SYSTEMATIC REVIEW

The role of oxidative stress in the pathogenesis of Alzheimer's disease

Filipcik P[1,3], Cente M[1,3], Ferencik M[1,2,3], Hulin I[4], Novak M[1,3]

Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia.
michal.novak@savba.sk

Abstract

Oxidative stress has been implicated in the pathogenesis of Alzheimer's disease (AD) as a relevant marker of neuronal degeneration. However it plays an important role not only in the pathogenesis of neurodegenerative diseases but also in other critical disorders like heart diseases, carcinogenesis and others. Oxidative stress is also associated with normal aging. In this review we discuss a crucial question: to what extent oxidative stress may be a causative factor in pathogenesis of AD type of neurodegeneration. The results of several recent epidemiological studies appeared to be controversial at this point. It is believed that antioxidant therapies may have beneficial effects at least in delaying disease progression and appearance of AD specific clinical symptoms. Since there is no cure for AD recently, healthy life style and antioxidants enriched nutrition (or even antioxidant therapy) may provide an effective way of fighting against this deleterious disease *(Ref. 102)*.
Key words: Alzheimer's disease, oxidative stress, tau protein, beta-amyloid, mitochondria, peroxisome.

Abbreviations:
AD – Alzheimer's disease, ROI – reactive oxygen intermediates, OXPHOS – oxidative phosphorylation, GSH – glutathione, $O_2^{\cdot-}$ – superoxide radical, $ONOO^-$ – peroxynitrite, $^{\cdot}OH$ – hydroxyl radical, SOD – superoxide dismutase, 4-HNE – 4-hydroxy-2-nonenal

The sporadic form of AD is multifactorial disease influenced by risk factors such as hypertension, diabetes, hypercholesterolemia and environmental stressors including oxidative stress. There is growing body of evidence in the recent literature that oxidative stress may be a major cause of neurodegeneration (Perry et al, 2002; Aliev et al, 2004; Moreira et al, 2005). Several epidemiological studies indicate that high dietary intake of vitamin C and vitamin E may lower the risk of Alzheimer's disease (Engelhart et al, 2002; Morris et al, 2002). In contrast, other studies have failed to detect beneficial effects of antioxidant therapies, and have concluded that the intake of carotenes, vitamin C and vitamin E was not associated with reduced incidence of AD (Luchsinger et al, 2003; Laurin et al, 2004). It appeared just recently in New England Journal of Medicine that, vitamin E has no benefit in patients with mild cognitive impairment (Petersen et al, 2005). It also has to be mentioned that, oxidative stress is associated with many different diseases, such as cancer (Toyokuni, 1998), autoimmune disorders (Gilgun-Sherki et al, 2004), diabetes (Matteucci et al, 2004), atherosclerosis and stroke (Madamanchi et al, 2005), supporting the view that oxidative stress is by-product of aging rather than the direct causative factor in pathogenesis of AD type neurodegeneration. The contribution of oxidative stress to neurodegeneration is not peculiar to a specific neurodegenerative disease. Oxidative stress has been found implicated not only in Alzheimer's disease but also in Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD). Recent data indicate that oxidative stress

[1]Institute of Neuroimmunology, Slovak Academy of Sciences, Bratislava, Slovakia, [2]Institute of Immunology, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [3]Centre of Excellence for Alzheimer's Disease and Related Disorders, Slovakia, and [4]Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Address for correspondence: M Novak, Inst of Neuroimmunology, Slovak Academy of Sciences, Dubravska cesta 9, SK-845 10 Bratislava, Slovakia.
Phone: +421.905609558, Fax: +421.2.54774276
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is one of the earliest events in pathogenesis of Alzheimer's disease (Nunomura et al, 2001), however the primary contribution of oxidative stress to disease development is still discussed among wide scientific community and promoter steps in AD pathogenesis are little known and are further intensively investigated.

Origin of oxidative stress – mitochondria

Discovery of mitochondrial genetics, biology and functional physiology were crucial key-stones for understanding roles and implications of processes running inside of mitochondria. Three of the most important aspects of mitochondrial oxidative phosphorylation (OXPHOS) for disease pathogenesis are:

– energy production,
– generation of reactive oxygen intermediates (ROI),
– regulation of programmed cell death (apoptosis).

The whole OXPHOS system includes the electron transport chain (ETC) components, ATP synthase, and the adenine nucleotide translocator (ANT) and is located in the inner mitochondrial membrane. Electrons from the organic acids oxidation are transferred through respiratory complexes resulting to generation of water. Released energy is used for electrochemical gradient formation, which is maintained by the proton pumps. Permanent flux of protons enables the ATP synthase catalyzed condensation of ADP and inorganic phosphate (Pi) to form ATP. Thereafter, the produced ATP is transported to cytoplasm by ANT in exchange for the ADP. However, during the transfers in respiratory chain, single electrons occasionally escape enzymatic control and combine with oxygen to create free oxygen radicals in 1 to 2 percent of all oxygen consumed (Cadenas and Davies, 2000). Because mitochondria use 90 percent or more of the cell's available oxygen to make ATP, they also generate more than 90 percent of the free oxygen radicals within the cell (Kidd and LeVine, 1986). OXPHOS is the primary endogenous source of reactive oxygen intermediates, which need to be detoxified in turn. For this purpose the mitochondria developed powerful antioxidant defenses consisting of several enzymes that are able to neutralize highly toxic radicals. However, the chronic exposure of cells to ROI might result into oxidative damage of intracellular molecules (nucleic acids, proteins and lipids) and thus leading to metabolic depression followed by cell death (Wallace, 1997 a).

Mitochondria as a major source of ROI are directly linked to apoptotic pathways. Abnormalities in cellular respiration (potential damage of mtDNA) are one of the most probable contributors to the onset of pathology in AD (Castellani et al, 2002; Eckert et al, 2003). Decreased ability of mitochondria to effectively transfer electrons through respiratory complexes leads to permeabilization of the inner mitochondrial membrane and release of Ca^{2+} from the organelle. Oxidative phosphorylation and energy production are consecutively uncoupled and depletion of ATP levels leads to further impairment of other Ca^{2+} regulation system, in the plasma membrane and the endoplasmic reticulum. Morphological alterations of mitochondria have been also observed, what may be related to metabolic and energy deficiency in neurons in Alzheimer's disease and other neurodegenerative disorders (Baloyannis et al, 2004), presumably suggesting oxidative damage in neurons in AD brains.

Oxidative radicals – mechanisms of oxygen radicals generation in Alzheimer's disease

Hypothesis about the contribution of trace elements to development of AD goes hand in hand with oxidative stress theories. Aluminium (Al), mercury (Hg), and iron (Fe) were thought to be the most relevant elements in AD (Markesbery et al, 1993). Through catalysis of free oxygen radical reactions is the iron probably the most important element. Redox potential of Fe^{2+}/Fe^{3+} iron is capable of transferring electrons and enabling the ROI generation. Oxidation of Fe^{2+} to Fe^{3+}, while transferring an electron to O_2 produces the superoxide radical ($O_2^{\cdot-}$).

A dismutation reaction of superoxide radical leading to the formation of hydrogen peroxide and oxygen can occur spontaneously or is catalysed by the enzyme superoxide dismutase (SOD). There are three distinct types of SOD classified on the basis of the metal cofactor: the copper/zinc (Cu/Zn–SOD, cytoplasmic), the manganese (Mn–SOD, mitochondrial) and the iron (Fe–SOD) isozymes (Bannister et al, 1987). Superoxide can act as either an oxidant or a reductant. It can oxidize sulphur, ascorbic acid or NADPH and it can reduce cytochrome c and metal ions. Superoxide forms the perhydroxyl radical ($^{\cdot}OOH$), which is a powerful oxidant (Gebicki and Bielski, 1981), but its biological relevance is probably minor because of its low concentration at physiological pH.

Hydrogen peroxide is noteworthy because it readily permeates membranes and it is therefore not compartmentalized in the cell. Numerous enzymes (peroxidases) use hydrogen peroxide as a substrate in oxidation reactions involving the synthesis of complex organic molecules. The well-known reactivity of hydrogen peroxide is not due to its reactivity *per se*, but requires the presence of a metal reductant to form the highly reactive hydroxyl radical, which is the strongest oxidizing agent known and reacts with organic molecules at diffusion-limited rates. The reaction of Fe^{2+}+ with H_2O_2 produces the highly reactive hydroxyl radical ($^{\cdot}OH$) via Fenton reaction. Fenton, more than hundred years ago, described an oxidizing potential of hydrogen peroxide mixed with ferrous salts (Fenton, 1894; 1899). In biological systems the availability of ferrous ions limits the rate of reaction, but the recycling of iron from the ferric to the ferrous form by a reducing agent can maintain an ongoing Fenton reaction leading to the generation of hydroxyl radicals. Haber and Weiss (1934) identified reaction resulting into $^{\cdot}OH$ formation through an interaction between $O_2^{\cdot-}$ and H_2O_2 in the presence of Fe^{2+} or Fe^{3+}.

Therefore, in the presence of trace amounts of iron, the reaction of superoxide and hydrogen peroxide will form the destructive hydroxyl radical and initiate the oxidation of organic substrates. Metals other than iron may also participate in these electron transfer reactions by cycling between oxidized and reduced states. Redox active metals, especially iron and copper, are

thought to play very important roles in homeostasis of intracellular processes, generating reactive radical species. Changes damaging balanced levels of these redox active metals lead to impaired cellular respiration, defects of antioxidant defence resulting into chronic imbalance of metabolic pathways affecting the lifetime of cells. Free oxygen radicals, such as superoxide ($O_2^{\cdot-}$), hydroxyl radical ($^{\cdot}OH$), perhydroxyl radical ($^{\cdot}OOH$), singlet oxygen, plus H_2O_2 and hypochloric acid (HOCl) are refered as reactive oxygen intermediates.

Nitrative stress in Alzheimer's disease

Contribution of nitrogen-derived oxidative stressor species in neurodegenerative disorders has been also reported. Nitric oxide ($NO^{\cdot}$) is a messenger synthesized in neurons through the activation of Ca^{2+}-dependent neuronal $NO^{\cdot}$ synthase (nNOS) after glutamate receptor stimulation (Garthwaite et al, 1988). Under certain pathophysiological circumstances involving glutamate receptor over-stimulation, $NO^{\cdot}$ biosynthesis may be exacerbated, causing neurotoxicity (Dawson et al, 1996). Moreover, excessive $NO^{\cdot}$ production in the brain leading to mitochondrial impairment and energy depletion is thought to be a contributing factor in the neuronal death observed in certain neurodegenerative diseases (Schulz et al, 1995). *In vitro* experiments of Almeida et al, 1999 using rat primary cortical neurons confirmed that NMDA-subtype glutamate receptors over-stimulation leads to the activation of nitric oxide synthase and thus to enhanced $NO^{\cdot}$ biosynthesis. Elevated levels of nitric oxide consecutively led to mitochondrial depolarization demonstrated as decrease of mitochondrial membrane potential associated with decreased oxygen consumption leading to neuronal ATP depletion. In agreement with this, mitochondrial depolarization in neurons have been claimed to be a target for glutamate-induced neurotoxicity, since accumulation of calcium after short-term exposure to glutamate has been detected (Khodorov et al, 1993). Nitric oxide can react with superoxide, at diffusion controlled rates, to produce an extremely strong oxidant, peroxynitrite ($ONOO^-$) (Beckman, 1996), which is 1000x more potent as an oxidizing compound than hydrogen peroxide. The expression of the inducible form of nitric oxide synthase (iNOS, also referred to as inflammatory NOS, or NOS^{-2}) has been characterized in numerous cell types as a consequence of the inflammatory processes that follow infection, disease, or tissue damage. In brain, glial iNOS expression has been described besides other kinds of diseases also in Alzheimer's disease (Wallace et al, 1997 b). In line with the hypothesis that iNOS may participate in the inflammatory pathomechanisms involved in AD, increased nitrotyrosine staining has been reported in AD brains, indicating sustained exposure and oxidative damage by peroxynitrite, an intermediate NO reaction product (Smith et al, 1997). One of the reasons for increased ROI production, especially superoxide, is that $ONOO^-$ inactivates both Mn and Fe superoxide dismutases, superoxide scavenging enzymes (Ischiropoulos et al, 1992), resulting in less scavenging of superoxide by superoxide dismutase and increased $ONOO^-$ production. $ONOO^{\cdot}$ can also affect cellular energy status by inactivating key mitochondrial enzymes (Radi et al, 1994) and can trigger intracellular calcium release from the mitochondria (Packer and Murphy, 1994).

Calcium homeostasis in Alzheimer's disease

One of the possible reasons of cellular calcium homeostasis dysregulation is impact of oxidative damage to proteins, membranes and nucleic acids, for example, by impairing the function of membrane Ca^{2+}-ATPases and calcium regulated enzymes such as calmodulin (Mark et al, 1997). The well-known role of calcium within the cell is to participate on many signaling pathways as a secondary messenger. Since the signaling pathways request balanced calcium levels, the perturbed calcium homeostasis in turn impairs the ability of cells to respond adaptively to environmental stimuli (Hubka, 2006), resulting in for example reduced glucose uptake. Pathogenic mechanisms that may result in perturbed cellular calcium homeostasis are shared in different disorders of aging. The most obvious shared mechanism involves increased oxidative stress, which can impair the function of key calcium-regulating proteins including Ca^{2+}-ATPases and calcium channels (Mattson, 1998). Experiments of Furukawa et al, 2003 using neuroblastoma cells overexpressing wild-type or N279K or V337M mutated tau revealed equal basal concentration of calcium in all cell lines. However, cells expressing the V337M tau mutation exhibited a much greater increase of Ca^{2+} in response to depolarization compared with cells overexpressing wild-type or N279K mutant forms of tau. Interestingly, treatment of V337M cells with microtubule-stabilizing agent, taxol, completely abrogated the enhanced Ca^{2+} response to depolarization. These data indicate a requirement for microtubule depolymerization in the mechanism whereby the FTDP-17 mutation V337M perturbs cellular calcium homeostasis. Age-related alterations in calcium regulation almost surely contribute to the decline in functional performance of the nervous system during aging. Moreover, by activating proteases and inducing oxidative stress, excessive increases in intracellular calcium levels may promote degeneration of neurons during normal aging (Mattson, 2002).

Impaired energy metabolism in AD

Reduction of ATP levels as well as metabolic defects in AD, have their origin in mitochondrial deficiency in the two key enzymes of the tricarboxylic acid cycle. Deficiency in several key enzymes of oxidative metabolism, including ketoglutarate dehydrogenase complex (KGDHC), pyruvate dehydrogenase complex (PDHC), two enzymes in the rate-limiting step of the tricarboxylic acid cycle, and cytochrome oxidase (COX), the terminal enzyme in the mitochondrial respiration chain that is responsible for reducing molecular oxygen has been found in brains of AD patients, suggesting defects in glucose metabolism (Gibson et al, 1998). Data from positron emission tomography (PET) consistently demonstrate reduced cerebral metabolism in temporal and parietal cortices in AD (Minoshima et al, 1997). Glucose metabolism of individuals with APOE4 genotype is diminished in vulnerable areas several decades before the appearance of clini-

cal manifestations including MCI (Reiman et al, 2004). Moreover, such cerebral metabolic rate abnormalities precede rather than follow any evidence for functional impairment by neuropsychological testing or of brain atrophy by neuroimaging (Baloyannis et al, 2004). Given the fact that impaired energy metabolism well precedes the any clinical symptoms and that oxidative stress is one of the earliest features of the disease, it is likely that mitochondria play a very proximal role in the pathogenesis of the disease (Nunomura et al, 2001). Bowling and Beal (1994) suggest that the age-related onset and progressive course of neurodegenerative diseases may be due to a cycling process between impaired energy metabolism and oxidative stress.

Role of peroxisomes in redox homeostasis

Peroxisomes are organelles with a single membrane present in vertebrate animal cells that compartmentalises enzymes peroxidase, catalase, D-amino acid oxidase, and to a lesser extent, urate oxidase. Their functions are not fully understood, but they participate in metabolic oxidations involving hydrogen peroxide, purine metabolism, cellular lipid metabolism (ß-oxidation of fatty acids) and gluconeogenesis. Peroxidases are a group of enzymes that catalyze oxidation-reduction reactions. As such, they are classified as oxidoreductases. Hydrogen peroxide is generated by superoxide dismutase during degradation of superoxide radical. Peroxidases reduce H_2O_2 to water while oxidizing a variety of substrates. Thus, peroxidases are oxidoreductases, which use H_2O_2 as electron acceptor for catalyzing different oxidative reactions. The overall reaction is as follows:

donor + H_2O_2 <=> oxidized donor + $2H_2O$

Catalase is the marker enzyme for peroxisomes and decomposes the H_2O_2 produced by the peroxisomal oxidases, including the acyl-CoA oxidase of the lipid ß-oxidation pathway (van den Bosch et al, 1992). Conversion of H_2O_2 to water and oxygen is performed by catalase:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Together with superoxide dismutases and the glutathione system, it forms the cellular defense mechanism against oxidative stress (Halliwell and Gutteridge, 1989). In mammalian brain catalase is the main enzyme that catalyzes the peroxidatic oxidation of ethanol to acetaldehyde, and its inhibition is associated with functional and metabolic disturbances of the CNS (Aragon and Amit, 1992). Catalase expression in brain was found significantly reduced in aging (Ciriolo et al, 1997) and as expected also in neurodegenerative disorders, including Alzheimer's disease (Aksenov et al, 1998). Peroxisomal enzymes urate oxidase and NADH oxidase generate superoxide radical as a consequence of the oxidation of their substrates (Fridovich, 1970). Peroxisomes also degrade fatty acids and toxic compounds and catalyze the first two steps in the synthesis of ether phospholipids, which are later used to build membranes. Peroxisomes are responsible for oxidation of long-chain fatty acids and thereby generating acetyl groups. Results of Stamer et al (2002) show, that the loss of peroxisomes from the neurites due to elevated tau expression is as damaging as the direct inhibition of catalase by the catalase inhibitor 3-aminotriazole (3-AT). By the same argument, the exclusion of mitochondria from the cell processes implies a local depletion of ATP. This might be bearable for a compact cell where ATP diffuses throughout the cytoplasm but becomes a problem in extended cell processes.

Intracellular markers of oxidative stress

Because of its high-metabolic rate, the brain is believed to be particularly susceptible to reactive oxygen intermediates (ROI), and the effects of oxidative stress on „post-mitotic cells" such as neurons might be cumulative. Oxidative stress acts on various levels within the cell and all cellular housekeeping macromolecules and structures are found to be impaired. Three major groups of biological molecules were observed modified by oxidative stress:

– nucleic acids (DNA and RNA),
– proteins,
– lipids.

DNA and RNA oxidation

Marker of DNA oxidation is demonstrated by increased levels of 8-hydroxy-2-deoxyguanosine (8-OHdG) and likewise the RNA oxidation marker is manifested by 8-hydroxy-2-guanosine (8-OHG). In a study of AD subjects compared to control subjects, there was a significant three-fold increase in mitochondrial DNA oxidation in parietal cortex in AD (Mecocci et al, 1994). There was a small, but significant, increase in oxidative damage to nuclear DNA, too. Similarly, Nunomura et al, 2004 investigated the oxidized RNA nucleoside 8-OHG levels in the frontal cortex of familial Alzheimer's disease (FAD) patients carrying the mutation in presenilin-1 (PS-1) or amyloid-ß protein precursor (AßPP) gene. They found a significant increase of 8-OHG reactivity in FAD individuals, when compared to controls. However, there was no difference between patients with PS-1 and the AßPP FAD mutation. Interestingly, cases of FAD showed increased levels of neuronal 8-OHG while having a lower percentage area of Aß42 burden. These findings indicate that oxidative stress is an early event involved also in familial AD. Further studies on the oxidative modifications of RNA and mtDNA in AD demonstrated localization to iron rich lysosomes called lipofuscins originating from the mitochondria. This indicates a disruption in mitochondrial function leading to accumulation of redox-active iron in the neuronal cytoplasm (Perry et al, 2002) and subsequent reactive oxygen species generation.

Protein oxidation

Oxidative damage to proteins results in the modification of amino acid residues. Significantly elevated levels of protein car-

bonyl and widespread nitration of tyrosine residues (Smith et al, 1997) were observed in brains of AD patients. Carbonyl derivates are one of the most extensively studied oxidative modifications. Some specifically oxidized proteins have been recently indentified by proteomics (Castegna et al, 2002) and interestingly, many are related to ATP generation. Therefore, oxidative modification may lead to metabolic impairment in AD. Moreover, cross-linking of proteins, by oxidative processes, may lead to the resistance of the lesions to intracellular and extracellular removal even though they are extensively ubiquitinated (Cras et al, 1995) and this resistance of neurofibrillary tangles to proteolysis might play an important role in the progression of AD (Smith, 1998). Equilibrium of oxidized protein level is formed by balance between rates of proteins oxidized and proteins degraded. Proteasomal pathway is the main protein degradation process, including degradation of oxidized and misfolded proteins as well. Aging-dependent loss of proteasomal function was reported in neurodegenerative diseases such as Alzheimer's disease and Huntington's disease. Presence of oxidative stress was also observed. Decrease in proteolytic potential becomes common during aging and goes hand in hand with greater risk for the damage associated with oxidative stress (Friguet et al, 2000).

Modifications of lipids by oxidative stress

During lipid peroxidation the non-saturated carbohydrate fatty acid side chains of membrane phospholipids are peroxidized, by formation of lipid peroxides. Peroxidation of cellular membrane lipids generates highly reactive aldehydes, such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA). Increased levels of 4-HNE have been observed in AD but also in PD, ALS and other neurodegenerative pathologies, confirming a pathophysiological role of oxidative stress in these diseases (Zarkovic, 2003). Increased concentrations of 4-HNE can further enhance cross-linking of damaged proteins. Conjugates of 4-HNE and protein have been reported to inhibit proteosomal function (Friguet and Szweda, 1997), and the proteosome itself might be target by direct attachment of 4-HNE to the proteasomes (Okada et al, 1999). Hansen et al, 1998 found that histones folding and protecting DNA contain significant amount of lysine and arginine (30—40 %). A reactive endproduct of lipid peroxidation, 4-hydroxynonenal can form a covalent adduct with cysteine, lysine, or histidine residues (Esterbauer et al, 1991). Since, 4-HNE has been found to be increased in AD brain, the glutathione transferase, an enzyme that protects neurons from 4-HNE damage has decreased activity in brains of AD patients (Lovell et al, 1998 b). Decreased ability of DNA to associate with the histones *in vitro* was observed by Drake et al (2004). This inhibition effect was caused by addition of 4-HNE, which binds to histones, altering the conformation of the histones and affecting the ability of histones to bind DNA. Additionally another, specific markers of lipid peroxidation are isoprostanes, which are isomers of the conventional enzymatically derived prostaglandins. Increased levels of specific isoprostane (iso-iPF2-VI) were detected as in brain regions of APP2576 mice as well as in plasma, urines and CSF of subjects with probable AD (Pracito et al, 2000; 2001), proposing an AD specific marker after oxidative stress exposure. Ultimately, the altered phospholipid composition as a result of oxidative stressors insult plays important role in intracellular pathology.

Peripheral markers of oxidative stress

Significant biological changes related to a condition of oxidative stress have been found not only in brain tissue, but also in peripheral tissue of AD individuals. Recently, increasing are studies dealing with the search for soluble peripheral biomarkers of oxidative stress in biological fluids, mainly cerebrospinal fluid (CSF), but also peripheral blood (serum/plasma) or urines or even in peripheral tissues themselves such as fibroblasts or blood cells. Cecchi et al (2002) showed clear increase in lipoperoxidation products, malondialdehyde (MDA), and 4-hydroxynonenal in fibroblasts and lymphoblasts of familial Alzheimer's disease (FAD) patients, compared to controls. 4-HNE was found increased in the CSF (Lovell et al, 1997) and plasma of AD patients (McGrath et al, 2001). Elevated levels of F2-isoprostane were observed in CSF (Montine et al, 1999), plasma (Waddington et al, 1999) and urines (Tuppo et al, 2001) of AD individuals. Using HPLC analysis Mecocci et al (1998; 2002) found a significantly higher lymphocyte concentration of the oxidized purine 8-hydroxy-2-deoxyguanosine (8-OHdG) at DNA level, besides a significantly lower plasma levels of antioxidants in AD compared to controls. Licastro et al (2001) detected increased glutathione peroxidase activity in plasma and erythrocytes of AD patients.

Tau protein and oxidative stress

An increase in oxidative stress is implicated not only in aging diseases of the brain, but in other organ systems as well, suggesting that oxidative stress is a normal by-product of aging and not a primary contributor to the development of AD. Nevertheless, through facilitating tau filament assembly (Gamblin et al, 2000), or by diminishing the capacity of the cell to degrade tau filaments, oxidative damage could contribute to tau-associated neurodegeneration. Study of Stamer et al (2002) showed, that overexpression of tau can adversely affect the ability of microtubule motors to traffic cellular organelles and vesicles along the microtubules. What is in direct relationship with enhanced oxidative stress susceptibility of tau overexpressing neuron, through lowered distribution of free radical scavenging organelles along neuronal processes. Analysis of brain proteome in P301L transgenic mice by David et al, 2005 revealed some down-regulated antioxidant enzymes (thioredoxin-dependent peroxide reductase, glutathione S-transferase and phospholipid hydroperoxide glutathione peroxidase (PHGPx). Alterations in the mitochondrial electron transport chain and up-regulated synaptic vesicle associated proteins were also reported. These data suggest mitochondrial dysfunction and compensatory changes related to mutated tau inhibition of transport along neurites. Tau

isoforms were found hypophosphorylated after short-term H2O2 treatment in hippocampal neurons cultured in vitro. Results of Zambrano et al, 2004 indicate that H_2O_2 treatment produces decrease in the phosphorylation levels of tau protein at epitopes (AT8, PHF-1) that have been shown to be dependent on cdk5 activity. Surprisingly, cdk5 activity was detected increased when compared to untreated controls. Thus, dephosphorylation of tau protein could indicate an initial response of cells against oxidative insults. Moreover, chronic and cumulative oxidative stress conditions in combination with other factors should favor cdk5 activity on tau protein that, together with other kinases, could lead to increased tau phosphorylation. Both tau and neurofilament protein appear uniquely adapted to oxidative attack due to their high content of lysine-serine-proline (KSP) domains. Exposure of these domains on the protein surface is affected by extensive phosphorylation of serine residues, resulting in an oxidative sponge of surface-modifiable lysine residues (Wataya et al, 2002). Since phosphorylation plays this pivotal role in redox balance, it is perhaps not surprising that oxidative stress, through activation of MAP kinase pathways, leads to phosphorylation (Zhu et al, 2000). Therefore, conditions associated with chronic oxidative stress are invariably associated with the extensive phosphorylation of cytoskeletal elements (Zhu et al, 2004). In the last few years, some research groups speculate with hypothesis of protective role for tau phosphorylation. This is supported by many facts including finding that embryonic neurons that survive after treatment with oxidants have more phospho-tau immunoreactivity relative to those that die (Ekinci and Shea, 2000). Findings of Nunomura et al, 2001 indicate that oxidative stress occurs early in Alzheimer's disease, significantly before the development of the pathology hallmarks, like neurofibrillary tangles and senile plaques. These relationships are more significant in ApoE4 allele carriers. Surprisingly, they found that increases in Aß deposition are associated with decreased oxidative damage. Moreover, neurons with neurofibrillary tangles (NFT) show a 40–56 % decrease in relative 8-OHdG levels compared with neurons free of NFT. These findings suggest that AD is associated with compensatory changes that reduce damage from reactive oxygen. However, functional exclusion of NFTs bearing neuron from "communication" is also possible, since Morsch et al (1999) calculated that this way affected neurons appear to survive for decades. By other words, impaired neurons might less participate on signal transduction, their function and metabolic activity may be lowered and oxidative stressors production as well decreased. Heme oxygenase-1 (HO-1) is an enzyme which catalyses the breakdown of heme to biliverdin with release of iron and carbon monoxide. HO-1 is over-expressed in the brains of AD patients. So it could be postulated that this would be one source of redox-active iron. Immunostaining of neurons demonstrated that HO-1 expression was co-localized to the neurofibrillary tangles (Schipper, 2004). However oxidative damage to nucleic acids was reduced in neurons exhibiting neurofibrillary tangles demonstrating an antioxidant mechanism for HO-1 and an involvement in tau proteins expression. Oxidative stress has been found increasingly implicated in a number of neurodegenerative disorders including AD, Parkinson,s disease (PD), amyotrophic lateral sclerosis (ALS), and others (Perry et al, 2002). Certainly there is enough evidence accumulated to suggest that mitochondrial abnormalities contribute to AD, oxidative stress and altered cellular respiration can therefore partially explain the genetic component of AD and the tauopathies. Moreover, caspases have also been shown to play a role in tau filament formation. Carboxy-terminal cleavage of tau by caspase 3, 8 or 9 can accelerate the rate of tau filament assembly in vitro (Gamblin et al, 2003).

Amyloid-ß and oxidative stress

It is also possible that the proteins that are involved in protofibril formation might themselves produce oxidative stress. Amyloid-ß has been demonstrated to produce H_2O_2 in cultured cells through metal ion reduction (Behl et al, 1994; Huang et al, 1999). Keil et al, 2004 investigated the effects of APP with double swedish mutation (K670M/N671L) expressed in PC12 cells. APPsw PC12 cells showed increased NO levels, decreased cytochrome C oxidase activity and reduced ATP levels. These data suggest possible links of amyloid ß-peptide and NO production with mitochondria failure. F2-isoprostanes, markers of oxidized prostaglandins, have also been shown to stimulate both generation (Qin et al, 2003) and aggregation (Boutaud et al, 2002) of Aß, possibly placing isoprostane accumulation upstream of Aß aggregation. This findings support the hypothesis that accumulation of Aß (and tau) is a result of oxidative stress, and that the pathological abnormalities attributed to oxidative stress precede both tau and ß-amyloid pathology (Smith et al, 2002). Research of novel therapeutic approaches centred on chelators with specificity for copper and iron ions is recently rising. Opazo et al, 2002 reported that Aß binds Cu^{2+} with very high affinity forming an allosterically cooperative Cu^{2+} coordination site that resembles superoxide dismutase 1. The Aß-Cu^{2+} complex is redox-active and produces H_2O_2 from O_2 through the reduction of Cu^{2+}. The metal dependence of the generation of H_2O_2 by Aß may be a target for AD therapeutics. Cu and Zn are markedly elevated in amyloid plaques (Lovell et al, 1998 a). Therefore it is significant that H_2O_2 generation by Aß in vitro is abolished by chelators (Huang et al, 1999), such as clioquinol, being reported recently, has in vivo efficacy in blocking brain Aß accumulation in transgenic APP2576 mice (Cherny et al, 2001).

Glutathione system

Glutathione (GSH) is employed in a number of cellular processes since, it is a very potent intracellular reductant, participating in catalysis, metabolism, transport and in the protection of cells against foreign compounds, free radicals and reactive oxygen compounds. It is the most abundant thiol-reducing agent in cells. GSH is a tripeptide that consists of glutamate, cysteine and glycine. It is an important antioxidant and essential cofactor for antioxidant enzymes. GSH is synthesized intracellularly by

consecutive actions of gamma-glutamyl cysteine synthetase (reaction 1) and GSH synthetase (reaction 2) (Anderson, 1997):

L-glutamate + L-cysteine + ATP ↔ L-gamma-glutamyl-L-cysteine + ADP + Pi (1),
L-gamma-glutamyl-L-cysteine + glycine + ATP glutathione + ADP + Pi (2).

Glutathione peroxidase (GSHPx) is found in the cytoplasm and mitochondria, which acts in concert with GSH to reduce H_2O_2 to H_2O. During this process GSH is oxidized to produce GSSG (oxidized GSH). GSHPx uses glutathione as a electron donor and is active with both hydrogen peroxide and organic hydroperoxide substrates. Glutathione reductase (GSHRd) is an NADPH-dependent enzyme that is involved in reduction of oxidized glutathione (GSSG) back to GSH. In glial and neuronal cells, glutamate induces GSH depletion and consequently apoptosis through endogenously produced active oxygen species, and apoptosis is accompanied by nucleosomal DNA fragmentation (Higuchi, 2004). Such apoptosis is also induced under GSH depletion, induced by L-buthionine-sulfoximine (BSO), an inhibitor of gamma-glutamyl cysteine synthetase (Higuchi and Matsukawa, 1999). As indicated above, function of glutathione system is to detoxify cellular peroxides and the ability of GSH strongly depends on activities of enzymes involved in glutathione generation and recycling maitenance. Interestingly, some contradictory results of activities and levels of glutathione peroxidase and glutathione reductase were reported in affected brain regions of AD patients (Pappolla et al, 1992; Lovell et al, 1995), suggesting impaired or upregulated antioxidative defence of neurons against oxidative stress. Together these data imply that oxidative stress plays an important role in the pathogenic process but that alterations in the glutathione system are secondary to other events leading to neurodegeneration.

Antioxidant therapies

Many reports showing decreased or unchanged vitamin levels in plasma, CSF or brain regions of AD patients have been published to date. Therefore, many studies of antioxidant treatments and increased dietary intakes of single vitamins have been performed over the past years. Nevertheless, the results coming from supplementation of antioxidants and increased dietary intake of vitamins in AD patients showed positive, but some neutral effects, too. Moreover enhanced toxicity at higher levels of antioxidants was also reported. Since, for example vitamin C is a very potent antioxidant, but under certain conditions behaves as a dangerous pro-oxidant. Combinations of vitamins, minerals and herbal antioxidants are likely to offer greater potential benefit for AD than any single antioxidant, especially if the agents work in different cellular compartments or have complementary mechanisms of action. Still, the optimizing steps of the dose, possible interaction toxicity or loss of efficacy studies will be necessary and crucial for treatment (Grundman and Delaney, 2002). Nevertheless, antioxidant therapy in Alzheimer's disease patients might be beneficial and can probably delay the disease progression, but the promises preventing the ongoing pathologic cascade of the disease are rather poor. Oxidative stress is common in many disorders, therefore we can not expect that treatment of this site of Alzheimer's disease will result in successful therapy. Pathophysiology of neurodegenerative diseases involves multiple pathways of neuronal damage, and therefore oxidative stress might take a place among multiple factors in this hypothesis. Nevertheless, it is insufficient as a driving force of neurodegeneration and an additional hit would be necessary for the onset of pathogenesis (Smith et al, 2005).

However, the importance of oxidative stress in disease pathogenesis is perhaps best highlighted by the fact that the risk factors for disease including age (Floyd and Hensley, 2002), apolipoprotein E genotype (Miyata and Smith, 1996) and amyloid-ß protein precursor and presenilin mutations (Cecchi et al, 2002; Marques et al, 2003) directly cause alterations in redox homeostasis. The contribution of oxidative stress to disease initiation in sporadic cases of AD is unclear and bears more questions about the mechanism of neurodegeneration (King, 2005).

Conclusions

Oxidative and nitrative stress is common hallmark of many, not only of neurodegenerative diseases. They have been implicated in the pathogenesis of Alzheimer's disease as a relevant marker of neuronal degeneration, what it supported by several animal models. Two most important subcellular organelles (mitochondria and peroxisomes) are involved as in generating of reactive oxidative radicals, subsequent regulation of threshold levels as in final scavenging of harmful agents. While it is unclear what is the initial source of oxidative stress in Alzheimer's disease, it is likely that the process is highly dependent on redox-active transition metals such as iron and copper, which are thought to play very important roles in homeostasis of intracellular processes, generating reactive radical species. Oxidative stress is one common component of a number of aging-related diseases. Cellular respiration leads to an increase in the production of reactive oxygen and nitrogen species. Such aggressive free radicals impinge upon the numerous proteins, lipids and molecules inside the cell. Over time, the cell's ability to compensate for the production of ROI/RNS is diminished, leading to an accumulation of abnormal proteins, a reduction in respiration and often activation of apoptosis. Brain metabolism was found diminished in AD and key mitochondrial and peroxisomal enzymes were shown reduced in brain as a consequence of oxidative stress. Tau and amyloid-ß, hallmark proteins of Alzheimer's disease, play important physiological roles related to oxidative stress. Observations of some research groups indicate that increased oxidative damage is an early event in AD that decreases with disease progression and lesion formation. These findings

correlate with decreased cellular respiration, which is major producer of oxidative stressors. Despite of not targeting direct cause of neurodegenerative disease, antioxidant therapies might have beneficial effects on oxidative homeostasis and can delay the onset of rapid pathological changes resulting in irreversible degeneratiohn of neurons.

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