Review

Oxidative stress hypothesis in Alzheimer's disease: a reappraisal

Domenico Praticò

Department of Pharmacology, Temple University, School of Medicine, Philadelphia, PA 19140, USA

Alzheimer's disease (AD) is the most common form of neurodegenerative disorder with dementia. In its sporadic form, AD results from the combination of genetic factors with different epigenetic events. Among them, oxidative metabolic reactions and their by-products have been consistently implicated in AD pathogenesis and represent the biological basis for the 'oxidative stress hypothesis' of AD. Numerous studies demonstrate that different biomarkers of oxidative-stress-mediated events are elevated in the AD brain. Studies in animal models of the disease with antioxidants report significant improvements of their AD-like phenotype. Although epidemiologic studies show that dietary intake of antioxidants reduces the risk of AD, clinical trials with antioxidants show only a marginal positive or no effect. These conflicting results have created a wave of criticism towards the oxidative stress hypothesis of AD. Here, I review the available data and discuss the necessary paths for a fair reappraisal of the hypothesis.

Introduction

As people worldwide live to an older age, dementia, of which the best-known risk factor is aging, has become a serious growing public-health problem. Alzheimer's disease (AD) is the most frequent form of neurodegenerative disorder associated with dementia in the elderly. Approximately 5% of AD is caused by missense mutations in the gene for either the Alzheimer amyloid β (Aβ) precursor protein (APP) or some of the enzymes involved in its metabolism (i.e. presenilin 1 and 2) [1]. However, the cause(s) of sporadic AD remains unclear, although it has long been proposed that abnormal metabolic oxidative reactions in the central nervous system (CNS) might have a pathological role. Thus, besides the pathological hallmarks of the disease, which include the accumulation of protein deposits in the brain as Aβ plaques and as neurofibrillary tangles (NFT), AD brains exhibit constant evidence of reactive oxygen species (ROS)- and reactive nitrogen species (RNS)-mediated injury [2] (Box 1). These reactive species are formed during normal metabolic reactions, are usually instable from a chemical point of view and are highly reactive (for this reason their levels are kept low by efficient antioxidant systems). The term antioxidant typically refers to a large and heterogeneous group of compounds that function by preventing the formation, detoxifying or scavenging of oxidant species. Antioxidants can be grouped under different criteria, such as vitamins (e.g. α-tocopherol, ascorbic acid and β-carotene), synthetic (e.g. butylated hydroxytoluene), natural (e.g. plant-derived polyphenols) and inorganic (e.g. selenium). Some antioxidants act as chain-breaking molecules because they prevent the propagation of or stop radical chain reactions (i.e. α-tocopherol). By contrast, other antioxidants such as glutathione peroxidase and catalase detoxify hydrogen peroxide (H_2O_2). This chemical reaction is crucial in cell biology because H_2O_2 in the presence of transition metals, such as Fe^{2+}, generate hydroxyl radicals ($OH^{\bullet}$) for which there is no known detoxifying system.

In some circumstances the production of oxidant species can exceed the endogenous antioxidant ability to destroy them and an oxidative imbalance occurs. This event results in cellular oxidative stress and subsequent molecular oxidative damage, which can translate into altered cellular functions and, as final result, cell death [3] (Box 2).

The CNS is very prone to oxidative imbalance because it is very rich in polyunsaturated fatty acids (PUFAs), has a high metabolic oxidative rate and has a high content of transition metals and ascorbate levels, which together act as potent pro-oxidants; but, by contrast, it possesses a relative paucity of antioxidant systems compared with other organs.

The source of oxidant species in the CNS includes altered mitochondrial function, the Aβ peptides themselves and the presence of unbound transition metals [4] (Box 2). All of these factors are not independent from each other, and it is plausible that, especially in the early stages of the disease process, Aβ could enter the mitochondria where it would increase the generation of ROS and induce oxidative stress. Interestingly, recent AD-brain post-mortem and AD transgenic-mice studies show that Aβ and APP can be found in mitochondrial membranes where they can block transport of protein and disrupt the electron-transport chain with final, irreversible cell damage [4]. Depending on the substrate attacked by ROS, oxidative stress will manifest as protein, DNA, RNA oxidation or lipid peroxidation. All of these signature markers of oxidative stress have been described in the AD brain, and this concept has been originally used to support the 'oxidative stress hypothesis' of AD [5–7] (Box 3). A simplistic interpretation of this hypothesis is that antioxidant therapies should be beneficial in AD treatment. However, conflicting data regarding this aspect have generated a wave of criticism toward the validity of the hypothesis. Considering that it is now 11 years since it was originally formulated, its reappraisal seems timely and appropriate to reach a fair conclusion on the 'case'.

Corresponding author: Praticò, D. (praticod@temple.edu).

0165-6147/$ – see front matter doi:10.1016/j.tips.2008.09.001 Available online 4 October 2008

Box 1. Molecular mechanism(s) and pathology in AD

Genetic mutations of the amyloid β (Aβ) precursor protein (APP) or presenilin-1 (PS1) increase the rate of Aβ formation. Oxidative stress can influence Aβ formation by increasing APP levels or, indirectly, Aβ processing by modulating the activity and levels of key enzymes such as β-secretase (BACE) and γ-secretase. Aβ itself has oxidant ability and, by inducing more oxidative stress, creates positive feedback on APP levels and on its proteoltyic pathway. The elevated levels of Aβ oligomers favor the phosphorylation of tau protein. With time, Aβ oligomers deposit in the extracellular space forming senile plaques (SPs), whereas inside neurons the hyperphosphorylated tau form neurofibrillary tangles (NFTs). Both lesions trigger further oxidative stress reactions and sustained inflammatory responses, which ultimately will translate into irreversible cell damage, slow degeneration and eventual cell death. These cell-biologic events will clinically manifest with progressive cognitive decline, early signs of dementia and, finally, full clinical AD (Figure I).

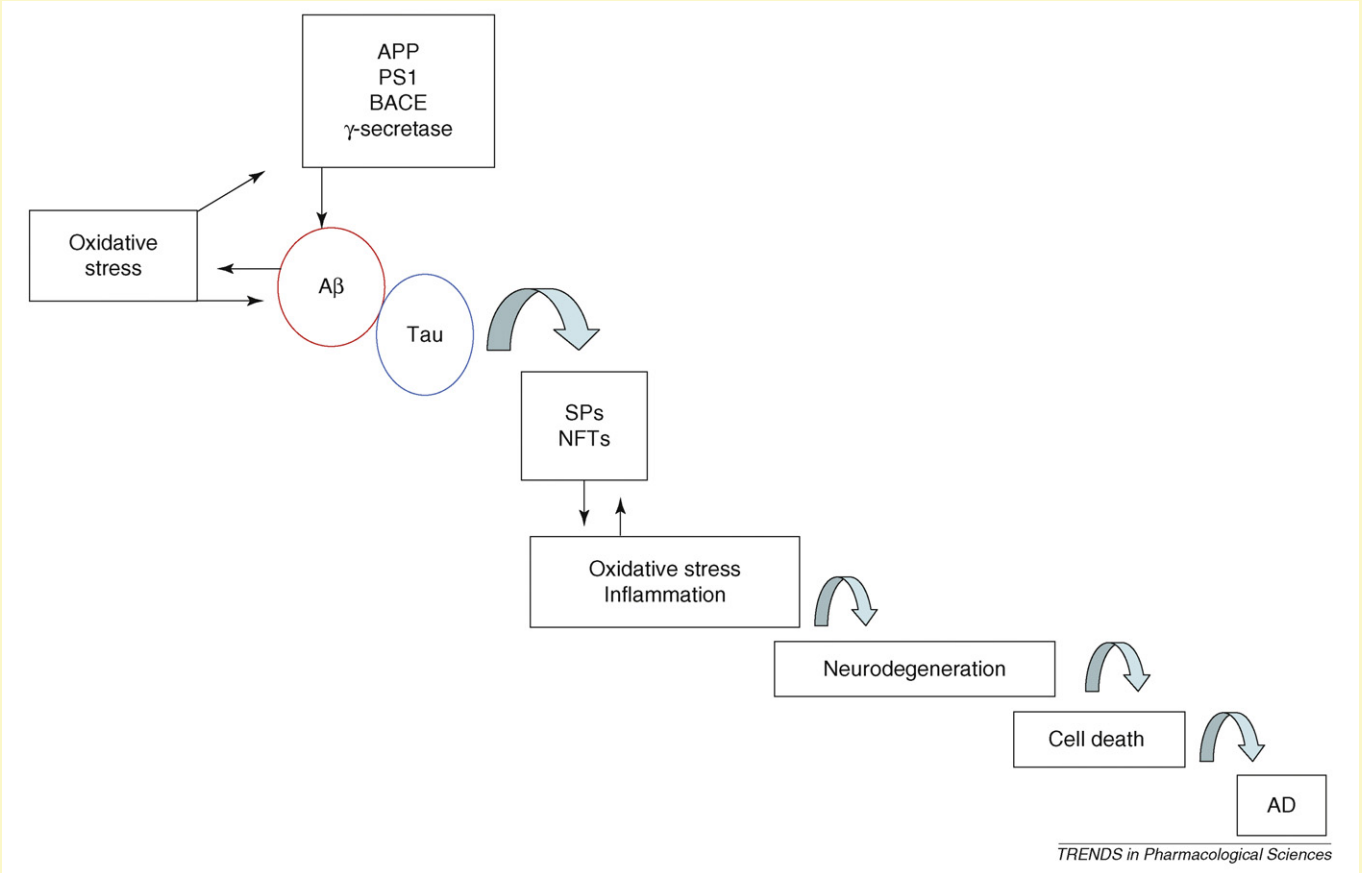

Figure I. Molecular mechanism(s) and pathology in AD.

With this concept in mind, first, the evidence for the presence of oxidative stress biomarkers in the CNS of human AD and animal models of AD-like pathology is presented. Second, the results of epidemiological and interventional studies in AD are summarized, and how they fit with the oxidative stress hypothesis of AD is explained. In the final part, both promises and caveats of these available data are weighted and future directions to be taken for addressing them are proposed.

Post-mortem human studies

Consistent data show that lipid peroxidation, the mechanism by which lipids are attacked by ROS, is increased in the brain of AD subjects compared with controls. Several biomarkers of this phenomenon have been used (Box 3), among them malondyaldehyde and 4-hydroxynonenal are increased in multiple brain regions and in cerebrospinal fluid (CSF) of AD subjects [8–11]. F_2-isoprostanes, prostaglandin-like compounds also formed by ROS-induced PUFA oxidation, are elevated in AD brain regions rich in the typical lesions such as frontal and temporal cortex and hippocampus but not in the cerebellum, an area typically without senile plaques or NFT [12,13]. A similar increase is seen in post-mortem ventricular CSF and lumbar CSF from AD and mild cognitive-impairment (MCI) patients compared with age-matched controls [12–14], and in brain tissues from MCI patients [15,16].

ROS-oxidized proteins such as carbonyls are increased in the frontal and parietal lobes and in the hippocampus of AD and MCI subjects [17–20]. Levels of 3-nitrotyrosine, another marker of protein oxidation (Box 3), are elevated in brains from MCI subjects compared with controls [21], who also manifest oxidative modification of several specific protein enzymes, including α-enolase and glutamine synthase [22].

ROS can attack nucleic acids causing oxidative modifications of purine and pyridine bases. Among them, the most popular base assayed is hydroxylated guanine, measured as the base 8-hydroxy-2-deoxyguanosine, which is increased in AD and MCI brain samples when compared

Box 2. *In vivo* sources of oxidant species

Oxidant species, which include reactive oxygen species (ROS) and reactive nitrogen species (RNS), can be formed outside the cell by UV irradiation, ozone, pollutants and cigarette smoke. Their formation can also be facilitated by genetic mutations. Membrane-linked sources of oxidant species include the NAD(P)H oxidase (NOx) and the cytochrome P450. Mitochondrial sources include the electron-transport chain (ETC) and the monoamino oxidase (MAO). Once the superoxide anion ($O_2^{\bullet -}$) is formed it is readily converted by manganese superoxide dismutase (MnSOD) to hydrogen peroxide (H_2O_2), which easily crosses the mitochondrial membrane. Cytosolic $O_2^{\bullet -}$ can also be converted by copper/zinc SOD to H_2O_2. Other cytosolic sources of oxidant species include xantine oxidase (XO), P450 reductases and nitric oxide synthase (NOS). The latter catalyzes the synthesis of nitric oxide ($NO^{\bullet}$), which can then react with $O_2^{\bullet -}$ to generate the potent pro-oxidant peroxynitrite ($ONOO^-$). Myeloperoxidase (MPO) uses H_2O_2 to make hypohalous acids (HOx). Finally, H_2O_2 can undergo Fenton reaction chemistry in the presence of metals such as iron (Fe^{2+}) to generate hydroxyl radical ($OH^{\bullet}$). Other sources not represented in the diagram are two main enzymes: the lipoxygenases and cylcoxygenases, which can oxidize membrane phospholipids generating potent biologically active oxidized lipids (i.e. prostaglandins and isoprostanes).

As a consequence of oxidative and/or nitrative stress, some proteins, lipids and nucleic acids are irreversibly modified, with subsequent reduction in the cell endogenous-antioxidant capacity (glutathione levels), inhibition of mitochondrial respiration and decline in ATP levels. Other consequences are Ca^{2+} dyshomeostasis (with further alteration in mitochondrial function), membrane damage and protein aggregation (with subsequent formation of insoluble aggregates that slow proteasome function). With time, these phenomena, if not properly counteracted, can cause progressive cell damage, degeneration and eventual cell death (Figure I).

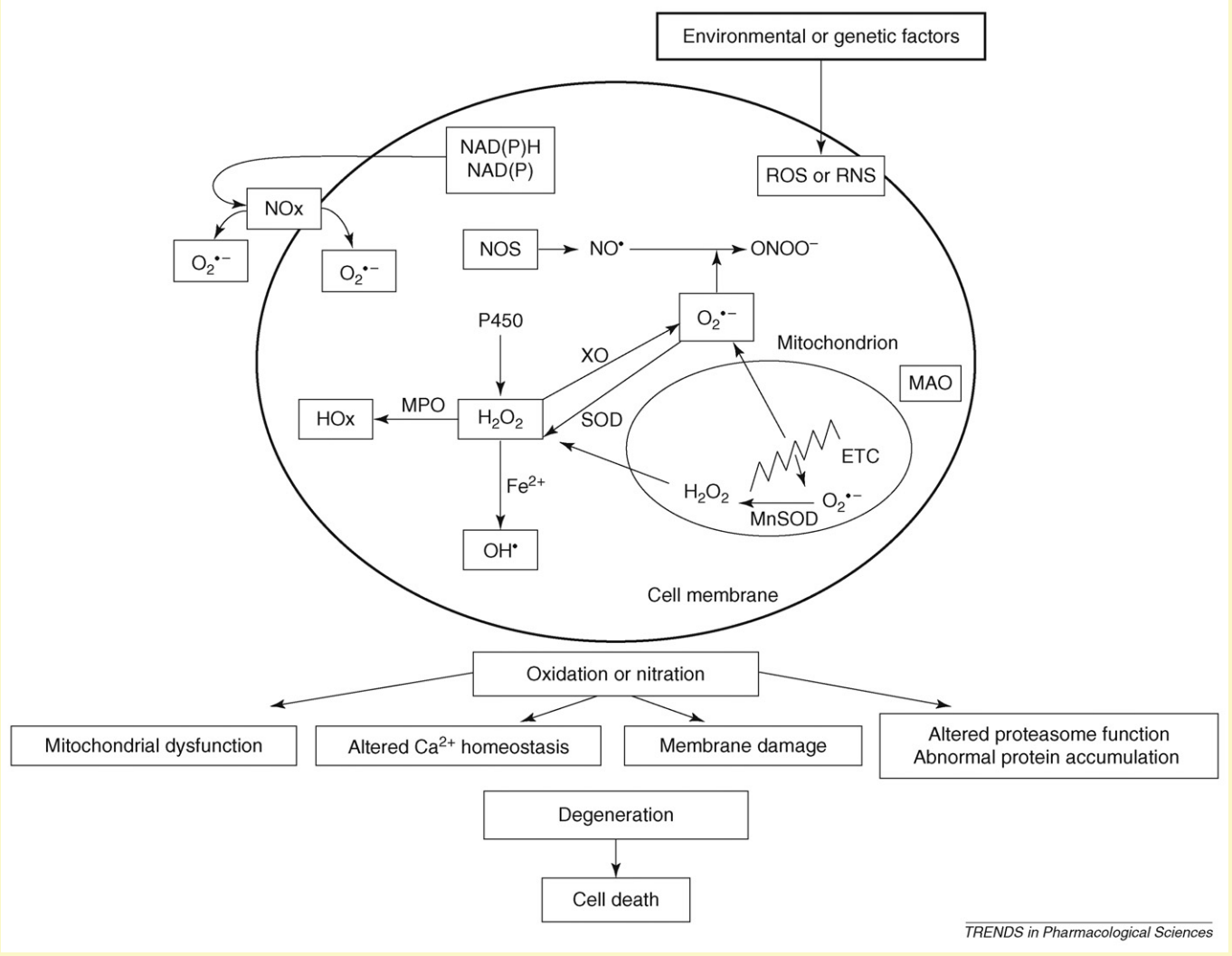

Figure I. *In vivo* sources of oxidant species.

with matched controls [23,24]. High levels of 8-hydroxyguanine (a marker of RNA oxidation) have also been reported in early AD and MCI patients [25,26].

Animal studies

Since the early nineties several animal models reproducing some of the AD pathologic hallmarks have been developed and are available to the scientific community. This fact enabled, for the first time, the opportunity to test the oxidative stress hypothesis of AD in an *in vivo* biologic system. Interestingly, most of the human data described so far have been confirmed in several experimental studies using some of these transgenic animal models. Thus, the Tg2576 mouse model of AD-like amyloidosis manifests

Box 3. Biomarkers of oxidative stress in AD brains

Lipid peroxidation

- Thiobarbituric-acid-reactive substances
- Malondyaldehyde
- 4-Hydroxy-2-nonenal
- Acrolein
- Isoprostanes
- Neuroprostanes

Protein oxidation

- Protein carbonyls
- Nitrotyrosine

DNA oxidation

- 8-hydroxy-2-deoxyguanosine
- 8-hydroxyguanosine

RNA oxidation

- 8-hydroxyguanine

evidence of brain lipid peroxidation [27], which is increased at a young age before the surge in Aβ levels and deposition [28,29]. In the same model there is a coincidental increase in nitrogen reactive species and antioxidant enzymes with the onset of Aβ deposition [30]. Similarly, the APP/PS1 double knock-in mice manifest features of lipid and protein oxidation at early stages of their phenotype [31–33]. Experimental thiamin deficiency, an established model of altered oxidative metabolism, exacerbates amyloid pathology in the Tg19959 mice by inducing oxidative stress [34]. Dietary copper, by stabilizing brain superoxide dismutase (SOD) activity, reduces Aβ production in the APP23 transgenic mice [35,36]. By contrast, dietary aluminum modulates brain amyloidosis by increasing oxidative stress in the Tg2576 mice [37].

A recent report shows that, in a triple transgenic mouse model (3xTg-AD), which develops plaques and NFTs, levels of antioxidants are decreased and, by contrast, lipid peroxidation is increased before the appearance of any AD-like pathology [38].

APP transgenic mice crossed with manganese SOD-heterozygous-deficient mice show increased brain lipid peroxidation, which associates with a significant increase in Aβ levels and plaque deposition [39]. By contrast, mice deficient for the α-tocopherol (vitamin E) transfer protein when crossed with the Tg2576 manifest earlier and more severe cognitive dysfunction and have more Aβ brain deposits, which are ameliorated by vitamin E supplementation [40]. Deletion of the prostaglandin E2 receptor (EP2) in the double mutant APP/PS1 mice results in less brain lipid peroxidation and a significant decrease in Aβ levels and deposition [41]. We have recently shown that pharmacological inhibition of the thromboxane receptor, TP, significantly reduces amyloid pathology in the Tg2576 mice by modulating oxidative stress [42].

Several studies using antioxidants have also been published in these AD models, all of which show a consistent beneficial effect towards their behavioral and amyloidotic phenotype. The curry spice curcumin, a well-known antioxidant and anti-inflammatory compound, significantly reduces oxidative stress and amyloid pathology in the Tg2576 [43]. Early vitamin E supplementation in the same model significantly reduces Aβ levels and deposition [44]. The same therapeutic regimen prevents the surge in amyloidosis and improves cognitive function after experimental traumatic brain injury, a known risk factor for developing AD [45]. Finally, melatonin, a drug with antioxidant properties, partially inhibits the expected time-dependent elevation in Aβ levels, reduces the abnormal nitration of proteins and increases survival in the Tg2576 mice [46].

Antioxidants and AD

Five cross-sectional studies have been reported so far. The majority of them (4 out of 5) show a direct relationship between antioxidant intake and better cognitive functions (Table 1). One study first reported an association between antioxidant use and a better performance on several cognitive tests. However, after adjusting the data for age, education and sex there was no significant difference in test performance between antioxidant users and nonusers [47]. Another study of >4000 elderly patients shows that decreased circulating levels of vitamin E, but not vitamin A, vitamin C, β-carotene and selenium, are consistently associated with decreasing memory levels [48]. The Honolulu-Asia Aging Study [49] reports that supplementary intake of vitamin E or C are both associated with a better cognitive performance, and the Chicago Health and Aging Project [50] reports that supplementary or dietary intake of vitamin E, but not vitamin C, is inversely correlated with cognitive decline. Finally, the Nurses' Health Study [51], which includes almost 15 000 women aged 70–79 years, shows that a long-term use of vitamin C and vitamin E supplements results in a better cognitive status.

By contrast with the cross-sectional studies, the prospective studies published so far present us with a more complex picture (Table 2). In one study, which followed a large sample size of individuals (>5000) for a mean of 6.0 years, dietary intake of vitamin E associates with a lower risk of developing AD [52]. By examining Canadians aged >65 years, a recent study concluded that antioxidant vitamin supplement affords a possible protective effect

Table 1. Cross-sectional studies of antioxidants and cognitive function

Study	Subjects	Findings	Refs
MoVIES[a] Project	$n = 1059$	Use of antioxidant supplements does not associate with a better cognitive performance	[47]
NHANES III	$n = 4809$	Lower levels of vitamin E associated with poor memory performance	[48]
Honolulu-Asia Aging Study	$n = 3385$	Supplementary intake of vitamin E or C associated with better cognitive functions	[49]
Chicago Health Aging Study	$n = 2889$	Vitamin E intake inversely correlated to cognitive decline	[50]
Nurse's Health Study	$n = 14\,968$	Supplementation with vitamin E and vitamin C associated with better cognitive function	[51]

[a]Monongahela Valley Independent Elders Survey.

Table 2. Prospective studies of antioxidants and risk of AD

Study	Follow-up	Findings	Refs
Rotterdam Study	6 years	Dietary vitamin E effective (more among current smokers)	[52]
Chicago Health Aging Study	3.9 years	Dietary vitamin E was effective only among non-apoE4 carrier	[54]
Washington Heights-Inwood Columbia Aging Project	4 years	No effect of vitamin E (diet or supplement)	[55]
Cache County Study	3 years	Vitamin E alone was not effective, but combined with vitamin C was effective	[56]
Honolulu-Asia Aging Study	30 years	Dietary vitamin E was not effective	[57]
Canadian Study of Health and Aging	5 years	Combination of vitamin E and vitamin C supplements and/or multivitamin consumption effective	[53]
Duke Established Populations for Epidemiologic Studies of the Elderly	10 years	No effect of vitamin C and/or vitamin E	[58]
Group Health Cooperative	5.5 years	No effect of supplemental vitamin E and vitamin C alone or in combination	[59]

Table 3. Controlled, randomized clinical trials with antioxidants

Study	Subjects	Findings	Refs
AD Cooperative Study	AD	Vitamin E delayed the time to institutionalization	[60]
AD Cooperative Study	MCI	No benefit of vitamin E on progression to AD	[61]
Age-Related Eye Disease Study	Elderly	No effects of a mix of antioxidants (vitamin C, vitamin E and β-carotene) on cognitive function	[63]
Women's Health Study	Elderly	No benefit of vitamin E on cognitive function	[64]

for cognitive decline [53]. Another report shows that dietary and not supplement-derived vitamin E intake associates with a lower risk for AD only in individuals who are not carrier of apolipoprotein E4 (apoE4) [54]. By contrast, two studies demonstrate no association between dietary or supplementary vitamin E intake alone and a decrease in the risk for AD [55,56]. Finally, in the Honolulu-Asia Aging Study [57], dietary intake of vitamin C, vitamin E, β-carotene or flavonoids does not associate with a reduced risk of late-life dementia. More recently, two other studies reported that the use of supplemental vitamin E and/or vitamin C did not delay the incidence of dementia or AD onset [58,59].

Clinical trials

High-dose vitamin E (2000 international units day^{-1}) was used in a double-blind, placebo-controlled, 2-year, randomized multicenter trial in AD patients. In this trial, although vitamin E did not influence the rate of decline of cognitive functions, it significantly delayed the time to institutionalization, loss of ability to perform basic activities and severe dementia [60]. Similar results were obtained when the selective monoamine oxidase B inhibitor selegiline, or a combination or vitamin E and selegiline, were administered [60] (Table 3). By contrast, the same dose of vitamin E had no benefit in subjects with a clinical diagnosis of MCI in a double-blind, 3-year follow-up study. Thus, the subjects receiving the antioxidant did not show any difference with placebo in preventing the progression from MCI to AD [61] (Table 3). However, in a follow-up publication the same authors show that, in a subgroup of these patients, the magnetic resonance imaging annualized-percentage changes of the volumes for some areas of the brain (hippocampus and entorhinal cortex) are less evident in the vitamin E than in the placebo group [62].

There are two other published negative randomized clinical trials of antioxidants and age-related cognitive decline [63,64]. All of these reports are ancillary studies to larger randomized controlled trials with an aging-related primary end-point. Although they do not directly investigate AD or MCI patients, they are important pieces of information because they inform us of the effects of antioxidants in preventing cognitive decline, a recognized prelude to MCI and then AD (Table 3).

Summary

Abundant human data consistently support the idea that oxidative stress occurs and is a constant feature of the AD brain pathology. Some recent evidence even indicates that this phenomenon is an early event and might have a pathological role in the pathogenesis of this disease. Experimental data from animal models of AD confirm the presence of oxidative stress during the early development of their phenotype. Genetic, dietary and pharmacologic approaches in these models also support the concept that oxidative stress has a contributing role to the pathogenesis of the disease. Human observational epidemiology studies are, in general, consistent with the hypothesis that there is an inverse relationship between antioxidant levels and intake, cognitive function and ultimately the risk of developing AD. However, randomized clinical trials with antioxidants do not fulfill the promises of those studies.

Does the latter observation mean that the oxidative stress hypothesis of AD is not valid anymore? Does it mean that oxidative stress does not play a functional part and is a simple secondary event in the pathogenesis of this complex disease? Based on the current knowledge, we do not have enough information to clearly answer those important questions.

There is no doubt that the conflicting data between the animal and the human studies, and within the human data *per se*, call for a rethinking of the oxidative stress hypothesis of AD. The most simplistic interpretation of the hypothesis would have predicted that, because oxidative stress is increased and has a pathological role in AD, antioxidants should be beneficial. Unfortunately, the scenario is not so simple and we need to critically look at the evidence we have.

If we look at the negative clinical trials it is curious that they all lack some important and necessary information

when an antioxidant therapy is administered, such as drug-level monitoring and/or a surrogate marker for an *in vivo* therapeutic effect of the drug of interest. In other words, we still do not know whether, in these studies, the subjects receiving the antioxidant(s) have circulating therapeutic levels of the substances received and, most importantly, whether the antioxidant(s) significantly reduces the *in vivo* oxidative stress levels, which is characteristic of the disease evolution.

Furthermore, the human studies with antioxidants have used different preparations of them (natural versus synthetic), a wide range of dosages and for a variable length of time. Taken together, these aspects make a comparison of the obtained results very difficult, if not impossible.

For all these reasons, the negative trials should be considered inconclusive and these examples unbalanced, and in consequence should not be considered to rule out the oxidative stress hypothesis of AD.

Conclusions: the road ahead

Here are summarized some basic aspects of oxidative biology, the knowledge of which is very important especially when we are dealing with a complex biologic system and multifactorial disease such as AD. They all need to be taken into account and addressed before ruling out or accepting the hypothesis and, most importantly, should serve as a guide for the future.

(i) It is possible that long-term exposure to antioxidants will be necessary to have an effect on the underlying pathologic process linked to changes in cognition, which takes decades (timing of the treatment).

(ii) Considering the complexity of the redox system *in vivo*, we will probably need better and more selective antioxidants. We will need molecules that can easily be delivered to neuronal cells and that will target specific sources of ROS, such as mitochondria. To this end, an exciting new research area is emerging aimed at developing mitochondria-targeted antioxidant therapies.

(iii) Before starting any antioxidant-therapy trial it will be extremely important to have information on the endogenous antioxidant levels of the participating subjects to identify 'responders' (subjects with low antioxidants) versus 'non-responders' (subjects with high or normal antioxidant levels) to a drug with antioxidant properties.

(iv) It will be imperative to monitor therapy compliance of the subjects enrolled to receive any drug with antioxidant activity. This goal can be reached only if we monitor not only the drug levels but also, most importantly, the *in vivo* antioxidant effect by measuring, before starting and during the treatment, surrogate markers of oxidative stress in each subject. These aspects will help in assessing and critically choose the best and most effective therapeutic dosage for each antioxidant selected.

(v) Because some antioxidants might also act as oxidants when oxidized, the use of a single molecule, like in two large randomized clinical trials [60,61], could not be sufficient to reach the best therapeutic effect. For instance, it is known that vitamin C is necessary to recycle the vitamin E radical (oxidized) to the reduced (antioxidant) form.

In conclusion, the oxidative stress hypothesis of AD is still very much alive and viable, but a great deal of work needs to be done to design future studies and appropriate clinical trials that will conclusively establish the role of oxidative stress in AD pathogenesis.

Acknowledgements

I regret that, owing to space limitation, I am unable to cite several important contributions. The work from my laboratory described in the article has been supported by the American Federation for Aging Research (www.afar.org), the Alzheimer's Association (www.alz.org) and the National Institute of Health (www.nih.gov).

References

1 Selkoe, D.J. (2007) Developing preventive therapies for chronic diseases: lessons learned from Alzheimer's disease. *Nutr. Rev.* 65 (Suppl. 64), S239–S243
2 Praticò, D. and Sung, S. (2004) Lipid peroxidation and oxidative imbalance: early functional event in Alzheimer's disease. *J. Alzheimers Dis.* 6, 171–175
3 Praticò, D. (2000) *In vivo* measurements of oxidative stress. *Lipids* 136 (Suppl. 36), S45–S47
4 Reddy, P.H. and Beal, M.F. (2008) Amyloid β, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. *Trends Mol. Med.* 14, 45–53
5 Markesbery, W.R. (1997) Oxidative stress hypothesis in Alzheimer's disease. *Free Radic. Biol. Med.* 23, 134–147
6 Butterfield, D.A. (1997) β-amyloid-associated free radical oxidative stress and neurotoxicity: implications for Alzheimer's disease. *Chem. Res. Toxicol.* 10, 495–506
7 Smith, M.A. *et al.* (2000) Oxidative stress in Alzheimer's disease. *Biochim. Biophys. Acta* 1502, 139–144
8 Lovell, M.A. *et al.* (1995) Elevated thiobarbituric acid-reactive substances and antioxidant enzyme activity in the brain in Alzheimer's disease. *Neurology* 45, 1594–1601
9 Subbarao, K.V. *et al.* (1990) Autopsy samples of Alzheimer's cortex show increased peroxidation *in vivo*. *J. Neurochem.* 55, 342–345
10 Markesbery, W.R. and Carney, J.M. (1999) Oxidative alterations in Alzheimer's disease. *Brain Pathol.* 9, 133–146
11 Lauderdack, C.M. *et al.* (2001) The glial glutamate transporter, GLT-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: the role of Aβ1-42. *J. Neurochem.* 78, 413–416
12 Montine, T.J. *et al.* (1998) Cerebrospinal fluid F_2-isoprostane levels are increased in Alzheimer's disease. *Ann. Neurol.* 44, 410–413
13 Praticò, D. *et al.* (1998) Increased F_2-isoprostanes in Alzheimer's disease: evidence of enhanced lipid peroxidation *in vivo*. *FASEB J.* 12, 1777–1783
14 Praticò, D. *et al.* (2002) Increase of brain oxidative stress in mild cognitive impairment: a possible predictor of Alzheimer's disease. *Arch. Neurol.* 59, 972–976
15 Keller, J.N. *et al.* (2005) Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology* 64, 1152–1156
16 Markesbery, W.R. *et al.* (2005) Lipid peroxidation is an early event in the brain in amnestic mild cognitive impairment. *Ann. Neurol.* 58, 730–735
17 Smith, C.D. *et al.* (1991) Excess brain protein oxidation and enzyme dysfunction in normal aging and Alzheimer disease. *Proc. Natl. Acad. Sci. U. S. A.* 88, 10540–10543
18 Hensley, K. *et al.* (1995) Brain regional correspondence between Alzheimer's disease histopathology and biomarkers of protein oxidation. *J. Neurochem.* 65, 2146–2156
19 Smith, M.A. *et al.* (1997) Iron accumulation in Alzheimer disease is a source of redox-generated free radicals. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9866–9868
20 Butterfield, D.A. *et al.* (2006) Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive

impairment: insights into the development of Alzheimer's disease. *Neurobiol. Dis.* 22, 223–232
21 Butterfield, D.A. *et al.* (2007) Elevated levels of 3-nitrotyrosine in brain from subjects with amnestic mild cognitive impairment: implications for the role of nitration in the progression of Alzheimer's disease. *Brain Res.* 1148, 243–248
22 Sultana, R. *et al.* (2007) Proteomic identification of nitrated brain proteins in amnestic mild cognitive impairment: a regional study. *J. Cell. Mol. Med.* 11, 839–851
23 Mecocci, P. *et al.* (1994) Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann. Neurol.* 36, 747–751
24 Wang, J. *et al.* (2006) Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. *J. Neurochem.* 96, 825–832
25 Nunomura, A. *et al.* (2001) Oxidative damage is the earliest event in Alzheimer disease. *J. Neuropathol. Exp. Neurol.* 60, 759–767
26 Ding, Q. *et al.* (2005) Ribosome dysfunction is an early event in Alzheimer's disease. *J. Neurosci.* 25, 9171–9175
27 Smith, M.A. *et al.* (1998) Amyloid-β deposition in Alzheimer' transgenic mice is associated with oxidative stress. *J. Neurochem.* 70, 2212–2215
28 Praticò, D. *et al.* (2001) Increased lipid peroxidation precedes amyloid plaque formation in an animal model of Alzheimer amyloidosis. *J. Neurosci.* 21, 4183–4187
29 Feng, Z. *et al.* (2006) Early melatonin supplementation alleviates oxidative stress in a transgenic mouse model of Alzheimer's disease. *Free Radic. Biol. Med.* 40, 101–109
30 Apelt, J. *et al.* (2004) Aging-related increase in oxidative stress correlates with developmental pattern of β-secretase activity and β-amyloid plaque formation in transgenic Tg2576 mice with Alzheimer-like pathology. *Int. J. Dev. Neurosci.* 22, 475–484
31 Matsuoka, Y. *et al.* (2001) Fibrillar β-amyloid evokes oxidative damage in a transgenic mouse model of Alzheimer's disease. *Neuroscience* 104, 609–613
32 Anantharaman, M. *et al.* (2006) β-amyloid mediated nitration of manganese superoxide dismutase. Implication for oxidative stress in a APP $^{NLh/NLh}$ x PS1 $^{P264L/P264L}$ double knock-in mouse model of Alzheimer's disease. *Am. J. Pathol.* 168, 1608–1618
33 Mohmmad Abdul, H. *et al.* (2006) Mutations in amyloid precursor protein and presenilin-1 genes increase the basal oxidative stress in murine cells and lead to increased sensitivity to oxidative stress mediated by amyloid β-peptide (1-42), H_2O_2 and kainic acid: implications for Alzheimer's disease. *J. Neurochem.* 96, 1322–1335
34 Karuppagounder, S.S. *et al.* (2008) Thiamine deficiency induces oxidative stress and exacerbates the plaque pathology n Alzheimer's mouse model. *Neurobiol. Aging* DOI: 10.1016/j.neurobiolaging.2007.12.013 (www.sciencedirect.com)
35 Schuessel, K. *et al.* (2005) Impaired Cu/Zn-SOD activity contributes to increased oxidative damage in APP transgenic mice. *Neurobiol. Dis.* 18, 89–99
36 Bayer, T.A. *et al.* (2003) Dietary Cu stabilizes brain superoxide dismutase 1 activity and reduces amyloid Aβ production in APP23 transgenic mice. *Proc. Natl. Acad. Sci. U. S. A.* 100, 14187–14192
37 Praticó, D. *et al.* (2002) Aluminum modulates brain amyloidosis through oxidative stress in APP transgenic mice. *FASEB J.* 16, 1138–1140
38 Resende, R. *et al.* (2008) Brain oxidative stress in a triple transgenic mouse model of Alzheimer's disease. *Free Radic. Biol. Med.* 44, 2051–2057
39 Li, F. *et al.* (2004) Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice. *J. Neurochem.* 89, 1308–1312
40 Nishida, Y. *et al.* (2006) Deletion of vitamin E enhances phenotype of Alzheimer disease mouse model. *Biochem. Biophys. Res. Commun.* 350, 530–536
41 Liang, X. *et al.* (2005) Deletion of the prostaglandin E_2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. *J. Neurosci.* 25, 10180–10187
42 Shineman, D.W. *et al.* (2008) Thromboxane receptor activation mediates isoprostane-induced increases in amyloid pathology in Tg2576 mice. *J. Neurosci.* 28, 4785–4794
43 Lim, G.P. *et al.* (2001) The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer' transgenic mouse. *J. Neurosci.* 21, 8370–8377
44 Sung, S. *et al.* (2004) Early vitamin E supplementation in young but not aged mice reduces Aβ levels and amyloid deposition in a transgenic model of Alzheimers' disease. *FASEB J.* 18, 323–325
45 Conte, V. *et al.* (2004) Vitamin E reduces amyloidosis and improves cognitive function in Tg2576 mice following repetitive concussive brain injury. *J. Neurochem.* 90, 758–764
46 Matsubara, E. *et al.* (2003) Melatonin increases survival and inhibits oxidative and amyloid pathology in a transgenic model of Alzheimer's disease. *J. Neurochem.* 85, 1101–1108
47 Mendelsohn, A.B. *et al.* (1998) Use of antioxidant supplements and its association with cognitive function in a rural elderly cohort. *Am. J. Epidemiol.* 148, 38–44
48 Perkins, A.J. *et al.* (1999) Association of antioxidants with memory in a multiethnic elderly sample using the Third National Health and Nutrition Examination Survey. *Am. J. Epidemiol.* 150, 37–44
49 Masaki, K.H. *et al.* (2000) Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. *Neurology* 54, 1265–1272
50 Morris, M.C. *et al.* (2002) Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. *JAMA* 287, 3230–3237
51 Grodstein, F. *et al.* (2003) High-dose antioxidant supplements and cognitive function in community-dwelling elderly women. *Am. J. Clin. Nutr.* 77, 975–984
52 Engelhart, M.J. *et al.* (2002) Dietary intake of antioxidants and risk of Alzheimer disease. *JAMA* 287, 3223–3229
53 Maxwell, C.J. *et al.* (2005) Supplemental use of antioxidant vitamins and subsequent risk of cognitive decline and dementia. *Dement. Geriatr. Cogn. Disord.* 20, 45–51
54 Morris, M.C. *et al.* (2002) Vitamin E and cognitive decline in older persons. *Arch. Neurol.* 59, 1125–1132
55 Luchsinger, J.A. *et al.* (2003) Antioxidant vitamin intake and risk of Alzheimer disease. *Arch. Neurol.* 60, 203–208
56 Zandi, P.P. *et al.* (2004) Reduced risk of Alzheimer disease in users of antioxidants vitamin supplements: The Cache County Study. *Arch. Neurol.* 61, 82–88
57 Laurin, D. *et al.* (2004) Midlife dietary intake of antioxidants and risk of late-life incident dementia: The Honolulu-Asia Aging Study. *Am. J. Epidemiol.* 159, 959–967
58 Fillenbaum, G.G. *et al.* (2005) Dementia and Alzheimer's disease in community-dwelling elders taking vitamin C and/or vitamin E. *Ann. Pharmacother.* 39, 2009–2014
59 Gray, S.L. *et al.* (2008) Antioxidant vitamin supplement use and risk of dementia or Alzheimer's disease in older adults. *J. Am. Geriatr. Soc.* 56, 291–295
60 Sano, M. *et al.* (1997) A controlled trial of seligiline, α-tocopherol, or both as treatment for Alzheimer's disease. *N. Engl. J. Med.* 336, 1216–1222
61 Petersen, R.C. *et al.* (2005) Vitamin E and donezepil for the treatment of mild cognitive impairment. *N. Engl. J. Med.* 352, 2379–2388
62 Jack, C.R., Jr *et al.* (2007) Longitudinal MRI findings from the vitamin E and donezepil treatment study for MCI. *Neurobiol. Aging* 29, 1285–1295
63 Yaffe, K. *et al.* (2004) Impact of antioxidants, zinc, and copper on cognition in the elderly. *Neurology* 63, 1705–1707
64 Kang, J.H. *et al.* (2006) A randomized trial of vitamin E supplementation and cognitive function in women. *Arch. Intern. Med.* 166, 2462–2468