Chapter 1

(Introduction)

1.1 Alzheimer's Disease

The incidence of Alzheimer's disease (AD), a leading cause of disability and death, is expected to grow rapidly over the next decade [Association 2019]. In AD Patient experiences irreversible dementia and increasing loss of cognition, eventually becoming entirely dependent on caretakers in everyday life. Thus, the financial, physical, and emotional burden placed on patients and caregivers can be overwhelming. AD was first discovered by Dr. Alois Alzheimer a German Psychiatrist (1864-1915) in 1906. He observed many abnormal clumps (presently referred to as Amyloid plaques) and some tangled bundles (known as neurofibrillary tangles or tau tangles) in a patient's (Augusta Deter, 1850-1906) brain tissue suffering from unusual mental illness and unpredictable behaviors. This led to the first case of AD. [Hippius and Neundörfer 2022].

The most common symptoms of AD are dementia-like symptoms and resemble memory impairment and decline in cognitive functions in people over the age of 60 years [DeTure and Dickson 2019]. Based on its prevalence, the disease can be divided into two types, i) familial AD (FAD) which involves the development of AD-like symptoms before the age of 60 whereas ii) sporadic AD (SAD) which appears in people over the age group of 65 years and is often associated with different gene mutations like mutations in presenilins (PSEN1 and PSEN 2) genes and amyloid precursor protein (APP) [Li et al. 2022, Petit et al. 2022]. The latter is accountable for less than 1% of the AD patient and is associated with several factors such as Apolipoprotein E (APOE) which is believed to be the most common for SAD and late onset of FAD [Dorszewska et al. 2016, Oumata et al. 2022].

The progress of AD is typically slow and spreads in three stages i.e. mild, moderate, and severe stages. Each stage represents different sets of symptoms, in the case of mild AD that includes lack of interest, mood swings, language problems, troubled writing and driving, depression, etc., and may last up to 2-4 years. These symptoms may

sometimes arise due to other medical conditions and don't confirm the actual presence of AD. In mild stages of AD, patients are observed with worsened memory loss which affects their daily chores. The mild stage may last up to 2- 10 years of the AD phase. While in the severe stage, the symptoms of the previous phases get combined with some other sever complications such as hallucination, seizures, weight loss, extreme mood swings, skin infections, and motor dysfunctions. The symptoms of the severe stage may last from 1-3 years and ultimately leads to the death of the patient [Braak and Braak 1997, Lyketsos et al. 2011, Reisberg et al. 1987].

The World Alzheimer's Report 2021, suggests that around 55 million people are positive dementia survivors, which is expected to be 78 million by the year 2030 [Gauthier et al. 2021]. Around 75% of dementia people are living without proper diagnosis which is believed to be as higher as 90% in low and middle-income countries due to lack of awareness. Among the total global population of dementia survivors, 60-70% is AD positive population which is expected to be 152 million by 2050. In India, the total population of the dementia patient by 2050 is expected to be 14 million out of which 60-70 % will be AD survivors [Patterson 2018]. This figure may even be larger in India as the elderly population is growing rapidly, lack of awareness, and most of the symptoms of AD are associated with dementia-like symptoms that occur in the aged population [Nandi et al. 2022].

1.2 Pathophysiology involved in Alzheimer's Disease

The exact cause of AD is still unknown owing to the multifaceted pathophysiology involved in the development and progression of the disease. Though several hypotheses have been proposed which includes, the cholinergic hypothesis that governs the lower acetylcholine (ACh) and an increased Acetylcholinesterase (AChE) levels in the synaptic cleft, Central nervous system (CNS) inflammation in response to activated microglial cells and astrocytes, activation of N-methyl-D-aspartate receptor (NMDAR),

Oxidative stress, cyclic-AMP-response element-binding protein (CREB) signaling pathways, apolipoprotein E4 (APOε4) gene transcription, accumulation of amyloid beta (Aβ) and its aggregates and hyperphosphorylated tau and formation of neurofibrillary tangles (NFTs), etc. The last two hypotheses are the most acceptable among all the existing hypotheses and are considered to be the "Gold standard" for AD development and progression.

1.2.1 Cholinergic Hypothesis

The cholinergic hypothesis is one of the important and most acceptable hypotheses to understand the development of AD, which came into existence in the 1970s and suggested that cholinergic neurons are affected in the early stage of AD [Perry et al. 1977, PERRY et al. 1978]. The loss of cholinergic neuronal function results in a decline in acetylcholine (ACh) synthesis in the forebrain (basal region) which induces cognitive deficit. The biochemical estimation of the AD brain reveals that the choline acetyltransferase (CAT) responsible for ACh synthesis gets significantly low in hippocampal and neocortical brain regions under AD-associated neurodegeneration [Perry et al. 1986]. The literature also suggested several cholinergic abnormalities such as muscarinic and nicotinic abnormalities, ACh synthesis and its release, choline, and axonal transport are some of the major contributors that led to the development of AD-associated behavioral dysfunctions [Terry and Buccafusco 2003].

The profound loss of cholinergic neurons is the most accepted and established pathophysiology of AD, which subsequently transforms into progressive decline in the later stage of the disease. ACh neurotransmitter plays a pivotal role in the regulation of cognitive functions, which is hydrolyzed by acetylcholinesterase (AChE) into choline and acetic acid. The low levels of ACh in cholinergic synapses cause cognitive dysfunction [Mesulam 2004]. The detailed cholinergic transmission is discussed in Figure 1.1 [Waiker et al. 2023]

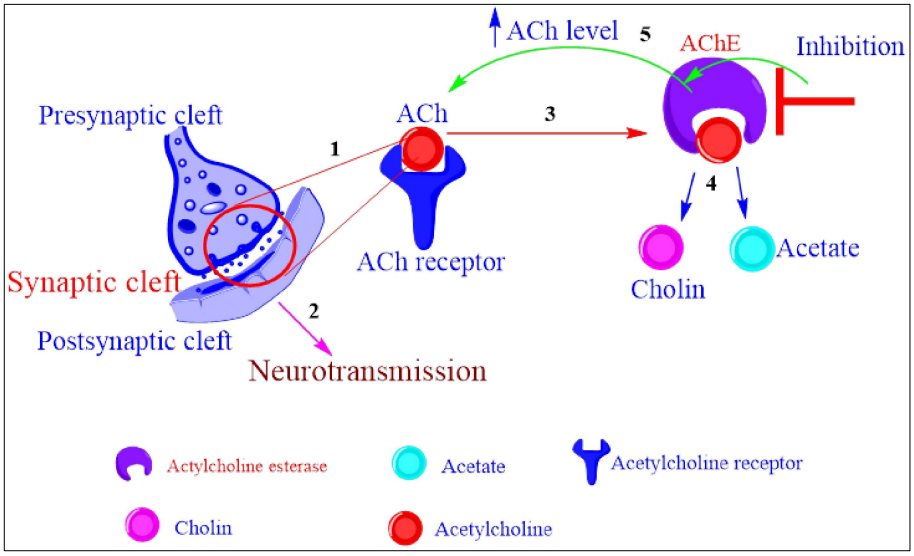


Figure 1. 1 Release of ACh and normal neurotransmission process. (1) Acetylcholine releases from presynaptic cleft and binds with the ACh receptor on the post synaptic cleft; (2) Normal neurotransmission process upon sufficient ACh present in the synaptic cleft (3) Hydrolysis of ACh by AChE to 4) choline and acetate; (5) Inhibition of AChE resulted in increased ACh level in synaptic cleft to maintain normal neurotransmission.

There are two types of acetylcholinesterase, AChE, and butyrylcholinesterase (BChE). The AChE is available in globular and asymmetric isoforms. The literature reveals that a profound loss of ACh globular G4 tetrameric form in AD conditions hence targeting its predominantly available G1 monomeric form could be a promising strategy in designing an AChE inhibitor [Sakayanathan et al. 2019]. BChE is a pseudo-neuronal ChE that regulates normal ACh level and maintain normal neurological functions [Greig et al. 2001, Kumar et al. 2018]. Both these enzymes are found to provoke catalysis of ChE hydrolysis [Massoulié et al. 1993]. AChE and BChE were also observed to promote Aβ-aggregation and the formation of neocortical Aβ-plaques and neurofibrillary tangles [Mushtaq et al. 2014]. Therefore, targeting both AChE and BChE (dual inhibition) could render the development and progression of AD in advance and late onset of the disease [Bartolini et al. 2003].

1.2.2 Amyloid beta (Aβ) Hypothesis

The Aβ hypothesis is one of the most acceptable hypotheses in AD development and progression for the last two decades [Verma et al. 2022]. The insoluble forms of sticky Aβ deposited outside of the neurons and form Aβ plaques, further these plaques get deposited in between the neurons and disrupt normal neuronal transmission. Some times these deposits are present in the synaptic cleft and interfere with the binding of the ACh to AChR and affect the neuronal transmission [Dinamarca et al. 2010]. Particularly the reduced neuronal communication resulted in impaired memory functions that lead to the development of AD. The presence of Aβ plaques also causes an activation of certain immune responses which results in inflammation and might damage the surrounding neurons [Yan et al. 2003]. The Aβ plaques can also be deposited around the blood vessels of the brain which can cause amyloid angiopathy that weakens the wall of the blood vessels and risk of hemorrhage or rupture of the blood vessels [Rozemuller et al. 2005].

Amyloid precursor protein (APP) is a membrane-bound enzyme present in the neuronal cell that is believed to help the neuronal cell grow and repair itself after an injury [Kojro and Fahrenholz 2005]. This enzyme like other enzymes gets used, broken down, and recyclized. Normally, the APP gets cleaved by α-secretase and BACE-1 to give soluble peptide fragments which are further degraded this process is called a non-amyloidogenic pathway whereas, the cleavage of APP by enzymes BACE-1 and γ-secretase results in the formation of insoluble sticky fragments called amyloidogenic pathway. These insoluble left-behind fragments lead to the formation of Aβ plaques [Hampel et al. 2021].

Therefore, designing BACE-1 inhibitors is always an interesting target to stop the progression of early and late onset of AD.

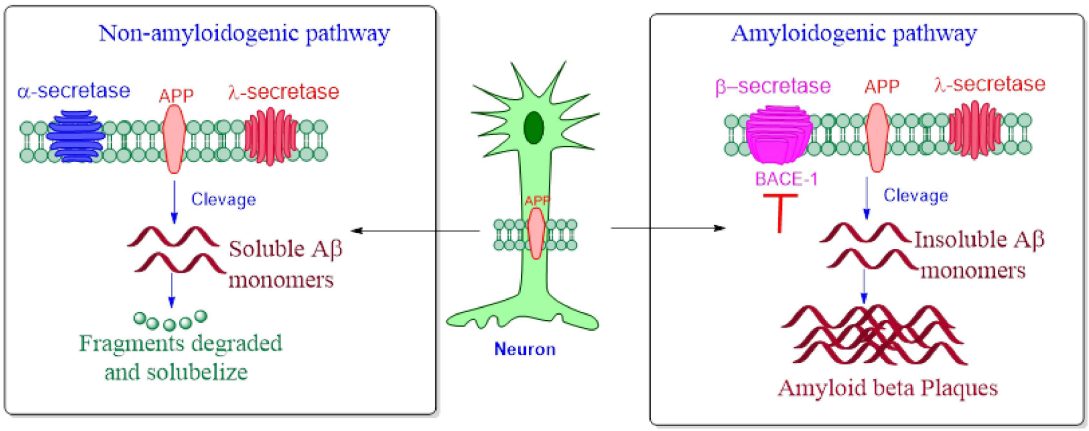


Figure 1. 2. Amyloidogenic (β-secretase assisted) and non-amyloidogenic (α-secretase assisted) pathways of Aβ formation.

1.2.3 Tau Hypothesis

Neurons are held together by their cytoskeleton which is made up of microtubules. The microtubules are track-like structures that essentially act like a mine cart shipping nutrients and molecules along the length of the neuronal cell [Drubin et al. 1986]. Tau is a protein called microtubule-binding protein which makes these tracks unbreakable. Generally, tau proteins exist in six isoforms with three binding domains: C-terminal (microtubule-binding domain), N-terminal projection, and a short tail sequence. It stabilizes neurons in normal conditions via phosphorylation in the axonal membrane. There are more than 85 phosphorylation sites available on the tau protein and mainly belongs to the family of serine, threonine, and tyrosine residues [Ittner and Götz 2011, Mietelska-Porowska et al. 2014].

Although it is believed that Aβ plaques build- up initiates pathways inside the neurons leads to activation of various enzymes and kinases i.e. tau protein kinase-I (TPKI), CDK-5, glycogen synthase kinase-3β (GSK-3β), microtubule affinity-regulating kinase (MARK), AMP-activated protein kinase (AMPK) extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), protein kinase-A (PKA), dual-specificity tyrosine-phosphorylation-regulated kinase-1A (Dyrk1A), mitogen-activated protein kinase

(MAPK), Src family non-receptor tyrosine kinase (SFKs), Ca^{2+}/calmodulin dependent kinase (CaMK), and Janus kinase (JNK).

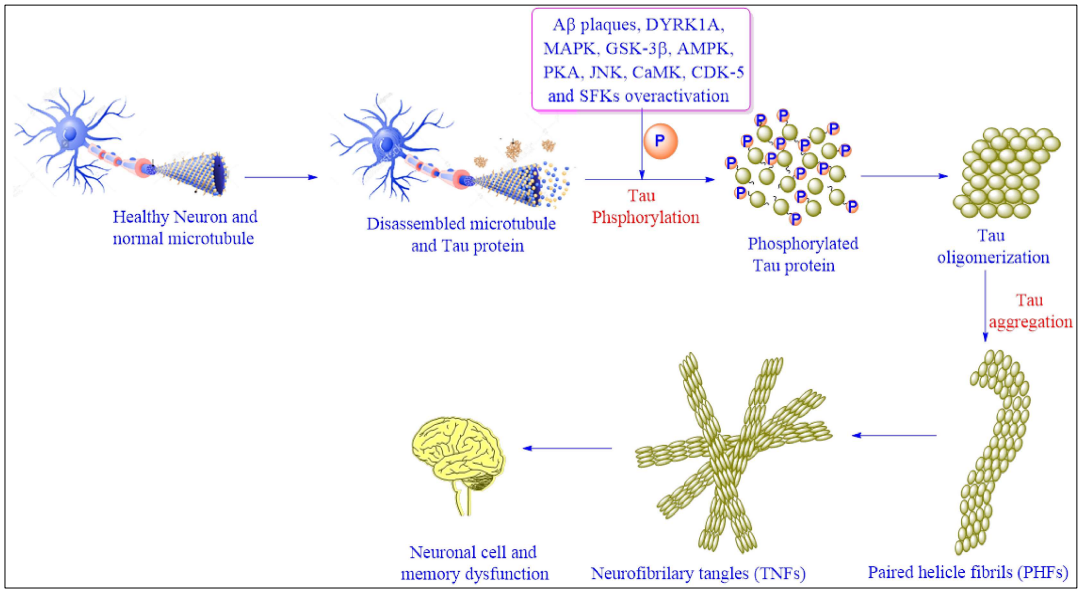


Figure 1. 3. Formation of neurofibrillary tangles (NFTs), and neuronal dysfunction associated with tau phosphorylation. DYRK1A: dual-specificity tyrosine-phosphorylation-regulated kinase-1 A, MAPK: mitogen-activated protein kinase: GSK-3β: glycogen synthase kinase-3β; AMPK: AMP-activated protein kinase: PKA: protein kinase-A; JNK: Janus kinase; CaMK: Ca^{2+}/calmodulin dependent kinase; CDK-5: cyclin-dependent kinase-5; SFKs: Src family non-receptor tyrosine kinases.

These kinases are responsible for the transfer of the phosphate group to the tau protein (tau hyperphosphorylation) and lead to its over-activation [Coman and Nemeş 2017]. Tau upon activation changes shape, stop supporting the microtubules, and clumps with other tau proteins or gets tangled and leads to another characteristic finding of AD-neurofibrillary tangles (NFTs) [Šimić et al. 2016]. Neurons with non-functioning microtubules and tangles do not pass signals as well, disrupt neurological plasticity, and lead to neurodegeneration and neuronal cell death [Grill and Cummings 2010a, b].

1.2.4 Excitotoxic Hypothesis

Glutamate is an important excitatory neurotransmitter involved in learning, memory, and maintaining neuronal synaptic plasticity [Olney et al. 1997]. Glutamate binds with the glutamate receptors present in the post-synaptic neurons. The glutamate receptor families can be divided into ionotropic and metabotropic receptors. Further, ionotropic can be divided into three sub families namely: NMDA receptor NR1, NR2A-D, and NR3A-B. While, the metabotropic receptor (G-protein coupled glutamate receptor) family can be divided into three subclasses: group1 (mGluRs1 & 5), group2 (mGluRs2 & 3), and group3 (mGluRs4 & mGluR6-8). The ionotropic receptors present on the post-synaptic sites mainly mediate fast excitatory transmission in neurons and glial cells [Esposito et al. 2013]. The ionotropic receptor, mainly the NMDAR subtype prevents the calcium ion entry in the cell by depolarization and closing of the magnesium ion-mediated cationic channels in the resting state of the cell. In the case of pathological conditions such as AD the hyper activation of NMDAR in response to Aβ causes the release of magnesium ions that allows the entry of calcium ions into neuronal cells via hyperpolarization-mediated opening of cationic channels [Texidó et al. 2011]. The increased influx of calcium ions in neuronal cells activates various metabolic pathways like GSK-3β, JNK, MAPK, etc. responsible for neuronal degeneration and synaptic loss. Thus, higher calcium influx inside the neuronal cells leads to hampered neurotransmission, neurodegeneration, and the development of cognitive dysfunction in the early onset of AD [Olney et al. 1997]. Thus N-methyl-D-aspartate (NMDA) attracts much attention due to its direct involvement in neurodegeneration.

1.2.5 Oxidative stress Hypothesis

To understand the role of oxidative stress (OS) in the AD brain, overwhelming evidence has been proposed which suggests that the AD brain is exposed to various oxidative stresses throughout the course of the disease. The OS condition in the brain causes low

levels of antioxidants and an enhanced level of reactive oxygen species (ROS) [Pratico 2008]. This imbalance in antioxidants and ROS leads to neuronal cell death. Other than the most acceptable hypotheses of AD (formation of Aβ plaques and NFTs) presence of OS is a widespread property of the AD brain [Resende et al. 2008]. The formation of free radical injuries alters the antioxidant levels such as catalase and superoxide dismutase in the brain that contributes to AD pathogenesis. The excited levels of lipid peroxidation and protein oxidation due to inconsistent free radical discharge contribute to cognitive impairments in the progression of AD. The accumulation of Aβ plaques, DNA oxidation, altered mitochondrial metabolism, lipid peroxidation, accumulation of transition metals (aluminum, mercury, zinc, copper, and iron) and trace elements disproportionately are also responsible for oxidative destruction and the development of AD [Allan Butterfield 2002, Ercal et al. 2001, Lin and Beal 2006, Stohs and Bagchi 1995].

1.2.6 Mitochondrial hypothesis

Mitochondria are the essential cellular organelle involved in ATP production via oxidative phosphorylation (OXPHOS) and involved in lipid metabolism as well as calcium signaling [Shoffner 1997]. Every tissue and organ of the body has its own energy requirements based on the functions it performs. However, tissues like the heart, neurons, kidneys, and liver have high levels of energy demand. Cellular senescence refers to the progressive deterioration of mitochondrial function with aging. Aging also contributes to the alterations in the components of the mitochondrial electron transport chain (ETC) [Swerdlow et al. 2014]. This may contribute to the overproduction of ROS and the underproduction of ATP in aged cells. The energy decline may result in the development of many age-related diseases like heart failure and AD [Espino de la Fuente-Muñoz et al. 2020, Stockburger et al. 2018].

Mitochondrial proton leak plays a major role in mitochondrial coupling efficiency as well as in the production of ROS. There are two types of proton leak: Basal and inducible. There are pieces of evidence showing that in aged cells pathological augmented and sustained basal proton leak contributes to a decline in respiratory efficiency due to increased mitochondrial load. Therefore, inhibiting the aging-induced pathological proton leak may be beneficial in rejuvenating mitochondrial ATP production [Silva et al. 2013b, Supnet and Bezprozvanny 2010, Yao et al. 2009].

1.2.7 Neuroinflammation

AD pathophysiology is mainly characterized by the two most acceptable hypotheses. The one involves the deposition of Aβ plaques in response to the amyloidogenic pathway of APP cleavage which left behind the insoluble fragments of Aβ peptides that aggregate themselves to produce Aβ plaques [Zhang and Jiang 2015]. The second involves the formation of NFTs in response to the hyperphosphorylation of tau which is a microtubule stabilizing protein. Upon hyperphosphorylation of tau, the detached tau proteins form the primary helical structures which finally aggregate to form NFTs [Uddin et al. 2020]. It has been observed that in response to the deposition of Aβ plaques and the formation of NFTs in the AD brain, astrocytes and microglial cells become activated. In response to activated astrocytes and microglia certain astrocyte-derived neurotoxin and cytokines release takes place and causes neurodegeneration and loss of neuronal plasticity [Liddelow et al. 2017, Mishra et al. 2020]. Several other traumatic brain injuries and metabolic disorders like diabetes mellitus and cardiovascular changes also confirm the greater risk of the development of inflammation, which leads to the development of memory dysfunction related to brain tissue atrophy. Thus, inflammation of brain tissue has an important role in developing early and late onset of AD [Ardura-Fabregat et al. 2017].

1.2.8 Dyrk1A hypothesis

Dyrk1A is a protein kinase overexpressed in AD, in reactive astrocytes and microglia, contributing to the pathology and progression of AD. The research supports the association of Dyrk1A with amyloid deposition, neurodegeneration, and dementia, especially in Downs syndrome and Parkinson's disease. Dyrk1A was found to be abnormally expressed in post-mortem AD brain slices, notably increased in reactive astrocyte lining and amyloid plaques [Ferrer et al. 2005]. Dyrk1A has been shown to phosphorylate AD proteins i.e. amyloid precursor protein, tau protein, and presenilin 1. In animal models of AD, Dyrk1A inhibitors improve amyloid pathology, motor function, memory, and most notably, neuroinflammation, a process associated closely with the activation of astrocytes and microglia [Branca et al. 2017, Souchet et al. 2019]. An understanding of how Dyrk1A is related to these pathologies will provide further validation of this protein as a drug target in AD [Lee et al. 2020, Melchior et al. 2019].

In AD and other neurodegenerative diseases, neuron loss underlies disease progression and leads to brain atrophy. to determine the role of Dyrk1a in such processes. The key finding is that lipopolysaccharide (LPS) activated microglia, through secretion of inflammatory cytokines; induce the activation of astrocytes, which kill neurons through the secretion of neurotoxic factors [Liddelow et al. 2017]. Three cytokines, released by activated microglia, were necessary and sufficient to induce an activated astrocyte state called A1: tumor necrosis factor-alpha (TNFα), interleukin 1 alpha (IL1α), and complement protein C1q. A1 astrocytes, produced by culture with the 3 cytokines, released soluble neurotoxins and induced neuron death in vitro. A1 astrocytes are found extensively in post-mortem AD brains. In further relevance to AD, the identity of the astrocyte-derived neurotoxin, while not conclusively known, may be an amyloid precursor protein, as shown in models of stem cell-derived astrocytes [Mishra et al. 2020].

1.2.9 Apolipoprotein E (ApoE) Hypothesis

The development of sporadic AD is a late onset of the disease where the exact cause isn't well defined. Though, it includes the combination of certain genetic and environmental risk factors and is associated with the majority of the cases [Boyles et al. 1989]. A gene namely: E-gene or APOE-e4 which is an e4 allele of apolipoprotein that has been identified and is believed that it's contributing to the increased risk in majority of the AD cases. The research supports that patients with inherited one e4 allele have a high risk for AD which is even higher for the patients with inherited two e4 alleles [Saunders et al. 1993]. Apolipoprotein E helps in Aβ breakdown while the e4 allele seems less effective as compared to other alleles APOE-e2 in doing this. Thus, e4 promotes Aβ aggregation and reduced Aβ clearance which leads to neuroinflammation and tauopathy-associated AD pathology [Corder et al. 1994].

1.3 Available Treatment for AD

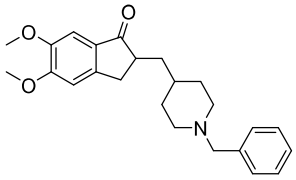
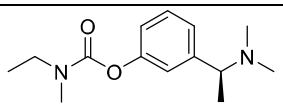
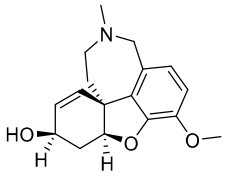
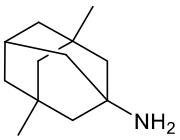
There is no disease-modifying therapy for AD available. Most of the treatment strategy only provides symptomatic relief to the patient. Certain FDA-approved medications such as AChE inhibitors (donepezil, rivastigmine, and galantamine) and NMDA receptor antagonists (memantine) are being used to provide modest symptomatic cognitive improvements and don't halt the disease progression. Recently, Aducanumab and Lecanemab (monoclonal antibodies) have been approved by FDA in an accelerated approval pathway as a disease-modifying therapy for AD. Aducanumab and Lecanemab both are associated with improvements in memory and cognitive impairments linked to Aβ plaques, though their use is still controversial in AD progression. (Table 1.1)

1.4 Newer therapeutic approaches to develop compounds for the treatment of AD

Over the past couple of decades, several efforts have been made to develop novel therapeutics for the effective treatment of AD. Several new drug candidates were

identified to treat AD but had to be eventually withdrawn from the clinical trials owing to their toxicity at a higher dose and lack of clinical efficacy.

Table 1.1 Chemical structures, mechanism, and adverse effects of USFDA-approved drugs for the treatment of AD.

Name	Structure	Mechanism	Adverse effects	Ref.
Donepezil		Cholinesterase inhibitor	Nausea, vomiting, loss of appetite, diarrhea weight loss, , weakness, drowsiness, dizziness, tremor, slow/irregular heartbeat, black stools, fainting, vomit that looks like coffee grounds, severe stomach/abdominal pain, trouble urinating, seizures..	[Wilkinson 1999]
Rivastigmine		Cholinesterase inhibitor		[Polinsky 1998]
Galantamine		Cholinesterase inhibitor		[Marco-Contelles et al. 2006]
Memantine		NMDA receptor antagonist	Body aches, dizziness, constipation, headache, and trouble breathing	[Mobius et al. 2004]
Aducanumab	Monoclonal Antibody (mAb)	Aβ-directed antibody	Brain edema, headache, micro hemorrhages, Fall, altered mental status, diarrhea, disorientation, confusion, delirium.	[Dhillon 2021]
Lecanemab	Monoclonal Antibody (mAb)	Aβ-directed antibody	Cerebral edema, micro or small hemorrhages, and mild to moderate infusion-related reactions	[Thambisetty and Howard 2023]

These include metrifonate, tesofensine, velnacrine, eptastigmine, and huperzine A [Imbimbo et al. 2000, López-Arrieta and Schneider 2006, Murphy et al. 1991, Yaari and Hake 2015]. Additionally, there are various disease-modifying candidates such as gantenerumab, crenezumab from Roche, solanezumab from Eli Lilly, and bapineuzumab from Pfizer have been reported to be unsuccessful in clinical trials [Mehta et al. 2017]. The diminished pipeline of new molecules presents an urgent need to identify novel therapeutic strategies for the development of neurotherapeutics against the progression of AD.

1.4.1 Multitarget approach

AD is multifaceted in nature with the involvement of diverse pathophysiological mechanisms, such as decreased ACh levels, augmentation in Aβ aggregation and plaque deposition, and increased expression of several factors such as BACE-1, tau hyperphosphorylation, oxidative stress, monoamine oxidase-B (MAO-B), phosphodiesterase (PDE) and cyclooxygenase 2 (COX-2). These diversified targets prompted the researchers to shift their focus from single targets to multiple targets.

The multi-targeting inhibitory approach is less selective in maintaining adequate potency for each target of interest. The multi-targeting approach may also require submicromolar potency for multiple targets to translate. Donepezil is a widely used FDA-approved drug that has inhibitory properties toward ChE enzymes and some associated functions and provides only symptomatic relief to the patient.

Multitargeted therapeutics bind to several targets simultaneously and affect multiple pathways at a single point in time. For example, ligands bound to peripheral anionic and catalytic anionic sites (PAS and CAS) of AChE could effectively inhibit the BACE-1 and Aβ aggregation [Chen et al. 2014]. Also, compounds with cholinesterase inhibition along with antioxidant and anti-Aβ aggregatory activities were observed intensively in the recent past [Jiang et al. 2011].

1.4.2 Computer-aided drug design approach

The computer-aided drug design (CADD) approach is extensively explored by drug discovery units of leading pharmaceutical and biotechnological companies. These approaches provide superiority and reliability to investigate and identify the promising drug candidate in the library of databases [Nadendla 2004]. The initial screening using computational methods can reduce the time and minimize the cost of the overall process. These computational methods can be divided into two broad classes: structure- and ligand-based drug design (SBDD and LBDD) also known as direct and indirect approaches, respectively.

The direct approach, i.e., SBDD is based upon the known 3D structure of the biological targets and discovering the molecules that satisfy some geometric constraints. Molecular docking and dynamics, X-ray diffraction (XRD), nuclear magnetic resonance (NMR), homology modeling, and molecular mechanics generalized Born surface area (MM-GBSA) are the tools that can be used in SBDD.

The indirect drug design approach, known as LBDD involves creating a lead molecule by comparing various structural characteristics of known active and inactive molecules. LBDD involves several tools, such as quantitative structure-activity relationship (QSAR), comparative molecular field analysis (CoMFA), comparative molecular similarity indices analysis (CoMSIA), pharmacophore modeling, high throughput virtual screening (HTVS) and combinatorial chemistry [Bacilieri and Moro 2006].

Indeed molecular modeling helps in the identification of moieties involved in the interaction with a particular protein and permits an understanding of the underlying molecular mechanisms responsible for specific biological activity. This knowledge of CADD helps to expedite the development of new active molecules to be successful drug candidates. However, as simulation accuracy is limited to the precision of the constructed models, computational simulations have to be evaluated against *in vitro*/*in*

vivo experimental outcomes to confirm the accuracy of the model and modify them if necessary, to yield better representations of the system.

1.4.3 Molecular hybridization

The molecular hybridization approach is a novel concept in drug design and discovery, which involves the hybridization of two or more pharmacophores in a single molecule with enhanced biological activity compared to individual parent molecules. The concept of molecular hybridization approach is now well adopted by medicinal and biological chemists to design novel compounds to improve affinity and efficacy toward biological targets with reduced toxic effects [Viegas-Junior et al. 2007].

In AD a multifaceted pathophysiology is involved, and very few commercial drugs are available for the treatment, which could provide symptomatic relief only rather than affecting the progression of AD. Therefore, pharmacophoric moieties of available therapeutics could be hybridized to improve the biological activity toward multiple targets, reduce toxicity, and improve the pharmacokinetic profile compared to parent molecules.

1.5 Design hypothesis in the present study

The multitarget directed ligand (MTDL) strategy has already proven itself as a reasonable proof of concept to overcome the progression of AD disease. In this work, an MTDL approach was adopted to design a candidate molecule that could interact with the multiple targets (Figure 1.4) mainly associated with AD. Most of the drugs regimen for AD, given to patients involves combinations of different medication which provides only symptomatic relief and also causes bioavailability complications, metabolism, drug-drug interactions, and mostly patient incompliance.

Considering the hypothesis as depicted in Figure 1. 4 it was assumed that simultaneous interventions of the multiple pathways involved in the development and progression of the disease could be achieved with a single agent that could inhibit the cholinesterase's

(AChE & BChE), along with A$\beta_{1\text{-}42}$ and tau aggregation reducing the neuro-inflammation developed in response to A$\beta_{1\text{-}42}$ and NFTs deposition. Beta secretase-1 (BACE-1) is an enzyme that induces the formation of insoluble A$\beta_{1\text{-}42}$ aggregates through catalytic proteolysis of amyloid precursor protein (APP). These A$\beta_{1\text{-}42}$ aggregates are responsible, in part, for neuritic injury and neuronal cell death. Thus, inhibition of BACE-1 and ChEs simultaneously through the MTDL approach may improve the learning and memory impairments and retard the progression of AD.

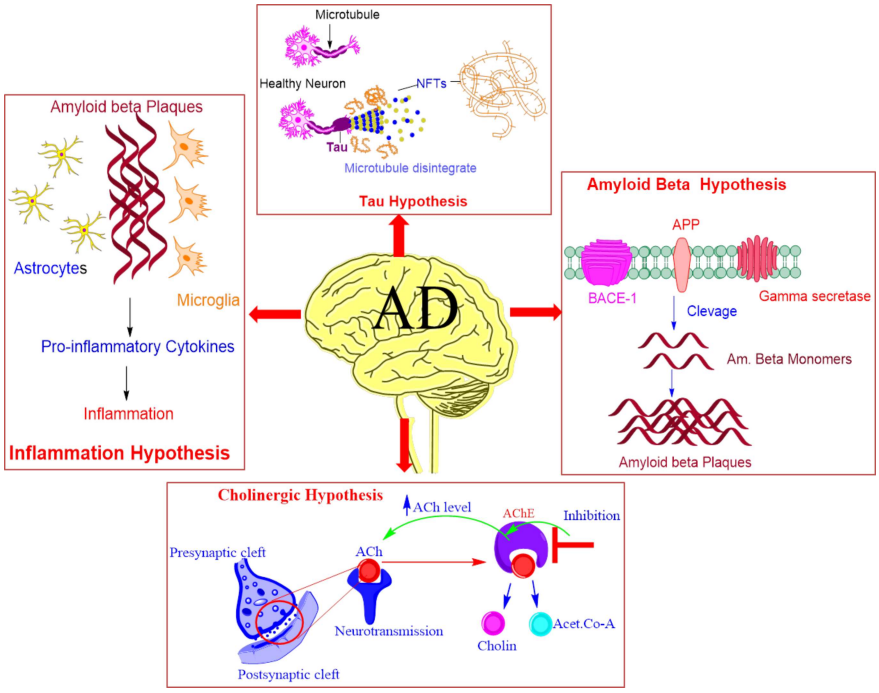


Figure 1. 4. Multitargeting inhibitory approaches for the treatment of Alzheimer's disease**.**

Using the molecular hybridization approach a novel series of compounds (series I) was designed with the assumption that the designed molecules will interact with the Catalytic anionic site (CAS) region (His447, Glu334, and Ser203) and the peripheral active site (PAS) region (Tyr124, Tyr72, Tyr341, Trp286, and Asp74) of the AChE and catalytic dyad (Asp32 and Asp228) region of BACE-1 enzyme, simultaneously. The Benzyl Piperazine moiety was selected owing to the protonation capabilities of its

protonated nitrogen atom at the physiological pH that will lead to enhanced BBB permeability.

In the second part of the thesis, an e-pharmacophore hypothesis of structure-based drug design was adopted. Two e-pharmacophore models (hAChE, PDB code: 4EY7, and hBACE-1, PDB code: 2ZJM) were generated using co-crystal structures. The top poses were selected based on their docking scores and interactions with the CAS and PAS site of the ChE and the catalytic dyad of BACE-1 enzymes.

Considering the in silico outcomes series-II compounds were designed and synthesized. Where 5-phenyl-1.3.4-oxadaiazole scaffold was kept as such (present in all five hits) and various substituted piperazines were introduced at the 3-NH position of the 1,3,4-oxadaiazole ring based on their docking and minimum energy scores.

The N-benzyl piperidine ring, a common feature in both the co-crystallized ligand complex of AChE and BACE-1 (donepezil and F1M) was also introduced in the molecules (series III**) to** investigate its efficacy over benzyl piperazine containing compounds. The oxadiazole moiety was selected for a new scaffold design since it occurs in all five selected hits obtained after the screening of the in-house database.