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Oxidative Imbalance in Alzheimer's Disease

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Abstract

Oxidative stress is a striking feature of susceptible neurons in the Alzheimer's disease brain. Importantly, because oxidative stress is an early event in Alzheimer's disease, proximal to the development of hallmark pathologies, it likely plays an important role in the pathogenesis of the disease. Investigations into the cause of such oxidative stress show that interactions between abnormal mitochondria and disturbed metal metabolism are, at least in part, responsible for cytoplasmic oxidative damage observed in these susceptible neurons, which could ultimately lead to their demise. Oxidative stress not only temporally precedes the pathological lesions of the disease but could also contribute to their formation, which, in turn, could provide some protective mechanism to reduce oxidative stress and ensure that neurons do not rapidly succumb to oxidative insults. In this review, we present the evidence for oxidative stress in Alzheimer's disease and its likely sources and consequence in relation to other pathological changes.

Index Entries: Oxidative stress; mitochondria; metal ion; hydrogen peroxide; Aβ, tau.

Introduction

Oxidative stress is defined as a breaching of the antioxidant defense system. Neurons of the central nervous system are subject to a number of unique conditions that make them particularly vulnerable to oxidative stress and its sequelae that can culminate in cell death *(1)*. This vulnerability is a consequence of the high energy and oxygen consumption rate, the high unsaturated lipid content of neuronal membrane, the high levels of transition metals, the relative scarcity of antioxidant defense systems compared with other organs, and the postmitotic nature of neuronal populations *(2)*. Because of

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their unique status, a balance between the production of reactive oxygen species (ROS) and antioxidant defenses is of particular significance in neurons. Under normal physiological conditions, damage produced by ROS is kept in check by an efficient array of antioxidant systems that display an impressive level of redundancy to provide multiple lines of defenses. However, in the case of age-related neurodegeneration, like that observed in Alzheimer's disease (AD), this balance between oxidative radicals and antioxidant defenses is altered, resulting in various forms of cellular and molecular damage. Although it is debatable whether oxidative stress is the cause or consequence of the disease, recent evidence suggesting that oxidative stress occurs earlier than all other known changes indicates a causative role in the pathogenesis of AD.

Oxidative Imbalance Is a Prominent Feature of AD

Individuals affected by AD show increased oxidative damage to every class of biological macromolecules, including sugar, lipid, protein, and nucleic acid *(3–5).* For example, direct protein oxidation/nitration of tyrosine and tryptophan residues is the most obvious form of protein damage resulting from oxidative imbalance and is widespread in AD susceptible neurons *(6–10).* The vast majority of oxidative-stress-dependent protein modifications, however, involve the adduction of products following the oxidation of carbohydrate or lipid moieties (glycoxidation and lipoxidation, respectively) and result in the formation of stable adducts called advanced glycation end products (AGEs) or advanced lipoxidation end products (ALEs) *(11).* AGE modifications are present on both neurofibrillary tangles (NFTs) and senile plaques in AD, whereas protein carbonyl modifications and 4-Hydroxynonenal (HNE) adducts (i.e., ALEs) are localized to both neuronal cell bodies and neurofibrillary pathology *(8,12–14).*

Analysis of the site of oxidative damage can provide clues to the mechanistic source of such damage. To address this issue, it is important to compare the differences noted when damage occurs to rapidly turned over biological macromolecules (active modification) vs damage to stable structures (accumulative damage), some of which arise by oxidative crosslinking. Obviously, the former represents the recent oxidative modification because it reflects the active modification that occurred within the lifetime of the modified macromolecules, whereas the latter represents the history of oxidative damage. Because the products of lipid peroxidation and glycation often yield crosslinked molecules that are resistant to removal, they can be used as markers for accumulative damage. Protein nitration, a non-crosslink-related oxidative modification of protein resulting from either peroxynitrite attack or peroxidative nitration, indicates more recent active modifications. What is remarkable when comparing these different markers is that highly stable modifications, involving crosslinked proteins, are predominantly associated with lesions *(8, 12–14),* whereas metastable modifications are more commonly associated with the cytoplasm of susceptible neuronal populations in AD *(7).* These findings point to the cytoplasm, not the lesions, as the source of ROS. Our observation that RNA, a rapidly turned-over cell component, is a target for oxidative stress provided another ideal marker for assessing recent active modifications. 8-Hydroxyguanosine (8-OHG), a nucleic acid modification predominantly derived from hydroxyl radical (·OH) attack of guanosine, is greatly increased in cytoplasmic RNA in vulnerable neurons *(15).* Ultrastructural analysis shows that most 8-OHG is in the endoplasmic reticulum, with the majority of mitochondria showing little 8-OHG *(3).* Because the ·OH can only diffuse nanometer distances and cannot permeate through the plasma membrane, the source of reactive oxygen must be in close physical proximity to the damage. Therefore, 8-OHG is likely to form at the site of ·OH production within the neuronal cytoplasm, a process dependent on redox-active metal-catalyzed

reduction of hydrogen peroxide together with cellular reductants such as ascorbate or superoxide. Although the cytosolic sites of damage seemingly exclude mitochondria, we suspect a more complex relationship that involves superoxide generated from mitochondria being dismutated to freely diffusible hydrogen peroxide that then interacts with cytosolic redox-active metals and other oxidative stress response elements to cause damage.

Although the increased oxidative damage specifically seen in vulnerable neurons is striking, similar increases in ROS might be occurring systemically in AD. In fact, a large body of evidence demonstrates lower plasma antioxidant levels and alterations in antioxidant enzyme activities in mild cognitive impairment (MCI) patients and patients at early AD stages *(16–20).* These findings suggest a systemic imbalance between ROS production and antioxidant defense systems in the plasma of AD patients and this is substantiated by increases in DNA, lipid, and protein oxidation products found in blood and cerebrospinal fluid (CSF) obtained from AD patients in comparison with controls *(21–23).* For example, HNE, a highly reactive aldehyde that is a byproduct of the oxidation of polyunsaturated fatty acids, has been found to be increased in AD brain tissues and in ventricular CSF *(24).* Increased levels of F2- and F4-isoprostane in CSF of patients with AD are also reported by various groups *(25–30).* More importantly, increased concentration of 8,12-isoprostane F2α-VI is detected in CSF, blood, and urine of MCI and AD patients and correlates with cognitive and functional impairments *(29,31).* Increased tyrosine nitration to proteins and DNA oxidation product (i.e., 8OHdG) are also found in CSF from AD patients *(32–34).* Reflecting such systemic oxidative imbalance in AD, we found oxidative damage in olfactory neurons and the surrounding epithelial cells from AD donors *(35),* and another group reported increased 8OHdG lymphocyte DNA content from AD donors *(36),* which inversely correlated with the plasma levels of several antioxidant carotenoids *(37).*

Mitochondrial Abnormalities, Metal Iron, and Oxidative Imbalance

One of the most striking features of the human brain is its respiratory requirements; 20 to 25% of total body basal respiration occurs in less than 2% of the body's mass that is occupied by the brain and most of that is in the even smaller mass occupied by neurons. The total dependence of the brain on oxygen is shown by the postischemic failure of neurons, indicating that even a transient interference with brain oxidative metabolism induces mental and neurological signs and symptoms as evidenced by the neuropsychiatric disorders associated with oxidative metabolism abnormalities *(38,39).* Abnormalities in oxidative metabolism occur in AD and a reduced rate of glucose metabolism is one of the best documented abnormalities in AD, which precedes, rather than follows, the clinical manifestation of the disease *(40–42).* The reduction of cerebral metabolism in AD places mitochondria at the center of this dilemma and, indeed, the activities of several important mitochondrial enzymes of oxidative metabolism are reduced in AD brain, including the α-ketoglutarate–dehydrogenase complex (KGDHC) and pyruvate–dehydrogenase complex (PDHC), two enzymes of the rate-limiting step of tricarboxylic acid cycle, and cytochrome oxidase (COX), the terminal enzyme in the mitochondrial respiratory chain that is responsible for reducing molecular oxygen *(43–49).* Importantly, the degree of dementia correlates much better with reductions in KGDHC activity than with the amount of senile plaques and NFTs in the brains of ApoE4-positive AD patients *(50).* Generally, the reduced activity of these key enzymes favors the aberrant production of ROS, especially in the form of superoxide. However, although there is increased production of superoxide in AD mitochondria, what is striking, as discussed earlier, is that there is virtually no overt oxidative modification to mitochondrial components but increased oxidative modification to the surrounding cytosolic com-

ponents, particularly in neurons with altered mitochondria *(3).* This suggests that there should be enhanced antioxidant mechanisms to remove superoxide from mitochondria and transfer the oxidative stress to cytoplasm. The Mn- and CuZn-superoxide dismutases (SOD2 and SOD1, respectively) are the major enzymes that remove superoxide by converting it to hydrogen peroxide. Although still controversial, several studies showed that SOD activity is elevated in susceptible neurons in AD *(51–56).* Furthermore, SOD1 activity is consistently increased by 50% in patients with Down syndrome resulting in an extra copy of the SOD1 gene located on chromosome 21 *(57).* Because Down syndrome is a disease that pathologically, and to some extent also clinically, resembles AD, this suggests that increased SOD activity might be involved in mediating AD-type pathology. Increased superoxide production coupled with elevated SOD activity would translate into elevated levels of hydrogen peroxide, which, unlike superoxide, can freely diffuse across the outer membrane of the mitochondria and greatly enhance oxygen radical burden in a compartment (i.e., the cytoplasm) having less protection from reactive oxygen than mitochondria. Although catalase, an enzyme found exclusively in the cytoplasm of the cell, can detoxify H_2O_2, studies of catalase activity in AD have not shown a consistent pattern *(58,59).* However, a more important parameter, the SOD/catalase ratio, in susceptible neurons was increased in AD neurons *(56,60)*, suggesting that elevated production hydrogen peroxide is not coupled with increased removal, a situation that would result in elevated hydrogen peroxide levels in the cytoplasm. Indeed, amyloid precursor protein (APP) transgenic mice do have increased levels of hydrogen peroxide *(61).*

Although the presence of hydrogen peroxide does not pose a direct threat to the cell, because it is not highly reactive, hydrogen peroxide can take part in chemical alterations to form radicals that are highly reactive to cellular macromolecules. For example, hydrogen peroxide can form hydroxyl radicals, the most highly reactive molecules of all categories of biomolecules, as a consequence of Fenton chemistry between reduced transition metals [usually iron(II) or copper(I)] and H_2O_2. Therefore, accumulations of hydrogen peroxide can become highly detrimental to the neuron if increases in H_2O_2 occur concurrently with alterations in metal homeostasis. Several studies have indicated a disruption of metal ion metabolism, especially iron and copper metabolism, in the brain of AD patients. For example, copper concentration in the CSF of AD patients has been found to be 2.2 times more elevated than in the control groups *(62).* Significantly elevated levels of iron were found in the amygdala, hippocampus, and olfactory pathway in AD compared to age-matched control subjects *(63–65).* Furthermore, iron regulatory protein (IRP)-2 is increased in AD and selectively associated with the pathological hallmarks of AD *(66).* Also, an increase in iron concentration with a concurrent decrease in ferritin is observed in AD brain *(67,68).* Iron regulatory protein (IRPs) are involved in the regulation of the expression of iron storage protein ferritin and transferring receptor at the mRNA level by interacting with a conserved RNA structure termed the iron-responsive element (IRE) so that intracellular iron homeostasis is maintained *(69).* Much data supports the theory that alterations in the IRP/IRE interaction are the cause for this observed disruption in iron homeostasis in AD *(66,70).* Such an increase in iron without an appropriate increase in ferritin to detoxify the iron would leave the neuron vulnerable to ROS. To explore the cellular location of redox-active copper and iron, we used the ability of redox-competent metals to catalyze the oxidation of a substrate in the presence of hydrogen peroxide via the Fenton reaction. After application of hydrogen peroxide to tissue sections with the oxidizable substrate diaminobenzidine, sites of redox activity are readily apparent and reside exclusively within the neuronal cytoplasm in AD *(71).* Chelation treatment with either deferoxamine

or diethylenetriaminepentaacetate (DTPA) blocks this activity, whereas subsequent reapplication of either copper or iron restores activity *(71).* The cytosolic localization of a transition metal that is close to oxidative modification is also confirmed by electron microscopy studies (Perry and Smith, unpublished results). This evidence not only clearly demonstrates that cellular redox activity is completely dependent on aberrantly accumulated exchangeable metals but also suggests that excess hydrogen peroxide from abnormal mitochondria is able to cause active oxidative modifications in the cytoplasm.

The stage is now set: Mitochondria abnormalities lie at the heart of increased oxidative stress in susceptible neurons in AD. However, what factor(s) is responsible for the mitochondrial abnormalities? Interestingly, we found a threefold to fourfold increase in the mitochondrial protein, COX-1, and mtDNA specifically in vulnerable neurons in AD *(3).* Although the possibility of a reduction of proteolytic turnover leading to accumulation of mitochondrial components cannot be ruled out *(72),* an alternative explanation is that mitochondria produce more mtDNA and proteins to prepare for division after susceptible neurons re-enter the cell cycle. Indeed, multiple lines of evidence show that many degenerating neurons in AD exhibit phenotypic changes characteristic of mitotic cells *(4,73,74).* For instance, several invariant disease-related alterations, including the phosphorylation of tau as well as the expression, phosphorylation, and metabolism of APP in degenerating neurons in AD, are also features of normal dividing cells *(73).* Further, various components of the cell cycle machinery are activated in susceptible neurons in AD, a significant fraction of which have successfully replicated nuclear DNA and even reached the G2-phase *(75–78).* As there is both division of organelle nucleoids and organellokinesis before mitosis, during late S-, G2-, and mitotic phases, mitochondrial proliferation is most evident *(79).* These events are crucial for the high energy demands required for cell division. However, as yet, there is no evidence suggesting a successful nuclear division or chromosome condensation in AD, implying that the neurons do not complete mitosis (M-phase). In fact, terminally differentiated neurons might lack the ability to complete the cell cycle such that they proceed variously though to a point prior to the actual event of cellular division, to a characteristic "molecular phenotype" representing a neuron arrested in a transitional phase of the cycling process *(74,80).* The interruption of the cell cycle would leave neurons with mitochondrial alterations and, therefore, very vulnerable to ROS and calcium imbalance to trigger mitochondrial turnover that then lead to increased mitochondrial degradation products, as observed in AD neurons *(3).* Ultrastructural analysis shows that autophagosomes, particularly the residual body of lipofuscin *(3),* contain abundant redox activity, consistent with the accumulation of metals, suggesting that mitochondrial enzyme turnover in lysosomes is the likely source of the increased metal ions in the cytoplasm.

Oxidative Imbalance and AD Pathology

Carbonyl-based damage is apparent in both senile plaques and NFTs and in the primary protein components of both *(8,12,81–84).* In more recent studies, we have demonstrated that the lesions are not only site of AGE accumulation but also continuing sites of glycation, because the initial Amadori product is closely associated with NFTs *(85).* Although it is clear that oxidative imbalance is a prominent feature of the AD brain, it is still not clear how oxidative stress is related to the hallmark pathologies (i.e., senile plaques and NFTs) of the disease. One important question is whether oxidative stress is secondary to these pathologies or is an important factor in the formation of these pathologies. To address this issue, it is important to investigate the temporal profile of these changes. Increased levels of isoprostane, a product of

polyunsaturated fatty acid oxidation, in living patients with MCI and probable AD suggest that lipid peroxidation is present at a very early stage, well before the end stage of the disease *(25,29,31).* Additionally, oxidative damage marked by lipid peroxidation, nitration, reactive carbonyls, and nucleic acid oxidation is increased in vulnerable neurons whether or not they contain NFTs *(15,86),* which suggests that increases in neuronal oxidative damage must precede neurofibrillary pathology formation. Moreover, a marked accumulation of oxidative active modification products (i.e., 8-OHG and nitrotyrosine) in the cytoplasm of cerebral neurons from Down syndrome patients who invariably develop AD symptoms, in their teens and twenties, temporally precedes amyloid-β (Aβ) deposition often by decades *(87,88).* That oxidative damage is the earliest event preceding the formation of pathologies is also confirmed in AD brains *(86).* This is further supported by the findings on Tg2576 APP transgenic mice model in which oxidative stress precedes Aβ deposition *(89,90).*

Emerging evidence supports an early role of oxidative stress in the disease, which raises one further question: Whether and how oxidative stress contributes to the formation of these lesions? Oxidative stress condition is known to increase Aβ production and deposition. For example, in vitro studies show that hydrogen peroxide upregulates both the secretion of Aβ in the cell medium *(91)* and levels of Aβ in the cell *(92),* which can be blocked by antioxidant treatment *(92).* Increased Aβ production induced by oxidative stress is likely the result of both increased synthesis of APP through the activation of AP-1 transcription factor *(93)* and increased generation of Aβ from APP through activation of β-secretase *(94).* Metal-catalyzed oxidation of Aβ induces its aggregation and, hence, its propensity to form amyloid fibrils and plaques *(95,96).* Moreover, some antioxidants like curcumin, wine-related polyphenols, and melatonin inhibit the progressive formation of β-sheets and amyloid fibrils *(97–99)* and even destabilize performed fibrils in vitro *(97).* Aβ production also increases in vivo after brain injury *(100–102)* and in response to ischemic/hypoxic injury and brain trauma *(103–105).* More importantly, even in Tg2576 APP transgenic mice, antioxidant treatment decrease overall levels of Aβ *(106–108).* This is also confirmed in other animal models of amyloidosis such as ApoE-deficient mice *(109).* As for neurofibrillary pathology, because of the lack of appropriate animal models until quite recently, major efforts have focused on in vitro tau phosphorylation and assembly. It is known that tau phosphorylation decreases its binding activity to microtubules that might be harmful to neurons *(110),* and various molecules that induce oxidative stress, such as acrolein, homocysteine, mercury, and Aβ peptides, increase tau phosphorylation in neuronal cells *(91,111–113)* and HNE inhibits tau dephosphorylation *(114).* Other groups found that hydrogen peroxide, ultraviolet (UV) light, menadione, and iron actually induced rapidly dephosphorylation of tau protein *(115–117).* The difference might be the result of different downstream signaling pathways involved. However, the long-term effects of oxidative stress, paralleling the situation in AD *(4),* have not been investigated. The role of oxidative stress in facilitating tau assembly is less controversial. Adduction of HNE to phospho-tau generates tau conformation defining the Alz50 epitope that is found in PHF-tau *(118)* and also facilitates the in vitro formation of filaments *(119,120).* Further, polyunsaturated free fatty acids, which are sensitive to oxidative changes and promote oxidation in other molecules, also promote tau polymerization *(121–123),* which can be prevented by the addition of free-radical scavengers *(124).* Importantly, covalent crosslinking resulting from oxidative stress favors the formation of polymers by promoting the formation of large aggregates and renders them resistant to cellular degradation mechanisms *(125).* Increasing evidence demonstrated that ROS and lipid peroxidation products directly modify and inhibit the proteasome, which likely contributes to elevated levels of aggregated proteins *(72).*

From the above-reviewed evidence, it can be surmised that oxidative stress plays a role in

the formation of the pathological lesions found in AD. However, what is the role of these lesions in relation to oxidative imbalance in AD brain? The simplest way to analyze this was to determine the spatio-temporal relationship between lesions and active oxidative modifications in the AD brain. Quantitative analysis of the extent of 8-OHG oxidation reveals that AD cases with the most extensive Aβ deposits show the lowest 8-OHG levels, and neurons containing NFTs have about half the levels of 8-OHG (i.e., active oxidative modification representing the current oxidative stress status), despite an obvious history of oxidative damage (AGE or lipid peroxidation). Together, these findings suggest that both Aβ deposition and NFTs are associated with reduced oxidative stress *(86,126).* To further examine the relationship of Aβ to oxidative damage, we investigated cases of Down's syndrome. In Down's syndrome, Aβ deposition follows, rather than precedes, increased 8-OHG, and, again as with AD, following Aβ deposition, 8-OHG levels decline to control levels (r = 0.98) *(87).* Because oxidative stress precedes the pathological lesions and there is an inverse correlation between oxidative stress levels and lesions suggests that lesion formation could be produced secondary to oxidative stress as a protective response to compensate for the primary insult that causes AD *(2,127).* Such a protective role is also supported by stereological studies that suggest that although marked neuronal loss (approx 60%) was identified in affected areas of AD brain, NFTs, if at all, might account for only a small proportion (approx 8%) of this loss *(128,129).* Actually, it was shown that NFT might not be obligatory for neuronal death at all because CA1 hippocampal neurons survive with NFT for decades *(130).* Therefore, it is tempting to suggest that the formation of AD pathologies could provide protection against oxidative stress, whereas those neurons without pathologies could rapidly succumb to oxidative insults. Indeed, lesions could reduce oxidative stress by sequestering free redox-active metals, which are increased in AD neurons and thereby modify metal-catalyzed redox activity *(71)* and act as a superoxide dismutase *(131)* or as a peroxidase *(7).* In fact, Aβ production and deposition appears to be a response to neuronal injury, rather than the mediator of such an injury *(103–105).* That Aβ levels in CSF of AD patients are inversely correlated with dementia severity is consistent with such a protective function *(132).* Indeed, recent reports support the view that Aβ peptides, which are normal physiological products, have a protective effect and, under certain conditions, can function as an antioxidant *(126,133–135).*

Conclusion

In summary, although AD is likely associated with multiple etiologies and pathogenic mechanisms, these all share the commonality of oxidative stress. Indeed, aging, a process during which free-radical-induced oxidative stress plays an important role, is, by far, the single largest risk factor of AD. Also, increased oxidative modifications to virtually every class of biological macromolecules are well-documented in AD patients and oxidative damage is the earliest event in AD that not only well precedes the occurrence of AD pathologies but could also directly and indirectly contribute to the formation of these pathologies. The source of oxidative stress in AD likely reflects the synergistic effects of abnormal mitochondria and disturbed redox-active transition metal metabolism. Given the presumably persistent nature of oxidative stress in AD and the genesis of such stress preceding AD pathologies, therapies involving the timely prevention of oxidative stress and effective removal of ROS at an early stage should be rigorously pursued as a preventative measure for this devastating disease.

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