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Scrutinizing the interactions of Cholinesterase Inhibitors with Amyloid Precursor Protein to Target Alzheimer's Disease

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Abstract

Alzheimer's disease is a fatal brain disorder. It is progressive cognitive disease as it destroys neurons, causing problems with memory, behavior, and finally hampers work. The role of amyloid plaques in the disease is mostly about the β-amyloid protein, which is part of much larger protein called Amyloid precursor protein (APP). Many drug targets have been identified for the disease like APP, tau protein, ubiquitins, all working in different pathways. But the oldest identified cause is APP and is yet under study. Here, we targeted APP with FDA approved cholinesterase inhibitors. Potency levels of these inhibitors were screened against APP and apolipoprotein E which can also be a novel suitable target. It was observed that these inhibitors act approximately the same way on APP. The active distances between these inhibitors and target protein sites were analyzed to screen the firmness of drug binding.

Keywords: β-amyloid protein, APP, tau protein, ubiquitins, cholinesterase inhibitors.

Introduction

Alzheimer's disease (AD), a neurodegenerative disease occurs by cognitive deterioration progressively, thus declining the daily activities. The ultimate cause of the disease is unpredictable (1). AD tends to run in families. In chromosomes 1, 14, 19, and 21 mutations occur and are believed to play a vital role in the disease. Among these PS1 (or AD3) on chromosome 14, and PS2 (or AD4) on chromosome 1 are the best known. The main causative protein responsible for AD i.e. Amyloid Beta Precursor Protein (APP or peptidase nexin-II) is encoded by chromosome 21, which is also the smallest human chromosome. Chromosome 19 encodes apolipoprotein E, a protein involved in triglyceride catabolism is also reported to be involved in AD (2, 3). Amyloid family proteins play a unique role in disease by forming lesions due to fragmented neurons (4).

It is known since a long time that AD prevails after unequal cleavage of APP into 2 subunits by β-secretase. Larger subunit remains in neuronal cell, which forms neurofibrillary tangles (NFTs) whereas smaller subunits are released into outer extracellular space forming amyloid plaques.

AD is the most prevalent neurodegenerative disorder, also being a major cause of Dementia (5). AD is caused by mutations in APP and presenilin genes, and it is a rare autosomal dominant .Both genes are linked to amyloid β (Aβ) peptide metabolism (6). Aβ is produced from APP by sequential cleavage involving β-secretase and γ-secretase released into the extracellular spaces (7). Formation of neurofibrillary lesions from degeneration of nerve cells in cerebral cortex and hippocampal formation is considered responsible for disease, which ultimately leads to the synaptic loss. In early stages, defect occurs by synaptic function correlated with loss of synapses and thus memory (8).

An amyloid plaque is the principal component present in the Alzheimer patients. Amyloid precursor protein (APP) is converted to amyloid beta-peptide by the proteolytic process which is involved in cell differentiation, cholesterol homeostasis and stress responses in cytoplasmic domain which anchors to a complex protein network that functions in axonal elongation, and neuronal cell migration. (9, 10). APP is processed by the following three proteases. The Table 1 below shows 3 proteases Alpha, Beta and Gamma respectively cleaving the peptide bonds between 612-613, 638-639 and 596-597. These details give the insight about the APP Processing mechanism of these 3 proteases.

Table 1: Proteases involved in the APP processing.

Proteases	Cleavage between amino acids
Alpha Secretase	612-613
Beta Secretase	638-639
Gamma Secretase	596-597

Meanwhile, it is also known that APP is a transmembrane protein that exists in different isoforms. It is composed of 39-42 amino acids. APP consists of large extra cellular transmembrane domains of about 616 amino acids. Structural domains folding has been identified in APP. Extra cellular region E1 contains many sub-domains, like growth factor-like domain and serine protease inhibitor domain. Intra cellular region E2 contains a coiled dimerization motif and binds proteoglycan in extra cellular matrix. Focusing on the molecular mechanism involved, it is observed that genetic mutation of gene located on chromosome 21 and 19 results in proteolysis of APP leading to generation of abnormal alpha and beta fragments. Deposition of alpha, beta or their integrated subunit leads to increased levels of intracellular Ca^{2++} in neurons. The increased level shows the activation of the protein kinase which phosphorylates the tau proteins and results in the formation of paired helical filaments seen in neurofibrillary tangles. Deposition of αβ-proteins and hyper phosphorylated tau result in plaque formation and neurofibrillary tangles. This ultimately results in death of nerve cells throughout the brain resulting in ultimate loss of functions. In AD brain cortex region, which is the centre of planning, thinking and remembering, generally gets shriveled (19). Hippocampus in brain then gets affected as it is the region playing a key role in storing information of new memories (13). It is also seen that there are three inhibitors of amyloid precursor protein, based on their cleavage site on APP. Following three are the categories of these inhibitors.

Beta Secretase inhibitors: Blocks the first cleavage of APP outside the cell.
Gamma Secretase inhibitor: Blocks second cleavage of APP in the cell membrane and stops the subsequent action of alpha-beta and its toxic fragments.
Cholinesterase inhibitors: The first FDA-approved Alzheimer's medications.

Donepezil is approved for treatment of all stages of AD. Rivastigmine and Galantamine are approved for the treatment of mild to moderate AD. These 3 cholinesterase inhibitors are commonly used for the treatment (9, 11, 14). Now pondering about treatment of this disease, we find that deficiency of acetylcholine and other neurotransmitters can also substitute as pharmacologic treatment of disease, which generally seeks to correct the histopathology of biochemical disarrangements. Prevention of breakdown of acetylcholine in the synapse is done by the cholinesterase inhibitors. Tacrine, the first cholinesterase inhibitor which is associated with hepatic toxicity can be consumed three times a day. Donepezil which should be used just once a day has side effects including nausea, vomiting, and diarrhea. Such drugs and their action mechanisms are summarized below in Table 2. Vitamin-E, estrogen, anti-inflammatory drugs, seligiline, and nicotine are the drugs which can act as neurotransmitter enhancers (6, 10, 11).

Table 2: Drugs and their general mechanism of action

Drug	**Mechanism of action**
TACRINE (cognex)	Cholinesterase Inhibitor
DONEPEZIL(aricept)	Cholinesterase Inhibitor
EPASTIGMINE	Cholinesterase Inhibitor
PHYCOSTIGMINE	Cholinesterase Inhibitor
XANOMELINE	Cholinergic Agonist

Rivastigmine, Metrifonate, Heptylphysostigmine, Memantine (20, 21), Reminyl, Donepezil are some of the drugs employed in treating AD. Especially Rivastigmine, Memantine, Donepezil drugs are generally considered because they inhibit the activity of APP cholinesterase inhibitors, FDA approved medications (12, 14, 18).

Materials & Methods

The target's sequence i.e. APP (Accession No. P56817) was retrieved from NCBI database (23) and suitable template pdb 2HIZ was retrieved from PSI-BLAST. They both were then aligned using CLUSTALW with alignment score of 2778 (24-26). Homology modeling was done through Modeller8v2 and resulted conformation was assessed using SWISS PDB (Spdb) VIEWER (27, 28). Resultant structure was validated by Structural Analysis Verification Server (29). The active sites of modeled structure were than determined by CASTp server through which erroneous amino acids were traced and their coordinates were then refined by AMBER Energy Minimization (30, 31). This resultant native state APP conformation is displayed in Figure 1. Then the docking analysis was carried out through HEX Docking Software (32).

Docking Methodology

Basic aim of molecular docking is to evaluate binding of geometrics of a putative ligand with target protein structure. Binding geometrics include positioning of ligand relative to the receptor and conformational states of ligand and receptor. Docking scheme has to strike a balance between achieving robustness, accuracy and also keeping the limited computational demands. Ideally available degree of freedom to ligand-protein system needs exploration. Conformations with minimum interaction energy have to be identified by the grid based molecular affinity potentials.

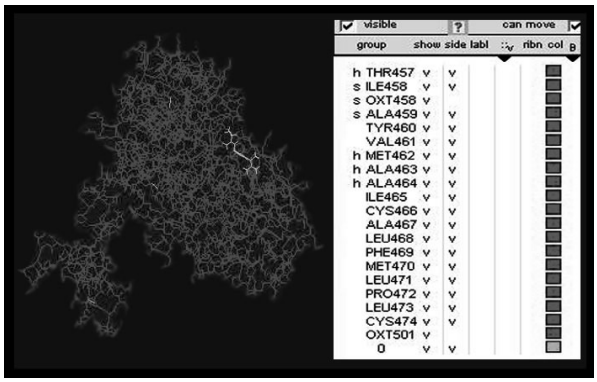


Figure 1: Best Predicted Native State Decoy of APP Protein Model in SPdb Viewer.

Here the substrate is considered as flexible while the protein is rigid. These methods search available databases for matches to an active site. The procedure is also known as virtual screening (16).

Results and Discussion

Active site is a region which is usually present in the receptor molecule, having the unique ability to bind a drug molecule effectively. Binding of the target molecule to the active site of receptor prevents binding of unwanted molecules which if bound would prevent action or metabolic functions of the receptor. Actively responsible residues in the active side of APP are highlighted below in Figure 2. The protein–ligand interactions can then be obtained as a result of docking thereby resulting in a docked structure in which the drug binds only to the active site of the receptor (15, 17).

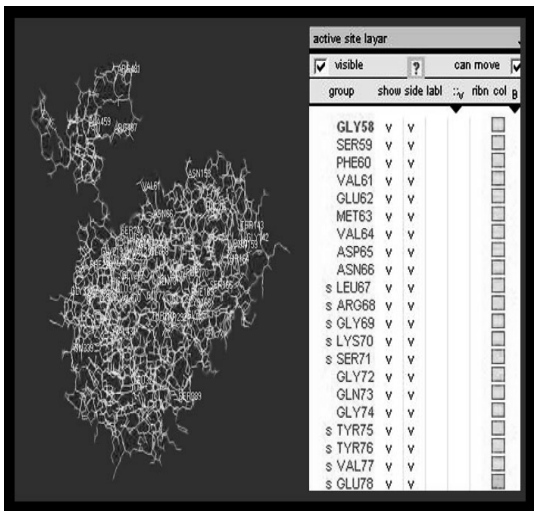
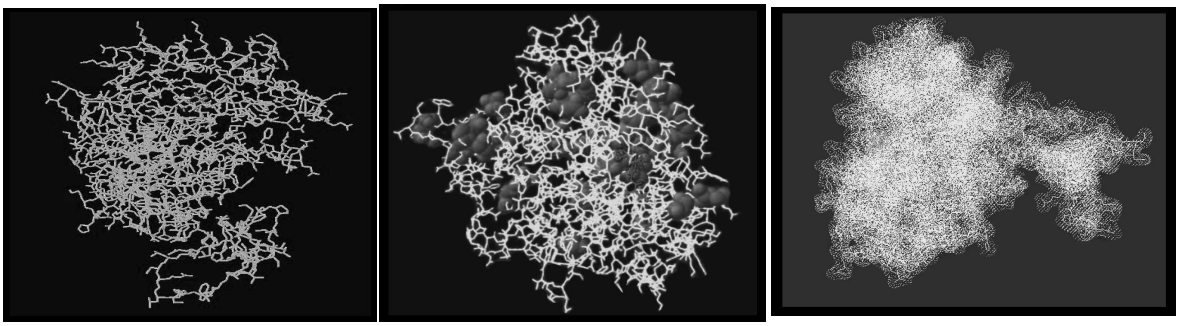


Fig.2 Residues involved in active side of APP shown in SWISSPdb viewer are highlighted here along with their respective loci on the Protein Structure.

The docking result of APP through Memantine, Donepezil and Rivastigmine drugs is displayed in docking snapshots as Figure 3.



(a) **(b)** **(c)**

Figure 3: Docking result of APP with a. Memantine, (shown as red pixels on displayed green APP protein) b. Donepezil (shown as Blue dots on yellow APP protein, showing that it binds at many sites) c. Rivastigmine (which is displayed as light red patch on APP).

Tabulation of bond lengths

After superimposition of template and target protein, integrated protein model coordinate file was obtained for the analysis for hot spots, i.e. most problematic amino acids were selected for the energy minimization step for generating overall stability of amino acids. Drugs selected for calculating bond energies are tabulated below in Table 3 and hence amino acids with lower E value were selected for docking between target and template.

Docking distance of Protein and Ligand was measured in A°s in HEX. For experimental drugs, it can be 5 A° maximum. Drugs which bind within this limit show that they are much stable and have effective binding. From docking results, it can be inferred that certain amino acids are responsible for activity of protein. Specificity and binding efficiency of drug can then be screened further. It was observed that Memantine and Donepezil bind at 4.13A° range and also they have lesser side effects when compared to others. These results are summarized in table 3. Thus, drug with the lowest possible interaction distance with the target protein or indirectly better binding affinity with the target protein should be taken for further research on drug development for AD.

Table 3: Drugs selected for calculation of Bond Energies between target protein and drug molecule. (a. MEMANTINE, b. RIVASTIGMINE, c. REMINYL, d. DONEPEZIL, e. METRIFONATE, f. HEPTYLPHYSOSTIGMINE)

Amino Acids	**Distance in A°**	**Amino Acids**	**Distance in A°**	**Amino Acids**	**Distance in A°**
1. His421 a	4.28 a	1. His421 b	8.28 b	1. Ser 42 c	8.34 c
2. Leu61	6.98	2. Phe123	7.98	2. ILe 325	7.86
3. Phe50	4.13	3. Phe23	5.13	3. Gly 62	5.13
4. Ile66	8.29	4. Val408	9.29	4. Phe 24	6.29
5. Leu26	4.36	5. Gly95	12.36	5. Leu 13	11.36
1. Phe 421 d	4.34 d	1. His 41 e	8.44 e	1. Leu 401 f	7.04 f
2. Ser345	7.66	2. Ser 25	7.66	2. ILe 225	6.66
3. Phe56	4.13	3. Phe 846	9.30	3. Gly 84	9.88
4. Leu34	5.29	4. Leu 470	8.99	4. His 40	5.99
5. Gly213	16.36	5. Gly 215	6.36	5. Phe 201	9.96

Conclusion

This work was carried out with the aim of developing suitable drug candidates for AD. The drug candidate chosen for testing here are analogues of commercially marketed drugs like RIVASTIGMINE, METRIFONATE, HEPTYLPHYSOSTIGMINE, REMINYL, MEMANTINE, DONEPEZIL. Docking target protein with these drugs infers that certain specific amino acids are responsible for activity of protein. Specificity and efficiency of drug molecule are thus determined. Results indicate that the drug with better binding affinity can be taken for further research. MEMANTINE and DONEPEZIL are found to have active sites as they had similar docking results against their target protein. Their average binding distance was found to be 4.13A° with the target protein binding site, while current experimental drugs have a binding distance of almost 5A°. This reduction in docking distance indicates better affinity of drug for the target protein, which further suggests that these experimental drugs could also be good drug candidates for the disease.

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