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# TARGET EVOLUTION AND THERAPY ADVANCEMENTS FOR ALZHEIMER'S DISEASE: A REVIEW

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## ABSTRACT

*Alzheimer's disease (AD) has engulfed most parts of the world and the number is expected to rise drastically in the near future. Consequently researchers are focusing on novel and better therapeutics to tackle this deteriorating condition. The current therapeutics offer only symptomatic relief leaving an incomplete cure. Given the complexity of the disease pathogenesis, a multi-target approach is the need. Although an efficient elucidation of disease etiology and pathology has served useful in drug discovery the final results in most cases are unconvincing. This article summarizes the different therapeutic strategies that can provide an insight in the treatment of AD.*

***KEY WORDS***

*Amyloid beta, oxidative stress, multi-target approach, pleiotropic ligands*

## INTRODUCTION

AD is a debilitating neurodegenerative disorder characterized by memory loss and cognitive impairment. The origin of AD was in 1906 when the Austrian physician Alois Alzheimer identified the two hallmarks of this disease-Neuritic Plaques (NP) and Neurofibrillary Tangles (NFT) using silver based histological staining. [1]

The characteristics of AD pathology include:

- Amyloid Cascade: The faulty processing of the Amyloid Precursor Protein (APP) causes toxic Amyloid beta peptide (Aβ) that gets accumulated extracellularly in a dense core to form NPs.
- Tau –related toxicity: A dysfunctional Tau protein when unable to stabilize microtubules gets aggregated to form NFTs.
- Neuronal loss: Cells in the cortex and hippocampus begin to die and large voids are created in the brain due to the shrinkage of the cortex. This causes loss of synaptic transmissions in association cortices leading to inefficiency in learning and memory.

**AD progression and neuropathology:**

The putative hypothesis of AD as of today is the amyloid beta and tau cascade. The amyloid-beta precursor protein (APP) under normal physiological conditions gets cleaved by the 3 secretases- α, β and γ secretase to form Amyloid beta (Aβ) peptides that help in transporting lipid molecules and act as transcription factors. [2]It also is important for suppressing synaptic activity or else excessive signal transmissions could lead to excitotoxicity. [3]However increased Aβ levels are known to accumulate and precipitate extracellularly to form a proteinaceous plaque core as follows.

1. The soluble Aβ proceeds to form fibrils via a partially folded intermediate through a

nucleation dependent polymerization reaction. This nucleation occurs only beyond a threshold concentration of soluble Aβ levels.

2. Fibrils mature to from a beta sheet configuration revealed through X-ray diffraction studies. This again proceeds via metastable intermediates such as oligomers and protofibrils.
3. Amyloid core thus consists mainly of these elongated sheets which further gets associated with macrophages and astrocytes.

Amongst the various peptides synthesized due to cleavage action of secretases, the $A\beta_{42}$ is most toxic as it is prone to fibril formation and these are proteolytic resistant. Mutations in APP or secretases can cause increased Aβ levels leading to early onset of AD. [4] Another process that is reported to take place concurrently and/or a downstream phenomenon of Aβ toxicity is the formation of NFTs. [5] These are aggregates of hyperphosphorylated tau protein that is unable to stabilize the tubulin of microtubules and hence gets disengaged from them misfolding into oligomers. The hyperphosphorylation is due to the increased levels of GSK-3β, a kinase that is responsible for phosphorylating tau protein.[6] Oxidative stress caused due to reactive oxygen species such as hydrogen peroxide is known to aggravate the neurodegeneration by lipid peroxidation and DNA cleavage. [7] Also the Apolipoprotein E (ApOE) has been identified as a genetic factor for AD. Individuals carrying the isoform ApOE4 are most associated with increased risk of developing AD. [1]

Diseases such as AD that are driven by an integration of several pathways are extremely difficult to manage. Consequently targeting these pathways requires a combined and carefully synchronized treatment regimen. Treatment of AD has changed several courses in the last few years. The same are discussed as follows:

- **First therapeutics: Symptomatic treatment**

1. **Acetylcholine esterase inhibitors:** The first approach was based on cholinergic hypothesis. As per this, AD causes tremendous loss of cholinergic neurons and neurotransmission. Acetylcholine esterase inhibitors (AChEI) restore the acetylcholine (Ach) levels and keep the neurons firing. [8] They either bind to the catalytic triad of ACh or to the acetylated ACh intermediate to prevent its deacetylation and enzyme reactivation. Physostigmine was the first AChEI to be investigated but failed phase 2 and 3 clinical trials due to narrow therapeutic index. Tacrine, the aminoacridine derivative was the first AChEI to be approved by FDA but was discontinued later due to hepatotoxicity due to its scaffold and short half life. Donepezil, an N-benzylpiperidine-indanone scaffold was the second drug to be approved developed through random screening. It prevents hepatotoxicity unlike Tacrine. Rivastigmine is a competitive and long acting drug and was reformulated in 2007 to a transdermal patch from an oral capsule for better efficacy. Another drug approved is Galantamine that has a MOA different than the rest. It binds to nicotinic ACh receptors modulating their action for Aβ phagocytosis. [9,10]

2. **NMDA blockers**: An increased sensitivity of the N-methyl-D-aspartate receptors (NMDA) for glutamate causes a Ca build up in cells leading to excitotoxicity. It has also been reported to cause an increase in toxic Aβ peptide. Further increased levels of Aβ are known to cause an increase in

glutamate levels. [11] The first NMDA antagonists were nimodipine and ifenprodil. However they blocked all NMDA receptor activity leading to no Ca influx and this caused the side effects of learning and memory dysfunction. The adamantane derivative memantine blocks receptor channel only when it is excessively open and at the same time maintains physiologically important receptor activity due to its rapid blocking/unblocking kinetics. [12] Currently memantine is given in combination with AChEIs for a synergistic effect. [13]

- **Agents targeting the amyloid and tau hypothesis:**

1. **BACE inhibitors**: BACE or the β-secretase enzyme cleaves APP to produce a 99-residue carboxy terminal fragment (C99) in the membrane. The BACE active site consists of two aspartate residues (Asp32 and Asp228) that interact with the aspartates of the APP substrate under acidic pH conditions. BACE is an aspartyl protease and inhibitors were designed based on structure –based semblance with other aspartyl protease inhibitors like renin and HIV. Molecules are developed that are small (to permeate the BBB) transition state analogues that mimic the tetrahedral intermediate formed during APP cleavage by BACE. [14, 15] Drug candidates undergoing clinical trials belong to the Hydroxyethylene, Hydroxyethylamine, acylguanidine and pyrimidinone classes. The challenge that BACE inhibitors portray is the small size of active site and consequently developing small non-peptidergic molecules to bind to it. [16]

2. **GS inhibitors and Modulators:** GS or the γ-secretase enzyme cleaves the C99 substrate at ε, ζ and γ sites to form varying lengths of Aβ. GS inhibitors thus prevent the cleavage at the ε site and stop the amyloidogenesis. Semagacestat (benzocaprolactum class) developed by Eli Lilly was a potential GS inhibitor whose phase 3 clinical trial had to be terminated due to excessive GI toxicity in Aug 2010. The reason was due to off-target selectivity since GS inhibition was found to affect the NOTCH signaling pathway. [17] A remedy to this solution is targeting selectively a subunit since GS has a heterogenous protein subunit composition exhibiting a heterogenous protein activity. Avagacestat (arylsulfonamide), developed by Bristol-Myers Squibb was a molecule that showed NOTCH-sparing activity due to a 137-fold increase in selectivity for APP over NOTCH substrate. But phase 2 clinical trials had to be terminated due to adverse nausea, vomiting and rash in patients. In response to the adverse effects of GS inhibitors, modulators are designed that cause a shift in the GS cleavage site to produce shorter non-toxic forms of Aβ peptides without inhibiting NOTCH or APP proteolytic processing. The first generation GS modulators (GSM) were the non-steroidal anti-inflammatory (NSAID) drugs-Ibuprofen, Sulindac and Indomethacin. A dose of 50mg/kg/day in transgenic mice showed 30-39% reduction in brain $A\beta_{42}$ levels. [16] The second generations GSMs were derived from NSAIDs that had no effect on COX activity. Tarenflurbil (Flurizan) was a notable compound developed by Myriad Genetics & Laboratories. However due to low brain permeability phase 3 clinical trial was terminated. To overcome this limitation a new molecule CHF5074 was developed and is currently in phase 2 clinical trial. All GSMs reduce $A\beta_{42}$ levels

without having any effect on total Aβ levels. [17]

3. **α- secretase Activators:** α-secretase cleaves APP to form an 83-residue carboxy terminal fragment (C83) in the membrane which on further action by BACE forms an amino-terminally truncated form of Aβ. Thus agents that shunt more APP down this α-secretase pathway make less substrate available for action by BACE and GS and decrease Aβ insults. [1] Etazolate is one such molecule (a selective $GABA_A$ receptor modulator) that has completed a phase 2 clinical trial. [18] Muscarinic and serotonin receptor agonists, statins and protein Kinase C activators are other classes of drugs under development. [19]

4. **Anti-Aβ aggregates:** This aspect deals with preventing Aβ aggregation that disrupts formation of soluble oligomers or plaques. A polyphenol from green tea, Epigallocatechin-3-gallate (EGCg) is undergoing a phase 3 trials and shows potential in anti-Aβ aggregation as well as stimulation of α-secretase activity. [20] Metal complexing agents such as clioquinol are known to chelate Zn and Cu to prevent them from inducing Aβ aggregation. [21] Active immunization (injecting Aβ peptides) or passive immunization (injecting anti- Aβ antibodies) is currently explored. Solanezumab is a humanized monoclonal antibody currently in phase 3 clinical trial that shows potential to binding soluble Aβ and preventing formation of NPs[22]. The serum amyloid protein (SAP) is known to bind Aβ deposits and prevent them for undergoing degradation. Agents that bind to SAP to form an undesirable complex which is immediately degraded by liver are being developed. [23,24,25] CPHPC, a potential candidate molecule that forms a decamer complex by bridging 2 pentameric SAP molecules is reported to decrease circulating SAP levels by 99% in humans due to its quick hepatic uptake thus making even less available to bind to Aβ. [26]

5. **GSK-3β inhibitors**: GSK-3 is known to phosphorylate tau protein. Increased GSK-3β is the isoform associated with AD. Inhibitors are designed that bind to the ATP active site of GSK-3β. [27] Lithium and Valproate were the first natural inhibitors however these lacked target selectivity and failed to show cognitive improvement in clinical trials. [28] Maleimide, indole,paullone and thiazole derivatives are undergoing clinical trials. [29] A major disadvantage of this class is that they target the ATP catalytic site that has a conserved sequence homology with other kinases of the same family and thus achieving selectivity is a challenge due to a similar 3D structure. [30] Hence there is a need to develop non-ATP inhibitors that can bind to an allosteric site.

6. **Inhibitors of tau aggregation**: Agents that bind to tau to prevent its aggregation into NFTs are being developed. One such example is Methylthioninium chloride (MTC) that releases soluble tau from NFTs in the form of monomers that can be easily proteolysed. [31]

7. **Anti-oxidants:** ROS can be modulated to decrease oxidative stress using anti-oxidants such as Vitamin E. [32]

8. **Neurotropin administration**: The nerve growth factor (NGF) plays an important role

in maintaining cholinergic function. However this peptide has an unfavorable size and polarity and BBB permeability is difficult. [33] Recently an adeno-associated virus is used to transfer the NGF gene that is injected into the brain of AD patient. This approach is currently in a phase 2 studies. [34]

9. **Harnessing receptors involved in ApOE metabolism**: The ApOE4 gene is a definite genetic marker for AD. [35] The ApOE4 is lipidated to form lipoprotein particles, a process regulated by nuclear receptors(RXR and LXR) that bind to Aβ to effect its clearance. Stimulating these receptors can help increase Aβ clearance. [36] A phase 2 clinical trial of FDA approved anticancer drug and an RXR agonist bexarotene has reported a reduction in Aβ plagues in an ApOE-dependant manner in amyloid mouse model. [37]

10. **Pleiotropic Therapy:** Considering the scores of pathways that drive AD multi-target drug ligands (MTDL) are designed that intervene several pathologies simultaneously.[38] According to studies, Docosahexanoic acid exerts its neuroprotective effects by acting as an anti-oxidant, increasing brain-derived neurotropin factor synthesis,anti-inflammatory activity, GSK3β inhibition as well as modestly suppresses Aβ production by altering secretase mobility. Another pleiotropic candidate drug is curcumin. It has been reported to posses Aβ and tau binding properties thus influencing their clearance, anti-oxidant and anti-inflammatory activity. [39] Translocator protein ligands (TSPO) too elicit such a neuroprotective effect due to an increased neurosteroidogenesis as reported by tests in transgenic AD mice. This is supported by prior studies in mice that have shown to reduce Aβ accumulation and improve memory on administration of testosterone and progesterone. [40]

## CONCLUSION

To date, treatment for AD relies solely on symptomatic relief offered by acetyl cholinesterase inhibitors and NMDA blockers making it an incurable disease. AD can be classified as a multi-factorial disease encompassing four cardinal pathways-cholinergic neuronal loss, amyloid and tau cascade, neuroinflammation and oxidative stress. The treatment approach has transcended from initial cholinergic hypothesis to recent amyloid and tau hypothesis. Instead of the conventional single target approach, research is focused in adopting MTDLs. Therapeutics need to be designed with the intent of slowing or even better reversing the disease progression. Such disease –modifying drugs need to possess the pharmacophores in a single molecule that shall act on various points in the disease progression. Another area of promise could be in developing therapies that can prevent the occurrence of AD in at-risk individuals. For this it is also necessary that diagnostic techniques be developed rigorously to detect AD in early stages. Thus a reinforcement of mechanism based approach is essential that can exploit multiple targets during any stage of AD.

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