ORIGINAL ARTICLE

In silico-guided Molecular Docking Analysis of Doxorubicin with Low-density Lipoprotein

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Abstract

Introduction: Brain tumor (BT), the most aggressive and difficult-to-treat malignancy, is the major cause of death in cancer patients. BT poses serious health concerns mainly because of their fast development and poor prognosis. BT includes a group of heterogeneous diseases, with unique biology that corresponds to the brain and its microenvironment. The brain contains many cell types distinct from those found elsewhere in the body, making it difficult to extrapolate the findings from brain cancers compared to other forms of cancer. Moreover, the anatomy of the brain presents challenges for treating both BTs and brain metastases. The brain is the hub of many proteins in which some of the proteins act as receptors for natural and synthetic ligands. Doxorubicin (DOX) is one of the most commonly used anticancerous drugs with high efficacy. **Materials and Methods:** *In silico* analysis provides key insights in designing effective drug delivery to the brain. Here, the molecular modeling package Schrödinger software was used to establish the specific interaction between DOX and low-density lipoprotein (LDL). **Results and Discussion:** DOX has good binding interactions (docking scores −8.526, −6.565, −6.667, and −7.040, respectively) with LDL (PDB ID: IN7D and 3M0C). The docking study of DOX found potent activity against BT with docking scores of −8.526, −6.565, −6.667, and −7.040. **Conclusion:** The present study may help medicinal scientists to formulate potent formulation against LDL receptor for BT targeting.

Key words: Brain tumor, Doxorubicin, Low-density lipoprotein receptor, Low-density lipoprotein

INTRODUCTION

In spite of the recent advances in brain research, disease related to central nervous system (CNS) remains the main cause of disability globally, accounting for prolonged hospitalization and care.[1] It constitutes a very tight microvascular system with additional properties such as non-fenestrated vessels, restricted movement of molecules and ions, and tight cellular junctions between blood and CNS.[2] The treatment of brain cancer is one of the most difficult challenges in oncology for mankind. The failure of chemotherapy is due to the inability of intravenously administered anticancer agents to reach the brain parenchyma. Brain cancers are the most formidable and difficult-to-treat disease in humans.[3] Achieving effective treatment with minimal side effects is one of the biggest challenges where blood–brain barrier (BBB) is the major problem in the treatment.[4,5] BBB works as a diffusion barrier that hampers the influx of different toxins, drugs, and molecules into the brain. Most of the drugs fail in early developmental phase due to poor BBB penetrating ability. Therefore, designing a suitable strategy for effectively deliver of anticancer agent into the brain is highly recommended and is the need of time.

Recent advancements in *in silico* and computational analysis have offered opportunities for designing and implantation of strategies for effective drug delivery to the brain.[6,7] *In silico* drug designing approaches play an important role in the identification and discovery of promising drug candidates.[8,9] It involves high-resolution screening of ligands to agonize or antagonize different protein structures.[10,11] *In silico*

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drug designing depends on the presumptions that candidate compounds have a strong affinity toward the target compound with lower side effects but possess strong absorption, distribution, and metabolism properties.[12,13]

Doxorubicin (DOX), an anthracycline drug, is one of the most commonly used chemotherapeutics with high efficacy and broad-spectrum usage.[14] Over the years, hundreds of DOX analogs have been tested for their biological properties, but only few have been approved for clinical use.[15] It intercalates and stacks between paired bases in DNA leading to the arrest of cell cycle. Occurrence of cellular resistance reduces the binding of drug to DNA using membrane efflux-transport mechanisms such as the P170 glycoprotein as well as intracellular vesicular trapping.

Low-density lipoprotein (LDL) acts as a ligand and binds to the LDLR in the brain through receptor-mediated endocytosis.[16] LDL is a substrate for steroid hormone production and is a primary transporter of cholesterol in the blood. It carries cholesteryl esters in the form of lipid protein.[17] In LDL protein, each molecule has an average mass of 3106 Da. The core of LDL contains 1500 cholesterol molecules that are connected to long-chain fatty acids. This consists of 800 phospholipids and 500 unesterified cholesterol molecules. The LDL molecule comprises 4563 amino acid residues.[18,19] It is an amphipathic molecule due to the hydrophobic core and having hydrophilic shell.[18-20]

A detailed and deeper understanding of molecular mechanism of DOX with LDL is important and significant for the effective delivery of this drug in target site. Therefore, exhaustive drug-ligand interaction screening was performed between an anticancer drug DOX (active compounds) on LDL protein using Schrödinger Software.

MATERIALS AND METHODS

Docking study using glide module of Schrödinger software

In glide (grid-based ligand docking with energetics), favorable interactions between a receptor molecule typically a protein and one or more ligand molecules are sought after. Each ligand must be a single molecule, although the receptor may include multiple molecules, such as a protein and a cofactor.[21] The docking modes for glide are stiff and flexible. For each input ligand, the flexible docking method automatically generates conformations. A ligand pose in flexible docking is the intersection of a ligand's position, orientation, and conformation with respect to the receptor. A set of hierarchical filters that evaluate the ligand's interaction with the receptor is applied to the ligand poses that glide creates. The initial filters use a grid-based approach based on the empirical ChemScore function to test the spatial fit of the ligand to the designated active site and examine

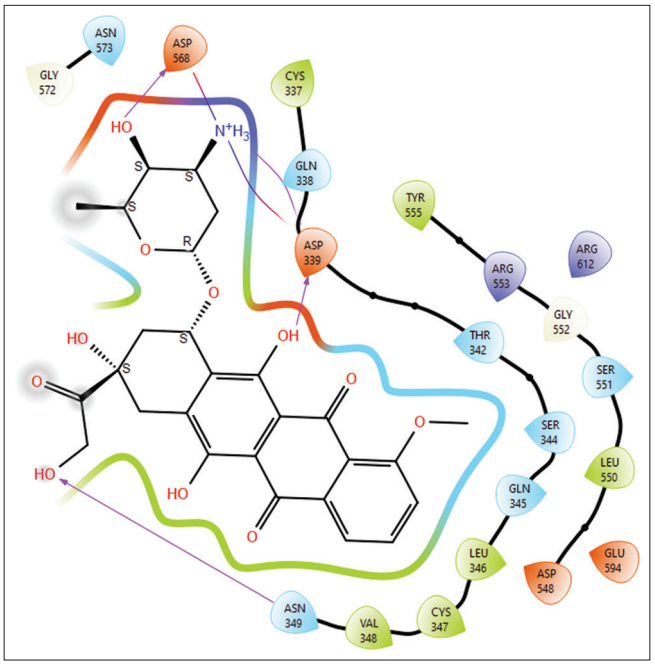

Figure 1: 3-D diagram showing binding interactions of doxorubicin with low-density lipoprotein protein (PDB ID: 1N7D)

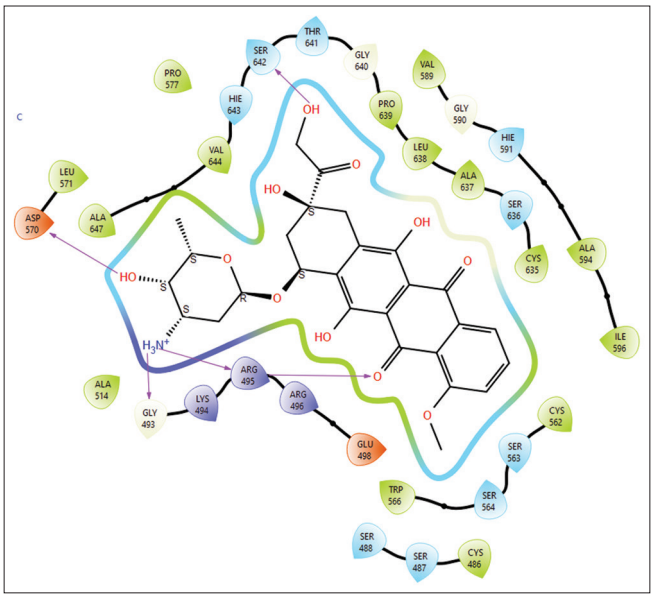

Figure 2: 3-D diagram showing binding interactions of doxorubicin with low-density lipoprotein protein (PDB ID: 3M0C)

the complementarity of ligand-receptor interactions.[22] Poses that pass these first checks move on to the algorithm's final stage, which entails evaluating and minimizing a grid approximation to the non-bonded ligand-receptor interaction energy from the OPLS 3e model. The positions with the least amount of energy are then scored at the end. The poses are scored by default using the GlideScore multiligand scoring mechanism developed by Schrödinger. A composite model score is then used to rank the poses of each ligand and to choose the poses that should be reported to the user if glide

Table 1: Docking scores of doxorubicin (active compounds) on low-density lipoprotein protein (PDB ID: 1N7D and 3M0C)

PDB ID	Docking model	Docking score
PDB ID: 1N7D	SP_1	−6.565
	SP_3	−6.667
	SP_4	−5.435
	SP_5	−3.974
PDB ID: 3M0C	SP_6	−7.040
	SP_7	−8.526
	SP_8	−7.193
	SP_9	−5.270
	SP_10	−6.559

score was used as the scoring algorithm.[23] Glide score, non-bonded interaction energy, and for flexible docking, extra internal energy of the produced ligand conformation are all combined by model.[24]

Protein preparation

The accuracy of glide results depends on the validity of the protein's initial structures. Schrödinger offers a full protein preparation facility in the Protein Preparation Wizard to ensure chemical accuracy and optimize protein structures for use with glide and other products. LigPrep by Schrödinger is a full-service ligand preparation facility, similar to that. Following protocol was implemented for protein and ligand structures using computational tools.

- Add a ligand/protein cocrystallized structure to Maestro by importing it from the PDB
- Multimeric complexes are made simpler. To prepare for glide, it is preferable to keep just one ligand-receptor subunit for computational efficiency. If the active site requires two identical chains, neither should be removed
- Choosing which waters to keep or remove. These fluids are distinguished by the presence of one oxygen atom and typically lack hydrogens. Waters that connect the ligand and the protein are occasionally maintained, but in general, all waters (apart from those coordinated to metals) are removed
- Adapt the cofactors, metal ions, and protein. Repairs should be made to structures that lack residues close to the active site
- The formal charges and ligand bond orders should be adjusted. Bonds between the ligand or a cofactor and a protein metal must be removed from complex structures
- The protein structure should be minimalized with caution. Using a user-selected RMSD tolerance, the minimization is constrained to the input protein coordinates
- Review the produced structures to ensure that water molecules are oriented correctly and that steric conflicts and H-bonding issues have been resolved.

Ligand preparation

The docked structures must accurately reflect the real ligand structures as they would look in a protein-ligand complex to produce the best results. With the use of 2D or 3D structures in the SD, Maestro, or SMILES formats, the Schrödinger ligand preparation product LigPrep can create high-quality, all-atom 3D structures for numerous drug-like compounds. The LigPrep procedure is made up of several processes that convert data, correct structures, create variants on structures, get rid of unnecessary structures, and optimize structures. Many of the steps are optional, and they can be changed using command-line arguments or choosing choices in the LigPrep panel. The steps are (1) convert structure format, (2) select structures, (3) add hydrogen atoms, (4) remove unwanted molecules, (5) neutralize charged groups, (6) generate ionization states, (7) generate tautomer, (8) filter structures, (9) generate alternative chirality, (10) generate low-energy ring conformations, (11) remove problematic structures, (12) optimize the geometries, and (13) convert output file.

Receptor grid generation

Several separate sets of fields that offer increasingly more precise scoring of the ligand poses are used to portray the shape and characteristics of the receptor on a grid. From the receptor grid generation panel, the receptor grid can be generated and set up. It is not possible to start a ligand docking job until the receptor grids have been produced.[25] A "prepared" structure, or an all-atom structure with the proper bond ordering and formal charges, is necessary for receptor grid formation. The force field utilized for grid generation is the OPLS 2005 force field, which has a wider variety of defined atom types and enables for accurate treatment of metals.

- The receptor grid generation panel: It has five tabs, which you use to specify settings for the receptor grid generation job. These are receptor, site, constraints rotatable groups, and excluded volumes.
- The receptor tab: In this tab, you define the part of the workspace system for which receptor grids should be calculated is defined you can also scale receptor atom van der Waals radii in this tab and choose whether to use partial charges from the force field or from the input structure.
- The site tab: The settings determine where the scoring grids are positioned and how they are prepared from the structure in the workspace.
- The constraints tab: It is used to define glide constraints for the receptor grids to be generated. Glide constraints are receptor-ligand interactions that you believe to be important to the binding mode, based on structural or biochemical data. Setting constraints enable glide to screen out ligands, conformations, or poses that do not meet these criteria early on in their evaluation for docking suitability.

- The rotatable groups' tab: The hydroxyl groups in residues such as Ser, Thr, and Tyr and the thiol group in Cys can adopt different orientations with different ligands. Glide can allow such groups to adopt different orientations when ligands are docked, to produce the most favorable interaction.
- The excluded volumes tab allows you to restrict ligands from filling specific areas of space in specified circumstances. For instance, you could want to prevent ligands from filling a pocket close to the active site if it is known that ligands would not bind there. The ligands will be prohibited from certain regions of space during docking using this tab to set them up.

Ligand docking

Glide ligand docking jobs require a set of previously calculated receptor grids and one or more ligand structures. If a correct Lewis structure cannot be generated for a ligand, it is skipped by the docking job. Glide also automatically skips ligands containing unparametrized elements, such as tin, or atom types not supported by the OPLS force fields, such as explicit lone pair "atoms." The ligand docking panel has several tabs: Ligands, settings, core, constraints, torsional constraints, and output. Molecular modeling studies were performed on the glide module of Schrodinger to investigate the potential interactions between most potent derivative and protein.

Validation of docking procedure

To make sure whether the docking procedure performed is correct or not, validation of the docking procedure was done by Auto Dock vina software. Before the docking of compounds in the datasets, the cocrystallized ligand present in the binding site of the protein was extracted and then redocked in the same binding site of the protein.

Docking study

Molecular docking studies of DOX were performed with receptor protein of LDL receptor (PDB ID: 1N7D and 3M0C) using glide module software (Schrodinger maestro v13.2). Protein data bank has been used for the procurement of protein structure. The protein was further processed through "protein preparation workflow" (Maestro wizard v13.2). The generating states and refinement step were used for improving the protein structure including optimization of H bonded groups, dehydration, and restrained minimization using default force field OPLS_3e. The minimized protein structure was used for the generation of grid around ligand molecule. Various docked ligand conformations were observed in docking results showing their binding energy scores. The ranking on the basis of scores was given representing high rank for lesser scoring conformation.[26,27]

RESULTS AND DISCUSSION

Nowadays, LDL is a protein that regulates interactions between cells and is one of the best ligands for targeting brain tumor (BT). Previous studies have demonstrated the potential for targeted treatment of malignant tumors, including brain cancer, but the underlying effector mechanism remains unclear. In this study, we analyzed the potential interaction between DOX and LDL for BT targeting using molecular docking. Molecular docking study was performed to examine the possible interactions between protein and potent ligands of the series using glide module of Schrodinger software. The inhibition of enzyme activity depends on the possible interactions of inhibitors with various amino acid residues of targeted protein of interest. Docking was performed for DOX to study the binding cavity of LDL receptor (PDB ID: 1N7D and 3M0C). The five-five grid for each LDL receptor (PDB ID: 1N7D and 3M0C) generate then SP docking perform on each grid generated. The H-bond is shown by purple arrows and π-π stacking interactions are shown by purple–green arrows Figures 1 and 2 . The compounds DOX showed that binding interactions with amino acid residues ASP 339, ASN 349, and ASP 568 of PDB ID: 1N7D with SP docking score values are −6.565 (DOCKING MODELSP_1) depicted in Table 1. The compound DOX showed that binding interactions with amino acid residues GLY 493, ARG 495, ASP 570, and SER 642 of PDB ID: 3M0C with SP docking score values are −8.526 (DOCKING MODELSP_7). These interactions were essential for LDL receptor inhibitory activity for BT targeting.

CONCLUSION

Molecular docking studies using Schrödinger software were performed between the DOX and the LDL receptor. Among that, LDL protein (PDB ID: 3M0CPDB ID: 1N7D) site numbers SP7 and SP3 showed significant binding interactions with the receptor; highest SP docking scores −8.526 and −6.667, respectively showed critical interactions with ASP570, GLY493, ARG49, SER642, ASP567, ASP339, and ASN349.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

REFERENCES

1. Kharya P, Jain A, Gulbake A, Shilpi S, Jain A, Hurkat P, *et al*. Phenylalanine-coupled solid lipid nanoparticles for brain tumor targeting. J Nanopart Res 2013;15:12.
2. Dixit N, Trivedi A, Ahirwar D. Polyethylene glycol-chitosan nanoparticle gel for the controlled nasal delivery of noscapine against glioma. Int J Appl Pharm 2022;14:153-61.
3. Mitusova K, Peltek OO, Karpov TE, Muslimov AR, Zyuzin MV, Timin AS. Overcoming the blood-brain barrier for the therapy of malignant brain tumor: Current status and prospects of drug delivery approaches. J Nanobiotechnol 2022;20:412.
4. Bors LA, Erdő F. Overcoming the blood-brain barrier. Challenges and tricks for CNS drug delivery. Sci Pharm 2019;87:6.
5. Li J, Zheng M, Shimoni O, Banks WA, Bush AI, Gamble JR, *et al*. Development of novel therapeutics targeting the blood-brain barrier: From barrier to carrier. Adv Sci 2021;8:2101090.
6. Parodi A, Rudzińska M, Deviatkin AA, Soond SM, Baldin AV, Zamyatnin AA Jr. Established and emerging strategies for drug delivery across the blood-brain barrier in brain cancer. Pharmaceutics 2019;11:245.
7. Sethi B, Kumar V, Mahato K, Coulter DW, Mahato RI. Recent advances in drug delivery and targeting to the brain. J Control Release 2022;350:668-87.
8. Brogi S, Ramalho TC, Kuca K, Medina-Franco JL, Valko M. *In silico* methods for drug design and discovery. Front Chem 2020;8:612.
9. Ridwan S, Erlina L, Bahtiar A, Sukmawati D. Identification and *in silico* analysis of inhibitor on the Wnt/β-catenin signaling pathway as potential drug for colon cancer. Int J Appl Pharm 2023;15:111-20.
10. Kumar S, Bhardwaj VK, Singh R, Das P, Purohit R. Identification of acridinedione scaffolds as potential inhibitor of DENV-2 C protein: An *in silico* strategy to combat dengue. J Cell Biochem 2022;123:935-46.
11. Jha NK, Kumar P. Molecular docking studies for the comparative analysis of different biomolecules to target hypoxia inducible factor-1α. Int J Appl Pharm 2017;9:83.
12. Zloh M, Kirton SB. The benefits of *in silico* modeling to identify possible small-molecule drugs and their off-target interactions. Future Med Chem 2018;10:423-32.
13. Singh R, Bhardwaj VK, Purohit R. Computational targeting of allosteric site of MEK1 by quinoline-based molecules. Cell Biochem Funct 2022;40:481-90.
14. Wander DP, Van Der Zanden SY, Vriends MB, Van Veen BC, Vlaming JG, Bruyning T, *et al*. Synthetic (N, N-dimethyl) doxorubicin glycosyl diastereomers to dissect modes of action of anthracycline anticancer drugs. J Organ Chem 2021;86:5757-70.
15. Van der Zanden SY, Qiao X, Neefjes J. New insights into the activities and toxicities of the old anticancer drug doxorubicin. FEBS J 2021;288:6095-111.
16. Seelig A. P-glycoprotein: One mechanism, many tasks and the consequences for pharmacotherapy of cancers. Front Oncol 2020;10:576559.
17. DeAngelis AM, Roy-O'Reilly M, Rodriguez-Oquendo A. Overview of the intersection of genomics of cholesterol metabolism and cardio-metabolic disease with reproductive health, especially in women. In: Translational Cardio-metabolic Genomic Medicine. United States: Academic Press; 2016. p. 251-82.
18. Wang S, Meng Y, Li C, Qian M, Huang R. Receptor-mediated drug delivery systems targeting to glioma. Nanomaterials 2015;6:3.
19. Chakraborty S, Cai Y, Tarr MA. *In vitro* oxidative foot printing provides insight into apolipoprotein B-100 structure in low-density lipoprotein. Proteomics 2014;14:2614-22.
20. Chu AC, Tsang SY, Lo EH, Fung KP. Low density lipoprotein as a targeted carrier for doxorubicin in nude mice bearing human hepatoma HepG2 cells. Life Sci 2001;70:591-601.
21. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol 2022;23:3-20.
22. Sethi A, Joshi K, Sasikala K, Alvala M. Molecular docking in modern drug discovery: Principles and recent applications. In: Drug Discovery and Development-New Advances. Vol. 2. London: Intech Open; 2019. p. 1-21.
23. Ban T, Ohue M, Akiyama Y. Multiple grid arrangement improves ligand docking with unknown binding sites: Application to the inverse docking problem. Comput Biol Chem 2018;73:139-46.
24. Guedes IA, Pereira FS, Dardenne LE. Empirical scoring functions for structure-based virtual screening: Applications, critical aspects, and challenges. Front Pharmacol 2018;9:1089.
25. Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ. Computational protein-ligand docking and virtual drug screening with the Auto Dock suite. Nat Protoc 2016;11:905-19.
26. Eldridge MD, Murray CW, Auton TR, Paolini GV, Mee RP. Empirical scoring functions: I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. J Comput Aided Mol Des 1997;11:425-45.
27. Brooijmans N, Kuntz ID. Molecular recognition and docking algorithms. Annu Rev Biophy Biomol Struct 2003;32:335-73

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