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Venomic and pharmacological activity of *Acanthoscurria paulensis* (Theraphosidae) spider venom

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ABSTRACT

In the present study we conducted proteomic and pharmacological characterizations of the venom extracted from the Brazilian tarantula *Acanthoscurria paulensis*, and evaluated the cardiotoxicity of its two main fractions. The molecular masses of the venom components were identified by mass spectrometry (MALDI-TOF-MS) after chromatographic separation (HPLC). The lethal dose (LD_{50}) was determined in mice. Nociceptive behavior was evaluated by intradermal injection in mice and the edematogenic activity by the rat hind-paw assay. Cardiotoxic activity was evaluated on *in situ* frog heart and on isolated frog ventricle strip. From 60 chromatographic fractions, 97 distinct components were identified, with molecular masses between 601.4 and 21,932.3 Da. A trimodal molecular mass distribution was observed: 30% of the components within 500-1999 Da, 38% within 3500–5999 Da and 21% within 6500–7999 Da. The LD_{50} in mice was 25.4 ± 2.4 μg/g and the effects observed were hypoactivity, anuria, constipation, dyspnea and prostration until death, which occurred at higher doses. Despite presenting a dose-dependent edematogenic activity in the rat hind-paw assay, the venom had no nociceptive activity in mice. Additionally, the venom induced a rapid blockage of electrical activity and subsequent diastolic arrest on *in situ* frog heart preparation, which was inhibited by pretreatment with atropine. In the electrically driven frog ventricle strip, the whole venom and its low molecular mass fraction, but not the proteic one, induced a negative inotropic effect that was also inhibited by atropine. These results suggest that despite low toxicity, *A. paulensis* venom can induce severe physiological disturbances in mice.

1. Introduction

According to the effects of venom in humans, accidents caused by spiders can be categorized in, at least, two distinct groups: those producing necrotic ulceration, and the ones that do not. Arachnidism produced by widow spiders (Theridiidae) will result in systemic symptoms but with minimal tissue damage. Envenomation by Agelenidae family (araneomorph funnel-web spiders, including hobo and grass spiders) results in severe tissue damage (Mattiello-Sverzut et al., 1998; Elston et al., 2000) and, in a minority of accidents, also systemic symptoms. Local necrosis and systemic symptoms are observed in the events incited by Sicariidae (*Loxosceles*; recluse and fiddlehead spiders) (Madrigal et al., 1972; Barbaro et al., 1992).

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Tarantulas (Theraphosidae, Mygalomorphae) bites are considered to be painful, but do not induce local necrosis or systemic effects (Saucier, 2004). With exception of bites by spiders of the genus *Atrax* (Hexathelidae), there are no records of serious accidents caused by Mygalomorphae spiders in human probably due to several factors such as venom low toxicity to humans, insufficient amount of venom injected, greater difficulty of Orthognatans' chelicerae in piercing the skin, and also because some species live in low frequented local by man (Lucas, 2003). Unless infection occurs, a slight inflammation at the puncture site can arise. Spiders of the family Theraphosidae have urticating hairs covering their bodies, which are brushed off by the spider as a mechanism of defense to deter predators. These hairs were found to induce local dermatitis in vertebrates, including humans (Shrum et al., 1999). The puncture wounds from the spider's fangs require local wound care, follow-up for signs of infection, short-term analgesia and a tetanus booster (Kelley and Wasserman, 1998; Shrum et al., 1999).

The spider venom is a diverse mixture of low molecular mass compounds (16% of all compounds), acylpolyamines (11%), linear peptides (6%), cysteine-knotted mini-proteins (60%), neurotoxic proteins (1%) and enzymes (6%) (Jackson and Parks, 1989; Kuhn-Nentwig et al., 2011). It is mainly used to paralyze prey and for defense, and contains toxins that affect the central or peripheral nervous systems. These neurotoxins have been identified mostly as acylpolyamines and peptides or proteins that act on membrane receptors or ion channels (see review Estrada et al., 2007).

The acylpolyamine toxins are low molecular mass compounds (<1 kDa) that appear to have evolved to specifically provoke rapid paralysis. Their complex structures are composed by a polyamine chain with a primary amino or a guanidine group at one end and an aromatic ring at the other. These compounds interact with multiple targets in the central and peripheral nervous systems of insects, and also in the CNS of mammals, whereas the main targets are ionotropic glutamate and nicotinic acetylcholine receptors (Kawai et al., 1982; Herold and Yaksh, 1992; Bixel et al., 2001).

Eight hundred curated sequences of protein toxins have been described for spider venom to date, among them approximately 20% corresponds to Theraphosidae spiders (available at ArachnoServer 2.0; Herzig et al., 2011). Most of these 200 peptides has 30–40 amino acid residues, three disulfide bridges and basic character (Escoubas and Rash, 2004), and are modulators of ion-channels, such as calcium, sodium, and potassium.

In this communication we report the results of proteomic and pharmacological characterizations of the venom extracted from the Brazilian spider *Acanthoscurria paulensis*.

2. Materials and methods

2.1. Spider collection

A. paulensis (Theraphosidae, Mygalomorphae) is a dark brown colored spider widely distributed in three Brazilian regions: South, Southeast and Midwest (Mello-Leitão, 1923; Lucas et al., 2010). The specimens used in the present work were collected in Brasília (Distrito Federal, Brazil) under the "Instituto Chico Mendes de Conservação da Biodiversidade" (ICMBio) license number 24227-1. Only adult male specimens were used in this study due to their availability in field at the time. The spiders were identified by Dr Paulo César Motta from the Laboratory of Arachnids (University of Brasília, Brasília, DF, Brazil) based on morphological characteristics.

2.2. Venom extraction and purification

The venom of eight adult male specimens of *A. paulensis* spiders was monthly obtained by electrical stimulation, solubilized in deionized water containing 0.12% trifluoroacetic acid (TFA) and centrifuged at 10,000 × *g* for 10 min. The soluble supernatant was immediately frozen, lyophilized and stored at −20 °C. The venom dry weight was determined in a high precision analytic balance. Aliquots of 5 mg of dried venom were solubilized in deionized water, centrifuged at 10,000 × *g* for 10 min and the supernatant was submitted to high performance liquid chromatography (HPLC), using a C18 reversed-phase semipreparative column (Jupiter 5 μm, 300 Å, 250 × 10 mm, Phenomenex) using a linear gradient from solution A (0.12% TFA) to 60% solution B (0.10% TFA in acetonitrile – ACN) run for 60 min after 10 initial minutes at 0% solution B with detection at 216 and 230 nm. The fractions eluted at a flow rate of 1.5 mL/min were individually and manually collected, vacuum dried and stored at −20 °C until use. In order to obtain the low molecular mass fraction (LMMF) and protein fraction (PF) for the evaluation of cardiotoxic activity, the fractions eluting from 0 to 35% solution B and from 35 to 74% solution B were separately collected. After removal of solvent, LMMF and PF were quantified by dry weight in a high precision analytic balance and stored at −20 °C until use.

2.3. Mass spectrometry

The molecular masses of the chromatographic fractions of *A. paulensis* venom were performed on an UltraFlexIII MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany). The samples were reconstituted in deionized water at variable concentrations and dissolved (1:3, v:v) in an α-cyano-4-hydroxycinnamic acid matrix solution (α-cyano-4-hydroxycinnamic acid at 5 mg/mL dissolved on acetonitrile, water, trifluoroacetic acid, 5:4:1, v:v:v) spotted in triplicate onto a sample plate and allowed to dry at room temperature. The MS spectra were acquired in both reflected and linear positive modes. Calibration of the system was performed using a mixture of the Peptide Calibration Standard and Protein Calibration Standard I for mass spectrometry (Bruker Daltonics, Germany). Spectra were processed with MassLynx™ 3.5 (Manchester, UK) and FlexAnalysis 3.3 (Bruker Daltonics, Germany).

2.4. Animals

Animals were contained in accordance with the ethical guidelines of the Brazilian Society for Neuroscience and Behavior, which follows the guidelines for animal care