Oxidative damage in Alzheimer’s disease

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Abbreviations

4-hydroxy-2,3-nonenal (HNE)
8-hydroxyguanine (8-OHG)
8-oxo-2’-deoxyguanosine (8-OHdG)
advanced glycation end products (AGEs)
Alzheimer’s disease (AD)
amyloid precursor protein (APP)
amyloid-β peptide (Aβ)
anion radical (O₂⁻)
apolipoprotein E (ApoE)
beta-secretase 1 (BACE1)
central nervous system (CNS)
cerebrospinal fluid (CSF)
creatine kinase (CK)
early onset Alzheimer’s disease (EOAD)
glutamine synthase (GS)
hydroxyl radical (OH)
inducible nitric oxide synthase (iNOS)
late onset Alzheimer’s disease (LOAD)
lipoxygenase enzyme (LOX)
low density lipoprotein (LDL)
malondialdehyde (MDA)
microtubuli-associated protein tau (MAP-tau)
mitochondrial DNA (mDNA)
neurofibrillary tangles (NFTs)
neuronal nitric oxide synthase (nNOS)
nitric oxide (NO)
N-methyl-D-aspartate receptor (NMDAR)
nuclear DNA (nDNA)
nuclear transcription factor kappaB (NF-κB)
paraoxonase 1 (PON1)
polyunsaturated fatty acids (PUFAs)
presenilin-1 (PS-1)
presenilin-2 (PS-2)
reactive nitrogen species (RNS)
reactive oxygen species (ROS)
superoxide dismutase (SOD)
thiobarbituric acid-reactive substance (TBARS)

Abstract

Alzheimer’s disease (AD) is the most common age-related neurodegenerative disease characterized by progressive loss of cognitive and intellectual functions, especially memory. The major neuropathologic hallmarks of AD consist of the presence of amyloid plaques and neurofibrillary tangles, and neuronal cell death. Convincing evidence demonstrates that oxidative stress is an early event in AD, and therefore may play a key pathogenic role in the disease. Indeed, the oxidative damage is evidenced by the large number of metabolic signs of oxidative stress as well as by biomarkers of oxidative damage to lipids, proteins, DNA and RNA, which are present in AD patients. Altered gene expression, heavy metals and the excessive production of nitric oxide are also implicated in AD pathogenesis. But the associations between these markers of free radical damage and the pathogenic cascades involved in AD are complex. The aim of this review is to focus on the involvement of oxidative stress in AD and to discuss its role in the progression of the disease.
Keywords

Alzheimer’s disease; oxidative stress; oxidative damage; peroxidation; protein oxidation; glycoxidation; DNA oxidation; heavy metals; nitrosative stress

Introduction

Dementia is a brain disorder that gravely affects a person's ability to carry out daily activities. The most common form of dementia among older people is AD, which involves the parts of the brain that control thought, memory, and language.

The socioeconomic costs of AD are a very serious and growing concern as our elderly currently represent the fastest growing segment of Western populations. Epidemiological studies show that globally, about 25 million people today have AD, with approximately 5 million new cases of dementia occurring every year, and with one new case being reported every 7 seconds\[^1, 2\]. Worldwide, the total number of people affected by AD is expected to double every 20 years to at least 81 million by 2040 with Western civilizations and developing countries at particular risk\[^1, 2\].

The two typical lesions that define AD are senile plaques composed of amyloid-β peptide (Aβ) and neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau\[^3\]. Senile plaques are observed in the interstice, between neurons. They measure between 20 and 100 microns and consist of a core which principal component is Aβ. This core is surrounded by a nest formed by degenerating neurites, activated microglia and astrocytes. Other substances that form the senile plaques are the alpha-synuclein (principal not amyloid component), alpha 1 antichymotrypsin, alpha 2 macroglobulin, apolipoprotein E (ApoE),
ubiquitin and the presenilins. Degenerative neurons are also distinguished around but not in contact with the plaques. Neurons present an accumulation of flame-shaped inclusions and sometimes form an elongated basket around the nucleus. The inclusions fill the cytoplasm, particularly in the soma and apical dendrite causing neuronal death mainly by apoptosis. Finally there are only remnants of the cytoskeleton. The NFTs are distributed in very characteristics areas as the entorhinal and perirhinal allocortex, hippocampus and the amygdala. The NFTs coincide with the senile plaques in frontotemporal regions but their presence is lower in not limbic structures. Besides those pathological hallmarks of the disease, AD brains show constant evidence of reactive oxygen species (ROS)- and reactive nitrogen species (RNS)-mediated injury[4].

This review focuses on the involvement of oxidative stress in AD pathology and discusses their potential interplay in its progression.

**Oxidative stress and AD**

In some circumstances the production of oxidant species can exceed the endogenous antioxidant ability to destroy them and an oxidative imbalance occurs. This event results in cellular oxidative stress and subsequent molecular oxidative damage, which can translate into altered cellular functions and, as final result, cell death[5].

The central nervous system (CNS) is very prone to oxidative imbalance because it is very rich in polyunsaturated fatty acids (PUFAs), has a high metabolic oxidative rate and has a high content of transition metals and ascorbate levels, which together act as potent pro-oxidants.
The source of oxidant species in the CNS includes altered mitochondrial function, the Aβ peptides themselves and the presence of unbound transition metals\cite{6}. All of these factors are not independent from each other, and it is plausible that, especially in the early stages of the disease process, Aβ could enter the mitochondria where it would increase the generation of ROS and induce oxidative stress.

Depending on the substrate attacked by ROS, oxidative stress will manifest as protein, DNA, RNA oxidation or lipid peroxidation. All of these signature markers of oxidative stress have been described in the AD brain, and this concept has been originally used to support the “oxidative stress hypothesis” of AD\cite{7-9}.

\textit{Lipid peroxidation in AD}

The reaction of the lipids with molecular oxygen is a process known as lipid peroxidation, a process that involves intermediate oxygen containing free radicals which attack hydrogen atoms from lipids, mainly polyunsaturated lipids, to form a peroxyl radical\cite{10}. The results of the lipid peroxidation could be structural damage to cellular membranes and the generation of oxidized products, some of which are chemically reactive and covalently modify macromolecules thought to be the main effectors of tissue damage\cite{11}. In blood circulation, the oxidation of unsaturated fatty acids carried in lipoproteins is believed to play a key role in the development and progression of AD\cite{12}. Senile plaques, NFTs and loss of neurons in the brains of AD are the results of abnormalities in lipid peroxidation and metabolism that may be caused, or increased by Aβ\cite{13, 14}. 
The extent of lipid peroxidation can be quantitatively assessed by measuring the levels of its end-products, e.g. malondialdehyde (MDA), by the thiobarbituric acid-reactive substance (TBARS) test, aldehydes and isoprostanes\(^{[15]}\).

The majority of studies have demonstrated higher MDA and/or TBARS levels in AD brains\(^{[16]}\), although others studies have found a reduction or no alterations\(^{[17, 18]}\). Some researchers have demonstrated a clear increase in TBARS production in the erythrocytes\(^{[19]}\), and in the MDA levels in the fibroblasts and lymphocytes\(^{[20]}\), but the plasma MDA levels in AD were found to be normal\(^{[21]}\).

The oxidation of PUFAs results in the production of multiple aldehydes, e.g. 4-hydroxy-2,3-nonenal (HNE), which can modify proteins, promoting protein aggregation\(^{[22]}\). Analysis of AD brains demonstrates an increase in free HNE in amygdala, hippocampus, and parahippocampal gyrus compared with age-matched controls\(^{[23]}\). This increased concentration corresponds with the regions showing the most striking histopathologic alterations in AD. A significant elevation of free HNE in ventricular cerebrospinal fluid (CSF) and serum provides a potential biomarker for AD\(^{[24, 25]}\). Moreover, HNE is elevated in neurons treated with Aβ(1–42)\(^{[26, 27]}\). HNE is able to inhibit the neuronal glucose and glutamate transporters, inhibition of Na-K ATPases, activation of kinases and dysregulation of intracellular calcium signalling, that ultimately induce an apoptotic cascade mechanism\(^{[28, 29]}\). Evidence shows that HNE are the major cytotoxic products of lipid peroxidation. Following lipid peroxidation, a 2-pentylpyrrole modification of lysine is the only presently known adduct that forms from the modification of proteins by HNE in AD cases. These findings, together with the recent demonstration that HNE is cytotoxic to neurons and that it impairs the function of membrane proteins including the neuronal
glucose transporter GLUT 3, indicate that HNE is a characteristic marker and a toxin leading to neurodegeneration in AD[30].

Isoprostan es are isomers of prostaglandins and are chemically stable end-products of the ROS-catalyzed oxidation of PUFAs. Increased levels of F2-isoprostan es have been detected in the AD brain[31] and in the urine of AD patients[31]. Enhanced levels of 8,12-iso-iPF2α-VI and iPF2α-III as specific F2-isoprostan es have also been observed in the CSF, blood and urine of AD individuals[32, 33], although other researchers could not confirm these findings as concerns the plasma and urine F2-isoprostan e concentrations[34].

ApoE is the principal apolipoprotein in the central nervous system, and it is the major apolipoprotein that is able to the lipid transportation and regulation of lipid metabolism. ApoE-dependent dendritic and synaptic regeneration may be less efficient with ApoE4, and this may result in age-related neurodegenerative changes. The increased risk of AD associated with ApoE4 may be modulated by diet, vascular risk factors, and genetic polymorphisms that affect the function of other transporter proteins and enzymes implicated in brain lipid homeostasis[35, 36]. Moreover, inheritance of the ApoE4 allele represents the strongest genetic risk factor for sporadic AD. Evidences suggest that ApoE isoforms may influence the cellular distribution of lipid peroxidation products in brain and may therefore contribute to the risk of AD associated with ApoE4[37]. Furthermore, the 12/15 lipoxygenase enzyme (12/15LOX) is increased in affected frontal and temporal regions of AD brains, and the activation of this enzyme occurs early in the course of AD, before the onset of dementia, implicating 12/15LOX-mediated lipid peroxidation in the pathogenesis of AD[38].
Protein oxidation in AD

Oxidative modifications of proteins are important in aging and age-related neurodegenerative diseases like AD\cite{7,39}, and research of protein oxidation is therefore essential for an understanding of how oxidative stress affects cellular functions and eventually leads to neuronal death.

ROS can attack protein side-chains and it results in the introduction of hydroxyl groups or in the generation of protein based carbonyls. Carbonyl groups are introduced in proteins by oxidizing amino acid residue side-chain hydroxyls into ketone or aldehyde derivatives\cite{40}. A variety of oxidative pathways lead to carbonylation of proteins\cite{41}. Carbonyl groups can also be introduced in proteins by direct oxidation of lysine, arginine, proline and threonine residues.

Increased reactive protein carbonyls were the first form of oxidative damage identified in AD\cite{42}, carbonyl-based damage was then detected in both senile plaques\cite{43,44} and NFTs\cite{43,45}, and in the primary component of tau protein\cite{45,46}. Protein oxidation is linked to the AD brain by an increase in carbonylated modified proteins\cite{47}. Studies have shown an increase in protein carbonyls in the hippocampus and parietal cortex, but not in the cerebellum, where there is less significant AD pathology\cite{47}.

The first use of proteomics to identify specifically oxidized proteins in the AD brain indicated several proteins as creatine kinase (CK) and glutamine synthase (GS)\cite{48}. These proteins are more affected by oxidation in AD, and consequently more prone to inactivation, representing a significant step in linking AD neurodegeneration with oxidative stress. Excess of protein oxidation and decreased activities of GS and CK have been found in the frontal and occipital lobes of normal aged subjects and AD patients as compared with
young controls\textsuperscript{[49]}. The finding that the GS activity was reduced in the frontal lobe in AD has suggested that the characteristic neuropathology of AD may represent a specific vulnerability of brain cells or areas to age-related oxidation. In accordance with this idea, the hippocampus and inferior parietal lobe in Alzheimer's patients had higher protein carbonyl contents and lower GS and CK activities than the cerebellum\textsuperscript{[47, 50]}, reflecting again that the increased of protein oxidation correlates with the AD histopathology.

Low density lipoprotein (LDL) oxidation is thought to be an important mechanism in many degenerative diseases including AD\textsuperscript{[51]}. Paraoxonase 1 (PON1) is a calcium-dependent esterase, which contributes to the antioxidant protection conferred by high density lipoprotein on LDL oxidation. In addition, PON1 is a potent cholinesterase inhibitor and an arylesterase that hydrolyzes paraoxon, an active toxic metabolite of parathion, thus to providing protection against organophosphate poisoning and metabolization of environmental neurotoxins that might be responsible for neurodegeneration with aging. It has been suggested that paraoxonase activity was significantly lower in AD patients\textsuperscript{[18]}. Increased protein nitration in the AD brain supports the notion that nitrosative stress also contributes to neurodegeneration in AD\textsuperscript{[52-54]}. Protein nitration also increases the susceptibility of brain proteins to proteasomal degradation\textsuperscript{[55]}. The overexpression of inducible nitric oxide synthase (iNOS) and neuronal nitric oxide synthase (nNOS) could be responsible for increased levels of RNS. Increased levels of nitrated proteins have been reported to be present in AD brain and CSF, implying a role for RNS in AD\textsuperscript{[52, 53, 56]}. Another potential mechanism of protein oxidation involves the toxic radical peroxynitrite, formed in the reaction of the superoxide anion with nitric oxide (NO)\textsuperscript{[57]}. Peroxynitrite formation may occur under conditions of oxidative stress in combination with the ongoing synthesis of NO\textsuperscript{[58, 59]}. Although the mechanism is not universally accepted, 3-NT
formation is thought to occur by the reaction of peroxynitrite with tyrosine residues of proteins[^57]. 3-NT and o-tyrosine are typical carbonyl end-products that are resistant to acid hydrolysis and are not normally present in proteins but are widely used as markers of protein oxidation[^15]. An increase in 3-NT immunoreactivity in neurons from AD brain was observed[^60], as well as elevated dityrosine and 3-NT levels in hippocampus, inferior parietal lobule, and neocortical regions of the AD brain and in ventricular CSF compared with aged matched controls[^60, 61]. Surprisingly, the level of 3-NT was found to decrease with the course of AD and lesion formation, suggesting that the histopathology of AD is linked to compensatory intracellular changes against oxidative stress[^62]. These observations indicate that oxidative stress on proteins is an early event in AD pathogenesis.

**Glycoxidation in AD**

Glycation has been held responsible for many age-related diseases[^63, 64]. Glycation of proteins starts as a non-enzymatic reaction with the spontaneous condensation of ketone or aldehyde groups of sugars with a free amino acid group to form a labile Schiff base. Then, a cascade of reactions results in the formation of advanced glycation end products (AGEs), e.g. pentosidine and N-epsilon-carboxymethyl-lysine[^65], which are composed of irreversibly cross-linked heterogeneous protein aggregates. AGE receptor, known as RAGE, is also a β-amyloid receptor[^64, 66]. The combination of AGE and RAGE can cause oxidative stress, as shown in the production of thiobarbituric acid-reactive substances, heme oxygenase-1, and the activation of nuclear transcription factor kappaB (NF-κB). β-amyloid binding with RAGE also induce the expression of macrophage-colony stimulating factor, which mediates microglial activation[^67, 68]. This introduces an inflammatory
pathway, according to a procedure linking oxidative processes and inflammation in AD\cite{69,70}. Furthermore, increased amounts of AGEs were noted in senile plaques and NFTs in AD\cite{44,71}, whereas the brain pentosidine and N-epsilon-carboxymethyl-lysine levels were demonstrated to be comparable in AD patients and controls\cite{72}, refuting the glycation hypothesis.

Extracellular accumulation of AGEs has been demonstrated in senile plaques in different cortical areas in primitive plaques and coronas of classic plaques, and this accumulation in AD is caused by an accelerated oxidation of glycated proteins (glycoxidation)\cite{73}. The major component of the NFTs, the microtubuli-associated protein tau (MAP-tau) has been shown to be subject to intracellular AGEs formation. MAP-tau can be glycated \textit{in vitro}, inhibiting its ability to bind to microtubules. In addition, MAP-tau isolated from brains of AD patients is glycated in the tubulin-binding region, giving rise to the formation of \textbeta-sheet fibrils\cite{74,75}. Some studies have shown the presence of AGEs in association with two major proteins of AD, A\textbeta\cite{44} and MAP-tau\cite{45,76}. This observation supports the argument that AGEs are implicated in the pathogenesis of AD\cite{77}.

In the AD plasma, elevated amounts of oxidized proteins\cite{78} and an enhanced protein glycation rate\cite{79} have also been reported, and AD patients exhibited increased populations of leukocytes expressing binding sites for monoclonal antibodies against RAGEs, A\textbeta and amyloid precursor protein (APP)\cite{80}. In contrast, elevated levels of AGE-associated parameters were not detected in AD blood, reflecting that the alterations in the CNS are not manifested in the periphery as regards AGEs\cite{81}.
ROS can also cause oxidative damage to nuclear and mitochondrial DNA. It includes base alterations, deoxyribose oxidation, single and double strand breaks, sister chromatid exchanges and DNA-protein crosslinks\[82\]. ROS, particularly hydroxyl radicals, interact with DNA generating various products of DNA bases, such as 8-oxo-2'-deoxyguanosine (8-OHdG), 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 8,5'-cyclo-2'-deoxyguanosine, 8-hydroxyadenine, 2-hydroxyadenine, thymine glycol, and cytosine glycol\[83\]. Guanine, because it has the lowest oxidation potential of the four DNA bases, is the most readily oxidized base and therefore the most commonly used analysis of DNA oxidation. 8-OHdG is the most predominant marker of in vivo oxidative DNA damage resulting from OH· attack on deoxyguanosine and can be measured by means of high-performance liquid chromatography with electrochemical detection or gas chromatography/mass spectrometry techniques\[15, 84\].

Increased oxidative damage to mitochondrial DNA (mDNA) has been observed in aging and in various regions of AD brain, indicating a role of DNA oxidation in AD pathology\[85\]. Similarly, nuclear DNA damage by ROS is increased in AD\[86, 87\]. Evidence also shows that oxidized DNA products are increased in the CSF of AD patients\[88\]. Previous studies have demonstrated a 2-fold increase in DNA strand breaks in AD brain that consequently results in depletion of energy stores and cell death\[89\]. DNA oxidation has been shown to rise with age, shown by an increase in 8-OHdG in the cerebral cortex and cerebellum brain regions\[90\]. Similarly both mDNA and nuclear DNA (nDNA) have increased 8-hydroxyguanine (8-OHG), 8-hydroxyadenine, and 5-hydroxymethyluracil in temporal, parietal and frontal lobes in AD brain\[86, 90\]. Overall, mDNA shows an approximately 10-fold higher
intensity of oxidized bases than nDNA. This result demonstrates that nDNA and mDNA are undergone to extensive oxidative damage in AD, which may contribute to the neurodegenerative pathology of this disease.

A direct effect of Aβ on DNA oxidation was also observed in neuronal PC12 cells. It has been suggested that free radicals generated by Aβ cause oxidative DNA alterations, including purine dimers[91].

An examination of the link between the extents of 8-OHdG, 8-OHG and the AD lesions at early AD stages indicated that markers of oxidative stress are present in those susceptible neurons without NFTs[92], confirming the primacy of oxidative stress, occurring decades prior to the disease onset. On the other hand, neurons containing NFTs showed a decrease in relative 8-OHG level as compared with neurons free of NFTs. These observations also point to that oxidative damage is an early event in the pathogenesis of AD, and that it decreases with disease progression, suggesting compensatory intracellular mechanisms that reduce oxidative stress[62].

Studies have shown 30-70% oxidation of the mitochondrial RNA in the frontal cortex of the AD brain in comparison to 2% oxidation in age-matched controls[93]. In addition, a specific increase in ribosomal RNA oxidation has also been shown in the inferior parietal lobule of AD brain compared to age-matched controls[94]. Increased levels of 8-OHG have also been reported in the hippocampus and cerebral neocortex of the AD brain, whereas the 8-OHG level in the cerebellum was not significantly altered compared with controls[92, 94, 95]. An increase in 8-OHdG has been identified not only in brain tissue but also in CSF from AD subjects[96]. RNA oxidation in the AD brain could render the cell incapable of initiating protein synthesis, hindering the cell's defense against further oxidative damage, an effect observed in AD[94].
Altered gene expression and oxidative stress in AD

The greater number of AD cases occur in the elderly, however, it is still unresolved whether AD is a disease of old age or whether it has earlier beginnings. Epidemiological studies have shown that patients with dementia are more likely to have had low scores on intelligence tests when they were children compared to people without dementia\[97\]. These findings show that AD patients may arrive at old age with significant predisposing deficits. Some studies point to a possible role for genetic changes on AD etiology.

Structural genomics studies have demonstrated that more than 200 genes might be involved in AD pathogenesis\[98\]. Also, the AD population presents a higher absolute genetic variation rate of 40–60% and AD patients differ in their genomic architecture from patients with other forms of dementia\[98\]. 5-10% of AD cases are of familial origin and involve mutations in genes associated with APP biosynthesis and proteolytic processing\[99-101\]. The genetics of AD have revealed that early (<60 years of age) onset AD (EOAD) is associated with APP or the presenilins, whereas the risk to develop late onset AD (LOAD) is linked to an ApoE polymorphism\[102\].

Genetic mutations leading to familial AD, EOAD and LOAD are also oxidative stress-causing mutations. Elevated vulnerability to oxidative stress-induced cell death and/or reduced antioxidant defenses have been demonstrated in cell lines expressing mutant human APP, presenilin-1 (PS-1), or presenilin-2 (PS-2)\[103-105\]; in transgenic mice expressing mutant human APP and/or PS-1 as well as knock-in mice expressing mutant human PS-1\[106-111\]; in fibroblasts and lymphoblasts from patients with familial AD with APP or PS-1 gene mutations\[20\]; and in cerebral cortex of autopsied brain samples from AD patients with APP or PS-1 gene mutations\[112, 113\]. Furthermore, increased PS-2 expression
increases DNA fragmentation and produces apoptotic changes\cite{114}, which are both important consequences of oxidative stress. Moreover, the possession of one or both ApoE ε4 alleles, a major genetic risk factor for LOAD and sporadic AD\cite{115}, is associated with oxidative stress. *In vitro*, ApoE shows allele specific antioxidant activity, with ApoE ε2 the most effective and ApoE ε4 the least effective\cite{116}. Oxidative injury in an ApoE genotype-dependent manner has been demonstrated in autopsy brain samples of AD\cite{117,118}. ApoE has been shown to be adducted with hydroxynonenal, a highly reactive lipid peroxidation product, in AD brains and CSF\cite{119}. Furthermore, ApoE is a strong chelator of copper and iron, both of which are important redox-active transition metals\cite{116}.

Interestingly, alteration in the gene MTHFR expression, coding for methylenetetrahydrofolate reductase, can influence homocystine levels, which may contribute to LOAD predisposition\cite{120}. Another suggested genetic risk factor, bleomycin hydrolase genotype, is also associated with alterations in redox homeostasis\cite{121}.

Genes related to oxidative stress and antioxidant pathways are generally induced in AD. For example, hemeoxygnase 1 is a 32-kDa stress protein that degrades pro-oxidant heme to antioxidant biliverdin, free iron and carbon monoxide\cite{122}. The induction of both mRNA and protein of hemeoxygnase 1 in cerebral cortex and vessels of AD suggests an antioxidant defense in AD that is mediated at the level of transcription\cite{123,124}. Expression of genes for antioxidant enzymes are also elevated in AD. For example, the gene expression of superoxide dismutase (SOD), catalase, glutathione peroxidase, and glutathione reductase are induced in brains from AD patients\cite{125}. These findings provide direct evidence that oxidative stress in AD increases relevant gene expression.

Beta-secretase 1 (BACE1) together with γ secretase liberates Aβ from APP\cite{126,127}. The activity of BACE1 increases in sporadic AD patients\cite{128,129}. Both BACE1 mRNA and
protein expression are elevated in vivo in the frontal cortex\textsuperscript{129}. The pathways regulating BACE1 have not been fully characterized. Oxidative stress is proposed to be one of the causes of elevated expression of BACE1 in AD\textsuperscript{130, 131}. Thus, one reasonable hypothesis is that oxidative stress elevates BACE1 which increases Aβ deposit and that will further overstate oxidative stress.

\textbf{Role of heavy metals in AD}

Most types of oxidative damage well-known in AD, including glycation, nucleic acid oxidation, lipid peroxidation, and protein oxidation, result directly or indirectly from metal-catalyzed OH\textsuperscript{-} formation. Consequently, it is not surprising that the loss of homeostasis of metals in the brain is accompanied by serious neurological consequences characterized with increased oxidative damage.

Copper, iron and zinc are the three most abundant metals in mammals, and play structural and catalytic roles in many enzymatic reactions. The catalytic activity of copper and iron is provided by their redox potential. However, this potential can also cause oxidative stress mainly via Fenton reaction\textsuperscript{132, 133}. On the other hand, zinc is redox inactive and usually plays a structural function in protein folding and stability and coordinating protein-protein interactions\textsuperscript{134}. Zinc also has antioxidant properties that enable it to modulate oxidative stress via protection of protein sulfhydryl groups\textsuperscript{135}.

An imbalance of metal homeostasis in the brain is thought to play an important role in the pathogenesis of AD\textsuperscript{136}. The mammalian brain contains a high concentration of copper, zinc and iron ions compared to other tissues, which reflects its high requirement in numerous metal-dependent enzyme and metabolic processes\textsuperscript{137}. The concentration of these
metals in the brain is firmly regulated at the level of the blood brain barrier\textsuperscript{138-140}. In AD brains considerable net increases in copper, zinc and iron have been reported compared to healthy age-matched controls. Systemic metal dyshomeostasis is also evident in AD patients with higher than age-matched normal levels of copper reported in both the CSF and serum\textsuperscript{141, 142}. By contrast, plasma zinc levels usually decrease with age\textsuperscript{143, 144} but there is evidence that plasma and CSF zinc levels are further depleted in AD patients compared to age-matched controls\textsuperscript{141, 145, 146}. Redox cycling between Cu\textsuperscript{1+}/Cu\textsuperscript{2+} and Fe\textsuperscript{2+}/Fe\textsuperscript{3+} promotes the activation of molecular oxygen, a process utilized by many enzymes including cytochrome-c oxidase, an integral part of the mitochondrial electron transport chain and ATP production\textsuperscript{147}. However, unregulated interaction of copper and iron with molecular oxygen also facilitates the generation of ROS. Levels of these metals are intrinsically high in the mitochondria, a highly oxygenated microenvironment that produces considerable levels of H\textsubscript{2}O\textsubscript{2}, which make it the most susceptible site for intracellular ROS generation\textsuperscript{148}. Mitochondrial dysfunction is a feature of normal aging and neurodegeneration, potentially due to an increase in ROS\textsuperscript{149}. However, redox active metals also provide catalytic function to antioxidant enzymes such as SOD. It is essential to have a response system to maintain a balance between the oxidative role of metals and the antioxidant defense. Certainly, these metals are kept within physiological limits by many metal-binding proteins that preserve metal homeostasis by regulating cellular metal absorption and efflux.

The superoxide anion radical (O\textsubscript{2}\textsuperscript{-}) is considered the primary ROS in biological systems and is generated mainly via metabolic processes in the mitochondria\textsuperscript{150}. The generation of highly reactive O\textsubscript{2}\textsuperscript{-} is susceptible to conversion to other secondary ROS, initially via a dismutation reaction catalyzed by SOD enzymes that produce H\textsubscript{2}O\textsubscript{2}. In the presence of
reduced metals (Cu$^{1+}$ and Fe$^{2+}$) H$_2$O$_2$ can generate highly reactive hydroxyl radicals (OH'). H$_2$O$_2$ is easily diffusible across biological membranes and H$_2$O$_2$ induced oxidative damage is associated with neurodegeneration. SOD enzymes work in concert with H$_2$O$_2$ removing enzymes to maintain essential but safe levels of cellular oxidants. In a normal healthy cellular environment excess H$_2$O$_2$ is usually removed by the action of catalases and glutathione peroxidases. However, these systems may become overwhelmed during neurodegeneration as levels of pro-oxidants rise. In the case of AD this is largely mediated by neuropathological increases in Aβ that can reduce Cu$^{2+}$ and Fe$^{3+}$ to generate H$_2$O$_2$ and downstream highly reactive ROS via Fenton and Haber–Weiss chemistry. The redox chemistry of Aβ has been comprehensively reviewed elsewhere\cite{14}. The presence of high concentrations of copper and iron in the vicinity of Aβ-rich plaques\cite{151}, provide the fuel for the generation of ROS that can affect lipid peroxidation and the formation of deleterious protein and DNA adducts\cite{152}. Although the affinity of Aβ to react with copper and iron put it in the pro-oxidant category it may, under certain conditions, also function as an antioxidant\cite{153}. For example, at physiological concentrations Aβ has been shown to be neuroprotective and neurotrophic to cultured cells\cite{154-156}.

Aluminum has been suggested as a causal factor in AD, in part because of reports showing the toxicity of aluminum, the elevation of aluminum concentrations in the brains of patients with AD, and an association between aluminum concentrations in water and the prevalence of AD\cite{157}. However, some studies clearly showed that the aluminum content is not elevated in the brains regions of AD patients that are selectively vulnerable to the neuropathologic changes associated with the disease\cite{158}.
Nitrosative stress and AD

NO is an important signaling molecule in the cardiovascular, immune and nervous systems, synthesized through converting L-arginine to L-citrulline by NOS. In the brain, NO is a multifunctional messenger molecule that plays an important role in learning and memory, and it regulates the expression of trophic factors that may be reduced with aging[159]. The excessive production of NO may also contribute to neuronal cell damage by a process termed “nitrosative stress”[160].

NO is highly diffusible and in the presence of O$_2^-$ can form highly RNS, including peroxynitrite. Peroxynitrite can react with vulnerable protein tyrosine residues in a reaction termed “nitration” which may contribute to neuronal injury[161]. Nitration of tau is thought to inhibit its ability to self-polymerize and nitrated tau has been reported in NFTs and neuritic plaques in AD[162].

Inflammatory stimuli can induce iNOS and activated microglia are a source of neurotoxic amounts of NO characteristic of several neurodegenerative disorders such as AD[160].

Another form of NO mediated nitrosative stress that has been implicated in AD is S-nitrosylation, which is a reversible modification of thiol groups of cysteine residues[163]. It can regulate a large range of biological processes including signal transduction, gene transcription and vesicle trafficking[161]. Like other forms of post-translational modification such as phosphorylation, it can be neurotoxic or neuroprotective, dependent on the target protein[161]. One of the first demonstrations that S-nitrosylation could be neuroprotective was its modulatory effect on N-methyl-D-aspartate receptor (NMDAR) activity of cultured rat neurons under conditions of excitotoxicity, thus preventing the excessive influx of Ca$^{2+}$[163]. Excessive Ca$^{2+}$ influx via NMDAR overstimulation can generate ROS and
activate cell death pathways and is thought to mediate neurotoxicity in numerous neurological and neurodegenerative disorders including AD\cite{164}.

An important role of Aβ in the production of NO has been reported in cell culture, transgenic mice, and human AD brain\cite{165-170}. Aβ-induced NO production by microglial cells has also been suggested to be one of the mechanisms of neuronal death in AD\cite{171}. On the other hand, decreased nitrite/nitrate levels in the frontal cortex of brains of AD patients compared to young and old individuals, and also a reduction in NO metabolites in the other cortical areas in AD as related to young controls has been reported\cite{172}.

Increased levels of other forms of nitrated proteins have been identified in AD brain\cite{52, 173} and an increase in nitrated proteins was also reported in patients with mild cognitive impairment, which is considered a transition state between cognitively normal and AD disease progression\cite{174}. Hence, protein nitration is not only an early marker of oxidative stress in AD, but it may be exacerbated by pathological increases of Aβ_{42}, which itself may form neurotoxic stable oligomers in the presence of Cu^{2+} and H_{2}O_{2}\cite{175}. Additionally, nitrosative stress has been associated with pathological perturbations to mitochondrial biogenesis\cite{176}.

**Conclusion**

Extensive scientific evidence suggests ROS-mediated oxidative damage to lipids, proteins, DNA and RNA in AD pathology. However, it is unknown whether oxidative stress is really one of the basic causes of the pathogenesis and neural damage in AD. Oxidative stress may act indirectly as a result of other pathologic processes. Even if it is not the specific cause of
the disease, oxidative stress is likely an essential factor in the development and progression of AD and, consequently, is a potential target for therapy.

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Conflict of Interest

The authors declare that they have no conflicting interests.

References


