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Docking of calcium-binding protein 1 of *Entamoeba histolytica* using FDA approved drugs

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ABSTRACT

Objective: To find out an alternative potential inhibitor of *Entamoeba histolytica* calcium-binding protein 1 (EhCaBP1) through *in silico* studies.
Methods: An attempt was made to find a new FDA approved, and cost effective alternative drug for amoebiasis. Sequence of the EhCaBP1 of *Entamoeba histolytica* was obtained through searching the UniProt database and protein BLAST was performed. The 3D structure of EhCaBP1 was retrieved from Research Collaboratory for Structural Bioinformatics and visualized using Discovery Studio Visualizer® 3.1. A total of 100 drugs were selected and docked using Patchdock and the different sorts of interactions with the target protein were studied using GS viewer, Ligplot and Discovery Studio visualizer.
Results: Among the 100 selected drugs, dolutegravir, cefazedone and ergotamine showed large number of interactions with the target protein.
Conclusions: The drugs cefazedone and ergotamine showed twenty and nineteen different sorts of interactions respectively with the target protein. These interactions may lead to metabolic changes and can subsequently stop the growth and cause the death of the parasite. Further investigations and experimental analysis are required to unveil the effects of these drugs.

1. Introduction

Entamoeba histolytica (*E. histolytica*) is a dysentery-causing protozoal parasite which infects a large number of vertebrates including monkeys, dogs, pigs, rats as well as human[1-3]. The infection caused by *E. histolytica* is the third major parasitic disease responsible for approximately 10 million deaths per year[4,5]. According to a recent research report, each year 50 million new cases of invasive amoebiasis occur globally[3,6]. The free living amebae is considered as a facultative opportunistic parasitic pathogen[1] which causes dysenteric stool, diffused abdominal pain, high fever and severe dehydration[3,7]. Dysentery, both bacterial and amoebic, is one of the most destructive diseases[8].

Amoebiasis is a cosmopolitan disease highly prevalent in Africa, Asia, India, South America and Mexico. Although these regions have improved their living conditions and level of sterilization, till now this disease is a major public health problem[9]. Infections caused by *E. histolytica* are more prevalent in children as compared to adults, and a study conducted in Bangladesh shows that at least 80% of children are infected with this parasite[10]. The infection persists in the areas where poverty rate is high[11].

The virulence factor of *E. histolytica* has the ability to destroy tissues through adhesion, host cell killing and extracellular matrix (ECM) proteolysis[12,13]. *E. histolytica* is composed of a specific galactose membrane termed as lectins which have the ability to attach to the host cell and are responsible for the production of host inflammatory response by provoking host cytokine production[8]. People who are new to endemic area are at higher risk to amoebiasis than local population due to differences in bacterial flora[8].

During its life cycle *E. histolytica* passes through several successive stages such as trophozoite, precyst, cyst, metacyst, and metacystic trophozoite[3,8]. *E. histolytica* is anaerobic but can tolerate small amounts of oxygen for short period of time, possibly associated with intestinal bacteria that salvage oxygen[1,14,15]. It can infect a large number of individuals but severe infections develop only in about 10% of the infected individuals[12].

Initial infection starts usually after two to six weeks of the ingestion of metacystic form via fecal-oral route. Acute infection

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leads to malaise, weight loss, severe abdominal pain, profuse bloody diarrhoea and fever, often leading to a misdiagnosis of appendicitis, especially in children[3,6,16]. Amoebic liver abscesses are formed because of toxin release and hepatocyte damage, which usually develop within five months after infection[3,17].

E. histolytica is capable of causing human amoebiasis when exposed to both glucose-poor environment in the colon and glucose-rich environment in liver[18]. Phagocytosis is required for the pathogenicity caused by *E. histolytica*. Ca^{+2} plays a vital role in the process of phagocytosis by interfering with cytoskeletal changes, affecting the actions of a number of contractile proteins[19]. During glucose starvation, *E. histolytica* down-regulates the expression of glycolysis related gene and uses amino acid as a source of energy[18]. To cause amoebic colitis, G-protein and EhCaBP1 play a vital role as signalling pathway[20]. Calcium (Ca^{+2}) is one of the most important and unique types of intracellular signal, which is responsible for controlling number of cellular processes in wide range. Cells become able to give response to an extracellular stimulus by a transient change in intracellular Ca^{+2} concentration which is in turn sensed by a specific protein called EhCaBP1[21].

E. histolytica requires energy as well as some virulence factors in order to escape the phagocytosis of host cell and intestinal invasion. Virulence factors include cysteine proteases, adherence lectin, and amoebapore pore-forming proteins while EhCaBP1 is responsible for signalling and pathogenesis[12,22]. EhCaBP1 is an essential protein, and down-regulation of its expression blocks proliferation of the parasite[21]. This protein has a variety of functions; for example, it combines with Rho family GTPases and acts as a master regulator of multiple key cellular processes such as cell division, transcription, and most prominently, dynamic reorganization of the actin cytoskeleton of the parasite[20].

Amoebiasis is generally treated with antibiotics such as tinidazole, metronidazole and nitroimidazole[23], but due to longer therapy time and repeated doses, the parasite becomes resistant slowly and gradually[24]. The development of new antiamoebic drug is very important, and vaccine development is required. The current study was conducted to find alternative, FDA approved and cost effective drug for amoebiasis.

2. Materials and methods

2.1. Selection of drugs

In the present study, a total of 100 drugs were selected for docking with EhCaBP1. The drugs were randomly selected from the FDA approved drugs which are currently used for other diseases and not for the treatment of *E. histolytica*.

2.2. Retrieval of protein sequence

Sequence of the EhCaBP1 was obtained through searching the UniProt database[25].

2.3. Running the NCBI protein BLAST

Protein BLAST was done to get all the related information of similar proteins and to select the appropriate and best structure[26]. The BLAST programme was used to understand sequences, evolutionary and functional relationships, and to find out the other members of the same families. The sequence obtained from UniProt was used to search the available 3D structure of *E. histolytica* calcium binding protein and we got it in PDB (accession number: 2NXQ)[27].

2.4. Retrieval of EhCaBP1 structure

The 3D structure of EhCaBP1 was retrieved from Research Collaboratory for Structural Bioinformatics (RCSB). The PDB ID of the selected protein was found to be 2NXQ, and the protein structure was in *pdb* format[28].

2.5. Visualizing the structure of protein by Discovery Studio (DS) visualizer

DS is a comprehensive platform used for examining and displaying molecular structures of proteins, nucleic acids, drugs, sequences, and other data of interest for researchers in biological sciences[29]. For visualizing the 3D structure of protein, DS Visualizer® 3.1 was used.

2.6. GS viewer 4.9

GS viewer is a multipurpose software used in the current study. It provides the key to identify different sorts of interactions of drugs with the target protein such as covalent interactions which are indicated by purple line, hydrogen bonds indicated by green line, and hydrophobic interactions shown by reddish line[30].

2.7. ChemSpider

Over 150 3D structures of drugs from Chemspider were retrieved. ChemSpider is a free chemical structure database providing fast text and structure search, accessing to over 35 million structures from hundreds of data sources[31].

2.8. PatchDock

PatchDock is a geometry-based molecular docking algorithm for small ligand and protein-protein docking[32]. We docked the potential drugs with protein using PatchDock server and then the top solutions were checked one by one in DS Visualizer, GS viewer, and Ligplot to observe the docked amino acids.

2.9. Checking the stereochemistry of ligand protein interaction while using Ligplot

The 2D representation of ligand-protein interactions was analysed by Ligplot to understand the bonding interaction pattern between the docked ligands and the protein amino acids, particularly the active sites residues. Ligplot is an important tool for understanding the hydrophobic interactions, hydrogen bonding interactions and covalent interactions[33].

3. Results

In the present study, 100 drugs were selected for docking with EhCaBP1, in which the following four drugs showed promising results.

3.1. Docking of dolutegravir with EhCaBP1

The docking of dolutegravir with EhCaBP1 showed 13 different kinds of interactions including covalent bonds, hydrogen bonds and hydrophobic interactions.

In our study, four residues (LYS 33, LYS 29A, LYS 29B, GLU 8) formed covalent bonds in which LYS 33 was the active site residue; three (LYS 28, LYS 29, and LYS 33) formed hydrogen bonds. Five hydrophobic interactions including Ile32, Ehe25, Ser27, Val26, Lys33 were also noted, in which LYS 33 was the active site residue (Figure 1).

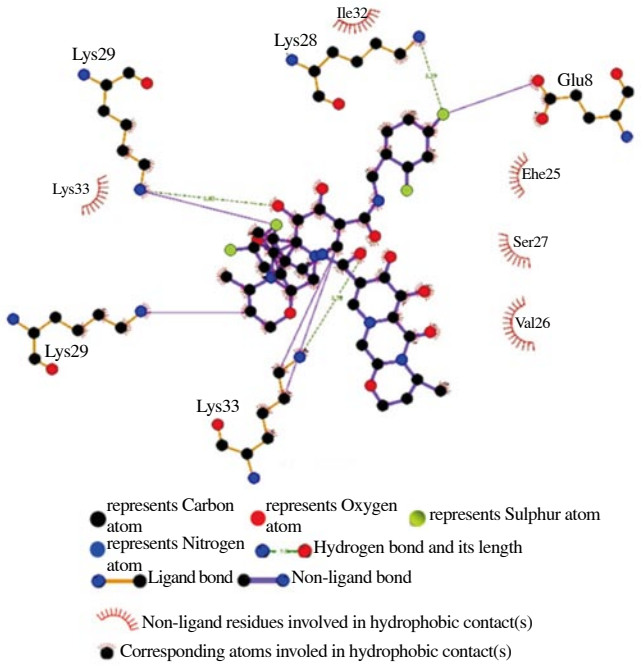

Figure 1. 2D schematic representation of dolutegravir with EhCaBP1 from *E. histolytica*.

3.2. Docking of cefepime with EhCaBP1

In this study, the docking of cefepime with EhCaBP1 showed 16 different types of interactions including covalent bonds, hydrogen bonds and hydrophobic interactions. Two residues (Gln 39, Ile 32A) formed covalent bonds out of which six single covalent bonds were with Gln 39, oxygen, nitrogen and carbon atoms and one with Ile 32. The residue that formed two hydrogen bonds was the active site amino acid (LYS 33). Six hydrophobic interactions (GLU8, Leu5, Phe25, Val26, LYS29, Leu37, Glu35, Leu40) were also noted (Figure 2).

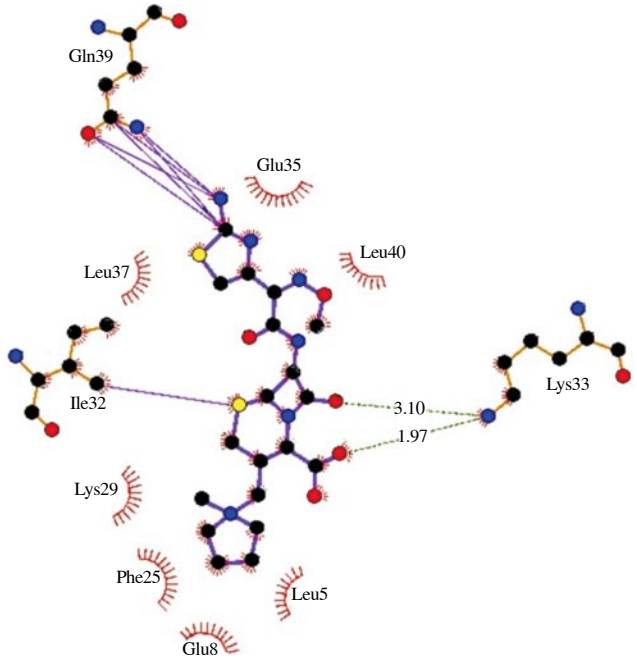

Figure 2. 2D schematic representation of cefepime with EhCaBP1 generated by Ligplot.

3.3. Docking with ergotamine

The docking result of ergotamine with EhCaBP1 showed 19 different sorts of interactions including covalent interactions, hydrogen bonding and hydrophobic interactions. The residues LYS33 and LYS29 formed six and three covalent interactions respectively whereas LYS28 formed one hydrogen bond. In this docking analysis, six hydrophobic interactions (Gln36, Lys33, Ile32, Phe25, Glu819, Lys29) were also noted (Figure 3), in which one (LYS33) was active site amino acid.

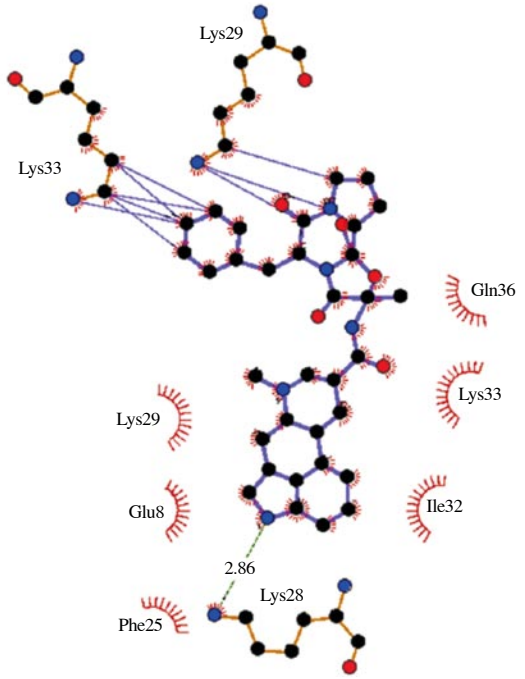

Figure 3. 2D schematic representation of ergotamine with EhCaBP1 generated by Ligplot.

3.4. Docking with cefazedone

Docking result with EhCaBP1 revealed twenty different sorts of interactions including covalent bonding, hydrogen bonds and hydrophobic interactions (Figure 4). Two residues LYS33 and SER44 formed three hydrogen interactions; whereas GLY67 and LEU37 formed nine covalent bonds and four residues (Phe61, Leu40, Ile41, Ile65) showed hydrophobic interactions.

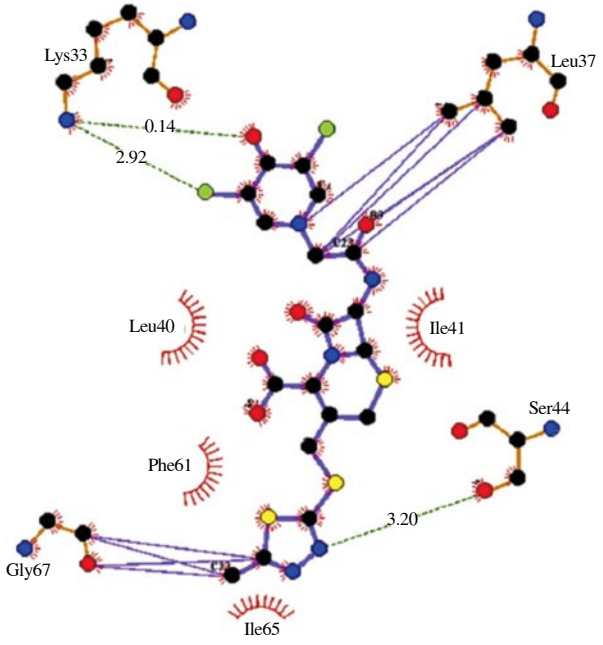

Figure 4. 2D schematic representation of cefazedone with EhCaBP1 generated by Ligplot.

4. Discussion

In this study, we have tried to find out a new FDA approved, and cost effective drug. The drugs which showed promising results included dolutegravir, cefepime, ergotamine and cefazedone.

Dolutegravir is an antiviral drug which is structurally diverse and can bind to various catalytic domains of various proteins[34]. In a study conducted in 2012, this drug was docked with HIV-I integrase and it was reported that it can cause inhibition[35], which is an attractive target for novel anti-AIDS agents. In another study, it was observed that this drug can form different sorts of interactions with Try99, Ala98, Glu170, Thr174, Gln168, and His171[36]. We have observed that dolutegravir can form several interactions with various amino acid residues of the EhCaBP1. To cause pathogenesis in animals and humans, *E. histolytica* requires not only energy but also EhCaBP1 for cell signalling[22]. These huge amount of interactions might bring enough conformational changes which may disturb the normal functions of the protein and the metabolic needs of *E. histolytica*. As a result of these changes cell signalling can be stopped, resulting in the minimal chances of liver amoebiasis, as the parasite can get entry into liver only by the process of cell signalling.

Cefepime is a fourth-generation cephalosporin with a pharmacokinetic profile similar to that of ceftazidime. This drug was previously docked with certain proteins and enzyme groups. The docking results of cefepime with CTX-M-9 showed a variety of interactions including ASP 101, ASN 136, LYS 137, ASP 101, GLU 166, SER 130, ASN 132, THR 235, SER 237, and ASP 240[37]. This drug was also docked with wild type ompF protein and several interactions with the residues were noted among them (D113, D121, E117, R42, R82, and R132)[38]. The changes that are showed by this drug in this present study might bring a lot of conformational changes which may damage the normal structure and functions of EhCaBP1.

Ergotamine is an FDA antipyretic drug which was previously docked with α-glucosidase, a key intestinal enzyme having clinical relevance in the treatment of diabetes mellitus[39].

The drug showed nineteen different sorts of interactions with the target EhCaBP1 which are enough to change the total chemistry of EhCaBP1. This drug also showed promising results with α-glucosidase and can possibly be used as an alternative drug for the treatment of amoebiasis at certain stages of the disease.

Cefazedone is an antibacterial drug, mostly effective against Gram positive bacteria. This drug disrupts the synthesis of the peptidoglycan layer which forms the bacterial cell wall[40]. Cefazedone is a heterocyclic compound which attracts the medicinal chemists due to its unique chemical properties and wide range of biological activities[41]. It is a cephalosporin type antibiotic which works similarly to penicillin and other antibiotics and is most frequently used in hospitals. It has a broad spectrum of activity against both Gram-positive and Gram-negative bacteria. Likewise, it also has good activity against anaerobes[42]. However, our study showed that this drug may be effective in future against enteric protozoan especially against *E. histolytica* and all those protozoan in which EhCaBP1 is present with the function of cell signalling and phagocytosis.

The study aimed to find out a potential inhibitor of calcium binding protein through *in silico* analysis along with the help of online docking tools, using a variety of FDA approved drugs. The drugs showing large number of interactions with EhCaBP1 included cefazedone and ergotamine. The former showed twenty while the latter showed nineteen different sorts of interactions with the selected protein. From the docking results it is evident that the target protein showed enough interactions with the possible drugs that may lead to metabolic changes and subsequently stop the growth and finally cause death of the parasite. Further investigation and experimental analysis are required to unveil the effects of these drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

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