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Research Article

INVESTIGATION OF POLYPHARMACOLOGICAL TARGETS OF CHOLINESTERASE INHIBITOR DRUG DONEPEZIL: A STRUCTURE BASED SYSTEMS BIOLOGY APPROACH

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ABSTRACT

Drug design and discovery is a process that may involve unintended drug-target interactions resulting in costly drug failures. Drug molecules interacting with multiple targets can lead to various side-effects, a phenomenon called adverse polypharmacology. In the present study, an *in silico* structure based systems biology approach was undertaken to identify off-targets of Donepezil, a drug that acts as a cholinesterase inhibitor (ChEI) which is prescribed for the treatment of Alzheimer's disease (AD). Donepezil is reported to cause side-effects that majorly affect gastrointestinal tissues resulting in gastrointestinal haemorrhage, bleeding, peptic ulcers and gastritis. Molecular off-targets of Donepezil were identified using Patch Search, which uses quasi-clique detection method based on local sequence similarity approach. The off-targets present in *Homo sapiens* having a good RMSD and high coverage value were docked with Donepezil using AutoDock. Two off-targets proteins namely N-alpha-acetyltransferase 60 (NAT) and Heparansulfate n-deacetylase/n-sulfotransferase were identified that have been previously reported to be involved in causing various gastrointestinal abnormalities and also being associated with *H. pylori*. *H.pylori* is a gram negative bacterium that has been reported to be associated with non-gastric diseases such as AD. Therefore, the result of the present study indicates that NAT and Heparan Sulfate n-deacetylase/n-sulfotransferase are probable off-targets of ChEI drug Donepezil prescribed for AD and the interactions of the off-targets with *H. pylori* may aggravate the aetiology of the side-effects of AD treatment with Donepezil.

Keywords: CCl_4, Polypharmacology, Alzheimer's disease, Donepezil, Acetylcholinesesterase, N-alpha-acetyltransferase 60 (NAT), Heparan Sulfate n-deacetylase/n-sulfotransferase, Molecular docking

1. INTRODUCTION

In recent years drug discovery and development are emerging to be more efficient due to development of applications based on computational methods. These computational approaches are anticipated to delimitate and converge the processes of chemical synthesis and biological testing of drugs along with reducing the total effective cost of the drug discovery process by diminishing conventional resource requirements. Conventional drug discovery approaches use 'one-drug-one-gene' model [1] which has shifted to a new paradigm called polypharmacology *i.e.* 'one drug and its multitudinous targets.' Polypharmacology has been defined as the propensity of small molecular fragments to interact with numerous targets. Polypharmacology is further divided into two categories: adverse poly-pharmacology which includes off-target binding and drug repurposing *i.e.* therapeutic polypharmacology [2].

Drugs that have successfully paved their way into the market with promising effects may also have to be withdrawn due to unforeseen side-effects. The two primary factors incumbent for drug failures are off-target binding and the lack of systems-level understanding of their mechanism of action. In some cases, an effect on an involuntary target might suggest new uses for an existing drug to deal with surrogate conditions. Additionally a drug which acts on multiple targets, simultaneously can be utilized for treatment of different diseases and can be safer than single-target drugs [3].

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease based on old-age related ailment which tends to worsen over time and is becoming a major health concern world over. It is a progressive disease, whose symptoms of dementia gradually worsen over a period of time ranging from mild memory loss in early stages, it gradually progresses to late-stage Alzheimer's where individuals start to lose the ability to carry on a conversation often failing to respond

to their environment. At present, there is no cure for AD, but current Alzheimer's treatment is reported to slow its progression [4].

New commercially available drugs for AD treatment include Cholinesterase inhibitors (ChEIs) are namely Donepezil, Galantamine and Rivastigmine which are known to be the first line of pharmacotherapy for the treatment of Alzheimer's disease [5]. Donepezil, a piperdine derivative, is commonly marketed under the trade name of Aricept. It is a centrally acting reversible acetylcholinesterase inhibitor and during AD therapeutic regimes it is reported to increase cortical acetylcholine [6]. Individuals with AD have been low levels of acetylcholine (ACh) which may be due to plaques that increase the activity of a chemical called acetyl-cholinesterase, which is involved in breaking down ACh. High activity of acetylcholinesterase has the overall effect of decreasing ACh levels, which contributes to the characteristic symptoms of AD [7]. Donepezil obviates the symptoms of AD by preventing hydrolysis of ACh, which in turn leads to an increased concentration of acetylcholine at cholinergic synapses [8].

Cholinesterase inhibitors are prescribed for the therapy for dementia associated with AD, in an endeavour to enhance the quantity of central nervous system acetyl-choline but have been reported to be associated with side-effects of nausea, diarrhea and weight loss [9]. Kok *et al.* (2015) have reported that ChEI, Donepezil increases gastric acid secretion causing stomach ulcers or induce elevated lesions in the upper gastrointestinal tract resulting in gastrointestinal bleeding (GIB) [10]. Thus, the objective of present study was to identify off-targets of Donepezil to account for the observed side-effects, using a systems structure biology approach.

2. MATERIAL AND METHODS

2.1. Mining of target protein of Donepezil (Aricept)

Recombinant human Acetylcholinesterase in complex with Donepezil (PDB ID: 4EY7 and ligand ID: E20) was searched using Drug and Drug target mapping tool of the Protein Data Bank (PDB) (https://www.rcsb.org/pdb/ligand/drugMping.do). Information about Donepezil such as its mode of action, metabolism, weight, SMILES, absorption *etc* were retrieved from DrugBank (https://www.drugbank.ca/) [11].

2.2. Identification of potential off-targets of Donepezil

Proteins with similar patches to PDB ID: 4EY7 were searched using PatchSearch tool (https://bioserv.rpbs.univ-paris-diderot.fr/services/PatchSearch/)with protein chain A to which the ligand Donepezil (ID: E20) binds. PatchSearch is based on the principle of Quasi-Clique detection outlook which is an efficient approach to recognize specific patches and it computes a similarity score between two binding sites to accurately align patches locally on a whole off-target surface [12].

2.3. Docking studies of drug Donepezil with its probable off-targets

Protein-ligand docking of probable off-target proteins was performed with using Autodock tool (version 4.2.6) (http://autodock.scripps.edu/) that uses the Genetic Algorithm (GA) based on Lamarckian approach and scoring function based on empirical free energy [13].

3. RESULTS AND DISCUSSION

The 2-D interactions of Donepezil (ligand ID: E20) bound with human Acetylcholinesterase were retrieved from PDB (ID: 4EY7). The off-targets of Donepezil were retrieved using online web portal PatchSearch with input given as Acetylcholinesterase protein chain A bound to Donepezil. A total 8308 protein hits were obtained ranked according to their chain, protein name, organism, coverage score, RMSD score and affinity. Out of these proteins, 3498 were present in *Homo sapiens* from which 295 proteins having low RMSD value (ideally should be less than 1.5 Angstrom or preferably less than 1 Angstrom [13]) and default coverage of 10 was shortlisted (Table 1).

Protein-ligand dockings were performed for top fifty shortlisted probable off-targets of *Homo sapiens* with Donepezil *i.e.* ligand ID: E20 1-benzyl-4-[(5,6-dimethoxy-1-indanon-2-yl)methyl] piperidine) using Autodock [14] are tabulated in Table 2. Binding energy (B.E), gives the energy value which expresses likeness of the ligand with which it binds the target protein. More negative binding energy corresponds to more likeness *i.e.* higher will be the affinity of the ligand/drug to bind with the receptor protein. The Inhibition constant [K_i] of a drug is the concentration required to produce half maximum inhibition on binding the receptor and final value is the total energy sum of changes of all energetic terms which are included in scoring function of ligand and protein upon binding, plus the changes upon binding of the entropic terms [15].

N-alpha-acetyltransferase 60 (NAT) coded by gene *NAA60*, is manifested by enteric bacteria such as *Klebsiellapneumoniae, Salmonella group B, E. coli* and

Helicobacter pylori (*H. pylori*). Meyer (1996) has reported the role of NAT in drug metabolism [23]. It is therefore hypothesized that during Donepezil treatment regimes of AD, NAT activity might be increased for metabolizing Donepezil. But due to similarity in the binding sites of Acetylcholinesterase and NAT the drug binds to NAT leading to gastrointestinal abnormalities. Contaldi *et al.* (2017) and Chung *et al.* (1998) have reported that *H. pylori* is associated with non-gastric diseases: AD, Parkinson's disease, atherosclerosis, and cardiovascular ischemia in addition to human stomach where it causes peptic ulcers [18, 24].

Table 1: Off-targets of Donepezil for *Homo sapiens* retrieved from Patch Search

PDB Id	Protein	RMSD (Å)	Score	Affinity
5HGZ	N-alpha-acetyltransferase 60	0.25	0.699	-1.72
4KTY	Coagulation factor XIII A chain	0.29	0.704	14.06
2I3Y	Epididymal secretory glutathione	0.46	0.618	-1.44
4V06	Tryptophan 5-hydroxylase 2	0.47	0.664	-0.89
1FVR	Tyrosine-protein kinase TIE-2	0.47	0.611	-1.27
5D5U	Heat shock factor protein 1	0.47	0.638	-1.86
4COS	Protein kinase C-binding protein 1	0.49	0.674	-1.22
2Q8R	CCL14	0.52	0.615	-1.91
3L9J	TNFALPHA	0.52	0.564	-0.54
2X5Y	Zinc finger CCCH-type antiviral	0.53	0.665	-0.39
2RKB	Serine dehydratase-like	0.53	0.625	-1.32
4IP8	Serum Amyloid A-1 protein	0.59	0.449	-1.20
2FG5	RAS-Related protein RAB-31	0.59	0.589	-1.36
5D3X	Phosphatidylinositol 3,4,5-trisphos	0.61	0.607	-1.78
1NF1	Protein (neurofibromin)	0.61	0.627	-1.88
4ZYO	Acyl-COA desaturase	0.62	0.614	-1.60
1XSL	DNA polymerase lambda	0.63	0.481	-1.65
1JL0	S-adenosylmethionine decarboxylase	0.63	0.451	-1.83
1I4D	Arfaptin 2	0.64	0.554	-1.78
3GF9	Intersectin 2	0.64	0.622	-1.40
1TNR	Tumor necrosis factor beta	0.64	0.563	-1.75
5EMO	KH domain-containing, RNA-binding	0.65	0.569	-1.94
2CUP	Skeletal muscle LIM-protein 1	0.65	0.373	-1.35
4ZDP	O-phosphoseryl-tRNA(SEC) selenium	0.65	0.599	-1.18
2L0T	Ubiquitin	0.65	0.541	-1.77
4Z54	Neuronal-specific septin-3	0.65	0.396	-1.94
2LSO	Histone H1X	0.68	0.579	-1.88
2B5I	Interleukin-2	0.69	0.584	-1.88
1NST	Heparan sulfate N-deacetylase/N-SUL	0.69	0.609	-1.80
1HRZ	Human SRY	0.71	0.564	-1.33
4NUA	Farnesyl pyrophosphate SYNTHASE	0.71	0.681	-1.82
2V70	Farnesyl pyrophosphate synthase	0.72	0.425	-1.49
2DLS	RHO guanine nucleotide exchange	0.72	0.321	-1.82
5DUI	Forkhead box protein O1	0.73	0.454	-1.82
4E9E	Methyl-CPG-binding domain protein 4	0.73	0.524	-1.68

Heparansulfate n-deacetylase/n-sulfotransferase, a protein coded by *NDST* gene is involved in the synthesis of Heparan sulfate which is a highly sulfated linear poly-saccharide ubiquitously present on the cell surface and in the extracellular matrix [25, 26]. Vega *et al.* (2014) reported that of *H. pylori's* outer-membrane proteins has the ability to recognise and adhere to HS of gastric mucosal protective lining which is the root cause of gastritis and ulcers [26].

The other top ranked off-targets obtained from Patch Search were not further considered as off-targets on account of poor docking energies and high inhibition constants even though some of them were found to be

responsible to cause reported side-effects caused by *H. pylori*.

Therefore, in the present study, we have been able to identify two off-targets proteins of AD drug Donepezil namely NAT and HS. Donepezil itself has been previously responsible to be associated with gastro-intestinal bleeding [16, 17] while in AD patients role of *H. pylori* in GI bleeding has also been reported [27-29]. It is therefore hypothesised that on Donepezil treatment it binds to off-targets HS and NAT leading to gastro-intestinal abnormalities which may also be aggravated due to activity of *H. pylori* following Donepezil exposure. Based on the results of the present study, it is postulated that NAT, Heparansulfate n-deacetylase/n-sulfo-transferase and AD may have a common link through gram negative bacteria *H. pylori* during AD treatment with Donepezil accounting for the reported side-effects of gastro-intestinal bleeding and other GI abnormalities due to off-target binding of Donepezil.

Table 2: Docking score of probable protein off-targets of Donepezil obtained from AutoDock.

PDB ID	Protein	Binding energy	Inhibition constant	Final value
4EY7	Human AchE	-11.10	4.97 nM	-14.142
5HGZ	N-alpha-acetyltransferase 60	-10.6	15.06 nM	-13.48
1NST	Heparansulfate n-deacetylase/n-sul	-9.97	15.06 nM	-13.477
2FG5	Ras-related protein rab-31	-9.71	49.16 nM	-12.699
5D3X	Phosphatidylinositol 3,4,5-trisphos	-9.71	2.45 uM	-12.699
2RKB	Serine dehydratase-like	-9.32	141.53 nM	-12.172
1HRZ	Human sry	-9.10	146.22 nM	-12.087
1JL0	S-adenosylmethionine decarboxylase	-8.60	212.40 nM	-11.842
4V06	Tryptophan 5-hydroxylase 2	-8.75	496.20 nM	-11.785
4Z54	Neuronal-specific septin-3	-8.64	386.10 nM	-11.777
4NUA	Farnesyl pyrophosphate synthase	-9.00	462.73 nM	-11.616
4IP8	Serum amyloid a-1 protein	-8.76	312.36 nM	-11.584
4KTY	Coagulation factor xiii a chain	-8.87	312.3 nM	-11.58
2DLS	Rho guanine nucleotide exchange	-7.95	974.90 nM	-11.104
4ZDP	O-phosphoseryl-trna(sec) selenium	-8.20	1.48 uM	-11.035
2Q8R	Ccl14	-8.07	967.90 nM	-11.031
3L9J	Tnfalpha	-7.66	2.38 uM	-10.784
2CUP	Skeletal muscle lim-protein 1	-7.75	2.45 uM	-10.747
4COS	Protein kinase c-binding protein 1	-7.85	2.09 uM	-10.742
3GF9	Intersectin 2	-7.62	1.77 uM	-10.687
4E9E	Methyl-cpg-binding domain protein 4	-7.80	2.62 uM	-10.661
2LSO	Histone h1x	-7.34	2.45 uM	-10.423
2X5Y	Zinc finger ccch-type antiviral	-7.35	4.16 uM	-10.183
2V70	Farnesyl pyrophosphate synthase	-7.25	6.46 uM	-10.146
1XSL	Dna polymerase lambda	-7.38	4.85 uM	-10.065
1NF1	Protein (neurofibromin)	-7.00	3.90 uM	-10.019
1FVR	Tyrosine-protein kinase tie-2	-6.93	7.45 uM	-9.909
1TNR	Tumor necrosis factor beta	-6.78	8.42 uM	-9.761
5DUI	Forkhead box protein o1	-6.62	22.66 uM	-9.510
5D5U	Heat shock factor protein 1	-6.42	13.99 uM	-9.354
1I4D	Arfaptin 2	-6.41	20.06 uM	-9.275
2B5I	Interleukin-2	-6.42	20.06 uM	-9.275
2L0T	Ubiquitin	-6.42	19.79 uM	-9.119
2I3Y	Epididymal secretory glutathione	-4.06	1.05 mM	-6.88

Binding energy in Kcal/mol. Green: The primary target of Donepezil (4EY7), Yellow: Off-targets with reported side effects involving Helicobacter pylori, Pink: Polypharmacological targets showing the side effects of gastrointestinal problems leading to stomach cancers caused by H. pylori.

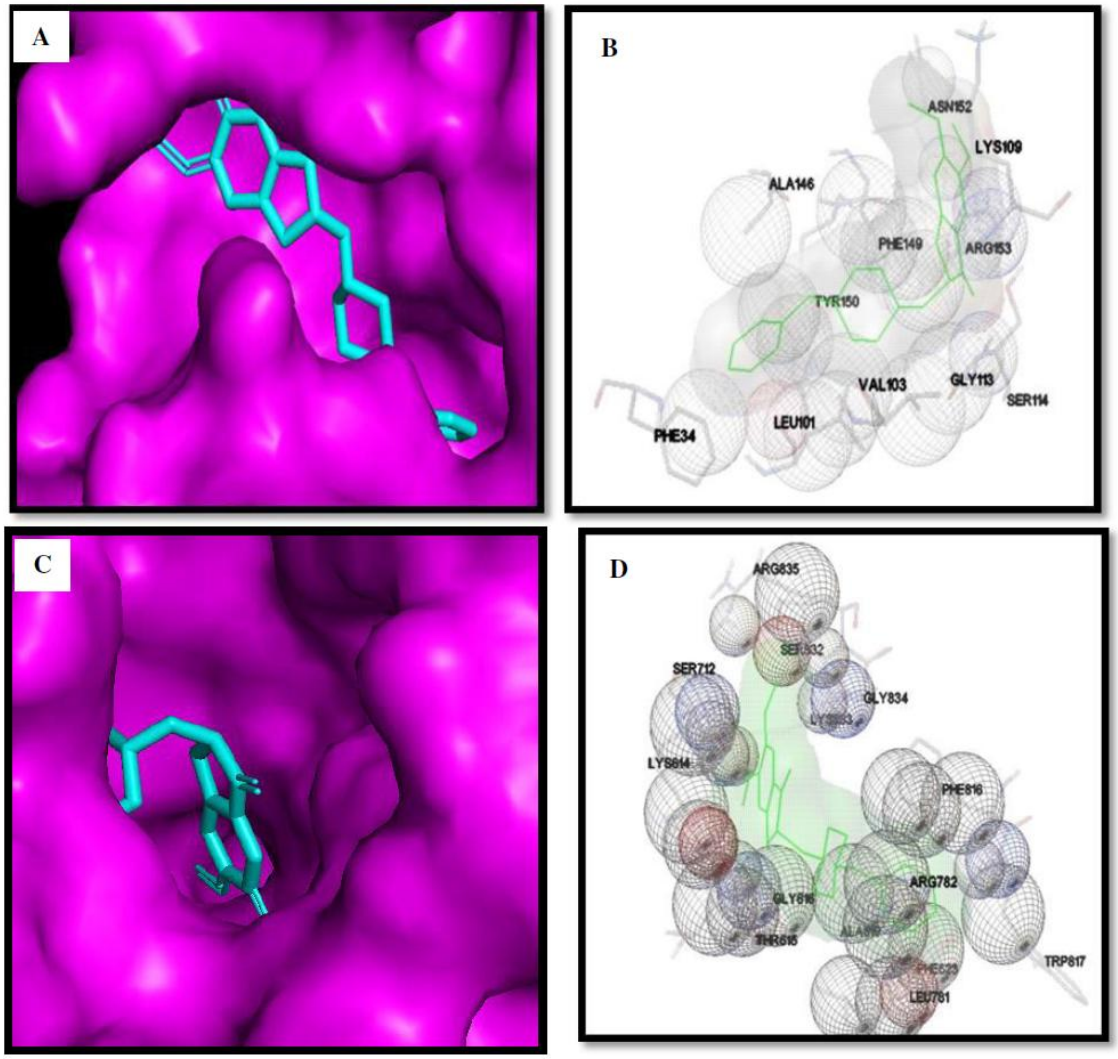

Fig.1: Docking pose and binding pocket residues of Donepezil (Ligand ID E20) with N-alpha-acetyltransferase 60 (NAT) (PDB ID: 5HGZ) (A & B) and Heparansulfate n-deacetylase/n-sulfotransferase(PDB ID: 1NST) (Figure C & D) visualized using AutoDock and PyMol

4. CONCLUSION

In the present study, N-alpha-acetyltransferase 60 (NAT) and Heparan Sulfate n-deacetylase/n-sulfotransferase have been identified as two off-targets of Donepezil, a drug prescribed for AD treatment. Both these proteins are reported to be associated with *H. pylori* which may further result in perturbations causing pathophysiological ramifications ranging from upper gastro-intestinal haemorrhage, bleeding (UGIB), peptic ulcers and gastritis which are reported to be associated with AD treatment regimes with Donepezil.

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Conflict of Interests

The authors declare that there is no conflict of interests.

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