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SHORT COMMUNICATION

Nucleotide binding domain 1 pharmacophore modeling for visualization and analysis of P-glycoprotein–flavonoid molecular interactions

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BACKGROUND: P-glycoprotein (P-gp) is a 170-kDa membrane protein. It provides a barrier function and help to excrete toxins from the body as a transporter. Some bioflavonoids have been shown to block P-gp activity.
OBJECTIVE: To evaluate the important amino acid residues within nucleotide binding domain 1 (NBD1) of P-gp that play a key role in molecular interactions with flavonoids using structure-based pharmacophore model.
METHODS: In the molecular docking with NBD1 models, a putative binding site of flavonoids was proposed and compared with the site for ATP. The binding modes for ligands were achieved using LigandScout to generate the P-gp–flavonoid pharmacophore models.
RESULTS: The binding pocket for flavonoids was investigated and found these inhibitors compete with the ATP for binding site in NBD1 including the NBD1 amino acid residues identified by the *in silico* techniques to be involved in the hydrogen bonding and van der Waals (hydrophobic) interactions with flavonoids.
CONCLUSION: These flavonoids occupy with the same binding site of ATP in NBD1 proffering that they may act as an ATP competitive inhibitor.

Keywords P-glycoprotein, Nucleotide-binding domain 1, pharmacophore model, flavonoid, competitive inhibition, herb-drug interaction

Introduction

P-glycoprotein (P-gp) is expressed in the cell membrane of various types of normal tissues, particularly in the brain cell, hepatocyte, kidney cell, and intestinal epithelium cell (apical site), where P-gp takes an action to extrude toxic substance and repels with drug absorption. Inhibition of P-gp action may lead to significant alterations in the conventional drugs' pharmacokinetic parameters and raising potential risks in occurring of drug-drug including herb-drug interactions (Li et al., 2014).

Many natural compounds including flavonoids have been elucidated their activities as modulators of P-gp by a mechanism of inhibiting the ATP binding site of the transporter (situated on the nucleotide binding domain 1; NBD1 and nucleotide binding domain 2; NBD2). Mechanism of actions of flavonoids to inhibit P-gp function, ordinarily is by blocking ATP binding site either competitively, non-competitively or allosterically that result in interfering with hydrolysis of ATP. Flavonoids are capable to negatively change ATPase action (NBD1 and 2 own an intrinsic ATPase activity) and, hence, inhibition of the ATPase activity of protein can interrupt P-gp efflux action (Lopez and Martinez-

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Luis, 2014).

Many researches regarding this transporter are only focused on mechanism of NBD2 inhibition, in spite of NBD1 inhibition is also a major mechanism that may result in a potential interaction with a P-gp substrate drug. Thus, the aim of this study was to develop *in silico* tools that can be used to screen the effects of flavonoids on drug absorption mechanism through P-gp indicating the potential drug interactions, so computational models like 2D and 3D structure-based pharmacophore models were developed based on the underlying mechanism of flavonoids as P-gp inhibitors through inhibition of ATP-hydrolysis by blockages (competition/allosteric inhibition) of ATP binding with NBD1. The models provided illustration whether each flavonoid bound to NBD1 of P-gp or not.

Receptor-ligand complexes were utilized to generate two and three dimensional structure-based pharmacophore models by interpreting molecular interactions between the receptor and ligand. The chemical attributes of complementarity disclosed by the molecular interaction are taken into account into the 2D and 3D structure-based pharmacophore models, along with the gap complementary between the receptor and the ligand. Therefore, in this study, the crystal structure of mouse P-glycoprotein; PDB code: 4Q9H (with the resolution of 3.40 Å) is utilized to generate a structure-based pharmacophore model of the flavonoid–protein interaction, forasmuch the transporter activity of the peptide can likewise be impeded by the flavonoids. The evaluation of the binding modes of bioflavonoids in this study should fill up the gap till more computational information on the binding is obtainable.

Materials and methods

Molecular docking

The putative binding site of flavonoids at the P-gp and their interactions were evaluated utilizing a molecular docking method to dock a flexible ligand to a rigid protein. The results obtained from the molecular docking including binding energies and validation have been published (Wongrattanakamon et al., 2016) and they disclosed exceedingly plausible sites for ligands and so this approach can be utilized for further applications to any drug transporters.

Briefly explanation regarding the method for molecular docking here, AutoDock 4.2.6 was utilized to dock a set of 25 known bioflavonoids including amorphigenin, epigallocatechin, rotenone, formononetin, chrysin, floretin (phloretin), afromosin, 6a,12a-Dehydroamorphigenin, catechin, (+)-12-hydroxyamorphigenin (dabinol), neohesperidin, sakuranetin, naringin, robinin, quercetin, naringenin, morin, epigallocatechingallate (EGCG), epicatechingallate (ECG), biochenin A, silymarin, hesperidin, demethylnobiletin, 5-hydroxy-3,6,7,8,3′,4'-hexamethoxyflavone (5HHMF), and nobiletin possessing experimental P-gp inhibitory activity. These bioflavonoids were obtained from previous publications (Zhang and Morris, 2003; Kitagawa et al., 2004; Chung et al., 2005; Gyémant et al., 2005; Martins et al., 2010; El-Readi et al., 2010). 2D structures were constructed using ChemBio-Draw Ultra 11.0 and later converted to 3D structures utilizing ChemBio3D Ultra 11.0. Energy minimisation of molecular 3D structures was also carried out utilizing the ChemBio3D Ultra 11.0 by MM2 forcefield with default setup until the minimum rms error became smaller than 0.100 kcal/mol Å.

These flavonoids were docked into NBD1 of mouse P-gp (PDB code: 4Q9H) to determine their binding modes. The volume of the grid was set to mantle the cytosolic domain containing ATP binding site on NBD1 and vicinity with a grid-spacing interval of 0.375 Å with dimension 126 × 126 × 126 Å. Molecular docking was carried out utilizing AutoDock via the Lamarckian algorithm and performed by default parameters with 50 runs, a population size of 250, 2 500 000 evaluations, and 27 000 generations per tested ligand for each cycle were employed throughout the study. A cut-off value 1 Å was used for clustering of ligand orientations into groups. AutoDockTools 1.5.6 and PyMol were utilized for visual inspection of the molecular docking result and graphical representations of all poses. An ultimate docked delegate of the potential binding mode of bioflavonoids was picked based on the selection of the compound having the lowest docked energy within the most populated cluster of the lowest possible energy (Badhan and Penny, 2006).

Structure-based pharmacophore modeling

The docked complexes of flavonoids at P-gp NBD1 were further examined using LigandScout software (Inte:Ligand version 3.12) (Wolber and Langer, 2005) to create schematic diagrams of protein–ligand interactions (binding modes). Pharmacophore models were created that pointed out certain amino acid residue atoms in NBD1 interacted with the ligand atoms. The interactions created by LigandScout were presented as four main features, namely hydrogen bond donors (HBD), hydrogen bond acceptor (HBA), hydrophobic interactions (H) and aromatic ring (Ar). The feature shown in green color is the HBD, red color is HBA, yellow color is H, and purple color is Ar. Pharmacophore modeling for P-gp inhibitors was advocated by the availability of 3D structural data on protein–ligand complexes. Molecular interactions of the ligands to any binding cavities at NBD1 were analyzed in order to identify key features for ligand binding (Fig. 1). 4Q9H including ligands were overlaid to illustrate ligand binding cavities (Fig. 2).

Pharmacophore modeling for P-gp inhibitors (flavonoids) was facilitated by the availability of 3D structural information on protein–ligand complexes. Molecular interactions of the ligands to any binding cavities at NBD1 were analyzed in

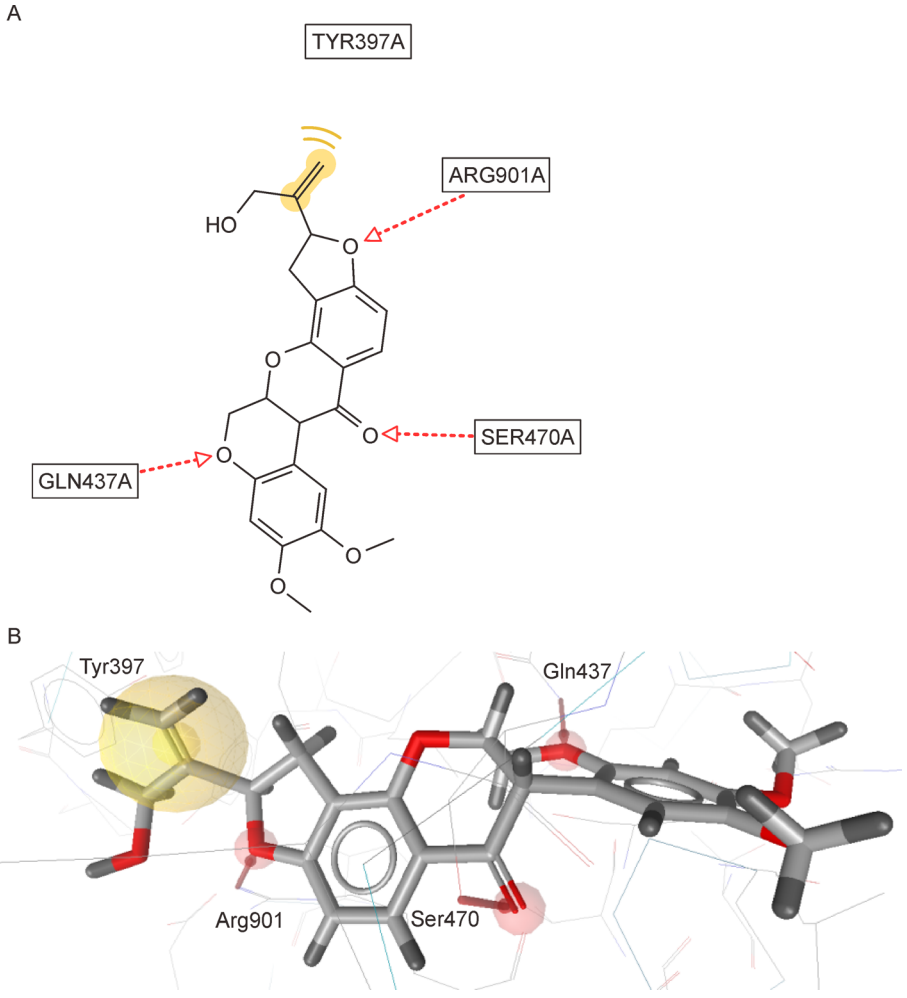

Figure 1 The example of pharmacophore models obtained from the docking complex illustrates the favorable binding position of amorphigenin with the lowest free energy of binding in the major active cavity of 4Q9H NBD1. 2D (A) and 3D (B) models show interactions between the amino acid residues and ligand formed in the cavity. Pharmacophore features in the models are color-coded: red–hydrogen bond acceptor, and yellow–hydrophobic interactions.

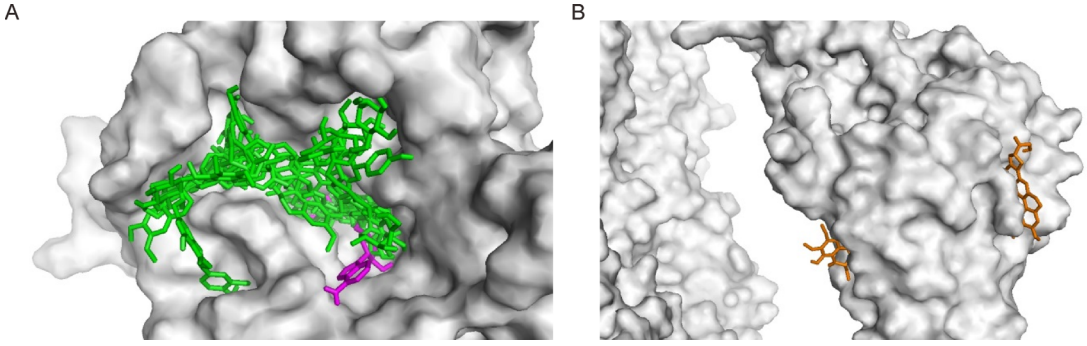

Figure 2 The binding patterns of the inhibitors on 4Q9H NBD1 (gray). (A) The binding cavity occupied by the most flavonoids (green) and ATP (magenta) is shown as a close-up inside NBD1 as the major binding site except (B) 6a,12a-dehydroamorphigenin and 5HHMF (orange) bind NBD1 at their own distinct cavities.

order to identify important features for ligand binding (Fig. 1). Additionally, the crystal structure of P-gp including ligands were overlaid to illustrate ligand binding cavities (Fig. 2).

Results

Our previous work (Wongrattanakamon et al., 2016) showed that, docking results of the 25 flavonoids against NBD1, the optimal pose of each compound with the lowest estimated free energy of binding ranging from −7.96 to −5.77 kcal/mol. Notably, the strong inhibitors exhibited more negative the estimated free energy of binding values but the weak inhibitors possessed lesser negative values. The docking result was contributed by accordance with the experimental flavonoid percentage of inhibitory efficiency. $R^2 = 0.8699$ was obtained (with a minimum of five points and higher than 0.6, a threshold routinely accepted to establish the goodness of structure-based models utilized in computational researches) suggesting that molecular docking approach to flavonoids using AutoDock at the ATP binding site of NBD1 is powerful and capable to predict potential herb-drug interactions via P-gp among flavonoids and its drug substrates.

At NBD1 (Fig. 2), the important binding site inside NBD1 was observed. Regarding this site, it was a shallow cavity with an opened wide mount that was considered as the major binding site. The most flavonoids including ATP (a substrate control) bound to this site and the amino acid residues around the binding cavity including Asp160, His162, Val164, Tyr397, Ser430 Gln434, Gln437, Arg463, Ile466, Val468, Ser470, Gln471, Glu472, Pro473, Val474, Leu475, Gly521, Glu522, Ala525, Leu527, Lys532, Arg539, Ser555, Glu898, Asn899, Phe900, Arg901, Thr902, and Ser905 took responsibilities in hydrogen bond formations with the ligands; Phe159, Val164, Tyr397, Val433, Leu439, Val468, Val474, Leu475, Val520, Ala540, Ala556, Leu557, Thr902, and Leu906 took responsibilities in hydrophobic interactions with the ligands; and Lys532, Thr902 and Leu906 took responsibilities in aromatic interactions (pi stacking) with the ligands. These amino acid residues of the major binding site played a key role in the molecular interactions with the most flavonoids and together with ATP. The interaction pattern of these most flavonoids as P-gp inhibitors corresponded with that of ATP as they occupied, first, the same amino acid residues like Ser430, Gln437, Ser470, Arg539, and Thr902 of ATP which is the natural substrate of P-gp NBD1 and second, the same binding cavity of the substrate at NBD1.

Additionally, in the bindings of 6a,12a-dehydroamorphigenin and 5-hydroxy-3,6,7,8,3′,4'-hexamethoxyflavone (5HHMF) with NBD1, ligand-transporter interactions occurred at their own distinct binding cavities. Regarding 6a,12a-dehydroamorphigenin, its binding site is quite far from the major binding site and only Met446 was responsible for hydrophobic interaction with the ligand. Regarding 5HHMF, its binding site is a wide mount pocket adjacent to the major binding site and only one interaction was formed by the hydrogen bond between Lys407 and the ligand. These van der Waals and polar forces were essential to support the interactions of two flavonoids in the active sites of NBD1.

One of the feasible mechanisms of P-gp inhibition may occur via blocking ATP hydrolysis at NBD1. Only few flavonoids bind P-gp at different (allosteric) sites. These P-gp allosteric inhibitors may induce a conformational change that remodels the shape of the active site and reduces the affinity of the P-gp's active site for ATP. Additional protein-ligand interaction data are in the Supplemental Material.

Discussion

Many previous reports indicated that flavonoids interact with P-gp and act as its inhibitors like genistein, morin, quercetin, chrysin, kaempferol and epigallocatechin bind to the transporter. The binding pocket for flavonoids was investigated and found these inhibitors compete with the ATP for binding site (as ATP competitive inhibitors) in NBD1 including the NBD1 amino acid residues identified by the *in silico* techniques to be involved in the hydrogen bonding and van der Waals (hydrophobic) interactions with flavonoids (Gadhe et al., 2013) and these reports are likewise consistent the result of this study suggesting one of the feasible mechanism of P-gp inhibition may occur via blocking ATP hydrolysis at NBD1. Only few flavonoids (6a,12a-dehydroamorphigenin and 5HHMF) bind P-gp at different (allosteric) sites. These P-gp allosteric inhibitors may induce a conformational change that remodels the shape of the active site and reduces the affinity of the P-gp's active site for ATP. The docking scores of both 6a,12a-dehydroamorphigenin and 5HHMF were agreeable with their experimental P-gp inhibitory activities (Wongrattanakamon et al., 2016) suggesting that a mechanism of action of these compounds still influenced with ATP binding site within NBD1 as allosteric inhibitors and finally prevented ATP to bind with its binding site.

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Compliance with ethics guidelines

Pathomwat Wongrattanakamon, Vannajan Sanghiran Lee, Piyarat Nimmanpipug and Supat Jiranusornkul declares that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

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