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12/15-Lipoxygenase Is Increased in Alzheimer's Disease

Possible Involvement in Brain Oxidative Stress

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Alzheimer's disease (AD) is a chronic neurodegenerative disorder that impairs cognition and behavior. Although the initiating molecular events are not known, increasing evidence suggests that oxidative stress could play a functional role in its pathogenesis. Lipoxygenase (LOX) enzymes by oxidizing polyunsaturated fatty acids synthesize hydroperoxyacids, which are potent pro-oxidant mediators. Because circumstantial evidence suggests that 12/15-LOX is a major source of oxidative stress, we investigated the protein levels and activity of this enzyme in different brain regions of histopathologically confirmed AD and control cases. Using quantitative Western blot analysis we demonstrated that in affected frontal and temporal regions of AD brains the amount of 12/15-LOX was higher compared with controls, whereas no difference between the two groups was detected in the cerebellum. This observation was confirmed by immunohistochemical studies. Levels of 12/15-hydroxyeicosatetraenoic acids, metabolic products of 12/15-LOX, were also markedly elevated in AD brains compared to controls. This increase directly correlated with brain lipid peroxidation, and inversely with vitamin E levels. Finally, genetic deletion of this enzyme *in vitro* resulted in a reduction of the cellular oxidative stress response after incubation with H_2O_2 or amyloid β. These data show that the 12/15-LOX metabolic pathway is increased and correlates with an oxidative imbalance in the AD brain, implying that this enzyme might contribute to the pathogenesis of this neurodegenerative disorder. *(Am J Pathol 2004, 164:1655–1662)*

Alzheimer's disease (AD) is the most common neurodegenerative disorder of the elderly, affecting ~6 to 8% all persons aged >65 years.[1] In addition to the presence of abundant senile plaques and neurofibrillary tangles, the AD brain exhibits evidence of oxidative damage and inflammation.[2,3] Lipoxygenases (LOXs) form a family of lipid-peroxidizing enzymes present in the plant and animal kingdoms, but not in bacteria or yeasts.[4,5] LOXs are non-heme iron dioxygenase enzymes that insert molecular oxygen into free and esterified polyunsaturated fatty acids.[6] The currently used nomenclature for LOX is based on their positional specificity of substrate oxygenation. For example, the 12-LOX oxygenates arachidonate at C-12 and catalyzes the formation of 12-hydroxyperoxyeicosa-tetraenoic acid (12-HPETE), which then is rapidly converted into 12-hydroxyeicosaenoic acid (12-HETE).[7] Interestingly, although some LOXs form exclusively one compound from arachidonic acid, others are classified as dual-specificity LOX [12-LOX (leukocyte type), 15-LOX-1] because they form two HPETE compounds at the same time. This latter group has been referred to as 12/15-LOX.[8] Thus, analysis of a cDNA encoding an arachidonate 12-LOX obtained from rat brain showed that it generates preferentially 12-HETE, but also 15-HETE.[9] 12/15-LOX has been described mainly in neurons and also in some glial cells throughout the cerebrum, basal ganglia, and hippocampus,[10,11] and its metabolic product levels are increased in an experimental model of brain ischemia-reperfusion injury.[12] Despite the fact that 12/15-LOX enzymatic activity, as well as protein and mRNA levels, have been well documented in the central nervous system (CNS), a specific biological role for this enzyme in the brain has yet to be established. Circumstantial evidence suggests that it may be involved in neurodegeneration,[13] by oxidizing fatty acids in the cell membranes, thereby contributing to *in vivo* oxidative stress.[14]

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Table 1. Demographic Data of the Cases Studied

Case	No.	Sex	Age (yr)	PMI (hr)	Duration (yr)	Criteria*	Stage*
AD	1	F	74	9	8	Probable	III-IV
	2	M	73	7	10	Probable	III-IV
	3	M	86	4	4	Definite	V-VI
	4	F	92	13	12	Definite	V-VI
	5	F	56	7	8	Probable	III-IV
	6	M	79	13	9	Definite	V-VI
	7	M	85	14	4	Probable	III-IV
	8	F	81	11	10	Definite	V-VI
	9	F	87	17	5	Probable	III-IV
	10	F	75	5.5	10	Probable	III-IV
			(79 ± 3)	(10 ± 1.3)	(8 ± 1)		
Controls	1	F	70	7			-
	2	M	60	5			-
	3	F	71	11			-
	4	F	87	15			-
	5	M	74	16			-
	6	F	98	10			-
	7	M	77	14			-
	8	F	80	8			-
	9	M	83	12			-
	10	M	67	12.5			-
			(76 ± 3.4)	(11 ± 1.1)			

*Criteria of AD are according to the NIA/Reagan criteria.[18] Stages are according the Braak and Braak's classification.[19] PMI; post mortem interval.

In the last decade consistent data have shown that oxidative stress is a feature of AD. Our recent work also provided strong support for the view that oxidative stress is an early event in AD, which is likely to play a more active role in its pathogenesis than previously hypothesized.[15,16] However, the source of oxidative stress in AD remains elusive.[17] Because 12/15-LOX could be an important mediator of the increased oxidative stress in the CNS of AD patients, we investigated whether this metabolic pathway is altered in AD brain. In the present study, we demonstrate for the first time that 12/15-LOX protein levels and enzyme activity are increased in affected frontal and temporal cortices of AD brains compared with controls, whereas unaffected brain regions (cerebellum) show similar values in AD and control brains.

Materials and Methods

Patients

Cases were randomly selected from autopsies performed at the University of Pennsylvania Alzheimer's Disease Center from patients with neuropathologically confirmed AD ($n = 10$), and normal control patients ($n = 10$) (see Table 1). Postmortem diagnostic evaluation of the patients and controls was always performed in accordance with previously described procedures and standard histopathological criteria that have been used in earlier studies from our laboratory.[15,18,19] Control patients had no history of dementia, other neurological diseases, or any systemic illnesses affecting the brain, and neuropathological examination did not reveal any significant abnormalities in these brains. All samples were coded, so their subsequent analysis was performed without knowledge of the age or diagnosis of the individuals from whom they were obtained or regional identity of the tissues. Frozen (−80°C), unfixed samples of mid-frontal, mid-temporal, and cerebellar cortices were used for Western blot studies, 12-HETE, 15-HETE, 8,12-*iso*-iPF$_{2\alpha}$-VI, and vitamin E level analyses (see Table 1). Paraffin-embedded immersion-fixed brain samples from different AD and control cases were used for immunohistochemical studies (see Table 3).

Biochemical Analysis

Samples were minced and resuspended in phosphate-buffered saline (PBS) containing 10 mmol/L ethylenediaminetetraacetic acid and 2 mmol/L BHT to prevent autooxidation. After homogenization, total lipids were extracted with a Folch solution, chloroform/methanol (2:1, v:v), the solution was then vortexed and centrifuged at 800 × *g* for 15 minutes at 4°C. An aliquot of the organic phase containing the extracted lipids was dried under nitrogen, then KOH (15%) was added and the mixture was incubated at 45°C for 45 minutes to effect hydrolysis and release of esterified 12-HETE, 15-HETE, and 8,12-*iso*-iPF$_{2\alpha}$-VI. Total 12-HETE and 15-HETE levels were assayed by liquid chromatography/mass spectrometry/mass spectrometry (LC/MS/MS). Briefly, a known amount of internal standards (d_8-12-HETE; d_8-15-HETE) (Cayman Chem., Ann Arbor, MI) was added to the samples, which were then subjected to solid phase extraction by using polymeric sorbent columns (Stratax, Phenomenex, Torrance, CA). The eluate was dried under nitrogen, resuspended in acetonitrile, and analyzed in a LC/MS/MS system, as previously described.[20] Briefly, a Micromass Ultima LC/Tandem MS was operated in the selected reaction-monitoring mode using argon as the dissociation gas, and collision energy of 14 V. The Shimadzu LC-

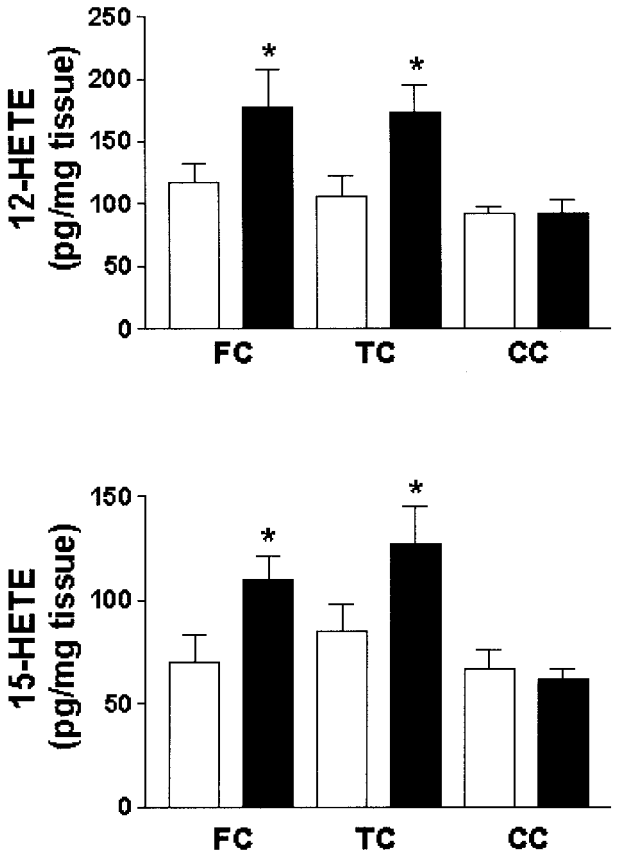

Figure 1. 12/15-LOX activity is increased in AD brains. Total 12-HETE and 15-HETE levels in frontal cortex (FC), temporal cortex (TC), and cerebellum (CC) of AD (**closed bars**) and control patient (**open bars**) brains ($P < 0.01$, $n = 10$ per group).

10Advp HPLC was operated at a flow rate of 0.2 ml/minute. The mobile phase was 65% A/35% B, where A = HPLC grade water (Burdick and Jackson, Muskegon, MI) and B = 95% acetonitrile/5% methanol. Both A and B contained 0.005% acetic acid, pH adjusted to 5.7 with ammonium hydroxide. Total 8,12-*iso*-iPF$_{2\alpha}$-VI levels were assayed by gas chromatography/mass spectrometry, as previously described.[15,16] Aliquots of the extracted organic phase were used to measure vitamin E levels by high-pressure liquid chromatography (HPLC) assay, as previously described.[21]

Biochemical and Quantitative Western Blot Analyses

Three regions of frozen, unfixed brain (mid-temporal, mid-frontal, and cerebellar cortices) were used for biochemical analyses, as described elsewhere.[22,23] Briefly, 1 g of each tissue sample was homogenized in buffer (750 mmol/L NaCl, 50 mmol/L Tris buffer, pH 7.6, and protease inhibitors cocktail), equal amounts of total protein (10 μg) were loaded in each lane and resolved on 10% sodium dodecyl sulfate gels (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) followed by Western blot analyses. Nitrocellulose replicas were probed with a rabbit polyclonal antibody raised against mouse 12/15-LOX (1:1000), which cross-reacts with human 15-LOX-1, but it does not with 5-LOX or P12-LOX.[24] For these reasons, as a positive control, purified recombinant human 15-LOX-1 (access no. MS23892) was loaded in the same gel. β-Tubulin was always used as an internal loading control. For quantitative immunoblot analysis, ^{125}I-labeled polyclonal rabbit antibody was used as the detection antibody.[22,23] Briefly, after the incubation with the primary anti-12/15-LOX antibody followed by extensive washing in Tris-buffered saline, the nitrocellulose replicas were incubated with 2 μCi/ml secondary radiolabeled antibody in buffer containing 0.4 mol/L of phosphate buffer, pH 7.6, 0.1% Triton, 50 mmol/L NaCl, 0.2 mmol/L ethylenediaminetetraacetic acid followed by an extensive wash in Tris-buffered saline buffer. The quantitative data were generated using ImageQuant analysis software in which the signal intensity correlates with pixel numbers, as described elsewhere.[22,23]

Immunohistochemistry

Tissue blocks from brains of different AD cases ($n = 9$) and controls ($n = 6$) were fixed in 70% ethanol in 150 mmol/L NaCl, pH 7.4, or 10% neutral buffered formalin. The clinical and autopsy data of this group are shown in Table 3. There was no significant difference in age, gender, or postmortem interval between these individuals and the patients described in Table 1. Tissue blocks were embedded in paraffin and cut into 6-μm-thick sections. The presence or absence of 12/15-LOX was monitored by immunohistochemistry using the same antibody against 12/15-LOX antibody used above,[24] diluted 1:100

Table 2. Levels of 8,12-*iso*-iPF$_{2\alpha}$-VI and Vitamin E in Mid-Frontal (FC), Mid-Temporal (TC), and Cerebellar Cortex (CC) of AD and Control Brains.

	AD			Control		
	FC	TC	CC	FC	TC	CC
8,12-*iso*-iPF$_{2\alpha}$-VI (pg/mg tissue)	305 ± 20*	310 ± 15*	105 ± 12	172 ± 18	185 ± 15	95 ± 12
Vitamin E (pg/mg tissue)	12 ± 1.6*	10 ± 1.2*	15.6 ± 1.5	20 ± 1.8	19 ± 1.5	17 ± 1.3

Results are expressed as mean ± SEM.
*$p < 0.01$ versus control.

in 2% donor horse serum, and in 0.1 mol/L Tris buffer, and by using the avidin-biotin complex method (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). Control for these experiments included applying the same procedures above except for substitution of the primary antibody with donor horse serum, or using a nonimmune rabbit serum in the initial incubation step instead. Extent of the immunopositive reaction in the brain sections was assessed semiquantitatively as 0 to 3+ as follows: 0, absent; 1+, modest; 2+, moderate; 3+, intense immunostaining.

Cell Culture

To study the cellular oxidative stress responses in the presence or absence of the 12/15-LOX enzymatic activity we used macrophages genetically deficient of this enzyme.[25] Resident peritoneal macrophages were isolated from 12/15-LOX-deficient (12/15-LOX$^{-/-}$) and wild-type littermates (12/15-LOX$^{+/+}$) mice, as previously described.[25] Cells were washed once in PBS, resuspended in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, and plated onto culture dishes. After 2 hours at 37°C in a 95% air, 5% CO_2 incubator, the macrophages were washed twice with Dulbecco's modified Eagle's medium to remove nonadherent cells. Cells were incubated with H_2O_2 (10 μmol/L) or A$\beta_{1\text{-}42}$ (1 μmol/L) for 24 hours. At this time point, supernatants were collected and analyzed for 8,12-*iso*-iPF$_{2\alpha}$-VI, 12/15-HETE levels as above described and interleukin (IL)-12p40 by a radioimmunoassay.

Statistical Analysis

Data are presented as mean ± SEM. Comparison between groups was performed by nonparametric one-way analysis of variance (Kruskall-Wallis test) with the Dunn's posttest. Only P values <0.05 were regarded as statistically significant. Correlations between 12-HETE, 15-HETE, other biochemical parameters, and the postmortem interval, age, stage, and duration of AD were examined by using linear regression.

Results

The clinical and autopsy data of the patient and control groups used for biochemical analyses are shown in Table 1. There were no significant differences in age, gender, and postmortem interval between the two groups. The levels of 12-HETE in the frontal cortex of AD brains were markedly elevated (180 ± 20 pg/mg tissue) compared to the normal control brains (117 ± 15 pg/mg tissue, $P < 0.01$). Increased 12-HETE levels were also detected in the AD temporal cortex relative to the controls (174 ± 20 pg/mg *versus* 106 ± 16 pg/mg tissue, $P < 0.01$) (Figure 1A). Similar results were obtained from assays of the levels of 15-HETE in the frontal cortex (110 ± 11 *versus* 70 ± 12, $P < 0.01$), and temporal cortex (130 ± 18 *versus* 85 ± 12, $P < 0.01$) (Figure 1B). Notably, levels of 12-HETE and 15-HETE were always lower in the cerebellum than in the neocortex of the AD brains, and there were no significant differences in these levels between AD and control cerebellum (Figure 1, A and B). No significant correlation was observed between age, postmortem interval, disease duration, or stage of the disease and the levels of 12-HETE and 15-HETE in both frontal and temporal cortex samples from AD brains (not shown).

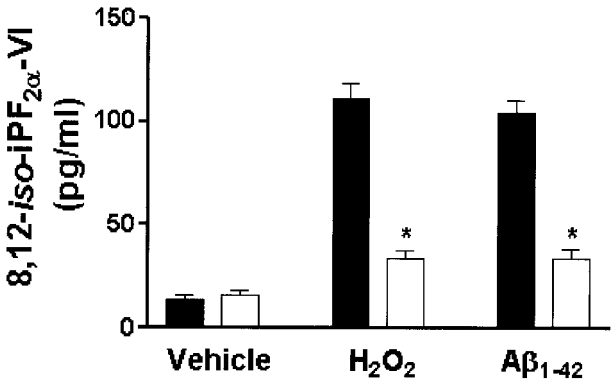

Figure 2. Absence of 12/15-LOX protects against oxidative stress. Macrophages from 12/15-LOX$^{+/+}$ (**open bars**) and 12/15-LOX$^{-/-}$ (**closed bars**) mice were isolated and incubated with H_2O_2 (10 μmol/L) or A$\beta_{1\text{-}42}$ (1 μmol/L) for 24 hours. Supernatants were collected and assayed for 8,12-*iso*-iPF$_{2\alpha}$-VI levels ($n = 4$ separate experiments; *, $P < 0.01$).

Confirming previous reports, we found that both frontal and temporal but not cerebellar cortex of the AD brains had significantly higher levels of total 8,12-*iso*-iPF$_{2\alpha}$, which is a specific and sensitive marker of lipid peroxidation (Table 2).[15,16] Next, we investigated tissue levels of vitamin E, and found that AD brains had much lower levels of this exogenous antioxidant compared with controls (Table 2). Moreover, we found a direct correlation between 12-HETE, 15-HETE, and 8,12-*iso*-iPF$_{2\alpha}$-VI levels ($r^2 = 0.71$, $r^2 = 0.68$; $P < 0.01$ for both), whereas an inverse correlation was observed between 12-HETE, 15-HETE, and vitamin E levels ($r^2 = -0.65$, $r^2 = -0.63$; $P < 0.01$ for both).

To more directly investigate the relationship between this enzyme and cellular oxidative stress responses, macrophages from 12/15-LOX$^{-/-}$ or wild-type 12/15-LOX$^{+/+}$ mice were incubated with H_2O_2 (10 μmol/L) or A$\beta_{1\text{-}42}$ (1 μmol/L), and supernatants collected 24 hours later. As expected, although the wild-type cells (12/15-LOX$^{+/+}$) incubated with H_2O_2 or A$\beta_{1\text{-}42}$ produced discrete amounts of both 12-HETE and 15-HETE, macrophages from 12/15-LOX$^{-/-}$ animals did not produce any detectable levels (not shown). As shown in Figure 2, incubation of 12/15-LOX$^{+/+}$ macrophages with both compounds significantly increased the amount of 8,12-*iso*-iPF$_{2\alpha}$-VI release in the supernatant. By contrast, 12/15-LOX$^{-/-}$ macrophages showed significantly much lower levels of this marker in response to these stimuli (Figure 2). To further support the biological relevance of the doses of agonists used we also measured levels of an inflammatory cytokine, IL-12p40. We found that although both stimuli induced a significant increase of Il-12p40 levels in wild-type cells, by contrast these levels were reduced by 60% in supernatants from 12/15-LOX$^{-/-}$

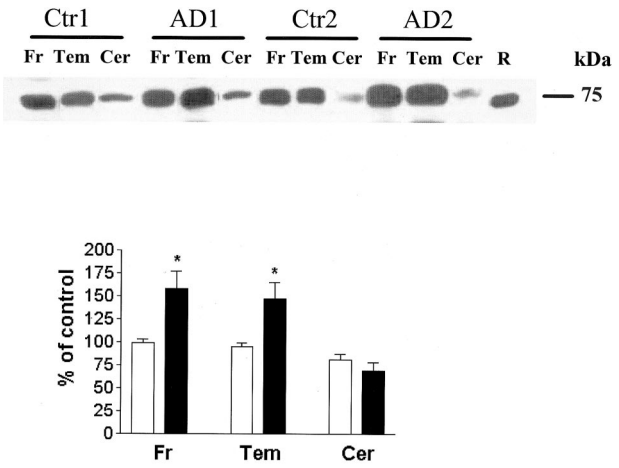

Figure 3. Distribution of 12/15-LOX protein in selected areas of AD and control brains. **A:** Representative Western blot analyses of 12/15-LOX protein in homogenates from two AD (AD1, AD2) and two control brains (Ctr1, Ctr2). Samples (10 μg protein) were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto nitrocellulose membrane, and probed with rabbit 12/15-LOX antiserum (Fr, mid-frontal cortex; Tem, mid-temporal cortex; Cer, cerebellum). As a positive control, purified recombinant human 15-LOX1 protein was used (R). **B:** Quantitative Western blot analysis of samples was performed using ^{125}I-labeled polyclonal rabbit antibody as the secondary antibody. Each sample was loaded in duplicates. Each bar represents a mean average from five AD (**closed bars**) and five control (**open bars**) samples and presented as a percentage of control samples (*, $P < 0.01$).

cells (not shown). No difference in lactate dehydrogenase levels, markers of cell damage, released in the supernatant was observed between the two cell types at the end of both stress challenges (not shown). Finally, addition of 12-HETE (1 μmol/L) or 15-HETE (1 μmol/L) to wild-type cells induced a significant increase in 8,12-*iso*-iPF$_{2\alpha}$-VI 24 hours later (not shown).

To determine the levels of 12/15-LOX we performed Western blot analyses in the same three brain regions (mid-frontal, mid-temporal, and cerebellar cortices) of AD and control brains ($n = 5$ per each group). We found that the amount of this protein enzyme in the mid-frontal and mid-temporal cortex was significantly increased in AD *versus* control brains (Figure 3A). Conversely, no significant difference in distribution of 12/15-LOX levels was observed between the cerebellum of AD and control patients (Figure 3A). Quantitative Western blot analyses were performed using ^{125}I-labeled secondary antibody. The results confirmed the selective increase of the protein and are summarized in Figure 3B, where each bar represents the relative amount of 12/15-LOX in the three different cortical areas compared to controls. No significant correlation between pathological severity of the disease or disease duration and amount of 12/15-LOX protein detected was found (not shown).

These biochemical findings were confirmed by immunohistochemical studies, which showed more intense 12/15-LOX immunoreactivity in brains from AD patients compared with corresponding control brain regions (Figure 4). 12/15-LOX immunoreactivity was notably more intense in the neuronal cell bodies and processes in the AD brains compared to controls, especially in areas with abundant dystrophic neurites and reactive astrocytes (Figure 4, c and d). Immunoreactivity for 12/15-LOX showed the highest intensity in neurons of the hippocampus and entorhinal cortex (Table 3). In general, we observed that the intensity of the immunoreactive products increased as disease progressed. However, semiquantitative analysis of the immunostaining pattern suggests that the early and moderate stages (Braak and Braak's[19] classification I to II, III to IV) had more intense positive reactions than the very advanced stages of the disease (Braak and Braak's classification V to VI) (Table 3).

Discussion

In the present study, we provided the first evidence showing that 12/15-LOX protein levels and enzyme activity are elevated in affected *versus* nonaffected areas of AD brains relative to age-matched control brains, where this evidence also correlated with the detection of increased lipid peroxidation. These findings support the hypothesis that increases in 12/15-LOX protein levels and enzyme activity may be linked to mechanisms of oxidative stress and neurodegeneration in AD.

Although the causative events responsible for neurodegeneration in AD are not completely known, it is increasingly evident that brain inflammation and oxidative stress are consistent pathological features of the disease. Aging is one of the strongest risk factors for developing AD, and oxygen-mediated events are considered possible mechanisms responsible for the increasing neuronal vulnerability in aging. LOXs are enzymes widely represented in the mammalian kingdom that can insert molecular oxygen into molecules of free and esterified polyunsaturated fatty acids and thereby synthesize several different biologically active eicosanoids. The biological significance of most LOX isoforms are far from clear, but two major functions have been ascribed to this large family of enzymes: formation of biological mediators/signal molecules, and modification of membrane structure (peroxidation reaction).[26]

One of the most abundant LOX isoforms in the CNS is 12/15-LOX, which has been described mainly in neurons and some glial cells throughout the cerebrum, basal ganglia, and hippocampus. Thus, previous works also showed that 12-HETE and 15-HETE are the principal LOX products in the CNS.[9–11] Originally, these metabolites had been proposed to play roles as second messengers in synaptic transmission and they were thought to be involved in learning and memory processes.[27] However, despite some circumstantial evidence suggesting that these enzymes might also play a role in neurodegeneration, a definite biological role for 12/15-LOX in diseases of the CNS has yet to be established.

Throughout the last 10 years oxidative stress has been implicated as a key event in AD pathogenesis.[2,17] Products of oxidative damage have been shown to be elevated in AD patients, including the nucleic acid oxidation product 8-hydroxy-2′-deoxyguanosine,[28] protein carbonyl oxidation products,[29] peroxynitrite-mediated damage products,[30] lipid peroxidation products such as F$_2$-isoprostanes[31] and 4-hydroxynonenal.[32] Additionally, levels of several endogenous and exogenous antioxi-

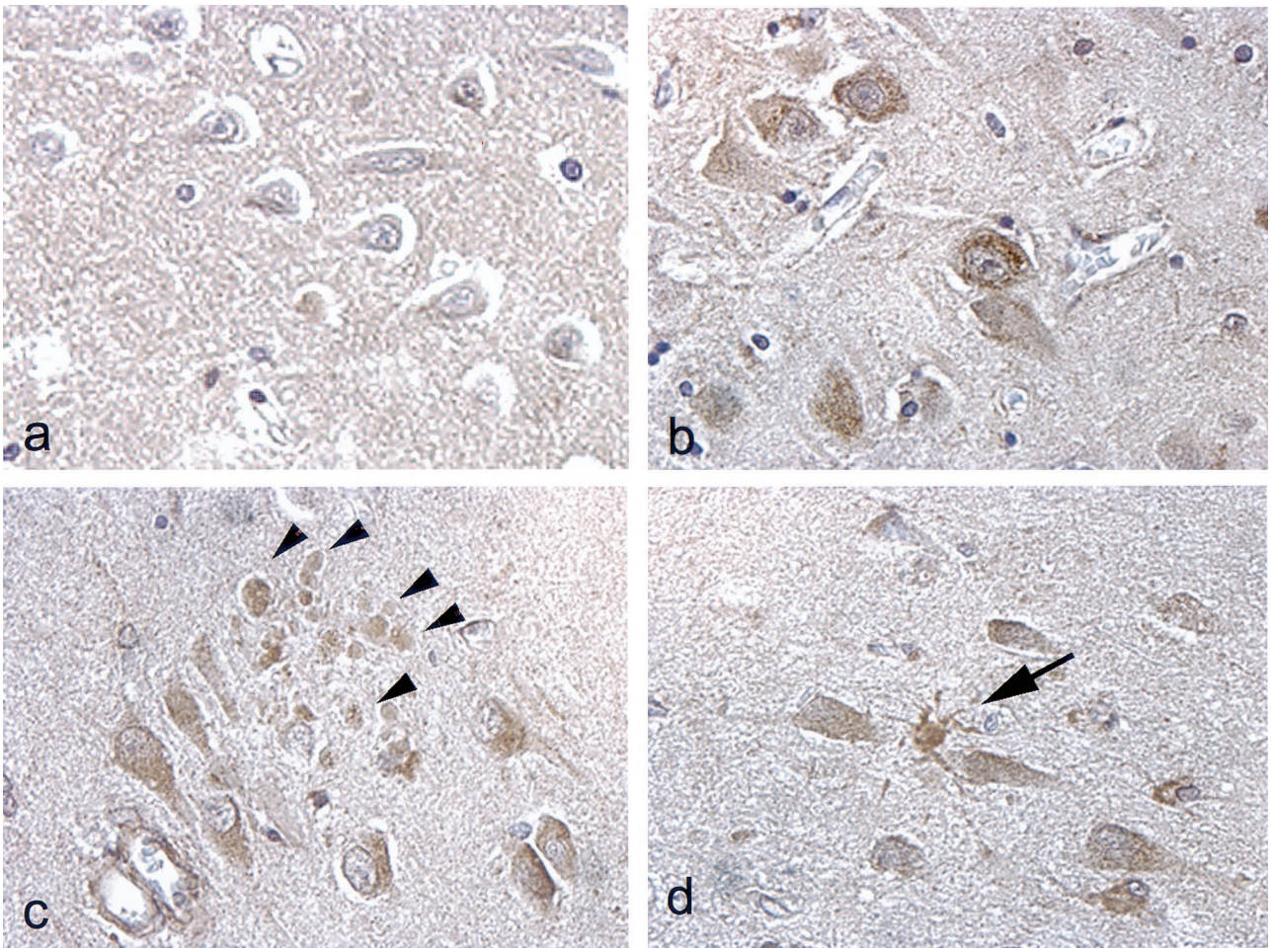

Figure 4. 12/15-LOX immunoreactivity in AD and control brains. Representative immunohistochemical analysis of hippocampus and entorhinal cortex sections from control patients (**a**) and AD patients (**b–d**) reacted with 12/15-LOX antiserum. Compared with controls, neurons in AD cases show intense stain in their cell bodies and processes (**arrowheads**), in addition some astrocytes (**arrow**) are also immunopositive for 12-LOX.

dants have been reported reduced in AD patients *versus* control brains.[33] Although the key mediators of the oxidative stress events in AD have not been fully elucidated, several different enzymatic and nonenzymatic mediators have been implicated in inducing a significant oxidative imbalance in the CNS of AD patients.

One of the most unique aspects of LOX enzymatic reactions is the fact that they may initiate and be directly involved in inducing structural or metabolic damage in cell membranes. In particular, once activated they produce specific hydroperoxy acids, which can provoke secondary oxygenation and induce an enzyme-catalyzed lipid peroxidation.[6] Supporting this hypothesis, we found that increases in 12/15-LOX protein levels and enzymatic products were associated with a significant elevation in 8,12-*iso*-iPF$_{2\alpha}$-VI levels in AD, which directly correlated with the high levels of 12-HETE and 15-HETE suggesting a common mechanism for the formation of these metabolites.

Because oxidative stress is a condition wherein the generation of reactive oxygen species exceeds the endogenous ability to destroy them, we also assayed the levels of vitamin E (one of the most potent exogenous antioxidants) in the AD and control brains studied here.[34] Compared with controls, the affected regions of the AD brains had much lower levels of vitamin E. From the present study, we cannot say whether the reduced vitamin E observed in AD brains derives from an increased utilization or a decreased intake. However, interestingly, we observed an inverse correlation of this vitamin with the levels of 12/15-HETE in these same regions, which would further support the hypothesis that an *in vivo* oxidative imbalance occurs in AD.[35]

Recent evidence from animal models and human studies suggest the novel concept that oxidative stress is an early functional event in AD pathogenesis.[36,37] Interestingly, semiquantitative analysis from our immunohistochemical studies indicated that the immunoreactivity was higher at the earlier rather than at the very advanced stages of the disease. This observation would support the hypothesis that the increase in 12/15-LOX protein levels and activity we observed in AD brains do not reflect nonspecific end-stage changes. It will be very important to determine whether this enzyme is also altered in other neurodegenerative diseases.

Several mechanisms have been implicated in the regulation of 12/15-LOX activity and expression levels in different cell types. Among them, different cellular

Table 3. Semiquantitative Analysis of the 12/15LOX Staining Distribution in AD and Matched Control Brains.

Disease	Criteria*	Sex	Age	PMI	CA4	CA3	CA2	CA1	Ent.Ctx.
AD	Possible	M	82	15	++	++	++/+++	++/+++	++/+++
	Possible	F	81	13	++	++	-/++	-/++	-/++
	Possible	F	83	7	++	++	+++	++	+++
	Probable	F	67	12	++	++	++	++/+++	++
	Probable	M	86	5	++	++	++	+/+++	-/++
	Probable	M	84	10	+	-/++	-/++	-/++	+
	Definite	F	74	14	-/+	++	++	++	+++
	Definite	F	86	4	-	+	-/++	-/++	-/+
	Definite	F	81	9	+/++	+/++	+/++	++	+/+++
			(80 ± 2)	(10 ± 2)					
Controls	N/A	F	67	5.5	-	-	-	-/++	+
		F	82	15	-/+	-/+	-/+	-	-/+
		M	80	12	+	+	-	-	-/+
		F	84	10-	-	-	-	-	-
		F	76	7	-	-/+	-	-/+	-
		M	74	11	-/+	-	-/+	-/+	-/+
			(77 ± 2)	(10 ± 2)					

* Criteria of AD are according to the NIA/Reagan criteria,[18] possible, probable and definitive correspond to I-II, III-IV, and V-VI in the Braak and Braak's classification, respectively.[19] Extent of immunostaining was assessed as: 0, absent; +, modest; ++, moderate; +++, intense. PMI; post-mortem interval.

stresses and inflammatory cytokines have been shown to induce 12/15-LOX activity and protein levels,[38] whereas there is evidence that AD brains are characterized by the presence of several of these mediators of inflammation.[3] Thus, it is possible that 12/15-LOX is augmented in response to cellular stress or other events leading to the development of AD. However, this increase in enzymatic activity could also facilitate progression of the disease and actually contribute to neuron loss. Given that oxidative stress is an early event in the pathogenesis of AD, it is possible that this enzyme is part of a self-propagating cycle of autocrine stimulation in the CNS that is associated with the induction of inflammatory mediators produced by microglia or astrocytes in response to the progressive accumulation of amyloid plaques. Cytokines, oxidants, and the same Aβ peptides could then stimulate neuronal 12/15-LOX, which by producing high levels of HETEs would further reinforce this vicious cycle of inflammatory and oxidative reactions. In support of this hypothesis, we found that cells lacking this enzyme by genetic deletion have a significantly reduced oxidative stress and inflammatory responses compared with cells that do possess the 12/15-LOX enzymatic machinery when challenged with agonists that have been implicated in the neuronal damage of AD. The possibility that this enzyme modulates oxidative stress responses also in the CNS is in line with our previous observation in an animal model of atherosclerosis, in which its absence decreased systemic lipid peroxidation and the development of the disease.[39]

In summary, the present study shows for the first time that the 12/15-LOX metabolic pathway is increased in AD compared with control aging brains, and that this increase is directly correlated with an oxidative imbalance in the CNS. Thus, drugs that specifically reduce or block the 12/15-LOX metabolic pathway activation may warrant consideration as potential therapeutic interventions for AD.

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