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In-silico and *in-vitro* evaluation of human acetylcholinesterase inhibition by organophosphates

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ABSTRACT

Organophosphates (OP) inhibit the acetylcholinesterase (AChE) activity and devastate the nervous system of pest however its mode of action is ubiquitous and acts similarly on human AChE (*h*AChE). Screening of OP was carried out by molecular docking with *h*AChE using Glide docking module of Schrodinger suite as the structural information of *h*AChE and OP together as co-crystal structure is rarely available. The docking was done at three different precision levels, high throughput virtual screening (HTVS), standard precision and extra precision. The ranking was done using over all binding energy i.e. dock score and molecular modelling generalized born surface area (MM-GBSA).

Investigation reported Tryptophan (Trp86) residue involved in most interactions by forming a π-cation interaction apart from Ser203 on anionic subsite of *h*AChE. The top rank ligand was Phoxim ethyl phosphonate (PEP) interacting with Trp86, Gly121 and Ser203. However contact with Gly121 was lost during simulation and Asp74 appeared and sustained. Molecular dynamic simulation (GROMACS 4.5.5) of *h*AChE-PEP complex for 4×10^4 pico-second with SPC16 water system at 310 K temperature explained the evident role of Trp86 in stabilizing the ligand at P-site of the enzyme. Asp74 and Tyr124 were noticed in conveying H-bonds. Trp86 has shown consistent and better stability of bond based on distance between residues and ligand. The top ranked OP i.e. PEP was used to establish a dose response relationship between OP and *h*AChE. PEP inhibits half of the enzyme activity at concertation of 29.99 μM (calculated by sigmoid plot) at $R^2 = 0.996$ and $P < 0.0001$.

1. Introduction

Acetylcholinesterase (AChE, EC 3.1.1.7) is a serine hydrolase enzyme mainly available at neuromuscular junctions and cholinergic brain synapses. Its biological role in the body is to terminate cholinergic impulse transmission by hydrolyzing the neurotransmitter acetylcholine (ACh) to acetate and choline (Manavalan et al., 1985; Gabriel and Soreq, 2006). The enzyme is monomer and has 12 stranded central mixed β sheets surrounded by 14 α helices. Human AChE (*h*AChE) has an ellipsoidal shape and the most remarkable feature of the structure is a deep and narrow gorge of nearly 20 Å long penetrating halfway into the enzyme which open out widely nearer to its base. The base is lined with aromatic amino acids which compose various subsites (Manavalan et al., 1985). The hydrophobic patch contains the choline binding site and another hydrophobic site the acyl pocket.

Since the nature of cholinergic neurotransmission in the animals is ubiquitous, AChE is the target of numerous pesticides, including organophosphates (OP) and carbamate insecticides. OP is esters or thiols derived from phosphoric, phosphonic, phosphinic or phosphor- amidic acid of broad class insecticides (Comroe et al., 1946; Rider et al.,1951).

AChE enzyme and its inhibitors are target to many X-ray crystallographic and molecular modelling studies. The X-ray crystal structures of AChE from different species have been documented in the protein data bank *e.g.* 1EVE (Kryger et al., 1999) however *h*AChE with OP as co-crystal structure is scarce. AChE generally accepts many substrates such as OP, aryl esters, selenoesters, carbamates, analogues of acetylcholine and numerous other substrate families (Quinn, 1987). Inhibition of AChE leads to accumulation of ACh at the synapses causing cholinergic hyper stimulation and neurotoxicity followed by loss of metabolic balance which may lead to death in absence of any effective prophylaxis (Haddad and Winchester, 1983). The OP exerts its main toxicological effects through non-reversible phosphorylation of esterase in the central nervous system. OP is substrate analogues to ACh and they enter into the active site of AChE like its natural substrate binding covalently to −OH group of Ser203. During acetylation, the OP splits and the enzyme is phosphorylated, phosphate radicals of OP bind covalently to the active sites of the cholinesterase, transforming them into enzymatically inert proteins (Namba et al., 1971; Worek and

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Aurbek, 2007).

Persistency of OP is less than that of organochlorine. They have shorter half-lives and reported to undergo degradation in sunlight and UV light (Jindal et al., 2000, 2007). However natural products are being used nowadays as substituents of OP to combat persistency of synthetic and chemical pesticides (Chauhan et al., 2016, 2018; Ranjan et al., 2016a,b).

Phoxim is an OP used to control parasite in the animals. Phoxim and its analogues have been studied for its structure activity relationships and reported LD_{50} in house fly and mouse as 5.9 mg/kg of body weight and 70 mg/kg of body weight respectively (Fukuto and Sims, 1971). In-silico studies on Phoxim ethyl phosphonate (here after PEP) have reported its lowest binding energy because of presence of multiple H-bond donor atoms. Molecular dynamic simulations have reported its interaction with Ser203 and Trp86 residues on *h*AChE. Ser203 is one of the key residue in phosphorylation/inactivation of AChE whereas Trp86 helps guard the inhibitor on the bottle neck on the binding gorge (Ranjan et al., 2015, 2016a,b, 2017).

AChE is highly catalytic in nature and its catalytic range is surpassed by astonishing catalysis rate of ACh hydrolysis (kcat = $10^4\,s^{-1}$) which is responsible for a 10^{13} fold increase over spontaneous ester hydrolysis (Malany et al., 2000). OP induced AChE inhibition can be express by the following equation:

$$E + PX \underset{k2}{\overset{k1}{\rightleftharpoons}} E^*PX \xrightarrow{k3} EP + X \quad (1)$$

Where, E is enzyme, PX is Organophosphate compound (P = phosphate group and X = leaving group on OP), E*PX is enzyme-OP complex in reversible state and EP is phosphorylated enzyme. k1 and k2 are the rates of reaction for first phase of the reaction which is reversible and k3 is rate of reaction which decides the conversion of the reversible enzyme-inhibitor complex to phosphorylated enzyme

Irreversible inhibition takes place in two steps. First step of the reaction is faster which inactivates the enzyme. This reaction is reversible and its effect is prevailing in the beginning of the inhibition. The second step is slower and irreversible inhibition which phosphorylate the enzyme and yields a very stable enzyme-inhibitor complex in which inhibitor (OP) is covalently bonded to the enzyme (Knežević-Jugović, 2008).

This reaction is time dependent irreversible and can be expressed as:

$$\ln\frac{E}{Eo} = -\frac{k_{3t}}{1 + K_I I(I)} \quad (2)$$

In above equation, $\frac{E}{Eo}$ is residual enzyme activity associated to initial enzyme activity (control) (Eo), KI is dissociation constant for enzyme-inhibitor complex (E*PX), k_1 is rate constant for formation of enzyme-inhibitor complex, k_3–the first-rate constant for the conversion of the reversible enzyme-inhibitor complex to phosphorylated enzyme and t–time interval after the enzyme and inhibitor mixing.

AChE is the one of best target of pharmaceutical interest in toxicological point of view. Its inhibition is one of the most successful strategies in the reinforcement of the cholinergic transmission. Most of our knowledge about *h*AChE inhibition is based on the model organism as the enzyme source; because of limited number of crystal structure of *h*AChE with OP.

This research has studied the simulated binding OP with *h*AChE and binding pattern of top docked OP. Molecular modelling generalized born surface area (MM-GBSA) score was used to assess the activity of bound OP. The top ranked OP (based on dock score) was used to establish a dose response relationship with *h*AChE.

2. Materials and method

The 3D model of *h*AChE (PDB ID 1B41) structure was retrieved from protein data bank (PDB) and OP were retrieved from PubChem database. The *h*AChE model obtained was derived by X-ray diffraction which has resolution of 2.76 Å.

2.1. Screening of OP and molecular dynamics studies

2.1.1. Preparation of protein target structure

The *h*AChE (PDB: 1B41), target protein which was complexed with a snake venom toxin fasciculin-II and other three ligands (resolution 2.76 Å) was obtained from the protein data bank in .pdb format. Structure was recovered from the *h*AChE model by removing crystal associated hetero atoms, ligands and water molecules. Structure was imported to Schrodinger suit, Maestro v9.5. Protein Preparation Wizard (Sastry et al., 2013) tool was used to optimize the structure. It comprised biological unit and assigned bond orders, zero order bonds to metals, formed disulfide bonds, deleted water molecules beyond 5 Å from hetero groups, generated metal binding states, added missing hydrogens, completed any missing side chains and loops and Protein preparation wizard. Protein preparation wizard has a refine function, which helped in optimization of H-bond network to fix the overlapping hydrogens. pH range was established to 7.0 and the structure was minimized by applying OPLS 2005 force field (Jorgensen and Tirado-Rives, 1988). Restrained minimization was used until the average root mean square deviation (RMSD) of the non-hydrogen atoms converged to 0.3 Å

2.1.2. Receptor grid preparation

Experimental facts about the active site of AChE points out the important residues such as Ser203, Glu334, and His447. Some of the previous work on *h*AChE suggest that the active site consists of 29 residues (Kryger et al., 2000; Zheng et al., 2009) including the triad of Ser203, Glu334, and His447, which act as charge relay system. Binding site is composed of two different pockets connected by a narrow gorge which is lined with 14 conserved aromatic amino acids. Residues on binding site are: Gln71-Tyr-Val-Asp-Thr-Leu76, Gly82-Thr-Glu84, Trp86- Asn-Pro88, Leu130, Tyr133, Glu202-Ser-Ala204, Trp286, Phe295, Phe297, Glu334, Tyr337-Phe338, Tyr341, Trp439, His447-Gly-Tyr449, and Ile451. Grid generation was performed using OPLS_2005.

2.1.3. Preparation of ligands

Structural files of commonly used OP were obtained from PubChem library (https://pubchem.ncbi.nlm.nih.gov/) (Kim et al., 2016). Over 200 different variant structures were used for screening. Prior to screening, preparation of OP structure was done using LigPrep module (Schrödinger Release, 2014) of Schrodinger suite (Schrödinger Release, 2014). Ligands were imported into Schrodinger workspace and each structure was neutralized, checked for any metal binding states, desalted, generated tautomers and 32 stereoisomers per ligand was allowed. Keeping in view of the flexibility of the rings present in each ligand and their possibility to change conformations during docking calculations, we have specified to generate one low energy ring conformation per ligand. Finally, each ligand was energetically minimized using OPLS 2005 force field.

2.1.4. Virtual screening and docking

The virtual screening was performed using Virtual Screening Workflow (VSW) module of the Schrödinger Suite 2014. This workflow includes LigPrep for ligand preparation, QikProp (QikProp, version 4.0) to filter out ligands based on properties, and Glide docking (Friesner et al., 2004) at the three precision levels, HTVS, standard precision (SP), and extra precision (XP). HTVS and SP modes were used for a large set of ligands and XP docking is more accurate than the above two methods. After ensuring that the protein and ligands were in the correct form for docking, the receptor-grid was used for this research is referred from the publication (Zheng et al., 2009), which includes Ser203, Glu334, and His447 forming a catalytic triad. Glide generates