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Review

Fatty Acid Oxidation in the Pathogenesis of Alzheimer's Disease

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Alzheimer's disease (AD) is the most common dementing illness of the elderly and is a mounting public health problem. Pharmacoepidemiological data, analytical data from human tissue and body fluids, and mechanistic data mostly from murine models all have implicated oxidation products of two fatty acids, arachidonic acid (AA) and docosahexaenoic acid (DHA), in the pathogenesis of neurodegeneration. Here we review the biochemistry of AA and DHA oxidation, both enzyme-catalyzed and free radical mediated, and summarize those studies that have investigated these oxidation products as effectors of neurodegeneration and biomarkers of AD. Given the evolving appreciation for toxicity associated with current pharmaceuticals used to block AA and DHA oxidation, we close by speculating on likely areas of future research directed at suppressing this facet of neurodegeneration. If successful, these interventions are unlikely to cure AD, but may check its explosive growth and hopefully reduce its incidence and prevalence in the elderly. ***(Am J Pathol 2005, 166:1283–1289)***

Alzheimer's disease (AD) is most commonly a disease of late life that derives from pathogenic processes underlying abnormal accumulation of amyloid-β (Aβ) peptides and hyperphosphorylated tau in certain regions of cerebrum. The etiology of late onset AD has been partially illuminated by several associated risk factors but likely is complex and multifactorial. Late onset AD represents a significant and growing public health burden, a silent epidemic currently affecting between 2.5 and 4 million people in the U.S. and more than 10 million people worldwide.[1,2] This epidemic is projected to grow significantly throughout the next generation with an estimated 8 to 12 million patients by the year 2050 in the U.S. alone. In addition to the untold suffering by patients and their families, AD is the third most costly medical condition in the U.S.[3–5] As the number of patients afflicted continues to mount, the need for safe and effective therapy to delay or avert AD will become imperative.[6]

Recent data suggest that two partially effective preventative classes of drugs already may have been identified: nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the cyclooxygenases (COXs), and antioxidants (AOs), which suppress free radical-mediated damage.[7–13] Of the AOs, the best studied is α-tocopherol, a lipid radical chain-terminating agent. It is critical to note that the apparent effectiveness for NSAIDs and AOs has been reproducibly observed for these classes of agents in epidemiological studies that measure subsequent risk of developing AD-type dementia.[7–12] In contrast, no effect or only modest effect from specific drugs within these classes has been observed in clinical trials of patients with established dementia.[13,14] Although there are several possible interpretations of these results, one is that at least some commonly used NSAIDs and AOs are effective at suppressing pathogenic processes of AD during latent or prodromal stages but are ineffective against clinically overt dementia. Although prevention trials for NSAIDs and α-tocopherol are one way to test directly this hypothesis, both recently have been challenged by unexpected toxicity from protracted exposure in the elderly.

In support of a mechanistic role for processes suppressed by NSAIDs or AOs in early phases of AD pathogenesis, transgenic mice that express mutant human amyloid precursor protein and accumulate Aβ deposits in brain with advancing age show significantly less Aβ accumulation when treated with NSAIDs.[15] Moreover, a variety of interventions have been reported to increase or decrease Aβ accumulation in transgenic mouse models of cerebral Aβ amyloidogenesis by promoting or suppressing free radical damage to brain.[15–18] Using different transgenic mice, others have shown that neuronal overexpression of one COX isozyme, COX-2, in brain leads to neurodegeneration and age-related cognitive

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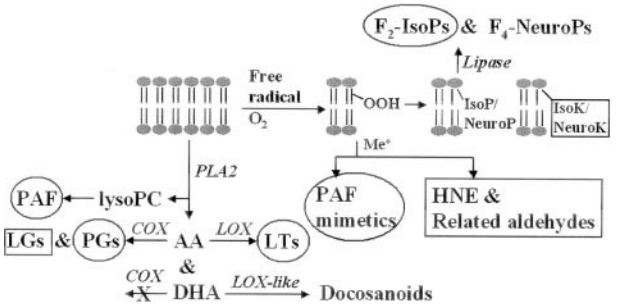

Figure 1. Phospholipid is acted on by PLA2 to liberate AA, DHA, and lysoPC that are then converted to a variety of biologically active metabolites via enzyme-catalyzed reactions. Alternatively, free radical-mediated attack on phospholipids followed by oxygen insertion generates lipid hydroperoxides that then may rearrange or fragment to produce a variety of products. Enzymes are listed in **italics**. **Circled** molecules are known to activate specific receptors. **Squared** molecules are chemically reactive and modify cellular nucleophiles.

deficits.[19] The major *in vivo* activity of the NSAIDs used in these studies is inhibition of both COX isozymes, although several alternatives have been proposed based on *in vitro* or cell culture data.[20–22] It is noteworthy that, despite many proposals for alternative actions of NSAIDs, we are aware of no data demonstrating major therapeutic action *in vivo* other than through COX suppression. For example, the recent proposal from cell culture data that NSAIDs may act via γ-secretase suppression[23] has not been supported by *in vivo* investigation.[24]

These reproducible and intriguing epidemiological data, in addition to the mechanistic data from animal models, have fueled substantial interest in polyunsaturated fatty acid (PUFA) oxidation, either enzyme-catalyzed or free radical-mediated, in the molecular pathogenesis of AD (Figure 1). Much of this recent investigation has focused on two PUFAs, arachidonic acid (AA, 20:4ω6) whose oxidation products are called eicosanoids, and docosahexaenoic acid (DHA, 22:6ω3) whose oxidation products are termed docosanoids. A critical distinction exists between AA and DHA. AA is evenly distributed in gray matter and white matter and among the different cell types in brain whereas DHA is highly enriched in neuronal membranes.[25,26] Thus, eicosanoids reflect oxidation reactions occurring in brain tissue, but not necessarily in neurons, while docosanoid formation is relatively specific for biochemical reactions occurring in neurons.

Enzyme-Catalyzed Oxygenation of AA and DHA

The ester that binds AA or DHA to the glycerol backbone of phospholipids is hydrolyzed by phospholipase (PL) A2 in response to a wide array of physiological and pathophysiological stimuli.[27] Although it is clear that products of enzyme-catalyzed oxygenation of hydrolyzed AA function as potent second messengers as well as autocrine and paracrine factors in the central nervous system, the physiological roles of liberated DHA and its docosanoid products are less clearly understood. There are two principal groups of enzymes that catalyze oxygenation of these hydrolyzed PUFAs to yield precursors of potent bioactive products. The first is the COXs that catalyze the formation of prostaglandin (PG) H_2 from free AA. The second is the lipoxygenases (LOXs) that catalyze the formation of hydroperoxides from AA and likely also DHA.[28] Although cytochrome P450s also may catalyze oxygenation of AA and DHA, the bioactivity of these compounds is less clear.

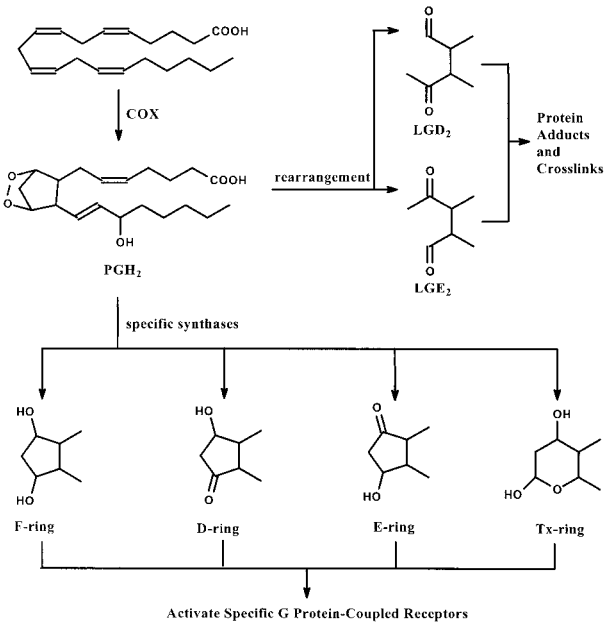

Figure 2. COX catalyzes the conversion of AA to PGH_2. Cell-specific synthases and isomerases catalyze the transformation of PGH_2 into other PGs or Tx that exert biological activity by binding to specific cell surface receptors. Alternatively, PGH_2 may rearrange to form LGs, γ-ketoaldehydes that irreversibly form adducts with protein lysyl groups and lead to protein-protein crosslinking.

Products of COXs and Their Bioactivity

Isozymes of COX catalyze oxygenation of hydrolyzed AA in what is the committed step in PG synthesis (Figure 2);[29] DHA is not a substrate for COX isozymes. A bis-oxygenase reaction in which AA plus two O_2 are converted to the bicycloendoperoxide intermediate PGG_2 is followed by a peroxidase step to generate PGH_2 with the release of an oxidizing radical.[30] PGH_2 exerts biological activity through three different mechanisms. First, PGH_2 may be further metabolized by a number of cell-specific synthases or isomerases to PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane (Tx) A_2, all of which exert potent autocrine and paracrine activities through stimulation of specific prostanoid cell surface receptors designated DP, $EP_{1\text{--}4}$, FP, IP, and TP, respectively.[31] It is through the coordinated cell-specific expression of these synthases or isomerases and receptors that products of COX achieve such a wide variety of actions in different cells and tissue. Second, PGH_2 itself is an agonist for TP. Finally, PGH_2 may rearrange to form levuglandins (LGs), highly chemically reactive γ-ketoaldehydes that form irreversible adducts with ε-amino groups of protein lysyl residues leading to protein-protein crosslinks;[32] indeed,

LGs significantly accelerate oligomerization of Aβ peptides *in vitro*.[33] One study has shown a specific approximately fivefold increase in cerebrospinal fluid PGE_2 in patients with early AD, none of whom were taking NSAIDs or aspirin.[34] We are unaware of any data exploring the time course of cerebrospinal fluid PGs in AD or its prodrome. Given the known and recently discovered toxicities associated with COX inhibitors,[35,36] future research in this area likely will concentrate on specific receptor antagonists. For example, microglia derived from mice lacking a specific PGE_2 receptor subtype, EP_2, display both enhanced phagocytosis of Aβ peptides in AD brain sections and decreased bystander damage to neurons.[37]

Products of LOXs and Their Bioactivity

LOXs are a family of cytosolic enzymes that catalyze the oxygenation of polyunsaturated fatty acids to form lipid hydroperoxides; for AA, these are called hydroperoxyeicosatetraenoic acids (HPETEs).[28] Different LOXs vary in the placement of the hydroperoxy group and are so named. Thus 5-LOX catalyzes the formation of 5-HPETEs, 12-LOX the formation of 12-HPETEs, and so on. HPETEs are unstable intermediates, like PGH_2, and are converted to potent autocrine and paracrine factors, their corresponding hydroxyl (HETEs), either nonenzymatically or by peroxidases. Products of 5-LOX may be further metabolized to leukotriene (LT) A_4, which can be subsequently hydrolyzed to LTB_4 or LTC_4. LTC_4 is metabolized via the mercapturic acid pathway to the cysteinyl-LTs (CysLTs): LTD_4, LTE_4, and LTF_4. Elements in this arm of LT biosynthesis are well known potent components of local inflammatory response and indeed comprise the slow reacting substance of anaphylaxis. The CysLTs activate two classes of receptors, $CysLT_1$ and $CysLT_2$, that are targets for antagonists being evaluated for efficacy in inflammatory diseases such as asthma.[38] In addition to LTs, LOX can catalyze the formation of lipoxins that function in the resolution of inflammatory responses and act via the ALX receptor.[38] In contrast to COX, hydrolyzed DHA apparently is a substrate for LOXs, at least in platelets and retina,[39,40] and perhaps also cytochrome P450s.[41] Although these oxygenated products of DHA have questionable biological significance in platelets, until recently virtually nothing was known about their potential neurobiological activity. One product of DHA oxygenation, 10,17-*S*-docosatriene, termed neuroprotectin D1 (NPD1), is formed from PLA2-hydrolyzed DHA by a LOX-like enzyme. NPD1 protects retinal pigment epithelial cells in culture from oxidative stress-induced apoptosis.[42] It is likely that NPD1 and other products of DHA oxygenation will soon be discovered to have potent neurobiological actions.

Unlike the COX pathway for which there is epidemiological and mechanistic data in support of a pathogenic role in latent or prodromal phases of AD, we are unaware of any epidemiological, pharmacological, or animal model data that yet point to a significant contribution of LOX pathway metabolites in AD pathogenesis; however, inhibitors of LT formation or receptor activation have not been in use for very long, so it is too early to discount this pathway. Nevertheless, 5-LOX is expressed in neurons, including the hippocampus, and its expression may increase with age.[43] One autopsy-based study has associated increased activity in 12/15-LOX pathways with late-stage AD.[44]

Free Radical Damage to AA and DHA

A central hypothesis for the pathogenesis of AD, supported by many experimental, autopsy, and clinical studies, is that increased free radical damage contributes to the initiation and progression of neurodegeneration, although the sources of this free radical stress remain an area of active investigation.[45] It is critical to note that unlike enzyme-catalyzed reactions described above, free radical damage is an indiscriminate process that will simultaneously modify multiple targets including nucleic acid, protein, and lipids.[46] PUFAs such as AA and DHA are among the most vulnerable targets for free radical damage, a process termed lipid peroxidation.[47] This complex process directly damages membranes and generates a number of oxygenated products that can be classified as either chemically reactive or relatively chemically stable products.[48]

Reactive Products of Lipid Peroxidation and Their Bioactivity

Recently, considerable progress has been made in understanding the potential contribution of chemically reactive products of lipid peroxidation to neurodegeneration. The presumed mechanism of action of all of these electrophilic products is adduction of nucleophilic groups in protein or nucleic acid. For example, adduction of a critical amino acid residue in an enzyme or transporter may lead to its dysfunction.[48–50] However, interpreting experiments that investigate the contribution of reactive products of lipid peroxidation to disease pathogenesis is limited by their lack of biochemical specificity.

Despite this limitation to understanding the precise biochemical mechanisms of action, many studies in a variety of model systems and autopsy-derived tissue have implicated reactive products of lipid peroxidation in the pathogenesis of AD.[51–57] One class of chemically reactive products of lipid peroxidation that have been studied in great detail is diffusible low-molecular weight aldehydes. By far, the most extensively studied of these are 4-hydroxy-2-nonenal, generated by peroxidation of ω-6 PUFAs like AA, and 4-hydroxy-2-hexenal, a product of peroxidation of ω-3 PUFAs like DHA.[58] Although the pathophysiological consequences of overproduction of 4-hydroxy-2-nonenal and 4-hydroxy-2-hexenal have been highlighted in numerous studies, it is noteworthy that these reactive aldehydes also are generated at low levels in all cells and appear to have a role in normal physiological signaling.[59] Indeed, several highly polymorphic enzyme systems have evolved apparently to metabolize specifically these lipid peroxidation products

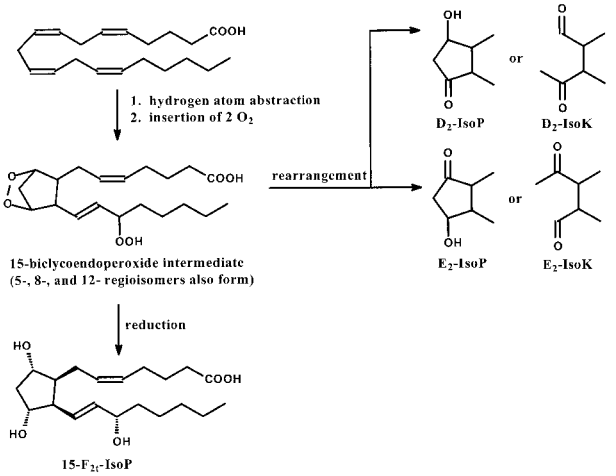

Figure 3. Free radical-mediated hydrogen atom abstraction from AA followed by insertion of 2 O_2 leads to generation of four regioisomeric endoperoxide intermediates, each of which may be reduced to form an F-ring or rearrange to form D-ring, E-ring, Tx-ring (not shown), or LG-like (IsoK) products. Similar reactions occur with DHA to generate NeuroPs and NeuroKs. Nomenclature for the IsoPs and NeuroPs follows conventions for PGs. Prostane rings are denoted D, E, F, or Tx and the subscripted number refers to the number of double bonds. The regioisomers are named by location of the side chain hydroxyl relative to the carboxyl. Default absolute configuration of side chain hydroxyl is S; R configuration is "epi" Side chains may be *cis*-or trans, denoted "c" or "t" in the subscript, with respect to cyclopentane ring hydroxyls. The structure of the most extensively studied IsoP, 15-F_{2t}-IsoP, is shown.

and thereby terminate their signaling or detoxify them;[60] two of these have been tentatively associated with an increased risk of AD.[61,62] Recently, another class of chemically reactive lipid peroxidation products has been identified: γ-ketoaldehyde isoketals (IsoKs) derived from AA and neuroketals (NeuroKs) derived from DHA.[63] These γ-ketoaldehydes are much more reactive with cellular nucleophiles than 4-hydroxy-2-nonenal or 4-hydroxy-2-hexenal and, unlike the structurally similar COX-derived LGs, IsoKs and NeuroKs, remain esterified to phospholipids. In light of the ability of LGs to significantly accelerate oligomerization of Aβ peptides *in vitro*,[33] IsoKs and NeuroKs are now being explored for related mechanisms of neurotoxicity.[64]

Stable Products of Lipid Peroxidation and Their Bioactivity

In the early 1990's Morrow and colleagues[65] demonstrated that free radical-mediated damage to AA followed by oxygen insertion and cyclization generated products that were isomeric to PG products of COX. These newly discovered compounds were termed isoprostanes (IsoPs) (Figure 3). There are three important differences between PGs and IsoPs. First, IsoPs are a large class of molecules consisting of 64 enantiomers contained within four regioisomeric families. Second, IsoPs are formed *in situ* while esterified to phospholipids and may be subsequently released by hydrolysis. Third, although some IsoPs do activate G protein-coupled receptors, the extent of their receptor-mediated activity remains unclear. What is clear is that predicting receptor activity based on similarity to isomeric PGs is limited. Since the discovery of potent renal vasoconstrictor activity for 15-F_{2t}-IsoP, there has been an explosion of interest in the PG receptor-mediated activity of IsoPs, especially effects of 15-F_{2t}-IsoP in vasculature, kidney, lungs, and platelets.[66] Much of the receptor-mediated activity of 15-F_{2t}-IsoP occurs via TP.[67] The contribution of IsoP-mediated receptor activation to neurodegenerative diseases is not known.

In addition to liberating diffusible reactive products of lipid peroxidation, fragmentation of lipid hydroperoxides can leave an abnormally shortened fatty acyl group, with or without an additional oxy function, esterified in the *sn*-2 position.[68] When this occurs with phosphatidylcholine (PC), the fragmented alkyl group may yield a mimic of platelet-activating factor, a potent autocoid that is formed by a two-step process: PLA2 hydrolyzes the fatty acid at *sn*-2 position which is then replaced with an acetyl group from acetyl-CoA. Indeed, more than one of these oxidatively modified PCs activates the platelet-activating factor receptor.[68] Other nonplatelet-activating factor-like phospholipids, including lysoPC, also are generated by lipid oxidation and have a variety of biological properties; however, very little is known about their activity in brain parenchyma.[69]

Quantitative in Vivo *Biomarkers of Lipid Peroxidation*

A final aspect to consider for lipid oxidation products is their use as quantitative biomarkers of free radical-mediated damage *in vivo*. Every lipid peroxidation product discussed above has been used as a measure of oxidative damage. Since we have reviewed this topic recently, we will not go into great detail here, however, an important point must be kept in mind.[45] The goal of a biomarker is to quantitatively reflect changes in free radical mediated damage. Interpretation of biomarkers that are chemically reactive or that are extensively metabolized has severe, inherent limitations. Consider 4-hydroxy-2-nonenal: although several robust methods exist for its quantification, how do you interpret a change in its concentration? Was it due to a change in lipid peroxidation, a change in the concentration or availability of intracellular nucleophiles like glutathione, a change in the rate of its metabolism by one of several highly efficient enzymes, or some combination of these?

Exquisitely sensitive assays have been developed for IsoPs and, because of the chemical stability and relatively limited metabolism of F_2-IsoPs *in situ*, they have emerged as a leading quantitative biomarker of lipid peroxidation *in vivo*.[70] F_2-IsoPs can be measured in tissue samples where this product of lipid peroxidation remains esterified to phospholipids. With respect to neurodegenerative diseases, a limitation of F_2-IsoPs is that they derive from oxidation of AA that is relatively uniformly distributed in gray matter and white matter as well as in neurons and glia. In contrast, products formed from DHA by identical chemistry, termed F_4-neuroprostanes (NeuroPs), provide a relatively selective window into oxidative damage to neuronal membranes.[71] This is an important

point for neurodegenerative research using specimens obtained at autopsy. Glia outnumber neurons by ~10:1 and in neurodegenerative diseases this ratio is even further skewed toward glia. F_4-NeuroPs are, as far as we are aware, the only measure of oxidative damage that is relatively selective for neurons.

Several groups have used measurements of hydrolyzed F2-IsoPs in body fluids in an attempt to quantify the magnitude of oxidative damage *in vivo*. In AD, there is broad agreement that cerebrospinal fluid F_2-IsoPs are increased in patients with mild dementia and even in individuals with prodromal dementia.[34,72–76] Similar to the attempts to identify peripheral biomarkers of other neurodegenerative diseases, attempts to use plasma or urine F_2-IsoPs in AD patients have not yielded reproducible results across centers using a variety of techniques.[73–81] This is perhaps not surprising given the small amounts of brain-derived F_2-IsoPs relative to peripheral organ-derived F_2-IsoPs and the many systemic, dietary, and environmental factors that modulate peripheral F_2-IsoPs independent of disease.

Other Diseases

It is important to note that the pathogenic pathways discussed above may contribute to AD pathogenesis but they are by no means specific to AD. Close parallels have been drawn in the pathogenesis of Parkinson's disease,[82] HIV-associated dementia,[83] and amyotrophic lateral sclerosis.[84] Some pathogenic overlap also exists for ischemic stroke, atherosclerotic vascular disease, diabetes, and arthritis. Although some view this lack of specificity as undermining a central role in AD pathogenesis, an alternative perspective is that these pathways represent fundamental and profoundly important mechanisms of tissue damage in multiple organs.

Summary

Interest in a pathogenic role for AA and DHA oxidation in AD is driven by compelling epidemiological data, associative data from autopsy samples and cerebrospinal fluid obtained early in the course of AD, and mechanistic data mostly from murine models. Given the known limitations of nonselective COX inhibitors,[35] the recently discovered limitations of COX-2 inhibitors,[36] and the recent (controversial) indication that very high-dose α-tocopherol may carry some toxicity,[85] the search is on for agents that suppress these pathways with minimal toxicity. For PGs and LTs, this will likely mean development of selective receptor antagonists. For AOs, this likely will mean a combination of dietary interventions, natural product supplements, and pharmaceuticals to achieve the desired effect with minimal toxicity. Analogous to treatments that suppress hypertension or hypercholesterolemia and thereby reduce vascular disease, these interventions will not cure AD but may check its explosive growth and hopefully reduce its incidence and prevalence in the elderly.

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