Psychiatria Danubina, 2013; Vol. 25, No. 4, pp 401-409 *Review*

THE OXIDATIVE STRESS HYPOTHESIS IN ALZHEIMER'S DISEASE

Manuela Padurariu[1], Alin Ciobica[2,3], Radu Lefter[2], Ionela Lacramioara Serban[1], Cristinel Stefanescu[1] & Roxana Chirita[1]

[1] *"Gr. T. Popa" University of Medicine and Pharmacy, Iasi, Romania*
[2] *"Alexandru Ioan Cuza" University, Iasi, Romania*
[3] *Center of Biomedical Research of the Romanian Academy, Iasi Branch, Romania*

received: 26.3.2013; revised: 14.8.2013; accepted: 10.9.2013

SUMMARY

Oxidative stress may be involved in many somatic and psychiatric pathological states including dementia. The hypothesis of oxidative stress involvement in dementia is supported by much scientific data through biochemical, genetic and molecular studies. Thus, there are many reports of an increased level of the markers for oxidative damage, alterations in the specific activity of the antioxidant system, mutations in specific genes, mitochondrial disturbances and also several connections between oxidative stress and amyloid plaques. Despite these evidence and clinical approaches in using antioxidant therapy in dementia treatment, studies have failed to prove a clear benefit for antioxidant treatment in dementia. Hence, there is a need for further research regarding antioxidant therapy in very early stages of dementia.

Key words: *Alzheimer – dementia - oxidative stress - antioxidant*

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General Aspects Regarding Oxidative Stress

According to the theory of free radicals, physiological aging could be viewed as a gradual, inevitable process, at least partially generated through the accumulation of certain oxidative lesions. The injuries caused by oxidative stress occur as a result of the imbalance between pro-oxidants and anti-oxidants. The imbalance, which may be equivalent to the loss of homeostasis, occurs by weakening the antioxidant barrier represented by enzymes and non-enzymatic antioxidant factors, which allows the accumulation of cytotoxic compounds, through an excess of pro-oxidant compounds that consume anti-oxidant reserves of the body (Sies 1997).

Under physiological conditions, unstable potentially cytotoxic molecules are produced, following the biochemical body reactions, called free radicals or reactive oxygen species (ROS). The natural response of the body consists in the local activation of anti-oxidant enzymes, which are designed to neutralize these compounds and thus restore homeostasis.

It should be noted that there are several types of free radicals depending on their structure, of which the most studied are: superoxide anion (O^{2-}), hydroxyl radical (HO^{-}), hydrogen peroxide (H_2O_2), nitric oxide (NO), peroxyl (ROO) and reactive aldehyde (ROCH). The difference between free radicals, which depends on their structural and biochemical features is crucial because it confers on the compound its oxidative power, i.e. its toxicity. From a biochemical point of view, free radicals are considered as atoms or molecules possessing a single electron structure. Thus, reactive oxygen species reach stable energy states by easily attaching to various molecules, in order to pair their single electrons through these oxidation reactions (Halliwell & Gutteridge 2007). Target molecules that may be present in various cellular structures such as cell membranes and intracellular membranes, DNA, proteins or carbohydrates, undergo significant structural and functional alterations in this "oxidative attack" hence endangering the overall cell functioning and viability.

The steady state of a compound is defined by a lower energy status, which in fact means greater biochemical stability. The oxidative reactions leading to compound stability are coupled with reduction reactions as so-called redox reactions. In these reactions the single electrons of free radicals couple/join with the electrophilic groupings of other reaction participants which form the target molecules. The participants in the reaction are the oxidizing compounds and the reducing compounds respectively (Evans 1993).

Depending on their oxidation power, there are two types of free radicals: free radicals with a lesser reactivity and more aggressive free radicals, with a larger reactivity. The less reactive oxidative agents are produced by normal aerobic metabolism, and they induce lesions that can be repaired relatively easily. In certain situations, which we will describe below, some less reactive species such as the superoxide radical, can become more aggressive compounds, such as the hydroxyl radical. This process unfolds through redox reactions as well, involving some micronutrients as cofactors. The hydroxyl radical possesses high cytotoxic and mutagenic capacity (Shukla et al. 2011).

Most free radical injuries concern lipidic structures, in particular the polyunsaturated fatty acids, which are

produced from lipid peroxidation reactions. Markers of this process, which can be relatively easily identified in various biological fluids, are represented by aldehydes. These form biologically active molecules that may be involved in further oxidation reactions, generating new oxidative damage. Among the most studied aldehydes are malondialdehyde (MDA), trans-4-hydroxy-2-nonenal (HNE), F2-isoprostane (F2-IsoPs) and thiobarbituric acid (TBARS) (Ferreiro et al. 2012). It should be noted that there also are other structures vulnerable to "oxidative attack", of which DNA and proteins are definitely worth mentioning. In this way, it can be explained how free radicals are involved in many diseases, including cancer and atherosclerosis, chronic inflammation and diabetes (Halliwell & Gutteridge 2007, Evans 1993). It is also known that oxidative stress plays a very important role in the etiopathogenesis of several neuropsychiatric disorders, including schizophrenia, Parkinson's Disease, Alzheimer dementia, anxiety or bipolar affective disorder (Uttara et al. 2009), as our group has also previously demonstrated (Padurariu et al. 2010a,b, Ciobica et al. 2010, 2011, 2012, Stefanescu et al. 2012).

On the other hand, the body possesses an arsenal of protection against oxidative stress, which under normal conditions is very effective. The antioxidant factors that form true protective systems of the body against the free radicals, are represented by antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase or aldehyde dehydrogenase and non-enzymatic antioxidant factors. The antioxidant enzymes catalyze the reaction of reduction of free radicals, which diminishes their power and hence oxidative cytotoxicity. Considering the enzyme superoxide dismutase (SOD), this enzyme acts on the superoxide anion (O^{2-}), producing from this reaction hydrogen peroxide (H_2O_2), which is a relatively more stable compound, and therefore has a lower oxidizing power. It is important to note that hydrogen peroxide is the only toxic compound with antioxidant capacity that does not have a free radical structure. Further, the enzymes glutathione peroxidase (GPX) and catalase assisted by various cofactors, transform H_2O_2 into H_2O (Bild et al. 2012). GPX enzyme has a much higher affinity for the H_2O_2 molecule than catalase and can detoxify even when found in very low concentrations (Chance et al. 1979).

This is not the only biochemical route of hydrogen peroxide, as in the presence of iron it can be transformed into the hydroxyl anion. The hydroxyl radical is known to be very toxic, showing the most increased pro-oxidant capacity of all SRO. So far no specific antioxidant system has been identified for it.

The enzymatic system is represented by proteins with enzymatic function that usually associate in complexes with various cofactors and microelements. For example, there are several SOD enzyme cofactors, including copper and manganese that form the enzymatic complexes CuZn-SOD and Mn-SOD. Catalase is an enzyme complex system of four protein subunits and four iron ions. Also, free iron promotes the formation of ROS, resulting in the generation of a highly toxic compound, the hydroxyl radical. Copper, in turn mediates lipidic peroxidation (Singh et al. 2004). Summarizing the aforementioned aspects, the oxido-reductive reactions (redox) of oxidative stress involve multiple participants: on the one hand pro-oxidant factors, represented by reactive oxygen species and some micronutrients and antioxidants compounds on the other, consisting in antioxidant enzymes, cofactors (such as iron, selenium, copper, zinc, manganese or coenzyme Q10), and non-enzymatic antioxidant factors.

Non-enzymatic factors may be considered homeostatic role molecules that act as "scavangers" towards the pro-oxidant compounds. Several antioxidant factors of endo- or exogenous origin have been described, including: uric acid, glutathione, lipoic acid, bilirubin, melatonin, ascorbic acid, beta-carotene, bilirubin, selenium, NADPH mannitol, benzoate, reduced CoQ10, or tocopherol. Of these, it appears that glutathione is the most important, reducing lipid peroxidation processes by directly blocking the activity of ROS. In addition, glutathione is important for maintaining vitamins E and C in reduced forms; this structure confers their antioxidant properties (Singh et al. 2004).

The Oxidative Stress Hypothesis in Alzheimer's Dementia

It seems that the brain is particularly vulnerable to oxidative stress, which is explained by its relatively low levels of antioxidants, high concentration of polyunsaturated fatty acids, along with an increased oxygen demand of the brain (Evans 1993).

There is relatively consistent evidence in the literature which showed that free radicals may be involved in the etiopathogenesis of Alzheimer dementia (Ferreiro et al. 2012, Padurariu et al. 2010a, Baldeiras et al. 2008, Greilberger et al. 2008). Hence, one can speculate accumulation of excess free radicals that stimulates excessive antioxidant defenses, leading to the depletion of the body's antioxidant reserves. In this context, studies show higher levels of lipid peroxidation products in the central nervous system and peripheral tissues both in patients with dementia of the Alzheimer type and mild cognitive impairment (Baldeiras et al. 2008, Greilberger et al. 2008). A reduction in the antioxidant enzymatic barrier reflected by a decreasing specific activity of the main antioxidant enzymes, glutathione peroxidase and superoxide dismutase (Padurariu et al. 2010a) has been demonstrated. Thus, increased production of reactive oxygen species, identified in dementia, could result in a "consumption" of too many antioxidants, and a lowering of the antioxidant system's capacity to protect the body against "oxidative attack". Thus, the reduction of the antioxidant filter allows the accumulation of new free radicals, thus maintaining a "vicious cycle" (Baldeiras et al. 2008). Excess free radicals can cause neurodegenerative pathological

changes of the type that can be explained partly at a biochemical level through lipid peroxidation reactions. Also, given that oxidative stress is associated with damage at the DNA level, we can mention also a DNA enzymatic repair system that identifies oxidized nitrogenous bases of the DNA structure, removes them and replaces them with unaltered nitrogenous bases (Atamna et al. 2000).

Other arguments supporting the theory of oxidative stress in dementia relate to increased redox active sources, such as some transition metals, in particular iron, in the early stages of Alzheimer's disease (Smith 2006). In this way, there are many studies that show the positive effect exerted by various copper or iron chelators (e.g. desferoxamina) (Huang et al. 2004), such agents presenting a therapeutic potential in Alzheimer's disease.

The causes that may increase free radical levels in the dementia of the Alzheimer type are varied. Accumulation of reactive oxygen species in Alzheimer's disease seems to have the following causes: mitochondrial dysfunctions, most likely leading to respiratory chain defects and consequently to the formation of excess oxygen free radicals, extracellular amyloid β (Aβ) deposits, which induce local inflammatory processes and activate microglia, which is another potential source of ROS. In addition, binding of redox active metals to Aβ deposits can induce a direct reaction of hydrogen peroxide formation (Beal 2005). Thus, free radicals can be produced by mitochondrial biochemical reactions, by microglial activation, generated by β amyloid plaques, but also in inflammatory reactions that have been identified in brains affected by dementia.

As is widely known, Alzheimer's disease is characterized at the histological level by so-called neurodegenerative plaques and neurofibrillary tangles. Thus, tau protein is normally associated with microtubules and localized at the axonal extensions of neurons; it binds to microtubules promoting their assembling and provides stability to the neuron. The hyperphosphorylated form of this protein is the main constituent of neurodegenerative plaques and neurofibrillary tangles. Senile plaques present in Alzheimer's dementia are composed of β amyloid, the amyloid precursor protein (APP), dystrophic neuronal extensions, activated microglia and reactive astrocytes (Behl 1997). Additionally, the formation of Aβ peptide occurs by proteolytic cleavage of its precursor APP.

Regarding the oxidizing ability of neurotoxic amyloid β, there is relatively consistent evidence to support it. In this regard, there have been reported effects of Aβ amyloid in the brain of animal models of Alzheimer's dementia that support its pro-oxidant role. In this regard there have been shown to be increases in lipid peroxidation by measuring the 4-hydroxy-2-nonenal and isoprostanoids, elevated levels of reactive oxygen species, increases in carbonylic proteins and decreases in the neurons survival ratio (Butterfield et al. 2002). Adding β-amyloid in neuronal cultures causes an increase in activity of the enzyme acetylcholinesterase together with the incubation of β-amyloid in the environment. On the contrary, it appears that the enzyme activity decreases if various antioxidants are added in the culture medium (Melo et al. 2003).

The most likely mechanism by which β-amyloid may increase oxidative stress *in vitro* refers to its ability to bind iron (Rottkamp et al. 2004). Thus, it has been suggested that a very large amount of iron could be bound at the neuronal RNA level, numerous studies indicating an oxidation process of RNA in patients with AD (Nunomura et al. 1999). Given its rapid turn-over, neuronal RNA has become one of the most used methods to observe the redox balance status and oxidative stress in the brain. This type of analysis has demonstrated new aspects of the pathogenesis of Alzheimer's disease, namely that oxidative stress may be the earliest element that indicates the risk of developing the disease.

The relationship between oxidative stress and amyloid plaques is not unipolar. It seems that oxidative stress may lead to intralysosomal induction of β-amyloid, being indirectly involved in the amyloid genesis. In addition, β-amyloid has the ability to destabilize lysosomal membranes, resulting in cell death. These latest findings show a clear link between oxidative stress and pathogenic macroautophagal processes in Alzheimer's disease (Zheng et al. 2007).

Additionally, the role of mitochondria in Alzheimer's dementia is not completely understood. The importance of mitochondria in the processes associated with Alzheimer's disease may be explained by its fundamental role in nerve cell survival through the control exercised both on energy metabolism and on various apoptotic pathways. In this way, it is considered that mitochondria is the most important place of producing ROS (Richter et al. 1995). Massive synthesis of free radicals is increased within the process of cellular aging, when both abnormalities in function and alterations of mitochondrial membrane integrity occur. Mitochondrial membrane defects are produced in turn by excess free radicals, the membrane structure being also a lipid nature and therefore highly susceptible to lipid peroxidation.

Mitochondria could be considered the central pawn in the Alzheimer type dementia. These generate many biochemical changes identified in the brains of the patients with dementia, which are explained, at least partly, by their connection to the oxidative stress and by their capacity to mediate intrinsic cellular apoptosis. In this way, morphological analysis showed a clear relationship between the reduction in the number and size of mitochondria and the appearance of Alzheimer's disease (Webster 2003). These changes explain the energy and metabolism deficiencies of neurons in the different neurodegenerative processes. The metabolic deficiencies which arise would generate, in addition to increased oxidative stress, disturbances of glucose metabolism and Ca^{2+}, which seem to be associated with

reduced neuronal span of life. The brain regions mainly concerned with these processes are: the hippocampus, the frontal cortex, the cerebellum, globus pallidus and locus coeruleus, all of them being very important areas for cognitive processes (Zhu et al. 2006). Basically, mitochondrial dysfunctions generate serious metabolic disturbances in cellular life that prevent normal functioning of neurons. In the case of severely affected neurons, mitochondria can initiate cell apoptosis. Moreover, a reduced number of neurons is a well-known feature of Alzheimer's dementia (Webster 2003).

Under physiological conditions superoxide radicals are produced mainly by the respiratory chain, but they can be also synthesized by the activated microglia. In fact, microglial activation represents a type of immune response to some brain lesions, a process that involves the generation of cytotoxic compounds such as superoxides that maintain a vicious cycle of neuronal damage (Nakajima et al. 2001).

The sources of reactive oxygen species are many and varied and have not yet been fully identified. The free radicals are the result of diverse physiological and pathological processes not only endogenous but also exogenous, such as aging, excessive caloric intake, infections, inflammatory states, environmental toxins, certain drugs, emotional or psychological stress, tobacco smoke, ionizing radiation, alcohol or unbalanced nutrition (Ranjana et al. 2012). Related to food, it is speculated that diets high in fat, processed foods and excess iron may increase the production of free radicals.

The causal relationship between oxidative stress and the changes identified in dementia has not yet been fully elucidated. It is not known which is the primary etiological factor, whether oxidative stress is a consequence of the degeneration processes of dementia or the oxidative compounds produce the characteristic lesions in dementia. One can speculate that at the basis of the neuronal alterations would sit the mitochondrial dysfunction which generates cytotoxic free radicals that would cause neuronal alterations and triggers apoptosis, thus explaining the massive neuronal losses in dementia (Webster 2003). Also, many theories consider the important role of cumulative oxidative stress in the development of these mitochondrial dysfunctions. In any case, it seems that oxidative stress, at least partly, explains the changes present in dementia. In this context, the subject of the exact relationship between dementia and oxidative stress remains open to new research ideas.

The Neurotoxicity of Oxidative Stress

Dementia in Alzheimer's disease is defined in clinical terms through a global, progressive and irreversible decline of the cognitive functions. These changes are explained by a massive and progressive destruction of the nerve cells caused by different neuropathological processes. The oxidative stress theory explains neuronal death as caused by free radicals that attach and change the composition of neuronal fat molecules, altering membrane fluidity and permeability and disturbing some of the membrane functions, such as transport and barrier-like functions. The consequences of these disturbances are directed mainly towards the traffic of Ca^{2+} ions that cross the membrane structure, with the alteration of the signal transduction processes (Rowan et al. 2004).

Besides, it is known that membrane lipids are among the most important targets of oxidative stress, which can be explained by the presence at this level of a higher number of double bonds in the fatty acid constituent structure.

It should be noted that among the fatty polyunsaturated acids in the brain the arachidonic acid and docosahexaenoic acid have the highest concentrations. Following the lipidic peroxidation reactions, unstable oxygen derivatives form, including the reactive aldehydes, which, in turn, act on the neighboring double bonds, generating a chain reaction that can complete with the peroxidation of all the unsaturated fatty acids in the membrane. These peroxidation byproducts are 4-hydroxynonenal or malondialdehyde (MDA). The latter is one of the most studied aldehydes. In structural terms, MDA is an organic compound with the chemical formula $CH_2(CHO)_2$, which is highly biochemically active and highly toxic. MDA participates in a wide variety of biochemical reactions of oxidation, interacting with proteins, resulting in protein oxidation end products, in analogy with advanced glycosylation end products. It also reacts with nitrogen bases, such as the adenosine and the guanosine in DNA structure, thus being involved in the mutagenic processes (Del Rio et al. 2005). Moreover, it is known that increased oxidative stress is associated with the development of neoplasms (Halliwell & Gutteridge 2007, Halliwell 2007, Reuter et al. 2010).

At the same time, active aldehydes can generate, following reactions with lipids, proteins such as lipofuscin, which accumulates in the neuronal cells, especially in the most metabolically active regions. Moreover, lipofuscin is considered the aging pigment, usually accumulating in old and highly worn cells (Porta 2002).

The high level of oxidative stress in dementia manifests and can be objectified by the detection of increased levels of resulting compounds through lipidic peroxidation, protein and DNA/RNA oxidation, advanced glycation end products and also by the determination of free radicals (Butterfield 2002).

As mentioned above, the oxidation processes affect proteins such as the cytoskeleton proteins or enzymes. Following oxidative degradation, protein structure and function suffers alterations by addition of carbonyl groups (aldehydes or ketones). The resulting carbonylic proteins have different biochemical properties. They become more hydrophobic and resistant to proteolysis, the difficult elimination leading to the accumulation of non-functional proteins (Massaad et al. 2011). Thus, the

proteins undergo alterations in their tertiary structure configuration, and the most significant results of protein degradation are translated, at cellular level, into affected mechanisms involved in energy production, disturbances in enzymatic activity and defects of the intercellular communication mechanisms via the carbonylic function of the proteins. Moreover, an increased carbonylic proteins level has been reported in the frontal and parietal lobes in Alzheimer's dementia (Hensley et al. 1996).

Also, the proteic structural alterations can accelerate the formation of oligomers or aggregates of toxic proteins that apparently contribute to pathologies commonly seen in neurodegenerative diseases such as Alzheimer's, Parkinson's or Huntington disease (Pérez et al. 2009).

Besides protein carbonylation, another cellular component affected by oxidative stress is cellular DNA, as we have mentioned before. DNA oxidative changes may include alterations varying from nitrogenous base losses to DNA repairing system damage. In this way, the highly toxic hydroxyl radical can easily access the cell nucleus causing the degradation of various nitrogenous bases such as guanine, adenine and pyrimidine, with the formation of toxic compounds such as hydrodeoxyguanosine, hydroxiadenine, peroxide thymine or glycol thiamine. These aspects are also confirmed by Gackowsk and his colleagues, who have demonstrated, in a study published in 2008, the occurence of the DNA oxidation reaction in individuals with dementia of mixed type, as opposed to the control group, by measuring the level of DNA oxidation products (8-oxo-2'-deoxyguanosine and 8-oxoguanine) in cerebrospinal fluid (Gackowski et al. 2008). Also, it has been stated that the presence of an enzyme DNA repairing system, that identifies oxidized nitrogenous bases from the DNA structure, removes them and replaces them with unaltered nitrogenous bases (Atamna et al. 2000).

Oxidative stress exerts also negative influences on the dopaminergic transmission system too, reducing thereby the individual's motivational state and causing serious cognitive deficiencies that characterize various dementias with dopaminergic implication (Luo et al. 2005).

In Alzheimer's disease, the most vulnerable cerebral areas are the entorinal cortex and CA1 region of the hippocampus (Karelson et al. 2001). In the literature it is stated that some neurons are more likely to be prone to "oxidative attack", which is explained by the fact that they have high levels of intrinsic oxidative stress. Thus, the most active neurons, which have long axonal extensions and are involved in many synapses, have high bioenergetic needs. If mitochondrial alterations occur, these neurons with increased energy requirements and therefore a greater number of mitochondria, are most vulnerable to oxidative stress. Among the neuronal groups showing higher levels of superoxide anion are included the hippocampal CA1 region, comparative, for instance, to CA3 region neurons. This group expresses higher concentrations of antioxidants and also genes involved in oxidative stress (Gandhi et al. 2012).

Other mechanisms of oxidative stress in dementia have also been described. In this regard, given the fact that there are β amyloid vascular deposits which can generate free radicals, this can facilitate their access to the vascular endothelium. The superoxides mechanism of action consists of nitrogen monoxide (NO) deprivation - nitrogen monoxide is both an important signaling factor for various cellular processes and mediator of vasomotricity - but also of NO turning into peroxynitrite, a strong oxidizing agent, which can be further a source of hydroxyl radicals (Massaad 2011).

Genetic Aspects of Oxidative Stress in Alzheimer's Dementia

In recent years there has been significant progress in understanding the relationship between oxidative stress and Alzheimer's type dementia. It is understood that this link is dynamic and present at multiple levels, and can be highlighted only indirect and sequentially. The genetic perspective on oxidative stress provides new data, while supporting the oxidative stress hypothesis in dementia.

Analyzes of the mutant APP gene (which is involved in the occurrence of some rare forms of familial Alzheimer and in the formation of β amyloid) shows a possible connection between the gene and the occurrence of oxidative stress in Alzheimer's disease (Yamada et al. 1999). The familial form of the disease, which has an early onset and is dominant autosomally transmitted, can be determined by the occurrence of mutations against the following genes: amyloid precursor protein gene (APP), presenilin 1 and presilin 2 (PS1, PS2) genes. Under normal conditions APP proteolysis is obtained by α-secretase, followed by γ-secretase and generates *non-amyloidogenic* fragments. In the case of APP mutations, the proteolytic cleavage is done by β-secretase and γ-secretase resulting in Aβ42 fragments with amyloidogenic properties, which are grouped into insoluble plaques. It seems that oxidative stress might alter APP processing by activating various signaling pathways (Shukla et al. 2011). It is also relevant that the gene encoding superoxide dismutase, SOD-1, is located on chromosome 21, where the APP gene is also located. Also, chromosome 21 is involved in Down's syndrome, which can lead among other complications to (the occurrence of) Alzheimer's disease (Webster 2003). It seems that within Alzheimer's disease an imbalance occurs between the SOD-1 gene and the antioxidant enzyme activity, which can lead to a dramatic increase in the concentration of free radicals. The balance is even more disturbed through the accumulation with aging of numerous somatic DNA mutations, which will further increase the harmful effects of free radicals, and will culminate with the onset of the disease.

Also, it seems that people who have C2 transferrin gene abnormalities, that cause improper coupling of iron and aluminum in the detoxification reactions of free radicals, are more likely to develop dementia (Bourdel-Marchasson et al. 2001).

On the other hand, oxidative stress may act on genes influencing their activity. Thus, some genes, like the E4 allele gene on chromosome 19, can be stimulated in the context of increased oxidative stress, leading to increased expression of E apolipoprotein, with negative effects on neuronal plasticity processes, such as learning and memory, which are, of course, seriously affected in Alzheimer's disease (Forero et al. 2006).

It seems that free radicals may act as mediators for the expression of genes as well. Thus, some studies show that genetic changes are present in certain areas of the brain and are rather the result of ROS mediated injuries, than primary genetic deficiencies and that the free radicals cause certain areas of the brain to be more susceptible to neurodegenerative processes (Aksenov et al. 1998).

Biomarkers of Oxidative Stress - Relevance in Alzheimer's Dementia

Since measuring free radicals is impracticable, oxidative stress determination is achieved by determining the resulting compounds from the oxidation processes in the various structures involved. Additionally, enzymatic determinations are performed to highlight the specific activity of various antioxidant enzymes. Determination of these biological indicators of oxidative stress can be achieved in various biological fluids such as blood, cerebrospinal fluid, urine, saliva and also at tissue level. In animal models of dementia, for instance, brain homogenate harvested from the concerned regions (Ciobica et al. 2009) is used. Obviously the preferable way is to use CSF or brain tissue, but this is difficult. On the other hand, access to blood or urine is much easier, but with greater risks of misinterpretation occurring, given that these fluids reflect the state of the entire body.

Previous studies showed an association between the amyloid plaques and the various oxidative stress markers, including mitochondrial and nuclear damaged DNA markers, lipid peroxidation markers for membrane lesions, and advanced glycation products. 8-oxo-2'-deoxyguanosine and 8-oxoguanine are the DNA oxidation markers, while the lipid peroxidation markers are represented by the 4-hydroxynonenal, malondialdehyde or the F2 isoprostanes. Furthermore, increases in the specific activities of antioxidant enzymes, such as SOD2 in the hippocampus, especially in CA1 region and the amygdala (Massaad et al. 2011) have been observed. Elevated activity of these enzymes could be explained by a compensatory activity type in the body. In other words, the body responds to the "oxidative attack" induced by free radicals by an increased synthesis of antioxidant enzymes to counteract the excess of ROS. Some studies have found low levels of nonenzymatic antioxidant factors (α-tocopherol and retinol), while others identified high concentrations of the elements iron, aluminum, mercury, with catalytic potential for the redox type reactions in patients with dementia (Webster 2003). In addition, high concentrations of non-enzymatic antioxidants in plasma are associated with better cognitive capacity (Bourdel-Marchasson et al. 2001).

Hence, Zhu in 2006 proposed the theory of "two-hits", which holds that the disease can be caused by both oxidative stress and disruptions of mitotic nature, and these factors might be used as indicators for the degree of development, and even for the occurrence of the disease (Zhu et al. 2006).

The establishment of biomarkers for identifying dementia in its preclinical stage is a priority given the development and prognosis of the disease. Considering the substantial evidence supporting the presence of increased oxidative stress in the early stages of the disease, to assign of a set of biomarkers taking into account the indicators of oxidative stress seems reasonable enough. Evaluation of oxidative status in patients with mild cognitive impairment who do not meet clinical criteria for dementia could be feasible in identifying the individuals at risk for dementia. Clinical trials demonstrate an increased level for the oxidative stress markers in patients with mild cognitive impairment similar to patients with dementia. These indicators relate to increased MDA and decreased antioxidant enzymes SOD and GPX, as our group previously demonstrated (Padurariu et al. 2010a).

Antioxidant Therapy

The numerous scientific observations supporting the involvement of oxidative stress in cognitive impairment and, in particular, Alzheimer's dementia, suggest the potential benefits of antioxidant therapy. Some current treatments accepted in Alzheimer's dementia have antioxidant properties (piracetam, ginkgo biloba or vitamin E). However, data from the literature are generally contradictory regarding the usefulness of these drugs in dementia and their use for disease prevention is extremely controversial, given some speculated side effects (Shukla et al. 2011).

It seems that the use of antioxidant compounds may also have a role in reducing Aβ amyloid-induced toxicity. These substances include blueberries, flavonoids, polyphenols, resveratrol, Ginkgo biloba extract, epicatechin, or melatonin. Also the use of EUK-8, a synthetic compound with a scavanger role against free radicals in *in vitro* conditions has proven effective against amyloid toxicity in cell cultures (Massaad et al. 2011).

Regarding the usefulness of vitamin C, this is considered the most important soluble antioxidant, which is able to neutralize ROS before the initiation of lipidic peroxidation. Also, vitamin E is an important liposoluble antioxidant that is useful particularly at the membrane level, where it protects polyunsaturated fatty acids against lipidic peroxidation (Singh et al. 2004). Although some studies report a reduction in the incidence and prevalence of dementia after supplementation with vitamins E and C, extensive meta-analytic investigations have not identified benefits for their recommendation in primary or secondary prevention of the disease (Boothby et al. 2005). Flavonoids are also effective antioxidants that act by chelating the trace elements involved in oxidative stress reactions.

Additionally, a Cochrane review of 36 studies that used *Ginkgo biloba* for the treatment of cognitive impairments and dementia showed that *Ginkgo biloba* is not associated with a consistent benefit, clinically significant for individuals with Alzheimer's disease (Birks et al. 2009). On the other hand, a European study showed equal efficacy between *Ginkgo biloba* (240 mg daily), Donepezil (5 or 10 mg daily) and a combination of the two (Winslow et al. 2011).

Regarding selenium, another antioxidant, studies have noted a decrease in its serum levels correlated with cognitive decline (Cardoso et al. 2010). However, extensive analysis on the usefulness of selenium in dementia demonstrated a lack of consistent clinical evidence to support the benefit of selenium supplementation in patients with Alzheimer's, and the absence of a significant decrease in brain, CSF, or blood selenium in patients with Alzheimer's disease (Cardoso et al. 2010). Still, other studies support a critical role of selenium in disease pathogenesis, suggesting a preventive role (Gao et al. 2007, 2012).

Another antioxidant, coenzyme Q10 (CoQ10) is an important cofactor in the electron transport chain, with antioxidant properties. In cell cultures, Coenzyme Q10 protects neuronal cells from oxidative stress, while in animal models of dementia and Alzheimer it reduces MDA, regulates SOD activity, reduces amyloid plaques and improves cognitive performance (Dumont et al. 2011, Yang et al. 2008). However, there is insufficient evidence in humans to deny or confirm the benefit of CoQ10 in dementia.

It seems that results are not encouraging for the other antioxidants as well. Meta-analyzes have not identified benefits in taking selegiline, nicergoline or piracetam, concluding that their administration is not recommended in Alzheimer's dementia (Hughes et al. 2007).

Given the experimental data that support the existence of increased oxidative stress in patients with Alzheimer's dementia, correlated with *in vitro* results, we consider extensive research regarding the benefits of antioxidants in patients with preclinical stage of dementia to be necessary.

Conclusion

Oxidative stress may be involved in many somatic and psychiatric pathological states including dementia. The hypothesis of oxidative stress involvement in dementia is supported by many scientific observations through biochemical, genetic and molecular studies. Thus, there are many reports of an increased level of the markers for oxidative damage, alterations in the specific activity of antioxidant system, mutations in specific genes, mitochondrial disturbances and also several connections between oxidative stress and amyloid plaques. Despite these observations and clinical approaches the use of antioxidant therapy in dementia treatment studies has thus far failed to prove a clear benefit for the antioxidant treatment in dementia. In conclusion, there is an increased need for further research regarding antioxidant therapy, probably in very early stages of dementia.

Acknowledgements: None.

Conflict of interest : None to declare.

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Correspondence:

Ionela Lacramioara Serban, MD, PhD
"Gr. T. Popa" University of Medicine and Pharmacy, Department of Physiology
700115 Iasi, Romania
E-mail: ilserban1@yahoo.com