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Original article

Use of ligand-based pharmacophore modeling and docking approach to find novel acetylcholinesterase inhibitors for treating Alzheimer's

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ABSTRACT

Alzheimer's disease is a neurological disorder in which the patient suffers from memory loss and impaired cognitive abilities. Though the main cause of the disease is not yet known, depletion of neurotransmitter at synaptic junctions, accumulation of insoluble beta amyloid plaques and neurofibrillary tangles are the main pathologies associated with it. The FDA approved drugs for alzheimer's belong to the category of acetylcholinesterase inhibitors. But most of the drugs have been observed to be associated with adverse side effects. In this study, we have developed a pharmacophore (responsible for interaction with acetylcholinesterase active site) based on the already existing drugs and drug candidates. This pharmacophore was used to search for novel AChE inhibitors with altogether different chemical scaffold using high throughput virtual screening and docking studies. Finally, we have reported two compounds, OPA and OMT, which possess high affinity for catalytic site of AChE enzyme and thus, can be considered as potential AChE inhibitors for the symptomatic treatment of Alzheimer's.

1. Introduction

The term "dementia" is used for a variety of neurological diseases with irreversible loss of neurons leading to impaired functioning of brain. It results in loss of memory and decline in cognitive abilities. There is an estimate that about 36 million people worldwide were living with dementia in 2010. This number is likely to increase up to 66 million by 2030 and is expected to reach around 115 million by 2050 [1]. Alzheimer's disease (AD) is the most common cause of dementia accounting alone for 60–80% of the total cases. It is a neurodegenerative disorder which progresses gradually making the patient incapable of performing even the very basic bodily functions, like walking and swallowing. One in every eighth American is suffering from AD, making it the sixth leading cause of death in United States [2].

The main cause behind AD is not yet known. But some abnormal brain pathologies, like overexpression of acetylcholinesterase enzyme leading to depletion of neurotransmitter at the synaptic junctions [3], extracellular accumulation of insoluble beta amyloidal plaques [4] and intracellular neurofibrillary tangles due to hyperphosphorylated tau protein [5] are the trademark of this disease. So far, most of the FDA approved drugs for the treatment of AD belong to the category of acetylcholinesterase inhibitors, which include tacrine [6], donepezil [7], galantamine [8] and rivastigmine [9].

Many different studies associate the deteriorated cognitive functions with lowering amount of acetylcholine (ACh) in AD patients [10]. ACh released into the synapse from one neuron binds to its receptor located on the postsynaptic membrane to transmit the signal to the next neuron. A serine protease, called acetylcholinesterase (AChE) is found at neuromuscular junctions and cholinergic brain synapses, which hydrolyze this neurotransmitter and yield choline and acetyl group and terminate the relaying signal [11,12]. The activity of this enzyme increases in AD patients, making it a promising target to be inhibited for symptomatic treatment of the disease.

Acetylcholinesterase is a 614 amino acid long protein found in muscles and nerves of vertebrates, including humans [13]. The hydrophobic active site of AChE is located at the bottom of a 20 Å deep gorge approximately in the centre of the molecule. It can be divided into several subsites, like catalytic traid (Ser203, His447, Glu334), oxyanion hole (Gly121, Gly122, Ala204), anionic subsite (Trp86, Tyr133, Glu202, Gly448, Ile451), acyl binding pocket (Trp236, Phe295, Phe297, Phe338), peripheral anionic subsite (Asp74, Tyr124, Ser125, Trp286, Tyr337, Tyr341) and other residues of the disulphide-linked loop (Cys69-Cys96) forming the omega group (Thr83, Asn87, Pro88) [14].

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Much progress has been made in the recent years for the generation of AChE inhibitors (ChEI). Physostigmine was the first ChEI investigated for the treatment of AD. Although it was able to pass through the blood-brain barrier (BBB), it had a short half-life with a narrow therapeutic index. Many side effects, such as nausea, vomiting, headaches, diarrhoea, and dizziness were also associated with its use. Hence, the drug was not approved for AD use [15]. The widespread use of first FDA approved AD drug, tacrine was also abandoned because of its adverse effects, like nausea, vomiting, dizziness, diarrhoea, seizures and syncope. It also had a difficult day dosing regimen and required periodic blood monitoring due to hepatotoxicity [16]. Metrifonate was another ChEI investigated for AD treatment. Although low risk side effects were observed with its short-term use, long-term admisitration caused respiratory paralysis and neuromuscular transmission dysfunction. Therefore, FDA submission was halted and the clinical trials were discontinued during phase III [17]. Similarly many of the proposed inhibitors are usually found associated with drug related or drug-induced toxicity during preclinical and clinical trials. Here the need arises for a fast and reliable technique to screen a huge number of chemical products available to explore their potential therapeutic activities to cut down on time and cost involved in *in vivo* identification of drugs.

Virtual screening is a computational approach to drug discovery that successfully complements high throughput screening (HTS) for hit detection [18]. Pharmacophore modeling is a powerful technique, which can be used to find novel ligands for a target receptor. A pharmacophore is defined as the 3D arrangement of features possessed by ligand molecule, which are important for its interaction with a target receptor in a specific binding site. When a set of active ligands is available, it is possible to compute their shared pharmacophore. The potency of pharmacophore-based virtual screening compared to other ligand-based screening approaches lies in its ability to detect a diverse range of putative active compounds with totally different chemical scaffolds. This increases the likelihood of some of the detected compounds to pass all the stages of drug development. The study presented here is an attempt to use ligand-based pharmacophore modeling combined with high throughput virtual screening and extra precision docking protocols to find natural products that could act as acetylcholinesterase inhibitors and could be used for the treatment of Alzheimer's disease.

2. Materials and methods

2.1. Preparation of ligand dataset for pharmacophore modeling

Three dimensional structures of sixteen different AChE inhibitors were retrieved from PubChem: physostigmine (CID_5983), phenserine (CID_192706), cycloartenol (CID_92110), cardanol (CID_11266523), tacrine (CID_1935), tolserine (CID_9928397), *p*-hydroxybenzoic acid (CID_135), ladostigil (CID_208907), donepezil (CID_ 3152), vaniloloside (CID_44577222), rivastigmine (CID_ 77991), huperzine A (CID_5912039), uleine (CID_252320), galanthamine (CID_ 9651), huperzine B (CID_5462442), galangin (CID_5281616). Pharmacophore modelling was carried out using Phase module of Schrodinger. The ligands were prepared using Prepare Ligands step of the Develop Pharmacophore Model panel in maestro. The preparation steps involved conversion of structures from 2D to 3D, addition of hydrogen atoms and counter ions, removal of crystal water molecules, generation of stereoisomers followed by energy minimization. A conformational search was run for the cleaned up ligands to generate a set of conformers for each ligand.

2.2. Development of pharmacophore model

Once the ligands were cleaned and conformations were generated, a set of pharmacophore features was used to create pharmacophore sites (site points) for all the ligands. It was performed using the Create Sites step of the Develop Pharmacophore Model panel. Phase supplies a built-in set of six pharmacophore features: hydrogen bond acceptor (A), hydrogen bond donor (D), hydrophobic group (H), negatively charged group (N), positively charged group (P), aromatic ring (R).

Now using the Find Common Pharmacophore feature, pharmacophores from all conformations of the ligands were examined. All pharmacophores containing identical sets of features with very similar spatial arrangements were then clustered together. If a given group was found to contain at least one pharmacophore from each ligand, it gave rise to a common pharmacophore, explaining how ligands bind to the receptor. These common pharmacophores were examined by applying a scoring procedure, which ranked all the hypotheses and identified the pharmacophore that yielded the best alignment of the chosen ligands. The highest scoring hypothesis was then used to search for matches in a dataset of known natural drug-like compounds.

2.3. Preparation of a phase database from a dataset of drug-like natural compounds

A large data set consisting of natural chemical compounds was downloaded from ZINC database (Irwin, 2005). The database was created and structures were added to it using Generate Phase Database panel. The natural compounds were converted to all-atom structures with reasonable 3D geometries. A whole set of conformations was generated for each compound with addition of site points to the structures for a given set of pharmacophore features.

2.4. Database screening using the selected pharmacophore hypothesis

The phase database prepared was screened for structures that match the hypothesis of the model. The search process consisted of two steps: finding and fetching. Firstly, the database was searched for 3D arrangement of pharmacophoric sites with similar site types and intersite distances in comparison to the selected hypothesis. After finding such a structure, its information was written to a match file. In the fetch step, the most relevant conformers, called hits, were retrieved from the database with the help of the match file and were then aligned to the hypothesis. All the hits above a certain fitness score were fetched and analyzed further.

2.5. Interaction analysis of the hits generated using in-silico docking studies

The hits generated were further investigated for their binding affinities and mode of interaction with human AChE using extra precision (XP) docking protocol of Glide, Schrodinger.

The crystal structure of AChE of human origin was downloaded for Protein Data Bank [PDB ID: 1B41] [19]. Crystal water molecules and all non-bonded heteroatoms, including the docked ligand were removed from the protein structure using Accelerys Viewerlite 5.0 [20]. The protein was prepared for docking studies using Schrodinger's protein preparation wizard [21]. Hydrogen bonds were added and optimized to the structure. Other preparation steps involved removal of bad contacts, optimization of bond lengths, creation of disulfide bonds, capping of protein terminals and conversion of selenomethionine to methionine.

A grid was generated at the active site of the prepared protein structure using the Glide docking module of Schrodinger [22,23]. The matches found in the database of natural compounds were then virtually screened against the prepared protein at desired grid coordinates using Glide model's XP docking protocols. The two top scoring compounds were then inspected for hydrogen