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Research Article

MOLECULAR DOCKING STUDIES OF ANDROGRAPHOLIDE WITH XANTHINE OXIDASE

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ABSTRACT

OBJECTIVE: The present work deals with the *in silico* docking studies of xanthine oxidase inhibitory activity of andrographolide. METHODS: The *in silico* docking studies were carried out using AutoDock 4.2. RESULTS: The docking energy of andrographolide showed binding energy -4.57 kcal/mol which had higher binding energy when compared to the standard febuxostat (-8.72 kcal/mol). Moreover, in andrographolide there were only 4 interactions of amino acid whereas in febuxostat there were 6 amino acid interactions. CONCLUSION: Molecular docking studies of andrographolide with xanthine oxidase enzyme exhibited binding interactions and warrants further studies needed for the development of potent xanthine oxidase inhibitors for the treatment of gout. These results clearly indicate that andrographolide have binding interactions with xanthine oxidase. Further investigations on the andrographolide compound and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of hyperuricemia and gout.

Keywords: Andrographolide, Xanthine oxidase, Molecular docking.

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].

Nowadays, the use of computers to predict the binding of libraries of small molecules to known target structures is an increasingly important component of the drug discovery process. There is a wide range of software packages available for conducting molecular docking simulations like, AutoDock and DOCK, GOLD, FlexX and ICM. AutoDock 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. Each docking is comprised of multiple independent executions of LGA and a potential way to increase its performance is to parallelize the aspects for execution [4].

Xanthine oxidase (XO) is a highly versatile enzyme that is widely distributed among different species from bacteria to man and within the various tissues of mammals. It is a member of group of enzymes known as molybdenum iron – sulphur flavin hydroxylases [5]. It catalyses the oxidation of hypoxanthine to xanthine which further reduce to uric acid, the final reactions in the metabolism of purine bases [6]. The accumulation of uric acid in the body is responsible for the formation of several diseases and thus it plays a vital role in producing hyperuricemia and gout [7]. Inherited xanthine oxidase reductase (XOR) deficiency leads to xanthineuria and multiple organ failure syndrome caused by the accumulation of xanthine in different tissues [8].

Andrographolide is a diterpenoid constituents present in *AP* and it was reported to possess anti-inflammatory activity. The stereochemistry of binding of the andrographolide on xanthine oxidase has not been characterized. Therefore, the structural models of the ligands in the xanthine oxidase binding sites has also been carried out, which may facilitate further development of more potent antihyperuricemic agents.

MATERIALS AND METHODS

Preparation of ligand structures

The small-molecule topology generator Dundee PRODRG 2 server [9] is used for ligand optimization, a tool for high-throughput crystallography of protein-ligand complexes which takes input from existing coordinates or various two-dimensional formats and automatically generates coordinates and molecular topologies suitable for X-ray refinement of protein-ligand complexes.

Preparation of target protein

Availability of several experimentally determined three dimensional structures of xanthine oxidase with PDB ID of 1APX was taken as the target protein for the docking studies (Figure: 1). Febuxostat provide an excellent basic for using structure-based approaches (Figure: 2) for the discovery of andrographolide, as a xanthine oxidase inhibitor (Figure: 3).

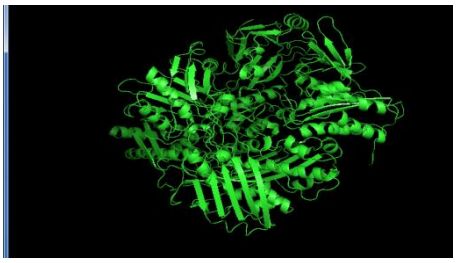

Figure 1: PDB format of Xanthine oxidase (Receptor)Ribbon like structure

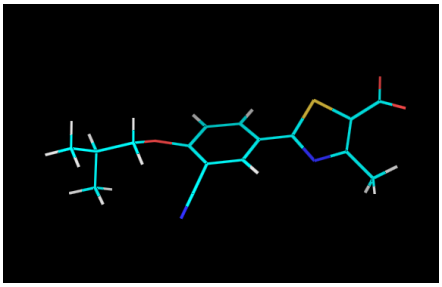

Figure 2: PDB format of febuxostat – linear chain structure

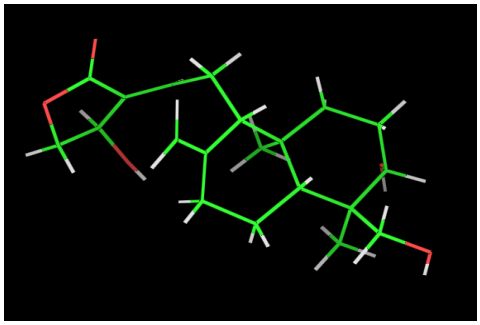

Figure 3: PDB format of andrographolide – linear chain structure

Binding site prediction

The probable binding sites of preferred target 1APX 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 favorable 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 favored high-affinity binding sites on the protein surface. These favorable 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 [10].

Protein-ligand interaction using autodock

The docking studies were conceded by Autodock tools [11, 12] (ADT) version 1.5.4 and autodock version 4.2 programs. The searching grid extended above the preferred target proteins; polar hydrogen was added to the ligand moieties. Kollman charges were assigned and atomic solvation parameters were added. Polar hydrogen charges of the Gasteiger-type were assigned and the non-polar hydrogen was merged with the carbons and the internal degrees of freedom and torsions were set. Andrographolide, a diterpenoid compound of *AP* were docked to target protein complex (XO) (1APX) with the molecule considered as a rigid body and the ligand being flexible. The search was extended over the whole receptor protein used as blind docking. Affinity maps for all the atom types present, as well as an electrostatic map, were computed with a grid spacing of 0.375 A°. The search was carried out with the Lamarckian Genetic Algorithm; populations of 150 individuals with a mutation rate of 0.02 were evolved for 10 generations. Evaluation of the results was done by sorting the different complexes with respect to the predicted binding energy. A cluster analysis based on root mean square deviation values (RMSD values), with reference to the starting geometry, was subsequently performed and the lowest energy conformation of the more populated cluster was considered as the most trustable solution.

RESULTS

The docked pose of xanthine oxidase enzyme with febuxostat as shown in Figure: 4 and andrographolide ligands as shown in Figure: 5 clearly demonstrated the binding positions of the ligand with the enzyme.

Analysis of the receptor/ligand complex models generated after successful docking of the andrographolide and febuxostat were based on the parameters such as, hydrogen bonds distance, amino acid interactions, binding energy and orientation of the docked compound within the active site. As a general rule, in most of the potent antihyperuricemic 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**, andrographolide showed binding energy -4.57 kcal/mol which had higher binding energy when compared to the standard febuxostat (-8.72 kcal/mol). Moreover, in andrographolide there were only 4 interactions of amino acid namely Gly798, Gln768, Phe799 and Ser1081 with hydrogen bonds distance 2.18 A°, 2.46 A°, 2.67 A° and 1.89 A° and RMSD value 2.01 A° whereas in febuxostat there were 6 amino acid interactions namely Gln1195, Thr1078, Gly1040, Arg913, Phe799 and Gln768 with hydrogen bonds distance 2.6 A°, 3.4 A°, 2.2 A°, 2.5 A°, 1.98 A° and 2.7 A° and RMSD value 1.216 A° respectively.

This proves that andrographolide consist of lesser xanthine oxidase inhibitory binding sites when compared to the standard febuxostat.

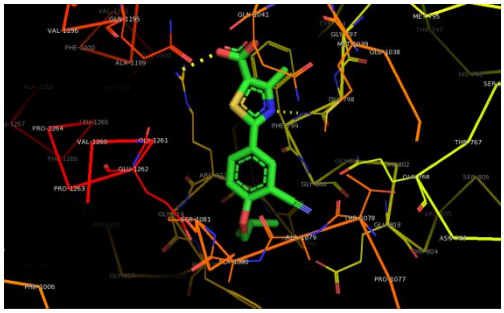

Figure 4: Febuxostat docked with xanthine oxidase

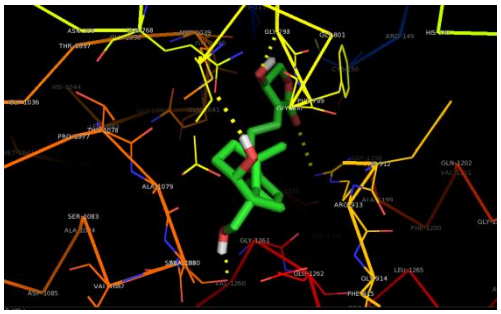

Figure 5: Andrographolide docked with xanthine oxidase

Table 1: Interaction of amino acids, H-bonds distance and energy value of XO-andrographolide and XO-febuxostat docked structure.

S.No	Receptor	Ligand	Interaction of amino acids	H-Bonds Distance (A°)	Energy value Kcal/mol
1.	Xanthine oxidase	Andrographolide	Gly798, Gln768, Phe799, Ser1081	2.18, 2.46, 2.67, 1.89	-4.57
2.	Xanthine oxidase	Febuxostat	Gln1195, Thr1078, Gly1040, Arg913, Phe799, Gln768	2.6, 3.4, 2.2, 2.5, 1.98, 2.7	-8.72

DISCUSSION

Molecular docking studies of andrographolide with xanthine oxidase enzyme exhibited binding interactions and warrants further studies needed for the development of potent xanthine oxidase inhibitors for the treatment of gout. These results clearly indicate that andrographolide have binding interactions with xanthine oxidase. Further investigations on the andrographolide compound and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of hyperuricemia and gout.

CONCLUSION

Molecular docking studies of andrographolide with xanthine oxidase enzyme exhibited binding interactions and warrants further studies needed for the development of potent xanthine oxidase inhibitors for the treatment of gout.

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REFERENCES

1. Cavasotto CN, Abagyan RA. Protein flexibility in ligand docking and virtual screening to protein kinases. J Mol Biol. 2004; 337: 209-225.
2. Apostolakis J, Pluckthun A, Caflisch A. Docking small ligands in flexible binding sites. J Comput Chem. 1998; 19: 21-37.
3. Seeliger D, Groot BL. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. J Comput Aided Mol Des. 2010; 24: 417-422.
4. Umamaheswari M, Madeswaran A, Asokkumar K, Sivashanmugam T, Subhadradevi V, Jagannath P. Discovery of potential xanthine oxidase inhibitors using in silico docking studies. Der Pharma Chemica. 2011; 3: 240-247.
5. Symons CRM, Taiwo AF, Petersen LR. Electron addition to xanthine oxidase. An electron spins resonance study of the effects of ionizing radiation. J Chem Soci. 1989; 85: 4063-4074.
6. Zarepour M, Kaspari K, Stagge S, Rethmeier R, Mendel R, Bittner F. Xanthine dehydrogenase AtXDH1 from Arabidopsis thaliana is a potent producer of superoxide anions via its NADH oxidase activity. Plant Mol Biol. 2010; 72: 301-310.
7. Egwim EC, Vunchi MA, Egwim PO. Comparison of xanthine oxidase activities in cow and goat milks. Biokemistri. 2005; 17: 1-6.
8. Borges F, Feranandes E, Roleira F. Progress towards the discovery of xanthine oxidase inhibitors. Curr Med Chem. 2002; 9: 195-217.
9. Schüttelkopf AW, van Aalten DM. PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. Acta Crystallogr D Biol Crystallogr. 2004; 60:1355-1363.
10. Laurie AT, Jackson RM. Q-SiteFinder: an energy-based method for the prediction of protein-ligand binding sites. Bioinformatics. 2005; 21: 1908-1916.
11. Sanner MF. Python: a programming language for software integration and development. J Mol Graph Model. 1999; 17: 57-61.
12. Thangaraj Sindhu, Sundaraj Rajamanikandan, Dhanapal Durgapriya, Jebamala Raj Anitha, Selvaraj Akila, Velliyur Kanniyapan Gopalakrishnan. Molecular dockingand qsar studies on plant derived bioactive compounds as potent inhibitors of dek oncoprotein. Asian J Pharm Clin Res. 2011; 4 (2): 67-71.