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Review Article

INHIBITION OF AMYLOID BETA FIBRILIZATION BY SMALL ORGANIC MOLECULES: AN IMPLICATION TO THERAPEUTIC ROUTE OF ALZHEIMER'S DISEASE

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ABSTRACT

The exact reason of Alzheimer's disease is still not understood but deposition of extracellular plaques formed by the aggregation of amyloid β peptide and intracellular accumulation of neuro fibril tangles (NFT) formed by phosphorylated tau protein are the two hall marks of Alzheimer's disease. Therapeutic route to Alzheimer's disease is still unknown. Studies with natural products, short peptides and synthetic organic molecules have identified a pool of small organic molecules with aggregation inhibitory activity. These molecules can be considered as lead compounds in the drug discovery of Alzheimer's disease.

Keywords: Amyloid β protein, Aggregation, Alzheimer 's disease, Small organic molecule.

1. INTRODUCTION

Neurodegenerative diseases significantly affect the quality of life of elderly people across the globe. Alzheimer's Disease (AD) and Parkinson's disease are the most common among the neurodegenerative diseases [1]. Loss of memory is the most prominent symptom of Alzheimer's disease. Degeneration of brain neurons causes gradual loss of movement, breathing, talking in AD patients [2]. Protein misfolding is the main reason of Alzheimer's disease. AD patients suffer from damage of brain cell neurons due to formation of extracellular plaques by aggregation of amyloid β protein and intracellular accumulation of neurofibril tangles (NFT) by tau protein [3, 4]. Prevalence of Alzheimer's disease has inspired scientific community of whole world to find therapeutic route to but till now very few medicines are available which can only treat the disease symptomatically and provide limited benefit. In this context, it is very much important to find ways to inhibit the aggregation process of amyloid beta and tau protein which can stop or postpone Alzheimer's disease. Many studies are taking place all over the world in which natural products, synthetically accessible small molecules and also peptides are being used as potential amyloid β inhibitors [5-9]. This article aims to review the *in vitro* and *in vivo* studies with small and simple organic molecules which show ability to suppress or postponed fibrillation process of amyloid β protein and thus can be very important in the path of drug discovery of Alzheimer's disease.

2. PROTEIN MISFOLDING

Proteins are workhorse of the living cell. They act as enzymes, hormones, neurotransmitters, nutrient storage, antibodies and many more to regulate the life of a living cell [10]. Proteins have marvelous versatility in their structure and keen specificity in their function. Structure and function of protein molecules are crucially related [11]. Specific function of protein molecules is completely governed by its correctly folded native structure. Most protein fold in the posttranslational period [12]. Protein disulfide isomerase (PDI) and peptidyl-prolyl cis-trans isomerase (PPI) have important role in the protein folding phenomenon [13, 14]. Chaperons assist significantly in correct folding of proteins. Chaperons can rescue incorrectly folded proteins to proper route of folding [15]. Beside chaperon, cell has its own quality control mechanism which discriminates between correctly folded and misfolded structures and ultimately degrades the misfolded protein into the amino acids [16, 17]. In spite of these protective mechanism, protein misfolding takes place within the life time of a cell. Misfolding can be induced by somatic mutations in gene sequence; error involved in transcription or translation;

failure of chaperone action; wrong post-translational modifications, inappropriate trafficking of proteins and structural alteration caused by environmental stress factors [18, 19].

Protein misfolding is currently linked to more than 25 diseases including well-known diseases such as cystic fibrosis, Alzheimer's disease, Parkinson's disease, as well as less familiar diseases such as Gaucher's disease, nephrogenic diabetes insipidus, and Creutzfeldt-Jakob disease [5, 9]. Misfolded protein causes disease either by loss of function (LOF) or by gain of function (GOF) [20]. Loss of function occurs when the misfolded protein fails to achieve its functional conformation and/or can not be recognized by the transport protein. This generally happens as a result of genetic mutation. Cystic fibrosis (CF), α1-antitrypsin deficiency and certain cancers are caused by loss of function of misfolded proteins [21]. On the other hand, gain of function (GOF) of misfolded proteins cause toxicity and adverse consequence. Misfolded proteins are very prone to aggregation because they interact improperly with solvent. Exposure of hydrophobic portions of protein, which remains buried in the core of correctly folded structure, leads to stickiness between hydrophobic patches of different molecules [22]. GOF results in aggregation of misfolded protein to form amyloid fibrils and thus triggers disease pathology. Neurodegenerative diseases such as Alzheimer's disease, Parkinson's Disease and several other types of amyloidosis are caused by GOF of the respective pathogenic proteins [23]. Neurodegenerative diseases generally affect aged people.

3. REASON BEHIND ALZHEIMER'S DISEASE

Exact cause of Alzheimer's disease is still unknown but two hall mark of Alzheimer's disease is extracellular deposition of amyloid β plaques and intracellular formation of neurofibril tangle by tau protein [3, 4]. In healthy brain neurons Amyloid precursor protein (APP) is cleaved in presence of two enzymes α secretase and ϒ secretase. This cleavage results in formation of a soluble peptide which can be later broken down to amino acids and can be recycled. But when β secretase replaces α secretase and accompanies ϒ secretase in the digestion process then the cleavage process produces insoluble peptide known as amyloid beta which clump together and forms amyloid beta plaques (ABP). Deposition of ABP in the extracellular region of brain neuron cells is the most common diagnosis in AD patients. ABP damage the brain and disrupt neuron function by different ways. Primarily if ABP is located between two neurons, then the signaling process of neuron is disrupted which leads to dementia [24]. ABP can also cause inflammation of brain. ABP may be accumulated on the outer side of blood vessel, which is known as angiopathy. Angiopathy will ultimately cause hemorrhage or the rupture of the vessel [25].

In the intracellular region neuro fibril tangle (NFT) is the cause of pathogenesis. Neuron cells are held together by cytoskeleton. Cytoskeleton is partly made by microtubules. Tau protein helps in the packing structure of microtubules. Formation pf ABP in extracellular region triggers a process that leads to phosphorylation of Tau protein [26]. Once phosphorylated, tau protein departs from the microtubule and ultimately phosphorylated tau proteins clump together to form NFT. Microtubule structure is weakened after losing tau protein and this finally leads to loss of signaling function [25].

4. SMALL MOLECULES WHICH CAN POSTPONE ALZHEIMER'S DISEASE

Search for small organic molecules, peptides, peptidomimetics and nanoparticles which can selectively prevent or interfere with the self-assembly process of amyloid β is an emerging field of study because this can develop new strategy in designing and synthesis of new pharmaceutical drugs to treat AD [9]. Increasing knowledge of high-resolution structure of amyloid β oligomers and atomic level binding information of amyloid β oligomer with its inhibitors has made this field more promising [27].

β- cyclodextrin is one of the first reported compound which shown to reduce Aβ 1-40 fibril formation [28]. In presence of β- cyclodextrin Aβ 1-40 showed a number of additional peaks in electrospray mass spectrum and this suggest that some kind of complexation between the two molecules inhibits the aggregation process.

A study with elderly leprosy patients reported absence of senile plaques in the brain of aged leprosy patients compared to age matched control [29]. Following this report two widely used anti leprosy drugs Dapsone and Rifampicin were tested for having anti fibrillogenic activity. It was found that Rifampicin (Fig. 1) inhibited aggregation and fibril formation of synthetic Aβ 1-40 in a dose dependent manner at reasonable concentration. However, dapsone exhibited no such effect [30]. The molecular mechanism of the aggregation inhibitory activity has been investigated. Some study suggested that Aβ 1-40 spontaneously fragments into free radical

peptides in aqueous solution and these could react with each other to generate covalently linked aggregate [31,32]. Rifampicin has a napthohydroquinone ring which can play as free radical scavenger. Studies with rifampicin analogues were also performed and the results indicated that inhibitory activity of rifampicin is related to its napthohydroquinone ring. Though all these studies were *in vitro* but it is known that rifampicin can cross the blood brain barrier in rat [33] and may be expected to penetrate brain of AD patients and interfere with amyloid plaques formation.

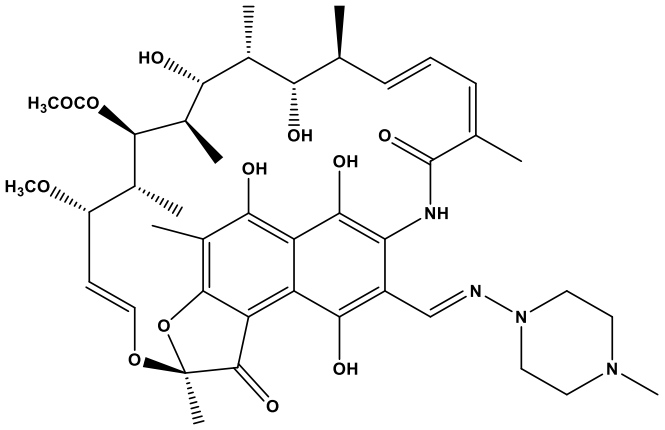
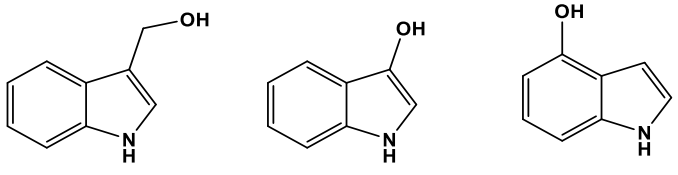


Fig. 1: Structure of Rifampicin

In vitro studies with nitrophenols in micromolar concentration level, exhibited ability to inhibit Aβ aggregation and also caused disaggregation of previously formed amyloid fibrils. Interestingly, nitrophenols also inhibited the formation of amyloid deposits in rat hippocampi in an in vivo model system of cerebral amyloid deposition. Nitrophenols are presumably hydrophobic enough to cross the blood brain barrier and access the central nervous system. It was suggested that nitrophenols in subtoxic concentration level could be used clinically to treat patients of amyloidosis. Moreover, nitrophenols can be a potent lead compound in the field of drug discovery for finding effective therapy for Alzheimer patients [34].

Many investigations with amyloid protein revealed very early intermediates as most cytotoxic in the aggregation process of amyloid protein. These studies encouraged researchers to find way to target the basic molecular recognition process to form these early intermediates [35-37]. Several short peptides were identified as potent aggregation inhibitor [38-41]. In another study a seven membered short peptide was found to form well ordered amyloid fibril [42]. Presence of a pair of phenylalanine residues in all these short peptides suggested that aromatic interaction may have played key role in the recognition process at very early stage of fibril formation. Replacement of phenyl alanine by tryptophan has shown remarkable change in amyloidogenic behavior of peptide fragments [43]. Inspired by all these findings Cohen and coworkers screened 29 indole derivatives and identified three hydroxy indole derivatives (Fig. 2) for having maximum potential to inhibit aggregation of amyloid fibril [44]. Though the inhibitory mechanism was not clearly understood but the presence of hydroxy group and the position of the hydroxy group was found to be very crucial for the inhibitory activity. The hydroxyl group is an electron donor and it alters electron density and a negative charge on the benzopyrrole ring. Additionally, the hydroxyl group can to interact with various functional groups by forming hydrogen bonds. Cohen and coworkers suggested that the hydroxyl group interacts with the backbone of the peptides and that the different electrondensity and negative charge on the benzopyrrole ring prevents the ability of another Aβ peptide to create a π-stacking interaction, hence preventing the aggregation. Nitrogen atom of the indole can participate in a NH….π hydrogen bond with the ring of another aromatic residue, which is energetically more favorable than typical nonbonding aromatic-aromatic interactions. The surface of amyloid oligomer has several NH….π hydrogen bonding sites. Therefore, this interaction can significantly contribute to the inhibitory activity. Small size of indole, hydrophobicity and high blood brain barrier permeability of other known indole derivatives recommend these hydroxy indoles to be considered as lead compounds in drug discovery.



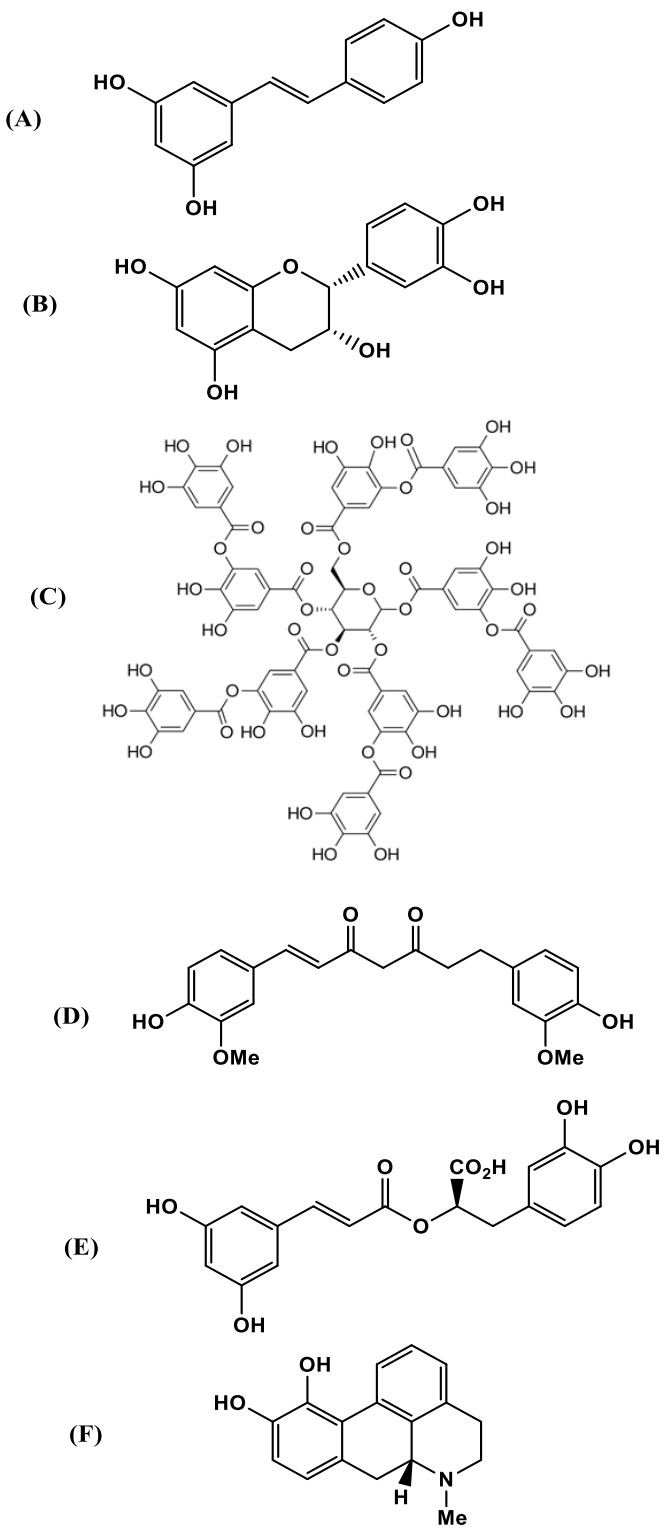
(A) (B) (C)

(A) indole-3-carbinol, (B) 3-hydroxyindole, and (C) 4-hydroxyindole

Fig. 2: Structures of three most effective amyloid aggregation inhibitors hydroxyindole derivatives

In the biological self-assembly, π stacking interaction is known to play crucial role [45, 46]. Stacking interactions may provide an energetic contribution as well as

directionality and orientation that are facilitated by the restricted geometry of planar aromatic stacking. π-Stacking interaction can accelerate formation of amyloid fibril in many cases [47]. Polyphenols are a large group of natural and synthetic small molecules that are composed of one or more aromatic phenolic rings. Natural polyphenols are known to act as free radical scavengers [48, 49] and have shown beneficial health-promoting effects [50]. Beside their anti-oxidant property, aromatic interaction between the phenolic compound and aromatic residues in the amyloidogenic sequence may direct the molecule to the amyloidogenic core, facilitate interaction and interfere with fibril assembly. Screening studies with a large no of polyphenols by different workers have identified many polyphenolic compounds as inhibitor of amyloid formation [51, 52]. Tagliazucchi and coworkers showed that resveratrol and catechin (Fig.3A, B) have protective synergistic effects against the cytotoxicity of β-amyloid1-41 [53]. Result of Yamada and coworkers exhibited that tannic acid (Fig.3C) is a potentialinhibitor of β-amyloid fibrillization. The authors showed a dose-response inhibition of β-amyloid assembly from monomeric β-amyloid into well-ordered fibrils [54]. In another study, Yamada and coworkers showed that Curcumin and rosmarinic acid (Fig. 3D, E) are efficient inhibitor of amyloid aggregation. In this study, they proposed that the compact and symmetric structure of curcumin and rosmarinic acid might play key role for specific binding of free β-amyloid and inhibition of its oligomerization [55].Callaway and coworkers reported apomorphine (Fig. 3F) to inhibit β-amyloid 1-40 aggregation. They screened several apomorphin derivatives and suggested that two hydroxy groups on the D-aromatic ring of apomorphine is crucial forthe inhibitory effectiveness of apomorphines. Comparing apomorphines with catechol which have low inhibition potency, the authors suggested that the additional ring structure of apomorphine makes it more hydrophobic, and hence increases its efficiency for binding with β-amyloid, which has a hydrophobic core [56]. Several studies have reported that polyphenols mostly inhibit formation of the assembly of large oligomers, and did not interfere with early nucleation events. This implies that polyphenols do not interact with amyloidogenic monomer but rather interact with amyloidogenic structures. Binding of polyphenol inhibitors are not sequence dependent but conformation dependent [57]. All these results recommend polyphenol compounds as potent therapeutic molecules for Alzheimer's disease.



(A) Resveratrol (B) Catechin (C) Tannic acid (D) Curcumin (E) Rosmarinic acid (F) Apomorphine

Fig. 3: Structures of polyphenols with ability to inhibit β amyloid fibrillization

In a study with the isomers of dihydroxy benzoic acid (DHBA) 2,3-, 2,5-, and 3,4-dihydroxybenzoic acid was

shown to dissociate biotinyl-Aβ (1-42) oligomers. Other isomers of DHBA, monohydroxy benzoic acid and unsubstituted benzoic acid showed no activity. None of the compounds blocked oligomer assembly, indicating that they do not interact with monomeric Aβ. A model for the mechanism of action of the DHBA dissociators proposes that these compounds destabilize oligomer structure promoting progressive monomer dissociation rather than fissioning oligomers into smaller, but still macromolecular, species [58].

Rizwan Hasan Khan and coworkers demonstrated that vitamin B12 can inhibit Aβ 42 amyloid fibrillation in a concentration dependent manner. Further, Vitamin B12 also provide protection against amyloid induced cytotoxicity in human neuronal cell line [59]. In another study they demonstrated that vitamin K3 also significantly inhibits fibril formation and inhibitory effect is dose dependent [60].

5. CONCLUSION

It is reported that nearly 40 million people are suffering from dementia caused by Alzheimer's disease globally and no proper therapeutic break through has still been developed. In this context search for potential inhibitors of amyloid aggregation is very much relevant. Ongoing research in this field is expanding the pool of small molecules which can interfere with the amyloid aggregation process. However, in most cases the mechanism of inhibitory action is not clearly understood. *In silico* studies with these small organic molecules may give insight into the molecular mechanism of aggregation inhibition. Detailed understanding of the mechanism of their action will facilitate drug designing and discovery of new pharmaceuticals. Moreover, most of these studies are in vitro. Much more in vivo research is required to validate those molecules as clinically useful. Studies with the toxic effects of these small molecules at different concentration level is also essential. The results reviewed in this paper can be useful to find a therapeutic solution of Alzheimer's disease.

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