Single-Cell Biology
ISSN: 2168-9431

Luna-Ramírez et al., Single Cell Biol 2016, 5:2
DOI: 10.4172/2168-9431.1000138

Mini Review Open Access

Scorpine-Like Peptides

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Abstract

Scorpine-like peptides are intriguing and unique compounds of scorpion venom. They possess two well-defined regions that confers them bi-functionality. The N-terminal region is similar to scorpion antimicrobial peptides lacking disulfide bridges, whereas the C-terminal region contains six cysteines forming three disulfide bridges that tightly bind the peptide. Scorpine-like peptides have shown activity against bacteria (i.e. B. *subtilis*, K. *pneumoniae*, P. *aeruginosa*), fungi and also as potassium channel blockers. Additionally, they have been successful in controlling malaria and some types of viruses.

Keywords: Scorpion venom; Peptides; Amino acids; Antimalarial activity

Introduction

Scorpion venoms are complex mixture of peptides with a variety of pharmacological functions, specially targeting membrane proteins and interfering with the membrane permeability for Na^+, K^+, Ca^{+2} and Cl^- in excitable and non-excitable cells [1,2]. Scorpion venoms also contain enzymatic and cytolytic compounds. In general, scorpion venom compounds can be classified in two groups: disulfide-bridged peptides (DBP) that represent neurotoxins [3] and non-disulfide-bridged peptides (NDBP) [4]. Interestingly, scorpine, a compound isolated from *Pandinus imperator* venom [5], contains a region belonging to the DBP and another belonging to the NDBP. Scorpine was shown to display antibacterial and anti-parasitic activities, but also to modify normal function of potassium ion channels. Scorpine-like peptides have been discovered in other species of scorpions, for example in *Hadrurus gertschi* [6,7], *Tityus costatus* [8], *Opisthacanthus cayaporum* [9], *Pandinus cavimanus* [10], *Euscorpiops validus* [11], *Urodacus yaschenkoi* [12], *Opistophthalmus carinatus* [13], *Heterometrus laoticus* [14] and *Vaejovis* species [15].

Structural Properties and Bioactivity of Scorpine-Like Peptides

The scorpine-like peptides belong to the third group of β-K^+ channel specific toxins (β-KTxs) and were first called 'orphan peptides' because they showed contrasting pharmacological activity due to their bi-functionality [6,13,16]. The N-terminal region of scorpine-like peptides have cytolytic or antimicrobial activity like the insect cecropins (20% identity) with an alpha-helical structure that moves freely [16,17], while the C-terminal region has K^+ channel blocking activity and it is tightly folded by three disulfide bridges exhibiting a "cysteine stabilized α/β motif" (CS-α/β) [18]. The C-terminal domain is characterized by a conserved sequence: $(x)_3CxA(x)_5GxCxHC(x)_3ExKxGxCHGTKCKC GxPLSY(x)_{1-4;}$ obviously containing 3 disulfide bridges and following completely the typical Cys pattern of invertebrate defensins discussed by Froy and Gurevitz [17] (Figure 1). This segment is responsable for the activity on potassium channels (Table 1).The biological activity of some of these peptides have been investigated (Table 1).

Antimicrobial and antiviral activity

Other examples of antimicrobial effect were reported. For instance, Opiscorpine showed anti-fungal activity against *Fusarium oxysporum*, a pathogen causing Fusarium wilt in many plants [13]. HgeScplp1 shows cytolytic activity at 200 nM in oocytes and erythrocytes and also inhibit the growth of *B. subtilis* at 2 μM [16]. In contrast, the recombinant

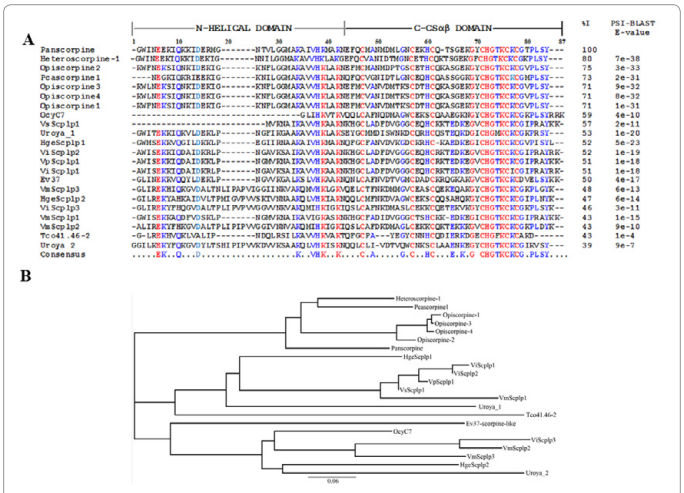

Figure 1: Multiple sequence alignment and phylogenetic analysis. A). All known scorpine-like peptipes were aligned with GENEIOUS. Conserved residues on all sequences are in red and conservative replacements are in blue. The N-terminal domain includes residues from 1-43 whereas the residues 44-87 belong to the C-Cαβ domain. Table 1 shows the accession numbers of each sequence to exception of peptides ViScplp1, ViScplp2 and ViScplp3 isolated from *Vaejovis intrepidus*; VpScplp1 from *Vaejovis punctatus*; VmScplp1, VmScplp2 and VmScplp3 from *Vaejovis mexicanus*; VsScplp1 from *Vaejovis subcristatus*, which were reported in Quintero-Hernández (2015). The percentage of identity was determined with LALIGN v.36.3.5 and the E-value with PSI-BLAST. B). The phylogenetic tree of scorpine-like peptides was constructed with the Neighbor-joining algorithm using GENEIOUS Software. Tree shows 22 scorpines grouped in three main clades. First clade similar to Heteroscorpine-1 grouping all the Opiscorpines, Pcascorpine and Panscorpine. Second clade consists of scorpines similar to Hge-scorpine-1, short-chain-scorpine, having a few Vaejovis scorpine-like-peptides and one from Urodacus. The last clade contains large-chain-scorpines similar to Hge-scorpine-2 grouping the remaining Vaejovis scorpine-like peptides and the long scorpine from Urodacus.

version of the scorpine-like Ev37 did not show any antimicrobial or hemolytic activity at 20 and 10 μM concentrations [11]. It remains to

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Received April 05, 2016; **Accepted** May 07, 2016; **Published** May 09, 2016

Citation: Luna-Ramírez K, Jiménez-Vargas JM, Possani LD (2016) Scorpine-Like Peptides. Single Cell Biol 5: 138. doi:10.4172/2168-9431.1000138

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Scorpion species	Name	Length (aa)	Biological effects	Reference**
Pandinus imperator	Panscorpine	75	Active against bacteria (*B. subtilis* and *K. pneumoniae*)	P56972, [5]
Heterometrus laoticus	Heteroscorpine-1	76	Active against *B.subtilis, K.pneumoniae* and *P.aeruginosa*.	P0C2F4, [14]
Opistophthalmus carinatus	Opiscorpine-1	76	The short synthetic peptide (20-54aa) has antifungal activity against *F. culmorum* (IC_{50}= 8.8 μM), *F. oxysporum* (IC_{50}= 10 μM) and bacteria *P. aeruginosa, E. coli.*	Q5WR03, [13]
	Opiscorpine-2	76	Active against fungi and bacteria*	Q5WR01
	Opiscorpine-3	76	Active against fungi and bacteria*	Q5WQZ7
	Opiscorpine-4	76	Active against fungi and bacteria*	Q5WQZ9
Pandinus cavimanus	Pcascorpine1	73	Active against fungi and bacteria*	H2CYP8, [10]
Opisthacanthus cayaporum	OcyC7_fragment	53	Active against fungi and bacteria*	C5J891, [9]
Hadrurus gertschi	HgeScplp1	76	HgeScplp1 has antibacterial activity against *B.subtilis*. Also it has hemolytic and cytolytic activities on oocytes and erythrocytes. The short peptide (29-76aa) blocks Kv1.1 (IC_{50}=185 nM) potassium channels. Shows a weak hemolytic activity	Q0GY40, [6,7]
	HgeScplp2	84	Active against fungi and bacteria*	P0C8W5, [7],
Urodacus yaschenkoi	Uroya_1	76	Active against fungi and bacteria*	L0G8Z0, [12]
	Uroya_2	83	Active against fungi and bacteria*	
Vaejovis intrepidus	ViScplp1	78	Active against fungi and bacteria*	[15]
	ViScplp2	78	Active against fungi and bacteria*	
	ViScplp3	85	Active against fungi and bacteria*	
Vaejovis punctatus	VpScplp1	78	Active against fungi and bacteria*	
Euscorpiops validus	Ev37	78	Selectively inhibits Kv1.3 channel (IC_{50}=0.95 μM).	P0DL47, [11]
Vaejovis mexicanus	VmScplp1	77	Active against fungi and bacteria*	[15]
	VmScplp2	84	Active against fungi and bacteria*	
	VmScplp3	85	Active against fungi and bacteria*	
Vaejovis subscristatus	VsScplp1	59	Active against fungi and bacteria*	
Tityus costatus	Tco41.46-2	68	Active against bacteria and may block Kv channels*	Q5G8A6, [8]

*By similarity to other sequences may have the same activity;
**Sequence references are databank accession numbers (either SwissProt, TREMBL or GenBank) as retrieved from www.ncbi.nih.gov/entrez (PDB codes, where available, are between parenthesis) or literature references.

Table 1: List of scorpine-like peptides obtained from the venom of various scorpions.

be shown whether the folding of the recombinant peptide is the correct one, necessary for activity. Scorpine was also demonstrated to affect viral replication in cell culture containing Dengue virus [19].

Antimalarial activity

Scorpine-like peptides have been used to control malaria, in special Scorpine, which has been recombinantly expressed in Anopheles gambie cells showing antibacterial activity against B. *subtilis* and K. *pneumonia*, at 5 and 10 μM, respectively. It also produced 98% mortality in sexual stages of *Plasmodium berghei* (ookinetes) at 15 μM and 100% reduction in P. *falciparum* parasitemia at 5 μM [19]. Moreover, the overexpression and secretion of Scorpine into the hemolymph from transgenic mosquitoes reduced sporozoite counts by 98% just a few days after a *Plasmodium*-infected blood meal, suggesting that it could be a powerful weapon for combating malaria [19,20]. Interesting results were obtained by heterologous expression of Scorpine in a strain of *Metarhizium anisopliae* fungus that can parasite mosquitoes. A fusion protein made with a repeat of peptides $[SM1]_8$ with Scorpine directs the product to the salivary glands of the mosquitoes [20]. Infection with these transgenic fungi reduces 98% of *Plasmodium* sporozoites in the salivary gland. The expression of those genes shortened the time taken to reduce transmission of malaria compared to wild type *M. anisopliae* and can reduce transmission rates even when the mosquito has an advanced infection. *M. anisopliae* can infect A. *gambiae*, A. *arabiensis*, A. *funestus* and other populations of mosquitoes. This transgenic strategy could have a wider impact. It could potentially be used to tackle other diseases spread by mosquitoes and other insects [20].

Modulation of activity of potassium channels

The C-terminal region of HgeScplp1 blocks Kv1.1 channel currents (IC_{50} 88 nM) [16] while the recombinant version Ev37 is able of inhibit the Kv1.3 channel current showing an IC_{50} value of 1 μM [11].

Mechanism of Action

The mechanism of action of scorpine-like peptides on microbes probably is similar to that described for the scorpion-AMPs. The latest are generally defined by containing 2-9 positively charged lysine or arginine residues plus hydrophobic amino acids. These residues confer physicochemical properties permitting interactions with microbial membranes and enabling their typically broad-spectrum antimicrobial activity by directly disrupting the membrane [21]. The membrane is disrupted in several stages; first the electrostatic interaction of AMPs with the polyanionic surface of lipopolysaccharide on the bacterial membrane causes an increase in surface area that weakens the bilayer. This alteration leads to pore formation causing the cell lyses. The disruption can be explained by diverse models (barrel stave, carpet model or torodial model) but merely forming a pore that creates an aqueous channel in the microbe membrane that leads to the loss of polarization, loss of intracellular cellular contents, disturbance of membrane function from lipid redistribution and finally death [21,22].

In the antimalarial activity both domains may be involved, the cytolytic activity of the N-terminal and the blocking effect of the C-terminal on potassium channels, although there are few studies on the subject but the mechanism of action has not been elucidated [20,23].

Concluding Remarks

Scorpine-like peptides are interesting compounds of scorpion venom that might aid against the fight towards multi-resistant drug bacteria, contribute to the understanding of potassium ion channels, control viral replication and help in combating malaria. The N-terminal region of scorpine-like peptides might provide antimicrobial and antifungal activities, whereas the C-terminal region might provide novel potassium channel modulators. The critical aspect would be to cleave the peptide in such a way to create (1) a potent AMP with the linear region of scorpine-like peptides and on the other hand, (2) a specific ion channel modulator that contains the motifs that provide the bioactivity.

Scorpines have shown promising antimalarial activity. By producing transgenic mosquitoes that express recombinant scorpines, the malaria parasite transmission can be interrupted. This effect is due to the bi-functionality of scorpine-like peptides. However, our understanding of the mechanism of action of scorpine-like peptides towards parasites and viruses remains unclear. All these evidences make scorpine-like peptides attractive compounds for further research and development into drug leads.

Acknowledgements

Partially supported by grants from SEP-CONACyT No. 237864 and DGAPA-UNAM IN203416 to LDP.

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