Reactive Oxygen Species Mediate Cellular Damage in Alzheimer Disease

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ABSTRACT: The two most striking features of Alzheimer disease are (i) the multitude of abnormalities affecting essentially every system and (ii) the strict age dependence. Recent work suggests that both features are linked to increased oxidative stress that damages lipids, proteins and nucleic acids and results in redox-active metal accumulations, mitochondrial damage and formation of advanced glycation endproducts. Interestingly, \(\beta\)-protein precursor, amyloid-\(\beta\), presenilins, and apolipoprotein E have all been linked to reactive oxygen species production or apoptosis, a process intimately associated with oxidative stress. In therapeutics, the commonality between a number of efficacious agents appears to be oxidative stress reduction. Therefore, we contend that oxidative stress is the element that links the multitude of changes in Alzheimer disease and that a reduction of oxidative stress will have a dramatic effect on reducing the incidence or progression of Alzheimer disease.

INTRODUCTION

A number of reports have established damage from reactive oxygen species (ROS) not only in the lesions of Alzheimer disease but also in neuronal populations affected in the disease (86,102–110). Now, studies are being focused on the relationship between oxidative stress and other factors that have been established by genetic or epidemiological studies that play an important role in Alzheimer disease. Specifically, we want to determine whether oxidative stress is a central process in neurodegeneration in Alzheimer disease or instead a result of the disease process (Figure 1).

The distinction of whether damage from oxidative stress occurs before neuronal destruction is essential to whether therapeutic reduction of oxidative stress will be efficacious.

Here we present evidence that oxidative damage is one of the earliest cytopathological markers of neuronal dysfunction in Alzheimer disease and, moreover, impinges on all proposed pathogenic risk factors and mechanisms implicated in Alzheimer disease.

Alzheimer Disease Involves a Multitude of Abnormalities

The pathological presentation of Alzheimer disease, the leading cause of senile dementia, involves regionalized neuronal death and an accumulation of intraneuronal and extracellular...
lesions termed neurofibrillary tangles and senile plaques, respectively (reviewed in (111)). Several independent hypotheses have been proposed to link the pathological lesions and neuronal cytopathology with, among others, apolipoprotein E genotype (17,84), hyperphosphorylation of cytoskeletal proteins (119), and amyloid-β metabolism (92). However, not one of these theories alone is sufficient to explain the diversity of biochemical and pathological abnormalities found in Alzheimer disease that involves a multitude of cellular and biochemical changes. Furthermore, attempts to mimic the disease by a perturbation of one of these elements using cell or animal models, including transgenic animals, do not result in the same spectrum of pathological alterations. The most striking case is that while amyloid-β plaques are deposited in some transgenic rodent models overexpressing β-protein precursor, there is no neuronal loss (44,45) — a seminal feature of Alzheimer disease.

Oxidative Theory of Aging

What many theories have failed to incorporate is that Alzheimer disease is a disease of aging (49) (Figure 2). Importantly, this holds true even in individuals with a genetic predisposition, i.e., those individuals with an autosomal dominant inheritance of Alzheimer disease or in individuals with Down’s syndrome who develop the pathology of Alzheimer disease. Therefore, age is a clear contributor in 100% of Alzheimer disease cases, whatever the genetic background. The free radical theory of aging (36) hypothesizes that the aging process is associated with (i) an increase in the adventitious production of oxygen-derived radicals, i.e., reactive oxygen species (ROS), together with (ii) a concurrent decrease in the ability to defend against such ROS, that together lead to the accumulation of oxidatively-modified macromolecules. We suggest that the decrease in ROS buffering capacity may also lead to a compromised ability to deal with abnormal sources of ROS such as those associated with genetic predisposition and/or disease status.

Sources of Reactive Oxygen Species

ROS production occurs as a ubiquitous byproduct of both oxidative phosphorylation and the myriad of oxidases necessary to support aerobic metabolism. In Alzheimer disease, in addition to this background level of ROS, there are a number of additional contributory sources that are thought to play an important role in the disease process: 1. Iron, in a redox-active state, is increased in neurofibrillary tangles as well as in amyloid-β deposits (28,110). Iron catalyzes the formation of •OH from H₂O₂ as well as the formation of advanced glycation end products. Furthermore, aluminum, which also accumulates in neurofibrillary tangle-containing neurons (28), stimulates iron-induced lipid peroxidation (69). 2. Activated microglia, such as those that surround most senile plaques (20), are a source of NO and O₂⁻ (16) which can react to form peroxynitrite, leaving nitrotyrosine as an identifiable marker (29,109). 3. Amyloid-β itself has been directly implicated in ROS formation through peptidyl radicals (10,39,87). 4. Advanced glycation end products in the presence of transition metals (see above) can undergo redox cycling with consequent ROS production (3,126,127). Additionally, advanced glycation end products and amyloid-β activate specific receptors, such as the receptor for advanced

![Fig. 2. Alzheimer disease, genetic as well as sporadic, always has a strict age related incidence.](image-url)
glycation end products (RAGE) and the class A scavenger-receptor, to increase ROS production (23,128). Abnormalities in the mitochondrial genome (18,22) or deficiencies in key metabolic enzymes (8,96,71,94,112) suggest that metabolic abnormalities affecting mitochondria may be the major and possible initiating source of ROS in AD.

**Oxidative Damage and Response**

An exact determination of the contribution of each source of oxidative stress is complicated, if for no other reason than that most sources have positive feedback. Nonetheless, the overall result is damage including advanced glycation end products (53,102,123,126), nitration (29,109), lipid peroxidation adduction products (63,86) as well as carbonyl-modified neurofilament protein and free carbonyls (99,105,107) with the involvement extending beyond the lesions to neurons not displaying obvious degenerative change.

Oxidative crosslinking makes proteins not only insoluble (reviewed in 104,108) but also resistant to proteolytic removal (21) by competitively inhibiting the proteosome (24). Therefore, oxidative crosslinking may be significant in the accumulation of ubiquitin conjugates in neurofibrillary tangles (65,72) in the face of numerous proteolytic activities, which are highly active against abnormal proteins (101). Indeed, it may not be coincidental that fibrillary inclusions found in neurodegenerative diseases other than Alzheimer disease are also extensively ubiquinated, e.g., Lewy/Pick bodies and Rosenthal fibers (25,55) and are also oxidatively modified (12–14).

The cytopathological significance of oxidative damage is seen by the upregulation of the antioxidant enzyme heme oxygenase-1 in neurons with NFT (78,89,103). Furthermore, in quantitative immunocytochemical studies of cases of Alzheimer disease, there is a complete overlap between heme oxygenase-1 and Alz50, an early marker of τ abnormalities, indicating that cytoskeletal abnormalities are associated with increased oxidative stress or vice versa (Smith and Perry, unpublished observation).

**Phosphorylation**

The mechanisms by which normal soluble cytoskeletal elements, such as τ and neurofilaments, are transformed into insoluble paired helical filaments is an important issue (91,108). Insolubility has been linked to the most well-known posttranslational change of τ—abnormal phosphorylation (27,31) and a number of specific kinases and phosphates have been implicated (reviewed in (119)). However, while increased phosphorylation decreases microtubule stability, a salient feature of the pathology of Alzheimer disease (1,2,43,73,75,76), NFT insolubility is not mediated by phosphorylation (108). Indeed, in vitro phosphorylation of normal τ or complete dephosphorylation of NFT has no effect on their solubility (27,31,33,108). Instead, recent studies suggest τ phosphorylation as found in Alzheimer disease may be part of a novel process similar to that seen during mitosis (74,79) suggesting that neurons affected in the disease might be abortively entering the cell cycle (60,61,122).

Phosphorylation is intimately tied to oxidative stress by the mitogen activated phosphorylation (MAP) pathway (34) as well as through activation of transcription factor NFκB (90). While there is controversy concerning the kinases involved in the phosphorylation of τ in Alzheimer disease, the MAP kinase pathway is implicated (41,52,120). In studies with antibodies specific to activated MAP kinase, we found activity in all pyramidal hippocampal neurons in cases of Alzheimer disease but none in young controls. In aged controls, active MAP kinase is found not only in the few Alz50- or phosphorylated τ (AT8)-positive neurons but also in many apparently normal neurons. Preliminary studies show many of the same neurons showing active MAP kinase show increased nitration. Therefore, abnormal phosphorylation of proteins in Alzheimer disease may be a consequence of oxidative stress. Moreover, it is perhaps not
surprising that, while all pyramidal neurons of the hippocampus show increased free carbonyls (107), lipid peroxide adduction (86) and nitrotyrosine (109), only a subset of neurons displaying overt degeneration also show increased phosphorylation (32,113). Heme oxygenase-1, an inducible antioxidant enzyme, also shows the same pattern of involvement since it accumulates concurrent with phosphorylated \( \tau \) (Smith and Perry, unpublished data).

**Amyloid-\( \beta \)**

The influences of amyloid-\( \beta \) and other genetic factors on Alzheimer disease may be through their effect on oxidative stress (Figure 3). A number of mechanisms have been invoked for the neurotoxicity of amyloid-\( \beta \) (129), reviewed in (46,87), including membrane depolarization (11), increased sensitivity to excitotoxins (51), and alterations in calcium homeostasis (56). However, the leading hypothesis is that neuronal damage by amyloid-\( \beta \) is mediated by free radicals and, as such, can be attenuated using antioxidants such as vitamin E (4,5) or catalase (54,130). Indeed, amyloid-\( \beta \) is reported to spontaneously generate peptidyl radicals (10,30,37,77). Further, mutations in \( \beta \)-precursor protein are associated with increased DNA fragmentation, possibly involving oxidative mechanisms (see below). Critically addressing whether amyloid-\( \beta \) initiates oxidative damage in Alzheimer disease requires careful study of the relationship of amyloid-\( \beta \) deposition and increased oxidative damage. In this regard, it will be extremely interesting to determine the oxidative status of the recently reported transgenic rodent models of amyloid-\( \beta \) deposition (26,40). Preliminary findings from our laboratory, in collaboration with Karen Hsiao and Miguel Pappolla, that indicate the full spectrum of oxidative changes of Alzheimer disease are found in neurons of transgenic mice with amyloid-\( \beta \) deposits (111).

**Genetic Factors**

Presenilins 1 and 2 (92,93) are genetic factors where the biological mechanism, although not established, may also involve oxidative damage. Increased presenilin 2 expression increases DNA fragmentation and apoptotic changes (124), both important consequences of oxidative damage. Apolipoprotein E, in brains and cerebrospinal fluid, is found adducted with the highly reactive lipid peroxidation product, hydroxynonenal (64). Furthermore, apolipoprotein E is a strong chelator of copper and iron, important redox-active transition metals (62). Finally, interaction of apolipoprotein E with amyloid-\( \beta \) only occurs in the presence of oxygen (116).
**Apoptosis**

In programmed cell death, i.e., apoptosis, cells are digested within their own membrane by proteases and nucleases as well as by increased ROS. However, without the full range of morphological changes, it is unclear whether DNA fragmentation is apoptotic or is, instead, mediated solely by oxidative stress (7). The relative contribution of oxidative stress- and apoptosis-related DNA fragmentation in AD is unresolved. Yet bearing on this issue, the relative infrequency of apoptosis defined by morphology (19,117) and the broad findings of fragmentation in all cells in cases of Alzheimer disease argues for widespread oxidative DNA damage rather than widespread apoptosis. Certainly, this interpretation is consistent with the oxidative nuclear damage in all cells of the brain in areas affected in Alzheimer disease (107,110).

**Therapeutics**

An important question in discerning whether reducing oxidative stress may have therapeutic value is whether it is a primary or secondary event in disease pathogenesis (57,106) (Figure 4). Recent evidence, reviewed above, supports oxidative damage as the earliest cytopathological and biochemical change of Alzheimer disease (53,86,102,104,107,109,123,126).

Agents that inhibit free radical formation (6,35,42,47,68,81,83,88,95,121,125), reduce both the incidence and the progression of Alzheimer disease (Figure 4) (9,15,38,48,50,58,66,67,70,80,82,85,95,97,98,114,115,118). This relationship, together with the efficacy of metal chelation treatment (59), strongly suggest that oxidative stress precedes cell and tissue damage and therefore agents that prevent oxidative damage show promise in the treatment of Alzheimer disease.

**CONCLUSION**

In summary, oxidative stress may underline all of the commonly accepted notions in Alzheimer disease pathogenesis, including hyperphosphorylation, apolipoprotein E genotype, and mutations of the β-protein precursor. Further studies, to examine the types and extent of oxidative damage, will undoubtedly identify which antioxidant agents will prove most efficacious in the treatment of Alzheimer disease.

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