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·Review·

Neuronal failure in Alzheimer's disease: a view through the oxidative stress looking-glass

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Considerable debate and controversy surround the cause(s) of Alzheimer's disease (AD). To date, several theories have gained notoriety, however none is universally accepted. In this review, we provide evidence for the oxidative stress-induced AD cascade that posits aged mitochondria as the critical origin of neurodegeneration in AD.

Keywords: Alzheimer's disease; amyloid-beta; free radicals; mitochondria; mitochondrial dynamics; oxidative stress

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder responsible for the cognitive deterioration of 26.6 million people worldwide. The prevalence is projected to increase by ~3 fold to 106.8 million by the year 2050, with 1 in 86 people living with AD and 59% of the cases in Asia[1]. Age is the primary risk factor for AD such that its incidence is 15% among individuals >65 years old and reaches ~50% among those >85[2]. While many specific aspects of AD have been documented, there has yet to be any clear understanding of its pathological initiation and progression. Molecular aberrations in the cell elicit neuronal failure through various well-established mechanisms, including oxidative stress[3], abnormal protein folding and aggregation[4], cell cycle dysregulation[5], mitochondrial dysfunction[6], synaptic failure[7, 8], inflammation[9], loss of calcium regulation[10], defective cholesterol metabolism[11, 12], vascular alterations[13, 14], and neurotrophin deprivation[11]. However, the causal relationships governing these factors remain unknown.

AD is characterized by the successive degeneration of neurons first in the entorhinal cortex of the mediotemporal lobe, followed by the CA1 region of the hippocampus, CA2/3, CA4, and the neocortex[15]. Despite this predictable vulnerability, the molecular mechanisms governing it are unclear.

Amyloid-β (Aβ) and the hyperphosphorylated form of the microtubule-associated protein tau are hallmarks of AD pathology, and are critically involved in neuronal death. While the function of Aβ is unclear, its accumulation into insoluble fibrils and plaques increases over the course of AD progression, causing a severe cellular burden. The Aβ peptide is formed by the proteolytic cleavage of its protein precursor, amyloid-β protein precursor (AβPP), *via* the sequential actions of β-site AβPP-cleaving enzyme 1 and γ-secretase, a protein complex with presenilins 1 and 2 at its catalytic core[11, 16]. Aβ is associated with multiple cascades that are thought to result in neuronal damage[17, 18] and as such, it is generally considered to be the primary mediator of neurodegeneration in AD[19]. However, the lack of therapeutic translation has become a major criticism of the amyloid cascade hypothesis[20].

The microtubule-associated protein tau is similarly associated with AD progression, and its accumulation in the form of neurofibrillary tangles (NFTs) strongly correlates

with dementia in AD[11, 21]. NFTs are used as a post-mortem diagnostic criterion for AD[22]. Tau is phosphorylated at serine and threonine residues that flank the microtubule-binding domain. Upon hyperphosphorylation, tau is unable to bind and stabilize cytoskeletal microtubules; instead it self-aggregates into NFTs in and around the cell[23].

AD is typically sporadic; nearly 90% of all cases are sporadic in nature and characterized as late-onset AD (LOAD), with an age of onset of ~65 years. Familial AD (FAD), on the other hand, is an early-onset, genetic condition caused by several known autosomal dominant mutations; its age of onset is typically ~40–60 years[24]. Mutations that yield FAD all involve or result in an alteration in the ratio of $A\beta_{42}$ to $A\beta_{40}$; this is perhaps the most touted evidence both for and against[25, 26] the amyloid cascade hypothesis[27]. Dominantly-inherited mutations in presenilin 1 (PS1), an essential component of the γ-secretase complex, account for the majority of FAD cases, and >170 such mutations in PS1 have been identified on chromosome 14[28]. In addition, mutations in presenilin 2 (PS2) (on chromosome 1[29]) and AβPP (on chromosome 21[30]) also elicit FAD, although they are less prevalent. These mutations also affect the $A\beta_{42/40}$ ratio. Importantly, both LOAD and FAD present identical brain lesions and patterns of neurodegeneration. Notably and somewhat contradictory, LOAD leads to a reduced $A\beta_{42/40}$ ratio, whereas FAD leads to an increase[31].

Neuronal Oxidative Stress: Sources and Vulnerabilities

Aerobic respiration, like any other biochemical or physical process, is not 100% efficient. The mechanism by which mitochondria process carbohydrates and establish a proton gradient for the synthesis of ATP (i.e., the tri-carboxylic acid (TCA) cycle and oxidative phosphorylation) involves the controlled transfer of electrons from strong reducing agents to strong oxidizing agents. Some of the free radicals thus generated escape; rather than appropriately transporting their electron to the next molecule in the cascade, they abandon the inner membrane of the mitochondrion and impose alterations on other macromolecules within the cell. These alterations are often detrimental: DNA/RNA oxidation may yield fragmentation and deficiencies in repair machinery[32, 33]; oxidative modification of enzymes and metabolic signaling proteins may lead to metabolic impairments[34]; and protein cross-linkages and an impaired proteolysis network resulting from oxidative modification may render proteins insoluble and prone to abnormal aggregation[35-37].

The ability of the cell to adapt to oxidative damage is robust, yet finite. Endogenous antioxidants are intended to sequester the formation of free radicals. Superoxide dismutase, for instance, catalyzes the conversion of the potent free radical superoxide to hydrogen peroxide and water. Other important oxidative stress-handling proteins include catalase, glutathione peroxidase, glutathione reductase, and heme oxygenase[38]. Under normal conditions, the majority of free radicals are sequestered within the mitochondria (i.e., their place of origin). Notably, however, three important factors (age, metabolic demand, and disease) exacerbate the vulnerability of cells to oxidative burden.

Statistically, the longer a cell is respiring, the more reactive oxidative species (ROS) go unsequestered within that cell. It is estimated that cells use 10^{13} molecules of oxygen on average per day, with 1% of the associated reactions producing unsequestered ROS. Therefore, 10^{11} molecules of ROS are generated in a given cell every day. Even with efficient antioxidant mechanisms, some ROS unavoidably escape and cause damage to the surroundings that may accumulate with time, especially in long-living cells. Age is thus a great risk factor for oxidative stress. Likewise, high metabolic activity, which requires more oxidative phosphorylation per unit time and thus involves a greater number of toxic species generated per unit time, also renders a cell more vulnerable to oxidative insults. In particular, the neuron, which requires energy for axonal transport, vesicular release, ion pump operation, electrochemical gradient maintenance, and the like, requires much more oxygen per unit time than any other cell type in the body. Metabolic demand is therefore another risk factor for oxidative stress. Disease conditions that produce mutations in cellular antioxidant defensive machinery provide a third risk factor for oxidative damage.

Altogether, the brain is tremendously vulnerable to oxidative damage. Although it constitutes only 2–3% of total body mass, 20% of the basal oxygen supply is used by the brain. Moreover, neurons are among the longest-living cells in the body and need the same metabolic

machinery for continuation of survival and functioning for decades. The presence of transition metals in the brain that catalyze oxidative reactions further increases the appearance of oxidative pathologies. Iron and copper, for instance, increase the likelihood of an oxidation/reduction reaction[39], and increases in the regional concentrations of these metals in brain compound the risk factors described above. Furthermore, the brain suffers a relative paucity of antioxidant systems as well as an increase in polyunsaturated fatty acids (prime targets for ROS)[40]. So an aging brain is tremendously susceptible to oxidative deterioration.

Oxidative Stress: a Prominent and Early Feature of Alzheimer's Disease

Free radical-induced damage to macromolecules has been well documented in AD. Deposition of redox-active ions, which are capable of generating the most damaging hydroxyl radicals through the Fenton reaction, is likely to exacerbate both the spectrum of molecules and the cellular areas affected. DNA and RNA oxidation, marked by increased levels of 8-hydroxy-2-deoxyguanosine and 8-hydroxyguanosine (8-OHG), suggest a higher frequency of DNA fragmentation and aberration in DNA repair[41]. Oxidative modification of metabolic proteins, such as creatine kinase BB, cytochrome c oxidase (COX), and ketoglutarate dehydrogenase complex (KGDH), has been demonstrated by elevated levels of protein carbonyl and nitration of tyrosine residues[42] and indicates impaired metabolic activity. Lipid peroxidation, marked by thiobarbituric acid reactive substances, malondialdehydes, 4-hydroxy-2-transnonenal (HNE), and isoprostane, as well as by altered phospholipid composition, suggests altered membrane integrity. Oxidative modification of sugars, marked by increased glycation and glycooxidation, indicates an impaired cellular ability to adequately process critical carbohydrates[43]. Each of these aspects is elevated in AD compared to control cases[42].

Moreover, mitochondrial abnormalities, attributed to oxidative stress-related damage, are well-established in AD. As stated above, specific enzymes involved in electron transport and the TCA cycle, such as KGDH and COX, are modified *via* ROS in AD[44,45]. Mitochondrial DNA (mtDNA) modification[46] and calcium dysregulation[47-49] occur in AD to a great extent, and increased numbers of mitochondria with broken cristae, altered size and shape, and aberrant intracellular localization are elevated in AD neurons[47, 50, 51]. These latter phenomena are the result of impaired mitochondrial dynamics that can be caused by and also amplify oxidative stress[52]. Mitochondrial fission and fusion, the ongoing processes that ensure organelle stability within the dynamic cellular environment, rely on critical membrane proteins. Fusion enables the exchange of lipid membrane in inter-mitochondrial components (i.e., mtDNA and fission/fusion proteins) such that the cell is populated by healthy, normal mitochondria[53]. Fission, on the other hand, coupled with fusion and autophagy, enables the sequestration and elimination of irreversibly damaged mitochondria and mitochondrial content[54]. These dynamics are the necessary means by which the cell dampens its inevitable accumulation of oxidative free radical-induced damage. Over the lifetime of a cell, however, oxidative damage and transcriptional errors (due to alteration of mtDNA) reach a critical threshold. Oxidative stress ultimately propagates throughout the cell as mitochondria become abnormally shaped and localized[47, 55-58].

Importantly, increasing evidence demonstrates that the oxidative modifications in vulnerable neurons in AD occur prior to the hallmark pathologies of the disease[35]. That is, the markers of oxidative damage are found in the cytoplasm of neurons prior to any indication of degeneration in AD brains[59, 60]. In fact, the hallmark pathologies such as amyloid plaques and NFTs may be an adaptation in response to elevated oxidative stress. More recent studies showed that patients who suffer from mild cognitive impairment (MCI), considered to be a prodromal stage of AD, are depleted in antioxidants along with increased lipid peroxidation in the plasma and lymphocytes[61]. Elevated protein/RNA oxidation and redox-active iron are also documented in the brains of MCI patients[32,62,63]. Transgenic mouse models of AD also demonstrate that oxidative damage precedes Aβ deposition[64]. The mitochondrial abnormalities described above provide considerable support for this primacy. Specifically, vulnerable neurons in AD and MCI exhibit severe metabolic deficiencies before any clinical manifestations of disease. Neuroimaging studies and neuropsychological tests have shown impaired cerebral metabolism prior to any evidence of functional impairment or brain atrophy induced by AD

pathologies[51, 65]. This metabolic impairment is likely the result of the alterations in mitochondrial dynamics and intracellular localization[66], which, in turn, increases the production of oxidative damage to mtDNA and membrane proteins leading to a vicious cycle.

Alzheimer-Specific Factors and Oxidative Stress

Besides the fact that oxidative stress characterizes aging, genetic and pathological factors associated with AD are also implicated in the regulation of oxidative stress. For example, presenilins are needed for the import of ~50% of cellular copper and zinc[67]. Increased presenilin 2 expression increases DNA fragmentation and induces apoptotic changes[68], which are important consequences of oxidative damage. The translation of APP is regulated by iron influx *via* an iron-responsive element RNA stem loop in its 5'-untranslated region[69]. APP is the dedicated ferroxidase in neurons responsible for the export of iron, and the elevation of redox-active iron in neurons in AD is likely due to the reduced ferroxidase activity of APP inhibited by zinc[70]. ApoE is a strong chelator of copper and iron, both of which are important redox-active transition metals[68]. ApoE is beneficial against free radicals as it reduces neuronal death caused by hydrogen peroxide and Aβ through antioxidant activity in an isoform-dependent manner, the E4 isoform being the least effective[71]. Interestingly, apoE is sensitive to attack by free radicals, and this sensitivity is also isoform-dependent, E4 being the most vulnerable[72]. Indeed, cerebral cortex samples obtained at autopsy from patients with APP or presenilin gene mutations or the apoE4 allele demonstrate higher oxidative stress[73, 74].

Aβ itself may cause oxidative damage in surrounding cells through, for example, activation of microglia and astrocytes that activate multiple ROS pathways[75], including upregulating L-tryptophan metabolism through the kynurenine pathway[76]. Several intermediates in this pathway, namely 3-hydroxykynurenine and quinolinic acid, are neurotoxic, and the Aβ-induced increase in their production subjects surrounding neurons to oxidative damage[77, 78]. Aβ-induced lipid peroxidation yields several reactive aldehydes (including HNE) that can harmfully modify membrane proteins. HNE production specifically disrupts iron homeostasis by impairing glucose transporters[79-82]. Iron and copper accumulation within neuritic plaques (mainly composed of Aβ) further facilitates free-radical generation[83]. When Aβ binds catalytic amounts of copper, hydrogen peroxide is generated, which contributes to oxidative stress as it produces hydroxyl radicals *via* the Fenton reaction.

Aβ has antioxidant effects intracellularly and may actually be secreted as a compensatory measure against oxidative stress[35, 84]. *In vivo* and *in vitro* studies of neuronal responses to oxidative stress indicate an increase in Aβ production[85], which is followed by a corresponding reduction in oxidative stress[86, 87]. Moreover, markers of oxidative stress, such as 8-OHG, are reduced in neuronal populations characterized by Aβ deposition, and elevated in vulnerable regions lacking Aβ[86, 87]. The data suggest that neurons may be salvaged from oxidative stress by Aβ.

Tau hyperphosphorylation is a pathological result of oxidative stress[59, 84, 87-89]. Interestingly, most neuronal loss during the course of neurodegeneration occurs where the levels of oxidative stress are highest, and the subsequent deposition of NFTs decreases these levels[59]. Of note, neurons with accumulated NFTs are able to survive for decades and become functionally integrated in cortical circuits[90, 91]. Phosphorylated tau antagonizes apoptosis by stabilizing beta-catenin[92]. Regardless, the abnormal accumulation of hyperphosphorylated tau in the form of NFTs occurs subsequent to oxidative stress-induced damage.

Oxidative Stress and Alzheimer's Disease: Ubiquity *versus* Specificity

Disease is defined by deficits in specific functional output, with the underlying anatomical and biochemical/structural changes elicited by insults and adaptations and/or failure of adaptations. Interestingly, oxidative stress is a prominent biochemical phenomenon not only in AD, but in almost all of the major neurodegenerative diseases, including Parkinson disease, amyotrophic lateral sclerosis, and Huntington disease[93]. Since oxidative stress contributes largely to aging, and age is the greatest risk factor for all neurodegenerative diseases, it is not surprising that oxidative stress is involved in pathogenesis of each of these diseases. However, given the different neuronal populations and distinct pathologies that characterize each

disease, the ubiquity of oxidative stress raises a question about the specificity of its involvement. In AD, for example, elevated oxidative stress (and neuronal damage) occurs in areas such as hippocampus and cortex, but not in the cerebellum, midbrain, or pons[42]. Similarly in Parkinson disease, enhanced oxidative stress primarily localizes to the substantia nigra and the basal ganglia[94]. If oxidative stress is indeed a fundamental pathologic process in these diseases, the region-specific neuronal deterioration demands explanation.

Although further study is necessary, it is likely that the specific characteristics of the neuronal populations involved contribute to such selectivity. Neurons with long axons and multiple synapses have higher metabolic demands that may render them more prone to oxidative attack at a steady-state level and thus become more vulnerable to additional disease-related changes. For example, CA1 neurons have higher levels of superoxide anion than parietal cortical or CA3 neurons, which is at least one of the reasons why they are more vulnerable to global ischemia-induced cell death[95]. Dopaminergic neurons are also exposed to high steady-state levels of oxidative stress produced by the metabolism of dopamine, which make them more vulnerable to insults that affect dopamine metabolism[96].

The particular vulnerability of a given individual to developing cumulative oxidative damage to the hippocampus (and thus suffering from AD) rather than the basal ganglia probably reflects a particular arrangement of predisposing genetic and environmental factors. Such initiating factors may not be a single event, but more likely a complex interaction between individual genetic/epigenetic backgrounds and the environment that ultimately determines the specific neuronal population affected. Overall, different diseases may display distinct oxidative stress patterns that likely will lead to a different homeostatic balance that is finely tuned to minimize cell death. More studies are needed to understand the role of oxidative stress in each disease.

The Age-Related Mitochondrial Cascade in Alzheimer's Disease

The evidence listed above reveals the following: (1) AD is primarily an age-related disorder; (2) the brain is the most metabolically demanding organ in the body; (3) free radical release invariably results from the tremendous amount of oxidation/reduction reactions within neurons; (4) resident antioxidant systems, though robust, are finite in capacity; (5) mitochondria are the metabolic center of the cell and experience the earliest detriments in AD; and (6) Aβ peptides and NFTs accumulate after oxidative stress has already incurred severe damage, and seem to be a compensatory response to cerebral oxidative stress.

Fitting the pieces together, we propose that over the course of a lifetime, statistically inevitable free-radical damage accumulates within mitochondria, affecting mtDNA, membrane proteins, and calcium homeostasis. The antioxidant response of neurons is able to hold back the effects of this stress for years or decades; metabolic deficits resulting from mitochondrial dysfunctions and perturbed localizations do not appear until adult life, and are the very first aspect of dementia. However, the increasing levels of transition metals and polyunsaturated fatty acids in the brain render neurons particularly susceptible to damage, and once mitochondrial mutations become widespread within the cell, compensatory responses, such as Aβ secretion, are initiated to defend against further damage. Eventually, secreted Aβ becomes subject to oxidative stress (i.e., dityrosine cross-linkages inhibit its solubility, promoting aggregation[62, 97]). The cascade worsens as Aβ induces neuronal defects, producing further oxidative stress, inflammation, and synaptic dysfunction. The well-established pathophysiological inclusions of AD, including cell cycle aberration, inflammation, synaptic dysfunction, and loss of calcium regulation, become increasingly relevant, and affected neurons ultimately die. Thus, after decades of stress and increasing malfunction, cognitive decline spreads and yields clinical AD (Fig. 1).

Though its logic is appealing, this description of AD, like all previous hypotheses posited as explaining the disease, suffers significant shortcomings. First, it does not adequately explain the predictable and consistent spread of neurodegeneration from the entorhinal cortex of the mediotemporal lobe, through the hippocampus, and into the neocortex. Perhaps this orderly passage reflects the metabolic demands of distinct regions within the brain; the entorhinal cortex may require the highest metabolic activity due to its involvement in memory formation and retrieval (an ongoing process throughout life). It is thus the first to be affected by oxidative stress accumulation that

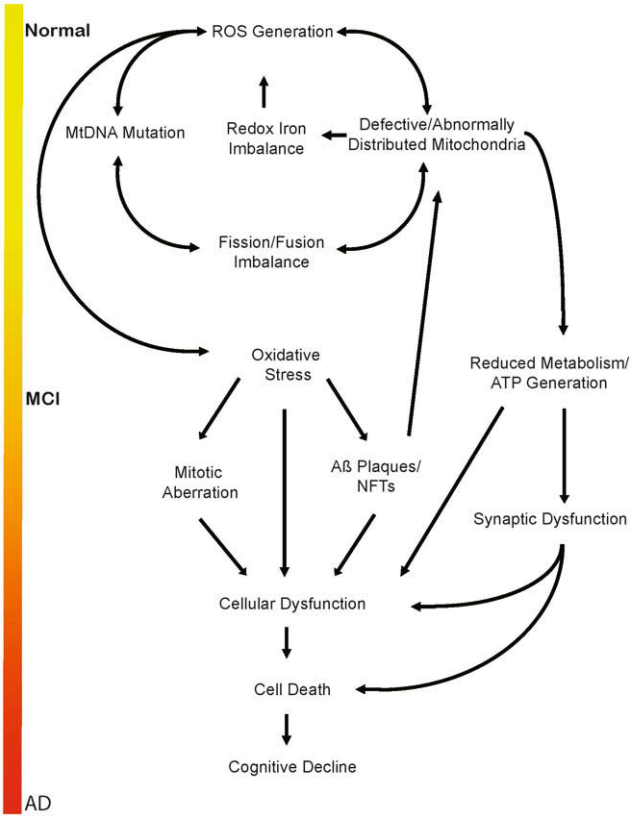

Fig. 1. Oxidative stress-induced cascade of Alzheimer disease (AD). The inevitable generation of free radicals within neurons eventually compromises the integrity of mitochondria, leading to a cascade of destructive events that elicits the hallmark features of AD. After years of accumulation, neuronal death and cognitive decline occur. MCI, mild cognitive impairment; NFTs, neurofibrillary tangles; ROS, reactive oxidative species.

leads to degeneration by a matter of simple probability. The remaining neurons in the pathway would suffer by connective association, and the disease would spread. Notably, this postulate lacks evidence, and its potential validity merits further investigation.

The mitochondrial cascade also seems to fail to explain the critical role of AD mutations in FAD and Down syndrome. A more careful look, however, ameliorates this misperception. In particular, the timeline of LOAD is such that Aβ accumulation and aggregation occurs late in adult life, causing deficits in cognition typically after age 65. The mitochondrial cascade attributes this latency to the prolonged period of oxidative stress accumulation, which takes years to have any metabolic deficits and subsequent Aβ secretion. Once secreted, Aβ, however, is oxidatively processed into insoluble fibrils, and damages surrounding cells *via* a variety of mechanisms. FAD represents a shortcut in this otherwise lengthy process. Mutations that increase the cleavage and processing of AβPP subject neurons to the early secretion and aggregation of Aβ. Thus, affected patients skip the necessary accumulation of mitochondrial damage, and with aberrantly produced Aβ, display clinical symptoms much earlier than LOAD patients, regardless of the endogenous oxidative environment. Still, little experimental evidence supports this postulate, but it seems quite likely given the mountains of data.

Therapeutic Implications and Conclusions

We are currently faced with a strong incentive to produce a therapeutic agent that prevents AD. Based on the evidence presented here, the most likely method of adequate prevention may come from antioxidation. Preventing the accumulation of damaging species within vulnerable neurons would ideally protect them from the deleterious cascades that ROS accumulation yields. Unfortunately, antioxidant therapies have had little success thus far[98]. It should be emphasized that the failure of antioxidant clinical trials does not necessarily nullify the contribution of oxidative stress to the disease; other factors such as patient selection (it would be preferable to select patients with low antioxidants) and monitoring/analysis of the responders (it would be preferable to monitor oxidative stress surrogate markers before and during the treatment to make certain of compliance) are important for clinical studies. Better antioxidants, especially those that target the major ROS production sites, are needed. Several agents under investigation, such as MitoQ, acetyl-L-carnitine, and α-lipoic acid, do show potential; however, an effective method of treatment is far from complete[47, 99-110]. Based on the many prospective studies and the complexity of the redox system *in vivo*, a balanced combination of several antioxidants may also be needed to have a significant effect on the prevention of AD, but it appears that not much has been done on this aspect. Although agents that ameliorate pathologies secondary to AD are under increasing scrutiny[22], these treatments would only slow the course of disease progression and could not achieve its full eradication. To do so, the community must acknowledge all

aspects of disease pathogenesis and establish an unbiased understanding of its progression. Only then can research be adequately focused such that appropriate therapeutic intervention is possible. We here pose the oxidative stress "looking glass" as our best hope.

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