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Original Research Article

An *in silico* study to find angiogenesis inhibitory role for *Naja oxiana* snake venom cytotoxins

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ABSTRACT

Cytotoxins especially CTX-I and CTX-II are components in the venom of cobra snakes. Cytotoxins as apoptotic agents are appropriate candidates to study of anticancer drugs. In this study we performed docking analysis of CTX-I and CTX-II against vascular endothelial growth factor (VEGF) and its main receptor (VEGFR2) that are the main key factors of angiogenesis. The results showed that CTX-I has higher binding affinity to VEGF and VEGFR2 compare to its native ligand, so it could be a suitable candidate to study anticancer drug.

Keywords: Cytotoxin; venom; *Naja oxiana* snake

INTRODUCTION

Six hundred species of snakes are venomous. These venomous snakes are identified by venom gland and classified to three main groups: *Viperidae*, *Elapidae*, and *Atractaspidinae*. Most of Cobra snake appertaining to the genus *Naja* is well-known example of *Elapidae* family [1]. Central Asian cobra, *Naja oxiana*,

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distributed in northeastern of Iran and some neighboring countries such as Afghanistan and northern Pakistan [2]. Transcriptome and proteome analysis of some cobra snakes showed that the cobra venom consist of mixture of various enzymes and toxic peptides like: phospholipase A2 (PLA2), l-amino-acid oxidase (LAAO), metalloproteinase, kunitz-type protease inhibitor, c-type lectin, three-finger toxins (3FTxs) and other toxins [3-7]. The highest percentage of venom component related to 3FTxs [3,4]. Three-finger toxins fall into neurotoxins and cytotoxins that the latter is the most in the venom composition. The structure of neurotoxins and cytotoxins are similar. However, they have different biological activity [8,9]. Mainly, cytotoxins have 62 amino acid residues that form three loops in their structure [8,9]. At high concentration, cytotoxins have cardiotoxic property and in low concentrations, they only increase heart rate [10]. A cytotoxin (NN-32) that was isolated from Indian cobra showed anticancer effect on leukemic cells through apoptotic pathway [11]. Furthermore, NN-32 reduced activity of vascular endothelial growth factor (VEGF), matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) and therefore showed anti-angiogenic activity on leukemic U937 cells [11]. VEGF and the main receptor, VEGFR2, are the key factors of angiogenesis. The VEGFR2 is the main receptor that overexpress in the most cancers. Binding of VEGF to VEGFR2 results in initiation of signaling pathway, cell growth, angiogenesis, invasion, and metastasis of cancer cells. Two cytotoxins (CTX-I and CTX-II) isolated from *Naja oxiana* venom and showed that they have anticancer activity through induction of apoptosis [12,13]. Nowadays, there is no study regarding anti-angiogenesis activity of CTX-I and CTX-II. In this study, we used bioinformatics approach to examine that the CTX-I and CTX-II could interact with VEGF and the receptor VEGFR2. Then, we performed docking analysis for the CTX-I and CTX-II with human VEGF and VEGFR2 using Hex 8.0 software.

MATERIALS AND METHODS

Structures

The 3D structures of cobra venom cytotoxin, CTX–I, CTX-II and complex of VEGFR2/VEGF were obtained through research collaboratory for structural bioinformatics protein data bank (RCSB-PDB). Minimization of energy was performed for all 3D structures to fix conformational problems associated with the X-ray or NMR structures [14]. All 3D

structures used to evaluate the protein structures of allowed and disallowed regions of residues.

Molecular docking

Docking was carried out by utilizing Hex 8.0 software as follows: 1- Docking of CT-I X (PDB: 1RL5) as ligand with molecule of VEGFR2 (PDB: 3V2A) as receptor, 2- CTII X- (PDB: 1FFJ) as ligand with VEGFR2 (PDB: 3V2A) as receptor, 3- CTX-I (PDB: 1RL5) as ligand with VEGF (PDB: 3V2A) as receptor, 4- CTTX-II (PDB id: 1FFJ) as ligand with VEGF (PDB: 3V2A) as receptor. In Hex, correlation was set to shape and electrostatics and other parameters as default of the software. The complex with minimum docking energy was identified to show the binding site residues in the interaction. The 3D structures of docked proteins were visualized by another scientific artificial reality application (YASARA) software.

RESULTS

Determination of the 3D structures of peptides and proteins

The three dimensional structure was obtained from PDB as shows in Figure 1, which was refined by energy minimization and evaluated by Ramachandran plot. Table 1 depicts the different minimizing energies related to the structures.

Docking analysis

Docking of CTX-I and CTX-II was performed with VEGF and VEGFR2 using HEX docking software. The software generated 500 matches and the docked putative conformations are explored on the basis of minimum energy. The conformation with lowest binding energy and related molecular interactions are identified. The energy of docked complexes are listed in Table 2.

According to the total free energy, CTX-I (PDB: 1RL5) showed the best interaction to VEGF (-43838.8 kJ/mol) and VEGFR2 (-25754.2 kJ/mol) as compare to interaction tendency of VEGF to VEGFR2 (-784.5 kJ/mol). The binding affinity of CTX-II to VEGF (-21710.2 kJ/mol) and VEGFR2 (-20284.4 kJ/mol) was higher than binding affinity of VEGF to VEGFR2 (-784.5 kJ/mol). The schematic view of docked complexe is shown in Figure 2.

Interaction analysis

The hydrogen bonding and hydrophobic interaction between CTX-I and CTX-II to

VEGF and VEGFR2 was identified by YASARA software. The residues involved in the interaction are shown in Table 3 and Figure 3.

Table 1. Various energies of the structures before and after energy minimization

Structures	1RL5		1FFJ		VEGF		VEGFR2	
Energy minimization KJ/mol	After	Before	After	Before	After	Before	After	Before
Bonds	39.27	143.07	32.22	142.64	190.85	556.48	123.12	332.92
Angles	228.59	253.56	200.84	170.56	1048.14	1219.12	677.30	768.17
Torsion	540.79	646.11	581.13	750.20	1598.03	1832.52	990.23	1104.75
Improper	82.70	79.73	60.50	75.52	283.57	204.92	185.86	131.35
Non-Bonded	-1665.76	77.23	1635.76	-832.16	-8487.05	3517.64	-5451.10	887.94
Electrostatic	1329.00	-1057.31	669.93	-449.22	-7465.92	-6347.09	-4803.58	-4032.93
Constraint	0.0000	0.0000	0.00000	0.0000	0.0000	0.0000	0.0000	0.0000
Total	554.60	142.40	3180.40	-142.43	-12832.35	983.6	-8278.15	-807.78

Total: Sum of minimized energies values in each column

Table 2. Total free energies of the interactions calculated by HEX

Structures	E_{Total}	E_{shape}	E_{force}	Bmp	RMS
1RL5 (ligand) with VEGFR2	-25754.2	-25754.2	0.00	-1	-1.00
1FFJ (ligand) with VEGFR2 (receptor)	-20284.4	-20284.4	0.00	-1	-1.00
1RL5 (ligand) with VEGF (receptor)	-43838.8	-43838.8	0.00	-1	-1.00
1FFJ (ligand) with VEGF (receptor)	-21710.2	-21710.2	0.00	-1	-1.00
VEGF (ligand) with VEGFR2	-784.5	-784.5	0.00	-1	-1.00

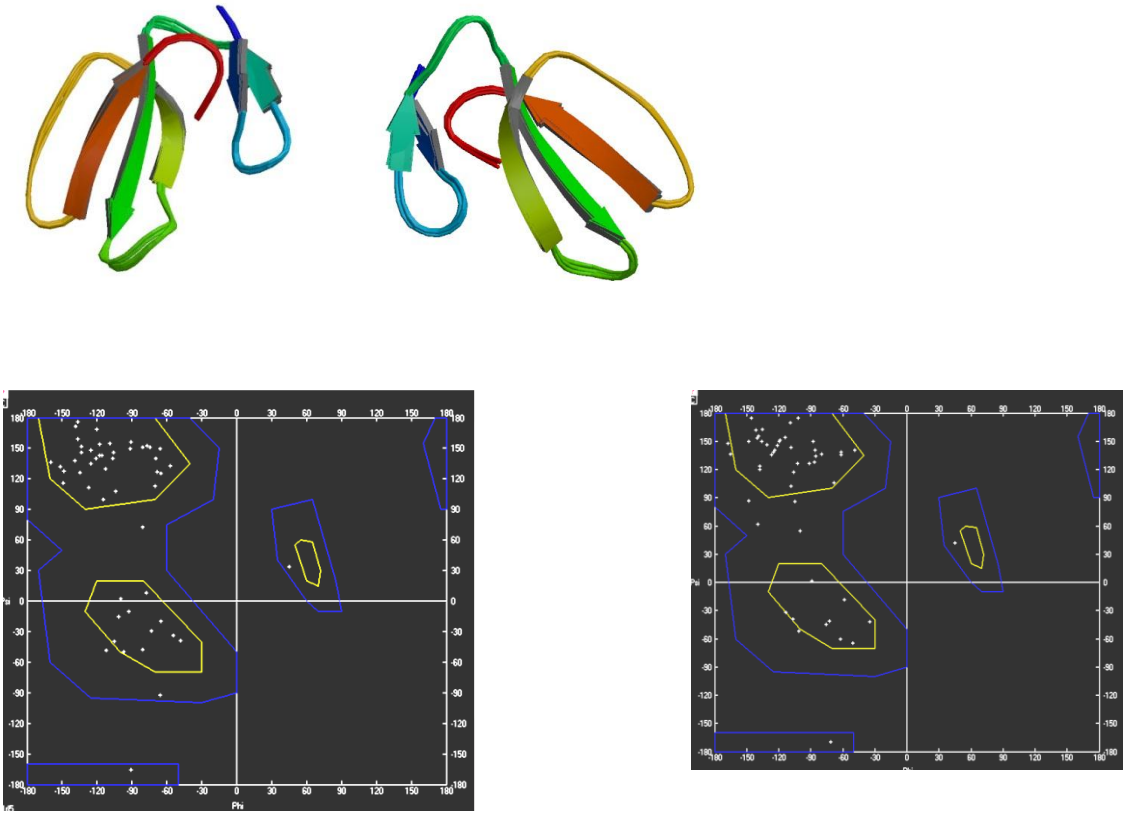

Figure 1. 3D structure of peptides: CTX-I (1RL5), CTX-II (1FFJ). The Ramachandran plot of CTX-I (1RL5), CTX-II (1FFJ).

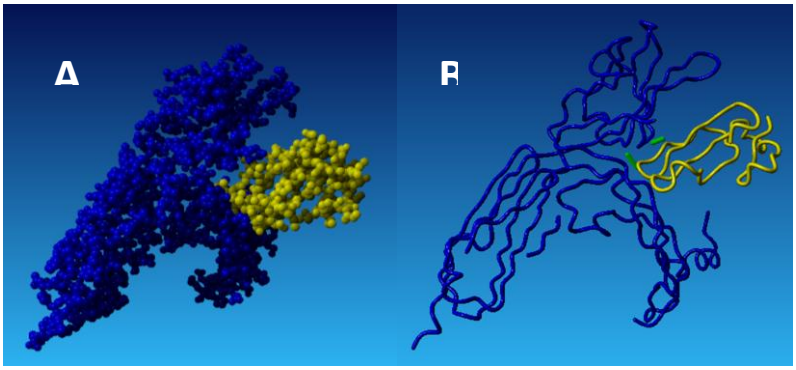

Figure 2. A: Docked complex of CTX-I (1RL5) and VEGF. B: Hydrophobic interactions.

DISCUSSION

This study showed that cytotoxin-I (CTX-I) and Cytotoxin-II (CTX-II) from *Naja oxiana* snake venom have a high binding affinity to both VEGF and VEGFR2. However, VEGFR2 are responsible for growth, development and angiogenesis of cancer cells. The interaction tendency of CTX-I to VEGF (-43838.8 kJ/mol) and VEGFR2 (-25754.2 kJ/mol) were higher than interaction tendency of VEGF to VEGFR2 (-784.5). In addition CTX-II showed high binding interaction to VEGF (-21710.21 kJ/mol) and VEGFR2 (-20284.4 kJ/mol) as compare to VEGF/VEGFR2 complex (-784.5). Previous studies suggest apoptogenic effects for CTX-I and CTX-II and make it a target for further studies. In this study, we design four docking complexes listed in Table 2. Hex software was used to evaluate the binding energy of polypeptides. As higher affinity related to lower energy, the results showed higher affinity for CTX-I. We suggest that cytotoxin-I (CTX-I) and cytotoxin-II (CTX-II) could be a powerful candidate for further studies to develop an anticancer agent.

Table 3. Amino acid residues involved in the interaction

	Structures	Residue in ligand	Residue in receptor
1	1RL5 (ligand) with VEGFR2	-	-
2	1FFJ (ligand) with VEGFR2	1-LEU-N	164-ARG-C 283-SER-N
3	1RL5 (ligand) with VEGF	45-ASN-OD1	132-GLN-N
4	1FFJ (ligand) with VEGF	-	-
5	VEGF (ligand) with VEGFR2	-	-

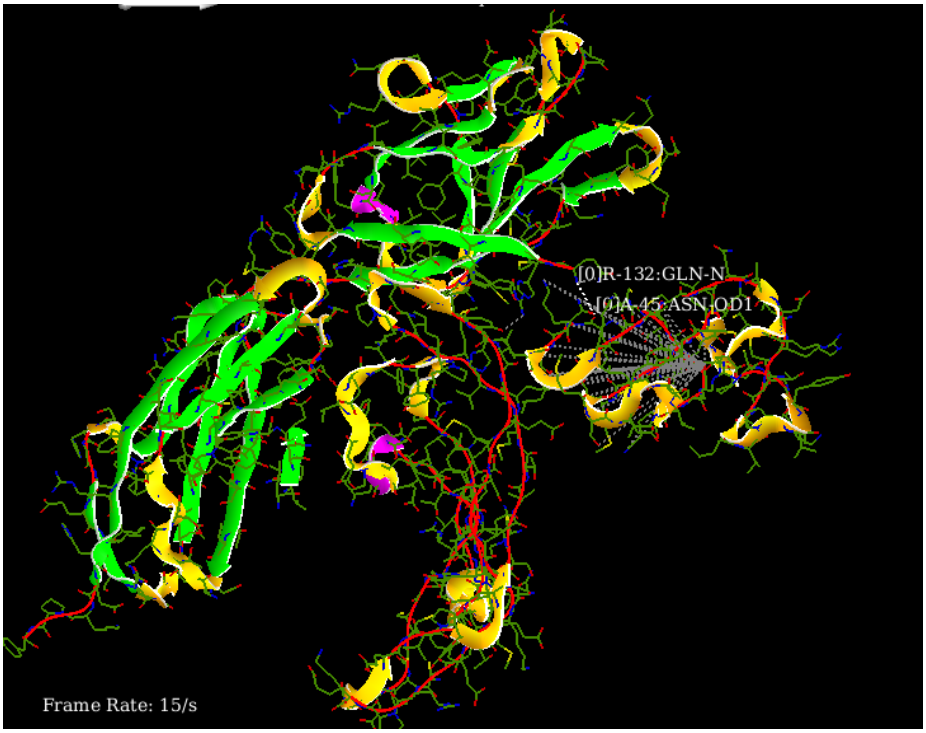

Figure 3. Amino acid residues involved in CTX-I (1RL5) as a ligand to VEGF as a receptor.

CONCLUSION

In conclusion, CTX-I and CTX-II which were isolated from venomous snake, *Naja oxiana*, showed high binding interaction to VEGF and VEGFR2 in docking analysis using HEX software. The therapeutic functions of the venoms that showed in the present study may lead to improve and design anti angiogenesis agents based on the structure of CTX-I and CTX-II and it could be a starting point of cancer studies.

REFERENCES

[1]. Vonk FJ, Jackson K, Doley R, Madaras F, Mirtschin PJ, Vidal N. Snake venom: from fieldwork to the clinic. *Bioessays*, 2011; 33(4): 269-79.

[2]. Wüster W, Thorpe RS. Asiatic cobras: Population systematics of the Naja naja species complex in India and central Asia, *Herpetological*, 1992; 48(1): 69-85.

[3]. Shan LL, Gao JF, Zhang YX, Shen SS, He Y, Wang J, Ma XM, Ji X. Proteomic characterization and comparison of venoms from two elapid snakes from Chin. *J Proteom,* 2016; 138: 83-94.

[4]. Jiang Y, Li Y, Lee W, Xu X, Zhang Y, Zhao R, Zhang Y, Wang W. Venom gland transcriptomes of two elapid snakes (Bungarus multicinctus and Naja atra) and evolution of toxin genes. *BMC genomics,* 2011; 12(1): 1-14.

[5]. Samel M, Tõnismägi K, Rönnholm G, Vija H, Siigur J, Kalkkinen N, Siigur E. L-Amino acid oxidase from Naja naja oxiana venom. *Comp Biochem Physiol B Biochem Mol Biol,* 2008; 149(4): 572-80.

[6]. Samel M, Vija H, Kurvet I, Künnis-Beres K, Trummal K, Subbi J, Kahru A, Siigur J. Interactions of PLA2-s from Vipera lebetina, Vipera berus berus and Naja naja oxiana venom with platelets, bacterial and cancer cells. *Toxins,* 2013; 5(2): 203-23.

[7]. Kulkeaw K, Chaicumpa W, Sakolvaree Y, Tongtawe P, Tapchaisri P. Proteome and immunome of the venom of the Thai cobra, Naja kaouthia. *Toxicon,* 2007; 49(7): 1026-41.

[8]. Fry BG, Wüster W, Kini RM, Brusic V, Khan A, Venkataraman D, Rooney AP. Molecular evolution and phylogeny of elapid snake venom three-finger toxins. *J Mol Evol,* 2003; 57(1): 110-29.

[9]. Kini RM. Molecular moulds with multiple missions: functional sites in three-finger toxins. *Clin Exp Pharmacol Physiol,* 2002; 29(9): 815-22.

[10]. Dufton MJ, Hider RC. Structure and pharmacology of elapid Cytotoxins. *Pharmacol Ther,* 1988; 36(1): 1-40.

[11]. Das T, Bhattacharya S, Biswas A, Gupta SD, Gomes A, Gomes A. Inhibition of leukemic U937 cell growth by induction of apoptosis, cell cycle arrest and suppression of VEGF, MMP-2 and MMP-9 activities by cytotoxin protein NN-32 purified from Indian spectacled cobra (Naja naja) venom. *Toxicon,* 2013; 65: 1-4.

[12]. Ebrahim K, Shirazi FH, Mirakabadi AZ, Vatanpour H. Cobra venom cytotoxins; apoptotic or necrotic agents. *Toxicon,* 2015; 108: 134-40.

[13]. Feofanov AV, Sharonov GV, Dubinnyi MA, Astapova MV, Kudelina IA, Dubovskii PV, Rodionov DI, Utkin YN, Arseniev AS. Comparative study of structure and activity of cytotoxins from venom of the cobras Naja oxiana, Naja kaouthia, and Naja haje. *Biochem,* 2004; 69(10): 1148-57.

[14]. Kaplan W, Littlejohn TG. Swiss-PDB viewer (deep view). *Brief Bioinform,* 2001; 2(2): 195-97.