In silico analysis of potential inhibitors of Ca\(^{2+}\) activated K\(^{+}\) channel blocker, Charybdotoxin-C from *Leiurus quinquestriatus hebraeus* through molecular docking and dynamics studies

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**ABSTRACT**

**Objective:** Charybdotoxin-C (ChTx-C), from the scorpion *Leiurus, quinquestriatus hebraeus* blocks the calcium-activated potassium channels and causes hyper excitability of the nervous system. Detailed understanding the structure of ChTx-C, conformational stability, and intermolecular interactions are required to select the potential inhibitors of the toxin.

**Materials and Methods:** The structure of ChTx-C was modeled using Modeller 9v7. The amino acid residues lining the binding site were predicted and used for toxin-ligand docking studies, further, selected toxin-inhibitor complexes were studied using molecular dynamics (MD) simulations.

**Results:** The predicted structure has 91.7% of amino acids in the core and allowed regions of Ramachandran plot. A total of 133 analog compounds of existing drugs for scorpion bites were used for docking. As a result of docking, a list of compounds was shown good inhibiting properties with target protein. By analyzing the interactions, Ser 15, Lys 32 had significant interactions with selected ligand molecules and Val5, which may have hydrophobic interaction with the cyclic group of the ligand. MD simulation studies revealed that the conformation and intermolecular interactions of all selected toxin-inhibitor complexes were stable.

**Conclusion:** The interactions of the ligand and active site amino acids were found out for the best-docked poses in turn helpful in designing potential antitoxins which may further be exploited in toxin based therapies.

**KEY WORDS:** Antidote, homology modeling, inhibitors, ion channel blockers, neurotoxin
otherwise called yellow scorpion, which produce a potent toxin called Charybdotoxin-C (ChTx-C), which greatly affects the Ca²⁺-activated K⁺ channels. It mainly causes the hyperexcitability of the nervous system especially heart beats of eukaryotes by ionic imbalance. Cysteine amino acids are conserved in all neurotoxins from animal origins, which are responsible for stability of the structure and function of toxins. ChTx-C is a small molecular weight protein with 37 residues, and it comes under the category of SCNs.[8] Of all the scorpion venom peptides that have been isolated, margatoxin (MgTx) and hongotoxin (HgTx) are among the most potent for Ca²⁺-activated K⁺ channel blocker (Kv1). It is reported that both the toxins inhibit Kv1.3 with picomolar affinities, whereas ChTx-C which will block only Kv1.3 in nanomolar affinity.[17] Several researches are going around the world in the field of toxins and it helps to design the better antidote for poisonous bites.

Clinically no inhibitor is used to antagonize ChTx-C directly; however, this study hypothesize that, if a molecule that competitively bind with the toxin and thereby reduce the probability of binding of the toxin with the channel and hence the toxin-induced changes or damages caused in the host organism may be reduced. Therefore in this work, computational prediction of protein structure provides reliable results when the suitable selection of the template structure. This research study will help us to identify the function of the ChTx-C and also identify the good inhibitors against yellow scorpion sting.

Materials and Methods

Comparative Modeling and Molecular Dynamics Simulation of Charybdotoxin-C

The three-dimensional structure of the target protein, ChTx-C was searched against structural database, protein data bank (PDB). As a result of structure search, there is no experimentally predicted structure available for ChTx-C, hence comparative modeling approach was employed. The computational prediction of protein structure provides reliable results when the suitable selection of the template structure.[12-14] The ChTx-C protein sequence was retrieved from Uniprot database (Uniprot sequence ID: P59944) (www.uniprot.org/). The sequence was formatted into fasta and template structure was searched using PDBSUM database (www.ebi.ac.uk/pdbsum). Template selection was made by considering percent identity, number of overlapping amino acids, Z-score, etc. Then the sequence alignment was done for template-target protein sequences using ClustalW tool (www.genome.jp/tools/clustalw/). Comparative modeling approach was employed to predict the three-dimensional structure of ChTx-C protein. The modeling of ChTx-C was done by satisfying the spatial restraint using Modeller 9v7 program.[13,14] The quality of the predicted three-dimensional structure was evaluated by analyzing their stereochemical and other structural properties using structure analysis and verification server (SAVES). A $\phi$ and $\psi$ of the predicted structure was calculated using Ramachandran plot of PROCHECK program.[15] As a result, it was found that few outlier amino acids residues were violating Ramachandran plot and present in the disallowed region, they were corrected using energy minimization techniques such as Steepest Descent and Conjugate Gradient. The stability of toxin protein was analyzed using DiAminoacid Neural Network Application (DiAANNA) server, which helps to predict the disulfide (S-S) connectivity patterns.[16] In order to find the atom level information and conformational stability, the predicted model of ChTx-C was allowed to MD simulation using Standard Dynamics cascade program available in simulation module of Accelrys Discovery Studio (ADS) 2.0.

Inhibitors Selection and Molecular Docking Analysis

Analogues of existing drugs used for scorpion bites were taken from the PubChem and Drug bank databases, and analogs search was set the threshold value to 90% similarity with core compounds. As a result of the search produced 133 chemical compounds. All retrieved compounds were used further for docking studies with ChTx-C. Molecular interaction studies were carried out using AutoDock 4.0 and initially, binding site of target protein was identified using Q-site finder and it was a cross checked with binding site prediction tool of ADS 2.0. As a result of binding search nearly ten binding pockets were identified, and best site for molecular docking studies was chosen based on site volume and key amino acids involved in toxicity.

Molecular Dynamics Simulation of Charybdotoxin-C and Inhibitor Complexes

Molecular dynamics simulations are important tools for investigating the physical basis of the structure and function of biological molecules.[17] MD simulation is one of major application used in this study to validate the homology model of predicted ChTx-C and docked complexes of ChTx-C-inhibitor complexes using several protocols of ADS simulation module. The standard dynamics cascad was used for MD simulation and constant-temperature and constant-volume ensemble (NVT) was used to control the temperature during the simulation process. The final result of production was analyzed their structural properties and strength of intermolecular H-bonding using analyze trajectory program of ADS 2.0. All selected best trajectory frames were saved for further structural superimposition of the native structure with simulated final structure using root mean square deviation calculation. Binding affinities and strength of binding of ChTx-C-inhibitor docked complexes were investigated by MD simulations using an all-atom force field with explicit water.

Results

Three-dimensional Structure Prediction and Validation and Molecular Dynamics Simulation of Charybdotoxin-C

The target protein, ChTx-C from L. quinquestriatus hebraeus, yellow scorpion sequence was retrieved from Uniprot database (http://www.uniprot.org/uniprot/) with the UniProt ID of P59944. The target protein is a 37 residues SCNs protein with the low molecular weight of 4318 Da. The target protein was used for template selection using PDBSUM database. The potassium channel/ChTx complex showed 91.7% identity with ChTx-C and it was selected as a template protein for modeling, and the PDB ID is 2A9H chain E.[18] Three-dimensional structure of ChTx-C was modeled using homology modeling method using automated modeling program, Modeller 9v7. The generated model was validated in SAVES program and the final validated model
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has 100% of amino acids in allowed conformation and the predicted structure has 91.7% of amino acids in the favored and additionally allowed regions of Ramachandran plot and the rest of the residues are found in the generously allowed region and none of the amino acids are found in disallowed region of Ramachandran plot [Figure 1]. The predicted and validated three-dimensional structure was allowed for MD simulation for 1 ns by setting the production type into constant volume ensemble (NVT) and temperature was set into 300 K, the remaining parameters were set as default value given in the ADS 2.0. The disulfide connectivity pattern of the target protein, ChTx-C was also analyzed using DiANNA server. This shows three disulfide connectivity, 1–11, 28–39 and 53–67 with the connectivity score of 0.99544, 0.99805 and 0.99584 respectively. It proves that the structure of the toxin is highly stabilized with six strong cysteine residues (S-S bonding). The detailed information of potential energy is given in Figure 2.

The sequence and structure are compared with margatoxin (MgTx) and hongotoxins. It indicates that the existence of proline at positions 10, 15 and 16 in margatoxin (MgTx) and hongotoxins significantly contribute for a conformational rigidity, which may have implications in the high binding affinity with the ion channel while the conformational flexibility in ChTx-C may be attributed to its reduced molar affinity.

Inhibitors Selection and Molecular Docking Analysis

Existing scorpion antidote compounds and their analogs were chosen for molecular docking analysis. Initially, all selected antidotes were retrieved from Drug Bank database, and analog search was performed using PubChem database. Molecular docking studies were carried out with selected inhibitors using AutoDock 4.0. From the selected ligands screened, 8 molecules have shown good docking scores and interactions. As a result of docking, a list of compounds namely mephenytoin, phensuximide, primidone, valproate, ethosuximide, fosphenytoin, lamotrigine and carbamazepine were shown good inhibiting properties with target protein[19] and the detailed interactions are given in Figure 3. The detailed interactions with binding energy, amino acids involved in H-bonding and their distances are given in Table 1. From the result of interaction analysis with ChTx-C, fosphenytoin and carbamazepine were shown good binding free energy.

Figure 1: Charybdotoxin-C modeling (a) Template-target alignment; (b) Predicted three-dimensional structure; (c) Ramachandran plot

Figure 2: (a) Potential energy profile; (b) root mean square deviation of modeled structure of Charybdotoxin-C over a period of 1 ns molecular dynamics simulation
Figure 3: The interactions of best inhibitors-Charybdotoxin-C docked complexes and the potential energy profile of (a) Mephenytoin; (b) Phensuximide; (c) Primidone; (d) Valproate; (e) Ethosuximide; (f) Dosephenytoin (g) Lamotringine; (h) Carbamazepine

Discussion

The resultant simulated structure of ChTx-C was obtained with the considerable decline of potential energy with a little deviation was observed in the three-dimensional structural conformation. The simulated structure showed a significant level of structural stability which can be used as a target protein for further interaction studies. The molecular interaction studies reveal that the interactions of toxin with the compounds are primarily of H-bonds formed with the binding site amino acids. The compounds primidone and lamotringine are found to have more number of hydrogen bonds with the toxin. Analysis of the interactions also reveals that the residues Ser 15, Lys 32 have significant interactions with selected ligand molecules and Val5, which have hydrophobic interaction with the cyclic group of the ligands and it may be speculated that when this drug like molecules bind with the toxins, eventually they may reduce the ability of the toxins to block the Ca²⁺ activated K⁺ ion channels and thereby help in preventing the severity of virulence. On the other hand residues such as Arg 25 and Lys 27 are involved in electrostatic interactions which are significant in toxin-channel interactions where it contributes considerably for the toxin specificity. Further, MD simulation performed for 500 ps revealed that throughout the period of simulation the energy level and hydrogen bonding interactions are found to be stable [Figure 3].
Molecular docking analysis of selected inhibitors

| Compound name | Inhibition constant (Ki) (μM) | Amino acids involved in intermolecular interactions | Intermolecular H-bonding distances (Å) | Binding energy (Kcal/mol) |
|---------------|-------------------------------|-----------------------------------------------|----------------------------------------|-------------------------|
| Mephenytoin   | 356.81                        | ASN4, VAL5                                    | 2.04                                   | −4.70                   |
| Phensuximide  | 591.53                        | ASN4                                         | 2.09                                   | −4.40                   |
| Primidone     | 766.75                        | ASN4, SER15, VAL5, LYS11                      | 2.01                                   | −4.25                   |
| Valproate     | 102.63                        | LYS32, THR8                                   | 1.74                                   | −5.44                   |
| Ethosuximide  | 539.67                        | VAL5                                         | 1.87                                   | −4.46                   |
| Fosphenytoin  | 45.84                         | VAL5, ASN4                                   | 1.99                                   | −5.92                   |
| Lamotrigine   | 866.76                        | VAL5, SER15, THR3, ASN4                       | 1.95                                   | −4.18                   |
| Carbamazepine | 74.12                         | VAL5                                         | 1.34                                   | −5.63                   |

**Conclusion**

Most of the animal SCNs cause severe detrimental effects to the nervous system by distressing ion channel proteins either by inhibition or by accelerating the ionic movements. ChTx-C is a toxin, which affects the Ca2+ activated K+ channels, which are considered to be important for neurotransmission and several other imperative biological roles. Charbdotoxin and Charbdotoxin-C share a significant structural and functional similarity.20,21 Even though some of the amino acid residues were found to be toxic, they make active site pockets. The predicted three-dimensional structure and several inhibitors and their analogs were used for this study to find the best antidote compounds for ChTx-C using various in silico approaches. In molecular docking analysis, a list of inhibitors namely fosphenytoin, carbamazepine, mephenytoin, lamotrigine, phensuximide, primidone, valproate, ethosuximide were showed very good interactions with binding site amino acids of ChTx-C. Hence, these selected inhibitors may work as the best antidote for yellow scorpion toxin. In order to find the suitability of selected inhibitors against ChTx-C further confirmation through in vitro studies are warranted.

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