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Research article

Using reverse docking to identify potential targets for ginsenosides

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ABSTRACT

Background: Ginsenosides are the main ingredients of ginseng, which, in traditional Eastern medicine, has been claimed to have therapeutic values for many diseases. In order to verify the effects of ginseng that have been empirically observed, we utilized the reverse docking method to screen for target proteins that are linked to specific diseases.

Methods: We constructed a target protein database including 1,078 proteins associated with various kinds of diseases, based on the Potential Drug Target Database, with an added list of kinase proteins. We screened 26 kinds of ginsenosides of this target protein database using docking.

Results: We found four potential target proteins for ginsenosides, based on docking scores. Implications of these "hit" targets are discussed. From this screening, we also found four targets linked to possible side effects and toxicities, based on docking scores.

Conclusion: Our method and results can be helpful for finding new targets and developing new drugs from natural products.

1. Introduction

Korean ginseng is well known as a medicinal herb that has widely been used in the traditional medicine field. Ginseng has pharmacological actions because of the saponin ginsenoside, about 40 types of which have been found. Depending on the method of production, ginseng can be classified into white ginseng (Ginseng Radix Alba) and red ginseng (Ginseng Radix Rubra). White ginseng is produced by air drying fresh ginseng, whereas red ginseng is produced by first steaming fresh ginseng and then air drying it [1]. Owing to this difference in production methods, red ginseng contains more Rg1 and Rb1 ginsenosides than white ginseng.

Virtual screening, which has become a *de facto* standard in modern-day drug discovery, is a computational method for identifying potent molecules binding to a specific target protein from a large and chemically diverse molecule library [2]. It is a one-target many-ligand concept [3]. Virtual screening-based drug designs have successfully resulted in some approved drugs in recent history. Central to virtual screening is a computational method called docking [4]. A docking program predicts the binding mode of a small molecule/target protein complex. In order to find the most plausible binding modes, a docking program ranks possible conformations using a scoring function.

Reverse docking is a recent method that does the opposite of virtual screening through the use of docking. One screens a database of target proteins against an active compound—the one-ligand many-target concept, and just as in virtual screening, uses docking to find correct binding modes for ligand–target protein complexes [3]. However, in reverse docking, target proteins for a given ligand rather than ligands for a given target protein are ranked. Utilizing the list of ranked target proteins, the relevance of a given ligand for particular diseases or its side effects can be estimated. Therefore, the reverse docking method is useful for drug repositioning [5], in which one looks for new targets of drugs already approved or of natural products the exact effects of which are not yet known [6].

Ginsenosides are known to have many therapeutic values: they have antiallergic, antioxidant, and immune-stimulatory properties, and can modulate blood pressure, metabolism, and immune functions. Ginsenosides are named according to their retention factor value in thin-layer chromatography. Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rg5, Rh2, Rh3, Rs3, and compound K are 20(S)-protopanaxadiols, and Re, Rf, Rg1, Rg2, Rg4, Rh4, and Rh5 are 20(S)-protopanaxatriols [7]. Furthermore, several ginsenosides, such as the ocotillol saponins F2, F3, F5, and F11 [8] and the pentacyclic oleanane saponin Ro [9] have also been identified. Recent studies have linked these ginsenosides to multiple bioactivities including neuroprotection,

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antioxidation, angiogenesis modulation, and cytotoxicity [10]. In this work, we utilized the reverse docking technique to elucidate and/or confirm therapeutic values and side effects of ginsenosides by screening a target protein database against them. Chen et al [11] have used a reverse docking method, called INVDOCK, to extract possible drug targets by predicting interactions between compounds including ginsenoside Rg1, which are found in medicinal plants, and human and mammalian proteins. However, in order to fully assess the therapeutic values of ginseng, one needs to analyze the full array of ginsenosides. We also utilized more comprehensive target protein databases developed recently. In terms of the reverse docking method, INVDOCK is proprietary and little is known about its performance as a docking method. In our work, the well-known commercial docking program Glide (version 6.7; Schrödinger, Inc., New York, NY, USA) was used as part of our protocol, making it easy to replicate. In addition to potential drug targets, we also examined interactions of ginsenosides with toxicity- and side effect-related target proteins. Details of the methodology are described in the Materials and methods section. Our analysis of the targets, which were found to interact with ginsenosides, is summarized in the Results section. Our results not only validate the previously identified therapeutic values of ginsenosides, but also give insights into the overlooked ones.

2. Materials and methods

2.1. Construction of drug target database

In this work, we used the Potential Drug Target Database (PDTD) as our drug target database [12,13]. The PDTD contains 1,207 entries covering 841 known and potential drug targets with known three-dimensional structures presented in the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank. The target proteins collected in the PDTD were selected from the literature and online databases, such as DrugBank and the Therapeutic Target Database [13]. We selected 529 entries, which are classified as therapeutic in the PDTD, and then constructed a drug target database for reverse docking. Furthermore, 549 kinase targets were collected from an online database and added to the drug target database [14]. Diseases related to the targets in our database are bacterial/fungal/viral/parasitic infections, blood and neuronal disorders, inflammation, renal disorders, cardiovascular disorders, gastrointestinal disorders, cancer, and kinase-related disorders (Table 1). Each record of a target was annotated by hyperlinks to other databases, such as DrugBank, Therapeutic Target Database, the Expert Protein Analysis System (ExPASy) proteomics server, and Kyoto Encyclopedia of Genes and Genomes (KEGG).

Table 1
Diseases related to target proteins in our database[1)]

(1) Bacterial infections (44)
(2) Diseases of the blood & blood-forming organs (51)
(3) Fungal infections (9)
(4) Disorders of gastrointestinal functions (15)
(5) Hormone- & hormone antagonist-related diseases (82)
(6) Immunomodulation (45)
(7) Inflammation (24)
(8) Neoplastic diseases (85)
(9) Parasitic infectious diseases (27)
(10) Renal & cardiovascular disorders (30)
(11) Diseases of the synaptic & neuroeffector junctional sites, central nervous system (25)
(12) Vitamin deficiency diseases (25)
(13) Viral infections (67)
(14) Kinase-related disorders (549)

[1)] Numbers in parentheses indicate how many of the targets are in our database

2.2. Selection of toxicity- and side-effect-related protein targets

In addition to the disease-related targets from PDTD, we included proteins that are linked to side effects and toxicities based on available data from the medical biochemistry literature. It is well known that the inhibition of some proteins important in normal cellular function may result in toxicity or side effects [15]. These proteins are involved in important cellular metabolism processes such as amino acid and nucleotide metabolism, the glycolytic pathway, and the urea cycle. There are 73 Protein Data Bank (PDB) entries for 20 types of proteins connected to toxicities and side effects.

2.3. Preparation of ginsenoside ligand and target structure

We prepared 26 kinds of ginsenoside structures [7] with known structures presented in the PubChem [16]. The structures were generated by the two-dimensional Sketcher in Maestro, after which LigPrep (version 3.4; Schrödinger Inc., New York, NY, USA) was used to convert them into three-dimensional structures. During the preparation, the force field was set to OPLS-2005, and all the combinations of stereoisomers were generated. They were assigned protonation states at pH 7.0. Our target database contained 1,078 protein structures, and the cocrystal complexes were downloaded from the RCSB Protein Data Bank. Missing residues and atoms of each protein structure were repaired using the Schrödinger Protein Preparation Wizard. The Protein Preparation Wizard automatically fixes incomplete residues and determines the tautomeric states of histidine, glutamine, and asparagine residues. Water molecules and cofactors were removed except for ones influencing the binding site. Hydrogen atoms were added, and the positions were optimized to 0.3 Å root-mean square deviation (RMSD) with heavy atoms fixed.

2.4. Reverse docking using Glide

All the reverse dockings in this research were performed with Schrödinger's Glide (version 6.7; Schrödinger Inc.). We used the standard precision mode of Glide, which is efficient and accurate for most of the targets [17,18]. Glide generates the possible binding modes of ligand–protein complexes and scores them with GlideScore, a mixture of interaction energy and parameter-based penalty functions that roughly represents binding energy. The reverse docking procedure was performed as follows: (1) using a Python script, we generated an input file for each of the 26 kinds of ginsenosides for docking with all the target proteins in our database; (2) using Glide, each ginsenoside was docked to all 1,078 target proteins; and (3) docking results were sorted by docking scores and arranged into a matrix form. The resulting docking profiles were clustered for further analysis.

3. Results

3.1. Reverse docking results

To identify the efficacy of the 26 kinds of ginsenosides, we performed reverse docking with a database of 1,078 target proteins. For a detailed analysis, we clustered the entire docking results by scores. Fifty-two percent of all target proteins were docked by one or more of the 26 ginsenosides. Rh4, in particular, interacted with 72% of the targets. The average GlideScore of the docked results was −5.285 kcal/mol. Rc had an average of −6.249 kcal/mol across all the docked targets, which was the highest. In addition, 336 target proteins interacted with more than 21 ginsenosides, which suggested that several ginsenosides can have activities toward a single target. For further analysis as to what kind of implications these data might have on the therapeutic value of ginsenosides, we

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