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A survey of the venom of the spider *Lycosa vittata* by biochemical, pharmacological and transcriptomic analyses

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ABSTRACT

Lycosa vittata, mainly distributed in the southwest of China, is a medium-sized and venomous spider, whose venom remains unexplored so far. This study aims to present an overview of the venom. It mainly consisted of diverse peptides and exhibited inhibitory effects on voltage-gated ion channels in rat dorsal root ganglia neurons, with a strongest inhibition on tetrodotoxin-sensitive and tetrodotoxin-resistant voltage-gated Na^+ channels. Interestingly, it exerted cytotoxicity to cancer cells, with approximately 10-fold selectivity on PC-3 over others, implying the existence of selective anti-PC-3 agents in the venom. Moreover, 51 toxin-like peptides were deduced from the venom gland transcriptome. Bioinformatic analyses suggested their structures might have some distinguished properties and their predicted functions were consistent with the venom activities. This study suggests that the venom is an attractive source of neurotoxins with therapeutic significance, and provides references for the structure and function investigation of specific toxins in the future.

1. Introduction

Spiders are the most diverse and successful terrestrial invertebrates excluding insects, and are also the most abundant terrestrial predators (Coddington and Levi, 1991; Saez et al., 2010). There are 44,906 described species in approximately 114 families to 2014, with an even greater number awaiting characterization (Platnick, 2014. The world spider catalog, version 13.5. American Museum of Natural History, is available online at http://research.amnh.org/iz/spiders/catalog/COUNTS.html). Spiders roam the world and have conquered most terrestrial environments (Dos Santos et al., 2009; Hu et al., 2014). One of the major contributors to the evolutionary success of spiders is their ability to produce complex venoms for predation and predator deterrence (King, 2004). Spider venoms are complex cocktails composed of a variety of chemical compounds, including salts, small organic molecules, peptides and proteins, but the major components are small and disulfide-rich peptides (Saez et al., 2010; Klint et al., 2012). These peptides target a diverse range of ion channels, cell receptors and enzymes in a wide range of vertebrate and invertebrate species (Liang, 2008; Saez et al., 2010). Therefore, the spider venom peptides exhibit attractive pharmacological activity, such as antiarrhythmic, analgesic, cytolytic, haemolytic, insecticidal, antimicrobial, antiparasitic, antitumor and enzyme inhibitory activity. In the past two decades, these fascinating bioactivities of venom peptides make spider venom an ideal natural source for the discovery of novel therapeutic leads (Escoubas and Bosmans, 2007).

The geographic distribution of *Lycosa vittata* is mainly the southwest of China, such as Tibet, Yunnan and Guizhou provinces. In this study, the spiders were collected in Nyangtri of Tibet. *L. vittata* mainly lives in valley and paddy fields, and feeds on insects. It is a medium-sized spider (Fig. 1A). The females are 14.5–16.5 mm in body length and 19–26 mm in leg span, and the males are 10.5–15 mm in body length and 18–25 mm in leg span. The spider is dark brown in color with tawny specks of its carapace. The chelicerae are brown and with long brown hairs.

The potential of spider venom components as pharmacological tools and drug leads attracts more and more researcher's attention, and this potential is conformed by an increasing number of publications (Escoubas and King, 2009; Diego-García et al., 2010;

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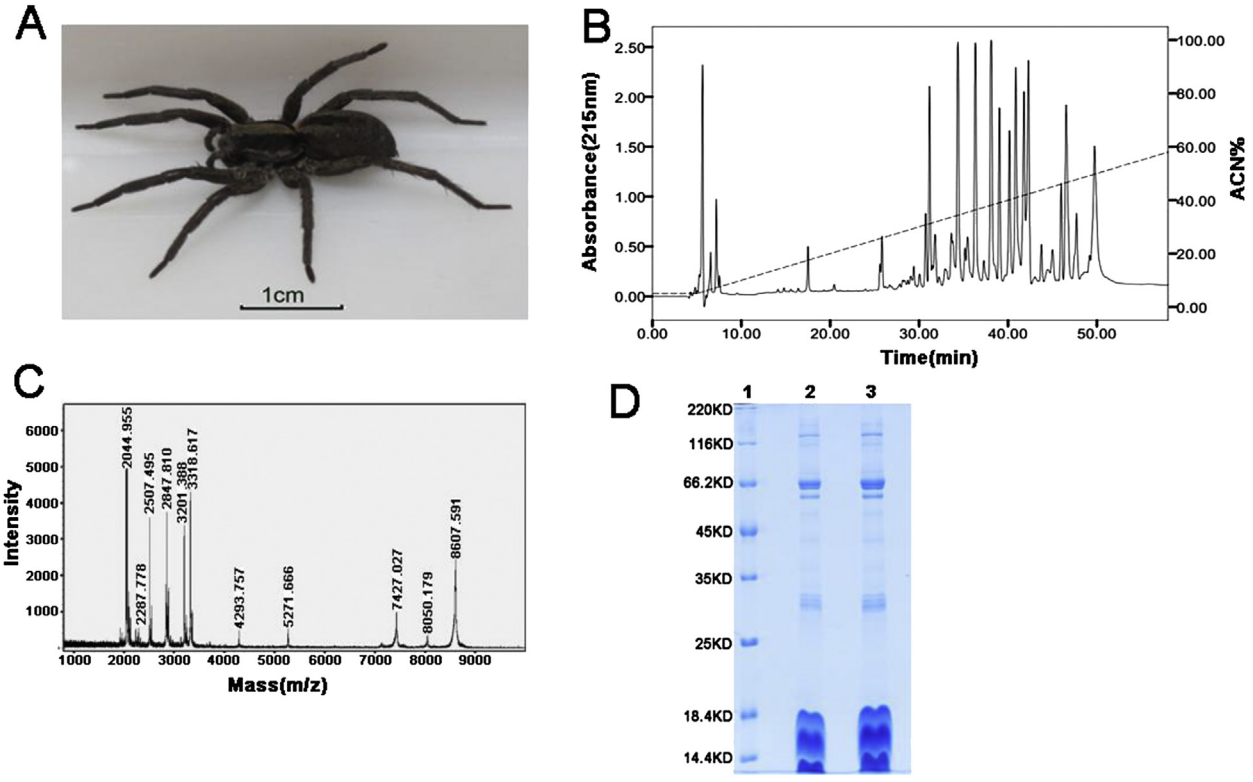

Fig. 1. A female *L. vittata* and the complexity of the venom peptides. A. A female *L. vittata* has a body length of 15 mm and a leg span of 25 mm. B. RP-HPLC separation of 1 mg of soluble venom from *L. vittata* in an analytical C18 column equilibrated with solution A (distilled water in 0.1% TFA), using a gradient from 5% to 50% of solution B (acetonitrile in 0.1% TFA) over 45 min with a flow rate of 1 mL/min. Absorbance was read at 215 nm. C. MALDI-TOF spectrum of *L. vittata* venom. D. SDS-PAGE of the venom of the spider *L. vittata*. 1, marker; 2 and 3, 70 and 140 μg venom, respectively.

Escoubas and Bosmans, 2007; King et al., 2008; Klint et al., 2012; Windley et al., 2012; Pineda et al., 2014; Kalia et al., 2015). However, the investigation of the gold mine of spider venom is only the tip of the iceberg. On this point in time, we investigated the biochemical and pharmacological properties of *L. vittata* venom and the transcriptome of the venoms glands. In the present study, we showed that the venom contains diverse peptides and possesses neurotoxic and anticancer activities, and we reported 51 peptide toxins deduced from the venom gland cDNA library of the wolf spider *L. vittata*. These results revealed the significant pharmacological activity and peptides diversity of the venom, and also provided the clues for screening pharmacological tools and drug leads from these peptides in future studies.

2. Materials and methods

2.1. Venom collection

The spider *L. vittata* were captured from the valley of Nyangtri of Tibet province in the southwest of China. Adult female *L. vittata* spider were maintained in plastic pails, and fed weekly with worms and given water daily. The venom was collected by using an electropulse stimulator to chelicere every two weeks as reported in our previous study (Wang et al., 2007). Venom was lyophilized and stored at −80 °C prior to analysis.

2.2. Toxin purification

The venom powder was dissolved with water to a final concentration of 10 mg/mL, centrifuged at 14,000 rpm for 30 min at 4 °C and filtered using a 0.22 μm microfilter (SJGVL04NS, Millipore). The venom solution was analyzed by RP-HPLC on a C18 column (phenomenex 100 Å, 4.6 mm × 250 mm, 5 μm) using a Waters Alliance 2695 (Milford, MA, USA). Venom components were eluted using a linear acetonitrile gradient (5%–50% acetonitrile/0.1% TFA in 45 min) at a flow rate of 1.0 mL/min (Liu et al., 2006). Fractions (1 mL/tube) were collected manually by monitoring the absorbance at 215 nm. Each fraction was lyophilized and reconstituted in 10 μL of 50% acetonitrile in water/0.1% TFA prior to MALDI-TOF-MS analysis.

2.3. MALDI-TOF-MS analysis

The venom was desalted with a ZIP-TIP and then analyzed using MALDI-TOF-MS (Ultraflex, Bruker Daltonics, Bremen, Germany). Ionization was achieved by irradiation with a nitrogen laser (337 nm) with a 20 kV acceleration voltage. α-Cyano-4-hydroxycimamic acid (CCA) was used as the matrix. Regarding the analysis the fractions from RP-HPLC, 1 μL of each fraction was applied to a well overlaid with 1 μL of CCA. Data were acquired using Bruker Daltonics Flexcontrol software (Bremen, Germany) and exported with Flex analysis software (Bremen, Germany). All analyses were conducted in a liner mode (Wang et al., 2013).

2.4. SDS-PAGE analysis

The venom powder was dissolved with water to a final concentration of 10 mg/mL, centrifuged at 14,000 rpm for 30 min at 4 °C and filtered using a 0.22 μm microfilter (SJGVL04NS, Millipore). The venom solution was analyzed by SDS-PAGE (15% separation gel