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Proposed Mechanism of Alzheimer's Disease: the Role of Oxidative Stress

A Review of Scientific Literature

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Abstract:

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that has become the sixth leading cause of death in the United States. The most notable neuropathological hallmarks of AD are the accelerated accumulation of β-amyloid deposits around neurons and the accumulation of neurofibrillary tangles (NFT) within neurons. These abnormalities block proteasome function, inhibit mitochondrial function, alter biometal levels, stimulate inflammatory processes, disrupt synaptic communication, and ultimately result in cell death. Mitochondrial dysfunction has been cited as an underlying cause of AD pathologies, specifically by enhancing the production and accumulation of β-amyloid. Once the β-amyloid oligomers form, they disrupt the Ca^{2+} signals of the astrocytes by forming calcium ion channels or influencing the existent calcium ion channels. The calcium disruption causes glutathione depletion, which leaves cells susceptible to further damage by oxidative stress. Oxidative stress, also enhanced by the mitochondrial dysfunction, has been extensively reported as having a major role in the development of neurodegenerative diseases. Thus, a vicious cycle ensues in which oxidative stress further enhances AD pathologies and further damages the neurons and astrocytes.

Introduction to Alzheimer's Disease

Alzheimer's Disease (AD) is the most common cause of dementia in elderly populations, accounting for 60 to 80 percent of all reported dementia cases. It is the sixth leading cause of death in the United States. This progressive neurodegenerative disorder is characterized by gradual memory loss, a decline in cognitive function and deterioration of physical function (1).

The greatest risk factor for Alzheimer's Disease is age- the majority of people diagnosed are over 65 years old. However, in about 5% of AD cases, early onset Alzheimer's Disease appears when a person is in their 40's or 50's. In the early stages of the disease, cognitive function declines and memory loss is mild. However, as the disease progresses over a few years, people eventually lose their ability to do daily tasks and engage in everyday conversations. The average life expectancy of someone diagnosed with AD is eight years. Ultimately fatal, this disease currently has no known cure (1).

The most notable neuropathological hallmarks of AD brains are the accelerated accumulation of β-amyloid deposits around neurons and the accumulation of neurofibrillary tangles (NFT) within neurons. β-amyloid are misfolded proteins that accumulate in the neuronal space of the brain. NFT are aggregations of hyperphosphorylated tau protein found accumulated inside the neurons of AD brains. These abnormalities block proteasome function, inhibit mitochondrial function, alter biometal levels, stimulate inflammatory processes, disrupt synaptic communication, and ultimately result in cell death (1).
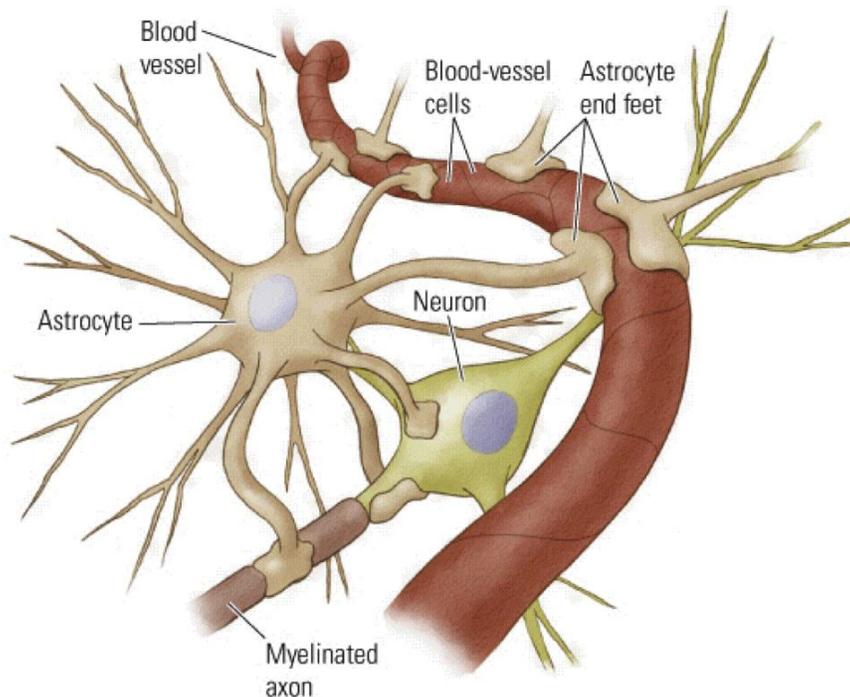
Neurons are the specialized cells in the brain responsible for the processing and transmission of information. The basic function of neurons- transmission of impulses- is disrupted by the AD pathology. Astrocytes, specialized cells also found in the brain, are responsible for supporting brain homeostasis. They primarily provide structural and metabolic support for the neurons. The function of astrocytes is also disrupted in many neurodegenerative diseases like AD. Apoptosis of astrocytes disrupts neurotransmitter homeostasis, synaptic transmission, and neuronal viability (2).

AD pathology begins in the entorhinal cortex of the brain, a region deep in the brain connected to the hippocampus. Then, it spreads to the hippocampus, the region of the brain that is involved in the processing of short-term memory and plays a major role in learning. Eventually, the AD progresses to other areas of the cerebral cortex. This includes, the medial parietal, lateral parietal, medial prefrontal, and medial temporal lobes. All of these areas of the brain play a role in episodic memory retrieval and encoding (3).

The progression of AD has been classified into three stages: a preclinical stage in which the disease is asymptomatic, a mild cognitive impairment (MCI) stage in which cognitive function declines, and a dementia stage (4).

It is believed that an AD brain begins deteriorating 10 to 20 years before any symptoms or clinically detectable signs emerge. During this preclinical stage, it is assumed that the AD pathology is causing damage to the brain, despite the fact that there are no manifested symptoms. As AD spreads throughout the brain, plaques and tangles develop, neuronal death occurs, brain shrinkage progresses, and more of the cerebral cortex is affected (5).

Cognitive decline is a normal consequence of aging. However, MCI is defined as a cognitive decline that is greater than expected for a healthy person of comparable age. MCI manifests itself in symptoms like: memory loss, confusion in familiar places, poor judgment, and personality changes. Although MCI is a step in the progression of AD, not all patients experiencing MCI will develop AD. Some patients with MCI never progress far enough to be diagnosed with dementia. Other MCI cases can develop into other dementia types, like Parkinson's disease or vascular dementia (6).

As AD progresses into the dementia phase, AD has spread to nearly all areas of the cerebral cortex controlling language, conscious thought, sensory processing, and reasoning. As the symptoms become more prevalent, the affected areas continue to shrink and the ventricles enlarge. Patients experience more severe symptoms, for example, shortened attention span, difficulty with language, loss of impulse control, and the inability to perform daily tasks. Ultimately, with severe AD, patients are entirely dependent on other people for their care. They have difficulty

swallowing, lose control of their bladder and bowels, and most frequently end up dying of aspiration pneumonia. This specific type of pneumonia develops as a result of the inability to properly swallow. The gradual progression of AD in a patient over the years results from a combination of genetic, biological, environmental, and lifestyle factors (5).

β-amyloid Deposits

β-amyloid is an insoluble, toxic polypeptide protein of about 39 to 43 amino acids. Unique to AD, β-amyloid accumulates into plaques and then into oligomers. These insoluble proteins clusters are found in the neuronal space of the brains of AD patients and block neuron function (7).

β-amyloid accumulates as a result of an error in a pathway that cleaves transmembrane Amyloid Precursor Protein (APP) into sAPPα. sAPPα is an important peptide fragment involved in promoting neuronal survival and growth. In the benign pathway, a protein called alpha-secretase cleaves APP. This releases sAPPα into the intracellular space. The remaining APP fragment, still embedded in the membrane, is cleaved by gamma-secretase. The smaller of the two fragments is released into the intracellular space, while the larger fragment enters the cytosol, eventually interacting with the nucleus (1).

In the alternative, harmful pathway, a third APP-cleaving enzyme, beta-secretase, cleaves the APP molecule at a different location, releasing a shorter sAPPα peptide. This leaves a larger polypeptide behind, still attached to the membrane. This segment of amino acids contains the entire β-amyloid sequence. Gamma-secretase then cleaves the remaining fragment, releasing the β-amyloid peptide into the intracellular space. These β-amyloid peptides begin to accumulate into insoluble oligomers and eventually form the plaques associated with AD (1). β-amyloid clearance by the central nervous system appears to be impaired in AD patients, further accelerating the accumulation of plaques (8).

β-amyloid oligomer density has a significant correlation with cognition performance. The aggregation of β-amyloid is neurotoxic. β-amyloid plaques have been directly associated with mitochondrial damage, oxidative stress, reduction in antioxidants, and destabilization of calcium ion homeostasis. In vitro, β-amyloid activates apoptotic pathways, ultimately leading to neurodegeneration. β-amyloid has been shown to interact with redox factor-1, which plays an important role in cell death signaling pathways and DNA repair (7).

One theory of β-amyloid toxicity is known as the "Amyloid Cascade Hypothesis". It assumes that β-amyloid is the main cause and driving force behind the AD pathologies in the brain. The idea is that β-amyloid oligomers form in the brain, disrupt the function of the neurons, and directly or indirectly kill the neurons.

According to this hypothesis, because β-amyloid formation is the cause of both familial AD (FAD) and late onset AD (LOAD), removing β-amyloid or preventing β-amyloid plaque formation is the best approach to preventing and treating AD (7). However, there is still no clinical evidence that indicates a strong correlation between plaque buildup and the degree of cognitive decline during AD. This would suggest that β-amyloid may not necessarily be the direct cause of the cognitive inhibition. Thus, there is still debate as to whether β-amyloid plaques are responsible for all neuronal damage associated with AD (8).

Neurofibrillary Tangles

The accumulation of neurofibrillary tangles within neurons is due to hyperphosphorylated tau proteins (7). Tau, or tubulin-associated unit, is a protein essential in the process of microtubule assembly. Tau protein is primarily found in the axons of the central nervous system neurons, but is also found in small concentrations in somatodendrite areas of neurons, in oligodendrocytes (cells responsible for axonal management and generation of myelin sheath), and in some non-neural tissues (9).

Tau proteins promote the assembly of microtubules and play a role in their stabilization. In normal cells, tau proteins are phosphorylated by protein kinases at multiple sites. The phosphorylation of the tau protein regulates its biological activity (9). In AD, an abnormal number of additional phosphates are added to the tau protein, causing the tau proteins to release from the microtubules. These hyperphosphorylated tau proteins bind with one another and form tangles within the cell, known as neurofibrillary tangles (NFT) (1). NFT are made up of abnormal filaments, wound helically around one another. The detachment of the tau proteins from the microtubules can cause the microtubules to disintegrate, collapsing the cell and disrupting the intercellular transport system. This phenomenon is not unique to AD. Unlike the β-amyloid plaques, neurofibrillary tangles are also observed in other neurodegenerative conditions like progressive supranuclear palsy, corticobasal degradation, and frontotemporal dementia. The presence of NFT alone is sufficient to cause dementia (9).

Braak and Braak used classical silver staining and immunohistochemical staining of hyperphosphorylated tau to show the gradual accumulation of NFT in neurons in AD patients. A clear association between NFT and cognitive impairment was found, further supporting the significance of tau pathology in symptoms of Alzheimer's Disease. The Braak staging system breaks down the progression of AD as associated with NFT into six distinct stages. The appearance of NFT in specific areas of the brain correlated with the impairment of specific brain related functions. For example, stage I and II, marked by NFT progression from the transentorhinal

region to the hippocampus, clinically correlates with the impairment of short term memory and with mild special disorientation. The research by Braak and Braak ultimately showed that the neuronal damage associated with AD begins well before clinical symptoms appear. Current research suggests this is due to the reduced ability of the neurons to get rid of misfolded, aggregated tau proteins that increase with advancing age (9). The accumulated NFT impair axoplasmic transport, the movement of proteins, vesicles, and organelles throughout the cell body. This ultimately leads to slower, impaired degradation of these abnormal accumulations of NFT (10).

Link Between NFT and β-amyloid Deposits

Although NFT and β-amyloid are the two hallmarks associated with AD, the exact connection between the two remains uncertain. Recent data has suggested that β-amyloid-induced neurotoxicity and synaptic failure results in tau phosphorylation. This would suggest that the β-amyloid somehow causes, or induces, the hyperphosphorylation of tau. Several studies support this hypothesis. β-amyloid plaques accelerate the formation of NFT in both tau transgenic mice and in cultured neurons. β-amyloid isolated from AD brains induced neuritic dystrophy resulting from abnormal tau phosphorylation (8).

Genetics of Alzheimer's Disease

Incidences of AD are classified into two different categories: Familial Alzheimer's Disease (FAD) and Sporadic Alzheimer's Disease (SAD). Early-onset AD is a rare form of AD that affects people between the ages of 30 and 60. Most of the early onset cases have genetic link. In some cases, early-onset AD is inherited as an FAD mutation. With these gene mutations, there is a clear genetic predisposition to developing AD. Usually, there is an autosomal dominant mutation in the amyloid precursor protein (APP) gene on chromosome 21, in the presenilin protein 1 (PSEN1) gene on chromosome 14, and/or in the presenilin protein 2 (PSEN2) gene on chromosome 1 (1). Mutations in PSEN1 and PSEN2 cause a partial loss of function in the gamma-secretase enzyme. This accelerates the production and accumulation of β-amyloid (20). Over 200 different mutations have been identified as being connected to FAD (1). These mutations contribute to this hereditary form of AD. The genes trigger the toxic β-amyloid cascade, which results in significant accumulation of the β-amyloid plaques (5).

Late-onset AD is much more common than early onset AD, accounting for about 95% of cases. Late-onset AD occurs in people over the age of 60. It is classified as SAD because mutations in the genes of FAD rarely play a role in the development of late-onset AD (5).

However, a gene called APOE, located on chromosome 19, has been linked to the development of late-onset AD (5). The APOE gene codes for a protein called Apolipoprotein E, which is the most prevalent lipoprotein in the brain (12). It is a major cholesterol carrier that regulates lipid transport and injury repair in the brain. It binds to several cell membrane receptors and delivers lipids to the cells. Apolipoprotein E has also been shown to bind to β-amyloid proteins and to NFT (13). Positron Emission tomography (PET) scans have shown that APOE is deposited in both NFT and β-amyloid plaques (12).

The APOE gene has three alleles, ε2, ε3, and ε4. The presence of the APOE ε4 allele results in a 2 to 3 fold higher risk of developing SAD. Furthermore, a person with two APOE ε4 alleles has an earlier onset and 12-fold higher risk of developing SAD than those individuals that are heterozygous, carrying only one APOE ε4 allele (3). On the other hand, individuals carrying the ε2 allele have a decreased risk of developing AD (13).

The exact cause of the link between the APOE alleles and AD remains unknown. However, the presence of the APOE ε4 alleles is clearly associated with the accumulation of β-amyloid and with hippocampus atrophy (3). APOE ε4 carriers also exhibit reduced glucose metabolism in neurons and increased inflammation in the brain (12). Although the exact mechanism of the Apolipoprotein E remains elusive, it has been hypothesized that the effects are related to this protein's regulatory role in tau phosphorylation, its function in the cell membrane repair and maintenance, and/or its affect on β-amyloid clearance (3).

Recent mice and human studies have shown that the presence of the APOE ε4 allele also has an effect on normal brain function, even in young subjects- both in mouse and human studies. Young mice without AD but with the presence of the APOE ε4 allele had task-specific spatial learning deficits. They also exhibited structural changes to presynaptic and postsynaptic compartments of the neurons, especially in the hippocampal regions. In human studies, young individuals with the APOE ε4 allele are less proficient at certain behavioral tasks than the APOE ε4 negative individuals. In addition, MRI scans show the inheritance of APOE ε4 causes specific changes in the brain activity in the medial temporal lobe (13). This research would suggest that the Apolipoprotein E protein plays a significant role in the cognitive abilities of a person.

Alzheimer's Disease Research: Obstacles and Limitations

The major obstacle scientists have in the research of AD is the lack of a sufficient in vitro or in vivo model. Currently, transgenic mouse models are used, which contain specific mutations in FAD genes. FAD genes are known to contribute to hereditary AD. However, even these models do not perfectly mimic a patient with

AD. A model with mutations in the in the APP or presenilin-1 or -2 gene can cause plaque formation as well as synaptic and memory deficits (14). However, these mutations fail to develop the NFT pathology and associated neuronal death. No model developed thus far has been able to replicate both β-amyloid and NFT pathology. Therefore, this complication has made it more difficult for researchers to pinpoint an exact mechanism of AD (3).

Oxidative Stress

Free radicals, specifically reactive oxygen species (ROS) and reactive nitrogen species (RNS), are an inevitable byproduct of biological processes. Free radicals are atoms or groups of atoms that have unpaired valence electrons, formed during many different reactions in the body. Although ROS and RNS play an important role in biological cell signaling, excess amounts of ROS or RNS ultimately damage cells by causing lipid peroxidation, DNA/RNA oxidation, and protein oxidation (15).

Living organisms have a natural, built in defensive system against damage caused by free radicals. Antioxidants are molecules that inhibit the oxidation of other molecules by scavenging for, and neutralizing free radicals. This prevents the free radicals from doing damage to the cells (15).

Oxidative stress is an imbalance between the production of free radicals and the capability of the cells to get rid of, or neutralize, the free radicals. Oxidative stress occurs when free radical production exceeds the ability of biochemical pathways to scavenge these free radicals. This can be a result of overproduction of free radicals, or not enough production of antioxidants. When cells are in a state of oxidative stress, they are very susceptible to damage (15).

Oxidative Stress and Alzheimer's Disease

ROS and RNS are unstable and are very reactive with other biomolecules. They can interact with and damage other biomolecules- lipids, proteins, and DNA/RNA. Increases in the amount of oxidative stress has already been linked with aging, as well as vascular diseases- both risk factors for AD. It has also been extensively reported that free radicals are pathologically important in the development of several neurodegenerative diseases, not just AD. Several studies have reported abnormally high levels of lipid peroxidation, DNA/RNA oxidation, and protein oxidation in AD patients (4).

Lipid peroxidation products are produced when lipids are attacked by ROS through a free radical reaction mechanism (4). The peroxidation of membrane lipids can impair cellular functions and cause the membrane to lose both fluidity and elasticity. Increased incidences of lipid peroxidation have been linked with many

health issues, including asthma and hepatic cirrhosis. Lipid peroxidation can be both mutagenic and carcinogenic (16). Specifically in AD, oxidized cholesterol has been shown to be a significant contributor to neuronal damage and the progression of AD pathology (15).

Protein oxidation can result from either direct attack by ROS or can result indirectly from glycoxidation and lipid peroxidation-lipid product binding. Protein oxidation damages the proteins and can cause a loss of function. The damaged protein molecules can interrupt a variety of cellular pathways and ultimately contribute to various diseases and disorders (16). High levels of protein oxidation has been found in the areas of the brain affected most severely by the AD histopathology- like the inferior parietal lobe and hippocampus. In contrast, there are low levels of protein oxidation in areas of the brain with low β-amyloid concentration, such as the cerebellum (15).

DNA and RNA can be damaged by oxidative species in three distinct ways: damage can be caused directly to the individual bases, damage can result in strand breakage, and damage can cause distortion of the helical structure of DNA. The modified DNA and RNA bases can result in a variety of genetic defects. The high prevalence of apoptosis observed in AD patients is directly triggered by DNA damage caused by oxidative stress (1).

The brain is particularly susceptible to oxidative stress because of the high rate of oxygen consumption by the cells of the brain. Of the oxygen respired by a person, 25% of it is utilized in the brain. This significant neuronal demand for energy is mostly due to the continued need to maintain ion gradients across plasma membranes. The high demand for oxygen increases the chance of creating free radicals, specifically superoxide (O_2^-), and hydroxyl (HO^-). Also, the brain has a high concentration of easily oxidized lipid species, which leads to higher rates of both lipid peroxidation and cholesterol oxidation. Beginning in the earliest stages of AD, the brain tissue is exposed to oxidative damage, this continues throughout the entire progression of the disease (15).

Within the brain, neurons are the cells that are most susceptible to ROS and RNS. The survival of these oxidized neurons depends on the antioxidant action of astrocytes. Astrocytes are cells that are important in providing structural and metabolic support for neurons (17). However, in comparison to other organs in the body, the brain has relatively low levels of antioxidants (18).

There are several studies that confirm a direct link between oxidative stress and AD. Increases in oxidative stress markers in the brain coincide with the progression of AD, suggesting that progression of AD might be related to the increase of ROS. The exact mechanisms underlying the disruption in ROS balance remain elusive. Support of oxidative stress as a cause of AD includes a study in

which levels of antioxidants decreased and extent of lipid peroxidation increased in triple-transgenic mice before the appearance of β-amyloid plaques and NFT. Furthermore, in vitro studies, oxidative stress has been found to directly promote both tau hyperphosphorylation and the aggregation of β-amyloid (17).

At the same time, there is a lot of evidence that oxidative stress is further enhanced by the tau hyperphosphorylation and by the aggregation of β-amyloid. As potent generators of ROS and RNS, β-amyloid plaques are a known promoter of oxidative stress. Within the sequence of β-amyloid, it has been suggested that the amino acid met-35 plays a role in promoting oxidative activity. When replaced by a cysteine residue in one study, oxidative stress induced by β-amyloid plaques dramatically declined (14). The formation of β-amyloid oligomers has been shown to further cause peroxidation by inserting themselves into lipid bilayers, causing oxidative damage to proteins and other biomolecules. It has also been observed in cultured neurons and in vivo mouse models that β-amyloid plaques cause an increase in oxidative stress that subsequently phosphorylates tau at the T231 residue (15).

Another indication of the link between AD and oxidative stress is the observation of decreased levels of antioxidants in patients with AD. In many reports, defects in the antioxidant defense mechanisms caused increased oxidative stress, further facilitating β-amyloid plaque deposition (15).

It has been suggested that β-amyloid deposition could be a protective response to neuronal damage (19). Because β-amyloid accumulation and oxidative damage are negatively correlated, β-amyloid plaques may be a compensatory response of the brain, intended to reduce oxidative stress. This theory could explain why immunization studies that decreased overall levels of β-amyloid plaques in AD brains show no improvement in cognitive function. To date, no treatments aimed at β-amyloid removal have been successful, suggesting that β-amyloid may not be the most appropriate therapeutic target (8).

Glutathione

Currently, definitively diagnosing a patient with AD is very difficult. Thus, determining specific biomarkers associated with AD, aside from β-amyloid and NFT will allow for early onset diagnosis and can potentially point to the underlying mechanisms of AD. Because of the short half-life of ROS, levels are difficult to measure directly. Measuring levels of antioxidants or antioxidant enzymatic activity can be used to determine about of ROS damage (4). Antioxidants are any substance that delays or prevents oxidation of substrates by scavenging oxidizing radicals or by regenerating oxidized biomolecules. Antioxidants play a key role in minimizing oxidative stress (18).

Studies looking at oxidative stress in AD patients have discovered decreased levels of glutathione (GSH), a specific antioxidant (20). Overall antioxidant levels in the brain are lower than other areas of the body. However, within the brain, GSH is the antioxidant present at the highest concentration. GSH plays a vital role in the maintenance of the function of brain cells. A decrease in brain level GSH has been associated with increased oxidative damage in the neurons. This ultimately increases apoptotic signaling and initiation of cell death. Both in vitro and in vivo studies have displayed a connection between GSH reduction and AD (17).

The application of β-amyloid to cell cultures led directly to GSH depletion in multiple types of cell cultures. Specifically, when β-amyloid was added to neurons and astrocytes, GSH depletion was induced (21). In an in vivo transgenic AD mouse model, the ratio of GSH to the oxidized form, glutathione disulfide (GSSG), in the blood, was monitored over time. The GSH/GSSG ratio in the brains of the mice decreased with the increase in AD pathology. This indicated a rise in oxidative stress. The GSH/GSSG ratio decreased before the onset of β-amyloid plaques. That was followed by an overall increase in the amount of GSSG but a continued decrease in the GSH/GSSG ratio. The increase in GSSG is a compensation mechanism for the increase in ROS. However, the relative levels of GSH continue to decrease (17).

There are several GSH related enzyme pathways that were also shown to change with AD pathology. Glutathione-S-transferase (GST) is an enzyme that catalyzes a reaction between a nucleophilic compound, such as 4-Hydroxynonenal (HNE), and GSH. The ultimate purpose of the enzyme is to detoxify the cells by getting rid of the GSH. It has been found that GST levels are significantly reduced in key areas of the brains of autopsied AD patients (22). In these same autopsied patients, mRNA levels of glutathione reductase enzymes, involved in GSH antioxidant activity with free radicals, are increased in certain areas of the brains. This mRNA increase is most likely the cell's way to attempt to compensate for the GSH depletion (17).

Specifically, mitochondrial GSH plays a critical role in the overall defense of a cell against oxidative stress. About 10-15% of the total cellular GSH is accounted for in the mitochondria. The mitochondria does not produce the GSH, rather it must be taken up from the cytoplasm first. When GSH is oxidized to GSSG, it cannot be exported from the mitochondria back into the cytoplasm. Ultimately, the presence of the highly reactive GSSG can inhibit mitochondrial function by interacting with proteins (18).

Changes in Intracellular Calcium and Glutathione

Recently, β-amyloid plaques have been shown to form pores in artificial membranes. β-amyloid has effects on ion selective channels, most notably, voltage gated calcium channels. It has been suggested that β-amyloid aggregations are directly inserted into the plasma membranes of astrocytes and initiate sporadic Ca^{2+} signals. The β-amyloid either forms calcium ion channels or influences the existent calcium ion channels. In the study, "Changes in Intracellular Calcium and Glutathione in Astrocytes as a Primary Mechanism of Amyloid Neurotoxicity", by Abramov et al. examines Calcium signal levels in cells treated and not treated with neurotoxic β-amyloid fragments (23).

The results show that β-amyloid causes sporadic fluctuations of Ca^{2+} in astrocytes, but not in nearby neurons. Proper maintenance of calcium levels in neurons is very important for neuronal signaling. Thus, the maintenance of proper calcium levels is also important for brain function, including the processes involved in learning and memory (23).

The increase in Ca^{2+} concentration observed in this study was not monotonic. The results showed transient Ca^{2+} signals, suggesting that the formed channels quickly dissociate or that there is a low probability that the β-amyloid will affect the calcium ion channels. It was previously found that the stability of β-amyloid in membranes is very easily disturbed. For example, it was found that in membranes that have increased levels of cholesterol, the beta sheet formation was favored over the alpha helix formation of β-amyloid (23).

These results would suggest that the β-amyloid affects the astrocytes, but not directly the neurons. However, astrocytes play a vital role in maintenance of the neurons, thus impaired neuronal viability was observed. Although astrocyte cell death was not directly caused by the insertion of β-amyloid into the membranes, calcium-dependent glutathione depletion was observed, indicating disturbed levels of antioxidants. GSH depletion leaves the cells more susceptible to damage by oxidative stress. Thus, this study ultimately concludes that the β-amyloid cause a direct decrease in GSH, by interrupting the calcium levels in the astrocytes (23).

Oxidative Stress and Mitochondrial Dysfunction

The progressive impairment of mitochondrial function has often been linked to normal aging process (24). Mitochondria are the major source of cellular energy. Dysfunctional mitochondria are less efficient at producing ATP and this subsequently leads to problems within the cells (3). Damaged mitochondria disrupt apoptosis-related proteins. As a result, dysfunctional mitochondria often result in increased rates of apoptosis (25).

Mitochondria are very vulnerable to damage by oxidative stress. Ultimately, oxidative stress has been labeled as one of the main contributors to mitochondrial dysfunction. There is much evidence that shows greater amounts of lipid peroxidation, proteins oxidation, and oxidative damage to mitochondrial DNA when compared with other organelles of the cell (25). Furthermore, mitochondria are also a major source of ROS generation. Electron leakage across the electron transport chain in the mitochondria produces superoxide anion, which accounts for 90% of endogenous ROS. Thus, damaged mitochondria are even more prone to generating ROS (4).

Several studies have found a significant amount of dysfunction in the mitochondria of neurons in patients with AD. This dysfunction is specifically linked with oxidative stress. Compared to an elderly control group, individuals with AD demonstrate significantly higher levels of oxidative damage in the mitochondria (25).

Almost all aspects of mitochondrial function are impaired in AD. One of the most documented abnormalities in AD is the reduced energy metabolism of neurons. This indicates a decrease in the ability of the mitochondria to produce ATP. Key enzymes for oxidative metabolism (i.e. pyruvate dehydrogenase complex, cytochrome oxidase, and α- ketoglutarate dehydrogenase complex) show reduced expression or reduced activity in AD patients (25).

Mitochondria also perform a central role in calcium homeostasis. As high capacity Ca^{2+} sinks, excessive uptake of Ca^{2+} has been associated with increased production of ROS, inhibited ATP synthesis, and increased signaling for apoptosis (25).

Mitochondrial Cascade Hypothesis

One group of researchers, Swerdlow, Burns, and Khan, have proposed a mechanism known as "Mitochondrial Cascade Hypothesis" that specifically links the deterioration of mitochondrial function with AD pathology. As stated above, mitochondrial dysfunction has already been linked with the normal aging process. However, this hypothesis suggests that the underlying cause of AD and AD pathology is an accelerated, or abnormal, deterioration of mitochondrial function. Once the mitochondrial dysfunction reaches a certain threshold, the histological changes associated with AD are triggered (4).

The Mitochondrial Cascade Hypothesis is broken down into three parts. Firstly, gene inheritance defines the baseline function of an individual's mitochondria. This means that the mitochondrion of each individual has an inherent level of function. Both parents contribute to an offspring's AD risk, but mothers contribute more since mitochondrial DNA is inherited maternally (4).

Secondly, the age at which mitochondrial deterioration manifest is affected by both environmental and inherited factors. Much research has concluded that mitochondrial changes are the main driving force behind many aging phenotypes. Thus, the belief is that the more durable the mitochondria, the slower the brain aging process (4).

Thirdly, both an individual's baseline mitochondrial function and their functional change rate influences the chronology of AD. For example, those individuals with low baseline function and fast rates of mitochondrial decline, would be expected to develop AD histology at a younger age than individuals with high baseline function and slow rates of mitochondrial decline (4).

This hypothesis expands on the previously defined "Amyloid Cascade Hypothesis" by suggesting that in late onset AD, it is mitochondrial dysfunction that affects APP expression and processing, as well as β-amyloid accumulation. This idea, that mitochondrial dysfunction is the root cause of AD, is supported by 1990s studies that showed mitochondrial dysfunction, as induced by toxins, pushed APP processing towards β-amyloid production. Furthermore, it is suggested that the mitochondrial dysfunction could produce other AD associated phenomena, like increased levels of oxidative stress markers, increased phosphorylation of the tau proteins, and increased inflammation in the brain (4).

Treatment Aimed at Alleviating Oxidative Stress: Orientin

Because of the clear link between oxidative stress and AD, recent studies have looked at using antioxidants as an effective treatment option for AD. In a 2015 study by Linju Yu, et al., the researchers studied the effectiveness of a therapy aimed at reducing mitochondrial dysfunction that is known to lead to oxidative stress in AD (25).

Erthyroid 2-related factor 2 (Nrf2), a transcription factor, enhances a cells defense against oxidative stress. Kelch-like ECH-associated protein 1 (Keap1) binds to Nrf2 to inhibit activation. Downstream of this activation, heme oxygenese-1 (HO-1) has been shown to play a neuroprotective role in the cytotoxicity induced by β-amyloid because of its redox properties. HO-1 is one of the most selective and sensitive antioxidant enzymes, known to play a significant large role in neuroprotection (25).

Nrf2 expression levels have been closely linked with AD brains. Nrf2 levels in the nuclei of the hippocampus are lower than normal in the brains of AD patients. At the same time, neurons that express Nrf2 are more protected from oxidative stress induced by β-amyloid. Furthermore, HO-1 mRNA and protein levels are significantly lower in AD patients (25).

Flavonoids, polyphenolic compounds found in plants, are regularly used in traditional medicine. Often cited at anticancer agents, recent studies in humans and animals have also suggested that flavonoids may prevent or delay neurodegeneration, especially in elderly populations (6). Orientin is a flavonoid compound that has anti-inflammatory and antioxidant properties. The data obtained by Linju Yu, et.al. showed that Orientin activated the Nrf2/HO-1 signaling. Thus, increased the levels of HO-1, increased the levels of Nrf2 and decreased levels of Keap1 inhibitor were observed. This decreased the β-amyloid-induced apoptosis in the brains of the transgenic mice. There was a decrease in overall neuronal death, reduction in ROS, and alleviation of memory and learning deficits. The results of this study suggest that Orientin might be a potential drug treatment for AD in the future (25).
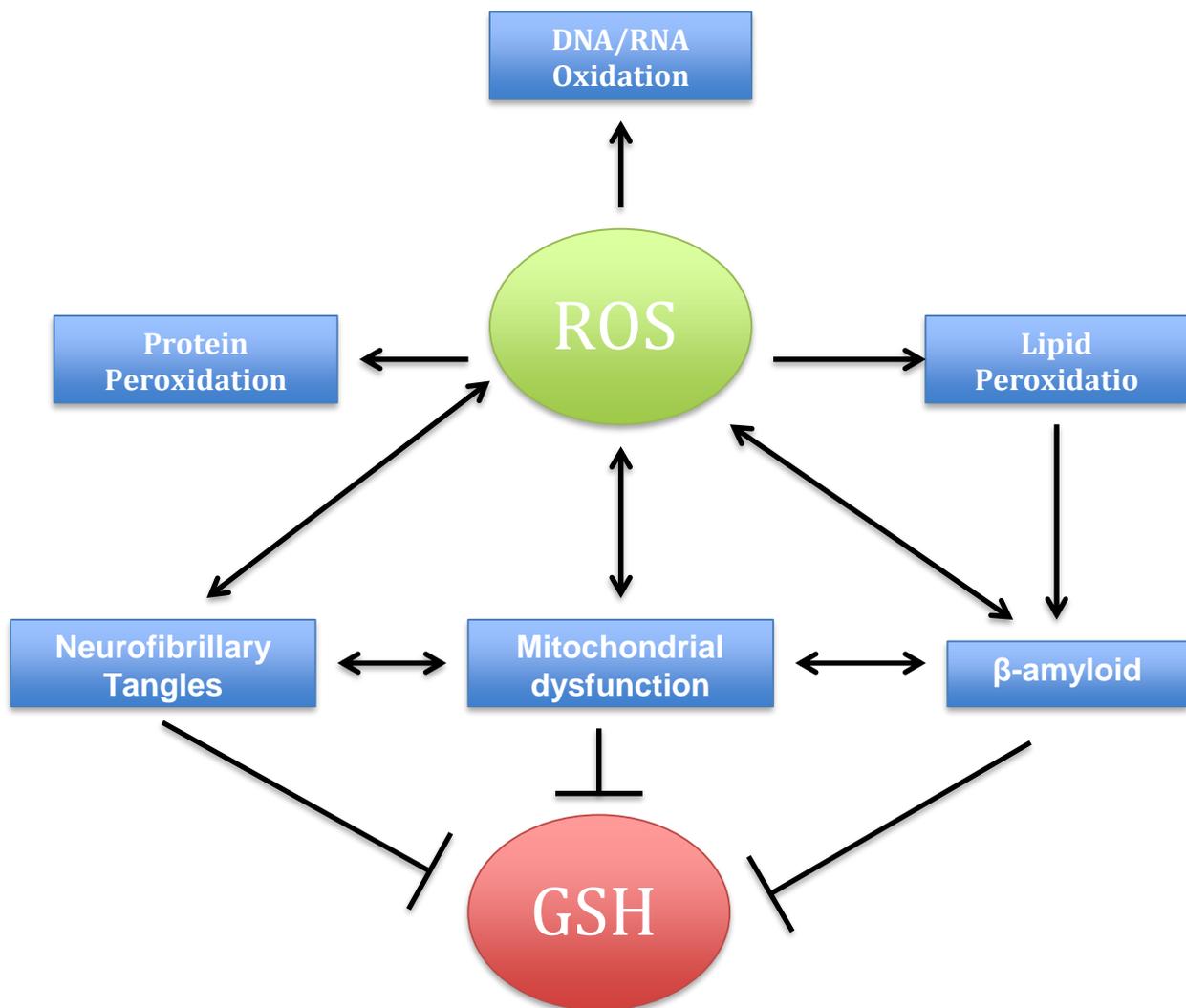
Treatment Aimed at Alleviating Oxidative Stress: Hesperidin

Hesperidin, a flavonoid found in citrus fruits, is already known to possess antifungal, antiviral, anti-inflammatory, and anticancer properties. In the in vitro studies, Hesperidin has also been shown to act as an antioxidant by scavenging for free radicals. Previous studies indicate that Hesperidin improved glucose utilization and protected neurons against β-amyloid-induced neuronal damage. In the paper by Dongmei Wang, et al., they look at how long term use of hesperidin affected memory loss, mitochondrial function, and oxidative damage in mouse models (26).

In summary, this study concluded that hesperidin reduced cognitive deficits and enhanced learning and memory in the mice. A reduction of oxidative damage was also demonstrated by the decrease in levels of hydrogen peroxide and malondialdehyde (MDA), both markers for oxidative stress. In addition, an increased level of glutathione was found in cells of the mice treated with Hesperidin compared to the control mice. Furthermore, the function of the mitochondria was restored, particularly in the electron transport chain enzymes, complexes I-IV. The exact mechanism underlying these effects is still unknown. However, it is most likely due to the antioxidant properties or alterations to the mitochondria (26).

Conclusion

The evidence presented in all the literature that was reviewed strongly supports the idea that the vicious cycle of oxidative stress plays a key role in nearly all aspects of AD. Oxidative stress damages mitochondria, inhibiting and altering their function. This further induces the formation of ROS. Possibly as an effort to compensate for oxidative damage, APP processing is pushed toward the formation of β-amyloid proteins. There is evidence that β-amyloid, which have also been shown to promote oxidative stress, causes the hyperphosphoylation of tau proteins. In addition, the β-amyloid disrupts the Ca^{2+} signaling in the brain, subsequently causing depletion of GSH antioxidants. The depletion of GSH further inhibits the ability of the brain to prevent oxidative damage. Thus, there is extensive evidence indicating the oxidative stress plays a significant role in Alzheimer's Disease. A summary of the role ROS plays in AD is outlined in the following figure:

The damage caused by oxidative stress, to the neurons, leads to protein oxidation, lipid oxidation, and DNA damage. All of these result in an increase in apoptosis, also seen in patients with AD. Proposed AD treatments aimed at mediating the mitochondrial damage and oxidative stress by the antioxidants Orientin and Hesperidin show significant results and promise for effective treatments for AD in the future.

My biochemistry classes, and my previous laboratory experience studying the effects of oxidative stress on HeLa cells, initially got me interested in the topic of oxidative stress. Oxidative stress is a known contributor to the progression of many diseases. Because Alzheimer's Disease is such a growing problem in the United States, I wanted to learn more about how oxidative stress contributed specifically to its pathology. Throughout the process of finding and reading these research papers, I learned how to effectively and efficiently digest, organize, and process the scientific information. Writing this senior honors project and presenting the information at the Celebration of Scholarship gave me invaluable experience in successfully communicating information to a wide variety of audiences. Furthermore, presenting the research paper about Orientin treatment by Linju Yu, et al. in my Honors Biochemistry 3 class allowed me to take the time to do an in depth study of the methods, results, and conclusions reached by the scientists. In summary, this literature review on the scientific evidence behind AD and oxidative stress led me to conclude that oxidative stress is a major contributor to, and result of, Alzheimer's Disease.

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