Toxicon 126 (2017) 51–58

Contents lists available at ScienceDirect

Toxicon

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

Some pharmacological effects of *Tityus obscurus* venom in rats and mice

Andria de Paula Santos-da-Silva [a, 1], Denise Maria Candido [b], Ana Leonor A. Nencioni [a], Louise F. Kimura [c, 2], José P. Prezotto-Neto [c, 3], Katia Cristina Barbaro [c], Hipócrates M. Chalkidis [d], Valquíria Abrão Coronado Dorce [a, *]

[a] Laboratory of Pharmacology, Butantan Institute, Av. Dr. Vital Brasil, 1500, 05503-900, São Paulo, SP, Brazil
[b] Laboratory of Arthropods, Butantan Institute, Av. Dr. Vital Brasil, 1500, 05503-900, São Paulo, SP, Brazil
[c] Laboratory of Immunopathology, Butantan Institute, Av. Dr. Vital Brasil, 1500, 05503-900, São Paulo, SP, Brazil
[d] Laboratory of Biological Research, Integrated Tapajos College/Amazon University FIT/UNAMA, Rua Rosa Vermelha, 335, 68010-200, Santarém, PA, Brazil

ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form
15 December 2016
Accepted 20 December 2016
Available online 22 December 2016

Keywords:
Scorpion venom
Tityus obscurus
Envenomation

ABSTRACT

There are a great number of studies about Brazilian scorpions. However, little is known about the venom of scorpions of northern Brazil, mainly about *Tityus obscurus*, which is responsible for the most number of accidents in the Amazon. Thus, this study aimed to evaluate some pharmacological effects of *T. obscurus* venom in rats and mice. In rats, the venom (10 mg/kg i.p.) caused hemorrhagic patches in the lung parenchyma but did not lead to pulmonary edema. There was a decrease in general activity, observed in the activity box after venom injection. The venom did not induce changes in the occurrence and intensity of experimentally induced convulsions, nor did it cause hippocampal neuronal loss. In mice, the LD_{50} obtained was 3.13 mg/kg (i.p.). Different doses of the venom (0.2; 1; 5; 10; 15 μg/30 μL per hind paw) induced edematogenic and moderate nociceptive activity in mice. The *Tiyus serrulatus* venom used as comparison caused more intense symptomatology in mice. Comparing to the venom of other *Tityus* scorpions of medical importance, that have convulsant and intense nociceptive effects and cause lung edema, as described in the literature, we can conclude that the venom of *T. obscurus* probably has different characteristics.

1. Introduction

Scorpions are widely distributed in the world, living in almost all biomes. They are classified into 16 to 19 families (Prendini and Wheeler, 2005; Sharma et al., 2015; Stockman, 2015; Stockman and Ythier, 2010). However, only 25 species from the family Buthidae are medically important, where they are responsible for envenomation cases.

In Brazil, *Tityus serrulatus*, *T. bahiensis*, *T. stigmurus* and *T. obscurus* are the main dangerous scorpions (Reckziegel and Pinto, 2014). In 2015, there were over 50,000 cases of scorpionism and 77 deaths (SINAN, 2015).

In the Amazon, greatest numbers of accidents are due to the sting of *T. obscurus* (Pardal et al., 2003). *T. obscurus* is known as the black scorpion because of its dark coloration in adulthood. However, when young, it has a pigmented body with light and dark spots, where it is confused with other species of scorpions found in the same region (Lourenço, 2002a, 2002b). According to Lourenço and Leguin (2008), *Scorpio (Atreus) obscurus* Gervais, 1843 is now known as the species *T. obscurus*, name which is currently valid and

* Corresponding author.
E-mail address: vdorce@butantan.gov.br (V.A.C. Dorce).
[1] Present address: Laboratory of Zoological Collections, Butantan Institute, Av. Dr. Vital Brasil, 1500, 05503-900, São Paulo, SP, Brazil and Post-Graduation Program in Zoology, Institute of Biosciences, University of São Paulo, Av. Prof. Lineu Prestes, 2415, 05508-900, São Paulo, SP, Brazil.
[2] Present address: Laboratory of Pain and Signaling, Butantan Institute, São Paulo, Brazil, Butantan Institute and Department of Immunology, Institute of Biomedical Sciences, University of São Paulo, Av. Prof. Lineu Prestes, 2415, 05508-900, São Paulo, SP, Brazil.
[3] Present address: Biotechnology Center, Nuclear and Energy Research Institute (IPEN/CNEN-SP), Av. Prof. Lineu Prestes, 2242, 05508-000, São Paulo, SP, Brazil.

http://dx.doi.org/10.1016/j.toxicon.2016.12.008
0041-0101/

considered in this work. These authors conducted a detailed study of all morphological characteristics of the original type material which confirmed that this species belongs to the genus *Tityus*. They verified the position of *T. obscurus* as a senior synonym of both *T. paraensis* Kraepelin, 1896 and *T. cambridgei* Pocock, 1897 (Pardal et al., 2014; Torrez et al., 2015).

The symptoms frequently observed after this scorpion sting are local and radiating pain, paresthesia, edema, erythema, sweating, piloerection and burning, besides minor systemic manifestations such as headache, agitation tremors, prostration (Pardal et al., 2014).

In Western Pará, Brazil, envenomation by *T. obscurus* is characterized by local effects such as pain and paresthesia. However, one predominant neurological manifestation which includes myoclonus, difficult ambulation and strong muscular contraction, referred to by patients as an "electric shock in the body". This symptom is not described in other regions of the Amazon, in accidents caused by the same scorpion species (Pardal et al., 2003, 2014).

Several studies describe the biochemical characterization of the toxins present in this venom, especially those that act on sodium and potassium channels, but little is known about the pharmacological characterization of *T. obscurus* venom (Batista et al., 2000, 2002a, 2002b; Guerrero-Vargas et al., 2012; Murgia et al., 2004).

Studies of biological and/or pharmacological activities of the whole venom or toxins in the venom will contribute to a better understanding of the pathophysiology of envenomation caused by the Amazon black scorpion and are considered highly relevant. Thus, the present study aimed to characterize the symptoms and behavioral effects of the crude venom of the *T. obscurus* scorpion in rats and mice.

2. Materials and methods

2.1. Animals

Male Swiss mice (18–20 g) and male Wistar rats (230–260 g) were used. These rodents were kept under standard vivarium conditions with free access to chow and water, housed under controlled temperature (20 ± 2 °C) and maintained on a 12/12 h light-dark cycle. All the experimental procedures were conducted with prior permission of the Institution's Ethics Committee for Experiments on Animals (Protocols number 679/09 and 769/10). The SISBIO/IBAMA provided animal collection permission (21483-2 and 20158-1), and the Council of Genetic Heritage Management provided authorization of access to genetic resources (CGEN-010803/2013-0).

2.2. Scorpion venoms

The scorpions *T. obscurus* were collected in the Amazon region, in Santarém and Belterra municipalities in Pará State. The animals were maintained in captivity in the arthropod vivarium of the Butantan Institute. *T. serrulatus* venom was supplied by the Venom Commission of the Butantan Institute. Both venoms were obtained by electrical stimulation of the telson. For experiments conducted in rats, lyophilized venom of *T. obscurus* scorpion diluted in 1.46% NaCl (concentration required to complete dilution of venom) was used. For experiments with mice, non-lyophilized *T. serrulatus* and *T. obscurus* venoms were diluted with PBS (pH 7.4) and centrifuged. The supernatant was aliquoted and stored at −20 °C until use. The protein was quantified by the bicinchoninic acid method (Smith et al., 1985), using bovine serum albumin (BSA) as the standard (Sigma-Aldrich, St. Louis, MO, USA).

2.3. Experimental procedures

2.3.1. Symptomatology and behavioral effects of T. obscurus scorpion venom

Rats were divided into six experimental groups (n = 5). A control group was injected with 1.46% NaCl intraperitoneally (i.p.). The other five groups were injected i.p. with the venom diluted in NaCl solution (1.46%) at doses of 0.5, 2, 5, 10 and 15 mg/kg. The injected volume was 0.1 mL/100 g of body weight of the animal. After injection, each animal was placed individually in transparent polyethylene boxes. The behavior and the symptoms of intoxication induced by the venom were observed and quantified within 6 h.

The 10 mg/kg dose of *T. obscurus* venom was chosen for all other experiments performed with rats because the main symptoms of the envenomation occurred at this dose.

2.3.2. Evaluation of the effects of the T. obscurus venom on lungs

Rats were divided into six groups: two injected with NaCl solution and four injected with *T. obscurus* venom (10 mg/kg) i.p. (n = 5). After injection, at intervals of 1 or 12 h for control groups and 1, 4, 6 and 12 h for experimental groups, the animals were anaesthetized in a CO_2 chamber and sacrificed by cervical vessels section for lung removal. The lungs were immediately weighed and the lung weight index (index = lung weight X 100/body weight) was calculated to determine the presence or absence of pulmonary edema (Sandoval and Dorce, 1993a).

For histological analyses, we used the lungs of the animals of the experiment in Section 2.3.1., which were injected with 10 mg/kg venom or NaCl solution. The animals were anaesthetized in a CO_2 chamber, perfused with formalin and the lungs were removed. They were fixed in formalin for seven days, dehydrated with ethanol, cleared with xylene and embedded in Paraplast®. The slides with sections of 10 μm thick of various regions of the lungs were stained with hematoxylin-eosin and examined under a light microscope (40× objective). Evaluation was performed by morphological comparison of lung sections between the experimental animals and the control group.

2.3.3. Effects of T. obscurus venom on general activity in the activity box

The two groups of rats were injected with NaCl solution or 10 mg/kg venom. The animals of one experimental group and one control group were tested alternately for general activity 5 min after injection and two other groups were analyzed 1 h after injection. The animals were observed for general activity in an activity box. This apparatus consists of a cage with two sensor groups, each one connected to a counter, where one measures only locomotor activity and the other measures general activity (horizontal locomotion and other movements) as described by Dorce et al. (2010). Animals of each group were placed individually in the activity box and observed for 10 min. After each animal observation, the box was cleaned with a 5% alcohol solution to prevent smell interferences with the next animal.

2.3.4. Evaluation of the effects of T. obscurus venom on seizure activity induced by pentylenetetrazol or picrotoxin

The effects of *T. obscurus* venom on seizure events were determined in rat models of epilepsy using the convulsants pentylenetetrazol (PTZ, 50 mg/kg i.p.) and picrotoxin (PTX, 3.5 mg/kg injected subcutaneously). The doses used were determined in our laboratory from previous tests based on literature data (Sandoval and Dorce, 1993b) and are the ones that cause seizures in about half of animals injected.

Groups of rats (n = 10) received NaCl solution + PTZ, 10 mg/kg venom + PTZ, NaCl solution + PTX, or venom (10 mg/kg) + PTX. So