Toxicon 118 (2016) 95–103

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Comprehensive analysis of venom from the scorpion *Centruroides tecomanus* reveals compounds with antimicrobial, cytotoxic, and insecticidal activities

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ARTICLE INFO

Article history:
Received 23 February 2016
Received in revised form
6 April 2016
Accepted 26 April 2016
Available online 27 April 2016

Keywords:
Antimicrobial activity
Cancer cell
Scorpion venom
Toxin

ABSTRACT

Centruroides tecomanus is a medically important scorpion of the state of Colima (Mexico). This communication reports the identification of venom components of this scorpion with biological activity over insects/crickets (*Acheta domestica*), crustaceans/fresh water shrimps (*Cambarellus montezumae*), and mammalians/mice (*Mus musculus*, strain CD1). It also describes the pharmacological effects on cell lines in culture (L5178Y cells, HeLa cells, HuTu cells and Jurkat E6-1 cells), as well as on several types of bacteria (see below). The soluble venom of this scorpion was fractionated by high-performance liquid chromatography (HPLC) and collected separately in twelve independent fractions collected over 60 min run (5 min time apart each other). The HPLC components of fraction VII were lethal to all three species used for assay. The IVth fraction had a toxic effect on freshwater shrimps. In this species, fractions VI, VII and VIII were all lethal. For crickets, fractions V and VI were toxic and fraction VII was lethal. In mouse, the lethal components were found in fraction VII, whereas fraction VIII was toxic, but not lethal, at the doses assayed. The molecular weight of peptides from the various group of fractions were identified by mass spectrometry determination. Components lethal to mice showed molecular weights from 7013 to 7487 Da. Two peptides were obtained in homogeneous form and shown to be lethal to the three species of animal used for assay. The soluble venom tested on L5178Y cell line survival was shown to be cytotoxic, at 10–100 μg/mL concentration, when compared to control murine splenocytes ($p = 0.007$). The soluble venom applied to Hela, Hutu and Jurkat cell lines did not show cytotoxic effects at these concentrations. On the contrary, it seems to have a proliferative effect. However the HPLC fractions I, III, VI and XII do have a cytotoxic effect on Jurkat E06-1 cells in culture at 200 μg/mL concentration. The antimicrobial activity of the venom fractions on *Staphylococcus aureus* (gram-positive), *Escherichia coli, Pseudomonas aeruginosa y Salmonella* spp (gram-negative) was measured, using the liquid inhibition growth system. The four strains of bacteria used were susceptible to fractions III and IV, affecting all four bacterial strains at concentrations below 5 μg/mL.

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http://dx.doi.org/10.1016/j.toxicon.2016.04.046
0041-0101/

1. Introduction

Scorpion venom contains a large number of different biologically active components that help ensure their survival, to defend against predators or capture prey. Within these components are proteins, peptides and enzymes, carbohydrates, free amines, nucleotides, lipids and other low molecular weight components with unknown function (Zlotkin et al., 1978; Possani, 1984). The molecules responsible for lethality in different experimental models of animals are toxic peptides having a molecular mass from 4 to 8 kDa, and are known to affect ion channel functions (Possani, 1984). On the other hand, there are reports of various peptides isolated from scorpions that have specificity for different species of organisms (mammals, insects or crustaceans). Some are toxic to more than one group of animals (Possani et al., 1999).

Isolation, chemical and functional characterization of scorpion venom components is expected to present an opportunity to discover new molecules of pharmaceutical interest. The idea is to identify their possible medicinal properties and verify their effectiveness. A variety of pharmacologically active components are known to occur in scorpion venoms, such as antimicrobial peptides (Zhao et al., 2009; Torres-Larios et al., 2000; Conde et al., 2000), insecticides (Ochola et al., 2007; Trung et al., 2006), antiepileptic peptides (Zhao et al., 2008), phospholipases (Valdez-Cruz et al., 2004), ion-channel specific toxins (Cestèle and Catterall, 2000; Gurrola and Possani, 1995; Corona et al., 2002; Rodríguez de la Vega and Possani, 2005), among others.

Mexico is highly biodiverse on scorpion species. Santibáñez-López et al. (2015) reported 281 distinct species of scorpions in Mexico, from which eight species, all from the genus *Centruroides* of the Buthidae family, are dangerous to humans (Dehesa-Dávila and Possani, 1994). One of them is *Centruroides tecomanus* (here abbreviated *Centruroides tecomanus*) which is distributed in the Western states of Mexico, endemic to the state of Colima, causing a high number of accidents to the human population (Chowell et al., 2006; Possani, 2005).

The venom composition of this scorpion started to be investigated 25 years ago (Possani et al., 1980). The authors reported gel filtration separation of the venom, followed by ion exchange chromatography and identification of the N-terminal amino acid of a toxic peptide. In addition, the authors reported the presence of hyaluronidase activity and circa 15 low molecular weight components positive to ninhydrin. Ramírez et al. (1988) using a similar chromatographic strategy reported the identification of 24 fractions, of which five of them were toxic to mouse. In the same year, Martin et al. (1988) determined the full amino acid sequence of a toxin whose physiological characterization showed that it affects the inactivation process of Na^+ currents. Valdez-Velázquez et al. (2013), described a proteomic analysis of this venom, and identified several peptides and proteins which correlated, with respect to molecular weight, with sequences obtained from a transcriptome analysis conducted after the construction of a cDNA library prepared from venomous glands of two individuals of the species *C. tecomanus.* At least 104 individual components were identified by mass spectrometry analysis and data obtained from the cDNA library. Comparing the deduced amino acid sequence with other known scorpion venom components suggested that several of the components could be Na^+- and K^+-ion-channel specific toxins. These findings support the intoxication symptoms described to occur when humans are stung by this scorpion. Among the sequences identified are also proteins involved in cellular processes, antimicrobial peptides, and other known venom components, proteins without defined function and sequences without similarity in databases. Among the cloned genes are those similar to enzymes with metalloproteinase activity.

This paper focuses on the identification of components from *C. tecomanus* venom, with an emphasis on components that affect insects, crustaceans or mammals, or that have antimicrobial or antiproliferative activities.

2. Material and methods

2.1. Venom source and purification procedures

The collection of scorpions of the species *C. tecomanus* took place in the community of Coquimatlán, state of Colima, Mexico (latitude 19°13′57.19″N; longitude 103°49′46.05″O; altitude 365 m above sea level). The venom of 200 scorpions was extracted by electrical stimulation (application of 15 V to the body of the animal). The venom was solubilized in 400 μL of double distilled water and centrifuged at 14,000 rpm for 15 min. The supernatant was recovered, lyophilized and kept at −20 °C. When needed aliquots were selected and used.

For purification a total of 2.3 mg protein of soluble venom, was applied into a C18 reverse-phase analytical column (250 × 10 mm) obtained from Vydac (Hisperia, CA, USA). The components were purified using a linear gradient of solvent A (water in 0.12% trifluoroacetic acid (TFA)) to 60% solvent B (acetonitrile in 0.10% TFA) for 60 min with a flow rate of 1 mL/min. The fractions were collected manually by monitoring the absorbance at 230 nm at intervals of 5 min apart, and then dried in a Savant Speed Vac SC210A apparatus (Albertville, MN, USA).

2.2. Mass spectrometry analysis

The relative amounts of protein/peptides were estimated based on the HPLC profile (integral area under the curve). The samples were diluted in 50% acetonitrile and 1% acetic acid to a final concentration of 0.1–0.5 μg/mL, and injected directly into an LC/MS system composed of a Finnigan LCQ^{Duo} ion trap mass spectrometer (MS) (San Jose, CA, USA) with a nanoelectrospray ionization (ESI) system.

2.3. Biological assays in different species (toxicity and lethality tests)

The toxicity and lethality tests of the twelve fractions obtained from HPLC separation (I-XII) were carried out on three different groups of organisms: mammals: albino mice (*Mus musculus*) of the strain (CD1) of approximately 20 g body weight; crustaceans:-freshwater shrimp (*Cambarellus montezumae*); insects:crickets (*Acheta domestica*). Bio-assays were conducted with 30 μg of each fraction injected into crustaceans and insects between the third and fourth abdominal segments, whereas for mice 50 μg of protein content was intraperitoneally injected. Control animals were injected with similar volumes of sterile water alone. The animals were kept under observation for a period of 24 h. Intoxication symptoms were said to be: “non toxic”, when the animals behaved like the controls; “Toxic” means that the animals showed symptoms such as: excitability, salivation, temporary paralysis, dyspnea and/or diarrhea, impairment of movements, lacrymation, respiratory problems in the case of mice, but recovered within 24 h, and “Lethal” when the animals showed symptoms of intoxication and died. The experiments were conducted in triplicate. The protocol used was approved by the Institutional Committee for Animal Welfare.

2.4. Cell culture

L5178Y/Tk±-3.7.2C (mouse lymphoma), HeLa (*Homo sapiens*,