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Review

Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death☆

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Abstract

Amyloid β-peptide [Aβ(1–42)] is central to the pathogenesis of Alzheimer's disease (AD), and the AD brain is under intense oxidative stress, including membrane lipid peroxidation. Aβ(1–42) causes oxidative stress in and neurotoxicity to neurons in mechanisms that are inhibited by Vitamin E and involve the single methionine residue of this peptide. In particular, Aβ induces lipid peroxidation in ways that are inhibited by free radical antioxidants. Two reactive products of lipid peroxidation are the alkenals, 4-hydroxynonenal (HNE) and 2-propenal (acrolein). These alkenals covalently bind to synaptosomal protein cysteine, histidine, and lysine residues by Michael addition to change protein conformation and function. HNE or acrolein binding to proteins introduces a carbonyl to the protein, making the protein oxidatively modified as a consequence of lipid peroxidation. Immunoprecipitation of proteins from AD and control brain, obtained no longer than 4 h PMI, showed selective proteins are oxidatively modified in the AD brain. Creatine kinase (CK) and β-actin have increased carbonyl groups, and Glt-1, a glutamate transporter, has increased binding of HNE in AD. Aβ(1–42) addition to synaptosomes also results in HNE binding to Glt-1, thereby coupling increased Aβ(1–42) in AD brain to increased lipid peroxidation and its sequelae and possibly explaining the mechanism of glutatmate transport inhibition known in AD brain. Aβ also inhibits CK. Implications of these findings relate to decreased energy utilization, altered assembly of cytoskeletal proteins, and increased excitotoxicity to neurons by glutamate, all reported for AD. The epsilon-4 allele of the lipid carrier protein apolipoprotein E (APOE) allele is a risk factor for AD. Synaptosomes from APOE *knock-out* mice are more vulnerable to Aβ-induced oxidative stress (protein oxidation, lipid peroxidation, and ROS generation) than are those from wild-type mice. Further, synaptosomes from allele-specific APOE *knock-in* mice have tiered vulnerability to Aβ(1–42)-induced oxidative stress, with APOE4 more vulnerable to Aβ(1–42) than are those from APOE2 or APOE3 mice. These results are consistent with the notion of a coupling of the oxidative environment in AD brain and increased risk of developing this disorder. Taken together, the findings from in-vitro studies of lipid peroxidation induced by Aβ(1–42) and postmortem studies of lipid peroxidation (and its sequelae) in AD brain may help explain the APOE allele-related risk for AD, some of the functional and structural alterations in AD brain, and strongly support a causative role of Aβ(1–42)-induced oxidative stress in AD neurodegeneration.
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Keywords: Amyloid β-peptide; Alzheimer's disease; APOE

1. Introduction

Alzheimer's disease (AD), the major dementing disorder of the elderly, affects more than four million persons in the United States of America. Aging is the chief risk factor for AD. Major pathological hallmarks of AD include loss of synapses and the presence of senile plaques (SP) and neurofibrillary tangles (NFT). SP consist of a highly dense core of the 39–43 amino acid peptide, amyloid β-peptide [Aβ(1–42)], surrounded by dystrophic neurites [35]. Based primarily on genetic evidence, Aβ likely is central to the pathogenesis of AD (reviewed in [87]). Further, the AD brain is under extensive oxidative stress indexed by protein oxidation, lipid peroxidation, DNA and RNA oxidation, advanced glycation endproducts, protein nitration, mitochondrial abnormalities, reactive oxygen species (ROS) formation, and other markers (reviewed in [13,14,58]).

The centrality of the 42-amino acid form of this peptide, Aβ(1–42), to the pathogenesis of AD was coupled to the extensive oxidative stress under which the AD brain exists in the Aβ-associated free radical oxidative stress model for neurodegeneration in AD brain [14,96]. In this model, Aβ-associated free radicals cause oxidative stress, including lipid peroxidation, in ways that are inhibited by free radical scavengers. In this review, free radical induced lipid

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peroxidation and its sequelae in AD brain will be discussed. Following a description of lipid peroxidation and its consequences, evidence that Aβ-associated free radicals cause lipid peroxidation will be outlined, and interspersed within the discussion of lipid peroxidation in AD brain, the last part of this review.

2. Lipid peroxidation and its consequences

Lipids are especially vulnerable to oxidative stress because of the unsaturated bonds in the fatty acid β-chain and high solubility of nonpolar, paramagnetic oxygen in bilayers [22]. Lipid peroxidation is initiated by a radical ($X^{\bullet}$) abstracting a hydrogen from an unsaturated carbon on a lipid acyl chain (Eq. (1)), resulting in a carbon-centered lipid radical ($L^{\bullet}$), the rate-limiting step. This lipid radical will then rapidly react with nonpolar, paramagnetic O_2, forming a peroxyl radical ($LOO^{\bullet}$) (Eq. (2)). The peroxyl radical can react with other nearby lipids and form a lipid hydroperoxide and another carbon-centered lipid radical (Eq. (3)), perpetuating the chain reaction. Lipid peroxidation is terminated by two of the peroxyl radicals quenching each other to form a nonradical and oxygen (Eq. (4)).

$$LH + X^{\bullet} \rightarrow L^{\bullet} + XH \quad (1)$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad (2)$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \quad (3)$$

$$LOO^{\bullet} + LOO^{\bullet} \rightarrow \text{nonradical} + O_2 \quad (4)$$

The number of lipids that are oxidized can be decreased by use of antioxidants such as the lipid soluble α-tocopherol (Vitamin E, TOH). A hydrogen on the α-tocopherol can actually be abstracted, forming the lipid hydroperoxide and a tocopheroxyl radical (Eq. (5)). The tocopheroxyl radical is relatively stable and does not cause further lipid peroxidation, i.e. Vitamin E is a chain-breaking antioxidant. Ascorbate (Vitamin C) can recycle the tocopheroxyl radical back to the tocopherol.

$$LOO^{\bullet} + TOH \rightarrow LOOH + TO^{\bullet} \quad (5)$$

(adapted from [22]).

Some lipid peroxidation products that are formed following free radical attack on the acyl chains are species with increased carbonyls, alcohols with conjugated double bonds (dienes), or free fatty acids. The lipid hydroperoxides can undergo various reactions and rearrangements forming aldehydes, specifically malondialdehyde, 4-hydroxynonenal, or acrolein [27]. The latter alkenal (2-propenal) can be formed by the incomplete combustion of plastics [22] and by the enzymatic action of alcohol dehydrogenase to oxidize allyl alcohol [2]. However, acrolein is more likely formed in vivo as a product of lipid peroxidation [22,23,93]. These unsaturated aldehydes (HNE and acrolein) are toxic and can act by modifying proteins by Michael addition to form a covalent adduct with cysteine, lysine, or histidine (Fig. 1) [17,23]. The adduct can cause conformational and structural changes to proteins, resulting in dysfunctional proteins and

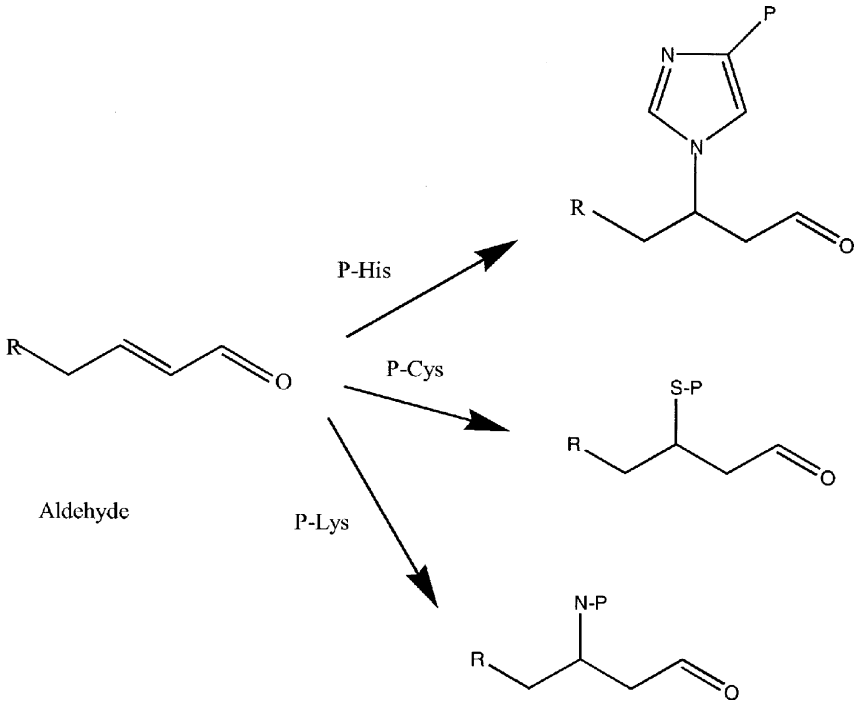

Fig. 1. Michael addition of an alkenal [HNE or acrolein] to protein lysine [P-Lys], histidine [P-His], or cysteine [P-Cys] residues resulting in the covalent oxidative modification of proteins following lipid peroxidation. Note that carbonyl groups are introduced to the protein as a consequence of this reaction.

neuronal death [76,91] and altering the membrane bilayer fluidity, causing increased rigidity.

The endogenous antioxidant, glutathione, blocks the damaging effects of these alkenals on synaptosomal proteins [76,77,91]. Both HNE and acrolein can diffuse from their point of production to cause oxidative modification of distant proteins [17].

Lipid peroxidation stimulates phospholipid lipase A_2 activity [72] releasing free fatty acids from the membrane bilayer. Recent studies suggest that such free fatty acids or other molecules, e.g. HNE, may cause polymerization of tau, a cytoskeletal protein that is the principal component of NFT [24].

Nonenzymatic, free radical-induced oxidation of arachidonic acid results in formation of F_2- and D_2/E_2-isoprostanes (IP), whereas analogous oxidation of docosohexanoic acid leads to F_4- and D_4/E_4-IP or neuroprostanes (NP). Both IP and NP are nonreactive, but serve as excellent markers of lipid peroxidation.

3. Aβ-induced lipid peroxidation

There is a growing body of evidence showing that Aβ peptide toxicity is mediated by free radical damage to cell membranes [8,13,14,56,83]. The concept that Aβ induces lipid peroxidation is a key component of the Aβ-associated free radical model for neurodegeneration in AD [10,13,96]. Consistent with a free radical process, Aβ causes lipid peroxidation in brain cell membranes that is inhibited by free radical antioxidants [3,8,10,13,16,37,44,55,56]. Moreover, the reactive alkenals, HNE and acrolein, are formed after Aβ addition to neurons [55], and these alkenals alter the conformation of membrane proteins [76,91] and are toxic to neurons [48,55,91]. Since Aβ aggregation likely is critical to the pathogenesis of AD, these results are consonant with the notion that Aβ-induced lipid peroxidation may in part account for neurodegeneration in AD brain.

Among the methods employed in our laboratory to monitor Aβ-induced lipid peroxidation is electron paramagnetic resonance (EPR) coupled with lipid soluble stearic acid spin labels [16]. The principle of the method is free radical-induced loss of EPR signal intensity from lipid-specific nitroxyl stearate spin labels (5- and 12-NS) in synaptosomal membranes due to interaction with Aβ-associated free radicals. The EPR signal of 12-NS, the nitroxide moiety of which is located deep in the lipid bilayer, was strongly decreased following addition of Aβ [8,16,18,37]. These findings, which support the hypothesis of Aβ-induced lipid peroxidation, are consistent with low-angle X-ray studies on Aβ [25–35] [61] or NMR studies on Aβ(1–42) [21], showing that the peptide is soluble in lipid bilayers with such orientation that the methionine-35 residue is deeply inserted into it. This amino acid residue presents a rich and interesting reactivity [86], and its involvement might account for the generation of Aβ-associated free radicals [11,21,96–98]. In addition, the lipophilic antioxidant, Vitamin E, was capable of inhibiting the Aβ-induced reduction of the 12-NS signal [37], confirming the radical nature of the process. Findings that Aβ [25–35], added to plasma or mitochondrial membranes of PC-12 cells over expressing Bc1–2, the gene product of which is thought to have antioxidant properties [32], did not lead to EPR stearic acid spin label signal reduction [8], additionally is consistent with the hypothesis of Aβ-induced lipid peroxidation.

The oxidative stress and neurotoxic properties of Aβ(1–42) likely derive from the single methionine residue at position 35 of this 42-mer [11,100]. Substitution of a carbon atom for the S atom of methionine, the only change in the approximately 4000-Da peptide, completely abrogates both properties of Aβ(1–42) [11,100]. Likewise, substitution of the already oxidized methionine sulfoxide for the S atom in the methionine residue of Aβ(1–42), again the only change in this peptide, also abrogates the ability of the resulting peptide to induce protein oxidation or neuronal death [97]. In Aβ(1–28) that contains the likely binding sites for redox metal ions (His 6,13,14), no ability to reduce these metal ions was found unless exogenous methionine was added [21]. This result suggests that Cu(II) bound to Aβ(1–42) interacts with methionine-35 residue to produce the free radicals associated with this peptide; however, the results also suggest, consistent with other studies [96], that in the absence of methionine in Aβ(1–42), redox metal ions play no role in the oxidative stress and neurotoxic properties of the peptide [11,21,96,100]. The methionine residue of Aβ(1–42) inserted into the neuronal lipid bilayer lies near the sites of unsaturation of lipid acyl chains [11,21]. Thus, formation of a sulfuramyl free radical in the methionine S atom [11,97] ($X^{\bullet}$ in Eq. (1) above) provides a means for H atom abstraction from unsaturated carbons in acyl chains of the phospholipids, with subsequent free radical formation on the lipids (see Eqs. (1)–(4) above).

3.1. Aβ(1–42) and brain aging

Senescence accelerated mice (SAMP8), whose life span is approximately 50% that of senescence resistant mice (SAMR1), has increased markers of oxidative stress in brain that are modulated by the brain-accessible spin trap, *N*-tertbutyl-α-phenylnitrone [17]. SAMP8 mice are known to deposit amyloid β-peptide [5], potentially coupling Aβ(1–42)-induced oxidative stress to brain aging in this rodent model of aging. More experimentation will be necessary to determine if this will turn out to be the case.

4. Lipid peroxidation in AD brain

Lipid peroxidation is widespread in AD brain and is detected with many markers.

4.1. Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) are one index of lipid peroxidation. However, TBARS is a nonspecific marker of membrane lipid peroxidation, likely due to reaction of thiobarbituric acid with nonlipid moieties. This may be the basis for disagreements in TBARS levels in different brain regions in AD. Increased in TBARS in AD frontal lobe but not in the cerebellum was reported [90], and a significant TBARS increase in sensory and occipital cortices was found in AD [4]. The inferior parietal lobe seemed to be affected by lipoxidation in one study [74], while a different study [46,47] showed statistically significant increased TBARS in hippocampus and cortex. A still different study [52] reported TBARS increase in all the regions of AD brain. Aβ(1–42) or Aβ(1–40) addition to synaptosomes were recently shown to increase TBARS [44,45].

4.2. Analysis of brain phospholipids

Compositional alterations in brain phospholipids in AD brain were reported. Polyunsaturated fatty acids (PUFA), including arachidonic and docosohexanoic acid, are abundant in brain and highly oxidizable. Therefore, arachidonic acid and docosohexanoic acid are vulnerable to free radical attack, and PUFA are predicted to decrease in AD brain, if lipid peroxidation were increased. In agreement with this prediction, several studies show a decrease in these fatty acids in AD [64,73]. The levels of phosphatidylinositol (PI) and phosphatidylethanolamine (PE), rich in easily oxidizable PUFA, were reportedly lowered in AD brain [79] as was PE-plasmalogen in AD brain [25]. Aβ added to synaptosomes led to free fatty acid release, primarily in the PE fraction, an effect blocked by the free radical scavenger Vitamin E [37], providing one possible mechanism for oxidative stress induced phospholipase A_2 activation in AD brain.

4.3. Formation of reactive aldehydes

Free radical attack on PUFA of phospholipids leads to multiple aldehydes with different carbon chain lengths, including acrolein and HNE [23]. These alkenals, though reactive, are longer lived than free radicals. Consequently, HNE is able to diffuse to sites distant from that of its formation [17]. HNE, an α,β-unsaturated aldehyde, is one of the major products of lipid peroxidation. The concentration of free HNE is elevated in multiple brain regions and in ventricular cerebrospinal fluid (CSF) in AD [57]. Protein-bound HNE also is elevated in AD [67,68,85], and may relate to apolipoprotein E (APOE) allele type [69,92]. The APOE e4 allele is a risk factor for AD, suggesting that the degree of expression of the lipid transporter APOE in brain might be associated with HNE production in AD.

Glutathione S-transferases (GST), which has high detoxifying activity against HNE [9], is significantly decreased in AD brain [47], consistent with the notion that a loss of protection against HNE might be correlated with subsequent protein modifications that lead to neuronal death. Glutathione is able to detoxify HNE [23,91]. HNE reacts with proteins, forming stable covalent adducts to histidine, lysine, and cysteine residues through Michael addition. Carbonyl groups are thereby introduced into proteins following oxidative damage, e.g. lipid peroxidation [23,91,94] (Fig. 1). In addition, HNE can inhibit synthesis of DNA, RNA, and proteins and alter activity of glycolytic, degradative, and transport proteins [23]. By disrupting Ca^{2+} homeostasis [55], reducing Na^+/K^+-ATPase activity [53,55], and imparing glucose transport [56], HNE is neurotoxic to rat hippocampal neurons. Aβ-associated oxidative stress leads to increased HNE [13,44,55], and given the increased deposition of this peptide in AD brain may be related to the mechanism by which HNE is increased in AD. Apoptosis in PC12 cells or neurons is induced by HNE [40,88], consonant with the concept that, in addition to direct ROS damage to neuronal membranes from Aβ, Aβ-induced HNE production may provide a secondary mechanism for Aβ-associated neuronal death.

Injection of HNE in rat forebrain selectively inhibited cholineacetyltransferase (ChAT) [7], the activity of which is greatly diminished in AD [35]. Consistent with this report, HNE is bound to ChAT in synaptosomes treated with Aβ(1–42), [13]. Conceivably, similar effects could account for the markedly decreased ChAT activity in AD brain.

HNE also may play a role in glutamate-induced neurotoxicity in AD. Glutamate is an excitotoxin that stimulates *N*-methyl-D-aspartate (NMDA) receptors resulting in increased intracellular Ca^{2+} and intracellular free radicals [41]. Normally glutamate is removed from outside the neuron by glutatmate transporters, particularly, the glial glutamate transporter, Glt-1 [51]. Conversion of glutamate to glutamine in a reaction catalyzed by glutamine synthetase (GS) is another means of removing this excitotoxic amino acid from outside neurons [17]. Aβ inhibits both glutamate uptake [28,29] and GS activity [1], and both processes are decreased in AD brain [12,13,60]. Based on blockage of Aβ-induced Glt-1 and GS inhibition by antioxidants or stress response proteins [1,26,28,29], Aβ effects on these glutamate systems likely involve free radicals. In the case of Glt-1, Aβ inhibition may occur by a mechanism that involves Aβ-induced lipid peroxidation and subsequent HNE modification to glutamate transporters [36,44]. Immunoprecipitation studies of HNE-modified proteins from AD and control brain suggested that glutamate-induced neurotoxicity in AD may be related to Glt-1 inactivation following HNE modification: AD Glt-1 had significantly more HNE bound to it than did this transporter in aged-matched neurologically normal controls (Fig. 2) [44]. Further, Aβ(1–42) addition to rodent synaptosomes induced HNE binding to Glt-1 (Fig. 2) [44], suggesting the possibility that these two observations are correlated. As noted above, GS provides a different mechanism for decreasing extraneural glutamate concentrations, and this enzyme is also inhibited

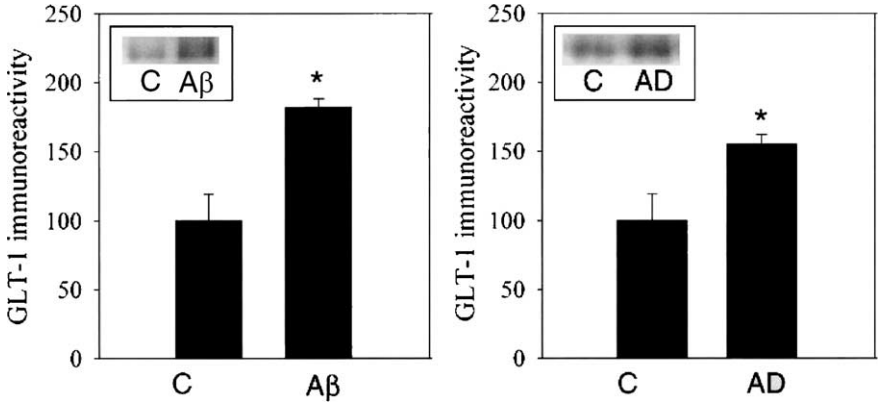

Fig. 2. HNE modifies Glt-1 after treatment of synaptosomes with Aβ(1–42) (left) and in the inferior parietal lobule of the AD brain relative to the respective control (C) (right). HNE-reactive proteins were immunoprecipitated with an anti-HNE antibody. The resulting immunoprecipitates were probed for Glt-1 by western blotting. For the Aβ(1–42) studies, $n = 3$, $P < 0.03$. For the AD studies, $n = 7$ for AD and $n = 4$ for control, $P < 0.03$, ANOVA. Data shown are mean ± S.E.M. Adapted from [44].

by Aβ in reactions that can be blocked by antioxidants [1,15,31]. Highly purified GS from AD brain is oxidized [15]. Predictably, GS has decreased activity in AD brain [30].

Due to its electrophilic characteristics and propensity to undergo Michael addition with cysteine, histidine, and lysine, acrolein is the most reactive of the α,β-unsaturated aldehydes produced by lipid peroxidation [23,93]. Acrolein alters the conformation of transmembrane and cytoskeletal synaptosomal proteins even at very low concentrations [76]. In AD brain, increased protein-bound acrolein and increased NFT-resident acrolein adducts were reported [20,48], and acrolein is toxic to primary hippocampal cultures [48]. The levels of acrolein found in AD brain were sufficient to cause increased protein carbonyl levels, when added to rodent synaptosomal membranes; however, if in vivo glutathione were elevated, complete protection against this concentration of acrolein-induced protein carbonyls resulted [76]. Other studies showed increased glutathione levels protected synaptosomal membranes against the oxidative stress induced by hydroxyl free radicals [77], peroxynitrite [38,39], HNE [91], and 3-nitropropionic acid [42]. Noting the decreased activity of glutathione S-transferase mentioned above, these findings may couple the observation that elevated glutathione protects synaptosomes against HNE and acrolein to the notion that increasing glutathione levels may be a potentially important therapeutic strategy in AD [101].

4.4. Isoprostanes

In agreement with other studies showing lipid peroxidation in AD brain, levels of IP are increased in AD CSF [71]. The levels of F_2-IP in AD lateral ventricular fluid were also significantly elevated, and the increase was related with the extent of degeneration and APOE4 genotype, but independent of the distribution of NFT [66]. In AD brain, IP and NP levels in vivo were quantified, showing an increase in total NP level, but not in total IP level [83]. Consistent with Aβ-associated free radical-induced lipid peroxidation [16], addition of this peptide to hippocampal neuronal culture significantly elevated IP [54].

4.5. APOE and oxidative stress

APOE is the predominant lipoprotein in the central nervous system [49] and has three major human isoforms designated e2, e3, and e4. Inheritance of the e4 allele of APOE is associated with an increased risk of the development of AD [84]. As noted above, increased oxidative stress is important in the pathogenesis of AD and may be APOE allele dependent: increased lipid oxidation in AD brain is associated with the presence of the e4 allele [66–68,81,82].

Knock-out (KO) mice lacking APOE have increased markers of oxidative stress [45,59], including greater basal levels of tyrosine nitration and isoprostane formation [50,62,70,80]. Genetically altered mice that express only the human APOE e3 or e4 alleles, coupled with oxidative challenge [19,89], suggest allele-specific (e3 > e4) antioxidant and HNE scavenging abilities [63,65,75,95].

APOE is associated with synaptosomes, and APOE-deficient synaptosomes are more susceptible to Aβ–induced oxidation [45,65], suggesting an antioxidant role for APOE. To investigate allele-specific vulnerability to Aβ(1–42)-induced oxidative stress, synaptosomes from *knock-in* mice expressing human APOE2, APOE3, or APOE4, with no mouse background APOE, were studied [43]. All markers of oxidative stress examined in synaptosomes, including those for ROS generation, lipid peroxidation, and protein oxidation, were significantly more elevated after Aβ(1–42) addition in samples from APOE4 *knock-in* mice relative to those from APOE2 or APOE3 mice (Fig. 3). The e4 allele of APOE is a risk factor for AD [84] and correlates with increased oxidative damage in AD brain. Aβ is deposited extensively in AD. These findings

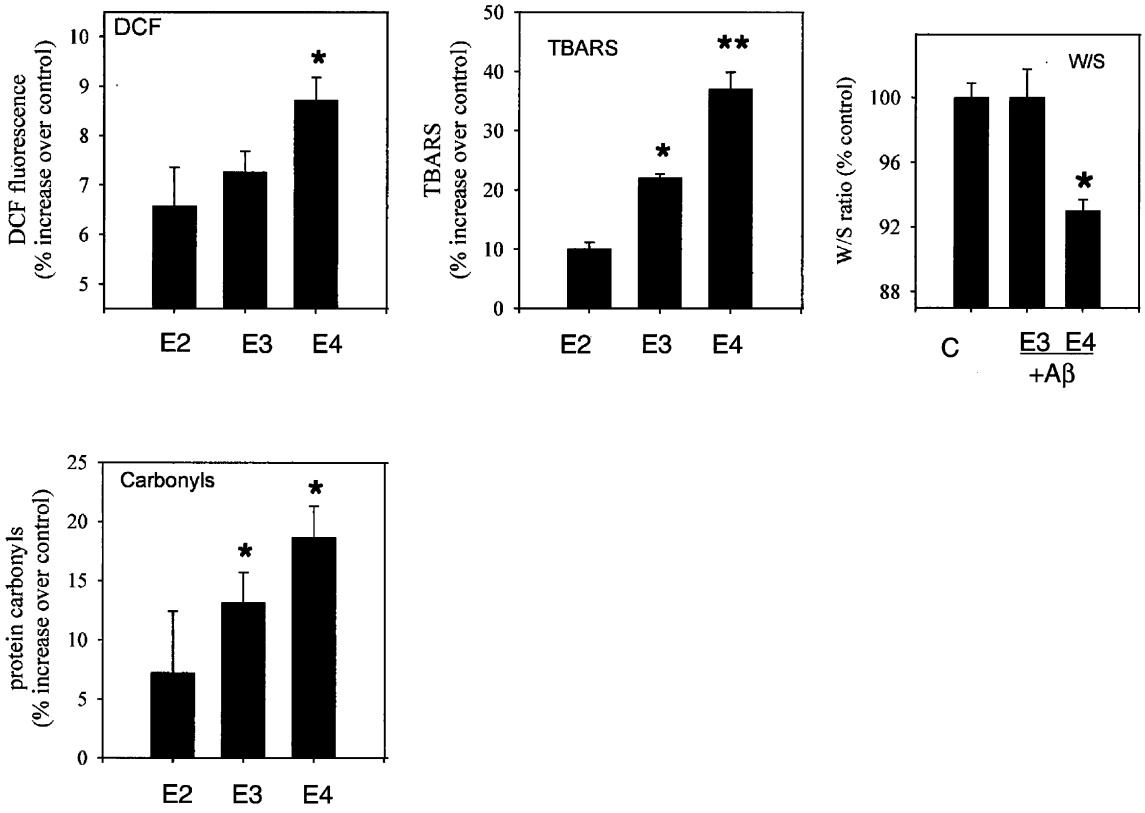

Fig. 3. APOE modulates Aβ-induced oxidative stress in an allele-dependent manner. Synaptosomes were loaded with nonfluorescent dichlorofluoroscein diacetate (DCFH-DA) (top left). Intracellular esterases produce the dianion, DCFH, that is unable to exit the cell. DCFH becomes fluorescent dichlorofluoroscein (DCF) after oxidation. Upon treatment of synaptosomes with Aβ(1–42), ROS increase significantly in all samples; however, ROS are significantly elevated in APOE4 synaptosomes when compared to APOE3 and APOE2 synaptosomes. Data are the mean ± S.E.M. ($n = 4$–6; $^{*}P < 0.05$ vs. e2 and e3, paired *t*-test). Aβ(1–42)-induced protein carbonyl formation in synaptosomal membranes is modulated by APOE in an allele-dependent manner (bottom left). Protein carbonyls are markers for protein oxidation and increase in synaptosomes upon treatment with Aβ-peptides. Allele-specific increases in protein oxidation correlate with increases in ROS and lipid oxidation and suggest that the e4 allele is more vulnerable than e2 or e3 to Aβ-induced protein oxidation. Data are the mean ± S.E.M. ($n = 4$–6; $^{*}P < 0.05$ vs. untreated synaptosomes, paired *t*-test). TBARS are markers for general lipid oxidation and increase in synaptosomes upon treatment with Aβ(1–42) (middle). Between each APOE allele, there are significant differences in TBARS with the e4 allele being most vulnerable to Aβ-induced lipid oxidation. Data are the mean ± S.E.M. ($n = 3$–5; $^{*}P < 0.05$, $^{**}P < 0.05$, paired *t*-test). Aβ(1–42) induces protein conformational changes in APOE4, but not APOE3, synaptosomal membranes (right). Changes in the motion of the protein specific spin label MAL-6 covalently bound to proteins are due to protein conformational changes and are manifested as changes in the *W/S* ratio. Because decreased *W/S* ratios correlate with increased protein oxidation [18], decreases in the *W/S* ratio are an indirect measure of protein oxidation. After a 2 h incubation at 37 °C with 10 μM Aβ(1–42), a decrease in the *W/S* ratio occurs in APOE4 synaptosomal proteins that is not observed in APOE3 synaptosomes, suggesting that APOE4 synaptosomes have an increased susceptibility to Aβ(1–42)-induced protein oxidation. Data are the mean ± S.E.M. ($n = 3$; $^{*}P < 0.05$, paired *t*-test). Adapted from [43].

may be related, i.e. Aβ(1–42)-induced oxidative stress is consistently greatest in synaptosomes containing the e4 allele of APOE (Fig. 3) [43]. Others showed that APOE e2 and APOE e3 bind HNE, whereas APOE e4, lacking cysteines at key resides, does not [65,75]. These ideas support the notion that the e4 allele of APOE may be less effective than either the e2 or e3 alleles in the modulation of Aβ-induced oxidative damage.

Alternatively, the APOE allele-specific modulation of Aβ(1–42)-induced oxidation in synaptosomes may be related to APOE-associated clearance and catabolism of Aβ. APOE binds Aβ in an allele-specific fashion [33], and the clearance of APOE/Aβ complexes is allele-dependent, based upon their respective affinities for the LRP receptor [6,34,78,99]. An inverse relationship between levels of Aβ and levels of APOE in the AD brain exists in an allele-dependent fashion. This is consistent with the concept that in the absence of APOE, Aβ would accumulate in the brain [84]. Competitive inhibitors of LRP enhance Aβ toxicity and abrogate protective effects of the e3 allele of APOE, likely by modulating the clearance of Aβ [33,95]. Thus, removal of extracellular Aβ by an LRP-mediated pathway may be a mechanism to prevent protein and lipid oxidation induced by Aβ, and may explain the differential increases in oxidative markers among the major alleles of APOE. The results from our laboratory and others are consistent with this hypothesis in that increased markers for protein and lipid oxidation correlate with APOE-mediated

uptake of Aβ, i.e. delaying the removal of Aβ results in increased oxidative damage.

Whether APOE can modulate Aβ(1–42)-induced oxidative damage in synaptic terminals by an antioxidant-related or Aβ clearance-related mechanism, APOE is important in maintaining synaptic integrity by modulating oxidative damage. Allele-dependent (e2 < e3 < e4) increases of several oxidative indices in synaptosomes suggest that the e4 allele of APOE is less effective in modulating this damage. These findings are particularly relevant to Alzheimer's disease where Aβ is overproduced, oxidative damage is evident, synapses are lost, and for which APOE e4 is a risk factor.

5. Conclusions

In aggregate, the studies summarized in this review suggest that Aβ(1–42)-induced lipid peroxidation, resulting in neurotoxic free radicals and reactive aldehydes, may be important in the neurodegeneration observed in AD brain. Further, the extensive lipid peroxidation in AD brain suggests that brain-accessible, lipid-resident endogenous and exogenous antioxidants that can block free radical-induced lipid peroxidation may be a promising therapeutic strategy in AD. In concert with this approach, raising the in vivo level of glutathione, that is able to protect neurons from HNE and acrolein [76,91], also may be a productive therapeutic route for this disorder [101]. Studies to test these ideas in animal models of AD are in progress.

The Baby Boomer cohort (many millions of people) moving through the population of the United States suggests that, in the absence of effective intervention, age-related neurodegenerative disorders, perhaps most especially AD, will become a major public health crisis in the not-too-distant future. Greater insight into the molecular basis of AD and how aging serves as a risk factor for this dementing disorder will be necessary to develop appropriate therapeutic strategies necessary to slow this inevitable outcome. Continued efforts to find the "accelerators and brakes" for AD will prove helpful. It is our view that the principal accelerator for AD is the excessive production of Aβ(1–42) with its consequent oxidative stress-induced neurotoxicity.

6. Note in Proof

Additional evidence for the role of Met-35 of Aβ(1–42) in the oxidative stress and neurotoxic properties of the peptide have been obtained [102]. Consistent with the thesis of this review, i.e., lipid peroxidation is an early event in the oxidative stress and neurotoxic properties of Aβ (1–42), substitution of negatively-charged Asp for Gly-37 in Aβ(1–42) completely abrogates these properties [103]. This result, in marked contrast to these properties induced by native Aβ (1–42) [13], is likely due to removal of then nearby Met-35 from the bilayer as a consequence of the negative charge on Asp-37, i.e., even though all the chemistry for Met-35-induced lipid peroxidation is present, there is no target for the free radical due to the putative removal of the Met from the bilayer.

Proteomics has been used for the first time to identify specifically oxidized proteins in AD brain [104,105]. Some of the excess carbonyl residues on these proteins conceivably may emanate from HNE or acrolein.

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