

Is antioxidant therapy effective to treat alzheimer's disease?

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative process associated with oxidative stress. In the past, it was claimed that all neuronal lesions involved in the onset and progression of AD were related to oxidative stress.

Today, we know that intracellular amyloid beta (Ab) could play a central role in the pathophysiology of the disease. Ab binds to heme groups in mitochondrial membranes causing electron transport chain impairment and loss of respiratory function. The experimental evidence of such oxidative stress leads to the basis for treatment of AD with antioxidants. Many clinical trials have been developed to clarify whether antioxidants are beneficial in AD treatment. However, the results obtained in no way confirm that antioxidants are an effective AD therapy. More research is necessary to clarify this point.

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DOI: 10.5530/ax.2011.4.3

OXIDATIVE STRESS AND ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a neurodegenerative disorder whose etiology is currently unknown. Many theories have been postulated attempting to explain the genesis and development of the disease. One of these theories with more experimental evidences is oxidative stress^[1,2]. Oxidative stress was defined by Sies in 1986, as "The production of reactive oxygen species in excess of antioxidant mechanisms"^[3]. A more modern definition is: "Altered homeostatic balance resulting from oxidant insult"^[4]. Oxidative modifications have been observed in all biomacromolecules in susceptible neurons in AD brains:

1. Lipids: lipid peroxidation is higher in pathological brains than in age-matched controls. Lipid oxidation has been revealed using different markers, such as thiobarbituric acid reactive substances (TBARS), malondialdehyde (MDA), 4-hydroxy-2-transnonyl (HNE)^[5] and isoprostane^[6].
2. Sugars: An increased glycation and glycooxidation in carbohydrates has been demonstrated in AD^[2,7-9].
3. Nucleic acids: oxidative markers can be found in both DNA (8-hydroxyl-2'-deoxyguanosine) and

RNA (8-hydroxyguanosine) molecules. RNA oxidation could cause faster degradation and poor protein translation^[10]. Mitochondrial DNA also shows serious oxidative damage. Mecocci *et al.* found a small but significant increase in oxidative damage to nDNA in patient brain samples and a highly significant threefold increase in oxidative damage to mtDNA in AD compared with age-matched controls^[11]. Later, the same authors found a significant reduction of mitochondrial membrane fluidity in AD^[12].

4. Proteins: protein nitration and carbonylation are indicative of oxidative modifications and both have been detected in AD patients^[13-15]. Some years ago, the group of Butterfield developed, a proteomic method to detect oxidative proteins specifically^[16-18]. Interestingly, they found oxidative modifications in many metabolic proteins and these correlate with the metabolic impairment shown in AD patients' brains^[19]. The authors found glycolytic enzymes and enzymes related to ATP synthesis in the group of metabolic oxidized. It is interesting that ubiquitinated proteins are increased in the disease^[20].

IS OXIDATIVE STRESS AN EARLY EVENT IN AD?

If oxidative stress is an important event in the pathophysiology of AD it would be relevant to know when the phenomenon begins. Free radicals may cause or be consequences of the lesions observed in AD. At present, we do not have a clear answer but we have substantial evidence that oxidative damage occurs very early in affected neurons. The group of George Perry and Mark Smith showed that brains from AD patients exhibit oxidative damage in vulnerable neurons before neurofibrillary tangles (NFT) appeared^[21,22]. Therefore, it seems that there is a temporal relationship between oxidative stress and cytoskeletal alterations. Using protein nitration or oxidized RNA (8OHG) as markers these authors showed that labeling precedes NFT formation. It thus seems that the oxidative processes occur initially in the neuronal cytoplasm and NFT formation could be due to either the accumulation of oxidized material or the fact that cytoskeletal proteins can take part in

oxidative adduction^[23]. On the other hand, the appearance of senile plaques also occurs after the oxidative stress process takes place. Down Syndrome patients have exhibited a marked increase in 8-hydroxyguanosine, advanced glycation endproducts, and nitrotyrosine, at ages preceding by decades amyloid-beta peptide ($A\beta$) deposition^[24,25].

MITOCHONDRIA AS SOURCE OF FREE RADICALS IN AD

It has been shown that $A\beta$ peptide causes a loss in mitochondrial function. The group of Oliveira from Coimbra found that $A\beta$ did not cause toxicity in cells depleted of mitochondria^[26]. Therefore, mitochondria seem to be a requirement for the intracellular toxicity of $A\beta$ peptide (see figure 1). We found that $A\beta$ causes direct damage to mitochondria resulting in increased production of reactive oxygen species (ROS). We

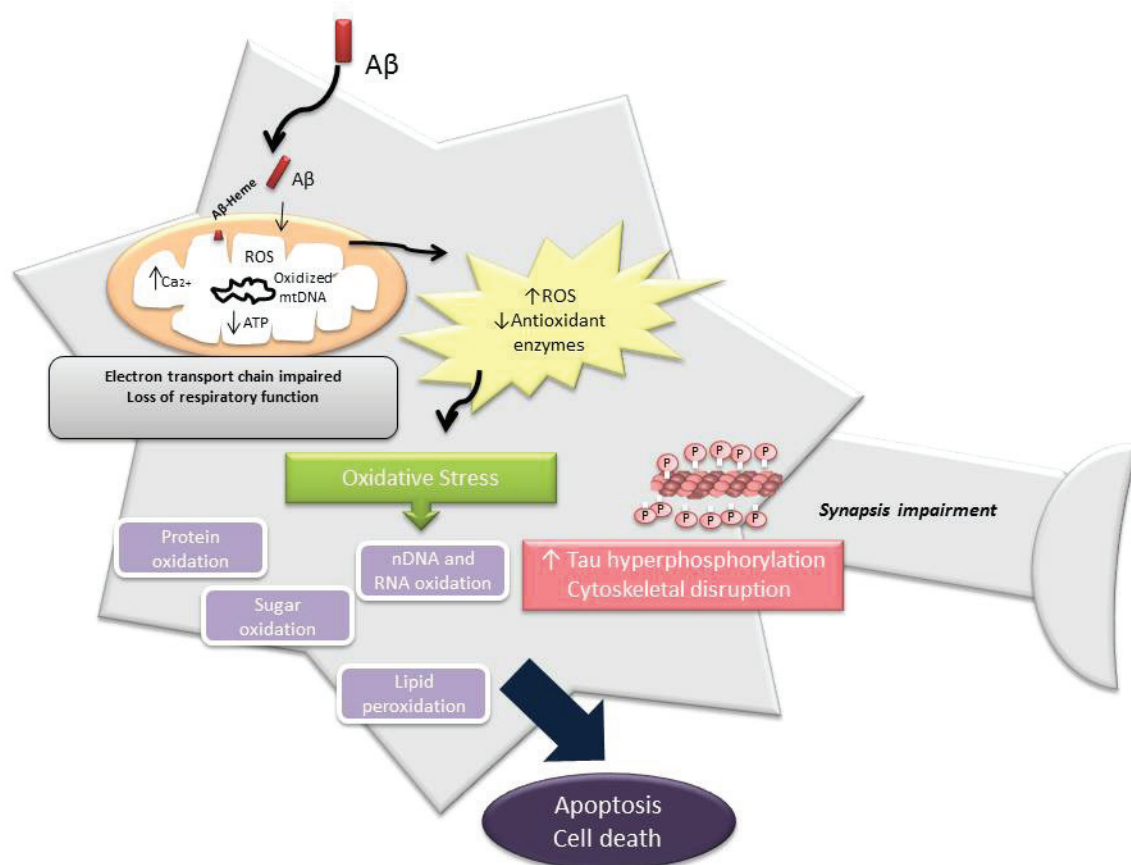


Figure 1. Rendition of the central role of mitochondria in ROS production in AD causing oxidative stress. This ROS production increases lipid peroxidation, protein oxidation, DNA and RNA oxidation and sugar oxidation. Cytoskeletal disruption causes synapses impairment. The global result is the induction of cell death by apoptosis in neurons.

confirmed the results of Oliveira showing that this was due to the loss of respiratory function and further extended the concept by finding that A β peptide interferes with heme by sequestering iron and therefore, rendering the heme complexes inactive^[27]. This interferes with the respiratory chain complexes and subsequently leads to an increased production of ROS and therefore, of oxidative stress. Oxidative stress leads to the release of cytochrome C then activating the internal pathway of apoptosis and providing a mechanistic insight for the apoptotic death of neurons caused by the interaction between A β peptide and mitochondria.

Moreover, cytochrome oxidase purified from AD patients' brain has a reduced activity^[27-29]. This could lead to increase the superoxide production by mitochondrial complex III. Two other mitochondrial enzymes, the pyruvate dehydrogenase complex and α -ketoglutarate dehydrogenase complex, have less activity than in controls^[30-32]. High levels of mitochondrial free radical production due to A β result in an increase in oxidized and nitrated proteins^[32].

On the other hand, there could be a link between the cytoskeletal abnormalities observed in AD (e.g., tau hyperphosphorylation) and mitochondrial abnormality. Tau hyperphosphorylation results in microtubular disruption and diminished axonal transport of mitochondria and consequently, increased mitochondrial turnover in the cell^[33]. In this context, our group showed that after incubation with A β , mitochondrial neurons aggregate around the nuclei and the cytoskeleton disrupts^[32-34].

ANTIOXIDANT TREATMENTS IN AD.

Many research projects have focused on antioxidant therapies in AD after evidence showing that ROS are involved in the early stages and evolution of the disease was reported. This review summarizes the results of these studies.

Polyphenols, Flavonoids, and Herbal Supplements

Resveratrol, a polyphenol present in red wine, may be a very effective modulator of AD development and progression^[35]. This compound could reduce oxidative stress, the secretion of A β , decrease inflammation, protect DNA-activating SIRT1, and decrease cell death^[36]. In a recent study, the capacity of resveratrol to remodel soluble oligomers, fibrillar intermediates, and amyloid fibrils into alternative aggregated species that are non-toxic^[37], was investigated.

In this respect, Hamaguchi *et al.* fed AD transgenic mice (Tg2576) with a mixture of phenolic compounds (curcumin, ferulic acid, myricetin, nordihydroguaiaretic acid, and rosmarinic acid) for ten months and concluded that administration of phenolic compounds prevented the development of AD pathology by affecting different A β aggregation pathways *in vivo*^[38]. One of these phenolic compounds, curcumin, was proposed by Zhang *et al.* as a therapeutic agent in the treatment of AD^[39]. However, in a six-month randomized, placebo-controlled, double-blind, pilot clinical trial with curcumin failed to improve cognitive performance in mild-to-moderate AD patients and did not decrease serum A β peptide or isoprostanes^[40]. The problem with curcumin and resveratrol is their bioavailability. They are poorly absorbed, rapidly metabolized, and quickly eliminated from the organism^[41,42]. Currently, a clinical trial in phase III with resveratrol is underway, sponsored by the Medical College of Wisconsin^[43] but results have not yet been published.

Another promising compound was the herbal product *Ginkgo biloba*. There are numerous studies *in vitro* and *in vivo* with experimental animals demonstrating the beneficial effects of *Ginkgo* in neuroprotection. Unfortunately, human studies have been discouraged. The Ginkgo Evaluation of Memory (GEM) study was a randomized, double-blind, placebo-controlled clinical trial with 3,069 participants. Compared with the placebo, the use of *Ginkgo*, 120 mg twice daily, did not result in less cognitive decline in older adults with normal cognition or with mild cognitive impairment^[44].

Hormones: Estrogens and Melatonin

The influence of estrogens on the brain and their decrease during menopause are of special interest, as it has been postulated that the decrease in levels of estrogens could affect cognitive functions in menopausal women^[45] that are important to verbal memory, working memory, and retrieval. Estrogen receptors are found in brain areas such as the hippocampus or the frontal lobes. Our group shown that oestradiol or genistein prevent the toxicity caused by A β peptide in primary cultured neurons from fetal rats^[32] and inflammation associated with astrocytes in culture^[46]. It is reasonable to think that estrogens may play an important protective role against the deterioration in cognitive functions that occur in AD. The Women's Health Initiative Memory Study (WHIMS), a substudy of the Women's Health Initiative (WHI), enrolled 7,479 women, and they were randomized to treatment with either unopposed estrogen (Premarin) if they were

hysterectomized, or a combination of Premarin and a progestational (Prempro) agent if they retained a uterus. The principal end point was both studies was dementia. The Prempro arm was stopped due to an increase in heart attacks and incidence of breast cancer as was the Premarin arm which was also stopped because of the number of strokes and the absence of cardiovascular benefit. Moreover, a more extensive analysis revealed an increase in the cumulative hazard for probable dementia^[47,48]. On the basis of this evidence, hormone replacement therapy with estrogens or progesterone is not indicated for the prevention of AD.

Melatonin secretion decreases in AD and this decrease has been postulated as being responsible for circadian disorganization, decrease in sleep efficiency, and impaired cognitive function seen in the patients. But a multicenter, randomized, placebo-controlled clinical trial coordinated by the National Institute of Aging in USA (the Alzheimer's Disease Cooperative Study) concluded that melatonin is not an effective soporific agent for patients suffering AD^[49].

On the other hand, melatonin prevented the cell damage to the hippocampus induced by the exposure to A β in organotypic hippocampal slides. In addition, melatonin significantly attenuated A β -induced phosphorylation of tau protein, and prevented GSK-3 β activation and neuroinflammation^[50]. At present, we do not have a clear idea about the beneficial effects of melatonin therapy in cognition, thus more clinical trials would be needed for clarification.

Vitamins

The authors of a project developed with 321 elderly men from the Veterans Affairs Normative Aging Study concluded that low vitamin B concentrations predict cognitive decline^[51]. Corroborating these results, another study in seniors showed that low vitamin B12 status and high serum folate was associated with cognitive impairment. When vitamin B12 status was normal, however, high serum folate was associated with protection against cognitive impairment^[52]. Vogiatzoglou *et al.* demonstrated that the decrease in brain volume was greater among persons with lower vitamin B12 status^[53].

Recent studies have revealed that disruption of vitamin A signaling observed in AD leads to A β accumulation and memory deficits in rodents. In this regard, AD transgenic mouse models revealed a robust decrease in brain A β deposition and tau phosphorylation after treatment with retinoic acids^[54]. This was accompanied by a significant

decrease in the APP phosphorylation and processing. The mice also showed decreased activation of microglia and astrocytes, attenuated neuronal degeneration, and improved spatial learning and memory compared with the vehicle-treated mice^[54]. Moreover, acitretin an analog of vitamin A, up-regulated α -secretase expression which results in a non-toxic processing of APP^[55].

Vitamin E is a powerful antioxidant and thus may cause beneficial effects and lower the progression of AD. Perkins *et al.*^[56] studied 4,809 elderly persons and observed that serum levels of vitamin E were lower in the elderly subjects than in young ones. Moreover, the lowering of vitamin E when normalized for plasma levels of cholesterol was consistently correlated with memory loss. A decisive paper published in 1997^[57] reported that vitamin E supplementation resulted in an improvement in the frailty of the patients but these authors did not observe a clear effect on cognition. Moreover, Tabet *et al.*^[58] revising data, concluded that there is not sufficient evidence to recommend treatment of AD with vitamin E. However, in all these studies the effect of vitamin E on redox status of the patients was never determined. In fact, it was always assumed that patients who were treated with vitamin E would improve their oxidative stress status. Thus we performed a study to correlate the administration of vitamin E to AD patients with the progression of the disease and with the blood oxidative stress status. We found that vitamin E does not cause a reduction of glutathione redox ratio in all patients. In those in whom it does not (non-respondent) vitamin E results in an even more pronounced loss of cognition than in patients treated with a placebo. For the patients who experimented a reduction in oxidative stress, cognition was maintained or slightly improved in the six months of the duration of the study^[59].

Dietary antioxidants

Finally, a mixture of antioxidants has been tested to reduce the detrimental effects of oxidative stress associated with AD. The Rotterdam Study included 5,395 participants who were monitored for the incidence of dementia during ten years and had dietary assessment. The authors concluded that high dietary intake of vitamin C and E may lower the risk of AD^[60]. But a more extensive analysis of the Rotterdam Study data (after multivariate adjustment) revealed that dietary intake levels of vitamin C were not associated with the risk of dementia and higher intake of foods rich in vitamin E may only modestly reduce AD^[61].

Concluding remarks

At present, we cannot conclude that antioxidant therapy is effective to treat AD. Moreover, the idea of antioxidant intake being completely safe is changing. Many antioxidants at high doses may not be beneficial, but may even be toxic. This is the case for vitamin E as some meta-analyses have concluded that supplementation with high doses may increase mortality^[62-64]. Our work has suggested that administering an antioxidant and following changes in cognition is not sufficient as there are patients whose antioxidant status does not change after administration of antioxidant vitamins^[59]. It seems that dietary antioxidants are safer but contribute very poorly to improve cognition.

If AD patients are in a very advanced phase of the disease when the neurologist diagnoses them it may be too late for treatment with antioxidants. In this respect, many authors indicate the fact that the disease begins decades before symptoms^[65]. Consequently, it is critical to find biochemical markers of the disease to detect it at the very early stages. This seems to be the only way to prevent, or delay, the onset of cognition impairment.

ACKNOWLEDGEMENTS

This work was supported by the following grants to JV: BFU2007-65803/BFI and SAF2010-19498 from the Spanish Ministry of Education and Science (MEC) co-financed with European FEDER funds; ISCIII2006-RED13-027 from the “Red Temática de investigación cooperativa en envejecimiento y fragilidad (RETICEF), and EU Funded CO STB35 and Prometeo 2010/074 from the Generalitat Valenciana. EG was the beneficiary of a Juan de la Cierva grant 2010-MICINN

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