



COMPARATIVE 20NS MOLECULAR DYNAMIC SIMULATION STUDIES AGAINST ACETYLCHOLINESTERASE RECEPTORS FOR HINOKITOL AND IMPERATORIN AS ANTIPARKINSONIAN AGENTS

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ABSTRACT

Parkinson's disease (PD) is the second most common age-related neurodegenerative condition caused by progressive dopaminergic neuron loss in the midbrain. This dopaminergic loss, tilts the balance towards acetylcholine, which also contributes to motor symptoms. Anticholinergics block the acetylcholine signaling helping to restore this balance. Among anticholinergics, plant secondary metabolites like Imperatorin (IMP) and Hinokitiol (HIN) reported for their anti-inflammatory, antimicrobial, as well as antioxidant activity were highly considered now a day as neuroprotectants. Amantadine (AMA), an aminoadamantane, well known for its inhibitory activity on acetylcholinesterase (AChE) receptor at therapeutic concentrations was used as standard. Thus, here, the inhibitory effect of HIN and IMP against Acetylcholinesterase receptors were studied for its molecular interactions using GROMACS molecular dynamics and Autodock. The results proved better Binding energy (B.E) score of -64.2793 kJ/mol with an inhibitory constant (K_i) of 28.84 μ M for HIN, indicating ability of HIN to act even at small concentrations. Moreover, reduction in AChE levels suggested HIN as a future drug of choice for Parkinsonism.

KEY WORDS

GROMACS 4.5.5, AChE, Hinokitiol, Imperatorin, Amantadine

INTRODUCTION

Among the neurodegenerative disorders reported clinically, Parkinson's disease (PD) owes the second most common chronic neurodegenerative condition in persons beyond sixty years. Medically, the progressive loss of dopamine producing nerve cells in a region of the brain called the substantia nigra results in a condition known as PD which is characterized by tremor, rigidity and bradykinesia affecting daily activities of mankind. Parkinsonism may even involve indicators including problems with sleep, mood as well as memory. With a worldwide prevalence of 30% [1], many older PD patients with more severe extrapyramidal signs also

suffer from cognitive impairment and dementia [2]. Furthermore, dementia can develop within 12 years of PD occurrence suggesting that it is a relatively common feature of the disease [Butter et al., 2008]. Approximately in 10 years of PD appearance, the patients are much prone to dementia [3]. Parkinson's disease dementia (PDD) usually expresses attention deficit, memory loss, and constriction of blood vessels [4]. In addition, they may have behavioral impairments like hallucinations, depression, apathy and anxiety [5,6,7,8]. The visual hallucinations occur in 48% of patients after the onset of Parkinsonian symptoms in PDD within a year [9]. At times, for PDD with a ratio of

21:3, the visual hallucinations become a solid prognosticator [10], which results mostly in Lewy body dementia. In a clinical observation, the cortical AChE, like cortical CAT activity, is decreased in Parkinsonian patients as a function of their degree of intellectual decline; a significant modification was detected between non-demented Parkinsonians and controls, between demented and non-demented patients. In the Cerebrospinal fluid, however, AChE levels in non-demented Parkinsonian patients were similar to those of controls, whereas in demented Parkinsonian patients, they were significantly higher than in non-demented patients [11].

The typical action of acetylcholinesterase (AChE) is the termination of synaptic transmitted signals at cholinergic synapses by rapidly hydrolyzing the neurotransmitter acetylcholine (ACh) [12]. In the brain striatum of humans, the purpose of DA is to excite and inhibit striatopallidal and striatonigral neurons. At the same time, this DA action is opposed by ACh from striatal cholinergic neurons [13]. The disruption of this balance between DA and ACh systems in both substantia nigra and striatum is supposed to perform a serious role in the pathogenesis of the neurotoxin-induced PD models [14].

HIN, also known as β -thujaplicin, an iron-chelating tropolone derivative found in the heartwood of cupressaceous plants [15] is known for its wide range of biological properties encompassing of antibacterial [16], antifungal [17], as well as antioxidant capacities [18]. HIN by thwarting the NF- κ B activity and PLC γ 2 and/or PKC cascades normalizes the immune cell function [19] and displays antiplatelet activity [20]. HIN even inhibits hydroxyl radical development by overpowering MAPKs and Akt activation. HIN possess a neuroprotective property against thromboembolic stroke in rats [21].

IMP, another bioactive furanocoumarin, used in traditional medicine for treatment of various diseases, including neurological diseases is found in various edible plants including *Angelica dahurica* and *Angelica archangelica* [22]. Wang [23] et al. 2013, reported that IMP by hypoxia re-oxygenation guards neuronal cells from apoptosis. A recent animal study also showed that IMP enhanced cognitive deficiency induced by scopolamine [24]. AMA, an aminoadamantane long known for its modest antiparkinsonian activity, has recently been shown to antagonize central nervous system dysfunction.

The cholinesterase inhibitors like donepezil has also been found to induce a favorable response in PD patients with dementia. This research implicates a potential role of AChE in the pathogenesis of PD, which should be further investigated. Despite such wide range of roles in signaling pathways, there is no report about the direct evidence of the neuroprotective effect of HIN on PD. However, pharmacokinetic studies have revealed that IMP is easily disseminated in the brain after oral administration [25,26], signifying its prospective for neurological disorder treatment. Likewise, recent findings specifically concluded the effect of AMA on levodopa-induced dyskinesias reduction. Furthermore, AMA may improve neuroleptic-induced tardive dyskinesias [27]. Thus, here, the inhibitory molecular interactions of HIN and IMP against Acetylcholinesterase receptors keeping AMA as standard were studied using Autodock v4.2 and GROMACS 4.5.5.

2. MATERIALS AND METHODS

2.1. Structure Generation

The three-dimensional structure of Acetylcholinesterase was obtained from Protein Data Bank (PDB id 1GQR). Co-crystallized ligand was identified and removed from the structure. The structure of the ligands were drawn using MarvinSketch [28]. The geometry optimization was performed using UCSF chimera [29] by adding Gasteiger atomic partial charges with 10,000 steps of energy minimization. The cleft where the co-crystallized ligand was bound was referred to as binding site.

2.2. Molecular docking

The docking studies was carried out using Autodock v4.2 [30]. The 3D structure of the receptor and the ligands were submitted in PDB format with default parameters essential hydrogen bonds, Kollman united atom charges and solvation parameters were added with Autodock Tools. The grid size was set to 40 x 40 x 40 xyz points with a grid spacing of 0.385 Å. The docking simulation was done using the Lamarckian genetic algorithm. The interaction analysis of the protein-ligand complexes and their amino acid positions were calculated and visualized through PyMol [31].

2.3. Molecular dynamic simulation

The simulation of the protein-ligand complexes were carried using GROMACS 4.5.5[32] with GROMOS 96 force field. The lowest energy complexes were selected

for molecular dynamics simulation. The ligand parameters were generated using PRODRG online server [33] with GROMOS force-field 43a1 framework. The protein-ligand complexes were solvated using a simple point charge water model immersed in a box with periodic boundary conditions with 0.5 nm distance from the protein to the box faces. Electrostatic energy was calculated using the particle mesh Ewald method. The cutoff distance of 1.0 nm for the calculation of the coulomb and Van der Waal's interaction, respectively was assigned. Energy minimization was done for 1,000 steps by the steepest descent method. After energy minimization, the systems were equilibrated under constant number of particles, volume, and temperature conditions for 2000 ps at 300K, followed by 2000 ps under constant number of particles, pressure, and temperature conditions. All the covalent bonds with hydrogen atoms were constrained using the Linear Constraint Solver algorithm (LINCS) [34]. The electrostatic interactions were treated using the Particle Mesh Ewald method. Finally, MD simulation was performed for 20 ns to check the stability of the complexes. The binding free energies of the complexes between ligands and Acetylcholinesterase were calculated from the snapshots of MD trajectories using the molecular mechanics Poisson Boltzmann surface area (MM/PBSA) method [35] is the widely used

method for binding free energy calculations. Using g_mmpbsa tool [36] the binding energies were calculated by taking the snapshots at an interval between 15 to 20ns MD simulation for each complex.

3. RESULTS

3.1. Autodock analysis

The AutoDock analysis proved that there are possible interactions like polar, nonpolar, Vander walls forces, pi-pi interactions as well as hydrogen bonds. As shown in Fig.1A, the H3N⁺ of AMA is able to form a hydrogen bond with serine at 78th position at the binding site of human AchE receptor, in a polar environment. In the same way HIN was forming prospective hydrogen bonds with glutamic acid at 199th position with its OH group (Fig.1B). The IMP on the other hand, formed hydrogen bonds with its oxygen in the imidazole ring with Histidine at 440th position (Fig.1C). Moreover, terminal hydroxyl groups formed hydrogen bonds with serine and glycine at 200th and 118th positions. The Binding energy (B.E) score of -64.2793 kJ/mol was found for HIN while IMP showed better score as -98.2875 kJ/mol. However, the inhibitory constant analysis of these drugs proved that the HIN with an inhibitory constant (K_i) of 28.84μM can work at the site of its action effectively even at small concentrations than IMP with a K_i of 2.92μM (Table. 1).

Table 1. Autodock v 4.2 docking scores of the drugs and its parameters.

Ligand	AutoDock 4.1 Score	estat	hb	vdw	dsolv	tors	Free energy binding (kcal/mol)	Inhibition Constant Ki (uM)
Hinokitol	-6.268	-0.3414	-0.5957	-6.7014	0.7739	0.5966	-6.19	28.84
Imperatorin	-7.6794	-0.0637	-0.9334	-9.5152	1.938	0.8949	-7.55	2.92
Amantadine	-7.4341	-1.9327	-1.1729	-5.6962	1.0693	0.2983	-7.29	4.53

3.2. Molecular Dynamic Simulation studies

The Fig.2&3 depicts the Root Mean Square Deviation (A⁰) and Energy (KJ/mol) variations for all the complexes from 1-20ns. The results disclosed that there were not asymmetrical fluctuations in the RMSD as well as Energy of the complexes throughout the 20ns simulations. The RMSD varied between 0.15 to 0.25A⁰, suggesting the negligible vibratory movements of the ligand-receptor

complexes, throughout the simulation period. The Binding Energy of the complexes also varied from -762000 to -756000 KJ/mol in 20ns, depicting the stability in energy of the complexes. The total Binding Energy for Hinokitol was found to be better (-64.2793 KJ/Mol) than Imperatorin (-98.2875 KJ/mol), when compared to positive control (Table 2).

Table 2. Binding energy scores of the selected drugs analysed using Autodock v 4.2

Energy (KJ/MOL)	Hinokitol	(+/-) dev/error	Imperatorin	((+/-) dev/error	Amantadine	(+/-) dev/error
van der Waal energy (Complex)	-128.769	+/- 1.100	-202.335	+/- 1.415	-93.366	+/- 7.743
Electrostatic energy (Complex)	-15.752	+/- 0.471	-3.026	+/- 0.916	37.122	+/- 72.774
Polar solvation energy (Complex)	46.227	+/- 0.776	41.145	+/- 0.972	12.511	+/- 79.088
SASA energy (Complex)	-10.479	+/- 0.065	-15.22	+/- 0.077	-10.812	+/- 4.717
Binding energy (Complex)	-108.791	+/- 1.244	-179.494	+/- 1.212	0	+/- 0.000
Total Binding energy (Complex)	-64.2793	+/- 0.5664	-98.2875	+/- 0.6613	-54.545	+/- 106.386

A) Amantadine, B) Hinokitol, C) Imperatorin

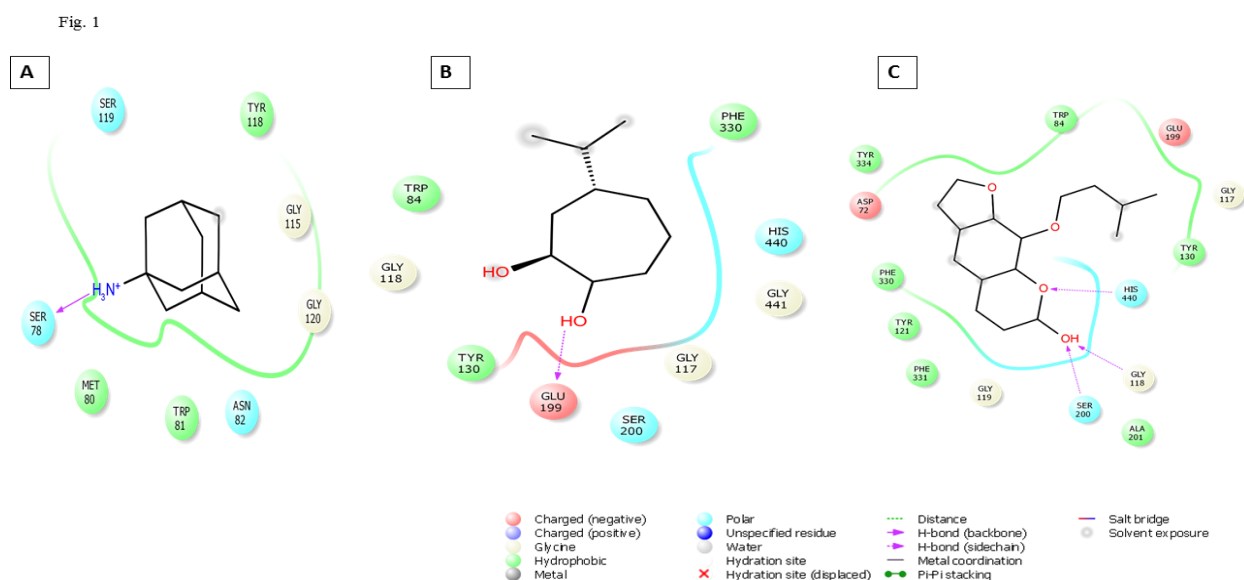
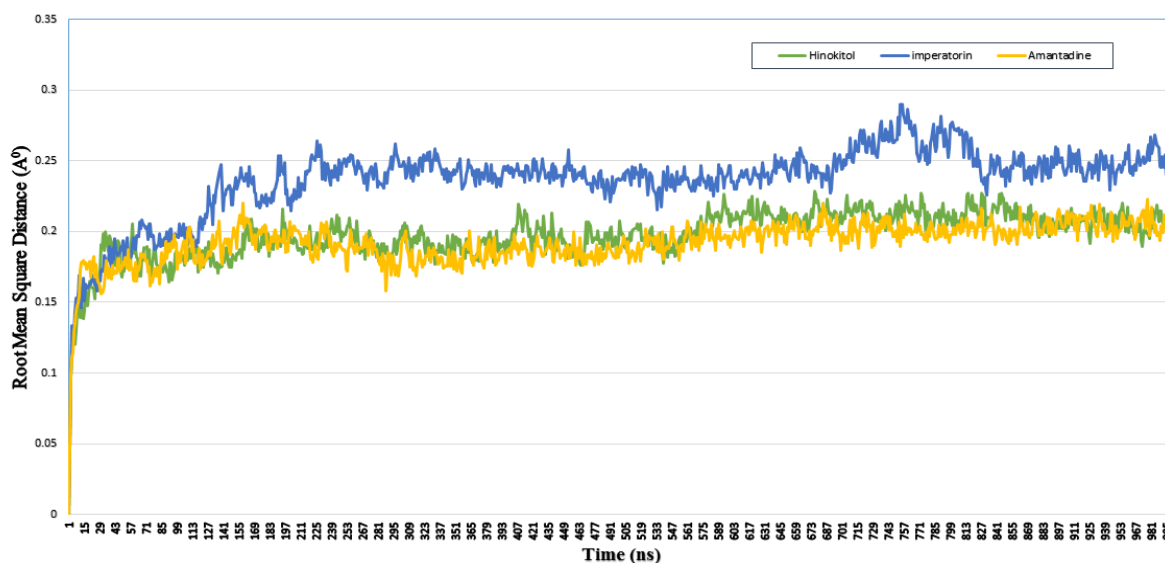
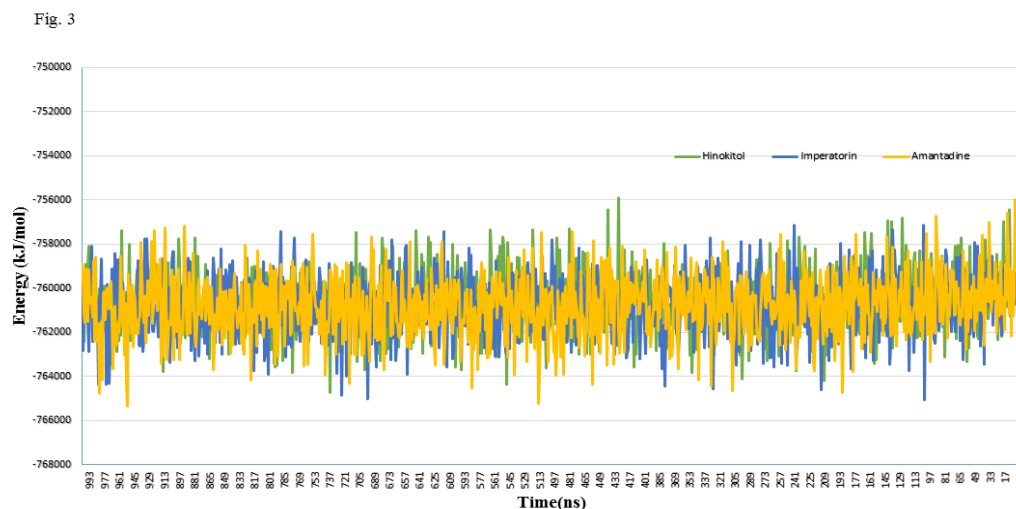
Fig. 1. Autodock interactive mechanisms of drugs with human Acetylcholinesterase receptors

Fig. 2. Root mean square deviation ($\text{\AA}$) of ligand-receptor complexes for 20 ns.


Fig. 3. Binding Energy (Kj/Mol) of ligand-receptor complexes for 20 ns.



4. DISCUSSION

The in-silico drug screening gained a great importance now a days in development of novel compounds owing to structures of molecular targets viz., enzymes or receptors providing possible candidates for supplementary medical authentication to make a time and cost effective research leading to drug discovery. Thus, there was an opportunity for developing complementary and alternative medicine widespread by prior in silico screening of novel AChE inhibitors [37]. Owing to this, the present in silico studies have revealed that the HIN has both polar and nonpolar region in its site of action like that of AMA. Moreover, the interacting hydrogen bonds errands the ability of the drug as a better choice as agonist for acetylcholine in acetylcholinesterase receptors at synaptic junction. Due to 6-OHDA toxicity, there will be a substantial decline in dopamine levels at the neuron junctions in substantia niagra [38], hence reducing the balance between dopamine and acetylcholine levels and leads to progressive lewy body formation as well as dementia. However, our molecular dynamics and Autodock studies have revealed that HIN helps in inhibition of action of AChE receptors, thus reinstating the equilibrium amongst acetylcholine and dopamine by dropping AChE and thereby increasing the dopamine levels indirectly in the substantia nigra (posterior tuberculum) of midbrain as well as striatum.

This study also becomes relevant when we consider the BBB crossing ability of IMP and HIN. Our earlier ADMET studies proposed that HIN has higher BBB crossing ability than IMP. The molecular dynamics simulation

studies have further narrow down the insights about the stability of the protein-ligand complexes at 20ns.

Abundant studies has established that AChE agonists displayed amazing neuroprotective effects through attenuation of oxidative stress and enhancement of antioxidant mechanisms. Therefore, both anti-AChE activity and antioxidative stress should be considered while designing drugs to treat neurodegenerative diseases like AD and PD. Anatomically, regions like cortical and hippocampal portions of brain are linked functionally with cholinergic mechanisms associated with cognitive functions. However, neurotransmission at circuitual, pathway as well as synaptic levels pay to ACh balance, leading to be responsible for spatial and episodic memory acquisition storage, working memory, attention, maintenance and retrieval, and other neurophysiological processes. The dorsal striatum primarily account for regulating the cognitive aspect which receives the response from the substantia nigral dopaminergic neurons for motor learning and recognition of the motor functions. Among brain structures, the striatum encompasses nearly the peak levels of ACh and DA. The two neurotransmitter systems interact both at the presynaptic and postsynaptic levels extensively in a bidirectional manner mediating the cognitive mechanisms by selecting motor reactions as well as its incentives [39] The anticholinergics especially HIN being our drug of choice may interact in a basal native concentration in the striatum in the low nanomolar range, which can shift to highers scales or fold increase upon activation of midbrain dopaminergic neurons. The regulation of striatal GABAergic circuits by

choline acetyltransferase (ChAT)-positive cholinergic interneurons had been suggested to offer strong integrative prospects based on interneurons networking participating not only in movements but also in fortification of related mechanisms. It has recently been identified that in addition to the GABAergic interneurons, $\alpha 7$ nAChRs are determined with interneurons with calcium-ATPase pump isoforms to launch critical links in postsynaptic regions [40].

Eventhough the mechanisms connecting the above considerations are quite uncertain, many incorporate attention deficits, altered motor coordination and dopamine-AChE level unbalance in both striatum and midbrain. Further research in animal models power additional clarifications in this issue and resolve contradictions. Furthermore, AChE receptors blockers might have damaging properties acting in these domains and attention should be hired in their solicitation in PD patients.

5. CONCLUSION

The study suggests that HIN can act as a neuroprotective drug by reducing the AChE levels in Substantia nigra of PD patients. The molecular dynamics study in turn proved the same by exhibiting its AChE receptor inhibition even at small concentrations at its site of action, than IMP.

6. CONFLICT OF INTEREST

The authors have no conflict of interest.

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