Molecular docking of some herbal-based potential anti-psoriasis agents with dihydrofolate reductase

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Abstract

Objectives: To find out the herbal inhibitor for Dihydrofolate reductase (DHFR) in replacements of some existing drugs like methotrexate to reduce side effects in psoriasis and also to cure psoriasis.

Methods: Computational simulation techniques are used for molecular docking of few herbals like beta-santalol, quercetin, carvacrol, curcumin and ferulic acid into DHFR by using AutoDock 4.0. Online tool and databases like Qsite-finder, PDB, and Chemspider are also used to retrieve active sites and 3D structures of enzymes and inhibitors. Parameters like binding energy, intermolecular energy, inhibition constant and H-bonding between ligands and target are used to determine extent of inhibition.

Results: This information could be supportive for new drug design for psoriasis, in which these potential inhibitors should interact strongly with above mentioned residues. In our study on the basis of minimum binding energy and inhibition constant we concluded that β-santalol was found to be best. β-Santalol interacted with DHFR and gives minimum binding energy - 6.85 Kcal/mol and inhibition constant 9.48 µm, it is also formed two H–bond with the active site residue Tyr121 of the DHFR. As this study utilized herbals which are unique with respect to inhibit DHFR and it could be better prospect on for methotrexate which is responsible for side effects if alone is used. This is the important initiation to in utilization of herbals for such target inhibition.

Conclusion: DHFR is an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid, using NADPH as electron donor. Tetrahydrofolic and its derivatives are essential for purine synthesis, which are important for cell proliferation and cell growth Therefore these herbals will block the reduction of dihydrofolic acid to tetrahydrofolic acid and inhibit the synthesis of nucleic acid precursor in not only psoriasis but in other such disease as well.

Keywords: Psoriasis, DHFR, Herbal Inhibitors, Drug Designing, Docking

1. Introduction

Psoriasis is a chronic, autoimmune disease that appears on the skin. It is a non-infectious skin disease characterized by well defined red, slightly raised plaques and papules with silvery scales. These patches, which are sometimes referred to as plaques, usually itch or feel sore. The biochemical basis for the pathogenesis of psoriasis, which is as equally varied as the genetic basis, can be attributed to both over-expression and under-expression of certain proteins in psoriatic lesions. The anomalies in protein expression can be divided into three areas: abnormal keratinocyte differentiation, hyper proliferation of the keratinocyte, and infiltration of inflammatory elements. Various markers of abnormal keratinocyte differentiation have been found, and all have implications in the pathogenesis of the disease. These include dihydrofolate reductase (DHFR). It is also documented that methotrexate during treatment some time induces keratinocyte differentiation may worsen the situation (Duvic \textit{et al.}, 1998; Schwartz \textit{et al.}, 1992; Kristian Reich & Ulrich Mrowietz, 2007).

Diseases like cancer and psoriasis are almost similar when they cause uncontrolled cell division due to over expression or over activity of enzyme like dihydrofolate reductase (EC 1.5.1.3). DHFR was the first enzyme targeted in chemotherapy. Several agents have been developed to target this key enzyme which is essential for synthesis of nucleic acids in cells (Ikram \textit{et al.}, 2011; Jordan \textit{et al.}, 2009). Several of them are in clinical trials and few of them are already in uses, which have been approved by the FDA. Therefore, drugs targeting DHFR is an exciting prospect in terms of prevention of psoriasis. DHFR reduces dihydrofolic acid to tetrahydrofolic acid (Fig.1). DHFR is essential in the pathway for DNA synthesis making it responsible for the growth and proliferation of cells that are characteristics in various diseases like psoriasis (Kristian Reich & Ulrich Mrowietz, 2007). This enzyme is a major target of sever-
al cyto-inhibiting and anti-proliferating drugs. These drugs are mainly the folate analogs such as methotrexate (4-amino-10-methylfolic acid) and aminopterin (4-aminofolic acid). Their inhibitory properties are due to similar in structure to that of the folic acid (Fig-2). Methotrexate is a competitive inhibitor of DHFR, because of the replacement of a 4-hydroxyl group of the vitamin, folic acid, by an amino group (Subramanian & Bernard, 1978; Takemura et al., 1999; Ramanan et al., 2003; Strojan et al., 2005; Slamon et al., 2006; Vivian Cody et al., 2009).

An examination of this NMR structure for the domain of DHFR showed the amino acid residues Ile-7, Leu-22, Phe-31, Phe-34, Arg-70, Val-115 and Tyr121 at its active sites. DHFR is an enzyme that reduces dihydrofolate acid to tetrahydrofolate acid, using NADP as an electron donor. Tetrahydrofolate and its derivatives are essential for purine synthesis, which are important for cell proliferation and cell growth (Takemura et al., 1999). A central eight-stranded β-pleated sheet makes up the main feature of the polypeptide backbone folding of DHFR (Jordan et al., 2009). Seven of these strands are parallel and the eighth runs antiparallel. Four α helices connect successive β strands (Slamon et al., 2006; Subramanian & Bernard, 1978).

Residues 9-24 are termed “Met20” or “loop 1” and, along with other loops, is part of the major sub domain that surrounds the active site. The active site is situated in the N-terminal half of the sequence, which includes a conserved Pro-Trp dipeptide; the tryptophan has been shown to be involved in the binding of substrate by the enzyme (Strojan et al., 2005). DHFR catalyzes the transfer of a hydride from NADPH to dihydrofolate with an accompanying protonation to produce tetrahydrofolate and NADPH is oxidized to NADP⁺. The high flexibility of Met20 and other loops near the active site play a role in promoting the release of the product (Takemura et al., 1999). In particular the Met20 loop helps stabilize the nicotinamide ring of the NADPH to promote the transfer of the hydride from NADPH to dihydrofolate (Subramanian & Bernard, 1978). The NADPH molecule forms nine hydrogen bonds to the protein. Seven of these are side chain hydrogen bonds: two to Arg44, two to Gln102, and one to each of Thr46, Ser63 and Ser64. The remaining two are to backbone atoms for residues Ile14 and Ala7. No specific hydrophobic interactions seem to exist (which is logical since this is a highly polar molecule) (Takemura et al., 1999 Ramanan et al., 2003; Vivian Cody et al., 2009 &2010).

Presently used drugs have lots of side effects on naturally dividing cells such as skin cells, hair cells etc. To minimize these effects, in this study we have tried five herbal molecules as inhibitor of the DHFR. These are β-Santalol, Curcumin, Quercetin, Ferulic Acid and Carvacrol. All these are various plant products, usually used for skin treatments. These were taken on the basis of lowest binding energy, inhibition constant, binding properties and orientation etc. We compare them with the methotrexate. Among these few has given as some astonishing results, which can be used as possible drug in place of methotrexate for cancer, psoriasis and other skin diseases. These inhibitors activity is studied through simulation techniques (Morris et al., 1998; Cole et al., 2005; Uthaman Gowthaman et al., 2008). Herbal molecules, which we have chosen for this study, are used to be a part of various skin related products. These molecules are documented and demonstrated in various anti-psoriatic and in anti-cancer biological activities.

2. Material and methods

The set of five ligand molecules studied in this work were retrieved from Chemspider chemical database (www.chemspider.com). Our inhibitors are β-Santalol ((2Z)-2-Methyl-5-(2-methyl-3-methylenebicyclo [2.2.1] hept-2-yl)-2-penten -1-ol) [Chemspider ID: 4444864], Carvacol (5-Isopropyl-2-methylphenol) [Chemspider ID: 21105867], Curcumin ((1E, 6E)-1,7-Bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) [Chemspider ID: 839564], Ferulic acid (2E)-3-(4-hydroxy-3-methoxyphenyl acrylic acid) [Chemspider ID: 393368] and Quercetin 2-(3, 4-Dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one [Chemspider ID: 4444864], Carvacol (5-Isopropyl-2-methylphenol) [Chemspider ID: 21105867], Curcumin ((1E, 6E)-1,7-Bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) [Chemspider ID: 839564], Ferulic acid (2E)-3-(4-hydroxy-3-methoxyphenyl acrylic acid) [Chemspider ID: 393368] and Quercetin 2-(3, 4-Dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one [Chemspider ID: 4444051] (Fig-3). The three dimensional structure of Dihydrofolate reductase (DHFR) of Protein [PDB: 1DLS] was obtained from Protein Data Bank (PDB) (Fig-4) (Protein Data Bank [http://www.rcsb.org/pdb/home/home.do]).

To find out the active site Q-SiteFinder online tool is used. Q SiteFinder uses the interaction energy between the protein and a simple van der Waals probe to locate energetically favorable binding sites (http://bmbpcu36.leeds.ac.uk/qsitefinder/help.html). Energetically favorable probe sites were clustered according to their spatial proximity. Clusters were then ranked according to the sum of interaction energies for sites within each cluster. In this way, it gives lot of active sites or binding pockets but we have chosen site 1 which is similar to the site used for methotrexate binding so that we can give the replacement of the drug on the basis of same active site binding.

In order to carry out the docking simulation, we used the AutoDock 4.0 suite as molecular-docking tool (Morris et al., 1998 & Cole et al., 2005). It is suitable software for performing automated docking of ligands to their macromolecular receptors. Typically, the ligands are substrates or drug candidates and the macromolecule is a protein of known three-dimensional structures. In this docking simulation, the target protein DHFR was kept as rigid. The ligands being docked were kept flexible, in order to explore an arbitrary number of torsional degrees of freedom in addition to the six spatial degrees of freedom spanned by the translational and rotational parameters. The Graphical User Interface program “AutoDock Tools” was used to prepare, run, and analyze the docking
simulations. Polar hydrogens were added into the receptor PDB file for the preparation of protein in docking simulation. This DHFR enzyme structure does not have any water molecules and/or ligands to remove from its PDB file and make a free receptor. Since ligands are not peptides, Gasteiger charge was assigned and then nonpolar hydrogens were merged. The rigid roots of each ligand were defined automatically instead of picking manually.

AutoDock requires pre-calculated grid maps, one for each atom type; present in the ligand being docked and its stores the potential energy arising from the interaction with macromolecule. This grid must surround the region of interest in the macromolecule. In the present study, the binding site was selected based on the amino acid residues, which are involved in normal function. Therefore, the grid was centered in the catalytic active region of active site motif and includes all amino acid residues that surround active site. The grid box size was set at 40, 40, and 40 Å\(^2\) (x, y, and z), though it was changed depending on the ligand size. AutoGrid 4.0 Program, supplied with AutoDock 4.0 was used to produce grid maps. The spacing between grid points was 0.375 Å. The Lamarckian Genetic Algorithm (LGA) was chosen to search for the best conformers.

During the docking process, a maximum of 10 conformers was considered for each compound. The population size was set to 150 and the individuals were initialized randomly. Maximum number of energy evaluation was set to 250000, maximum number of generations 1000, maximum number of top individual that automatically survived set to 1, mutation rate of 0.02, crossover rate of 0.8, Step sizes were 0.2 Å for translations, 5.0° for quaternions and 5.0° for torsions. Cluster tolerance 0.5Å°, external grid energy 1000.0, max initial energy 0.0, max number of retries 10000 and 10 LGA runs were performed (Morris et al., 1998; Cole et al., 2005). All the AutoDock docking runs were performed in Intel inside core i5 laptop @ 3.0 GHz of hp, with 4 GB DDR RAM. Auto-Dock 4.0 was compiled and run under Windows 7 operating system in cygwin environment.

To analyzing the docking results read a docking log or a set of docking logs (By convention, these results files have the extension “.dlg”). During its automated docking procedure, AutoDock outputs a detailed record to the file specified. The output includes many details about the docking which are output as AutoDock parses the input files and reports rank, binding energy, Ki, intermolecular energy, internal energy, torsional energy, unbounded extended energy, cluster rms, reference rms, donor and acceptor hydrogen, hydrogen bonding energy, phi value and theta value. After the input phase, AutoDock begins the specified number of runs. It reports which run number it is starting; it may report specifics about each generation.

3. Result and Discussion

Existing NMR structure of protein DHFR was downloaded from PDB [PDB ID: DHFR]. An examination of this NMR structure for the domain of DHFR showed the amino acid residues Ile-7, Leu-22, Phe-31, Phe-34, Arg-70, Val-115 and Tyr121 at its active sites. Active site is also obtained by using Q-SiteFinder online tool. DHFR is an enzyme that reduces dihydrofolate to tetrahydrofolate, using NADPH as electron donor. The criteria which we have followed in the selection of ligands include targeting DHFR to replace methotrexate for inhibition of this enzyme and also to inhibit NADPH binding to the enzyme so that enzyme activity can be inhibited (Takemura et al., 1999; Vivian Cody et al., 2009 & 2010).

DHFR inhibitors include β-Santalol, Curcumin, Quercetin, Ferulic Acid and Carvacrol were retrieved from Chemspider database. All the ligands and DHFR were confirmed to have minimized energy based on the final binding energy obtained from the docking results (Table 1). Interactions are based on H-bonding between donor and receptor (Table-2). Molecular docking simulations were conducted with the AutoDock 4.0 software suite. 10 Docking runs were performed for each of the five different ligands into active site of DHFR protein. For each docking simulation, the activity inferred by inhibitory docked models, in which the docked conformers of ligands and DHFR would permit active interaction at active site (Fig-5).

3.1 Docking of β-Santalol

The docking results of β-Santalol are good enough to consider it is a possible drug. Docking simulation of β-Santalol into the active site of the DHFR produced 10 clusters of conformers using RMSD-tolerance of 2.0 Å out of 10 docking runs (Table 1) in run no. 8. The conformation of the #1 ranked (run8) 10 clusters were favored in that structure of 10 runs of DHFR. It has been observed that one inhibitory docking mode was found in each cluster rank, which would explain the inhibitor effect of the enzyme. In this clusters, there were, two H-bonds form between active site amino acid of DHFR Tyr121 and Hydrogen (Table-2, fig-5B) of the β-Santalol and second H-bond between the Tyr121 and Oxygen of β - Santalol with lowest binding, intermolecular energy and inhibition constant which is lesser than other ligands used in this study.
Table 1. Binding parameters of docking results showing various parameters like binding, intermolecular energy and inhibition constant of the docking results

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ligand</th>
<th>NO. of Cluster</th>
<th>Cluster Rank</th>
<th>Run</th>
<th>Lowest Binding Energy (kcal/mol)</th>
<th>Intermolecular Energy (kcal/mol)</th>
<th>Inhibition Constant (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β-Santalol</td>
<td>10</td>
<td>1</td>
<td>8</td>
<td>-6.85</td>
<td>-8.34</td>
<td>9.48</td>
</tr>
<tr>
<td>2</td>
<td>Ferulic acid</td>
<td>09</td>
<td>1</td>
<td>8</td>
<td>-5.65</td>
<td>-7.14</td>
<td>72.07</td>
</tr>
<tr>
<td>3</td>
<td>Quercetin</td>
<td>07</td>
<td>1</td>
<td>4</td>
<td>-6.00</td>
<td>-7.79</td>
<td>39.69</td>
</tr>
<tr>
<td>4</td>
<td>Curcumin</td>
<td>01</td>
<td>2</td>
<td>9</td>
<td>-4.96</td>
<td>-6.45</td>
<td>232.15</td>
</tr>
<tr>
<td>5</td>
<td>Carvacrol</td>
<td>01</td>
<td>3</td>
<td>1</td>
<td>-5.20</td>
<td>-5.80</td>
<td>153.55</td>
</tr>
</tbody>
</table>

3.2 Docking of Curcumin

Docking simulation of curcumin into the active site of the DHFR produced 3 clusters of conformers (Table 1). The conformation of the #1 ranked (run4) 10 cluster were favored in that structure of 10 runs of DHFR. The conformation of the curcumin in the DHFR was also highly energetically and statistically favored. It was observed that one inhibitory docking mode was found in each cluster rank, which would explain the inhibitor effect of the enzyme. It is also forming one H-bond with the active site residue Arg70 of the enzyme (Table-2, fig-5C). In this interaction ligand oxygen is bonded with the hydrogen of the enzyme with lowest binding, intermolecular energy and inhibition constant.

Table 2. Interaction between ligands, target showing donor, acceptor of H-bond, and their binding energies

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Inhibitor</th>
<th>H-Bond (Donor)</th>
<th>H-Bond Acceptor</th>
<th>Length (Å)</th>
<th>Theta value</th>
<th>Phi value</th>
<th>Binding energy kcal/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>β-Santalol</td>
<td>D:LIG1:H</td>
<td>A:TYR121:OH</td>
<td>2.163</td>
<td>157.959</td>
<td>100.279</td>
<td>-4.938</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D:LIG1:O</td>
<td>A:TYR121:HH</td>
<td>2.163</td>
<td>157.959</td>
<td>100.279</td>
<td>-4.938</td>
</tr>
<tr>
<td></td>
<td>Acid</td>
<td>D:LIG1:O</td>
<td>A:TYR121:HH</td>
<td>2.097</td>
<td>146.500</td>
<td>145.072</td>
<td>-2.529</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D:LIG1:H</td>
<td>A:ILE16:O</td>
<td>2.166</td>
<td>124.127</td>
<td>130.151</td>
<td>-0.447</td>
</tr>
<tr>
<td>3</td>
<td>Carvacol</td>
<td>D:LIG1:H</td>
<td>A:ILE16:O</td>
<td>2.166</td>
<td>124.127</td>
<td>130.151</td>
<td>-0.447</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D:LIG1:H</td>
<td>A:THR56:O</td>
<td>2.123</td>
<td>125.276</td>
<td>87.037</td>
<td>-1.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D:LIG1:H</td>
<td>E1A:GLU30:O</td>
<td>1.953</td>
<td>149.128</td>
<td>166.595</td>
<td>-2.305</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D:LIG1:H</td>
<td>E1A:ASP21:O</td>
<td>1.941</td>
<td>140.533</td>
<td>121.484</td>
<td>-0.982</td>
</tr>
<tr>
<td>5</td>
<td>Curcumin</td>
<td>D:LIG1:O</td>
<td>A:ARG70:HH</td>
<td>2.117</td>
<td>133.76</td>
<td>58.442</td>
<td>-0.975</td>
</tr>
</tbody>
</table>

3.3 Docking of Quercetin

Docking simulation of Quercetin into the active site of the DHFR produced 7, 2 and 1 clusters of conformers (Table 1). The conformation of the #1 ranked (run4) 7 cluster were favored in that structure of 10 runs of DHFR. It was observed that one inhibitory docking mode was found in each cluster rank, which would explain the inhibitor effect of the enzyme. It is also forming one H-bond with the active site residue Arg70 of the enzyme (Table-2, fig-5E). Though among these interacting amino acids Val115 is the only binding pocket amino acid and glu30 forming two H- bonds with the ligand, situated very near to the phe31 and phe34 of binding domain. Interestingly it is also showing pi-pi interaction with active site amino acid phe34.

3.4 Docking of Ferulic acid

Docking simulation of Ferulic Acid into the active site of the DHFR produced 9 clusters of conformers (Table 1). The conformation of the #1 ranked (run2) 9 cluster were favored in that structure of 10 runs of DHFR. In this result no further rank is obtained for...
any other conformation. In #1 ranked cluster, there were, three H-bonds form (Table-2, fig-5D) between tyr121 oxygen and hydrogen of ligand, second between the Tyr121 hydrogen and oxygen of ferulic acid and Ile16 oxygen and hydrogen of ligand with lowest binding, Intermolecular Energy and inhibition constant.

### 3.5 Docking of Carvacol

Docking simulation of carvacol into the active site of the DHFR produced 10 clusters of conformers (Table 1). The conformation of the #1 ranked (run6) 10 cluster were favored in that structure of 10 runs of DHFR. In #1 ranked cluster, one H-bond form (Table-2, fig-5A) between Ile16 oxygen and hydrogen of ligand with lowest binding, intermolecular energy and inhibition constant.

**Fig.1.** Reduction of dihydrofolate to tetrahydrofolate catalyzed by DHFR where NADPH is oxidizing into NADP by DHFR.

**Fig. 2.** Similar structure of dihydrofolic acid (top) and methotrexate (Bottom), therefore methotrexate can be used as a competitive inhibitor to DHFR.

### 4. Conclusion

In this work, a molecular docking simulation study was undertaken. It is to investigate the binding mechanism of various herbal inhibitors. These inhibitors are more or less structurally similar to methotrexate, which is a competitive inhibitor of dihydrofolate reductase (DHFR). It is to enable the finding of potential anti-psoriatic drugs. We determined the energetically favored docking sites for the inhibitors by using AutoDock 4.0. Our docking results explain that the numbers of clusters with each ligand vary in the range from three to ten for enzyme (Table 2), indicating that the binding specificity of each ligand is varying in DHFR. In this study, the H-bonds made important contributions to the interactions between ligand and enzyme.

From the frequency of residue’s occurrence in the formation of hydrogen bonding, it is evident that active site amino acids play an important role due to the formation of both H-bond donor and acceptor. Ile16, Asp21, Glu30, Thr56, Arg70, Val115 and Tyr121 take part in the hydrogen bonding with comparatively high frequency. Tyr121 is of great concern in anti-psoriasis drug design, which is believed to be one of active site member in DHFR. It is significant to have this Tyr121 locked such that its function is depressed. This information could be supportive for new drug design for psoriasis, in which these potential inhibitors should interact strongly with above mentioned residues. In our study on the basis of minimum binding energy and inhibition constant we concluded that β-santalol was found to be best. β-Santalol interacted with DHFR and gives minimum binding energy - 6.85 Kcal/mol and inhibition constant 9.48 µm, it is also shown H–bond interaction with the target (Table-2, fig-5B). Therefore the above mentioned inhibitors will block the reduction of dihydrofolic acid to tetrahydrofolic acid and inhibit the synthesis of nucleic acid precursor.
Fig. 3. Two dimensional molecular structures of the ligands/inhibitors- (A) Carvacrol (B) β–Santalol, (C) Ferulic Acid, (D) Quercetin (E) Curcumin

Fig. 4. Dihydrofolate Reductase in rainbow color ribbon model. Image obtained from PDB.
Fig. 5. Docking Pictures of Target and Ligands. A) Carvacrol, B) β-Santalol, C) Curcumin, D) Ferulic Acid, E) Quercetin.

Note: The green dot lines denote the H-bonds. All the amino acid residues which involved in molecular interaction are shown in wire frame model and colored by atom types in which hydrogen is colored white, carbon gray, oxygen red, nitrogen blue, and sulfur yellow. Ligands are shown in wireframe model with rainbow colors.

5. References


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