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Chemical Underpinnings of Alzheimer's Disease Symptoms and Causative Factors: A Systematic Review

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ABSTRACT

The brain depends on a complex network of chemical interactions to maintain its homeostasis and functionality. Accordingly, the pathology of neurodegenerative diseases, such as Alzheimer's disease (AD), has been shown to disrupt the chemical mechanisms that support the brain to carry out its degenerative effect. However, the nature of these disruptions remain elusive. AD is biologically defined by the deposition of Amyloid-Beta (Aβ) plaques and tau neurofibrillary tangles, and has been shown to be strongly correlated with the expression of the Apolipoprotein E4 (ApoE4) allele. Recent findings in the field of neurochemistry indicate the chemical underpinnings of hallmark signs of AD. This review will discuss the process of pathogenesis of AD, from the formation to the causal effects of AD markers, through a chemical lens. The fibrillization of Aβ isoforms as well as the fluent molecular mixing of tau isoforms and hyperphosphorylation of tau observed in mouse lines are discussed as precursor processes to Aβ plaques and tau neurofibrillary tangles. Furthermore, the pathology of Aβ plaques through redox chemistry in the brain, tau neurofibrillary tangles through microtubule disassembly, and ApoE4 through reactive oxygen species (ROS) formation are also presented. Current therapeutic approaches targeted towards specific facets of AD, such as Aducanumab, have found moderate success in treatment, and this progress indicates neurochemical pathways as a potential target for future therapeutic procedures to counteract the degenerative effects of AD.

Introduction

Alzheimer's disease (AD) is the leading cause of dementia in the elderly and afflicts over 36 million people worldwide[1]. Despite the dire consequences presented by the disease, no effective treatment exists. In the absence of effective treatment coupled with demographic shifts, the prevalence of AD is projected to increase to epidemic levels within the next few decades. Modern research suggests that there are three main facets of AD: Amyloid-Beta (Aβ) plaques, the ApoE4 genotype, and tau neurofibrillary tangles.

The extracellular accumulation of Aβ plaques was among the first symptoms to be recorded of the disease, and has come to be largely associated with AD development and pathology. While studies have proposed its correlation to larger-scale effects such as cerebrovascular dysregulation and neuron death[2], the precise mechanism by which plaque formation manifests and causes its degenerative effect relies on the electrochemical redox reactions that support the brain's homeostatic functions. Specifically, the regulation of free radicals such as reactive oxygen species (ROS) production through transition metal ions present in brain tissue is disrupted.

Reactive oxygen species are a wide class of oxygen-derived free radicals that play a dual role in maintaining and disrupting homeostasis. In abundance, ROS interferes with neurovascular coupling via the nitric oxide signaling pathway leading to decreased cerebral blood flow (CBF) and, over an extended period of time, extensive brain tissue

damage[2,3]. Furthermore, the generation of ROS has also been shown to be coupled with the formation of lipid peroxidation products as well as protein carbonyls, both of which result in the free radical attack of DNA, proteins, and other lipids in the brain. However, the NADPH oxidase (NOX) mediated release of ROS is also a key propagator in the innate immune inflammatory response to foreign, invading microbes[4]. As a result, a delicate balance exists between the necessary production and effective clearance of ROS in the brain.

In fact, the ApoE4 genotype is also able to utilize ROS formation in brain perivascular macrophages (PVM) to cause neurovascular dysfunction[5]. Carrying the ApoE4 gene significantly increases the likelihood of developing late-onset AD by not only increasing ROS production, but also mediating Aβ pathology, which is similarly related to free radical attack[6]. Furthermore, the ApoE4 genotype has also been shown to disrupt lipid homeostasis in the brain, causing detrimental cellular accumulation of cholesterol.

In addition to Aβ and ApoE4, the aggregation of insoluble filaments of the microtubule-associated protein, tau, into neurofibrillary tangles is the other major culprit in AD pathogenesis[7]. Under normal conditions, tau regulates the assembly and structure of microtubules. However, in AD and other neurodegenerative diseases, "pathological" species of the tau protein (p-tau) becomes abnormally hyperphosphorylated, disrupting its regulatory function and causing free tau proteins to aggregate into neurofibrillary tangles[8]. The pathology of p-tau is closely linked to neurovascular coupling, similar to Aβ and ApoE4, and dampens nitric oxide production by decoupling nitric oxide production from the activation of *N*-methyl-d-aspartate receptor (NMDAR)[9].

Lastly, the process of fibrillization serves as a crucial mechanism for mediating the degenerative effect of Aβ and p-tau, both of which involve insoluble aggregates of diseased proteins. The fluent mixing and fibrillization of protein isoforms (structurally different proteins of similar function) in tau neurofibrillary tangles (3R and 4R isoforms) and in Aβ plaques (Aβ40 and Aβ42 isoforms) are vital steps leading to the pathology of these factors and remain an important consideration for potential therapeutic approaches[10,11].

Amyloid Beta

Among all markers for Alzheimer's disease, Amyloid-Beta plaques are the most widely recognized. Aβ is formed by the cleavage of Amyloid Precursor Protein (APP) by β- and γ-secretases. The imperfect cleavage of APP by γ-secretases leads to two major isoforms of Aβ: Aβ42 and Aβ40. Despite being very similar in structure, Aβ42 has been shown to be far more toxic compared to Aβ40[11]. An increased Aβ42:Aβ40 ratio has been suggested to increase the likelihood of early-onset familial AD cases[12], as well as increased neurotoxicity[13]. On the other hand, a decreased Aβ42:Aβ40 ratio has been shown to significantly decrease Aβ deposition and subsequent plaque formation[14].

The key difference in toxicity lies in Aβ42 and Aβ40's differing fibrillization properties. Proteins begin by forming into "initial aggregates", or oligomers, from which fibrils are ultimately formed[15]. The process of fibrillization has been shown to begin with a "lag phase", which involves the oligomeric assembly of diseased proteins, before progressing to constructing the fibrils[15]. However, this "lag phase" can be skipped in the presence of fibril seeding, or pre-formed oligomers[15]. Evidence suggests that Aβ42 seeds are far more effective in spurring rapid fibrillization of Aβ42 oligomers compared to Aβ40 seeds with Aβ40 oligomers[15]. Studies of the deposition of Aβ42 oligomers support the idea that Aβ42 seeding is far more effective than the presence of other monomers or even other mature fibrils. As a result, the presence of Aβ42 in a mixture is able to accelerate the fibrillization far more than a pure Aβ40 mixture, which is unable to capitalize on the advantages of seeding in mitigating lag time in fibril aggregation.

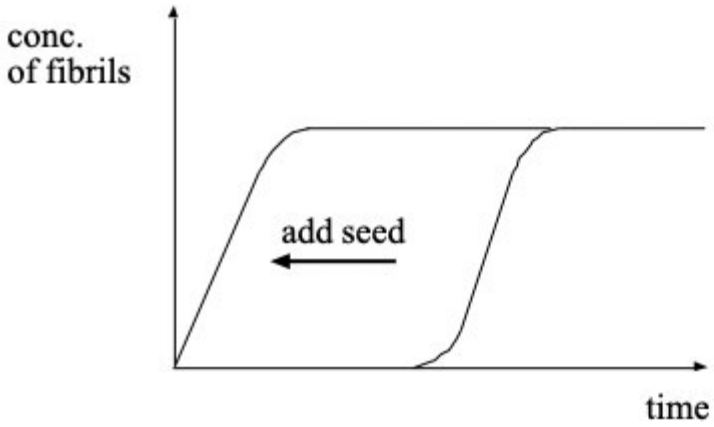

Figure 1. The addition of a fibril seed during Amyloid-Beta fibrillization eliminates the lag phase[15].

It has been well documented that AD patients have significantly decreased abilities to clear Aβ. In a healthy patient, soluble Aβ proteins are naturally cleared via Aβ-degrading enzymes such as Neprilysin and Insulin-Degrading Enzyme[16]. However, the accumulation of insoluble variants of Aβ plaques undermines homeostatic pathways for clearance and allows Aβ to carry out its neurodegenerative effect unhindered.

The actual pathogenesis of Aβ in AD depends on the oxidative stress resulting from the dysregulation of the redox chemistry of transition metal ions such as Copper, Zinc, and Iron. The brain naturally accumulates these metal ions, and normally relies on antioxidants to closely control the formation of reactive chemicals[17].

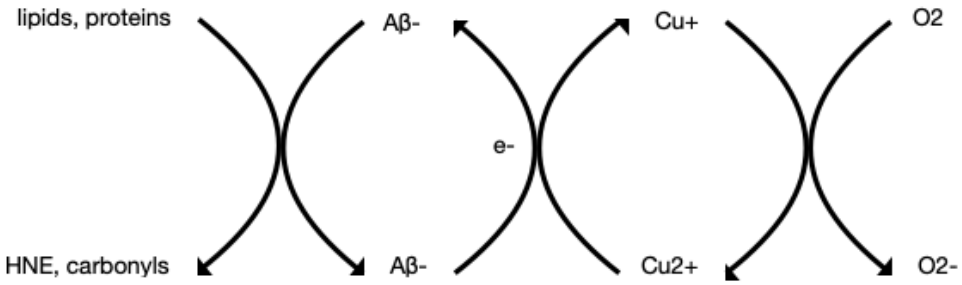

Figure 2. Amyloid-Beta mediated reduction of Cu2+ results in the formation of Amyloid-Beta free radicals that can extract protons from lipids and proteins to form highly electrophilic species (HNE and carbonyls, respectively). Cu+ formed via Amyloid-Beta mediated reduction reacts with molecular oxygen to form ROS.

In the case of overaccumulation of insoluble Aβ plaques, Aβ mediates the excess formation of ROS along with other reactive species such as lipid peroxidation products like 4-Hydroxy-2-nonenal (HNE) and protein carbonyls[18,19].

Reactive oxygen species are oxygen-derived free radical compounds that are crucial to homeostasis. ROS are inherently unstable and highly reactive free radicals. A free radical can be defined as any molecular species that has an unpaired electron in its orbital. The presence of this "free radical" allows the species to act as either an oxidizing or reducing agent and react with biologically important molecules such as DNA, proteins, lipids, and carbohydrates. The human immune system uses the deleterious effects of ROS in its innate inflammatory response to pathogenic microbes. Phagocytes release free radicals to lyse the contents of these foreign microbes.

However, in excess, ROS can disrupt neurovascular coupling by interfering with nitric oxide signaling pathways. As the most energy-demanding organ in the body, the brain's homeostasis depends on neurovascular coupling. Neurovascular coupling connects neural activity and cerebral blood flow (CBF), and relies on signaling molecules, such as nitric oxide, to couple changes in neural activity to changes in CBF. ROS such as the superoxide ion O2-, can react with NO to form peroxynitrite (ONOO-), effectively intercepting vasodilation signals meant for smooth muscle surrounding vessels to the brain.

Lipid peroxidation products such as HNE similarly disrupt key chemical bonding. The unique chemical structure of HNE results in an overwhelmingly electrophilic nature of the molecule that demonstrates high reactivity towards nucleophilic thiol and amino groups[20]. This reactivity allows HNE to significantly alter protein function by interfering with the cross-linking that occurs in the form of disulfide bridges between thiol groups, maintaining the tertiary level of structure in proteins. Hence, the reactive nature of HNE allows it to potentially react with a wide range of biologically important molecules, including DNA via epoxidation and amino acids via Michael addition (notably, thiol groups in cysteine) and Schiff base formation[20].

Protein carbonylation is another major marker of oxidative stress. Carbonyl formation can result from direct ROS free radical attack, as well as through reactions with lipid peroxidation products such as HNE and free reducing sugars (glycation)[21], all of which are indicative of oxidative stress mediated by Aβ.

Unlike lipid peroxidation products and protein carbonyls, the formation of ROS through Aβ requires a mediating-step. In fact, evidence suggests that Aβ causes the reduction of molecular oxygen by first reducing Cu2+, which in turn reduces oxygen to its free radical form[17]. The transition metal ion Cu2+ has a high affinity for the Aβ protein and is able to exist at multiple oxidation states, which allows it to mediate the reduction of oxygen relatively easily. The final addition of the free radical to O2 produces a highly reactive molecule that contributes to the oxidative stress in AD.

Iron, another abundant transition metal in the body, is also capable of serving as a mediator for the amyloid cascade, and has shown high affinity for the Aβ42 *in vitro*[22]. However, Fe ions are unlikely to react with Aβ *in vivo*, as a result of being associated with ferritin in the neuritic processes of the plaque[17]. Some studies in the past have shown a correlation between iron levels and amyloidogenesis[23,24], but it remains unclear whether Fe directly interacts with Aβ, and whether the correlation is a result of a secondary effect via other processes[25].

In contrast to iron and copper, zinc attenuates insoluble Aβ plaque growth and undermines its neurotoxic effect. Similar to Cu and Fe, Zn has a high binding affinity for Aβ and can occupy the multiple valence states characteristic of transition metals. However, the binding of Zn2+ to Aβ leads to the redirection of plaque assembly towards less toxic amorphous aggregation[26]. This process, acting in tandem with existing clearance enzymes in the brain, slows down insoluble plaque deposition. In fact, zinc supplementation has been shown to slow the rate of cognitive decline in AD patients[27]. Furthermore, past studies have suggested that Zn2+ can undermine Aβ pathogenesis by competing with Cu2+ and silencing its binding and subsequent reduction[28].

ApoE4

The Apolipoprotein 4 (ApoE4) allele of the ApoE gene has been shown to be the strongest genetic risk factor for AD. ApoE is normally responsible for lipid metabolism and is an essential factor in regulating the clearance and accumulation of bodily fats as necessary. Specifically, the expression of the ApoE gene in neurons and other brain inhabitant cells controls the transport of cholesterol and phospholipids throughout the brain. Cholesterol is a key building block for myelin and, as a result, brain cholesterol levels have been shown to affect signal transduction and synaptic plasticity[29]. In the absence of cholesterol, myelin formation and repair is significantly hindered and neuronal pathways are disrupted. However, intracellular buildup of excess cholesterol has also been shown to negatively affect myelination due to aberrant deposition of cholesterol in oligodendrocytes[30]. Therefore, the close regulation of cholesterol is essential for maintaining proper synaptic transmission of neuron signals, making ApoE a valuable facet in AD pathology, which undermines neuronal connections and framework.

ApoE exists in three main isoforms (ApoE2, ApoE3, ApoE4), all of which have vastly different effects on AD pathology. While ApoE4 increases the risk of developing AD, ApoE3 shows no effect towards AD development, and ApoE2 has even been shown to decrease the risk of AD. Notably, the ApoE4 genotype severely undermines cholesterol efflux from brain cells, while ApoE3 is more efficient than ApoE4, and ApoE2 is more efficient than ApoE3[31]. One proposed explanation for these discrepancies is in the differential formation of cholesteryl esters, which are used for the transport and storage of cholesterol. Cholesteryl esters are present in much higher levels in mice models of ApoE4 compared to ApoE3 and ApoE2[30], which indicate an increased storage of cholesterol in lipid droplets in the brain. This suggests the implication of cholesterol esterase, the enzyme that hydrolyzes cholesteryl esters to form cholesterol and fatty acids, in ApoE4-mediated AD pathology.

Beyond the abnormal alterations to regular protein activity in ApoE4, the pathology of the diseased protein also contributes to AD by inducing oxidative stress and neurovascular dysregulation via ROS generation. Similar to the ROS formed in Aβ pathology, free radical attack on signaling molecules such as NO cause significant chemical alterations, such that they are unable to reach their target or produce their desired effect.

Past studies have shown that ROS generation in the hippocampus of mice expressing the human ApoE4 isoform is significantly increased compared to wild type and ApoE3 controls[5]. However, although it is well established that the ApoE4 is correlated with increased ROS generation, the exact mechanism by which this occurs remains unclear.

One of the main hypotheses for ROS generation in ApoE4 models is that the ApoE4 allele mediates and contributes to the pathology of Aβ. Mice models of ApoE4 revealed that the diseased protein interacts with and relies on the binding with the Low Density Lipoprotein receptor-related protein 1 (LRP1) to contribute to the aggregation and deposition of insoluble Aβ40 and Aβ42[6]. Specifically, ApoE4 disrupts cell receptor-mediated natural clearance processes of Aβ by competitively binding to heparin sulfate proteoglycans (HSPGs) and interacting with LRP1. Both HSPGs and LRP1 normally contribute to enzymatic clearance of soluble Aβ. However, once insoluble Aβ plaques have fully formed, they are unaffected by enzymatic and other control factors that clear away soluble forms of Aβ, accelerating the pathological process. This hypothesis therefore indicates that the Aβ plaques, not the ApoE4 protein itself, is the source of ROS.

Another possible mechanism by which ApoE4 may contribute to ROS generation is by affecting calcium signaling through LRP1. In addition to being a receptor for ApoE, LRP1 is also a receptor for pore-forming subunit ligands of voltage gated calcium ion channels[32]. Specifically, the attachment of the α2δ-1 subunit ligand to LRP1 enhances the expression of the entire functional complex (Ca2+ gated ion channel) through LRP1's association with the receptor-associated protein chaperone[32]. Furthermore, Ca2+ signaling activates the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOX), the main source of ROS in many cells[33]. Through this proposed mechanism, ApoE4 induces ROS generation independently of Aβ by activating NADPH oxidases through calcium signaling.

Although it remains obscure the exact source of ROS in models of ApoE4, past evidence suggests both an Aβ-dependent and Aβ-independent pathway, of which either, or even both, could possibly contribute to the observed hippocampal oxidative stress.

Tau

The spread of tau pathology in AD is caused by the accumulation of neurofibrillary tangles of the insoluble tau protein. As a microtubule-associated protein in its healthy form, tau plays an essential role in synaptic plasticity, cell signaling, and the assembly and maintenance of microtubules in the brain. The transformation of tau from its healthy form into its diseased form of neurofibrillary tangles relies on the hyperphosphorylation of the protein, which marks the beginning of its pathological development.

Microtubules are made up of tubulin dimers structured in linear protofilaments that wrap around a hollow core. Under healthy conditions, tau stabilizes microtubules at microtubule binding repeats (domains), but the phos-

phorylation of tau induces the dissociation of tau from microtubules at microtubule binding repeats. Hyperphosphorylated tau becomes completely dissociated from microtubules and organizes into paired helical filaments (PHFs), which in turn aggregate into tau oligomers and neurofibrillary tangles[34].

Microtubules are formed by the polymerization of tubulin dimers[35]. In a healthy brain, tau contains microtubule binding domains made up of three to four sequence repeats each that promote its association with tubulin[36]. However, the hyperphosphorylation of tau results in the alteration of these domains such that they no longer associate with tubulin and maintain its protofilament structure. Under physiological conditions, the tau protein exists in its soluble form that can be easily regulated through clearance and formation pathways that exist in the brain[37]. However, the hyperphosphorylation of tau induces the formation of insoluble filaments, not only disrupting the healthy functions of the protein, but also sprouting its own unique pathology that contributes to neuron death in AD.

Hyperphosphorylation of the tau protein is caused by the deregulation of certain protein kinases. Although many protein kinases are capable of phosphorylating tau *in vitro*, *in vivo* studies have identified cyclin-dependent kinase 5 (CDK5)[38], glycogen synthase kinase-3 beta (GSK3β)[39], and extracellular signal-regulated kinase 2 (ERK2)[40], as the 3 key agents in tau hyperphosphorylation. Interestingly, the activity of these three kinases have also been shown to serve as a link between tau and Aβ pathology. Cultured neurons experienced activation of CDK5, GSK3β, ERK2, which contributed to tau neurofibrillary tangle formation via phosphorylation of tau[34].

The way in which soluble hyperphosphorylated tau aggregates into the characteristic neurofibrillary tangles in AD relies on the molecular incorporation of different isoforms of the tau protein. The microtubule-associated protein tau (MAPT) gene encodes for six different isoforms of the tau protein. Alternative splicing of the exons on the encoding gene differentiates the isoforms by the presence of either 1 or 2 N-terminal repeats and of either 3 or 4 C-terminal microtubule binding repeats[41]. The six isoforms are 0N3R, 1N3R, 2N3R, 0N4R, 1N4R, 2N4R, where 3R isoforms contain 3 C-terminal microtubule binding repeats and 4R isoforms contain 4 C-terminal microtubule binding repeats. For tauopathies, the molecular composition of 3R and 4R isoforms differentiate one another in their unique yet related pathologies. Corticobasal degeneration, globular glial tauopathy, and progressive supranuclear palsy all only involve 4R isoforms, while Pick's disease involves only 3R isoforms[42]. However, AD has been shown to exhibit fluent molecular mixing between both isoforms[10]. Evidence from cryogenic electron microscopy analysis suggests that the core pair of tau protofilaments in the insoluble filament types present in AD allows 3R and 4R isoforms to be indiscriminately added without change in its conformation[43]. This supports the idea that propagation of tau fibrils in AD rapidly accelerates after initial seeding as a result of the inherently stable addition of all isoforms. In fact, intracerebral injection of initial AD tau filaments was found to induce the formation of neurofibrillary tangles in wild-type mice with no genetic predisposition for AD[43]. Moreover, the ability to indiscriminately add all isoforms also suggests that the prevalence of AD among other forms of dementia may be partially rooted in its nature of incorporating all isoforms instead of selective addition. Rarer forms of dementia such as corticobasal degeneration and Pick's disease involve only either 3R or 4R isoforms in their models of tauopathy.

Once insoluble filaments have formed and advanced into neurofibrillary tangles, tau can no longer be easily cleared naturally by the brain. With little to no regulation on its presence and growth, tau induces the uncoupling of neuronal nitric oxide synthase (nNOS) from postsynaptic density 95 (PSD95)[9]. PSD95 is a scaffolding protein that plays a critical role in organizing the framework for postsynaptic signaling complexes, anchoring glutamate receptors and vascular signaling enzymes. Specifically, it has been shown that glutamatergic synaptic activity induces functional hyperemia through the glycine-glutamate *N*-methyl-d-aspartate receptor (NMDAR), which is linked to nNOS via PSD95. Hence, PSD95 serves as a key intermediate between the glutamate and nitric oxide signaling pathways that together define neurovascular coupling.

By uncoupling nNOS from PSD95, tau effectively disconnects the chemical signaling pathway that connects glutamate and glycine signalers to NO formation. The activity of the enzyme nNOS in forming NO is unpaired from brain communications via glycine and glutamate for increased blood flow. Significant differences in changes in CBF due to stimuli were reported in mice expressing the mutated human tau form P301S as well as mice expressing the

P301L mutation in comparison to wild-type mice[9]. These attenuated changes in CBF can be attributed to the uncoupling of nNOS from PSD95.

Discussion

Alzheimer's disease is one of the greatest public health challenges of the century that the world faces. As of today, it remains one of the only leading causes of death with no definitive cure. There has been progress on therapeutics, and several ways of identifying symptoms and predicting onset through genetic testing exist. Past studies have shown that changes in lifestyle can improve brain health and affect AD onset. However, current treatment methods are only capable of slowing the progression, but no complete cure or prevention exists. Moreover, these methods only target specific facets of the disease, such as Aβ plaque deposition and formation.

Perhaps the largest challenge in seeking to cure AD is in the multifaceted nature of its pathology. Aβ, tau, and ApoE4 are completely distinct in their normal functions, and yet their pathologies in the context of AD are closely interrelated. From the way in which they are formed to their resulting effects, the three facets of the disease contribute to one another and cause similar effects. ApoE4 has been hypothesized to contribute to Aβ deposition, accelerating the formation of insoluble plaques, and is also capable of forming ROS independent of Aβ. Aβ proceeds through an independent transition metal-mediated pathway that results in ROS formation similar to ApoE4. Tau neurofibrillary tangles similarly affect smooth muscle dilation in a separate dissociation mechanism that dampens the formation of nitric oxide, which both ApoE4 and Aβ then interfere with post-formation through ROS generation.

Future therapies aimed towards curing AD must be able to address multiple facets at once by targeting intersecting pathways for pathology. Therapies targeted towards specific symptoms of AD can find only limited success, and an inability to fully cure or prevent the disease. Despite the hurdles to drug development that exist, a potent and overarchingly effective therapeutic is vital to the treatment of existing patients as well as the prevention of AD for those with high risk factors.

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