OXIDATIVE MARKERS AND ANTIOXIDANT DEFENCES IN PATIENTS DIAGNOSED WITH PROBABLE ALZHEIMER DISEASE

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Summary

INTRODUCTION: Increasing evidence suggests that oxidative stress is associated with aging and several neurodegenerative diseases, including Alzheimer's disease (AD), although the role of oxidative stress in the aetiology of the disease is still unclear. OBJECTIVE: To evaluate oxidative damage levels and antioxidant activity in subjects diagnosed as probable Alzheimer patients. METHODS: Levels of malondialdehyde (MDA), advanced oxidation protein products (AOPP), glutathione reduced (GSH) and activity of the antioxidant enzyme Cu/Zn Superoxide Dismutase (SOD) were measured in plasma of 25 AD patients and 30 healthy aged-matched controls. RESULTS: The level of MDA was significantly higher (p=0.024) in plasma of AD patients (0.91+/-0.37), compared to control individuals (0.65+/-0.28). SOD activity was significantly (p=0.001) lower in AD (26.00+/-8.22) compared with the controls group (32.0+/-4.53). There were no significant differences in GSH and AOPP levels, in plasma of AD patients. CONCLUSIONS: Results presented here shows that oxidative status is actually affected in AD patients. Our findings underline the role of free radical in the pathogenesis Alzheimer disease.

Key words: Alzheimer, oxidative stress, neurodegenerative diseases

Introduction.

Alzheimer's disease (AD) is one of the most common dementing disorders and has profound medical and social consequences. The initiating molecular event is unknown, and its pathophysiology is highly complex. However, free radical injury appears to be a fundamental process contributing to the neuronal death seen in this disorder, and many studies using surrogate markers of oxidative damage have provided evidence supporting this hypothesis (1). In addition, recent molecular, cellular, and gene expression studies have revealed that amyloid beta-protein enters mitochondria, induces the generation of free radicals, and leads to oxidative damage in post-mortem brain neurons from AD patients and in brain neurons from cell models and transgenic mouse models of AD. In the last three decades, tremendous progress has been made in mitochondrial research and has provided significant findings to link mitochondrial oxidative damage and neurodegenerative diseases such as AD (2).

Methods

Our study included twenty-five patient diagnosed as probable Alzheimer, using NINCDS-ADRDA criteria, and thirty healthy subjects as controls. Analyzed subjects age range was between 65 and 80. All individuals were interviewed about their medical history and were physically examined. In addition, routine biochemical studies were performed. Subjects were excluded if they were considered to be malnourished and with evidence of other significant medical problems or if they were taking antioxidant supplement. Patient inclusion criteria were the ability to give written consent and it was made clear to patients that they may leave the study without compromising their care at any point. Experimental design was conformed according to the ethical guidelines of the "World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects" adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964.

The fasting venous blood was drawn from AD patients and healthy volunteers around 9:00 am and the plasma was separated immediately by centrifugation. Lipid peroxidation product, MDA, was measured using a chromogenic assay according to Esterbauer H and Cheeseman KH. This assay is based on the reaction of a chromogenic reagent (N-methyl-2-phenylindole in acetonitrile); with malondialdehyde (MDA) yield a stable chromosphore with a maximum absorbance at 586 nm. Assay detection limit is 0.1µmol/L and an interassay variation is lower than 5% (3). Determination of AOPP was performed as described by Witko-Sarsat (4), with slight modifications. Volumes were changed in order to use a Genesys spectrophotometer, which uses cuvettes instead of plates. 100 µl of plasma or 100µl of PBS, as blank, were mixed with 900 of phosphate buffer (PH= 7.4), 100 µl of chloramine T (0-100 µmol/l) were used for calibration purposes. Later, 50 µl of 1.16 M KI and 100 µl of acetic acid were added, and absorbance at 340 nm was measured immediately after. Concentration of AOPP is expressed in chloramine units (µmol/L). Reduced glutathione level (GSH) was estimated spectrophotometrically by Sedlak and Lindsay's method, using Ellman's reagent [5,5-dithio-bis(2-nitrobenzoic acid); DTNB]. Interassay variation coefficient was 4.0% (5). Superoxide Dismutase (SOD) activity was assayed according to the method of Marklund and Marklund, considering that one unit of SOD activity is the amount of the enzyme that inhibits the 50% auto oxidation rate of pyrogallol, and was expressed as Units/ml (6).

Results

AD patients had significantly elevated levels of MDA, compared to controls, and showed a markedly decreased Superoxide Dismutase activity; though there wasn't significant difference in AOPP and GSH plasmatic concentrations (Table I).

Table I. MDA, Malondialdehyde, AOPP, Advanced oxidation protein products, Cu-Zn SOD, Cu-Zn, Superoxide Dismutase GSH glutathione reduced. Figures parameters are means ± SD.
∗ Significant at $p< 0.05$.

	AD Patient	Control	p
MDA (µmol/L)	0.91 ± 0.09	0.65 ± 0.05	0.024*
AOPP (µmol/L)	22.7 ± 6.6	22.8 ± 2.3	0.52
Cu-Zn SOD (U/ml)	26.0 ± 1.6	32.9 ± 0.8	0.001*
GSH (µmol/L)	20.1 ± 2.5	26.0 ± 2.3	0.23
N	25	30	

Discussion

Production of ROS throughout cells and tissues normally occurs as a byproduct of oxidative phosphorylation and oxidases which support aerobic metabolism. Increasing evidence demonstrates that oxidative stress causes damage to cell function with aging and is involved in a number of age-related disorders including atherosclerosis, arthritis, and neurodegenerative disorders (7). In AD, there are a number of additional ROS sources which can play a role in the disease process: i) redox-active iron, at increased levels in tangles and plaques, can catalyze the formation of from HO• from H_2O_2, as well as the formation of advanced glycation endproducts (AGE); ii) activated microglia, such as those surrounding senile plaques, can produce nitric oxide and O , which can subsequently react to form peroxynitrite, with nitrotyrosine as the identifiable marker; iii)) β-amyloid has been directly implicated in ROS formation by means of peptidyl radicals; iv) AGE, in the presence of redox-active metals such as iron, can undergo redox cycling resulting in ROS production. AGE can further increase ROS formation by activation of specific receptors, such as the receptor for AGE (RAGE) and the class A scavenger-receptor. β-amyloide is also capable of this receptor activation; and (v) mitochondrial metabolic abnormalities, such as changes in the mitochondrial genome or deficiencies in key metabolic enzymes, may be a major initiating source of ROS in AD (8).

We evaluated some oxidative stress biomarkers in plasma of AD patients, particularly markers of lipids and proteins damage. In our study, MDA levels were significantly high in AD patients compared with the control group, which means that these patients were exposed to oxidative stress via lipid peroxidation. Similar results were reported by Marcus *et al*, in 1998 (9), and agree with other findings in brain tissues and cerebrospinal fluid of AD patients (10-13).

Advanced oxidation protein products were described by Witko-Sarsat for the first time in 1998. Such products are generated by the action of chlorinated oxidants, mainly hypochlorous acid, produced by myeloperoxidase in activated neutrophils (4). Several researcher reports protein damage, but AOPP levels have not been evaluated previously in this illness.

Antioxidant enzymatic defenses play an important role in scavenging free radicals produced under oxidative stress conditions. In the AD brain, the activity of the antioxidant proteins catalase, Superoxide Dismutase (SOD), glutathione peroxidase and glutathione reductase are increased in the hippocampus and amygdale (9).Our data reveals that SOD activity in AD patients was significantly low compared with controls, indicating decreased antioxidant defense against elevated lipid peroxidation processes, in these patients. Rinaldi et al. reported antioxidant enzymatic activity diminished in AD patients compared to controls (14).

Glutathione plays important roles in antioxidant defense, nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation). Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases, including Alzheimer's disease (15). Most researchers have observed that glutathione levels decreased with ageing (16-18), evaluated population is composed for elder people. Results presented here, shows that GSH levels were quite low in AD patients and controls, that is why, in order to find statistically relevant differences, it would be necessary to increase the sample size.

Conclusion

We conclude AD patient possess high lipid peroxidation levels compared to controls. Antioxidant defense system in normal aging is able to compensate such peroxidation effects; however, in patients with Alzheimer disease, this compensation mechanism is insufficient. Our results underline the outstanding role of ROS in progression of this neurodegenerative disease.

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