



**ALZHEIMER'S DISEASE AND ITS POTENTIAL DRUG TARGETS -
REVIEW ARTICLE**

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder in which the death of brain cells causes memory loss and cognitive decline, i.e., dementia. However even with it being very common no effective drug has been introduced or developed to fight against it. There are several targets in AD which has been worked on, prominent being Acetylcholinesterase, NMDA (N-methyl D-aspartate), BACE 1 (beta-site amyloid precursor protein cleaving enzyme 1) and GSK3 Beta. But the drugs developed against these targets have seen partial success in decreasing or halting Alzheimer's disease. Many molecules have failed in the clinical trials on various parameters like efficacy and safety. Ayurvedic medicine is a system of traditional medicine native to India, and ayurvedic practitioners have developed a number of medicinal preparations and surgical procedures for the treatment of various ailments. There are certain plants referred to as "nervines" that are known to have good neuroprotective properties. There have been many studies on therapeutic application of these plants against Alzheimer's. Computational studies on the bioactive compounds from these Ayurvedic plants can help us identify potential molecules as potent drug molecules against the targets of Alzheimer's disease.

Keywords: Alzheimer's disease, targets, Ayurvedic, Bioactive compounds, computational drug discovery.

1. INTRODUCTION

Alzheimer's is a progressive neurodegenerative disease that results in loss of memory, cognitive function and mental impairment. It is the most common form of dementia and Alzheimer's is a potential healthcare problem seen in the elderly in most of the developed countries and in many developing countries. According to the World Alzheimer's report 2015 there are 4.1 million people affected with dementia in India and 22.9 in Asia. According to their estimate the incidence of dementia is going to increase drastically in Asia. It has predicted that the number of people affected by Dementia will be 67 million by 2050⁽¹⁾. The key events that lead to Alzheimer's are the changes in the neuronal structures or death of neurons in the brain. Hyperphosphorylation of Tau proteins lead to formation of Neurofibrillary tangles thereby modifying the microtubules in neurons which

destabilize the neuronal structure eventually leading to cell death and the APP processing of beta secretase enzyme leading to formation of solid forms called amyloid plaques blocking the neuronal transmission at the synaptic cleft. ⁽²⁾⁽³⁾ These two major physiological changes are believed to be central dogma of the Alzheimer's disease pathology.

Research on development of drugs against Alzheimer's disease has been happening since decades and yet there has been very few drugs approved by the FDA. The drugs which have been approved too have many limitations as far as the efficacy and safety of these drugs are concerned. ⁽⁴⁾ This dearth of therapeutics in regards to Alzheimer's has drawn attention of the medical community and new methods of drug discoveries are being looked at. New strategies are being looked at modification of disease pathology instead of halting disease progression. ⁽⁵⁾

In silico based drug discovery is a promising strategy for development of therapeutics for diseases like Alzheimer's. Computational drug discovery has gained scope in every aspect of drug discovery due to the increase in data available on protein structures and small molecules. Computational drug discovery basically involves two methods, Structure based drug design and Ligand based drug design. Various techniques like Molecular Docking, Pharmacophore modeling, Conformation analysis, Virtual library creation and screening, etc. are used to identify potential drug candidates against targets. ⁽⁶⁾ The vast amount of resources and databases available has increased the efficacy of computational techniques in Drug Discovery.

Ayurvedic medicine is an ancient medical practice that involves use of plant extracts and herbal formulations as therapeutics against many diseases. Ayurvedic literatures note certain plant species as *Medhya Rasayanas* or 'Nervines' which are brain tissue specific and are well known to reduce neuronal damage and increase neuron development. These are also known to improve memory and cognitive function. ⁽⁷⁾ There has been many photochemical research on the medicinal plants and the bioactive compounds in these plants like Flavonoids, Tanins, etc have shown good pharmacological properties. These studies indicate the potential therapeutic possibilities of many compounds against Alzheimer's and their drug likeliness. ⁽⁸⁾

Here in this review, we discuss the major disease targets involved in the Alzheimer's disease pathology and the existing research on drug development against these targets. We summarize the limitations of the current drug molecules and also look upon the possibilities bioinformatics in drug discovery and development of novel drugs against Alzheimer's disease from the bioactive compounds from the ayurvedic plants.

Drug Targets

ACETYLCHOLINESTERASE

Acetylcholinesterase (AChE) is an enzyme that causes the termination of neurotransmission at cholinergic synaptic junctions. This enzyme causes fast and rapid hydrolysis of Acetylcholine which is a common neurotransmitter. The action of AChE causes reduction in cholinergic activity is correlated with the degree of cognitive impairment and is associated with decreased levels of the neurotransmitter acetylcholine (ACh). Thus, AChE inhibitors are used to inhibit the action of AChE. This further helps us to reduce the effects caused by AChE and decreases the neuro-degeneration ⁽⁹⁾.

AChE has a primarily hydrophobic active gorge into which the ACh molecule diffuses and is cleaved. AChE contains two active sites where the ACh binds and is hydrolyzed to choline and acetate, thereby terminating its neurotransmitter function ⁽¹⁰⁾. AChE is highly selective for ACh and has greater catalytic activity at low ACh concentrations ⁽¹¹⁾. Both in vitro and in vivo data suggest that AChE behaves as a potent amyloidogenic factor and modulates the toxicity of amyloid fibrils ⁽¹²⁾. AChE inhibitors prevent the hydrolysis of ACh after its release from the terminals of cholinergic neurons. While AChE inhibitors are only indicated as symptomatic therapy for AD, increasing

evidence supports a role for these agents in altering the natural course of the disease. The peripheral anionic site (PAS) of AChE, the sub-site located at the entrance of the active site gorge, mediates substrate inhibition of AChE at higher doses⁽¹³⁾.

Few of the many drugs used in the treatment of AD are mainly focused on inhibition of AChE activity namely, donepezil, rivastigmine, and galantamine. Rivastigmine binds directly to the catalytic site and forms a relatively stable acyl-enzyme intermediate that does not undergo hydrolysis for many hours. Although donepezil partly covers the PAS region of AChE when it binds to the AChE catalytic site, PAS mediated⁽¹⁴⁾. A β fibrillization is not apparently blocked by donepezil. This induces a strong dose-dependent increase of enzyme activity, possibly due to its non-competitive action. Galantamine causes significant but less marked increases in AChE activity than donepezil. Galantamine has a competitive action that depends not on the absolute concentration of the drug but more on the relationship with the substrate concentration.

The main drawbacks of these drugs are that amyloidogenic properties of AChE may not be blocked. In spite of the approval of these drugs and are in commercial use there are few drawbacks as mentioned above which needs further research and study for the development of a more efficient drug for the inhibition of AChE.

NMDA RECEPTOR

NMDA receptors have received much attention over the last few decades, due to their role in many types of neural plasticity and their involvement with respect to excitotoxicity on the other hand. There is a great interest in developing clinically relevant NMDA receptor antagonists that would block excitotoxic NMDA receptor activation, without interfering with NMDA receptor function. This is needed for normal synaptic transmission and plasticity. The recent analyses point to neurosteroids as NMDA receptor inhibitors which have desirable properties. These compounds cause voltage independent NMDA blockage, hence in-turn reducing the chances of Alzheimer's disease. Importantly, neurosteroids are also characterized by use-independent unblock, compatible with minimal disruption of normal synaptic transmission. These neurosteroids have very good neuroprotective properties that play a very important role in blocking NMDA receptor.⁽¹⁵⁾

Senescent synaptic function is observed as a shift in Ca²⁺-dependent synaptic plasticity and similar mechanisms, these contribute the early cognitive functions of AD. In the case of aging, intracellular redox state mediates a shift in Ca²⁺ regulation including N-methyl-d-aspartate (NMDA) receptor hypofunction and increased Ca²⁺ release from intracellular stores to alter synaptic plasticity. Thus ageing process helps us understand the early symptoms of ageing. The main focus of this is to provide an update on mechanisms that contribute to the senescent synapse and possible interactions with AD-related molecules, with special emphasis on regulation of NMDA receptors.⁽¹⁶⁾

Memantine, an uncompetitive glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonist, is widely used as medication for the treatment of Alzheimer's disease (AD). It has been reported that memantine reduces amyloid- β peptide (A β) levels in both neuronal cultures and in brains of animal models of AD. However memantine was not able to collectively reduce the effects of AD. Collectively, our results indicate that chronic treatment with memantine reduces the levels of A β both in AD models and in aged animals, and that memantine affects the endocytosis pathway of APP, which is required for β -secretase-mediated cleavage. This leads to decrease in APP, hence reducing AD.⁽¹⁷⁾

An *in silico* study was performed by checking the reactivity of bioactive compounds from *Withania somnifera* and NMDA. It was predicted that close to 25 phytochemicals pass the Blood Brain Barrier (BBB), has mutagenicity, druglikeness and good Human Intestinal Absorption properties. Further, molecular docking was performed to know whether these phytochemicals inhibit the GluN2B containing NMDARs or not. The results suggest that Anaferine, Beta-Sitosterol, Withaferin A, Withanolide A, Withanolide B and Withanolide D inhibit GluN2B containing

NMDARs through allosteric mode similar to the well known selective antagonist Ifenprodil. These phytochemicals have good neuroprotective and therapeutic properties and hence can be used as good NMDA antagonists.⁽¹⁸⁾

GLYCOGEN SYNTHASE KINASE 3 (GSK3)

Glycogen synthase kinase 3 (GSK3) is an ingrained active, proline-directed serine/threonine kinase that plays a vital part in a number of physiological processes ranging from glycogen metabolism to gene transcription. GSK3 also plays a pivotal role in the pathogenesis of both sporadic and familial forms of Alzheimer's disease (AD), this observation that has led us to coin the 'GSK3 hypothesis of AD'. According to this hypothesis, excessive-activity of GSK3 accounts for memory infirmity, tau hyper-phosphorylation, increased β -amyloid production and local plaque-associated microglial-mediated inflammatory responses; all of which are major characteristics of AD. If our 'GSK3 hypothesis of AD' is substantiated and GSK3 is indeed a causal mediator of AD then inhibitors of GSK3 would provide a novel avenue for therapeutic intervention in this devastating disorder. Hence by understanding the in-depth nature of the structure it would give us an overall opportunity to decrease the chances of AD caused by excessive-activity of GSK3.⁽¹⁹⁾

Hyperphosphorylation of tau forming neurofibrillary tangles is a key event in the pathogenesis of AD. MiR-124-3p belongs to micro RNA, it is known to play a role in AD, the extent is still unknown. However when there is excess of GSK3beta there is a decrease in MiR-124-3p. Along with this decrease in MiR-124-3p in micro RNA, there is also a decrease of it being expressed in N2a/APP695 cells plus there is an increase in abnormal hyperphosphorylation of Tau. Excess of GSK3beta leads increase in expression levels of Caveolin-1, phosphoinositide 3-kinase (PI3K), phospho-Akt (Akt-Ser473)/Akt, phospho-glycogen synthase kinase-3 beta (GSK-3 β -Ser9)/GSK-3 β in N2a/APP695swe cells. MiR-124-3p inhibits abnormal hyperphosphorylation of Tau by regulating Caveolin-1-PI3K/Akt/GSK3 β pathway in AD. Hence this reveals that miR-124-3p may play a neuroprotective role in AD, which may provide new ideas and therapeutic targets for AD.⁽²⁰⁾

We utilized virtual screening to search databases with the potential for it to be used as drugs targeting GSK-3 β kinase. Virtual screening of close to 1.1 million compounds in-house and ZINC databases was conducted using an optimized computational protocol called GOLD docking protocol. . Of the top-ranked compounds, 16 of them underwent a luminescent kinase assay and a cell assay using HEK293 cells expressing DsRed-tagged Δ K280 in the repeat domain of tau (tauRD). The compounds VB-003 (a potent GSK-3 β inhibitor) and VB-008 (AM404, an anandamide transport inhibitor), with determined IC50 values of 0.25 and 5.4 μ M, respectively, was found to reduce and decrease the Tau aggregation. Both compounds increased expression of phospho-GSK-3 β (Ser9) and reduced endogenous tau hosphorylation at the sites of Ser202, Thr231, and Ser396. In the Δ K280 tauRD-DsRed SH-SY5Y cells, VB-008, but not VB-003, enhanced HSPB1 and GRP78 expression, increased Δ K280 tauRD-DsRed solubility, and promoted neuronal outgrowth. VB-008 performed best to the end of the present study. The identified compound VB-008 may guide the identification and synthesis of potential inhibitors analogous to this compound. This in-turn helps to inhibit the excessive production of GSK3beta.⁽²¹⁾

BACE1

β -site APP cleaving enzyme 1 (BACE1) is the β -secretase enzyme which generates Amyloid β (A β) peptides which accumulate as fibrillary plaques in the neuronal cells. These lead to neural degeneration, neuroinflammation, neuronal dysfunction and death which are the central cause of pathogenesis in Alzheimer's Disease. Mutation in three genes (amyloid precursor protein (APP) and presenilin 1 and 2 (PS1 and PS2)) causes early-onset of Familial AD (FAD) by increasing the A β peptide formation⁽²²⁾. A β peptides are produced by a single copy gene on chromosome 21, where it is encoded as an internal peptide within a large precursor protein referred to as the amyloid β protein

precursor (APP)⁽²³⁾. It is generated by cleavage of the APP by β -secretase and γ -secretase in the amyloidogenic pathway.

The normal pathway that undergoes is the non-amyloidogenic pathway where the α -secretase and γ -secretase cleave the APP to produce soluble peptide fragments. In the amyloidogenic pathway β -secretase cleave the APP at the Asp+1 residue of the A β sequence to form the N-terminus of the peptide. The structure is left with a membrane-bound carboxyl terminal fragment (CTF), C99. The C99 is cleaved by γ -secretase to generate the C-terminus of the A β . The cleavage is not specific and hence leads to generation of A β 40 or A β 42 peptides. These are insoluble peptides which accumulate as fibrillary plaques⁽²⁴⁾.

To reduce the levels of A β , the activity of BACE1 must be inhibited. There have been many inhibitor drugs which are being tested clinically for inhibition of this enzyme. Many companies like Eli Lilly and Roche developed inhibitor drugs which later failed during clinical trials. The drug AZD3293 developed by Eli Lilly and AstraZeneca has reached the phase 3 of the clinical trials. It is important to understand the efficacy and degree of BACE1 inhibitors, the way they should be administered and also the side effects that can be caused by the inhibitors of BACE1⁽²⁵⁾. The novel way of developing inhibitors for BACE1 can be achieved by means of various computational methods. Computational tools are used to model and change the configuration of novel inhibitors of BACE1 and their validation⁽²⁶⁾.

As a novel approach, extracts from plants have been used to identify the inhibitors for BACE1. Certain inhibitor compounds such as Danshen, Chicoric acid, Tannic acid have been isolated from various natural plant sources. These have been tested *in vivo* which have shown positive results by showing memory improvement and also decrease in levels of A β in the brain^(27,28,29).

CONCLUSION

Alzheimer's disease is predicted to be one of the leading cause of death in the world by 2050 and the disease incidence is increasing exponentially. This pose a major problem for all the countries and their governments as this puts a large burden over the economies due to the cost involved in management of patients and the treatment involved. Therefore, there is a dire need for development of efficient drugs against Alzheimer's disease with sufficient efficacy as well as safety.

The failure of potential drug candidates in clinical trials and toxic side effects of already approved drugs has led researchers look at new strategies and novel targets for developing drugs against Alzheimer's. Multi target drug development and computational drug discovery are the prominent strategies being looked upon. Multi target drug development focus on increasing the safety of drug candidates by decreasing the incidence of various toxic side effects. Computational drug discovery has broadened the perspective of lead selection as it has enabled researchers to select or validated molecules from enormously large databases of molecules available. Various tools used for *in silico* studies help reduce the resources spent and time involving drug discovery.

Acetylcholinesterase, NMDA glutamate receptor, GSK3beta and BACE1 have been established as the major protein molecules that are involved in the Alzheimer's disease pathology. Various experiments have proved their importance in disease incidence and these have been the prime drug targets for many pharmaceutical companies. Ayurvedic medicine literature point at the possibilities of developing novel lead molecules from the various bioactive compounds present in Ayurvedic plants. These compounds have been studied to be neuroprotective and known to improve cognitive functions, etc. So this approach of identifying drug candidates with Ayurvedic literature as a basis can give us good lead on developing novel efficient drugs against Alzheimer's disease.

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