ELSEVIER

Biochemical Pharmacology 63 (2002) 563–567

Biochemical Pharmacology

Commentary

Alzheimer's disease and oxygen radicals: new insights

Domenico Praticò*

Department of Pharmacology, Center for Experimental Therapeutics School of Medicine, University of Pennsylvania, 421 Curie Blvd., BRB 2/3, Room 812, Philadelphia, PA 19104, USA

Abstract

Alzheimer's disease (AD) is the most common form of neurodegenerative disease, with dementia, in the elderly. In addition to the presence of senile plaques and neurofibrillary tangles, the AD brain exhibits evidence for oxygen radical-mediated damage, a situation commonly known as oxidative stress. However, the ability to directly implicate this mechanism in AD has been a difficult task for several reasons. First, most of the analytical approaches used to investigate oxidative stress turned out to be unreliable. Second, the majority of the published studies have been performed in post-mortem tissues with advanced disease, leaving open the question as to whether oxidative stress is an early event or a common final step secondary to the degenerative process. The discovery of the isoprostanes, recent studies performed in living patients, and the development of transgenic animal models of AD-amyloidosis are three important factors that are helping us to better understand and define the role that oxygen radicals might play in AD pathogenesis. Here we review some of the most recent works that have supported the importance of oxygen radical-mediated damage in AD. The accumulated information points toward an earlier involvement than previously thought of oxidative stress in the pathogenesis of the disease, making this a potential target for therapeutic intervention, especially in subjects at high risk for developing AD.

Keywords: Alzheimer's disease; Oxygen free radicals; Oxidative stress; Isoeicosanoids; Amyloid; Transgenic animals

1. Introduction

AD is the most common neurodegenerative disorder of the elderly. Clinically, it is characterized by progressive memory loss, decline in language skills, and other cognitive impairments. AD affects approximately 4 million people in the US, with an incidence rate from 0.5% per year at 65 years to 8–10% at age 85 years [1,2]. While early-onset familial AD is caused by multiple mutations in different genes, late-onset AD, which is sporadic and accounts for 90% of total patients, is probably due to the effects of genetic risk factors combined with different epigenetic events [3]. Although the initiating causes leading to AD are unknown, it is clear that its pathophysiology is complex and most likely involves multiple distinct and overlapping pathways of neuronal damage [4]. This is supported by the fact that in addition to the pathological hallmarks of the disease, which include senile plaques and neurofibrillary tangles, AD brains exhibit a number of other abnormalities: loss of synapses, gliosis, microglia activation, signs of inflammation, and damage secondary to oxygen radicals [5–7]. Oxygen radicals are chemically unstable and highly reactive compounds, which are formed during normal cellular metabolism. Due to their reactivity, they can be responsible for cellular and tissue damage anytime their generation exceeds the endogenous ability to destroy them. This condition is also known as oxidant or oxidative stress.

2. Oxidative stress and AD

A role for oxidative stress has been widely discussed in the pathogenesis of AD [6,8,9]. However, the ability to implicate this mechanism directly has been confounded mainly by two factors: the evanescent nature of the oxygen radicals, and the use of non-specific analytical approaches [10]. Isoprostanes are members of a complex family of lipids, isomers of conventional enzymatically derived prostaglandins, which are produced by oxygen radical-catalyzed peroxidation of polyunsaturated fatty acids [11]. Most of the work has been focused on a group of isomers of

* Tel.: +1-215-898-6446; fax: +1-215-573-9004.
E-mail address: domenico@spirit.gcrc.upenn.edu (D. Praticò).

Abbreviations: AD, Alzheimer's disease; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances; $iPF_{2\alpha}$, isoprostane-$F_{2\alpha}$; CSF, cerebrospinal fluid; Aβ, amyloid beta peptide; WT, wild type.

0006-2952/02/$ – see front matter
PII: S0006-2952(01)00919-4

the prostaglandin $F_{2\alpha}$, called $iPF_{2\alpha}$, and an abundant literature has established that their measurement provides a reliable marker of *in vivo* lipid peroxidation and oxidative stress [12]. The brain is particularly vulnerable to oxygen radical-mediated damage because of its high energy and oxygen consumption rate, and its abundance of peroxidizable fatty acids. In addition, it contains high levels of transition metals, which can catalyze the formation of oxygen radicals, and a scarcity of antioxidant defense systems compared with other organs [13]. Thus, oxidative damage to brain tissue manifests itself predominantly as lipid peroxidation, and in the last decade, although not always consistently, the majority of published studies have provided evidence for this [6,8,9]. In this commentary, I will review recent data supporting a role for oxygen radicals in AD, with particular emphasis on lipid peroxidation in its pathogenesis.

3. Human studies

An impressive number of studies have been published on oxidative injury and lipid peroxidation in AD. Most of them have been biochemical or histological studies performed on AD post-mortem brain tissues. Historically, malondialdehyde (MDA) and (thiobarbituric acid reactive substances) TBARS assays have been the first and probably the most popular techniques employed to quantitate, biochemically, lipid peroxidation in AD. The majority of these investigations have shown higher MDA and/or TBARS levels in AD than in control brains [14–16]. However, it is now widely accepted that while these assays work well when applied to *in vitro* systems, they are not reliable in complex biological systems [17]. Another by-product of the oxidation of polyunsaturated fatty acids that has been quantified is 4-hydroxynonenal. This highly reactive aldehyde has been found elevated in AD brain tissue as well as in ventricular cerebrospinal fluid (CSF) [18,19]. However, since its levels are generally low and there is a need for large quantities of samples in order to measure it, its use in biological systems has been relatively limited.

Initially, we investigated post-mortem brain tissues from AD patients and compared them with tissues from patients with Parkinson's disease or schizophrenia, or with brains from neurological normal controls [20]. We found that two distinct isoprostane isomers, $iPF_{2\alpha}$-III and $iPF_{2\alpha}$-VI, were elevated markedly in both frontal and temporal poles of brains affected with AD but not in the other groups. Remarkably, no such difference was observed in the cerebellum, an area traditionally devoid of the pathological hallmarks of the disease. Levels were also elevated in the ventricular CSF of AD patients compared with that of the non-AD group [20]. This study confirmed that oxidative stress is a feature of AD, and at the same time clearly indicated that it is not a diffuse process, but rather localizes in areas affected by the disease. Interestingly, it showed that isoprostane levels are readily detectable, not only in AD but also in controls, and that determination of their levels in CSF could be potentially exploited to develop tests reflecting disease activity in living patients with AD. However, our study, like any of the others performed in AD post-mortem brain tissues, has still left open the important question of whether or not oxidative stress is a component early in the evolution of AD or is a final common step of the neurodegenerative process. Several studies have attempted to measure serum and plasma levels of MDA in living patients with AD, but conflicting results have been reported [21–23].

In 1999, a study suggested for the first time that the increase in lipid peroxidation is present before the end-stage of the disease. Total CSF isoprostane-$F_{2\alpha}$ ($iPF_{2\alpha}$) was higher in patients with probable AD than in controls, but no correlation was found with age, cognitive performance, or other considered variables [24]. We confirmed and extended this observation in living subjects with clinical diagnosis of AD [25]. Urine, plasma, and CSF were collected from patients with probable AD and matched controls. We found that, compared with controls, patients with a clinical diagnosis of AD had increased CSF, plasma, and urinary levels of a major isoprostane, 8,12-*iso*-$iPF_{2\alpha}$-VI (Fig. 1) [25]. Urinary and circulating levels of this isoprostane directly correlated with levels in the CSF of AD patients, suggesting a common origin and mechanism of formation: brain oxidative stress. Further, we found a significant correlation between the level of 8,12-*iso*-$iPF_{2\alpha}$-VI in the CSF and the severity of the dementia in AD patients as measured by two of the most common cognitive tests: the mini-mental state examination and the dementia severity rating scale. Previous works have shown that CSF tau protein levels increase and the percentage ratio of amyloid β 1–42 ($A\beta_{1-42}$) decreases with the progression of AD [26,27]. Remarkably, we observed a direct correlation between CSF 8,12-*iso*-$iPF_{2\alpha}$-VI levels and CSF tau and an inverse correlation with CSF $A\beta_{1-42}$ in AD patients [25]. These findings suggest that elevation of isoprostane not only reflects an increase in central nervous system oxidative damage but also correlates with the progression of the disease. In summary, our study strongly supports the hypothesis that oxidative stress occurs early in the course of this dementing disorder, thereby implicating it as a potential contributor to brain degeneration in AD. Further, the fact that urine correlated with CSF levels offers for the first time the potential for using a non-invasive tool to investigate brain oxidative damage and monitor therapeutic responses in AD. The peripheral increase in isoprostane levels in AD was confirmed recently by another group [28]. Finally, to further confirm that brain oxidative stress is an early event in AD, we assayed levels of 8,12-*iso*-$iPF_{2\alpha}$-VI in young patients with Down's syndrome. These subjects suffer from a variety of symptoms secondary to extra gene copies present on an extra chromosome 21 [29]. One of

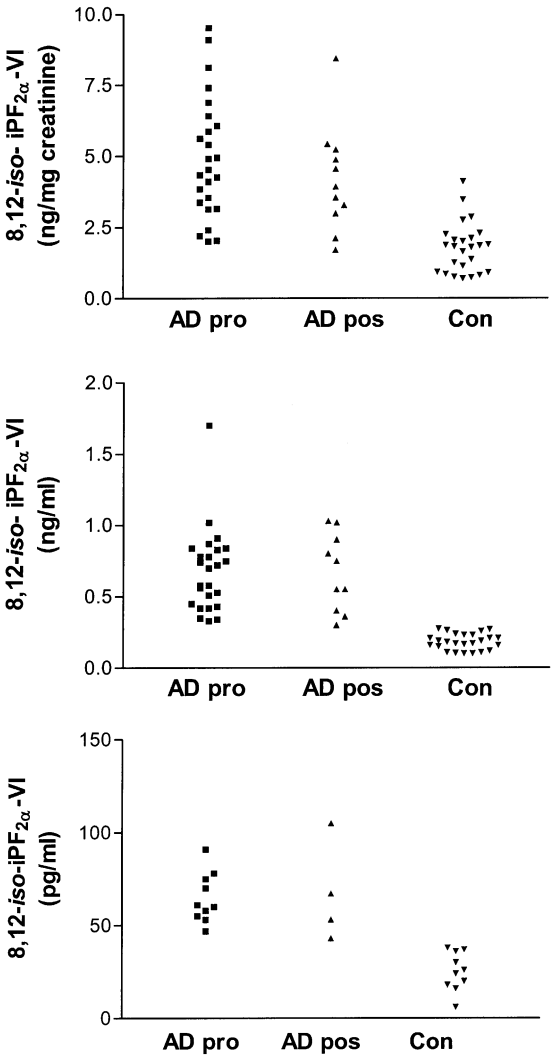

Fig. 1. Urinary (top), plasma (center), and CSF (bottom) 8,12-*iso*-iPF$_{2\alpha}$-VI levels in patients with probable (pro) or possible (pos) Alzheimer's disease (AD) and age- and sex-matched controls (Con). Reprinted with permission from[25]. Copyright @ 2000 John Wiley & Sons Inc.

these genes is the one that codifies for the amyloid precursor protein (APP). This would increase the concentration of amyloid beta peptide (Aβ) in the brain of these subjects and account for the precocious AD-like pathology and dementia they develop later in life. We found elevated 8,12-*iso*-iPF$_{2\alpha}$-VI levels in urine samples of subjects with Down's syndrome compared with those of matched controls, which correlated with the duration of the disease [30].

4. Transgenic animal studies

An ideal transgenic mouse model for AD should mimic the age-dependent accumulation of amyloid plaques, neurofibrillary tangles, and neuronal cell death, and should also display memory loss and behavioral deficits. Several transgenic mouse lines have been established in the last 5 years, all of which manifest a time-dependent increase in Aβ accumulation in the brain, and some of which also display behavioral impairments [31]. Even though not perfect, these animal models are valuable tools to study pathogenetic mechanisms leading to and/or following Aβ deposition *in vivo*. This and the availability of specific and sensitive assays for measuring oxidative stress are now allowing us to better define the functional role of oxidative stress and lipid peroxidation in AD.

Initially, two studies reported that aged transgenic mice, over-expressing the double Swedish mutant of APP (APPswe 695), also known as Tg2576 mice, have immunohistochemical evidence of oxidative stress in brain tissue [32,33]. Again, from these studies it is not possible to distinguish whether oxidative stress is an event that precedes, coincides, or follows Aβ accumulation. Recently, we completed a study with the same model. We performed a time-course experiment using these mice and wild type (WT) littermates as controls starting at 4 months, an age when there is no sign of Aβ deposition, until 18 months of age, when Aβ levels are very high and abundant plaques are present in their brains [34]. Urine and plasma were collected at monthly intervals and assayed for 8,12-*iso*-iPF$_{2\alpha}$-VI levels. No difference was found in urinary levels between 4- and 7-month-old Tg and WT mice. However, these levels significantly increased in Tg mice starting at 8 months of age, and reached a 3-fold increase by 10–11 months of age [35]. Similar results were observed for plasma levels; again there was no difference until 7 months of age. In contrast, Tg mice had higher levels than WT mice starting at 8 months until the end of the study, i.e. 18 months of age. Small cohorts were killed at 4, 8, 12, 15, and 18 months of age, and their brains harvested for 8,12-*iso*-iPF$_{2\alpha}$-VI, Aβ levels, and immunohistochemistry. Homogenates from the cerebral cortex and hippocampus of Tg mice had higher 8,12-*iso*-iPF$_{2\alpha}$-VI levels than those from WT mice starting at 8 months of age (Fig. 2). In contrast, a surge in Aβ levels as well as plaque deposition in Tg mice occurred later at 12 months of age. A direct correlation was found between 8,12-*iso*-iPF$_{2\alpha}$-VI and Aβ_{1-40} ($r^2 = 0.77$, $P < 0.001$), and Aβ_{1-42} ($r^2 = 0.64$, $P < 0.001$). Finally, we monitored brain Aβ plaques using immunohistochemistry. No immunoreactivity was observed in any WT brains. Starting at 12 months of age, Tg2576 mice had only a few scattered Aβ deposits in the cerebral cortex and hippocampus, which became abundant by 18 months of age. Taken together, these results clearly show that lipid peroxidation is elevated in the brains of young Tg mice, and that, most importantly, this elevation precedes the surge in Aβ levels and amyloid plaque formation. This suggests that oxidative damage to the brain may contribute to the pathogenesis of AD independent of amyloid accumulation. These findings support the hypothesis that oxygen radical-mediated lipid peroxidation plays an earlier role than previously anticipated in the pathogenesis of AD. A recent study confirmed this hypothesis by showing that

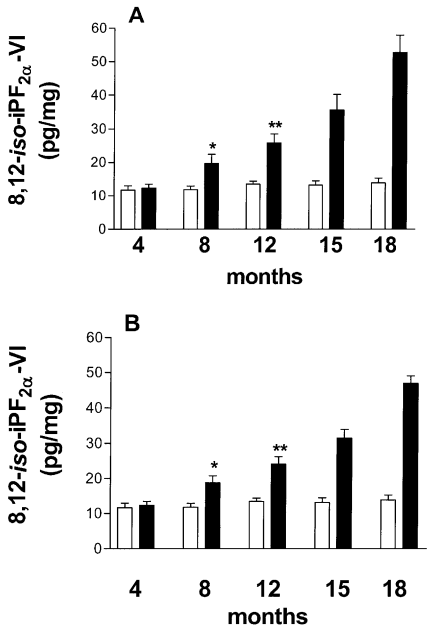

Fig. 2. Total brain cortex (A) and hippocampus (B) levels of 8,12-*iso* $iPF_{2\alpha}$-VI in Tg2576 mice (filled bars) and WT littermates (open bars) at different ages ($^{*}P < 0.01$, $^{**}P < 0.003$). Values are means ± SEM, $N = 6$. Reprinted with permission from[35]. Copyright @ 2001 Society for Neuroscience.

feeding Tg2576 mice the antioxidant curcumin reduces oxidative stress and amyloid pathology in the brain [36].

5. Conclusions

AD is a challenging brain disorder with enormous medical and social consequences. The fact that age is a risk factor in AD has provided initial support to the hypothesis that oxygen radicals, like in the aging process, could be involved. Although AD is likely to be associated with multiple etiologies and mechanisms, it is evident that oxidative stress is part of it. For years we have been accumulating evidence that oxidative stress is a feature of AD. However, only recently with the development of sensitive and specific assays, studies in living patients, and transgenic models of the disease has new light been shed into the central issue of whether oxidative stress is a result of the pathology of AD or serves as an initiator of the pathological damage. Today we have enough data to state that oxidative stress is an early event in AD and is likely to play a more active role in the pathogenesis than previously hypothesized.

Acknowledgments

Part of the research described in this article has been funded by the American Heart Association and the National Institute of Health. I wish to thank Barbara Dallao and Alfredo Praticò for their long-term support and encouragement.

References

[1] Clark CM. Clinical manifestation and diagnostic evaluation of patients with Alzheimer's disease. In: Clark CM, Trojanowski JQ, editors. Neurodegenerative dementias: clinical features and pathological mechanisms. New York: McGraw-Hill, 2000. p. 95–114.
[2] Martin JB. Molecular basis of the neurodegenerative disorders. N Engl J Med 1999;340:1970–80.
[3] Selkoe DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. Nature 1999;399:A23–31.
[4] Masliah E, Licastro F. Neuronal and synaptic loss, reactive gliosis, microglial response and induction of the complement cascade in Alzheimer's disease. In: Clark CM, Trojanowski JQ, editors. Neurodegenerative dementias: clinical features and pathological mechanisms. New York: McGraw-Hill, 2000. p. 131–46.
[5] Praticò D, Trojanowski JQ. Inflammatory hypotheses: novel mechanisms of Alzheimer's neurodegeneration and new therapeutic targets? Neurobiol Aging 2000;21:441–5.
[6] Praticò D, Delanty N. Oxidative injury in diseases of the central nervous system: focus on Alzheimer's disease. Am J Med 2000;109:577–85.
[7] Praticò D. The *in vivo* measurement of the redox state. Lipids 2001;36:545–7.
[8] Smith MA, Rottkamp CA, Nunomura A, Raina AK, Perry G. Oxidative stress in Alzheimer's disease. Biochim Biophys Acta 2000; 1502:139–44.
[9] Christen Y. Oxidative stress and Alzheimer's disease. Am J Clin Nutr 2000;71:621S–9S.
[10] Gutteridge JMC, Halliwell B. The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem Sci 1990;15:129–36.
[11] Praticò D. F_2-isoprostanes: sensitive and specific non-invasive indices of lipid peroxidation *in vivo*. Atherosclerosis 1999;147:1–10.
[12] Praticò D, Lawson JA, Rokach J, FitzGerald GA. The isoprostanes in biology and medicine. Trends Endocrinol Metab 2001;12:243–7.
[13] Floyd RA. Antioxidants, oxidative stress, and degenerative neurological disorders. Proc Soc Exp Biol Med 1999;222:236–45.
[14] Lovell MA, Ehmann WD, Butler SM, Markesbery WR. Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. Neurology 1995;45:1594–601.
[15] Marcus DL, Thomas C, Rodriguez C, Simberkoff K, Tsai JS, Strafaci JA, Freedman ML. Increased peroxidation and reduced antioxidant activity in Alzheimer's disease. Exp Neurol 1998;150:40–4.
[16] Plamer AM, Burns MA. Selective increase in lipid peroxidation in the inferior cortex in Alzheimer's disease. Brain Res 1994;645: 338–42.
[17] Gutteridge J. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995;41:1819–28.
[18] Markesbery WR, Lovell MA. 4-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. Neurobiol Aging 1998;19:33–6.
[19] Lovell MA, Markesbery WR. Ratio of 8-hydroxyguanine in intact DNA to free 8-hydroxyguanine is increased in Alzheimer disease ventricular cerebrospinal fluid. Arch Neurol 2001;58:392–6.
[20] Praticò D, Lee VMY, Trojanowski JQ, Rokach J, FitzGerald GA. Increased F_2-isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation *in vivo*. FASEB J 1998;12:1777–83.
[21] Jeandel C, Nicolas MB, Dubois F, Nabet-Belleville F, Penin F, Cuny G. Lipid peroxidation and free radical scavengers in Alzheimer's disease. Gerontology 1989;35:275–83.
[22] Kalman J, Kudchodkar BJ, Murray K, McConathy WJ, Juhasz A, Janka Z, Lacko AG. Evaluation of serum-lipid-related cardiovascular

risk factors in Alzheimer's disease. Dement Geriatr Cogn Disord 1999;10:488–93.

[23] Ahlskog JE, Uitti RJ, Low PA, Tyce GM, Nickander KK, Petersen R, Kokmen E. No evidence for systemic oxidative stress in Parkinson's or Alzheimer's disease. Mov Disord 1995;10:566–73.

[24] Montine TJ, Beal MF, Cudkowicz ME, O'Donnel H, Margolin RA, McFarland L, Bachrach AF, Zackert WE, Roberts LJ, Morrow JD. Increased CSF F_2-isoprostane concentration in probable AD. Neurology 1999;52:562–5.

[25] Praticò D, Clark CM, Lee VMY, Trojanowski JQ, Rokach J, FitzGerald GA. Increased 8,12-*iso*-iP$F_{2\alpha}$-VI in Alzheimer's disease: correlation of a non-invasive index of lipid peroxidation with disease severity. Ann Neurol 2000;48:809–12.

[26] Kanai M, Matsubara E, Isoe K, Urakami K, Nakashima K, Arai H, Sasaki H, Abe K, Iwatsubo T, Kosaka T, Watanabe M, Tomidokoro Y, Shizuka M, Mizushima K, Nakamura T, Igeta Y, Ikeda Y, Amari M, Kawarabayashi T, Ishiguro K, Harigaya Y, Wakabayashi K, Okamoto K, Hirai S, Shoji M. Longitudinal study of cerebrospinal fluid levels of Tau, Aβ1-40, and Aβ1-42(43) in Alzheimer's disease: a study in Japan. Ann Neurol 1998;44:17–26.

[27] Arai H, Terajima M, Miura M, Higuchi S, Muramatsu T, Machida N, Seiki H, Takase S, Clark CM, Lee VMY, Trojanowski JQ, Sasaki H. Tau in cerebrospinal fluid: a potential diagnostic marker in Alzheimer's disease. Ann Neurol 1995;38:649–52.

[28] Tuppo EE, Forman LJ, Spur BW, Chan-Ting RE, Chopra A, Cavalieri TA. Sign of lipid peroxidation as measured in the urine of patients with probable Alzheimer's disease. Brain Res Bull 2001;54: 565–8.

[29] Coyle JT, Oster-Garnite ML, Gearhart JD. The neurobiologic consequences of Down syndrome. Brain Res Bull 1986;16:773–87.

[30] Praticò D, Iuliano L, Amerio G, Tang LX, Rokach J, Sabatino G, Violi F. Down's syndrome is associated with increased 8,12-*iso*-iP$F_{2\alpha}$-VI levels: evidence for enhanced lipid peroxidation *in vivo*. Ann Neurol 2000;48:795–8.

[31] Janus C, Chishti MA, Westaway D. Transgenic mouse models of Alzheimer's disease. Biochim Biophys Acta 2000;1502:63–75.

[32] Pappolla MA, Chyan YJ, Omar RA, Hsiao K, Perry G, Smith MA, Bozner P. Evidence of oxidative stress and *in vivo* neurotoxicity of β-amyloid in a transgenic mouse model of Alzheimer's disease. Am J Pathol 1998;152:871–7.

[33] Smith MA, Hirai K, Hsiao K, Pappolla MA, Harris PLR, Siedlak SL, Tabaton M, Perry G. Amyloid-β deposition in Alzheimer transgenic mice is associated with oxidative stress. J Neurochem 1998;70:2212–5.

[34] Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficit, Aβ elevation, and amyloid plaques in transgenic mice. Science 1996;274:99–102.

[35] Praticò D, Uryu K, Leight S, Trojanowski JQ, Lee VMY. Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer's disease. J Neurosci 2001;21:4183–7.

[36] Lim GP, Chu T, Yang F, Beech W, Frautschy SA, Cole GM. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 2001;21:8370–7.