Ezetimibe Repurposing: An In-Silico Testing of its Potential Anti-giardia Activity

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Ezetimibe Repurposing: An In-Silico Testing of its Potential Anti-giardia Activity

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Abstract
Giardia leads to human parasitic disease, and it is highly prevalent in the developing countries. The current medication used to treat giardia is associated with numerous side effects. Moreover, the problem of the emerging giardia resistance given the limited number of anti-giardia agents. Ezetimibe is a cholesterol lowering agent, and it acts to inhibit cholesterol absorption. Giardia needs cholesterol for its survival. It is also known that Giardia depends on external sources of cholesterol. Interfering with the cholesterol uptake pathway might be a promising approach in Giardia therapeutics. Searching for new potential therapeutic targets and agents was the main objective of this research. The hypothesis we proposed is that Ezetimibe might have an inhibitory and/or killing effect on Giardia. Our aim was to test in-silico the potential anti-giardia activity of this drug and the possible targets. Literature as well as public databases search was done to find possible targets for Ezetimibe in Giardia. 3D Models for the found potential targets were generated using comparative modeling software. Models refinement and assessment were also done. Using Surflex-dock the possible binding of Ezetimibe to the giardia targets was tested. We found that a putative Lecithin-cholesterol acyltransferase (LCAT) and a putative Giardia lamblia low-density lipoprotein receptor related protein could be possible targets for this drug. The docking score on LCAT was fair. By binding to one or both of these proteins, Ezetimibe might be able to affect Giardia viability. In vitro validation of these findings is yet to be done.

1 Introduction

Giardia lamblia (aka: Giardia intestinalis, Giardia doudenalis) is an intestinal parasite of worldwide distribution with different prevalence and incidence numbers. In 2004, Giardiasis enlisted in the WHO neglected diseases initiative1. According to Centers for Disease Control and Prevention, in the developed countries, where hygiene and water systems are fairly set up, Giardia infections reach up to 2% in adults and 8% in children while a 33% of people in developing countries are infected with Giardia2.

Recently a number of studies conducted to investigate the prevalence of intestinal parasites among food handlers in many countries. In Sudan3 and western Iran4, Giardia was the second most prevalent parasite while in Turkey5, Jordan6 and northern Iran7 it was the most prevalent parasite with a percentage of >50% of food handlers in Northern Iran.

Many chemotherapeutic agents have been used to treat giardiasis like 5-nitroimidazole and others8,9, but their side effects represent great limitations10. Metronidazole is considered a first choice drug, but the issues of toxicity and the emerging parasite resistance are two big concerns11. Although the relationship of Metronidazole - cancer has not been yet proved to be cause-effect, but the risk is alarming12,13.

Giardia is known to be highly dependents on external sources for lipids14-16 and cholesterol17,18. Cholesterol is also important for Giardia encystation19. What is more interesting was the finding that the usage of fibrates (Fenofibrate) which is thought to increase bile cholesterol content has resulted in having the giardia being able to colonize the colon20. Another interesting research findings relevant to this point was that Terbinafine is effective against Giardia21. Terbinafine is an inhibitor of the squalene mono-oxygenase; a key enzyme in cholesterol synthesis.
Here we are in line with the idea of limiting the amount of cholesterol available to Giardia might be a better option to treat Giardiasis. We also thought of the cholesterol lowering agents that are available and approved for human use. In this work, we selected Ezetimibe (C23H24F2N2O2) which is a well-known cholesterol absorption inhibitor22-24.

1.1 Ezetimibe receptor

Research on Ezetimibe’s blockade mechanism of cholesterol absorption in human found many receptors to be as targets for this molecule. Niemann-Pick C1-like 1 (NPC1L1) is known to be involved in cholesterol absorption and many research results concluded that Ezetimibe exerts its cholesterol lowering action through interfering with this pathway25-27. Some researchers28 pointed out that many other receptors but not NPC1L1 are involved in cholesterol absorption. Scavenger receptor class B member 1 (SR-BI) is also known to play a bigger role in cholesterol absorption and also found to be sensitive to Ezetimibe blocking29. Werner Kramer et al30 found Aminopeptidase N (CD13) to be the molecular target to Ezetimibe. Similarly, lecithin-cholesterol acyltransferase (LCAT) activity has been found to be decreased by Ezetimibe31.

1.2 Giardia cholesterol absorption

M. R. Rivero et al. identified the Giardia protein GL50803_113565 to be a putative Giardia lamblia low-density lipoprotein receptor related protein (LRP)15. They named it as GILRP. They found that this protein is involved in intracellular lipoprotein uptake.

2 Materials and Methods

The work was completely achieved in-silico; the materials used are mainly software, web servers and databases. More explanation is to be mentioned in the relevant points.

To find an in-silico evidence for the potential anti-giardia activity of Ezetimibe, we had to search for possible targets. We followed two strategies for finding targets. One strategy was to search for Giardia proteins that are homologous for Ezetimibe’s targets in human. We searched for all the possible targets that are known to be acting as Ezetimibe receptors in human. The sequences of these receptors (proteins) were obtained from the NCBI database.

Two Giardia assemblages; assemblage A and assemblage B is known to infect human. PSI-BLAST32 was employed to search for the Giardia homologs of these proteins in these assemblages. The scoring matrix set to BLOSUM50 and the remaining parameters as default.

The second strategy was to search in the literature for protein that is known to be involved in cholesterol uptake.

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2.1 Models prediction, refinement and assessment

The protein sequences in FASTA format were retrieved from the NCBI (National Center for Biotechnology Information) [http://www.ncbi.nlm.nih.gov/].

I-TASSER23 was employed for model and binding site prediction. Rampage34 was used for structure validation and Chiron35 which employs discrete molecular dynamics simulation to resolve steric clashes was used for models’ refinement. For GL113565, we did also topology prediction using TOPCONS36.

2.2 Docking

Docking was performed using the molecular modeling suite; Sybyl-x 2.1.1. The docking mode was set to Surflex-Dock GeomX. The binding sites were based on research findings in the literature as well as prediction. For LCAT, amino acids predicted by I-TASSER to be probably involved in binding sites were used for the docking of Ezetimibe.

For GILPR, researchers15 have identified two possible regions in the protein to be as the binding sites R1 (Amino acids 134 to 151) and R2 (Amino acids 178 to 200). We used them in addition to those predicted by I-TASSER. We have used these amino acids residues to generate the protomol; which is a specified space for docking i.e. binding site37.

3 Results

Previous research found that four human proteins25,26,30,31,38 are known to be acting as targets or their activity is affected by Ezetimibe. These are: NPC1L1, SR-BI, Amino peptide N (CD13), and LCAT.

With the search setting illustrated in the method (default PSI-BLAST with the scoring matrix set to BLOSUM50), we found that Giardia has no homologous proteins to NPC1L1, SR-BI, and Amino peptide N (CD13). Only it has homologous protein to human LCAT. In the literature15, Giardia protein GL50803_113565 is found to have a role in lipoprotein endocytosis. The model generated is illustrated in figures 1 and 2, and the topology is in figure 3. The result of models assessment and validation based on Ramachandran plot analysis is presented in Table 1.

Docking scores for Ezetimibe in both of the two models’ different binding sites have been illustrated in Tables 2 and 3. 2D depiction of the binding poses of the highest scores in both of the protein is shown in figures 5 and 6. Figure 7 is showing 3D binding of Ezetimibe on LCAT.
Estimated TM-score = 0.64±0.13;

*TM-score: Template modelling score.

Fig 1: 3D model of LCAT. The figure shows the 3D structure model of LCAT as predicted by I-TASSER

Estimated TM-score: 0.59±0.14

Fig 2: 3D Model of GILPR. The figure shows the 3D structure model of GILPR as predicted by I-TASSER

Table 1: Ramachandran plot analysis of the two proteins models before and after energy minimization

<table>
<thead>
<tr>
<th>Ramachandran Plot analysis</th>
<th>I-TASSER model for GILPR</th>
<th>I-TASSER model Lecithin-cholesterol acyl transferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Refinement</td>
<td>After Refinement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of residues in favored region (~98.0% expected)</td>
<td>63.1%</td>
<td>73.7%</td>
</tr>
<tr>
<td>Number of residues in allowed region (~2.0% expected)</td>
<td>26.7%</td>
<td>19.3%</td>
</tr>
<tr>
<td>Number of residues in outlier region</td>
<td>10.2%</td>
<td>7.0%</td>
</tr>
</tbody>
</table>
Fig 3: Topology prediction of GILPR. The figure illustrates the topology prediction of GILPR with only one transmembrane i.e. single-spanning membrane protein (TM-helix (OUT->IN)). The prediction is almost in total agreement with 5 other predictions (OCTOPUS, Philius, PolyPhobius, SCAMPI, and SPOCTOPUS). Apparently there is no detectable homologous transmembrane regions in the PDB.

Table 2: Docking score of Ezetimibe in 5 binding sites in lecithin-cholesterol acyltransferas

<table>
<thead>
<tr>
<th>Docking site (Amino acids residues involved in binding sites)</th>
<th>Docking score</th>
</tr>
</thead>
<tbody>
<tr>
<td>149,150</td>
<td>5.9080</td>
</tr>
<tr>
<td>134,195,200</td>
<td>4.5959</td>
</tr>
<tr>
<td>208,211,213,216,218</td>
<td>3.6072</td>
</tr>
<tr>
<td>162,163</td>
<td>3.5836</td>
</tr>
<tr>
<td>350,368</td>
<td>3.0204</td>
</tr>
</tbody>
</table>

Table 3: Results of docking score of ezetimibe in GILPR

<table>
<thead>
<tr>
<th>Docking site (Amino acids residues involved in binding sites)</th>
<th>Docking score</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1: 134-151</td>
<td>4.3956</td>
</tr>
<tr>
<td>155,169,178</td>
<td>4.1928</td>
</tr>
<tr>
<td>R2: 175-200</td>
<td>3.5307</td>
</tr>
<tr>
<td>363,365,370,372,380,381,382</td>
<td>3.3899</td>
</tr>
<tr>
<td>152,175</td>
<td>2.5488</td>
</tr>
</tbody>
</table>

4 Discussion

Generally, it is widely acknowledged that 3D models for target proteins of ≤ 30% sequence identities (Twilight zone) to the template are not highly reliable and do not qualify for further studies like docking. But I-TASSER predicted models for twilight zone proteins have recently been found to produce docking results similar to those produced when using crystallographic structure. Moreover, TM-Score of the two models is above 0.5 which means the predicted models have similar folds to those in the PDB.

The refinement of the models through energy minimization resulted in fair improvement of the overall quality of the models.

The topology of GILPR indicates that this protein has one transmembrane which is in agreement with the general topology of LRP (Low density lipoprotein receptor-related proteins) as predicted by M. R. Rivero. But at the same time it is not in agreement with the tertiary structure model predicted by I-TASSER. This shows that this protein needs more research to fully be characterized.

Many binding sites were investigated for docking Ezetimibe as shown in table 2 and table 3 for LCAT and GILPR, respectively. The docking scores were not high enough to indicate a good binding or a biological activity except for only one site. This site is the area of amino acid residues 149 and 150 in LCAT, into which the docking score was 5.9080.
Fig 4: Chiron clash energy minimization summary; where red represents the class energy for the modelled structure, the green is for the final structure after minimization and the black is for a set of high resolution structures.
5 Conclusions

From this work, Ezetimibe might have anti-giardia activity probably through binding to LCAT. In vitro testing of this action is currently on going, moreover other pharmacologically related molecules will also be tested. With regard to cholesterol uptake in Giardia; though not yet fully understood but targeting this pathway might help produce more effective better-tolerated Anti-giardia drugs.

6 Acknowledgment

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7 Competing interests

We have no conflict of interest.

8 Author’s contributions

RA participated in the design of the study, carried out the in-silico analysis, interpreted the results, and prepared the manuscript. FF participated in the design of the study and was responsible for its coordination, interpreted the results independently of the first author, and contributed in the preparation of the manuscript. All authors read and approved the final manuscript.

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