Screening from the World's Largest TCM Database for Against H1N1 Virus

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Abstract

The swine influenza virus (H1N1) 2009 pandemic highlights the importance of having effective anti-viral strategies. Recently, oseltamivir (Tamiflu) resistant influenza viruses are identified; which further emphasizes the urgency in developing new antiviral agents. In influenza virus replication cycle, viral surface glycoprotein, hemagglutinin, is responsible for viral entry into host cells. Hence, a potentially effective antiviral strategy is to inhibit viral entry mechanism. To develop novel antiviral agent that inhibits viral entry, we analyzed 20,000 traditional Chinese medicine (TCM) ingredients in hemagglutinin subtype H1 sialic acid binding site found on H1N1 virus. We then performed molecular dynamics simulations to investigate receptor-ligand interaction of the candidates obtained from docking. Here, we report three TCM derivatives that have high binding affinities to H1 sialic acid binding site residues based on structure-based calculations. The top three derivatives, xylopine_2, rosmaricine_14 and rosmaricine_15, all have an amine group that interact with Glu83 and a pyridinium group that interact with Asp103. Molecular dynamics simulations show that these derivatives form strong hydrogen bonding with Glu83 but interact transiently with Asp103. We therefore suggest that an enhanced hemagglutinin inhibitor, based on our scaffold, should be designed to bind both Glu83 and Asp103 with high affinity.

Keywords: swine influenza virus, H1N1, molecular dynamics, traditional Chinese medicine (TCM), Tamiflu, hemagglutinin

Introduction

Hemagglutinin is the key glycoprotein responsible for influenza viral entry into the host cell. This protein binds to sialic acid-containing residues found on host surface and facilitates receptor-mediated endocytosis (1). The virus-containing endosome then fuses with a lysosome, in which the low pH triggers the refolding of hemagglutinin structure, resulting in protrusion of fusion peptide responsible for bringing viral and host plasma membrane into proximity (2).

Base on the influenza virus entry mechanism, inhibition of hemagglutinin by blocking its sialic acid binding site can be a novel treatment for influenza infection, in addition to antiviral drugs such as oseltamivir and zanamivir. Both oseltamivir and zanamivir are neuraminidase inhibitors that prevent influenza viral progeny from leaving the host (3). However, these two antivirals could be ineffective in late stage of influenza infection due to the build up of viral count in host body (4). Furthermore, some influenza viral strains resistant to oseltamivir have already been reported (5). Therefore, the hemagglutinin inhibiting strategy may offer not only an alternative treatment, but also an effective treatment for drug-resistant viral strains.

In this study, we focused our research on the H1 serotype of hemagglutinin found on H1N1 virus. More specifically, we have applied structure-based drug design - docking screening and molecular dynamics simulation – to investigate the interactions between H1 sialic acid binding site and candidate compounds isolated from traditional Chinese medicine. In the past, many studies have demonstrated that TCM is resourceful in bioactive compounds, including those for anti-inflammation, anti-depressant, and nephrolithiasis prevention (6-8). Furthermore, most bioactive compounds from TCM have not been investigated. Both docking and molecular dynamics simulations have been widely applied before for studying protein behaviors and for designing new therapeutics (9-28). Some studies may use chemical compounds as the potent ligand for designing drug by molecular

simulation (29-61). Nevertheless, we are one of the pioneers in applying structure-based approaches to TCM researches.

Methods

Homology Modeling

Hemagglutinin protein model was obtained through homology modeling. We specifically chose H1 serotype for investigation. A sequence alignment of the template (PDB ID: 2WRG (62)) to target sequence (GenBank association ID: CY063825) was performed to evaluate the similarity and identity. A high sequence identity indicates a more reliable homology model. Both ClustalW (63) and MODELLER (64) were used for sequence alignment and homology modeling. The modeling result is further verified with Ramachandran plot (65) and Verify-3D (66). Ramachandran plot shows all possible conformation of dihedral angles. Verify-3D evaluates compatibility of each residue against the three-dimensional structure.

Docking

A total of 20,000 compounds from TCM Database@Taiwan (http://tcm.cmu.edu.tw) were docked into the H1 sialic acid binding site. The ligand docking site was determined by using the binding location of N-acetyl-D-glucosamine (NAG) found at the receptor-binding domain of H1. NAG was co-crystallized with the hemagglutinin protein and was used as a control. LigandFit (67) of Discovery Studio 2.5 was used for docking screening. In LigandFit, proteins are held rigid while ligand conformations generated from Monte Carlo simulation are screened based on shape-matching. Ligand poses generated were minimized inside the binding pocket, using Steepest Descent and Conjugate Gradient under CHARMm (Chemistry at HARvard Molecular Mechanics) force field. Ligands were scored by DockScore, which approximates ligand binding affinity based on sum of ligand-receptor interaction energy (total of van der Waal energy and electrostatic energy) and ligand interaction energy (67). NAG was used as control and was re-docked back into the binding

site for assessing binding affinity. The DockScore of NAG was used for filtering TCM compounds.

De Novo Design

Leading compounds from docking were taken for *De Novo* design using Discovery Studio 2.5. The *De Novo* Evolution protocol utilizes Ludi algorithm (68) to calculate interaction sites suitable for hydrogen bonding or hydrophobic interaction. Ludi fragments that bind to the residues at the interaction site or fit into the interaction site are connected to existing scaffolds. The generated TCM derivatives were evaluated based on the interaction between the added fragments and H1 serotype of hemagglutinin. The derived compounds were then screened using Lipinski's Rule of Five (69) to evaluate drug-likeness. Interactions between drug-like substances and H1 sialic acid binding site were then evaluated using docking techniques.

Molecular Dynamics Simulation

Top three TCM derivatives and the control were selected for molecular dynamics simulations. Each protein-ligand complex was solvated in a water box with periodic boundary condition. Each protein-ligand complex was first energetically minimized by using 500 steps of Steepest Descent and 500 steps of Conjugate Gradient method. Each system was then heated from 50K to 310K without constraint for 50 ps. The equilibration step was conducted for 200 ps without constraint. The final production step was conducted for 20 ns in NVT ensemble with snapshots save every 2.5 ps. The SHAKE algorithm was applied to restraint bonds containing hydrogen atoms. The time step was set to 1 fs. Particle Mesh Ewald (PME) method was used to calculate electrostatic interaction. Root mean square deviation (RMSD), hydrogen bond frequency, energy trajectory, and hydrogen bond distance were calculated for analyzing the ligand-protein system.

Results

Homology Modeling

We have used the H1 serotype (PDB: 2WRG) of hemagglutinin as the template for homology modeling. Our protein model has a sequence identity of 85.5% and a similarity of 94.5% (Fig. 1). High number of sequence identity and similarity indicates that the alignment and the resultant model can be reliable.

The quality of the protein model was evaluated using Ramachandran plot. Majority of residues are in the favored regions, while 3% of residues are in the disfavored regions and 7% in the semi-allowed regions (Fig. 2). We also closely investigated all the deviations in the Ramachandran plots and found that majority of deviations resided far away from the binding site. Thus, no modifications were made on these deviations.

Verify-3D was used to evaluate the stereochemical quality of the homology model. Most of residues had score greater than 0 (Fig. 3). Only few residues are in the negative region in the Verify-3D plot. These residues were not closed to the binding site residues (Arg238, Asn104 and Asn81).

Docking and De Novo Design

To identify potential TCM inhibitors for hemagglutinin, we performed docking screening using the H1 homology model. DockScore algorithm was used to rank the TCM ingredients (Table 1). Glycyrrhizic acid, cynarin, rosmarinic acid and ergotamine were on top of the list, each with elevated DockScore over 90. LigScore 1, LigScore2 and –PMF were also calculated for readers' interests. LigScore 1 and LigScore 2 predict binding affinities by evaluating van der Waal energy and ligand-receptor attractive force, while also include desolvation penalty (70). PMF calculates the sum of pairwise interaction terms in the receptor-ligand complex (71).

To further determine the binding affinity to hemagglutinin, compounds with DockScore higher than the control were taken for *de novo* design. The result derivatives were re-docked back to H1 sialic acid binding site. The top three leading compounds, shown in Table 2, all have elevated DockScore compared to the scores of their parental

compounds. The structures of the control and the top three derivatives are shown in Table 3. Of the parental compounds, xylopine can be isolated from *Guatteria amplifolia* (72) and rosmaricine can be obtained from rosemary, *Rosmarinus officinales* (73).

The docked poses of the control and the top three derivatives are shown in Fig. 4. The derivatives (xylopine_2, rosmaricine_14 and rosmaricine_15) share similar binding poses, where the added pyridinium ring interacts with Asp103 and the protonated amine group found on the original scaffold interacts with Arg238. The control has a totally different docking pose compared to the derivatives. Unlike the derivatives, the control only interacts with Arg238.

Molecular Dynamics Simulation

The top three derivatives and the control were taken for molecular dynamics simulations. The root mean square deviations, RMSDs, of the H1-ligand complexes, shown in Fig. 5 (top), demonstrate the stability of the complexes. All complexes reach equilibrium by 8 ns. The RMSDs of the ligands are shown in Fig. 5 (bottom) suggest that all ligands bind to the receptor over the course of simulation. The total energy trajectories show that the rosmaricine_14-H1 complex has the lowest total energy than the other complexes after 3 ns (Fig. 6). All the complexes, however, become energetically stable after 8 ns of simulation.

To analyze the interaction between TCM derivatives and H1 serotype of hemagglutinin, we calculated hydrogen bond frequency and the interaction distances between the receptor and the ligands at the sialic acid binding sites. The molecular dynamics simulation result for the control NAG shows a transient hydrogen bond interaction between NAG and Asp103 from 1.7 ns to 16.4 ns (Fig. 7). In addition, there is also transient interaction between NAG and Asn104. The distance between NAG and Arg238 appears to be the most stable, with an average of 1.59 Å (Table 4). For xylopine_2, hydrogen bonds to Glu83 and Asp103 can be identified from docking (Fig. 4 (b)). The interaction of Xylopine_2 to Glu83, however, is

continuous throughout the simulation with an average distance less than 2.5Å (Table 5). Xylopine_2 also has transient interaction with Asp103, but the distances fluctuate periodically over time (Fig. 8). As shown in Fig. 9 and Table 6, rosmaricine_14 forms a stable hydrogen bond interaction with Glu83 throughout the 20 ns simulation. Rosmaricine also forms interactions to Asp103, Ser100 and Arg238. However, these interactions appear to be transient and only seen at the beginning of the simulation run. For rosmaricine_15, stable interactions are only observed at Glu83 and Asp103 (Fig. 10 and Table 7).

Discussions

Our docking screening method gave three derivatives, xylopine_2, rosmaricine_14 and rosmaricine_15 that form hydrogen bond networks with Glu83 and Asp103 at the H1 sialic acid binding site. The control NAG, however, differs form the derivatives in having interaction to Arg238 and transiently to Asp103 and Asn104. The interactions on Glu83 are conserved and stable throughout the simulation time for all derivatives. On other hand, the hydrogen bonds on Asp103 are different for NAG and for each derivative. The control forms hydrogen bond interactions to Asp103 from 1.7 ns to 16.4 ns (Fig. 11(a) and (b)); this interaction, however, ceases after 16.4ns (Fig. 11(c)). The binding pose of NAG, after molecular dynamics simulation, returns to the initial conformation, suggesting that the NAG-Asp103 bound state may not be energetically stable. For xylopine_2, hydrogen bond interactions occur via the added pyridinium group to Asp103, but the hydrogen bond distances fluctuate periodically overtime. For rosmaricine_14, the hydrogen bonds formed on Asp103 occur initially between Asp103 carboxylate side chain and H55 on rosmaricine_14, but later shift to H29 and H32 on rosmaricine_14. This shift is attributed to the change in Asp103 conformation; as illustrated in Fig. 12. For rosmaricine_15, the ligand interaction on Asp103 is relatively weaker, with an average interacting distance of 3.02 and 3.56 Å. The inconsistent hydrogen bonding between TCM derivatives and Asp103 side chain could be an undesirable factor for drug binding. We, therefore, proposed that a

potential hemagglutinin inhibitor, in addition to have strong hydrogen bond interaction to Glu83 should also establish continuous interaction to Asp103. Moreover, addition of amine functional group and a pyridinium group to candidate compound can potentially enhance the binding strength to H1 sialic acid binding site, as indicated in Fig. 13.

Conclusion

Our results from docking suggest that xylopine_2, rosmaricine_14 and rosmaricine _15 are lead compounds for inhibiting hemagglutinin binding to cell surface sialic acid-containing molecules. Molecular dynamics simulation shows that the binding site residue, Asp103, is highly flexible and can be an undesirable factor for ligand binding. Our derivatives have continuous interactions on Glu83 but not on Asp103 during the simulation run. We, therefore, suggest that a potential hemagglutinin inhibitor can be designed based on our candidate compounds but have improved affinity for Asp103. We also suggest that an inhibitor should have an amine and a pyridinium group in the scaffold for strengthening the interaction with binding site residues.

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