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Research Article

Structural Prediction and Comparative Molecular Docking Studies of Hesperidin and L-Dopa on A-Synuclein, MAO-B, COMT and UCHL-1 Inhibitors

Priya Nagappan*,Vijayalakshmi Krishnamurthy

Department of Biochemistry, Bharathi Women's College, Chennai, India

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ABSTRACT

Parkinson disease (PD) is due to oxidative stress and excitotoxicity leading to depletion of neurotransmitters like dopamine, epinephrine, nor-epinephrine, serotonin, abnormal ubiquitination and mitochondrial dysfunction. Aim and Objective: The present work deals with the *insilico* docking studies of target proteins such as α- synuclein, MAO-B and COMT, UCHL-1 inhibitors with hesperidin and L-Dopa. Methods: The *insilico* docking studies were carried out using AutoDock version 4.2. Results: The docking energy of hesperidin with α- synuclein showed binding energy -1.0 kcal/mol whereas L-Dopa showed binding energy -4.44 kcal/mol. Hesperidin with MAO-B showed binding energy -6.26 kcal/mol whereas L-Dopa showed binding energy -4.4 kcal/mol. Hesperidin showed binding energy -2.47 kcal/mol with COMT whereas L-Dopa showed binding energy -5.22 kcal/mol. Hesperidin with UCHL-1 showed binding energy -6.08 kcal/mol whereas L-Dopa showed binding energy -4.24. Conclusion: These results clearly indicate that the flavonoid hesperidin have similar binding sites and interactions with α-synuclein, MAO-B, COMT, UCHL-1 compared to the L-Dopa the standard drug.

Keywords: α- synuclein, MAO-B, COMT, UCHL-1, hesperidin, L-Dopa

INTRODUCTION

Drug design is an important tool in the field of medicinal chemistry where new compounds are synthesized by molecular (or) chemical manipulation of the lead moiety in order to produce highly active compounds with minimum steric effect[1]. New drug discovery is considered broadly in terms of two kinds of investigational activities such as exploration and exploitation[2]. Docking of small molecules in the receptor binding site and estimation of binding affinity of the complex is a vital part of structure based drug design[3]. AutoDock version 4.2 is the most recent version which has been widely used for virtual screening, due to its enhanced docking speed. Its default search function is based on Lamarckian Genetic Algorithm (LGA), a hybrid genetic algorithm with local optimization that uses a parameterized free-energy scoring function to estimate the binding energy.

Neurodegenerative disorders are a group of devasting disorders of the central nervous system, in which progressive loss of structure and function of neurons including neuronal death is observed. The age dependent neurodegenerative disease include Parkinson disease and Alzheimer disease[4], which are caused by genetic and environmental influences[5] and lead to the accumulation of protein aggregation thereby causing oxidative stress and inflammation[6]. Symptoms of PD are tremor, rigidity, akinesia, bradykinesia and postural instability[7]. The genes responsible for the cause of disease includes α-synuclein (SNCA), Parkin (PARK 2), Leucine Rich Repeat Kinase 2 (LRRK-2), PTEN-induced putative kinase 1 (PINK-1) ubiquitin carboxyl–terminal esterase L1(UCHL-1) and DJ-1 (PARK 7)[8,9].

Hesperidin, a bioflavonoid is an abundant and inexpensive by-product of citrus family. A deficiency of these substances in the diet has been linked with abnormal capillary leakiness as well as pain in the extremities causing aches, weakness and leg cramps at night. The dopamine precursor levodopa (L-Dopa) is proved to be a powerful drug for PD. To increase the action of L-Dopa and to control the catabolism of L-Dopa, the adjuvant like inhibitors of peripheral L-Amino acid Decarboxylase (AADC), COMT (or) MAO-B can be supplemented[10]. Therefore in order to overcome the side effects of synthetic drugs, flavonoids like hesperidin can be used as substituents. Therefore the present study was focused to screen hesperidin for the inhibitory activity of α- synuclein, MAO-B, COMT and UCHL-1 using molecular docking studies.

MATERIALS AND METHODS

Preparation of ligand structure

Before docking partial atomic charges are applied to each atom of the ligand. AutoDock ligands are written in files with special keywords recognized by AutoDock. The root is rigid set of atoms, while the branches are rotatable groups of atoms connected to the rigid root. The

*Author for Correspondence

TORSDOF for a ligand is the total number of torsions that only rotate hydrogen's. TORSDOF is used in calculating the change in free energy caused by the loss of torsional degrees of freedom upon binding. After, all the above conditions are set the ligand is saved in "pdbq" format.

Preparation of target protein

Availability of several experimentally determined 3D structures of α –synuclein with PDB ID: 1XQ8, MAO-B with PDB ID 2V5Z, COMT with PDB ID 3BWY, UCHL-1 with PDB ID 2ETL was taken as the target protein for the docking studies. L-Dopa provides an excellent basic for using structure-based approaches for the discovery of hesperidin, as a target protein inhibitor. The active sites of α-synuclein, MAO-B, COMT and UCHL-1 were identified using Q-site finder. The ligands were drawn using ACD chemsketch and then converted into PDB format using open babel tool. The 3D structures α- synuclein, MAO-B, COMT and UCHL-1 were docked with hesperidin and standard L-Dopa using AutoDock software. The results obtained were then analysed using Acceryls discovery studio visualizer.

Binding site prediction

The probable binding sites of preferred target protein α-synuclein, MAO-B, COMT and UCHL-1 receptors were searched using Q-site finder to predict the ligand-binding site. It works by binding hydrophobic probes to the protein, and finding clusters of probes with the most favourable binding energy. These consist of active sites on protein surfaces and voids covered in the interior of proteins. The individual probe sites relate most closely to the favoured high-affinity binding sites on the protein surface. These favourable binding sites relate to locations where a putative ligand could bind and optimize its Vander Waals interaction energy. Q-site finder includes a graphical user interface, flexible interactive visualization, as well as on the fly computation for user uploaded structures. It is important to keep the predicted ligand-binding site as small as possible without compromising accuracy for a range of applications such as molecular docking, de novo drug design and structural identification and comparison of functional sites[11].

Molecular docking using AutoDock

AutoDock version 4.2 was used for docking simulation[12-14] which employs the preparation of receptor by adding hydrogen's and assigning kollman charges followed by conversion of PDB file to pdbqt. Ligands were assigned with Gasteigerb charges and nonpolar hydrogen docking simulations were run using Lamarckian Genetic algorithm (LGA) which is known to be the most effective and reliable method of AutoDock. The obtained conformations were then summarized collected and extracted by using AutoDock tool. The protein α-synuclein, MAO-B, COMT and UCHL-1 was prepared for molecular docking by adding all hydrogen atoms using standard procedures. The binding energy and inhibitory constants were observed for each ligand protein complex.

RESULTS

The selected bioflavonoid hesperidin and the synthetic drug L-Dopa were docked in the active site of optimized and energy minimized α-synuclein, MAO-B, COMT, UCHL-1 and the results were analysed to identify natural compounds with good inhibitory activity considering the interactions binding energy and inhibitory constant. The compounds had very good interaction with active site residues and also low inhibitory constant. The interactions of the hesperidin and L-Dopa with specific receptors are shown in the figure 3-10. Similarly interactions of amino acid and H-Bonds distance and energy value of α-synuclein, MAO-B, COMT and

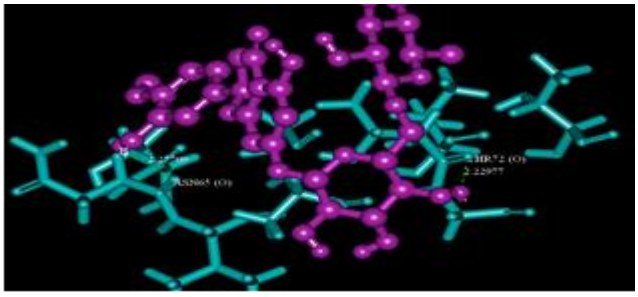

Figure 1. Interactions between α-synuclein and Hesperidin Visualized using Acceryls Discovery Studio Visualizer

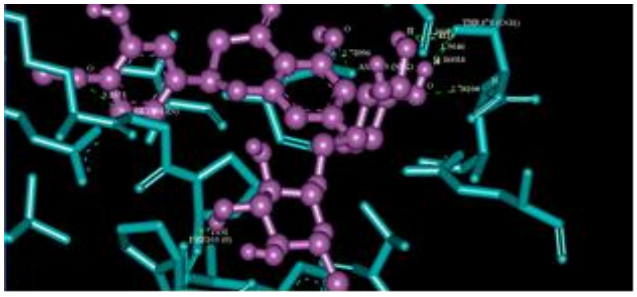

Figure 2. Interactions between MAO-B and Hesperidin Visualized using Acceryls Discovery Studio Visualizer

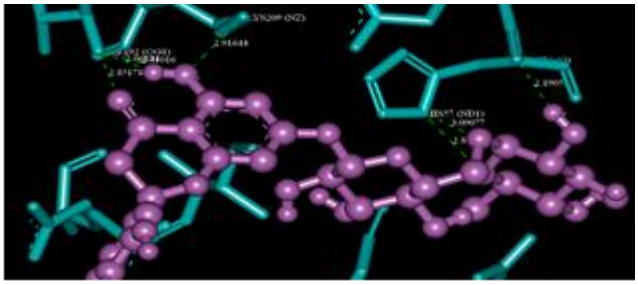

Figure 3. Interactions between COMT and Hesperidin Visualized using Acceryls Discovery Studio Visualizer

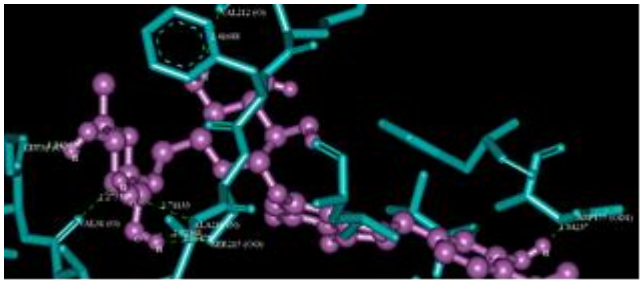

Figure 4. Interactions between UCHL-1 and Hesperidin Visualized using Acceryls Discovery Studio Visualizer

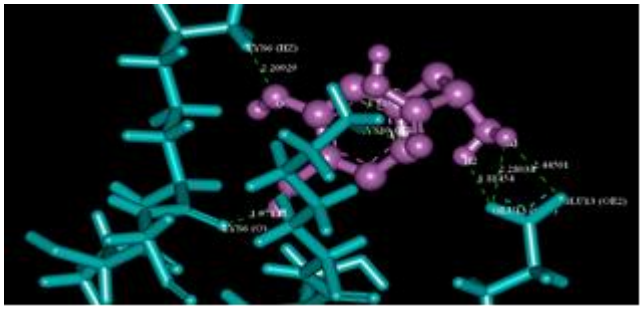

Figure 5. Visualizing Hydrogen Interactions between α-synuclein and L-DOPA Using Acceryls Discovery Studio Visualizer

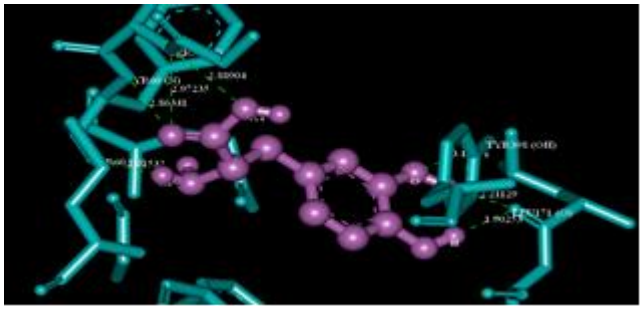

Figure 6. Visualizing Hydrogen Interactions between MAO-B and L-DOPA Using Acceryls Discovery Studio Visualizer

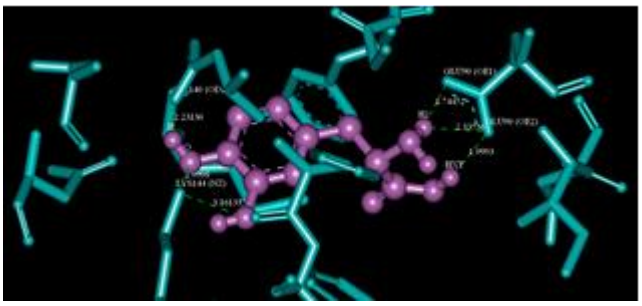

Figure 7. Visualizing Hydrogen Interactions between COMT and L-DOPA Using Acceryls Discovery Studio Visualizer

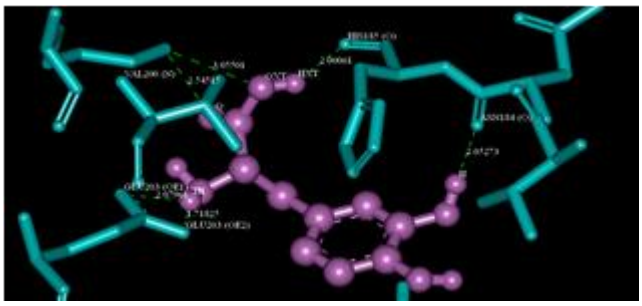

Figure 8. Visualizing Hydrogen Interactions between UCHL-1 and L-DOPA Using Acceryls Discovery Studio Visualizer

Table 1: Interaction of amino acids, H-Bonds distance and energy value of α-synuclein, with hesperidin and L-Dopa

S.No	Receptor	Ligand	Interaction of amino acids	H-bonds distance (A°)	Energy value kcal/mol
1	α –synuclein	Hesperidin	ASN 65, THR 72	2.27, 2.22	-1.0
2	α –synuclein	L-Dopa	LYS6, LYS6, LYS10, LYS10, GLU13, GLU13, GLU13	2.20, 1.97, 1.88, 1.98, 2.44, 2.28, 1.81	-4.44

Table 2: Interaction of amino acids, H-Bonds distance and energy value of MAO-B, with hesperidin and L-Dopa

S.No	Receptor	Ligand	Interaction of amino acids	H-bonds distance (A°)	Energy value kcal/mol
1	MAO-B	Hesperidin	ASN203, THR478, THR 478, THR 478, THR478, THR478, GLY101, PHE103	2.78, 2.79, 2.86, 1.96, 2.26, 2.48, 3.16, 1.71	-6.26
2	MAO-B	L-Dopa	SER59, SER59, TYR60, TYR60, TYR398, LEU171, LEU 171	2.88, 2.97, 2.86, 2.01, 3.13, 2.21, 1.90	-4.4

Table 3: Interaction of amino acids, H-Bonds distance and energy value of COMT, with hesperidin and L-Dopa

S.No	Receptor	Ligand	Interaction of amino acids	H-bonds distance (A°)	Energy value kcal/mol
1	COMT	Hesperidin	GLU56, HIS57, HIS57, LYS209, THR192, THR192, THR192	2.19, 3.09, 2.83, 2.91, 2.81, 2.06, 2.85	-2.47
2	COMT	L-Dopa	LYS144, LYS144, ASP140, GLU90, GLU90, GLU90	3.16, 2.99, 2.23, 2.33, 1.99, 1.74	-5.22

Table 4: Interaction of amino acids, H-Bonds distance and energy value of UCHL-1, with hesperidin and L-Dopa

S.No	Receptor	Ligand	Interaction of amino acids	H-bonds distance (A°)	Energy value kcal/mol
1	UCHL-1	Hesperidin	LEU32, VAL31, ALA216, SER215, SER215, VAL212, ASP155	1.84, 2.27, 2.71, 2.92, 2.06, 2.41, 1.84	-6.08
2	UCHL-1	L-Dopa	GLU203, GLU203, ASN184, HIS185, VAL200, VAL200	2.07, 1.71, 2.05, 2.00, 3.05, 2.54	-4.24

UCHL-1 with hesperidin and standard L-Dopa are shown in table 1-4.

DISCUSSION

The docked pose of α- synuclein, MAO-B, COMT and UCHL-1with hesperidin and L-Dopa is shown in figure 3-10 and this clearly demonstrated the binding positions of the ligand with the protein target. Analysis of the receptor/ligand complex models generated after successful docking of the hesperidin and L-Dopa were based on the parameters such as hydrogen bonds distance, amino acid interactions, binding energy and orientation of the docked compound with the active site. As a general rule, in most of the potent therapeutic agent, both hydrogen bond and hydrophobic interactions between the compound and the active sites of the receptor have been found to be responsible for mediating the biological activity.

As shown in Table 1 hesperidin showed binding energy -1.0 kcal/mol and standard L-dopa -4.44 kcal/mol. Moreover, in hesperidin there were only 2 interactions of amino acids namely ASN65, THR72 with hydrogen bonds distance 2.27Aº, 2.22Aº whereas in L-Dopa there were 7 amino acid interactions namely LYS6, LYS6, LYS10, LYS10, GLU13, GLU13, GLU13 with hydrogen bonds distance 2.20Aº, 1.97Aº, 1.85Aº, 1.98Aº, 2.44Aº, 2.28Aº, 1.81Aº respectively. Table 2 reveals that hesperidin showed binding energy -6.26kcal/mol and L-Dopa -4.44 kcal/mol. Hesperidin had 8 amino acid interactions namely ASN203, THR478, THR478, THR478, THR478, THR478, GLY 101, PHE103 with hydrogen bonds distance 2.78Aº, 2.79Aº, 2.86Aº, 1.96Aº, 2.26Aº, 2.48Aº, 3.16Aº, 1.71Aº where as in L-dopa there were 7 amino acid interaction interactions namely SER59, SER59, TYR60, TYR60, TYR60, TYR398, LEU 171, LEU171 with hydrogen bonds distance 2.88Aº, 2.97Aº, 2.86Aº, 2.01Aº, 3.13Aº, 2.21Aº, 1.90Aº respectively. Table 3: Shows that hesperidin showed binding energy -2.47kcal/mol and L-dopa -5.22 kcal/mol with COMT. Moreover in hesperidin there were only 7amino acid interactions namely GLU56, HIS57, HIS57, LYS209, THR192, THR192, THR192 with hydrogen bonds distance 2.19Aº, 3.09Aº, 2.83Aº, 2.91Aº, 2.81Aº, 2.06Aº, 2.85Aº respectively. Table 4 reveals that hesperidin showed binding energy -6.08kcal/mol and L-Dopa -4.24 kcal/mol. With hesperidin there were 7amino acid interactions namely LEU32, VAL31, ALA216, SER215, SER215, VAL212, ASP155 with hydrogen bonds distance 1.84Aº, 2.27Aº, 2.71Aº, 2.92Aº, 2.06Aº, 2.41Aº, 1.84Aº whereas in L-dopa there were 6 amino acid interactions namely GLU203, GLU203, ASN 184, HIS185, VAL200, VAL200 with hydrogen bonds distance 2.07Aº, 1.71Aº, 2.05Aº, 2.00Aº, 3.05Aº, 2.54Aº respectively. Hesperidin has a potent invitro antioxidant [15]. Hesperidin shows neuroprotective effect on induction with 6-OHDA induced Parkinson model[16]. Molecular docking studies of hesperidin with α-synuclein, MAO-B, COMT and UCHL-1 exhibited binding interactions and warrants further studies for the development of potent α- synuclein, MAO-B, COMT and UCHL-1, inhibitors for the treatment of Parkinson disease.

CONCLUSION

The results of the present study clearly demonstrated that hesperidin and standard L-Dopa had inhibitory activity against α- synuclein, MAO-B, COMT and UCHL-1 inhibitors for the treatment of Parkinson disease. These results clearly indicate that the flavonoids hesperidin has similar binding sites and interactions with α- synuclein, MAO-B, COMT and UCHL-1 as that of the standard L-Dopa. This *insilico* studies by hesperidin clearly showed the inhibition of α- synuclein, MAO-B, COMT and UCHL-1. Further investigations on the compound hesperidin and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of Parkinson disease. Hesperidin showed good inhibitory activity against MAO B and UCHL-1 whereas L-Dopa had better activity against α- synuclein and COMT. Further studies on animals model has to be carried out in future to justify the same.

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