifically associated with amyloid cores, but were detected in dystrophic neurites surrounding the amyloid core (Calingasan et al. 1999b). Although both the HNE-derived hemiacetal and pyrrole products were strongly associated *with APOE4* isoform expression in LAD subjects (Montine et al. 1997a; Montine et al. 1997b) it remains unclear if acrolein staining is associated with *APOE4* expression. Collectively, these studies suggest that ARA peroxidation is strongly associated with pyramidal neurons and quantification of extractable levels of α, β-unsaturated aldehydes likely reflect neuronal lipid peroxidation.

Spectrophotometric measurement of TBA-adduct levels were elevated in the inferior temporal gyrus (ITG) (Palmer and Burns 1994), the amygdala (AMY) and hippocampus/ parahippocampal gyrus (HPG) (Lovell et al. 1995), as well as multiple neocortical brain regions including the frontal, parietal, temporal, and occipital cortices (DiCiero Miranda et al. 2000) of LAD subjects compared to age-matched NC subjects. In contrast, mean levels of HPLC-identified TBA-MDA adducts were not significantly different in the frontal lobe (FL), SMTG, middle temporal gyrus (MTG), or HIP of LAD subjects compared to age-matched NC subjects (Lyras et al. 1997). Although HPLC- based quantification allows exclusive quantification of TBA-MDA adducts, levels were quantified in tissue samples with prolonged postmortem intervals (PMI) (average 20.9 to 46.7 hrs), potentially skewing the results (Lyras et al. 1997). Furthermore, levels of TBA-adducts were significantly influenced by *APOE* genotype with higher levels associated with heterozygous and homozygous APOE4 expression in LAD subjects (Ramassamy et al. 1999).

Mean levels of HNE, but not the straight chain aldehydes, propanal, butanal, pentanal, hexanal, quantified by HPLC coupled with fluorescence detection were significantly elevated in the AMY and HPG of LAD subjects compared to age-matched NC subjects with short PMIs (<3 hrs) (Markesbery and Lovell 1998). In a study of short PMI vCSF, levels of extractable HNE, quantified by HPLC/fluorescence, were significantly elevated in LAD subjects compared to NC subjects (Lovell et al. 1997). Additionally mean levels of the α, β-unsaturated aldehyde, acrolein, were significantly elevated in the AMY and HPG of LAD subjects compared to age-matched NC subjects with short PMIs (<3 hrs) (Lovell et al. 2001). While the literature clearly demonstrates elevated levels of lipid peroxidation during LAD, these studies failed to address whether lipid peroxidation is also associated with MCI or PCAD subjects. Therefore, to address whether lipid peroxidation is associated with the pathogenesis of AD, more recent studies have included subjects associated with the prolonged prodromal phases described earlier.

Mean levels of extractable acrolein and HNE in the HPG and SMTG, quantified by LC-MS/MS, were elevated in both MCI and early AD (EAD) compared to age-matched NC subjects (Williams et al. 2006). Elevations reached statistical significance in the HPG and SMTG of EAD subjects but only in the SMTG of MCI subjects compared to age-matched NC subjects (Williams et al. 2006). Similarly, mean levels of extractable HNE were significantly increased in the HPG and SMTG of both early disease stages compared to age-matched NC subjects (Williams et al. 2006). Furthermore, median levels of extractable acrolein but not HNE, quantified by GC-MS, were significantly elevated in the HPG but not the SMTG of PCAD subjects compared to age-matched NC subjects (Bradley et al. 2010).

Collectively, elevated levels of extractable acrolein and HNE suggest that ARA lipid peroxidation is associated with both the earliest clinically and pathologically identifiable stages of AD progression, particularly in the HPG. Additionally, mean levels of extractable acrolein and HNE quantified by LC-MS/MS in MCI subjects were comparable to levels quantified in EAD subjects (Williams et al. 2006) suggesting that lipid peroxidation is a phenomenon that is initiated early and remains a key feature even at later stages of disease progression. In contrast to earlier studies demonstrating lipid peroxidation only in brain regions associated with AD pathology, later studies show levels of extractable acrolein and HNE were elevated in the cerebellum, reaching statistical significance in both EAD and MCI subjects (Williams et al. 2006). However, levels of extractable acrolein were significantly reduced in CER of PCAD subjects (Bradley et al. 2010). Collectively, these challenge the establishment of the CER as an internal control and suggest that LPO of ARA is a global brain event that may precede development of AD-related pathology.

While quantification of ARA LPO secondary by-products has taken center-stage, recent quantification of the DHA-derived LPO secondary by-product, HHE has become of interest. Quantification of extractable HHE levels by GC-MS in the HPG and SMTG of PCAD, MCI, LAD, and age-matched NC subjects showed elevated median levels in a disease progression related manner in the HPG, reaching statistical significance in PCAD and LAD subjects compared to age-matched NC subjects (Bradley et al. 2012). Similar to levels of extractable acrolein and HNE previously discussed, median levels of extractable HHE were comparable in the HPG of both PCAD and LAD subjects suggesting that like ARA LPO, DHA LPO is an early and sustained event during the pathogenesis of AD. Similar to previous studies of extractable acrolein and HNE, median levels of extractable HHE were significantly elevated in the CER of LAD subjects compared to age-matched NC subjects (Bradley et al. 2012). Unlike previous studies of extractable acrolein and HNE that were not correlated with pathological burden, levels of extractable HHE quantified in the HPG significantly correlated with Braak staging scores (Bradley et al. 2012) suggesting that peroxidation of DHA may be related to NFT formation.

Biologically active electrophilic α, β-unsaturated aldehydes form physiological irreversible adducts with nucleophilic side chains of amino acid residues, particularly the sulfhydryl group of cysteine, the imidazole moiety of histidine, and the ε amino group of lysine via Michael's addition reactions (reviewed in (LoPachin et al. 2009)). Therefore, total levels of protein-bound α, β-unsaturated aldehyde adducts may serve as an additional LPO index. Protein-bound HNE adducts are the most commonly studied protein α, β-unsaturated aldehyde adduct. Similar to extractable levels of HNE, mean levels of total protein-bound HNE are significantly greater in the HPG of PCAD (Bradley et al. 2010), the HPG and IPL of subjects with MCI (Butterfield et al. 2006), and the IPL of EAD subjects (Reed et al. 2009) compared to age-matched NC subjects. Additionally, mean levels of protein-bound HHE were elevated in the HPG of both early stages of disease progression, MCI and PCAD, as well as LAD compared to age-matched NC subjects (Bradley et al. 2012). Elevated levels of protein-bound α, β-unsaturated aldehydes during the earliest clinical and pathological stages of disease progression suggest proteins are subject to modification early in the disease progression.

While indirect immunochemical quantification provides evidence of elevated protein-bound α, β-unsaturated aldehydes in neocortical regions associated with AD progression, it does not help to identify specifically modified proteins. However, redox proteomics has proven to be useful in the identification of α, β-unsaturated aldehyde modified proteins (Dalle-Donne et al. 2005), including HNE modified proteins. In a study of the HPG and IPL of LAD subjects, significant HNE modifications of 7 proteins involved in energy production, structural integrity, antioxidant defense, and excitotoxicity were reported (Perluigi et al. 2009). Although protein expression was independent of HNE modification, the activities of ATP synthase and aconitase but not manganese superoxide dismutase (MnSOD) were significantly diminished in LAD subjects compared to age-matched NC subjects (Perluigi et al. 2009). Similarly, elevated HNE modification of α-enolase and MnSOD in the IPL of EAD subjects compared to age-matched NC was reported (Reed et al. 2009) corresponding to significantly decreased enzymatic activity of both α-enolase and MnSOD in the IPL of EAD subjects compared to age-matched NC subjects (Reed et al. 2009). Furthermore, Reed *et al*. identified 7 proteins in the HPG of MCI that exhibited significant increases in HNE modification including lactate dehydrogenase (LDH) and ATP synthase, compared to age-matched NC subjects (Reed et al. 2008). As in previous studies LDH activity was significantly reduced by 40% and ATP synthase activity was significantly reduced by 35% in the HPG of MCI subjects compared to age-matched NC subjects (Reed et al. 2008). However, the degree of HNE modification is not limited to clinical phases of AD progression but has also been reported in PCAD subjects compared to age-matched NC subjects (Aluise et al. 2010). Collectively these studies suggest key proteins involved in neuronal communication, protein synthesis, energy metabolism, structural integrity, and antioxidant defense are subject to HNE modification early in the progression of AD and indicate that HNE modification may play a role in the modulation enzymatic efficiency. Loss of enzymatic function early in the progression of AD, as previously discussed, may contribute to dysregulation of cellular processes and ultimately neurodegeneration (reviewed in (Butterfield et al. 2002; Sultana et al. 2013)).

Multiple deoxynucleotide adducts are generated by the LPO secondary by-products acrolein, HNE, and MDA *in vitro* (reviewed in (Burcham 1998)). P^{32}-postlabeling of nuclear DNA (nDNA) demonstrated that the HNE guanosine-derived adduct, 6(R/S)-[1(R/S)-hydroxyhexanyl]-8(R/S)-hydroxy 1, N^2-propano-2′-deoxyguanosine 3′-monophosphate (HNE-dG), was not significantly altered in either the HIP or parietal cortex in LAD subjects compared to age-matched NC subjects (Gotz et al. 2002). Levels of HNE-dG quantified by LC-MS/MS were comparable to levels quantified by P^{32}-postlabeling but were not significantly different in the either the HPG or IPL of LAD subjects compared to age-matched NC subjects (Liu et al. 2006).

LPO by-products: Antemortem

An early study by Feillet-Coudray et al. reported comparable plasma concentrations of 8-epi$PGF_{2\alpha}$, measured by commercially available ELISAs, in probable AD and age-matched NC subjects (Feillet-Coudray et al. 1999). Similarly, Mufson *et al*. reported that geometric means of 8-epi$PGF_{2\alpha}$ were significantly different in either the plasma or urine of MCI or probable AD subjects compared to age-matched NC subjects (Mufson and Leurgans 2010).

In contrast, mean urinary levels of the F_2-IsoP, 8-iso-$PGF_{2\alpha}$-III, measured by commercially available ELISAs, were elevated in probable AD subjects compared to age-matched NC subjects, although the changes did not correlate with MMSE scores (Tuppo et al. 2001). Concentrations of 8,12-iso-$iPF_{2\alpha}$-IV, measured by GC-MS, were significantly elevated in urine, plasma, and lumbar CSF (lCSF) of probable AD subjects compared age-matched NC subjects (Pratico et al. 2000). Furthermore, urinary and plasma levels of 8,12-iso- $iPF_{2\alpha}$-IV collected at time of lumbar puncture were significantly correlated with CSF levels suggesting that peripheral fluids may reflect the central nervous system, although only CSF levels were correlated with cognitive decline (MMSE scores) (Pratico et al. 2000). In a follow-up study of an expanded subject cohort as well as subjects with MCI, significantly elevated levels of 8,12-iso- $iPF_{2\alpha}$-IV in urine, plasma, and lCSF were found in both MCI and probable AD subjects compared to age-matched NC subjects (Pratico et al. 2002). Furthermore, urinary and plasma levels measured in MCI were significantly lower compared to probable AD subjects (Pratico et al. 2002) suggesting that 8,12-iso-$iPF_{2\alpha}$-IV can differentiate between MCI and LAD subjects. Consistent with the previous study, urinary and plasma levels exhibited strong correlations with measurable lCSF levels (Pratico et al. 2002). Urinary levels of 8-iso-$PGF_{2\alpha}$ were reduced by ~26% in probable AD subjects who were treated for 6 months with α-tocopherol at 400 mg/day (Guan et al. 2012).

Mean levels of serum hydroperoxides, quantified by colorimetric detection of *N*,*N*-dimethyl-para-phenylenediamine radical cations, were elevated in a disease-dependent manner reaching statistical significance in stable MCI and probable AD subjects, but not MCI subjects who later converted to probable AD compared to age-matched NC subjects (Cervellati et al. 2014). Consistent with increased hydroperoxide levels, mean serum levels of TBARs were significantly elevated in probable AD subjects compared to age-matched NC subjects (Padurariu et al. 2010). Additionally, plasma and serum levels of TBA-adducts were significantly elevated in probable AD subjects compared age-matched NC subjects independent of gender (Aybek et al. 2007; Puertas et al. 2012) but was independent of *APOE4* expression (Aybek et al. 2007). In contrast, plasma levels of TBA-MDA adducts, quantified by HPLC, were not significantly different in probable AD compared to age-matched NC subjects (Casado et al. 2008; Polidori et al. 2004) but were significantly elevated when stratified by age (Casado et al. 2008). Although, plasma MDA concentrations are significantly elevated in probable AD subjects, levels were also significantly elevated in subjects with vascular dementia (VaD) (Casado et al. 2008; Gustaw-Rothenberg et al. 2010) but to a significantly greater extent in LAD subjects compared to VaD subject (Gustaw-Rothenberg et al. 2010). Meta-analysis of serum and plasma TBA-MDA and TBARs concentrations showed a significant 24% increase in probable AD subjects (n = 1098) compared to NC subjects (n = 1094) (Schrag et al. 2013). Significantly elevated serum concentrations of TBA-adducts were observed in MCI subjects compared to age-matched NC subjects, but were significantly lower compared to probable AD subjects (Padurariu et al. 2010). Similarly, plasma concentrations of TBA-MDA adducts, quantified by HPLC, were significantly elevated in a disease-dependent manner, whereas levels in MCI subjects were significantly greater than age-matched NC subjects although significantly lower compared to probable AD subjects (Torres et al. 2011). Conversely in another study, MDA

levels in MCI subjects were comparable to age-matched NC subjects (Martin-Aragon et al. 2009).

Despite extreme biological reactivity, α, β-unsaturated aldehydes have been identified in biological accessible fluids. In a study of plasma, significantly elevated levels of HNE but not MDA were observed in probable AD subjects compared to NC subjects (McGrath et al. 2001). While both plasma and lCSF HNE concentrations were significantly elevated in probable AD subjects compared to age-matched NC subjects levels were not significantly correlated (Selley et al. 2002). The wide range of concentrations observed in both probable AD and age-matched NC subjects as well as the lack of relationship with CSF severely limits the potential utility of HNE as a diagnostic tool.

A series of more recent studies indicates that measurable acrolein adducts in plasma, lCSF, and urine may enhance the diagnostic accuracy traditional Aβ biomarkers in detection of MCI and LAD subjects. Quantification of protein-bound acrolein, specifically the FDP-lysine protein adduct, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{–}40,}$ quantified by ELISAs in plasma specimens from 50 MCI subjects, 70 probable AD, and 33 NC subjects showed levels of the protein-bound acrolein and the $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio were significantly increased in both MCI and probable AD subjects compared to age-matched NC subjects (Waragai et al. 2012). Unfortunately, overlap of levels precluded differentiation of MCI and probable AD subjects (Waragai et al. 2012). To determine if CSF levels of protein-bound acrolein could distinguish MCI and probable AD subjects, levels of the protein-bound FDP-lysine adduct, $A\beta_{1\text{-}42}$, and $A\beta_{1\text{-}40}$ were quantified by ELISA in lCSF collected from 40 MCI and 54 probable AD subjects. Although mean levels of protein-bound acrolein were not significantly different between MCI and probable AD subjects, levels of $A\beta_{1\text{-}40}$, the $A\beta_{1\text{-}40}/A\beta_{1\text{-}42}$ ratio, and the $A\beta_{1\text{-}40}$/protein-bound acrolein ratio were significantly higher in MCI subjects compared to probable AD subjects. Furthermore, Z-scores, a reflective measure of atrophy severity in a defined volume of interest including the hippocampus as measured by MRI inversely correlated with CSF concentrations of $A\beta_{1\text{-}40}$, the $A\beta_{1\text{-}40}$/protein-bound acrolein ratio, and the $A\beta_{1\text{-}42}$/protein-bound acrolein ratio. Conversely, MMSE scores were positively correlated with CSF concentration of $A\beta_{1\text{-}40}$, the $A\beta_{1\text{-}40}$/protein-bound acrolein ratio, and the $A\beta_{1\text{–}42}$/protein-bound acrolein ratio (Mizoi et al. 2014). Significant correlations with both clinical and pathological assessments of AD progression suggest that measurable levels of $A\beta_{1\text{-}40}$ and protein-bound acrolein normalized levels of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ are viable biomarkers that closely mirror disease progression. While both CSF levels of $A\beta_{1\text{-}40}$ and the $A\beta_{1\text{-}40}$/protein-bound acrolein ratio provide clear delineation between MCI subjects and probable AD subjects, CSF collection is a highly specialized invasive procedure limiting the usefulness for both diagnostic and routine monitoring of disease progression.

In an attempt to quantify levels of lipid peroxidation in a more readily accessible biological fluid levels of 3-hydroxypropyl mercapturic acid (3-HPMA), a metabolite of acrolein-glutathione conjugate and amino acid-bound acrolein FDP-lysine adduct were quantified in urine from 22 MCI, 32 probable AD, and 22 age-matched NC subjects. Levels of creatinine (CRE) normalized 3-HPMA and amino acid-bound acrolein were significantly decreased in pooled MCI and probable AD subjects compared to age-matched NC subjects. Furthermore, urinary levels of CRE normalized 3-HPMA and amino acid-bound acrolein levels were

significantly reduced in probable AD subjects compared to the MCI subjects. Declining 3-HPMA/CRE and amino acid-bound acrolein/CRE levels correlated with declining MMSE scores and higher clinical dementia rating scale sum of boxes scores but not severity of brain atrophy reported as Z scores (Yoshida et al. 2015). Although levels of 3-HPMA/CRE and amino acid-bound acrolein/CRE levels did not correlate with AD-associated atrophy, they did correlate with declining MMSE scores suggesting measurable levels 3-HPMA/CRE and amino acid-bound acrolein/CRE are viable markers for the clinical detection and monitoring of clinical progression.

Summary

Results from the studies discussed clearly show markers of LPO are elevated in diseased brain regions of not only LAD subjects, but also subjects in the earliest clinically and pathologically detectable stages along the disease continuum. Collectively, these studies suggest LPO may contribute to the disease pathogenesis early in the prodromal phase. Equally important, levels of multiple LPO biomarkers, including F_2-IsoPs, MDA, and protein-bound acrolein adduct in readily accessible biological fluids (serum, plasma and urine) are significantly altered in both MCI and LAD subjects compared to age-matched NC subjects. Furthermore, study of multiple biological fluids within a single cohort has demonstrated that plasma, serum, and urinary levels are directly related to CSF levels suggesting peripheral fluids may reflect ongoing LPO in CNS. While additional work is needed in larger well defined cohorts focusing on the relationship between LPO biomarkers and key AD features, including cognitive decline and brain atrophy, these studies suggest that quantification of LPO biomarkers in peripheral fluids may enhance early detection of subjects during the prodromal phase.

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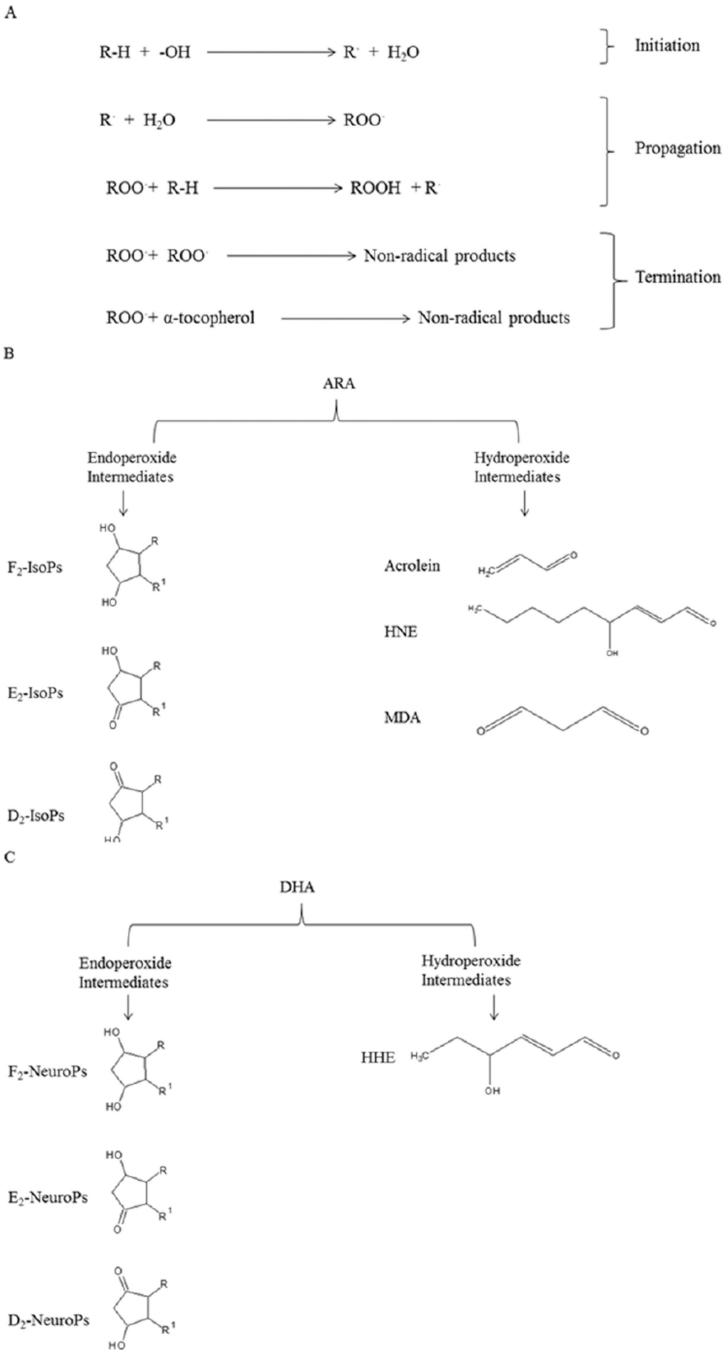

Fig 1.

Schematic of lipid peroxidation (A), representative ARA (B) and DHA (C) derived LPO secondary by-products

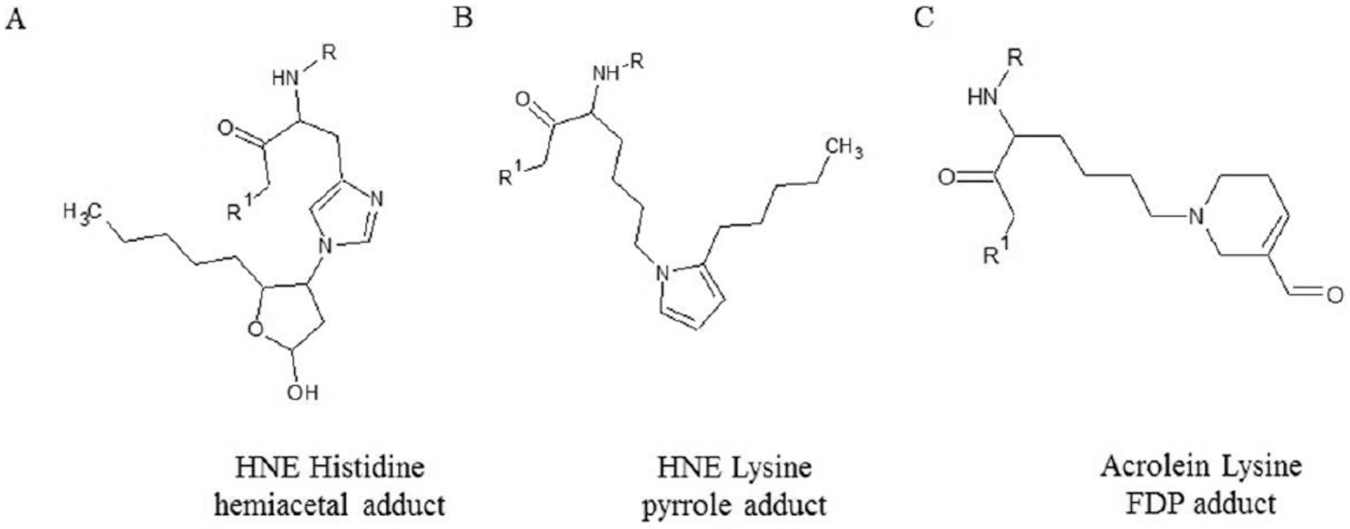

Fig 2.

The HNE histidine-derived hemiacetal adduct (A), the HNE lysine-derived pyrrole adduct (B) and the acrolein lysine-derived adduct N^{ε}-(3-formyl-3, 4-dehydropiperidino)-lysine (FDP-lysine) (C)