Computer Aided Drug Designing for the Disease Cholera

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Abstract:
Cholera is caused by infection of the large intestine by vibrio cholera bacteria, which secrete a toxin that inflames the large intestine and prevents it from reabsorbing water. The illness often is mild, but it can be severe. In about 1 out of 20 cases, severe diarrhea and vomiting lead to rapid loss of water from the body, known as dehydration without treatment death can occur within hours. The protein involved in pathogenesis of the disease were identified and appropriate target was selected Protein APAG was selected as target. The sequence of the protein structure was retrieved and Homology modeling was also performed. The possible ligand molecule for the protein target was chosen from the drug bank and medicinal plant compounds ligand structure was also designed. The target and ligand docking studies was done and based on the studies it was concluded that natural compound CONESSINE HYDROBROMIDE (Holarrhena Antidysentrica) showed better docking than synthetic drug ligands. Pencilamine, caffeine, lapachol, curcumin and apomorphine are the docked ligands. Pencilamine and apomorphine was found to be effective natural drug than other for cholera disease.

Keywords: Conessine hydrobromide, Apag protein, Holarrhena dysentrica, Homology modeling, Docking, vibrio cholera.

I. INTRODUCTION

Robert Koch (1843-1910) enjoyed worldwide fame, including acknowledgement of his discovery in 1882 of the tubercle bacillus that caused tuberculosis and in 1884 the cholera bacillus, Vibrio cholera which cause the disease cholera. Cholera is an infectious disease that causes severe watery diarrhea, which can lead to dehydration and even death if untreated. It is caused by eating food or drinking water contaminated with a bacterium called Vibrio cholera. Cholera has been nicknamed the “blue death” because a person’s skin may turn bluish-gray from extreme loss of fluids. Modern sewage and water treatment have virtually eliminated cholera in industrialized countries. But cholera still exists in Africa, Southeast Asia and Haiti. (Feb 1, 2020) The disease is most common in places with poor sanitation, crowding, war, and famine. Common locations include parts of Africa, south Asia, and Latin America.(www.WHO.int) Dr. MINTZ: Well, boiling water is a very effective way to disinfect the water. And it will not only kill Vibrio cholera, the bacteria that causes cholera, but it's a right way to make sure your water is free of any pathogen, any living organism that could cause infection or illness. Rhinelander, suggested in July of 1832 that cholera could be treated by the infusion of saline solutions into the victim's veins. Such a treatment along with a regimen of attendant antibiotics is the preferred therapy for modern cholera victims. When treated early the fatality rate is very low. There's a vaccine for cholera, but most people don't need it. It's usually only recommended if either: you're travelling to an area where cholera is common and you'll be visiting remote places without access to medical care.Feb 18, 2020 Areas affected by cholera epidemics. In 2015, cholera outbreaks were reported in several African countries notably in Democratic Republic of the Congo (DRC), Kenya, Malawi, Mozambique, Nigeria, Somalia, South Sudan, and Tanzania. The outbreak in DRC affected 32 districts.(www.cdc.gov) In 1849, a cholera epidemic that was sweeping through Britain reached West Riding Asylum in Wakefield, West Yorkshire. The deadly disease soon spread through the wards. Searching for the source of the outbreak, over a century and a half later, a care-home owner in Devon – alarmed by the fact that local care homes could admit residents with COVID-19 – expressed his fears in a strikingly similar way. Care homes are the epicenter of the COVID-19 pandemic in the UK. Compared to all other settings, they have seen the biggest relative increase in deaths since the start of the outbreak(www.who.int).

Figure 1. vibrio cholera bacteria
SYMPTOMS

- Dry mouth
- Dry skin
- Abdominal cramps
- Excessive thirst
- Low urine output.
- Leg cramps
- Dehydration
- Diarrhea has a “fishy” odor

DIAGNOSIS

Cholera is diagnosed by checking a person’s stool for VIBRIO CHOLERAE bacteria.

TREATMENT

People with cholera need fluids and salt to replace those lost through diarrhea and vomiting. The person may be asked to drink large amounts of a solution that is made from a prepackaged mixture of sugar and salts mixed with water. If the person is too sick to drink, fluids can be given intravenously. Without such treatment, severe cholera can kill up to half or more of those who become ill. With prompt treatment, however, 1 percent of people with cholera die. Sometimes antibiotic medications are given as well to decrease the length and severity of the illness.

PREVENTION

Cholera is usually transmitted through contaminated water or food. Outbreaks can occur in any part of the world where water supply, sanitation, food safety, and hygiene are inadequate.
- Drink only boiled water or water that has been treated with chlorine or iodine.
- Eat only thoroughly cooked food and are still hot, or fruit that you have peeled yourself.
- Avoid undercooked or raw fish or shellfish.
- Make sure all vegetables are cooked properly, avoid salads.
- Avoid foods and beverages from street vendors.
- Give liquid bland foods, lemon, onions and mint to the patient.
- Vegetables and fruits must be washed with solution of potassium permanganate.
- New Vaccines for cholera are available and appear to provide a somewhat better immunity and fewer side-effects than the previously available vaccine.
- Health education aimed at behavior change is thus an important component of cholera prevention and control.

GENERAL DRUGS FOR THE DISEASE CHOLERA

General Drugs Preferred For the Disease Cholera

1. Osalazine (Dipentum)
2. Selegiline (Eldepryl)
3. Cymetine (Emsam)
4. Penicillamine (Zelapar)

Figure 2. General drugs

FEATURES ABOUT SELECTED PROTEIN

ROLE OF ENVELOPE PROTEIN APAG IN CHOLERA

Thus the protein involved in the cholera sequence was identified.

RETRIEVAL OF PROTEIN SEQUENCE SWISSPROT

SWISSPROT is a database which strives to provide a high level of annotation (such as the description of the function of a protein, its domains structure, post-translational modifications, variants, etc.), a minimal level of redundancy and a high level of integration with other databases.

Figure 3. Apag protein sequence

In figure 3 the sequence for protein named PROTEIN APAG involved in disease cholera was retrieved from SWISS—PROT database.

HOMOLOGY MODELING

Homology modeling, also known as comparative modeling of protein, refers to constructing an atomic-resolution model of the “target” protein from its amino acid sequence and an experimental three-dimensional structure of a related homologous protein (the “template”). Homogenous modelling is likely to be useful in modelling ligand docking (drug design).

SWISS PDB VIEWER

Swiss-PdbViewer (aka Deep View) is an application that provides a user friendly interface allowing to analyze several
proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. SwissPdb viewer is tightly linked to Swiss model, an automated homology modelling (Drug designing tool).

**GROMOS 43 B1**

GROMOS™ is an acronym of the GROningen MOlecular Simulation computer program package, which has been developed since 1978 for the dynamic modelling of (bio)molecules. GROMOS 43 is a force field version which helps in evaluating the energy of a structure as well as repairing the molecule through energy minimization.

**RAMACHANDRAN PLOT**

Ramachandran plot was originally developed in 1963 by G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan, is a way to visualize energetically allowed regions for backbone dihedral angles \( \psi \) against \( \phi \) of amino acid residues in protein structure.

**MODEL VERIFICATION**

WHATCHECK is a protein verification tool of WHATIF. WHATIF is a versatile protein structure analysis program that can be used for mutant prediction, protein model verification, molecular graphics etc. After modeling the quality of the protein was also checked. Bond length and bond angle for the protein APAG was also analyzed using WHATCHECK.

**PROCHECK**

Procheck is a program for accessing the “stereo chemical quality “of a given protein structure. The aim of the procheck is to access the normal or the unusual geometry of the residues in the given protein structure compared to the stereo chemical parameters derived from the well-defined high resolution structures. The input to the procheck is a single file that contains the co-ordinates of our protein structure. The output comprises a number of plots, together with a detailed residue-by-residue listings, main chain properties, disordered geometry ad planar groups. SAVES is a structure analysis and verification server is a metaserver for analyzing and validating protein structures. It provides various verification programs like WHAT-CHECK, ERRAT, VERIFY-3D, PROVE.

**Amino acid residue involved in protein**

**Figure 5. Ramachandran plot**

In Figure 5 the energy of the protein was minimized and a refined structure of the receptor was obtained.

**Figure 6. Model verification**

In figure 6 the Protein Model was verified using WHATCHECK.

**Figure 7. Score Chart**
Based on the above result, figure 7 represents that the selected protein APAG have a good quality function and 90% of the most favored region.

CHI1-CHI2 PLOTS
The Chi1-Chi2 plots show the chi1-chi2 side chain torsion angle combinations for all residue types whose side chains are long enough to have both these angles. The shading on each plot indicates how favorable each region on the plot is; the darker the shade the more favorable the region.

SIDE, MAIN CHAIN PARAMETERS:
Protein side chains help to stabilize the tertiary structure of a protein.

BOND LENGTH AND BOND ANGLE
The bond length is the distance between the two nuclei that are connected to each other. It changes based on hybridization, number of bonds, type of bond, and size of atoms. The bond angle is the angle between two bonds on the same atom. If the bond length and bond angle of the protein was low it will affect the stability of the protein.

Bond length and Bond angle was found to be good for the predicted protein APAG.

SELECTION OF LIGAND
A ligand is an ion, a molecule or a molecular group that binds to another chemical entity to form a larger complex that can be
described in terms of non-covalent interactions and the nature of the orbitals used in bond formation. When a protein binds to another molecule, the molecule may be referred as ligand. The site where the ligand is bound is known as the binding site or active site of the protein. In order for a molecule to be classified as a ligand for a protein, several weak interactions such as hydrophobic, Vanderwaals, and hydrogen bonding must take place simultaneously. Therefore the binding of a ligand by a protein is generally quite specific.

**SELECTION OF NATURAL DRUGS**

1) Using DRUGBANK five natural drug compounds were selected for the disease cholera.

2) It’s chemical structure, description, molecular weight, IUPAC name, physical and chemical properties for the selected drug molecules was also analyzed.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>INHIBITOR</th>
<th>MOLECULAR WEIGHT</th>
<th>MOLECULAR FORMULA</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>CAFFEINE</td>
<td>200.24</td>
<td>C_{8}H_{11}N_{2}O_{3}</td>
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<tr>
<td>2</td>
<td>CURCUMIN</td>
<td>398.54</td>
<td>C_{13}H_{20}N_{2}O_{4}</td>
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<tr>
<td>3</td>
<td>LAPACHOL</td>
<td>335.60</td>
<td>C_{14}H_{14}O_{9}</td>
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<tr>
<td>4</td>
<td>PENICILAMINE</td>
<td>210.654</td>
<td>C_{6}H_{12}N_{6}O_{4}</td>
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<tr>
<td>5</td>
<td>APOMORPHINE</td>
<td>225.2046</td>
<td>C_{8}H_{11}N_{3}O_{5}</td>
</tr>
</tbody>
</table>

**Table 1. Selected Drug Compounds**

The inhibitor is a substance which slows down or prevents a particular chemical reaction or other process or which reduces the activity of a particular reactant, catalyst, or enzyme (www.sciencedirect.com). There are two kinds of inhibitors, reversible and irreversible inhibitors. Reversible inhibitors slow down a chemical reaction, but do not stop it completely. Irreversible inhibitors prevent an unwanted reaction from occurring (www.pubmed.com).

**Table 2. Selected Natural Drug**

The inhibitor is a substance which slows down or prevents a particular chemical reaction or other process or which reduces the activity of a particular reactant, catalyst, or enzyme (www.sciencedirect.com). There are two kinds of inhibitors, reversible and irreversible inhibitors. Reversible inhibitors slow down a chemical reaction, but do not stop it completely. Irreversible inhibitors prevent an unwanted reaction from occurring (www.pubmed.com).

Two natural inhibitors were selected to find the active site present in the APAG protein.

**APAG PROTEIN STRUCTURE**

The apap domain is a ~125 amino acids domain present in bacterial apapG proteins and in eukaryotic F-box proteins. The domain is named after the bacterial apapG protein, of which it forms the core. The domain also occurs in the C-terminal part of
eukaryotic proteins with an N-terminal F-box domain. The Salmonella typhimurium apaG domain protein corD is involved in Co (2+) resistance and Mg (2+) efflux. Tertiary structures from different apaG proteins show a fold of several β-sheets (see The apaG domain may be involved in protein-protein interactions which could be implicated in substrate-specificity. apaG protein (CHAIN-A and CHAIN-B) have only one glycerol molecule so that cholera causing vibrio cholera bacteria cannot grow. In general glycerol was used to grow bacteria. Figure 18, 19 and 20 represents crystal structure of protein, sequence analysis, identity, annotation and presence of glycerol molecule was also found. (NCBI database)

Figure 18. protein structure (CHAIN-A and CHAIN-B)

Figure 19. protein sequence analysis

Figure 20. Sequence Annotation

IDENTIFICATION OF ACTIVE SITE

WHAT IF

WHAT IF is a versatile protein structure analysis program that can be used for mutant prediction, structure verification, molecular graphics, active site prediction etc.

ACTIVE SITE

The active site is the region of an enzyme where substrate molecules bind and undergo a chemical reaction. The active site consists of amino acid residues that form temporary bonds with the substrate (binding site) and residues that catalyse a reaction of that substrate (catalytic site). (www.webmd.com)

Figure 21. Active Site

ACTIVESITE OF MODELED PROTEIN

The amino acids present in the active site of the protein was identified using WHATIF interface.

The amino acids present in the active site of ‘protein apag’ was found to be GLU73, HIS29, LEU41, LEU42, LEU43, GLU44, TRP45, ARG9, VAL91, ALA92, SER93, THR94, GLN97, SER34, ASP12, MET45, GLN234, GLU132, LEU110, GLY111, PHE119, GLY120, LEU121, ILE122, LEU123, HIS124, ASP125, GLY126, VAL144. Figure 22 represents the active site of protein.

Figure 22. Active site of protein

DOCKING OF MODELED PROTEIN WITH LIGAND

Docking the modeled protein with the appropriate ligand using ARGUSLAB software.

ARGUSLAB

The Argus Lab molecule builder allows to construct new molecules and to modify existing molecules. It is a very useful, highly featured and easy to use molecular modelling, graphics and drug design program. The program contains two docking engines and a simple scoring function, based on an enhancement
of the x-score method. Figure 23 and 24 represents the docking of a modeled protein with natural drug penicillamine and apomorphine.

**Figure 23. Ligand protein docking (natural drug penicillamine)**

**Figure 24. Ligand protein docking (natural drug apomorphine)**

**Docking Score for Apomorphine and Pencillamine**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Score 1</th>
<th>Score 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apomorphine</td>
<td>-6.23 kcal/mol</td>
<td>-6.07 kcal/mol</td>
</tr>
<tr>
<td>Pencillamine</td>
<td>-6.22 kcal/mol</td>
<td>-6.17 kcal/mol</td>
</tr>
</tbody>
</table>

**Table 4. Docking score.**

**Visualization of Docked Drug Molecule**

**Swiss-pdb viewer**

Swiss-pdb viewer is used to visualize the docked drug molecule. It is a user-friendly interface allowing several proteins at the same time. The amino acid mutation, h-bonds, angle and distance between two atoms were easily obtained. Based on the docking score best ligand was selected. The docked molecule was visualized and the receptor-ligand binding site was also studied. Figure 25 Active site of penicillamine and amino acid residues Figure 25 and 26 represents the docked natural drug compound and the amino acid residues involved for docking.

**Figure 25 Active site of penicillamine and amino acid residues**

**Figure 26. Apomorphine active site and amino acid**

**Protein-Ligand Docking**

**Protein–ligand docking** is a molecular modelling technique. The goal of protein–ligand docking is to predict the position and orientation of a ligand (a small molecule) when it is bound to a protein receptor or enzyme.[1] Pharmaceutical research employs docking techniques for a variety of purposes, most notably in the virtual screening of large databases of available chemicals in order to select likely drug candidates. (Wikipedia)

**Figure 27. Ligand pose energy (apomorphine)**

**Figure 28. Ligand pose energy (Penicillamine)**

Figure 27 and 28 represents the ligand and drug bonding site with score (Arguslab software).

**Conessine Hydrobromide**

Conessine is a plant steroid alkaloid that acts as a potent and specific antagonist of histamine H3 receptors. Conessine displayed high affinity at both rat and human H3 receptors (pKi = 7.61 and 8.27) and generally high selectivity against other sites, including histamine receptors H1, H2, and H4. Conessine was found to efficiently penetrate the CNS and reach very high.
brain concentrations. Although the very slow CNS clearance and strong binding to adrenergic receptors discouraged focus on conessine itself for further development, its potency and novel steroid-based skeleton motivated further chemical investigation. Modification based on introducing diversity at the 3-nitrogen position generated a new series of H3 antagonists with higher in vitro potency, improved target selectivity, and more favorable drug-like properties. Conessine also has high affinity for the adrenergic receptors. Conessine has been shown to possess anti-malarial activity. In India conessine finds therapeutic use for treatment of dysentery and helminthic disorders.(https://drugs.ncats.io/drug/N9EQE108I5).

**HOLARRHENA ANTIDYSENTERICA**

*Holarrhena antidysenterica* (L.) Wall. Ex A. DC. Is a medicinal plant abundantly found in India. Its uses are mentioned in the classical Ayurvedic literature and by many folklore claims. The plant is also of extreme economic importance. Its seeds are mainly used as an antidiabetic remedy. It is also used to treat jaundice, dysentry, diarrhea.(https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5628520/).

Figure 29 and 30 represents the target protein docking with the natural drug compound shows good docking score.

**II. RESULTS AND DISCUSSION**

Cholera is caused by infection of the large intestine by vibrio cholera bacteria which secrete a toxin that inflames the large intestine and prevent it from reabsorbing water. The disease leads to rapid loss of water from the body known as rehydration without treatment death can occur within hours. The protein involved in pathogenesis of the disease were studied and the appropriate target was selected protein Apag was selected as target. The possible ligand molecules for the protein target was chosen from drug bank and five compounds were selected two natural drug and three synthetic drugs. The docking scores for synthetic and natural compound interaction were analyzed based on the docking scores it was concluded that the natural compounds penicilamine and apomorphine have a good docking score when compared to other three compounds. So, when Apag protein combined with penicilamine and apomorphine can be used to treat cholera. The natural compound CONESSINE HYDROBROMIDE (*Holarrhena Antidysenterica*) also shows better docking result when compared to synthetic drug. So, the natural compound conessine can also be used treat cholera.

**III. REFERENCES**

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