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CRITICAL REVIEW

Tau and beta-amyloid in Alzheimer's disease: Theories, treatments strategies, and future directions

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ABSTRACT

Alzheimer's disease is a severe neurodegenerative disease characterized by the deposition of neuritic plaques on neuronal membranes and the formation of neurofibrillary tangles within neurons. Several proteins, such as amyloid beta and tau, play major roles in the pathophysiology and progression of Alzheimer's disease and are important factors to consider when developing novel therapeutics. The following review outlines the current understanding of the role these peptides play in Alzheimer's and the state of therapeutics that target them. Contrary to previous theories, it is now understood that soluble amyloid beta and tau proteins are more neurotoxic than the insoluble aggregates they form. Treatments that target the individual biosynthetic pathways of these neurotoxic proteins have been ineffective. Thus, new therapies must overcome challenges associated with pharmacokinetics and clinical research design.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative condition resulting in ~3.5% of all human deaths — a statistic projected to triple by 2050 as a result of aging populations.[1-3] While it is typically characterized by severe memory loss, AD also results in linguistic, visuospatial, and cognitive deficits, primarily due to damage of the hippocampus.[4] AD commonly affects those greater than 65 years old.[5] Therapies have focused on inhibiting the accumulation of amyloid and tau proteins, but these treatments have been ineffective.[6-8] The objective of this review is to discuss the current understanding of the roles and mechanisms of key proteins and oligomers in the pathophysiology of AD, address the shortcomings of current treatment strategies, and offer insight into future research and therapeutic directions.

AMYLOID PROTEINS

Amyloid beta (Aβ) peptides are among the most studied proteins implicated in the pathophysiology of AD. They form the basis of one of the most popular hypotheses regarding AD pathology, the amyloid cascade hypothesis, which suggests that Aβ proteins aggregate into plaques on neuronal membranes, leading to a series of pathological changes that ultimately result in the clinical symptoms of AD.[7] These proteins are produced through a series of biosynthetic secretory pathways beginning with the proteolytic cleavage of the amyloid precursor protein (APP), encoded by a highly conserved gene located on chromosome 21.[9,10] APP is an integral protein with a single transmembrane domain that is normally cleaved by α-secretase, an enzyme that releases the soluble extracellular domain of APP.[11] This pathway is non-amyloidogenic, as APP is cleaved at a residue contained within the Aβ primary sequence.[12] In AD, however, APP is instead cleaved extracellularly by β-secretase and in the transmembrane region by γ-secretase, an oligomeric enzyme complex composed primarily of presenilin proteins.[13,14] These presenilin proteins and the transmembrane APP cleavage for which they are responsible are essential in the development of AD.[15] The reason for this remains elusive. This abnormal pathway is generally understood to be the mechanism by which Aβ proteins are derived from APP.[4] Aβ exists in several forms, the most toxic of which contain 40 and 42 amino acids, known as Aβ40 and Aβ42, respectively. These forms are present in both human blood and cerebrospinal fluid (CSF).[16]

Soluble Aβ oligomers form fibrils and plaques through a well-described process known as nucleation-dependent polymerization (Figure 1). Initially, individual monomers interact with each other through hydrogen bonding and Van der Waals forces, gradually forming larger polymers. Since this nucleation phase is thermodynamically unfavourable, it is the rate-limiting step in the polymerization process. After a nucleus of adequate size has formed, monomers can interact at several sites allowing a complete fibril to form rapidly.[17] The rate-limiting step can be overcome by adding already-formed nuclei, known as seeds, to a solution of Aβ monomers, thus significantly accelerating fibril polymerization.[18,19] These polymers grow continuously, eventually adhering to neuronal membranes in large, complex structures known as neuritic plaques.

Several mechanisms have been proposed to explain the neurotoxicity of Aβ. While it was once thought that plaques were the primary toxic species in AD, it has recently been demonstrated that soluble Aβ40 and Aβ42 oligomers are in fact more responsible for cell death than their aggregated counterparts.[20,21] These soluble Aβ species exhibit a secondary structure consisting of either two hydrophobic β-pleated sheet regions or the formation of a β-barrel, which cause cell death by inserting into neuronal membranes and destabilizing them.[22,23] Additionally, it has been proposed that Aβ oxidatively damages membrane lipids, prematurely triggers neuronal apoptotic mechanisms, and opens calcium channels in neuronal membranes leading to electrical imbalances and subsequently cell death.[24-26] Ultimately, fewer neurons result in fewer synapses and decreased cognitive function.

In summary, Aβ is a class of proteins produced by proteolytic cleavage of the transmembrane APP by β- and γ-secretases. Through the formation of fibrils, plaques, and soluble oligomers, Aβ plays a major role in neuronal cell death and is one of several proteins implicated in AD.

TAU PROTEINS

In addition to Aβ, tau proteins have been significantly studied and play a more important role in AD. Tau describes a family of six protein isoforms that result from alternative splicing of the *MAPT* gene located on human chromosome 17.[27,28] These isoforms may vary, with an additional 29- or 54-amino acid sequence found at the N-terminus of certain isoforms implicated in AD.[27]

Due to its mostly hydrophilic nature, tau does not exhibit a compact secondary structure similar to many other proteins and is naturally unfolded.[29,30] These physical properties make tau unique among proteins and are important to consider when developing a biochemical understanding of AD.

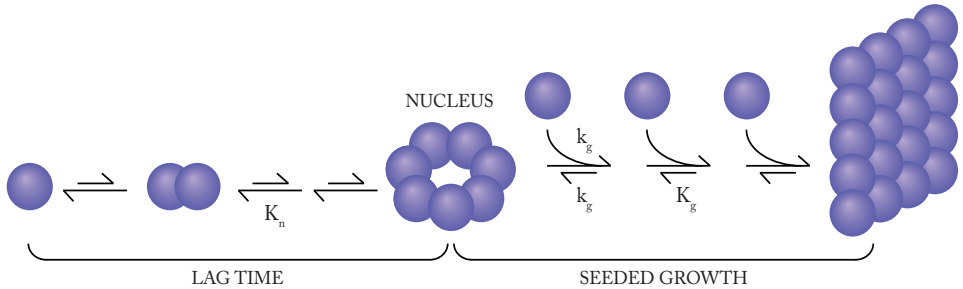
Unlike Aβ, the role of tau proteins in healthy physiology is well understood. These proteins are essential for the stabilization of microtubules in neurons, which facilitate intracellular transport and neurotransmission.[31,32] Microtubules are polymers of the dimeric protein tubulin, and are regulated through two distinct phases: a shrinking phase known as catastrophe, whereby tubulin subunits are removed from one end, and a growth phase known as rescue, whereby tubulin subunits are added to the opposite end.[33] Tau slows the rate of catastrophe and accelerates rescue by binding to individual tubulin dimers and inducing conformational changes that strengthen interactions between the various tubulin units.[34-36] This allows microtubules to grow to considerable lengths, which is particularly important in neurons, where microtubules must facilitate the transport of vesicles over great distances down the axon.

The role of tau in AD has been widely studied and involves several different signalling pathways. Under normal conditions, tau and other microtubule-associated proteins are phosphorylated to decrease their affinity for tubulin, thereby detaching them from microtubules. This is advantageous in certain circumstances since microtubule-associated proteins often serve as obstacles for motor proteins as they transport cargo over microtubules.[37] Tau in AD is hyperphosphorylated at several sites, which excessively decreases its affinity for tubulin and limits its ability to stabilize microtubules.[38-40] The shift of this balance towards the hyperphosphorylated state has been observed *in vivo*, suggesting that this process in AD may be the result of an imbalance between phosphatases and kinases.[41,42] Tau in AD is also subject to cleavage by several proteases which releases protein fragments that may further decrease the ability of tau to stabilize microtubules, resulting in neuronal death.[43,44]

Both hyperphosphorylation and proteolytic cleavage of tau cause it to be detached from microtubules and increase its propensity to aggregate into insoluble structures known as neurofibrillary tangles (NFTs).[45] NFT formation is similar to Aβ aggregation in several ways: it follows a nucleation-dependent mechanism that can be accelerated through the addition of seeds, and it is likely that these large aggregates are less neurotoxic than the soluble oligomers and monomers from which they are formed.[46-49]

Tau, like Aβ, is an important protein implicated in the pathophysiology of AD due to its ability to form aggregates via hyperphosphorylation. However, there are significant differences between NFT and Aβ processes, such as the fact that NFTs are cytosolic while Aβ aggregates are extracellular.[50] The exact mechanisms of tau toxicity are unclear, although it is likely that hyperphosphorylated and aggregated tau causes neuronal death by failing to support microtubules.[51]

FIGURE 1. A simple nucleation-dependent polymerization mechanism.



Monomers (depicted as violet spheres) are in equilibrium with small oligomers in the thermodynamically unfavourable nucleation phase shown on the left (K_n<<1). When a nucleus of adequate size forms, monomers can interact at various sites and the process becomes favourable (K_g>>1), leading to a period of rapid growth, shown on the right.[17] The rate limiting step can be skipped entirely by adding exogenous nuclei to a solution of monomers.[18,19]

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TREATMENT APPROACHES

Several therapies have been developed that attempt to disrupt the protein pathways involved in AD (Table 1). The amyloidogenic pathway is frequently the target of such therapies, many of which inhibit β- and γ-secretase. Initial studies on AD in β-secretase knockout mice models showed significantly reduced blood and CSF Aβ concentrations. However, neuronal myelination in these mice was significantly impaired, suggesting that β-secretase plays a role in healthy physiology.[52,53] In spite of this, pharmacological β-secretase inhibitors have been developed, which have proven promising *in vitro* but do not translate to clinical benefits.[54-57] Similarly, γ-secretase inhibitors have demonstrated significant *in vitro* reduction of Aβ, but have shown no clinical benefit despite decreasing plasma Aβ concentration.[58-61] This points to a more complex role of Aβ in AD that is not yet fully understood.

Some drugs adopt the opposite approach; rather than inhibit β- and γ-secretases, they stimulate α-secretase. As described above, APP can be cleaved through an amyloidogenic pathway that involves β- and γ-secretase or a non-amyloidogenic pathway that involves α-secretase.[12,13] By activating protein kinase C, drugs such as bryostatin and semagacestat enhance α-secretase activity, leading to decreased Aβ production *in vitro*.[62,63] Early clinical trials demonstrate significant cognitive improvement and no adverse side effects, but also report potential bioaccumulation at higher doses.[64] Therefore, α-secretase stimulation is a promising avenue of research and warrants further exploration.

Tau, however, has not been a therapeutic target in AD, likely because research focused on Aβ for several decades without considering the possibility that tau may be a key driver.

The development and testing of novel treatments for AD currently face many challenges. Firstly, any drug that aims to treat AD must cross the blood-brain barrier (BBB), a tight membrane of epithelial cells that cover much of the brain and spinal cord.[65] A great deal of research is focused on bypassing the BBB, employing strategies such as encapsulating drugs in modified red blood cells or creating openings with ultrasound.[66,67] Additionally, given that current AD diagnosis is based primarily on cognitive tests and that AD progresses slowly over an extended period, designing effective clinical trials is difficult.[68] Challenges associated with drug delivery, AD diagnosis, and clinical research design combined with the complexity of AD pathophysiology make drug discovery challenging.

CONCLUSION

Tau, Aβ, APP, and the various secretases, proteases, and kinases that regulate their synthetic pathways have all been investigated as potential drug targets in AD, with limited success. The chemical processes of this disease, such as the nucleation-dependent polymerization of both tau and Aβ proteins, the destabilizing effects these processes have on neuronal membrane, and their related neurotoxicity, are crucial concepts in understanding AD. Novel therapeutics will need to address the relationships between these complex pathways, as well as overcome difficulties associated with drug delivery, clinical research, and the inherent complexity of AD pathophysiology.

TABLE 1. Summary of AD therapies targeting protein pathways

Treatment	Mechanism	Trial Stage	Reference
LY2886721	BACE1 inhibitor	Phase I: Safety and efficacy	May 2015[54]
Verubecestat	β-secretase inhibitor	Phase I: Safety and efficacy	Egan 2019[56]
Lanabecestat	β-secretase inhibitor	Phase I: Safety and efficacy	Wessels 2019[57]
Semagacestat	γ-secretase inhibitor	Phase III: Superiority	Doody 2013[59]
LY450139	γ-secretase inhibitor	Phase II: Tolerance	Fleischer 2008[60]
LY450139	γ-secretase inhibitor	Phase II: Tolerance	Siemers 2006[61]

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