In silico Analysis for Predicting Fatty Acids of Black Cumin Oil as Inhibitors of P-Glycoprotein

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ABSTRACT

Background: Black cumin oil is obtained from the seeds of Nigella sativa L. which belongs to family Ranunculaceae. The seed oil has been reported to possess antitumor, antioxidant, antibacterial, anti-inflammatory, hypoglycemic, central nervous system depressant, antidepressant, and immunostimulatory activities. These bioactivities have been attributed to the fixed oil, volatile oil, or their components. Seed oil consisted of 15 saturated fatty acids (17%) and 17 unsaturated fatty acids (82.9%). Long chain fatty acids and medium chain fatty acids have been reported to increase oral bioavailability of peptides, antibiotics, and other important therapeutic agents. In earlier studies, permeation enhancement and bioenhancement of drugs has been done with black cumin oil. Objective: In order to recognize the mechanism of binding of fatty acids to P-glycoprotein (P-gp), linoleic acid, oleic acid, margaric acid, cis-11, 14-eicosadienoic acid, and stearic acid were selected for in silico studies, which were carried out using AutoDock 4.2, based on the Lamarckian genetic algorithm principle. Materials and Methods: Template search with BLAST and HHblits has been performed against the SWISS-MODEL template library. The target sequence was searched with BLAST against the primary amino acid sequence of P-gp from Rattus norvegicus. Results: The amount of energy needed by linoleic acid, oleic acid, eicosadienoic acid, margaric acid, and stearic acid to bind with P-gp were found to be ~10.60, ~10.48, ~9.95, ~11.92, and ~10.37 kcal/mol, respectively. The obtained data support that all the selected fatty acids have contributed to inhibit P-gp activity thereby enhances the bioavailability of drugs. Conclusion: This study plays a significant role in finding hot spots in P-gp and may offer the further scope of designing potent and specific inhibitors of P-gp. Key words: Binding affinity, black cumin oil, fatty acids, in silico analysis, P-glycoprotein

SUMMARY

• Generation of 3D structure of fatty acid compounds from Black cumin oil and 3D homology modeling of Rat P-glycoprotein as a receptor.

INTRODUCTION

Black cumin oil is a fixed oil and generally regarded as safe by the Food and Drug Administration. The oil is obtained from the seeds of Nigella sativa L. (Ranunculaceae), an annual flowering plant. Nigella is indigenous to South-west Asia and especially found in the Mediterranean region. In India, N. sativa is found as a weed in Punjab, Himachal Pradesh, Bihar and Assam and commonly known as “Kalajira” or “Kalongi.” The seeds are considered carminative, stimulant, diuretic, emmenagogal, and galactagogal, whereas their oil is applied externally for skin eruptions as antiseptic. Seed oil is beneficial to treat eczema and boils and to prevent cold symptoms. The seed oil has been reported to have antitumor, antioxidant, antibacterial, anti-inflammatory, hypoglycemic, central nervous system depressant, antioxidant, and immunostimulatory activities. These activities have been attributed to the fixed oil, volatile oil, or their components. Seed oil consisted of 15 saturated fatty acids (17%) and 17 unsaturated fatty acids (82.9%). Linoleic acid (50.2%), oleic (19.9%), margaric acid (10.3%), cis-11, 14-eicosadienoic acid (7.7%), and stearic acid (2.5%) were the major components. P-glycoprotein (P-gp), an ATP-dependent active transporter belongs to ABC transporter superfamily, occurs not only in cancer cells but also in the plasma membrane of many normal tissues. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms. For reprints contact: reprints@medknow.com

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as a possible site of interaction during the intestinal absorption. Improved clinical efficacy of various drugs observed by P-gp inhibition in intestine, brain, liver, and kidneys, which has been hypothesized and emphasized by many researchers in recent years. Long chain fatty acids (oleic and linoleic acid) and medium chain fatty acids (caprylic and capric acid) have been reported to increase oral bioavailability of peptides, antibiotics, and other important therapeutic agents. The oral bioavailability of cinnarizine was greatly enhanced by oleic acid. A concentration-dependent increase in the oral bioavailability of polar high molecular weight drugs such as glycyrrhizin in rats has been found with fatty acids. Fatty acids have also been reported to produce a dose-dependent increase in the concentration of norfloxacin in rabbits. Fatty acids act as absorption enhancers by increasing the fluidity of the apical and basolateral membranes. Nigella oil interacted with carvedilol and amoxicillin when co-infused and increased the permeation and absorption across the gut wall. The hexane extract of Nigella seeds affected the intestinal absorption that might be attributed to the presence of fatty acids in it. Linoleic acid, oleic acid, margaric acid, cis-11, 14-eicosadienoic acid and stearic acid were identified as main fatty acids. Although these studies lack information on their exact mechanism of action, a great interest is growing in order to understand the molecular mechanisms. Most of the drugs inhibit P-gp function by blocking drug binding sites and enhance the bioavailability. Then, the question is raised how the inhibitors are separated at the molecular level and block the binding sites of P-gp. Molecular docking is a method, which predicts the preferred orientation of two molecules when bound to each other and form a stable complex. Docking is frequently used to investigate the binding affinity and activity of the small molecule candidates to their protein targets receptor of known three-dimensional (3D) structure. Thus, in the present study, we did a molecular docking analysis to investigate the mechanism how the fatty acids of black cumin oil inhibit the multi-drug resistance transporter P-gp at the molecular level and increase bioavailability of drugs.

**MATERIALS AND METHODS**

**Three-dimensional modeling of rat P-glycoprotein receptor**

**Template search**

Template search with BLAST and HHblits has been performed against the SWISS-MODEL template library (SMTL, last update: October 08, 2014, last included protein data bank (PDB) release: October 03, 2014). The target sequence was searched with BLAST against the primary amino acid sequence of P-gp from Rattus norvegicus (Uniprot ID: P43245) contained in the SMTL. An initial HHblits profile has been built using the procedure outlined in Remmert et al. followed by one iteration of HHblits against NR20. The obtained profile has then been searched against all profiles of the SMTL. A total of 3270 templates were found.

**Template selection**

For each identified template, the template’s quality has been predicted from features of the target template alignment. The templates with the highest quality have then been selected for model building. After analyzing obtained results, we have selected PDB ID: 3G60 (ABCB1 A of Mus musculus) as a template for the 3D model building of Rat P-gp.

**Model building**

Models are built based on the target template alignment using Promod-II. Co-ordinates which are conserved between the target and the template are copied from the template to the model. Insertions and deletions are remodeled using a fragment library. The side-chains are then rebuilt. Finally, the geometry of the resulting model is regularized by using a force field. In case loop modeling with ProMod-II does not give satisfactory results, an alternative model is built with Modeller.

**Model quality estimation**

The global and per-residue model quality has been assessed using the QMEAN scoring function.

**Model validation**

The model validation completed by rampage Ramachandran Plot analysis server. We have found that 88.5% (1097) residues were lying in favored region, 8.5% (105) were in allowed region and 3.0% (37) were in the outlier region.

**Preparation of receptor molecule**

Modeled 3D structures of rat P-gp were submitted to minimization process. Chimera 1.10 was used for energy minimization, removal of steric collision with the steepest descent steps 1000, steepest descent size 0.02 Å, Conjugated gradient steps 1000 and the conjugate gradient step size 0.02 Å for the conjugate gradient minimization.

**Preparation of three-dimensional structure of ligand**

The .mol files of fatty acids from black cumin oil Linoleic acid, oleic acid, margaric acid, cis-11, 14-eicosadienoic acid and stearic acid were obtained from ChemSpider database. They were converted it into .pdb files using Accelrys Software Inc., Discovery Studio Modeling Environment, Release 4.0, (San Diego: Accelrys Software Inc, 2013). Discovery Studio makes it easier to examine the properties of large and small molecules. Further, the ligands were submitted for minimization using Chimera version 1.10 (Chimera development by the UCSF Resource for Biocomputing, Visualization, and Informatics is funded by the National Institutes of Health) using with Genetic Algorithm Steps 2000 and 0.5 grid units Optimized.

**Docking studies**

Docking studies were performed by MGL tools version 1.5.6 Autodock 4.2 (MGLTools is a software developed at the Molecular Graphics Laboratory (MGL) of The Scripps Research Institute for visualization and analysis of molecular structures) and Cygwin interface was used in the Microsoft Windows 7 professional service pack 1, operating System on Intel® (Microsoft Corporation) i5, 3230M CPU at 2.60 GHz, 64-bit and 4.0 GB of RAM of Lenovo machine. We adopted molecular docking methods followed by searching the best conformation of P-gp and natural compounds complex based on total internal binding energy. Water molecules were removed from the protein structures before docking and hydrogen atoms were added to all target proteins. Kollman united charges, and salvation parameters were added to the proteins. Gasteiger charge was added to the ligands. Grid box was set to cover the maximum part of proteins and ligand. The values were set to 60 Å x60 Å x60 Å in X (25,427), Y (38,034) and Z (98,248) axis of a grid point. The default grid points, spacing, was 0.375 Å. Lamarckian Genetic Algorithm (LGA) was used for proteins ligands flexible docking calculations. The LGA parameters such as population size (ga_pop_size), energy evaluations (ga_num_generation), mutation rate, crossover rate and step size were set to 150, 2500000, 27000, 0.02, 0.8 and 0.2 Å, respectively. The LGA runs were set at 10 runs. All 10 conformations of the receptor and ligands complex were analyzed and the interactions and binding energy of the docked structure using Accelrys Software Inc., Discovery Studio Modeling Environment, Release 4.0, (San Diego: Accelrys Software Inc, 2013) also graphics generated by PyMol.
RESULTS

Detailed information of selected compounds and *in silico* results were documented in Table 1 and 2. Results showed that fatty acids exhibited interactions with P-gp and were found to bind easily in the active site with a slight conformational difference [Figures 1-4]. The amount of energy needed by Linoleic acid, Oleic acid, Margaric acid and Stearic acid to bind with P-gp were found to be −10.60, −10.48, −9.95, −11.92 and −10.37 kcal/mol respectively. All compounds showed binding energy values ranging between −11.92 to −9.95 kcal/mol. In the formation of complex for fatty acids with P-gp, involved amino acids were Ser221, Pro222, Ile224, Gly225, Ser228, Ala229, Lys233, Tyr302, Tyr309, Ile337, Leu338, Thr341, Ile344, Gly345, Ala348 for Linoleic acid, Ser221, Pro222, Ile224, Gly225, Ser228, Ala229, Ala232, Lys233, Tyr302, Tyr309, Ile337, Leu338, Thr341 for Oleic acid, Ser221, Ile224, Gly225, Ser228, Ala229, Lys233, Tyr302, Tyr309, Ile337, Leu338, Thr341, Ile344, Gly345, Ala348 for Margaric acid, Ile217, Leu218, Ser221, Pro222, Gly225, Ser228, Lys233, Tyr302, Tyr309, Ile337, Leu338, Thr341, Ile344, Gly345, Ala348 [Figure 1] for Eicosadienoic acid and Thr198, Gly202, Ser221, Gly225, Ser228, Ala229, Lys233, Tyr302, Tyr309, Ile337, Leu338, Thr341, Ile344, Ala348 for Stearic acid, respectively. Some amino acids are found to be common for all compounds such as Ser221, Pro222, Ile224, Gly225, Ser228, Ala229, Lys233, Tyr302, Tyr309, Ile337, Leu338, and Thr341. Linoleic acid and Eicosadienoic acid involved in the building of 2 hydrogen bonds with the minimum distance of 1.70361 Å, while Oleic acid, Margaric acid and Stearic acid involved in the formation of three hydrogen bonds with the minimum distance of 1.63207 Å. Inhibition constant was also predicted for fatty acids, which bring about additional information along with energy values. Inhibition constant for Linoleic acid, oleic acid, margaric acid, cis-11, 14-eicosadienoic acid and stearic acid were found to be 32.46 µm, 65.36 µm, 159.33 µm, 9.48 µm and, 130.78 µm, respectively [Table 2].

DISCUSSION

Linoleic acid, oleic acid, margaric acid, cis-11, 14-eicosadienoic acid and stearic acid were selected for *in silico* docking studies to understand the mechanism of binding interaction between fatty acids and P-gp.
Unfortunately, the 3D structure of rat P-gp was not available in the PDB so we have modeled the structure using homology modeling approach from SWISS-MODEL server. The most suitable templates were searched using the BLAST program again PDB. We selected PDB ID: 3G60 (ABCB1 A of Mus musculus) as a template for the 3D model building of rat P-gp. The model quality estimation was done by the Q-mean scoring function. The obtained QMEAN Z-score was 8.46 kcal/mol. Further, the model was validated by RAMPAGE (Ramachandran plot) analysis server. We have found that 88.5% (1097) residues were lying in the favored region, 8.5% (105) were in allowed region and 3.0% (37) were in the outer region. Active sites of proteins are often associated with structural pockets in the protein. The identification of such substrate binding sites in enzymes helps us to understand their binding interactions with substrates and other small molecules. The drug binding site of P-gp was taken from the template structure that was used for homology modeling. The important parameters determined were binding energy, inhibition constant and intermolecular energy. All selected fatty acids involved in building of hydrogen bonds. Hydrogen bond interactions play a significant role in predicting the binding affinity and help in describing drug permeability. The obtained data supports that all the selected fatty acids have contributed to inhibit P-gp activity thereby enhances the bioavailability of drugs. Eicosadienoic acid has a highest binding affinity with P-gp as the amount of energy needed to bind with P-gp was lowest (−11.92 kcal/mol).

CONCLUSIONS

Complete understanding of P-gp efflux mechanisms would offer an opportunity for not only enhancing the bioavailability of life-saving drugs such as paclitaxel and saquinavir but also improve their pharmacokinetics. This manuscript provides details of interactions between fatty acids and P-gp varying in their chemical nature and binding affinity, which has helped in understanding the mechanism how fatty acids inhibit P-gp through binding and increase the bioavailability of drugs. This study may offer further scope of designing potent and specific inhibitors and play a vital role in finding hot spots in P-gp.

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Conflicts of interest

There are no conflict of interest.

REFERENCES

1. Burdock GA. Encyclopedia of Food and Color Additives. Boca Raton, FL: CRC Press; 1997.
2. Kirtikar KR, Basu BD. Indian Medical Plants. Delhi, India: Sri Satguru Publications; 2000.
3. Dwivedi SN. Herbal remedies among tribals of Sidhi district of Madhya Pradesh. J Econ Taxon Bot 2004;28:675-86.
4. Dwivedi S, Kaul S, Pandey D, Shrivastava S, Dwivedi SN. Satus and conservation strategies of endangered and vulnerable medicinal plants. Planta Med 2007;3:13-5.
5. Worthen DR, Ghosheh OA, Crooks PA. The in vitro anti-tumor activity of some crude and purified components of blackseed, Nigella sativa L. Anticancer Res 1998;18:1527-32.
6. Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res 2000;14:323-8.
7. Morsi NM. Antimicrobial effect of crude extracts of Nigella sativa on multiple antibiotics-resistant bacteria. Acta Microbiol Pol 2000;49:63-74.
8. Nair MK, Vasudevan P, Venkitanarayanan K. Antibacterial effect of black seed oil on Listeria monocytogenes. Food Control 2005;16:395-8.
9. Rathee PS, Mishra SH, Kaushal R. Antimicrobial activity of essential oil, fixed oil and unsaponifiable matter of Nigella sativa L. Indian J Med Res Pharm Sci 1982;44:8-10.

10. Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of Nigella sativa and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med 1995;61:93-6.

11. Al-Hader A, Aql M, Hasan Z. Hypoglycemic effects of the volatile oil of Nigella sativa. Int J Pharmacogn 1993;31:96-100.

12. Khanna T, Zaid FA, Dandiya PC. CNS and analesis studies on Nigella sativa. Fitoterapia 1993;64:407-10.

13. Mansour MA, Givavi OT, El-Hadhey T, El-Khateb AS, Al-Shabanah OA, Al-Sawaf HA. Effects of volatile oil constituents of Nigella sativa on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone. Res Commun Mol Pathol Pharmacol 2001;110:239-51.

14. Salem ML, Hossain MS. In vivo acute depletion of CD8(+) T cells before murine cytomegalovirus infection upregulated innate antiviral activity of natural killer cells. Int J Immunopharmac 2000;22:707-18.

15. Amin S, Mir SR, Kohli K, Ali B, Ali M. A study of the chemical composition of black cumin oil and its effect on penetration enhancement from transdermal formulations. Nat Prod Res 2010;24:1151-7.

16. Ambudkar SV, Dey S, Hvy cyna CA, Ramachandra M, Pastor I, Gottesman MM. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol 1999;39:361-98.

17. Dietrich CG, Geier A, Oude Elferink RP, ABC of oral bioavailability: transporters as gatekeepers in the gut. Gut 2003;52:1788-95.

18. Varma MV, Ashokraj Y, Dey CS, Panchagnula R. P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. Pharmacol Res 2003;48:347-59.

19. Kang MJ, Cho JY, Shim BH, Kim DK, Lee J. Bioavailability enhancing activities of natural compounds from medicinal plants. J Med Plants Res 2009;3:1204-11.

20. Tokumura T, Tsumura Y, Tatsuki K, Kayano M, Machida Y, Nagai T. Enhancement of the oral bioavailability of cinnarizine in oleic acid in beagle dogs. J Pharm Sci 1987;76:286-8.

21. Bernet LZ, Isumi T, Zhang Y, Silverman JA, Wacher VJ. Intestinal MDR transport proteins and P-450 enzymes as barriers to oral drug delivery. J Control Release 1999;62:25-31.

22. Sasaki K, Yonebayashi S, Yoshida M, Shinizu K, Aotsuka T, Takayama K. Improvement in the bioavailability of poorly absorbed glycyr rhizin via various non-vascular administration routes in rats. Int J Pharm 2003;265:95-102.

23. Dos Santos I, Faivaz F, Laguery AM, Bonini F. Improvement of norfloxacin oral bioavailability by EDTA and sodium caprate. Int J Pharm 2003;260:1-4.

24. Chi SC, Park ES, Kim H. Effect of penetration enhancers on flurbiprofen permeation through rat skin. Int J Pharm 1995;126:267-74.

25. Ali B, Amin S, Ahmad J, Ali A; Mohd Ali, Mir SR. Bioavailability enhancement studies of amoxicillin with Nigella. Indian J Med Res 2012;135:555-9.

26. Amin S, Kohli K, Khar RK, Mir SR, Pilak KI. Mechanism of in vitro percutaneous absorption enhancement of carvedilol by penetration enhancers. Pharm Dev Technol 2008;13:533-9.

27. Kitchen DB, Decornez H, Furr JR, Bajajrat J. Docking and scoring in virtual screening for drug discovery: methods and applications. Nat Rev Drug Discov 2004;3:935-49.

28. Ats chul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389-402.

29. Su L, Jordannan P, Muk DD, Mathur PP, Cheng YH, Moh KW, et al. Role of P-glycoprotein at the blood-testis barrier on adjudin distribution in the testis: a revisit of recent data. Adv Exp Med Biol 2012;763:318-33.

30. Remnant M, Bieger A, Hauser A, Söding J. Hbiibits: lightning-fast iterative protein sequence searching by HMM-HMM alignment. Nat Methods 2011;9:173-5.

31. Guex N, Peitsch MC. SWISS-MODEL, and the Swiss-PdbViewer: an environment for comparative protein modeling. Electrophoresis 1998;19:2714-23.

32. Sali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. J Mol Biol 1993;234:779-815.

33. Benkert P, Basirni M, Schwede T. Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics 2011;27:343-50.

34. Wang J, Wolf RM, Caldwell JW, Kollman PA, Case DA. Development and testing of a general amber force field. J Comput Chem 2004;25:1157-74.

35. Wang J, Wang W, Kollman PA, Case DA. Automatic atom type and bond type perception in molecular mechanical calculations. J Mol Graph Model 2006;24:247-60.

36. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera – a visualization system for exploratory research and analysis. J Comput Chem 2004;25:1605-12.

37. Morris GM, Goodsell DS, Halliday RS, Huey R, Hart WE, Belew RK, et al. Automated docking using Lamarckian genetic algorithm and an empirical binding free energy function. J Comput Chem 1998;19:1639-62.

38. Rarey M, Kramer B, Lengauer T, Klebe G. A fast flexible docking method using an incremental construction algorithm. J Mol Biol 1996;261:470-89.

39. Goodsell DS, Morris GM, Olson AJ. Automated docking of flexible ligands: applications of AutoDock. J Mol Recognit 1996;9:1-5.

40. Desai PV, Raub TJ, Blanco MJ. How hydrogen bonds impact P-glycoprotein transport and permeability. Bioorg Med Chem Lett 2012;22:6540-8.

41. Available from: http://www.chemspider.com/Chemical-Structure. 4444106.html. [Last accessed on 2014 Nov 22, 13:27].

42. Available from: http://www.chemspider.com/Chemical-Structure. 393217.html. [Last accessed on 2014 Nov 22, 13:33].

43. Available from: http://www.chemspider.com/Chemical-Structure. 10033.html. [Last accessed on 2014 Nov 22, 13:35].

44. Available from: http://www.chemspider.com/Chemical-Structure. 9658485.html. [Last accessed on 2014 Nov 22, 13:39].

45. Available from: http://www.chemspider.com/Chemical-Structure. 5091.html. [Last accessed on 2014 Dec 22, 05:36].