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Oxidative Stress and DNA Oxidative Damage in Alzheimer's Disease

NTIKBASANI G.* , KOROS C.** , BOVIATSIS E.***, KONTZOGLOU C****, DONTAS I.*****, PERREA D.*****

Abstract

Alzheimer's disease (AD) is the most prevalent form of dementia and its prevalence is expected to increase. Increasing evidence implicates oxidative damage as a mediator of toxicity in Alzheimer's disease. There is some evidence of increased oxidation of DNA (Deoxyribo- Nucleic Acid), RNA (Ribo-Nucleic Acid), proteins, lipids and carbohydrates in AD brain and in addition, some oxidative stress markers have also been found to be increased in cerebrospinal fluid, blood and urine of AD patients although the results remain contradictory. This article incorporates a literature review of oxidative stress issues in Alzheimer's disease and put emphasis on DNA oxidative damage.

Key Words: oxidative, markers, 8-hydroxy-2-deoxyguanosine, Gas chromatography/mass spectrometry, immunofluorescence.

Definition of oxidative stress

The term oxidative or oxidant stress refers to the state, where the free radicals are in excess of the antioxidant defense mechanisms.[8]

*Deparment of Neurosurgery, NIMITS Hospital, Athens, Greece
**Department of Neurology, Aiginitio Hospital, Athens, Greece
***Department of Neurosurgery, Evangelismos General Hospital, Athens, Greece
****3rd Department of Propaedeutic Surgery, Medical School, University of Athens, Laiko General Hospital, Athens, Greece
*****Laboratory for Experimental Surgery and Surgical Research, N. S. Christeas Medical School, National and Kapodistrian University of Athens, Athens, Greece

Relationship between oxidative stress and abeta amyloid cascade hypothesis

Alzheimer's disease (AD) is the most prevalent form of dementia and its prevalence is expected to increase.[9] According to the reviewed literature, there are a lot of interactions between Aβ and the molecules of oxidative cascade. Increased oxidative stress accelerates the accumulation of amyloid proteins in Alzheimer's disease.[10] There is growing body of evidence that oxidative stress mediated by Aβ, possibly involving oligomerization of Aβ.[11] A lot of groups found that Aβ is able to accumulate in mitochondrial membrane and subsequently induce mitochondrial dysfunction and ROS (reactive oxygen species) production.[12] As it has been shown in cultured neuronal cell models, Aβ binds to peptide- binding alcohol dehydrogenase (ABAD) in mitochondria, thus degenerates ABAD and degenerated ABAD enhances the production of lipid peroxidation products. [13]

Moreover increased lipid peroxidation may lead to increased Aβ(1-42) production and both lipid peroxidation products and Aβ(1-42) are able to lead to neuronal apoptosis.[14] Several studies have shown that Aβ itself was associated with protein oxidation in neuronal culture in vitro that could be blocked by antioxidants [15]. Aβ interacts with the receptor for advanced glycation end products and as a consequence it has pro-oxidant effects on neural, microglial and cerebrovascular cells.[10]

Based on the results of recent studies , it has been proposed that Aβ accumulation in neuronal cells correlated with neuronal iron homeostasis disruption and implicated in the process of oxidative stress in AD. [16] Iron can promote the cleavage and synthesis of Aβ precursor protein in an oxidative stress- mediated pathway and Aβ can be oxidatively modified by metal- catalyzed hydroxyl radicals and become more water- insoluble and resistant to the protease. [17]

Finally an interesting theory of Zhu and colleagues is the " Two- Hit" hypothesis, according to which the

early and progressive oxidative damage to neurons creates a " oxidative steady state" in order to protect the cell, but eventually the cell becomes vulnerable to additional insults, such as Aβ deposition.[18]

Biomarkers of oxidative stress in AD brains

The free radicals are highly unstable and able to react with other biomolecules such as proteins, DNA and fatty acids [8]. As suggested in all the reviewed literature, the interaction of free radicals with biomolecules results in the formation of molecules which constitute oxidative stress markers. Oxidative stress biomarkers in AD brains are distinguished according to the biomolecule class, disturbed as follows: lipid peroxidation markers (thiobarbituric- acid-reactive substances, malondyaldehyde, 4-hydroxy-2-nonenal, acrolein, isoprostanes, neuroprostanes), protein oxidation markers (protein carbonyls, nitrotyrosine), DNA oxidation markers (8-hydroxy-2-deoxy-guanosine, 8-hydroxyguanosine), RNA oxidation markers (8-hydroxy-guanine) [19].

Oxidative damage of DNA

a) CNS cells affected by oxidative stress:

Neurons are prone to oxidative damage of DNA, and their repair mechanisms are less effective and function only in the transcribed genome.[20]

Oxidative stress causes apoptosis in the glial cells, the oligodendrocytes, the microglia and the astrocytes, as shown in the DNA end-labeling technique.[21]

b) DNA oxidative damage types:

Increased 8-OHdG (8-hydroxy-2-deoxyguanosine) (Oxidative marker for DNA) and decreased repair of DNA in CSF (celebrospinal fluid) [22]. Also increased fragmentation (DNA break-up) in AD, caused by oxidative strand cleavage and the repair of oxidative base modification, erroneously attributed to apoptosis [23]. The DNA oxidation may cause strand breaks, sister chromatid exchange, DNA-protein cross linking, and base modifications [8].

c) Matching base damage with DNA source:

Increase in 8-OHdG present in nuclear and mitochondrial AD brain DNA while an increase in 5-OHU(5-hydroxyuracil) , 8-OHdA(8-hydroxyadenine) and 5-OHdC(5-hydroxycytosine), has been identified in nuclear brain fractions in subjects with A.D.. [8]

d) DNA damage type depending on the free radical type acting upon it:

The hydroxyl radical affects multiple bases.8 Hydroxyl radical, being the most reactive species interacts with C-8 of guanine (8-oxo-guanine), which is one of the most commonly found oxidized bases in DNA [24]. ROS, peroxynitrite and nitric oxide may lead to DNA cleavage and actually ROS cause DNA cleavage during hydroxylation of guanine and methylation of cytosine. [25] Peroxynitrite forms 8-nitroguanine, 8-oxoguanine and single strand break.26]

e) Where do we look for 8OHdG increase?

In peripheral blood lymphocytes of AD patients (with HPLC <high performance liquid chromatography> + electrochemical detection), in brain tissue of AD patients (mtDNA (mitochondrial) and nDNA (nuclear) are affected) and in DNA from ventricular CSF [27].

f) DNA oxidation assessment methods:

Using HPLC there was identified an increase in 8-OHdG, mtDNA and nDNA in brain fractions˙ in old age, not accompanied by dementia, there is an increase in 8OHdG, mtDNA and nDNA [28]. The lack of istones in mtDNA and diminished capacity for DNA repair render mtDNA an easy target for oxidative stress3. HPLC+ECD (electrochemical detection) are used for the measurement of 8-oxo-dG (8-oxo-deoxyguanosine). [29] HPLC can be used as quantitative method and solely for the detection of oxidized mtDNA. [30] The measurement of 8oxodG(8-oxo-deoxyguanosine) is performed in hydrolysed DNA using HPLC with electrochemical detection or to analyse for 8-oxo-guanine with GC-MS (gas chromatography-mass spectrometry). [31] Aruoma et al. (1997) [32] reported that: the DNA oxidation can be assessed in urine with HPLC and electrochemical detection, where we find high concentrations of 8-OHdG, 8OHdA, and 7-methyl-8-hydroxyguanine. In general, in the assessment of DNA oxidation, the most commonly used indicator is 8OHdG and

while HPLC+ECD is a very sensitive technique, it could underestimate the actual amount of DNA 8-OHdG due to the processing method.[32] They also mention that HPLC has the disadvantage of being very specific for only one lesion of DNA. [33] Gas chromatography /mass spectrometry is a sensitive method detecting oxidative adducts in all 4 DNA bases simultaneously. [28] In a mixture of mitochondrial and nuclear DNA from parietal lobe samples in AD patients examined with the above method, 8-hydroxyadenine, 8-hydroxyguanine, 5-hydroxycytosine were found elevated, while in nuclear DNA, examined with this method from frontal and parietal lobe and cerebellum of AD patients, there was a statistically significant increase in 5-hydroxyuracil, 8-hydroxyadenine, 8-hydroxyguanine (with the increase in 8-hydroxyguanine being most evident), in frontal, parietal and temporal lobe in AD patients compared to normal subjects in the control group, while similarly there was a statistically significant increase in 5-hydroxyuracil in the cerebellum.[28] Gas chromatography/mass spectrometry with selective ion motoring determines the levels of free 8hydroxydeoguanine (as a hydrolysis product) and the bound (to intact DNA) in the ventricular CSF in AD patients and according to a study, the bound molecule shows a statistically significant increase, while the free is found decreased in AD patients compared to the control group, while it is suggested using the ratio of bound molecule to the free compound (representing the repair product) as an indicator for the disease progress and the efficacy of antioxidant interventions. [34] The same method has been used for the measurement of 8OHdG in urine with detection limit of 1.8 pmol [32]. The 8-hydroxydeoxyguanosine in urine is probably not affected by diet, but it is unknown if some quantity of 8-hydroxydeoxyguanosine is metabolized into other products in humans [32]. Gas chromatography/mass spectrometry uses as internal standards stable-isotope-labeled analogs of the modified bases and while it has an advantage over other methods as it allows for a more accurate quantification of the DNA damage and recognizes the type of the responsible damage radical (e.g. oxygen radical oxidizes guanine, peroxynitrite forms 8-nitroguanine, hydroxyl radical oxidizes the 4 bases), it has the disadvantage of possible overestimation of 8-hydroxyguanine (when DNA is heated, there should be no oxygen in the preparation stage) [32]. ELISA(enzyme-linked immunosorbent assay): It can determine changes in the level of 8-oxo-deoxyguanine, when doubled, it requires a relatively complex sample preparation, it can measure 8-oxo-deoxyguanine in urine, although its values in urine may be affected by the general metabolic rate [29]. In some cases 8hydroxy-deoxyguanosine is found rising several-fold with age, as it constitutes an indicator of DNA oxidative damage [35]. Immunofluorescence can be applied for mitochondrial DNA 8-OHdG [30]. In situ hybridization for mitochondrial DNA and immune-cytochemistry against 8-hydroxy-2-guanosine, have been used for the detection of the mitochondrial DNA damage in various cells e.g. in vascular wall cells. [36] There is a report of an effort to count cells positive to the immunocytochemistry method against 8-hydroxy-deoxyguanosine. [37]

Conclusions

Oxidative or oxidant stress occurs every time that free radicals are in excess of the antioxidant defense mechanisms and it is considered to be an interesting, pathophysiologic step of Alzheimer's disease, because according to the reviewed literature: i) there are important interactions between Aβ and the molecules of the oxidative cascade, which concern the Aβ [1-42] production, the Aβ accumulation, the Aβ oligomerization, the ROS production, the lipid peroxidation products creation, the APP cleavage and synthesis, the Aβ's ability to dissolve in water, the Aβ resistance to protease. ii) oxidative stress results to production of molecules, which constitute oxidative stress markers. DNA can be modified by oxidative stress and conclude to strand breaks, sister chromatid exchange, DNA- protein cross links, and base modifications, such as increase in 8-OHdG, which is present in nuclear and mitochondrial DNA from brain tissues and ventricular CSF of AD patients as well as in DNA from peripheral blood lymphocytes of AD patients. According to the reviewed literature two common used techniques to measure 8oxodG are HPLC+ ECD and GC-MS.

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