Theories of Alzheimer's disease: amyloid hypothesis, blood-brain barrier hypothesis and cholinergic hypothesis

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Abstract. Alzheimer's disease (AD) is the biggest drain on society's resources in the developed world. AD accounts for between 60 and 80 percent of dementia cases in older persons, making it the most prevalent cause of dementia. This paper analyzes three theories and experiments about AD, which are amyloid hypothesis, blood-brain barrier (BBB) hypothesis and cholinergic hypothesis. The amyloid hypothesis suggests that cognitive decline in patients is caused by the accumulation of amyloid beta (Aβ), which creates plaques that block the efficient transmission of neuronal signals. The BBB hypothesis suggests that the breakdown of the integrity of the BBB allows harmful substances and immune cells to enter the brain, causing inflammation and neuronal damage, which leads to cognitive decline in Alzheimer's patients. The cholinergic hypothesis proposes that AD is caused by the nervous system's reduced production of the neurotransmitter acetylcholine.

1 Introduction

Alzheimer's disease, commonly known as AD, is a neurodegenerative aliment that affects the brain and progresses over time. It is divided into sporadic Alzheimer's disease (SAD) and hereditary Alzheimer's disease (FD), of which SAD accounts for 95% of all cases. It causes a steady deterioration in various human functions like memory, cognitive function, and functioning in every day. When the condition worsens, patients lose function and eventually die, with an average life expectancy of three to five years after diagnosis. There is currently no treatment to stop or reverse Alzheimer's, only to delay its symptoms. Illness makes patients increasingly need to be cared for by others, placing a burden on the caregiver's mental, economic, and physical levels. The most important risk factors of AD are the advanced age. After the age of 65, the disease is much more common, and the risk keeps increasing as people age exponentially. As the population ages, AD will place a growing economic burden on the world. Finding effective means to treat or delay the development of AD has become an important topic. These hypotheses include amyloid peptide hypothesis, BBB hypothesis, cholinoxic hypothesis, calcium balance hypothesis, mitochondrial cascade hypothesis and heavy metal hypothesis. Considering that sporadic AD accounts for the vast majority of Alzheimer's cases, this paper presents several mainstream hypotheses and practical of the pathogenesis and experimental evidence for SAD.

2 Amyloid hypothesis

2.1 The introduction of Amyloid Hypothesis

The Amyloid hypothesis in AD is one of the theories that seeks to explain the mechanisms of AD. The amyloid beta (Aβ) buildup is thought to be a key factor in the etiology of the illness. The Amyloid Hypothesis stands out as a prominent work that seeks to elucidate the disease's etiology and pathogenesis.

Aβ is a normal consequence of brain activity. In general, there will be no problems with the production and clearance of Aβ. However, in the situation of AD, a disruption in this process occurs, leading to the accumulation of Aβ plaques in the brain. These plaques, which are abnormally shaped with various protein fragments, are created as a final product of the buildup of Aβ. These plaques frequently build up between nerve cells, impairing the efficient signal transmission. The coordinated network of neurons is essential for memory and cognitive processes. When there are Aβ plaques built up, distinctive cognitive deterioration is seen in Alzheimer's patients.

Amyloid Precursor Protein (APP) is a single-pass transmembrane protein expressed at high levels in the brain and metabolized by and secretase. When APP is cleaved by these secretases, neurotoxic peptides are released, which accumulate into oligomer aggregate [1]. However, the capacity of the metabolism to break down Aβ is reduced in senior individuals or those with

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pathological conditions, and Aβ peptides may build up (Fig. 1) [1]. The major components of the accumulated Aβ including 40 and 42 amino acid residues. Elevated Aβ 42 concentrations or ratios lead to the formation of Aβ amyloid fibrils. As amyloid fibrils build up, they eventually transform into senile plaques that induce neurotoxicity, trigger a cascade of neuroinflammatory responses, and cause the loss of neuronal cells and neurodegeneration. There are a few APP mutations at the secretase cleavage site and are associated with increased synthesis of Aβ 42 [2].

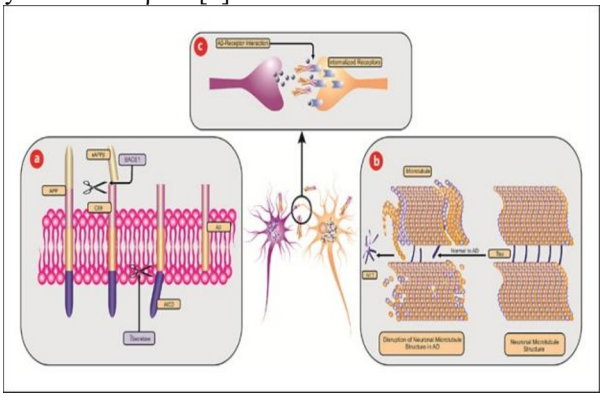

Fig. 1. Major features of AD within the neuronal system [1]

2.2 The possible treatment based on amyloid hypothesis

2.2.1 LDL receptor related protein (LRP1) receptor mediated uptake and degradation of Aβ

LRP1 is an endocytogenic receptor belonging to a group of low-density lipoprotein receptors. These cell surface receptors are involved in processes such as cholesterol metabolism, intracellular transport and cellular conduction. LRP1 plays an important role in Aβ clearance through receptor-mediated endocytosis in neurons [3]. To determine the metabolism of LRP1 in Aβ in human neuroblastoma NTERA-2(NT2) cells, the siRNA of LRP1 in NT2 cells was knocked down. The results showed that the associated Aβ levels were significantly reduced in LRP1-inhibited NT2 cells compared to control cells, and Aβ clearance was reduced. These results suggest that LPR1 is involved in the uptake and subsequent degradation of Aβ in neuronal cells. Conditional removal of LRP1 from mouse neurons resulted in increased levels of Aβ in the brain, while in vivo microdialysis studies showed that neuronal LRP1 knockout impaired Aβ clearance in mice's interstitial fluid.

2.2.2 Aβ degrading enzyme

Metabolic markers in living mice showed that the transfer of newly generated Aβ in the brain was very rapid. Therefore, the use of Aβ-degrading proteases helps to regulate the level of Aβ in the Brain. Among proteases that appear to degrade only Aβ in its monomeric state and proteases that degrade peptides in oligomeric and or highly clustered (fibrous) forms, the latter has received less attention, possibly because Aβ peptides undergo structural changes due to polymerization into starch-like fibers, thereby developing resistance to A range of proteases.

Enkephalinase (NEP), also known as neutral endorphins, is a type II membrane protein that hydrolyzes circulating bioactive peptides, including enkephalin, cholecystokinin, neuropeptide Y, and substance P. Studies have shown that NEP mRNA levels in AD hippocampus are lower than mRNA levels in control brain neurons [4].

Insulin-degrading enzyme (IDE) is A 110kDa zinc metal endopeptidase that hydrolyzes a variety of regulatory peptides, including insulin, islet glucagon, atrial natriuretic factor, transforming growth factor alpha, beta-endorphin, islet amyloid polypeptide, Aβ, and APP intracellular domain (AICD). In A mouse experiment with IDE gene disruption, mice with low IDE function had impaired in vivo degradation of Aβ, insulin, and ALCD, and exhibited some of the signature features of AD and DM2, namely A slow increase in brain Aβ, as well as hyperinsulinemia and diabetes. This confirms the key role of IDE in the metabolism of A by others in vivo [5].

3 BBB structure and function: the gatekeeper of the CNS

3.1 Introduction to BBB theory

The BBB is a complex and dynamic interface that separates the central nervous system (CNS) from the systemic circulation. Comprised of specialized endothelial cells interconnected by tight junctions, the BBB acts as a selective barrier, regulating the passage of molecules, ions, and immune cells between the blood and the brain parenchyma [6]. Pericytes, astrocytes, and microglia contribute to BBB maintenance and function, providing structural support and actively participating in signaling pathways that influence barrier integrity [7]. Their interactions with endothelial cells, the primary barrier-forming cells, help maintain the selective permeability of the BBB, protecting the brain from harmful substances while allowing essential nutrients and molecules to pass through. Understanding the roles of these cell types in BBB function is crucial for advancing our knowledge of brain health and addressing conditions like AD where BBB dysfunction is implicated.

3.2 Disrupted BBB's transport on the clearance of Aβ peptides

Aβ, a peptide derived from APP, is a central player in AD pathology. Efflux transporters, including P-glycoprotein and the receptor for advanced glycation end products (RAGE), are involved in Aβ transport across the BBB. Impaired function of these transporters compromises the efflux of Aβ from the brain, contributing to its accumulation within the CNS [8].

Here's how BBB dysfunction affects Aβ clearance: (1) Reduced Efflux Transporters Activity: The BBB employs efflux transporters, such as P-glycoprotein (P-gp), to actively pump waste products, including Aβ, out of the brain and into the bloodstream. Dysfunction of these transporters can impede the removal of Aβ from the brain. When efflux transporters fail to function properly, Aβ accumulates in the brain's interstitial fluid (ISF), increasing the risk of aggregation into plaques. (2) Increased Permeability: BBB dysfunction often leads to increased permeability of the barrier. Gaps or leaks in the BBB allow molecules, including Aβ, to pass more easily between the blood and the brain. This increased permeability can facilitate the entry of peripheral Aβ from the bloodstream into the brain, adding to the Aβ burden in the brain. (3) Neuroinflammation and Oxidative Stress: BBB dysfunction can trigger neuroinflammatory responses and oxidative stress in the brain. These processes can damage neurons and contribute to the release of Aβ from neurons into the ISF. Inflammatory molecules can also impair the function of BBB transporters and further disrupt Aβ clearance mechanisms. (4) Impaired Perivascular Clearance: The BBB is closely associated with a perivascular system known as the glymphatic system. This system helps clear waste products, including Aβ, from the brain during sleep. BBB dysfunction can impair glymphatic function, reducing the efficiency of Aβ clearance through perivascular pathways. (5) BBB Aging and Aβ Clearance: Age-related changes in the BBB, including increased permeability and reduced transporter function, can contribute to age-related Aβ accumulation. As people age, the BBB may become less effective at maintaining Aβ homeostasis, allowing Aβ to accumulate more readily.

Overall, the dysfunction of the BBB's transport mechanisms disrupts the finely-tuned balance of Aβ production and clearance in the brain. This disruption leads to the accumulation of Aβ peptides, which can aggregate into toxic plaques, contributing to the pathogenesis of AD. Understanding how to restore or enhance BBB function may hold promise as a therapeutic approach to address Aβ accumulation and slow the progression of AD.

3.3 Vascular risk factors

Endothelial Cell Damage: Hypertension, characterized by elevated blood pressure, exerts excessive force on the BBB's delicate endothelial cells. This can lead to endothelial cell damage, compromising the integrity of tight junctions that normally seal the gaps between these cells, thereby increasing BBB permeability.

Oxidative stress and inflammation: Hypertension and diabetes can trigger oxidative stress and inflammation throughout the vascular system, including within the brain. These processes can damage BBB components, including endothelial cells and surrounding astrocytes, which play a role in BBB maintenance.

Altered cerebral blood flow: Hypertension disrupts the regulation of cerebral blood flow, potentially leading to fluctuations in blood flow that can weaken the BBB. Insufficient blood flow can deprive the BBB of essential nutrients and oxygen needed to maintain its structural and functional integrity.

Hyperglycemia: Diabetes, especially when poorly controlled, leads to hyperglycemia (high blood sugar levels), which can damage endothelial cells of the BBB. Hyperglycemia-induced oxidative stress can impair tight junctions and promote inflammation, compromising BBB function.

Lipid accumulation: Hypercholesterolemia, characterized by elevated levels of cholesterol in the blood, can lead to the accumulation of lipids in blood vessel walls, including those in the brain. This lipid buildup can disrupt the BBB's structural integrity and exacerbate inflammation.

Neuroinflammation: chronic inflammation associated with these vascular risk factors can activate microglia and other immune cells within the brain. These immune responses can further disrupt the BBB and compromise its selective permeability.

Aβ accumulation: Interestingly, BBB dysfunction can also lead to the accumulation of Aβ peptides in the brain. A disrupted BBB may allow more Aβ to enter the brain or hinder its removal from the brain, contributing to Aβ plaque formation, a hallmark of AD.

In summary, these risk factors induce endothelial dysfunction and oxidative stress, disrupting the intricate balance of the BBB and facilitating the entry of harmful molecules into the brain [9].

3.4 Therapeutic Implications and Future Directions

Understanding the pivotal role of BBB dysfunction in AD opens doors to potential therapeutic interventions. Strategies aimed at preserving BBB integrity, such as modulating tight junction proteins or enhancing efflux transporters, and during that process, several mechanisms come into play as followed.

Astrocyte-Endothelial Cell Interactions: Astrocytes, a type of glial cell in the brain, play a crucial role in BBB regulation. They release signaling molecules such as transforming growth factor-beta (TGF-β) and angiopoietin-1 (Ang-1), which promote the expression and proper assembly of tight junction proteins like claudins and occludin [10].

Endothelial Cell Signaling: Endothelial cells themselves can respond to various signaling molecules and pathways that influence tight junction protein expression. For instance, endothelial cells can sense and respond to changes in shear stress and neuroinflammatory signals like tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β) that may disrupt tight junctions [11].

Transcriptional Regulation: Transcription factors like Krüppel-like factor 2 (KLF2) and peroxisome proliferator-activated receptor gamma (PPAR-γ) regulate the expression of tight junction proteins by binding to their respective promoter regions.

4 Acetylcholine hypothesis

4.1 Introduction of Acetylcholine hypothesis

Acetylcholine is the major neurotransmitter in the brain that active in the cortical part of the brain, the basal ganglia part, and the base of the forebrain. Acetylcholine is a common neurotransmitter in the central and limbic systems, and a neurotransmitter that has excitatory effects on central neurons. Acetylcholine helps control the contraction and relaxation of muscles in the motor system to make movements and is a major neurotransmitter in the autonomic nervous system. In addition, acetylcholine can dynamically adjust the cortical network to a state suitable for learning and plays an important role in memory formation. Acetylcholine has been found to be deficient in the brains of Alzheimer's patients, and acetylcholinesterase inhibitors have been shown to be effective in improving memory in patients. Fig. 2 shows the key steps in the synthesis, release and reuptake of the neurotransmitter acetylcholine [12].

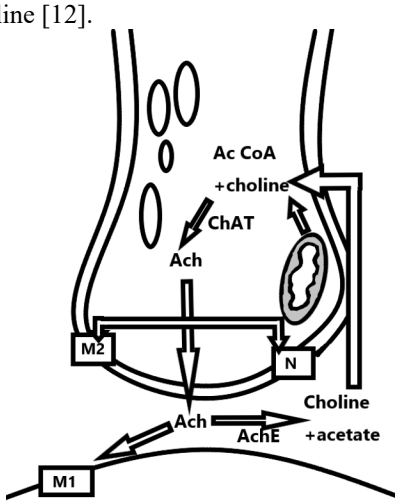

Fig. 2 The synthesis of neurotransmitter acetylcholine [12]

Choline is an important substrate for acetylcholine synthesis. Acetyl-coenzyme A (Ac-CoA) is produced by the breakdown of glucose (a carbohydrate) by glycolysis (Krebs cycle) and is essential for the synthesis of acetylcholine along with choline acetyltransferase (ChAT). When the neurotransmitter acetylcholine is released at the synapse, it binds (activates) postsynaptic receptors (M1) and transmits signals from one neuron to another. Excess neurotransmitters in the synaptic cleft are broken down by acetylcholinesterase (AChE) into choline and acetate, which are recycled back into acetyl-CoA through uptake mechanisms [12].

Researchers found that in spatial tests of mice, their behavior was significantly correlated with the concentration of endogenous cholinergic energy in the hippocampus, and the M1 muscarinic acetylcholine receptor played an important role in regulating the hippocampus and shaping plasticity functions, thereby causing disorders of memory and spatial functions. Therefore, the relationship between cholinergic system and AD lesions can be inferred [13]. The cholinergic hypothesis proposes that AD is caused by the nervous system's reduced production of the neurotransmitter acetylcholine. The source of corticocholinergic innervation is the Meynert basal nucleus (NBM) in the basal forebrain, which is severely neurodegenerative in the Alzheimer's brain [14]. Cholinergic antagonists can impair memory, while agonists can enhance memory. Memory and cognitive decline in Alzheimer's patients are positively correlated with a decrease in acetylcholine in the brain.

4.2 Practical cholinergic theory

Acetylcholinesterase inhibitors (AChEIs) can increase the concentration of acetylcholine at M-AChRs and N-AChRs by inhibiting the degradation of acetylcholine within synapses, thereby increasing cognitive performance and improving memory in AD patients.

A 2017 CHEI drug treatment trial included 16,106 AD patients and showed that Cholinesterase inhibitors improved cognitive function [15], overall condition, and functional ability, and mortality was slightly lower in the experimental group than in the placebo group. However, the lower symptom improvement and higher all-reason discontinuation rates compared to placebo implied a lower risk-benefit ratio for CHEI. Side effects of taking CHEI medications may include nausea, vomiting, abdominal pain, headache, muscle cramps, loss of appetite, diarrhea, insomnia, slow heartbeat, and fainting.

Cholinesterase inhibitors currently approved by the FDA for the treatment of mild and moderate AD include tacrine, donepezil, rastigmine, and galantamie.

4.2.1 Tacrine

Tacrine is the first acetylcholinesterase inhibitor to treat AD. It can effectively inhibit the breakdown of acetylcholine in the brain, increase acetylcholine levels, and delay the symptoms of dementia. Tacrine was approved by the US FDA in 1993, but has hepatotoxicity and side effects such nausea, diarrhea, insomnia, vomiting, fatigue and lack of appetite. In a 12-week controlled trial of tacrine in the treatment of AD [16], 468 patients received Tacrine or placebo and were tested after 12 weeks using the AD Assessment Form and impressions of clinical overall changes in cognitive components and clinician ratings. The results showed that after 12 weeks, the patients who took tacrine had improved ADSD cognition, clinician scores and nursing staff scores.

4.2.2 Donepezil

Donepezil is the second-generation central acetylcholinesterase inhibitor. It can inhibit brain acetylcholinesterase with high specificity and reversibly. Cortical neurons can be protected from glutamate neurotoxicity by increasing the expression of neuronal α4β2-nAChRs and α7-nAChRs. In addition, it can reduce apoptosis caused by glutamate. In a 12-week trial [17], 134 Alzheimer's patients were randomly assigned Donepezil or placebo. As a result, patients improved in all areas of the neuropsychiatric scale except elation.

4.2.3 Rivastigmine

Rivastigmine is a brain-selective formate cholinesterase inhibitor. In 2000 and 2007, the US Food and Drug Administration approved the use of oral formulations and transdermal patches, respectively. The drug is not metabolized by the liver or P450s and is well tolerated in mild and moderate AD. It inhibits both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) associated with impaired cholinergic neurotransmission in AD. A trial involving 3,450 people with mild to moderate AD lasting 12 weeks to 52 weeks reported that cabalatin (orally or transdermal) was beneficial in patients with mild to moderate AD [18].

4.2.4 Galantamine

Galantamine is an alkaloid isolated from the flowers and bulbs of Caucasian snow lotus and Amaryllidaceae, and can also be produced synthetically. It has acetylcholinesterase inhibitory properties and is approved by the US Food and Drug Administration for the treatment of moderate dementia. Acetylcholinesterase inhibitors may cause symptoms in some people, but these drugs still improve memory and cognition in people with AD. In a six-month multicenter [19], double-blind trial, 636 patients were given either placebo or galantamine. The results showed that the galantamine treatment group produced a better effect than placebo on clinician interview change impression and caregiver impression (CIBIC-plus), with no change from baseline in ADAS-cog/11 and DAD scores in the Galantamine treatment group at month 12.

5 Conclusion

The amyloid hypothesis posits that the accumulation of Aβ plaques in the brain plays a central role in AD. Through a comprehensive review of in vitro and animal model studies, we have observed substantial evidence linking Aβ to neuronal toxicity and cognitive decline. The development of amyloid-targeted therapies and recent advances in neuroimaging techniques have further underscored the importance of this hypothesis in Alzheimer's research. The BBB hypothesis has emerged as a crucial area of investigation, focusing on the permeability of the BBB and its potential role in AD pathogenesis. The experiments discussed in this thesis have highlighted the significance of compromised BBB integrity in facilitating the entry of harmful substances into the brain, including neuroinflammatory factors. These findings underscore the need for therapeutic strategies that address BBB dysfunction to mitigate Alzheimer's progression. While the cholinergic hypothesis, which centers on the depletion of acetylcholine in the brain, has been supported by pharmacological interventions and neurotransmitter studies. The cholinesterase inhibitors, used to boost acetylcholine levels, have demonstrated symptomatic relief in Alzheimer's patients. However, ongoing research seeks to refine and expand our understanding of the cholinergic system's role in Alzheimer's pathology.

While AD is a multifactorial condition with complex interplay among various pathological processes, their respective experimental validations underscore the importance of continued investigation into these areas, not only to advance our knowledge but also to inform the development of novel therapeutic strategies. Ultimately, a holistic approach that considers the intricate relationships among these theories may hold the key to more effective interventions and, eventually, a cure for AD.

6 Author contribution

All the authors contributed equally, and their names were listed in alphabetical order.

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