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In Silico Screening of Chemical Inhibitors to Cardiotoxins of Snake Venoms

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Abstract

Cardiotoxins (CTXs) are principal toxic components of snake venoms and the protein toxins exhibit a wide range of biological activities such as systolic heart arrest, membrane depolarizations and lysis of erythrocytes by interacting with components on cell membranes. In the present study, lead chemical inhibitors to the CTX1 (an S-type CTX from *Naja naja*) and as well to the CTX VI (a P-type CTX from *Naja atra*) were screened by means of high-throughput virtual screening method. Despite similar three-dimensional folds of the protein toxins, they showed differential binding interactions with the chemical molecules and the differential binding interactions could be attributed to the differences in the structural contexts of the surface regions for the two protein toxins. Moreover, a comprehensive analyze on the data suggested that compounds such as Tricyclene, Myrcene, Physagulin D (1->6)-β-D-glucopyranosyl-(1->4)-β-D-glucopyranoside and Forsythoside would act as lead inhibitors to the CTXs. And, implications of the study for designing small molecular inhibitors to the CTXs of snake venoms are also concisely discussed.

Keywords: Antidote, cardiotoxins, chemical inhibitors, hemolysis and snake venoms.

INTRODUCTION

Snake venoms are a mixture of protein toxins with diverse biological activities [1,2]. Of the many protein toxins, cardiotoxins (CTXs) and neurotoxins (NTXs) are principal components for lethal actions of the snake venoms [3,4,5]. Both the CTXs and NTXs are belonging to 'three-finger toxin' (TFT) superfamily and they are also paralogous proteins [1,3,6]. While the CTXs exhibit cell lytic activities, the NTXs target inhibition of nicotinic acetylcholine receptors [1,7, 8]. Moreover, the CTXs are most abundant three-finger toxin of snake venoms and the proteins toxins belonging to TFT superfamily are reported to present in all venomous snakes [6,9]. However, to the best of our knowledge, there were no small molecular inhibitors to the CTXs as per the literature available to date. In these contexts, it is worthy of mentioning that the author's laboratory has been recently identified a few small molecular inhibitors to the CTXs by means of an array of computational methods and the inhibition potentials of the compounds have also been experimentally validated by examining the hemolytic activities of the CTXs treated with the compounds at various concentration [*Biswajit Gorai and Thirunavukkarasu Sivaraman. In silico screening of crucial residues for hemolytic activities of cardiotoxin 1 from Naja naja and in vitro validations. Int. J. Biol. Macromol. (2016), in press*].

In the present study, a small molecular database, NPACT (http://crdd.osdd.net/raghava/npact), consisting of several hundreds of natural compounds was subjected to high-throughput virtual screening using molecular docking strategies against CTX1 (cardiotoxin 1 from *Naja naja*) and CTX VI (cardiotoxin VI from *Naja atra*). The CTX1 and CTX VI are belonging to S-type and P-type cardiotoxins implying that the hemolytic efficiencies of the toxins are likely to be different from each other [10,11]. Interestingly, the top-ten chemical inhibitors identified for the CTX1 and the CTX VI were quite different from one another especially from their structural standpoints. Meanwhile, both the CTXs showed two distinct binding sites for the chemical compounds and overall structural contexts and chemical environments of the binding sites were found to be similar in both the toxins. The differential interactions of the structurally similar proteins towards the chemical molecules and implications of the study on developing potent inhibitors to the CTXs have also been briefly discussed.

METHODS

The primary sequences of the CTX1 from *Naja naja* and the CTX VI from the *Naja atra* were retrieved from UniProt database (http://www.uniprot.org). The three-dimensional (3D) structure (PDB ID: 1UG4) of the CTX VI was retrieved from the PDB database (http://www.rcsb.org/pdb/) and experimental 3D structures for the CTX1 have not yet been deposited in the PDB. Hence, the 3D structures of the protein were homology modeled and validations of the 3D structure have been elaborately described elsewhere by the authors [12]. The 3D structures of both the CTX1 and the CTX VI were then subjected to molecular dynamics simulations in near physiological conditions (pH 7, 310 K, 1 atmospheric pressure and explicit water system consisting of 0.1 M ionic strength) for 25 ns using GROMACS 4.5.5 [13,14] and average structures calculated for the proteins from their respective equilibrium phases of the simulations were used for studying interactions of the proteins with chemical molecules of the NPACT database [15].

The NPACT (http://crdd.osdd.net/raghava/npact) database consisted of downloadable 950 natural chemical molecules as of October 2016 and all the molecules were retrieved, processed and stored in different file formats (sdf, pdb & mol2). Overall binding energies of the chemical compounds with the CTX1 and CTX VI were calculated through unbiased molecular docking strategy using iGEMDOCK [16,17] with default settings except population, generations and solutions: the population size, number of generations and number of solutions were set as 200, 70 and 5, respectively in the present study. Non-covalent interactions between the top-ten chemical molecules depicting higher binding affinities with the CTXs were scrutinized using molecular visualization tools such as PyMol 0.99rc6 and Schrödinger suite 9.3, USA.

RESULTS AND DISCUSSION

The CTXs are single polypeptide chain consisting of 59-62 amino acids with four conserved disulfide bonds and an all β-sheet proteins [18,19,20]. Hemolytic activities of the CTXs have been well documented in the literature and on the basis of their hemolytic potencies the CTXs could be classified into two types: P-type and S-type CTXs. The P-type CTXs and the S-type CTXs have invariably 'proline' and 'serine' residues at positions 30 and 28, respectively in their primary structures [21,11]. It has also been demonstrated that, while both types of CTXs interact strongly with anionic phospholipids, only the P-type CTXs showed strong perturbation with the zwitterionic phospholipids [22,23]. However, there were no straightforward correlations between the hemolytic activities and 3D folds of the CTXs [10, 24]. The CTX1 and CTX VI considered in the present study are belonging to the S-type and P-type, respectively and the rationalization for selecting the CTX1 and CTX VI as representatives of the S-type and P-type CTXs have been well described elsewhere by the authors [10].

Of the 950 plant-derived compounds, the CTX1 showed stronger binding affinities with the top-ten compounds such as Tricyclene, Limonene, Alpha-spinasterol, Beta-pinene, Beta-phellandrene, Ocimene, Myrcene, Menthol, Menthone and Gamma-caryophyllene. The binding affinities of the compounds were ranged from -128.1 kcal/mol to -114.1 kcal/mol and two-dimensional structures of the compounds are depicted in the Figure 1. Interestingly, the binding surfaces of the top-ten ligands on the CTX1 were not identical to one another. In other words, the CTX1 showed two distinct binding regions: one of the two binding regions was constituted by residues such as Lys2, Cys3, Leu6, Tyr11 & Arg58 and an another binding region was constituted by residues such as Leu20, Tyr25, Lys30, Lys44, Lys50 & Glu52. Of the top-ten chemical compounds, compounds such as Tricyclene, Limonone, Alpha-spinasterol, Beta-pinene, Beta-phellandrene, Ocimene, & Menthone were found to interact with the former binding region of the CTX1 and compounds such as Myrcene, Menthol & Gamma-caryophyllene were found to interact with latter binding region of the CTX1 (Figure 2).

In the case of the CTX VI, chemical compounds such as Physagulin D (1->6)-β-D-glucopyranosyl-(1->4)-β-D-glucopyranoside, Lobatosides C, Forsythoside, Isoverbascoside, Muricatetrocins B, Dehydrotomatine, Squamostatin A, Sanggenon D, Indicanine D & Withanoside IV were found to have stronger interactions with the protein toxin and binding affinities of the compounds were calculated to be -127.4, -127.2, -124.6, -124.6, -120, -118.6, -118.4, -117.3, -117.1 & -116.7 kcal/mol, respectively (Figure 3). Like the CTX1, the CTX VI also showed two distinct binding sites for the ligands: one binding site was constituted by residues such as Asn4, Gln5, Phe10, Tyr11, Thr56, Asp57, Arg58 & Asn60 and the another binding region was constituted by residues such as Leu20, Val41, Cys42, Pro43, Lys44, Ser45, Ser46, Lys50, Val52 & Asn55. While compounds such as Physagulin D (1->6)-β-D-glucopyranosyl-(1->4)-β-D-glucopyranoside, Lobatosides C, Muricatetrocins B, Dehydrotomatine, Sanggenon D, Indicanine D & Withanoside IV depicted stronger interactions with the former binding site, the other three compounds such as Forsythoside, Isoverbascoside & Squamostatin A depicted binding affinities with the latter binding region described above for the CTX VI (Figure 4).

It is interesting to note that both the CTX1 and CTX VI showed two distinct binding sites for the chemical compounds screened in the present study. In both the proteins, one binding site was constituted by residues from Strand I, II, & IV and as well from residues from C-terminal regions of the proteins; the another binding site was constituted by residues from Strand III & V and as well residues from the long loop connecting strand IV and V of the proteins. However, the two distinct binding sites of the CTX VI were larger than the counter parts of the CTX1. It is also wondering to note that the top-ten chemical compounds screened for the CTX1 and for the CTX VI were different from one another (Figure 1 & 3). Meanwhile, the binding affinities (-128.1 to -114.1 kcal/mol) of the CTX1 with its top-ten compounds and the binding affinities (-127.4 to -116.7 kcal/mol) of the CTX VI with its top-ten compounds were very similar to each other. In this background, the differential binding interactions of the structurally similar protein toxins with the diverse chemical molecules could be presumably attributed to the differences in the structural contexts of the surface regions for the two protein toxins.

CONCLUDING REMARKS

In the present study, the NPACT database consisting of 950 plant-derived natural compounds was screened using HTVS method in conjunction with molecular docking technique in order to identify efficient inhibitors to the CTX1 and CTX VI. A comprehensive analyze on the dockings data suggested that compounds such as Tricyclene, Myrcene, Physagulin D (1->6)-β-D-glucopyranosyl-(1->4)-β-D-glucopyranoside and Forsythoside would act as lead inhibitors to the cardiotoxins of snake venoms. It is also believed that the present study will be very useful in designing specific small molecular inhibitors to CTXs in the near future, which in turn may pave the way towards an efficient 'combination therapy' in the treatment of snakebite.

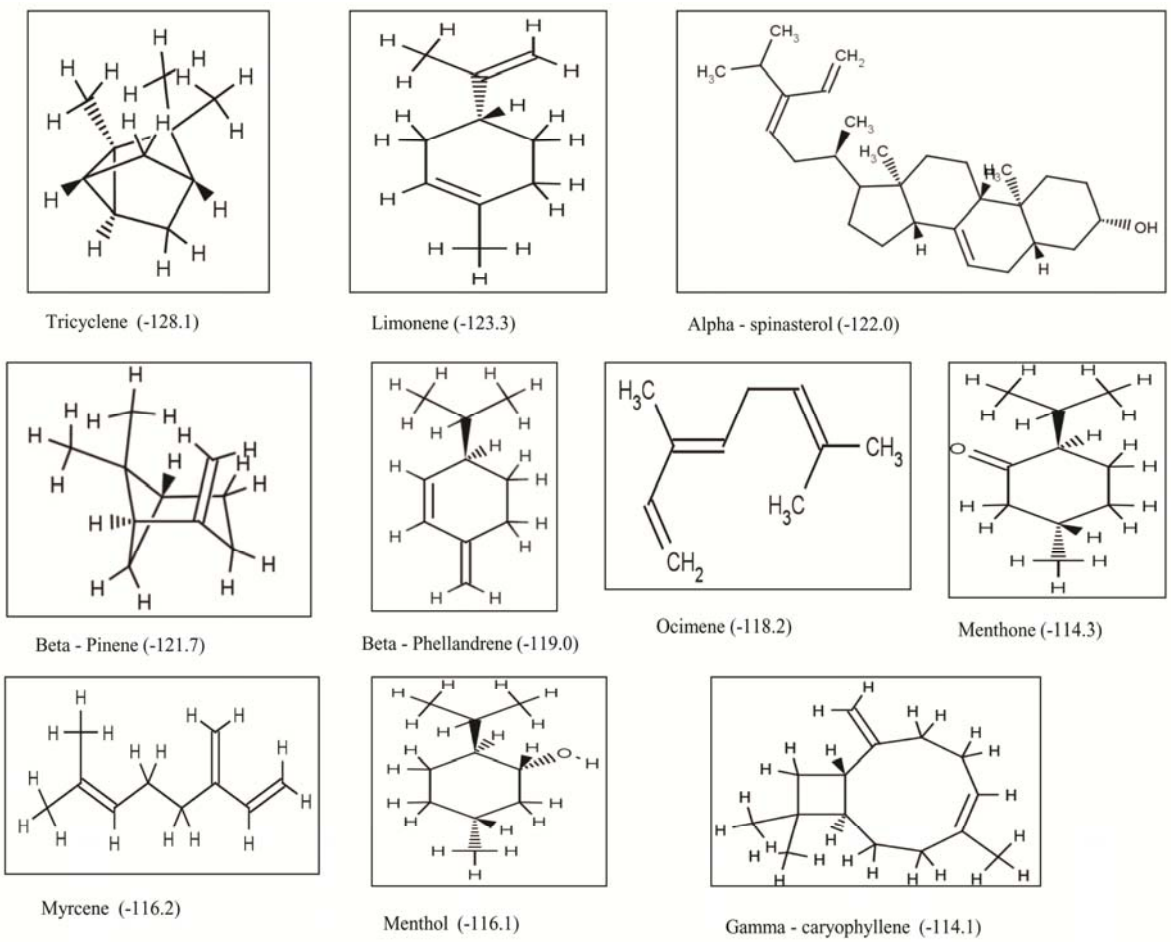

Figure 1: Two-dimensional structures of the top-ten chemical inhibitors to the CTX1 (calculated docking energies (kcal/mol) are given in parenthesis for each molecule).

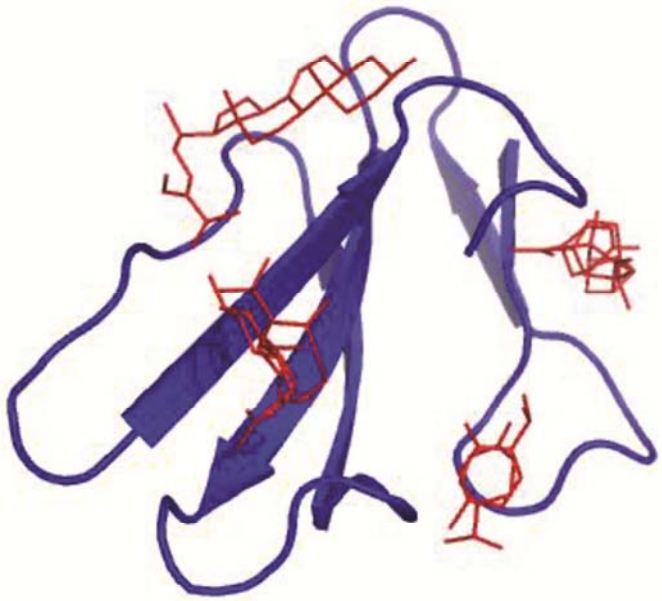

Figure 2: Pictorial illustrations of the binding locations of the top-ten chemical inhibitors on surface regions of the CTX1 from Indian cobra (*Naja naja naja*) venom are shown.

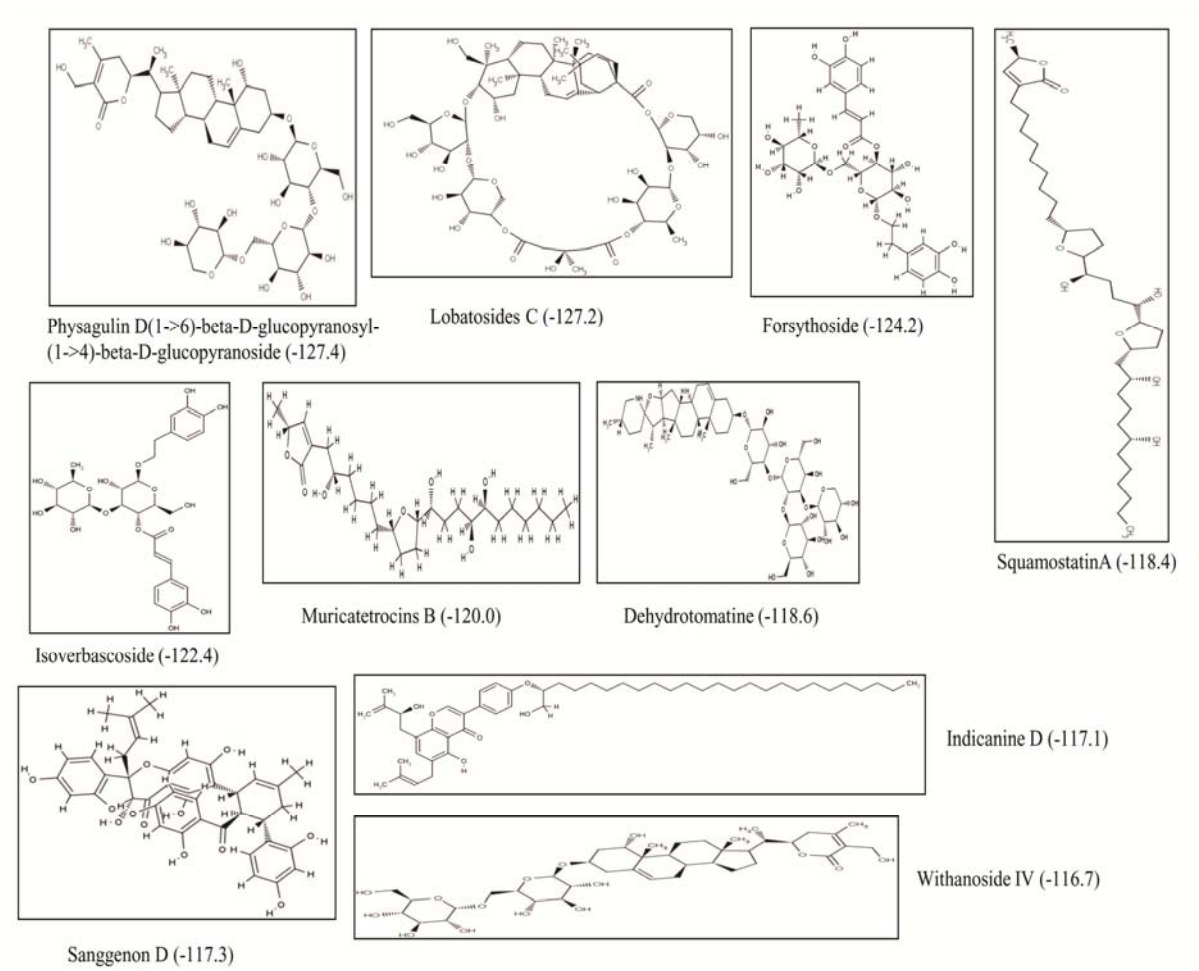

Figure 3: Two-dimensional structures of the top-ten chemical inhibitors to the CTX VI (calculated docking energies (kcal/mol) are given in parenthesis for each molecule).

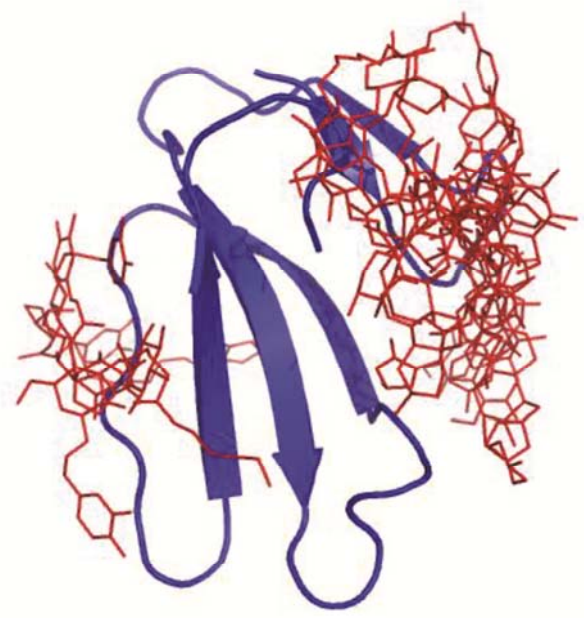

Figure 4: Pictorial illustrations of the binding locations of the top-ten chemical inhibitors on surface regions of the CTX VI from Taiwan cobra (*Naja naja atra*) venom are shown.

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