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Involvements of the Lipid Peroxidation Product, HNE, in the Pathogenesis and Progression of Alzheimer's Disease[A]

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Abstract

Alzheimer's disease (AD) is an age-related neurodegenerative disorder. A number of hypotheses have been proposed to explain AD pathogenesis. One such hypothesis proposed to explain AD pathogenesis is the oxidative stress hypothesis. Increased levels of oxidative stress markers including the markers of lipid peroxidation such as acrolein, 4-hydroxy-2-*trans*-nonenal (HNE), malondialdehyde, etc. are found in brains of AD subjects. In this review we focus principally on research conducted in the area of HNE in the central nervous system (CNS) of AD and mild cognitive impairment (MCI), and further we discuss likely consequences of lipid peroxidation with respect to AD pathogenesis and progression. Based on the research conducted so far in the area of lipid peroxidation, it is suggested that lipid accessible antioxidant molecules could be a promising therapeutic approach to treat or slow progression of MCI and AD.

Keywords

lipid peroxidation; Alzheimer disease; HNE; amyloid beta-peptide; proteomics; oxidatively modified proteins

Alzheimer's disease (AD) is an age-related neurodegenerative disorder characterized histopathologically by the presence of senile plaques (SP), neurofibrillary tangles (NFT), and synapse loss [1]. Though AD has been known for more than a century, the exact mechanism (s) of its pathogenesis still largely remains unknown. A number of hypotheses such as amyloid cascade, excitotoxicity, oxidative stress, and inflammation hypotheses, etc., have been proposed for AD pathogenesis; however, none of these hypotheses clearly account totally for all aspects of the disease. One such hypothesis proposed to explain AD pathogenesis is the oxidative stress hypothesis [2-6] . Oxidative stress is defined as an imbalance in the levels of oxidant (reactive oxygen species (ROS)/ reactive nitrogen species (RNS)) and antioxidant defense systems. This increase in the ROS/RNS may further lead to the damage of biomolecules leading to loss of function and consequently to cell loss, one of the key observations in AD brain.

[A]This paper is dedicated to the life and legacy of William R. Markesbery, MD, who died January 30, 2010, and who contributed enormously to the concept of brain oxidative stress in Alzheimer disease.

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The presence of high levels of unsaturated lipid content coupled to high oxygen utilization, high level of redox metal ions, and relatively poor antioxidant systems makes brain particularly vulnerable to oxidative damage. In the case of AD, amyloid β-peptide (40-42 amino acids) [Aβ(1-40) or Aβ(1-42)], a main component of SP, is generated by the proteolytic cleavage of amyloid precursor protein by the action beta- and gamma-secretases. Aβ(1-42) has been shown to induce oxidative stress in both *in vitro* and *in vivo* studies [7,8]. Aβ(1-42) exists in various aggregated states such as monomers, oligomers, protofibrils, and fibrils, among which the oligomeric form of Aβ(1-42) is considered as highly toxic [9]. The increased toxicity associated with oligomeric Aβ(1-42) may be attributable to its ability to reside in the lipid bilayer, where lipid peroxidation can occur. Indeed, Mattson and co-workers showed that addition of Aβ (1-42) to neurons directly lead to formation of HNE [10], as did our laboratory [12].

Extensive experimental evidence from our laboratory and others showed that methionine at residue 35 of Aβ(1-42) is particularly important for its oxidative role [4,11,12]. Methionine can undergo one-electron oxidation to form a sulfuranyl radical cation, which has the ability to abstract an allylic H-atom from the unsaturated acyl chains of lipid molecules, thereby leading to the initiation of lipid peroxidation processes (Figure 1) [13,14]. Methione 35 in Aβ (1-42)-mediated lipid peroxidation requires a helical secondary structure of the peptide. This secondary structure, of course, is the case for most proteins that are bilayer-resident. Indeed, NMR studies showed that Aβ(1-42) is helical when solubilized in micelles (reviewed in [9]). Like any alpha-helix, every fourth amino acid interacts with each other (i+4 rule of helices). In particular, the backbone carbonyl of Ile-31 is located within a van der Waals distance of the S-atom of Met-35 in Aβ(1-42). Since O is more electronegative than S, electron density of the lone pair of electrons on S in Met are drawn away from the S and toward the O, making these electrons more vulnerable to a one-electron oxidation to form the sulfuranyl radical cation. This radical can abstract a labile allylic H atom from an unsaturated acyl chain of lipids forming a carbon-centered free radical. The latter can immediately bind paramagnetic and non-polar oxygen to form the peroxyl free radical, which, in turn, can abstract another acyl chain-resident labile allylic H-atom, continuing the chain reaction. Note that there is a large amplification effect of a free radical on Aβ(1-42) that is mediated by the chain reaction within the lipid phase of the membrane. Note also (Figure 2) that the lipid acyl hydroperoxide formed by these reactions can lead directly to HNE. The acid formed on the sulfuranyl radical by abstraction of a labile allylic H-atom from lipid acyl chains has a pKa of -5; hence, any base, including water, can remove this H^+, resulting in reduced Met again. That is, the Met acts as a catalyst for lipid peroxidation. This chemistry is discussed in greater detail in [9].

Recent studies reported the presence of Aβ(1-42) in mitochondrial membranes [15,16]. Hence, Aβ(1-42) may initiate lipid peroxidation in the mitochondrial membrane by similar processes as discussed above that may not just lead to alterations in lipid components of the membrane, but affect proteins embedded in the membrane. Consequently the process of lipid peroxidation may lead to alteration in membrane fluidity and eventually lead to alterations in membrane functions. In the case of mitochondria, alteration in the membrane may lead to leakage of apoptosis-inducing molecules such as cytochrome C from the mitochondria, and also cause functional alterations of proteins involved in the electron transport system, all of which may lead to increased release and production of RNS and ROS. In addition, an *in vivo* study showed that increased lipid peroxidation leads to up regulation of BACE1 expression, which may lead to increased Aβ(1-42) production [17]. Lipid peroxidation products and Aβ(1-42) have been shown to induce JNK pathways, leading to neuronal apoptosis [18].

As noted above, lipid peroxidation leads to the production of HNE [Figure 2], malondialdehyde, and the α,β-unsaturated aldehyde, acrolein, which are diffusible and highly reactive with other biomolecules and, consequently, neurotoxic [19]. Two additional markers of lipid peroxidation are known, i.e., isoprostanes and neuroprostanes, that are products of

arachidonic and docosahexaenoic acid oxidation, respectively, the latter fatty acid being neuronal specific [20]. The aldehydic products of lipid peroxidation are highly reactive and covalently bind to proteins through Michael addition to protein cysteines, lysines, and histidines, altering their structure [21] and function [14,22,23] (Figure 3). Previous studies showed that the levels of free HNE and acrolein are increased in AD brain [24,25].

Lipid Peroxidation in AD Brain

Lipid peroxidation products including free HNE, acrolein, neuroprostanes, isoprostanes are elevated in AD brain [26-28]. A recent study reported increased levels of specific HNE-histidine Michael adducts in AD hippocampus compared to age-matched controls [29], confirming our earlier findings [14]. Further, Lui et al., [30] showed that HNE also can covalently modify the histidine side chains of Aβ, leading to increased aggregation of this peptide.

Increased levels of the GSH-HNE Michael adduct (HNE-GSH) were found in the AD hippocampus, and substantia innominata, entorhinal cortex, frontal and temporal cortex, as well as cerebellum [31]. In normal cells the HNE-GSH adducts are eliminated by multidrug resistant protein 1 (MRP-1); however, in AD brain, glutathione *S*-transferase (GST) and MRP-1 were found to be HNE- modified, which might account for loss of GST activity in AD [32, 33], and contribute to the increased levels of HNE and accumulation of HNE-protein adducts. In contrast, the expression and activity of aldehyde dehydrogenase, which converts HNE into an acid (which then abrogates its Michael addition properties), was reported to be altered in AD brain [34] . Further, AD brain demonstrated decreased activity and HNE modification of the proteasome, which in turn leads to increased accumulation of cytotoxic biomolecues, thereby increasing neuronal cytotoxicity [32,33,35]. In addition, neprilysin (NEP), a major protease that cleaves Aβ *in vivo*, also has also been shown to be HNE- modified in the brain of AD subjects [36].

The levels of F(2)-isoprostanes [F(2)-IsoP], F(4)-neuroprostane [F(4)-NP] and isoprostane 8,12-iso-iPF2(α)-VI were found to be increased in AD brain compared to controls [37,38]. Another product of lipid peroxidation, MDA, was found to be significantly increased and colocalized with SP and NFT in AD brain [39]. Moreover, the levels of MDA correlated with the decreased activity of superoxide dismutase (SOD) [40]. Pamplona et al., (2005)[41] also found increased amounts of the direct oxidation of amino acids, glycoxidation and lipoxidation in AD brain as indicated by significantly decreased levels of docosohexanoic acid, a good substrate for lipid peroxidation, and increased concentrations of glutamic and aminoadipic semialdehydes, N^{ε} - (carboxymethyl)-lysine, N^{ε} -(carboxyethyl)-lysine, and N^{ε} - (malondialdehyde)-lysine. Additional markers of protein oxidation were identified as neurofilament L, α-tubulin, glial fibrillary acidic protein, ubiquinol-cytochrome *c* reductase complex protein I, and the β-chain of ATP synthase, which are targets of N^{ε} - (malondialdehyde)-lysine formation.

Liu et al (2006) [42] reported increased levels of HNE bound to 2′- deoxyguanosine (HNE-dG) in AD brain compared to controls. In contrast, a previous study by Gotz et al., [43] reported no difference in the levels 1,N2-propanodeoxyguanosine adducts of HNE (HNE-dGp) in the AD brain compared to controls.

A CSF study by Pratico et al., [44] demonstrated increased levels of the isoprostane 8,12-iso-iPF2(α)-VI in cerebral spinal fluid (CSF) in AD. These researchers also showed that AD patients with a ventriculoperitoneal (VP) shunt had a 51% decrease in lipid peroxidation products after a year, a finding that led these researchers to suggest that improving CSF drainage may remove the end products of lipid peroxidation from the CSF and lead to decreased damage to brain lipids. In another study, the levels of CSF F2-isoprostanes (IsoPs), which were

reported to be reduced upon treatment of AD patients with α-tocopherol and vitamin C, suggesting that antioxidants may be a promising therapeutic approach to treat AD [45].

Lipid Peroxidation in MCI

Mild cognitive impairment (MCI) is arguably the earliest form of AD. Brain from MCI subjects showed increase levels of TBARs, MDA, free HNE and protein-bound HNE [46-48]. Other makers of lipid peroxidation, i.e., F(2)-IsoP, F(4)-NP and acrolein, were also found to be significantly increased in MCI brain [37,47]. Further, in MCI, Pratico et al., [49] reported elevated levels of the F_2-isoprostane 8,12-iso-iPF2(α)-VI in CSF, plasma, and urine and suggested that this isoprostane could be used as a potential marker to identify MCI individuals who are at higher risk of progressing to AD.

HNE-Modified Proteins in AD and MCI Brain

Proteomics studies from our laboratory have reported a large number of proteins in AD brain that showed increased levels of protein-bound HNE, including: ATP synthase, α-enolase, aconitase, aldolase, glutamine synthetase (GS), MnSOD, peroxiredoxin 6, dihydropyriminidase related protein-2 (DRP-2), and α-tubulin [50]. These proteins play important roles in regulating various cellular functions such as glucose metabolism, glutamate levels, antioxidant defense systems, axonal growth, and structural functions, all of which are reported to be altered in AD brain. Some of these HNE-bound proteins identified by proteomics in AD brain were previously found to be either nitrated or carbonylated in AD [51-55]. Further, proteomics studies in MCI identified increased levels of protein-bound HNE for 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 [56]. The MCI HNE-modified proteins regulate various cellular functions such as glucose metabolism, protein synthesis, protein structural changes, lipid metabolism, antioxidant defense systems, and axonal growth. Some of the proteins that showed increased HNE modification in MCI are also found to be HNE modified in AD brain, consistent with the notion that these proteins may contribute to the progression of MCI to AD. Further, by using immunoprecipitation techniques, HNE-bound p53 levels were reported to be elevated in AD brain, while MCI brain showed a trend toward increase HNE levels [57]. HNE modification of proteins has been shown to lead to loss of protein function. The appearance of common targets of HNE-modified proteins in AD and MCI brain suggests that these proteins may be key players in AD pathogenesis.

Phospholipid Asymmetry and AD and MCI

Lipid peroxidation within the cell bilayer can also affect the distribution of membrane phospholipids, as previous studies have reported an abnormal composition of aminophospholipids in AD brain [58]. In particular, the asymmetric distribution of the anionic aminophospholipid phosphatidylserine (PtdSer) has been shown to be significantly altered by lipid peroxidation products, such as HNE and acrolein, produced in the bilayer [13,24,47, 59-66] as a result of the highly oxidative environment that is a hallmark of MCI and AD pathology. Normally PtdSer can be found sequestered to the cytosolic, inner-leaflet of the lipid bilayer, an asymmetric distribution that is selectively regulated by the ATP-dependent, membrane-bound, aminophospholipid translocase, flippase, which unidirectionally transports PtdSer inward against its concentration gradient [67]. Collapse of PtdSer asymmetry results in the outer-leaflet exposure of this phospholipid, which signals induction of early apoptosis and is crucial for selective recognition and mononuclear phagocytosis of target cells by macrophages and fibroblasts in the periphery or microglia in the brain [59-63,68]. Moreover, exposure of PtdSer to the outer-leaflet has been shown to affect activity of membrane receptors and transport proteins, as well as signal transduction and cellular morphology [69-71].

Therefore, asymmetric distribution of phospholipids, like PtdSer, within the bilayer is critical to the maintenance of cellular homeostasis.

Because the oxidative modification of proteins and lipids by ROS and/or RNS during disease progression ultimately results in diminished and/or complete loss of protein function [2], it is likely that oxidative modification of flippase and/or PtdSer by HNE or acrolein in the bilayer induces PtdSer asymmetric collapse [13,24,47,59-66]. By diffusing from their formation sites, these reactive alkenals could react via Michael addition with flippase, covalently binding a critical cysteine residue of its primary structure, disrupting its translocase activity [24,47,59, 65,67]. Furthermore, considering that externalization of PtdSer signals early apoptosis, exposure of PtdSer to the external bilayer leaflet may also result from the activation of pro-apoptotic proteins in MCI and AD. Indeed, reports demonstrate loss of flippase activity accompanied by the subsequent outer-leaflet exposure of PtdSer occurs downstream of caspase-3 activation, which is found to be increased in MCI and AD brain [72-78]. On the whole, however, it is evident that PtdSer asymmetric collapse is an important aspect of neurodegeneration of MCI and AD brain, regardless of exposure route. Considering PtdSer asymmetric collapse signals the initiation of early apoptotic events within the cell, it appears that PtdSer externalization could be a potential link to those patients with MCI that may eventually develop AD.

Conclusions

A large body of evidence, as discussed above, demonstrates the specific role of HNE modification of proteins in AD progression and pathogenesis and suggests that lipid accessible antioxidant molecules could be a promising therapeutic approach used to treat MCI and AD. However, Vitamin E studies in clinical trials of AD and MCI have failed, possibly explained based on the lack of reducing equivalents given with Vitamin E (e.g., GSH or Vitamin C). Alternatively, the present antioxidant status of subjects treated with antioxidants generally was not assessed. One could imagine that subjects with high antioxidants status might be non-responders to farther antioxidant treatment. Lastly, it is conceivable that antioxidant trials require a significantly long treatment to show efficacy. [79-81]. The lack of protection of antioxidants such as Vitamin E may also be an indication that lipid peroxidation is not a key process in the progression of disease. However, given that lipid peroxidation is prevalent in brain and CSF of subjects with amnestic MCI (which has no dementia), as discussed above, such a conclusion seems unlikely to be correct. Further studies are in progress to better understand the role of lipid peroxidation in the pathogenesis and progression of AD.

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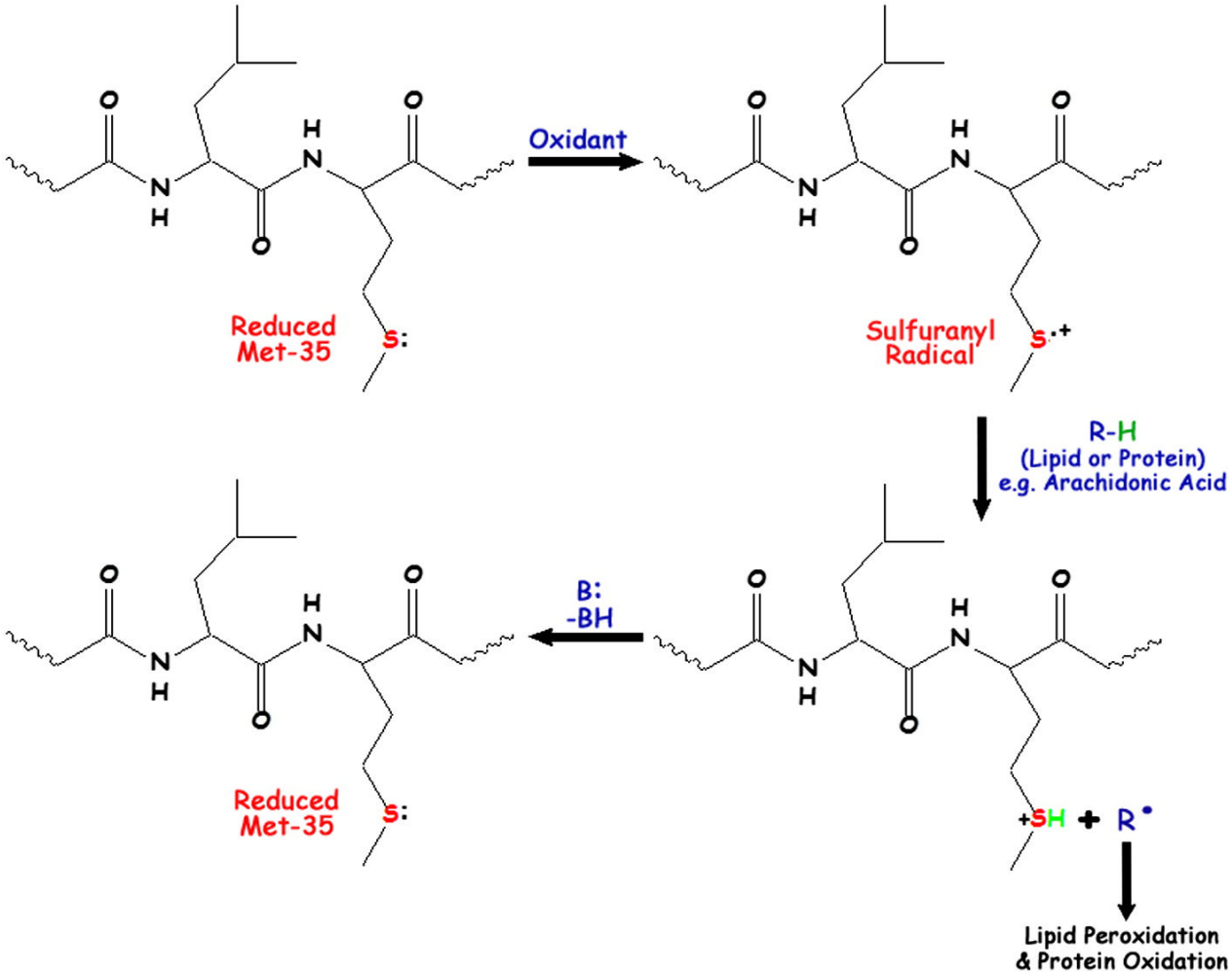

Figure 1. Involvement of Met-35 of Aβ(1-42) in lipid peroxidation

The S-atom of Met-35 of the Aβ(1-42) peptide can undergo one-electron oxidation to form a sulfuranyl radical cation within the bilayer, which has the ability to abstract a labile, allylic H-atom from the unsaturated acyl chains of lipid molecules, leading to initiation of lipid peroxidation processes [13,14]. Like most integral membrane proteins, α-helical Aβ(1-42) adheres to the i+4 rule, causing the backbone carbonyl oxygen of Ile-31 on Aβ(1-42) to draw the electron density of the Met-35 S-atom toward itself, making the S-atom more vulnerable to oxidation and subsequent formation of the sulfuranyl radical cation. The sulfuranyl radical can, in turn, abstract allylic H-atoms from neighboring fatty acyl chains within the bilayer, forming a fatty acid carbon-centered free radical that can immediately bind paramagnetic, non-polar oxygen (O_2) to form a peroxyl free radical. The peroxyl radical then abstracts another labile H-atom from nearby fatty acyl chains, perpetuating the catalytic chain reaction initiated by Met-35 of the Aβ(1-42) peptide. Because Met-35 is inevitably reduced back to its starting state, this reaction can begin again, amplifying the neurotoxic affects of the Aβ(1-42) peptide within the cell.

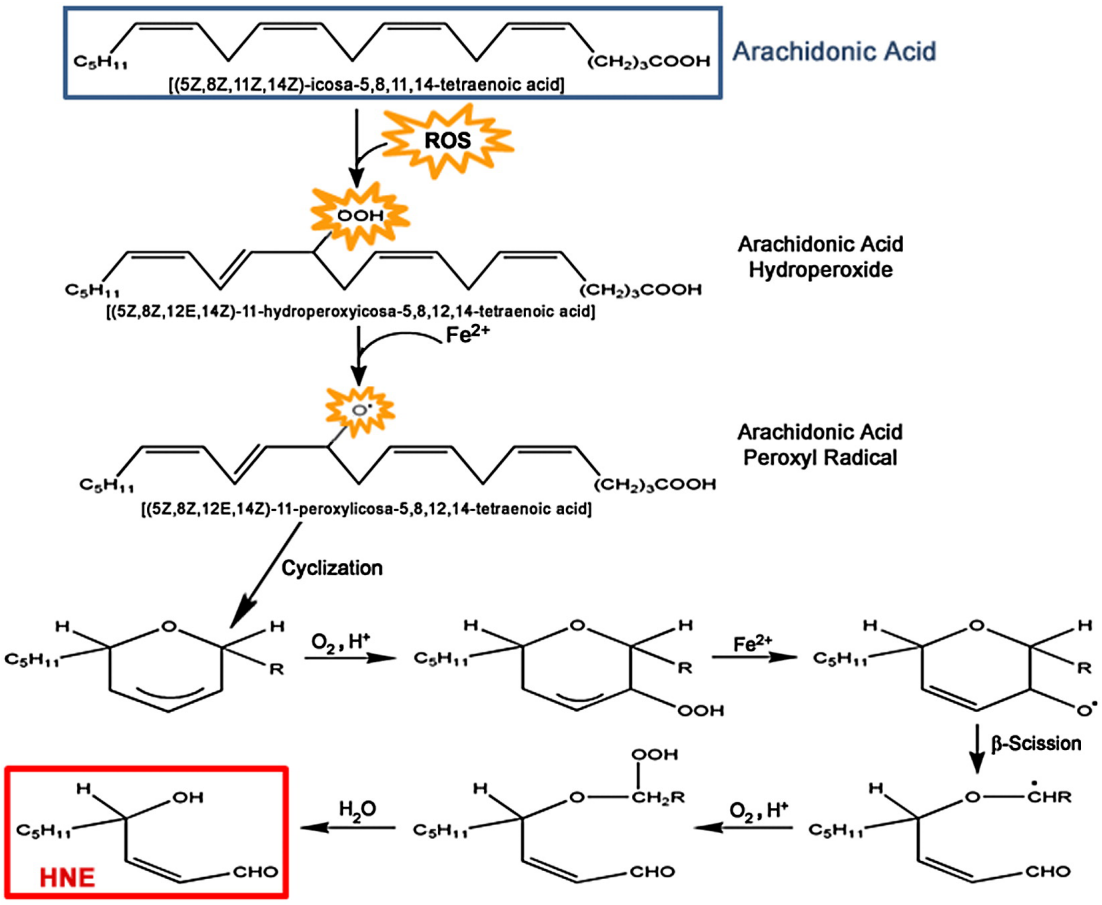

Figure 2. Formation of 4-hydroxy-2-trans-nonenal (HNE) from arachidonic acid

The catalytic conversion of the Aβ(1-42) Met-35 S-atom to the sulfuranyl radical cation leads to the abstraction of a labile, allylic H-atom from unsaturated fatty acyl chains within the bilayer. In particular, arachidonic acid is a common fatty acid within the bilayer that is readily oxidized to produce one of the highly reactive lipid peroxidation products, HNE. Reactive oxygen species (ROS) other than Aβ(1-42) can also oxidize arachidonic acid to form a reactive hydroperoxide intermediate that is quickly converted to a peroxyl radical by Fe^{2+} (Fenton chemistry). The highly reactive peroxyl radical causes a molecular rearrangement which cyclizes the radical, arachidonic acid intermediate. Further oxidation and Fenton chemistry results in the β-scission of the cyclized intermediate, causing eventual formation of HNE.

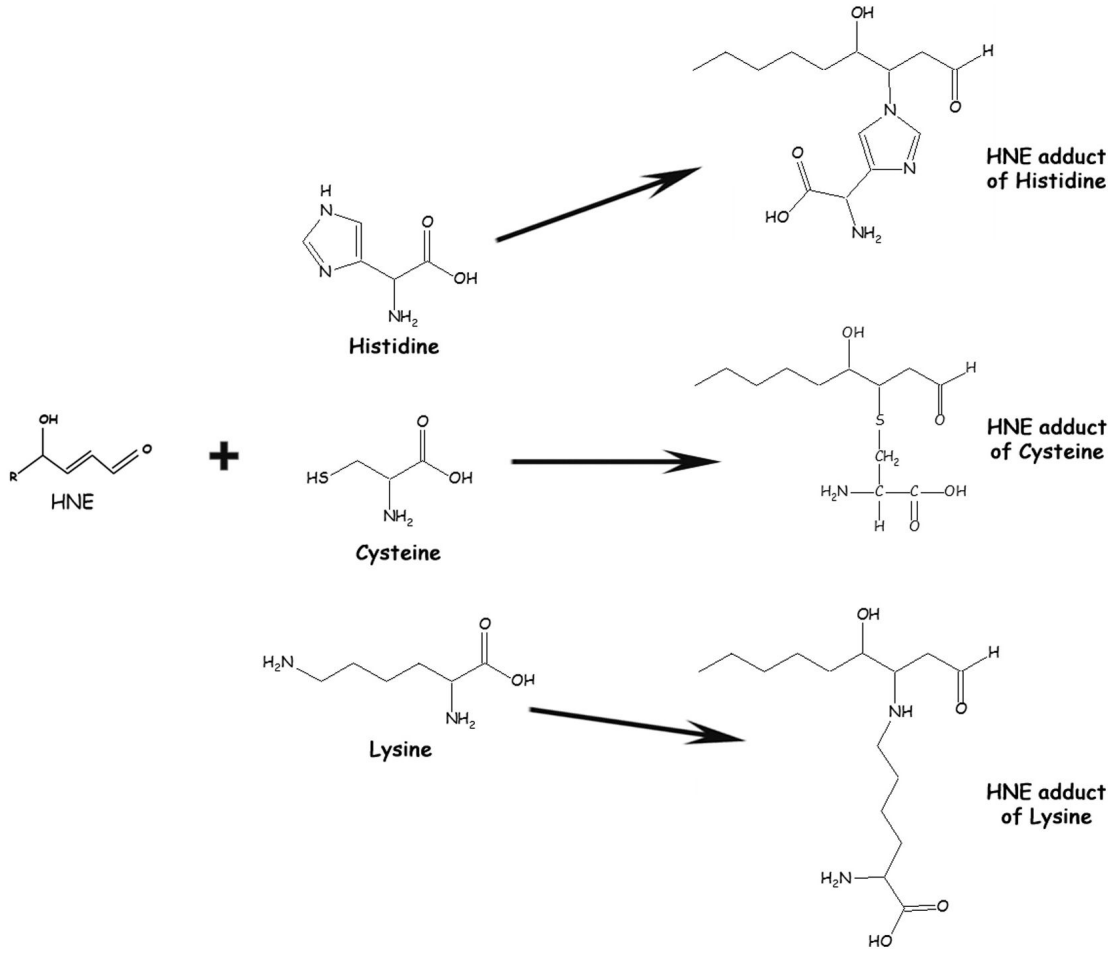

Figure 3. 4-hydroxy-2-trans-nonenal (HNE) adduction of lysine, histidine, and cysteine

HNE is a membrane diffusible, highly reactive alkenal species (formed by oxidation of arachidonic acid) that can readily oxidize other biomolecules, contributing to AD neurotoxicity [19]. HNE can covalently binds to lysine, histidine, and cysteine residues of proteins via Michael addition, forming adducts that are known to change the structural conformation [21] and function of proteins [14,22,23]. Resultant HNE-adduct structures with Lys, His, and Cys are shown.