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Local inflammatory reaction induced by *Scolopendra viridicornis* centipede venom in mice

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ABSTRACT

Centipede envenomation is generally mild, and human victims usually manifest burning pain, erythema and edema. Despite the abundance and ubiquity of these animals, centipede venom has been poorly characterized in literature. For this reason, the aim of this work was to investigate local inflammatory features induced by *Scolopendra viridicornis* centipede envenomation in mice, evaluating edema formation, leukocyte infiltration, production of inflammatory mediators, and also performing histological analysis. The highest edematogenic activity induced by the venom, determined by plethysmometry, was noticed 0.5 h after injection in mice footpad. At 24 h, edema was still detected in animals that received 15 and 60 μg of venom, and at 48 h, only in animals injected with 60 μg of venom. In relation to leukocyte count, *S. viridicornis* venom induced cell recruitment, mainly neutrophils and monocytes/macrophages, in all doses and time periods analyzed in comparison with PBS-injected mice. An increase in lymphocytes was detected especially between 1 and 24 h at 60 μg dose. Besides, eosinophil recruitment was observed mainly for 15 and 60 μg doses in early time periods. Edema formation and cell recruitment were also confirmed by histological analysis. Moreover, *S. viridicornis* venom stimulated the release of IL-6, MCP-1, KC, and IL-1β. Conversely, *S. viridicornis* venom did not induce the release of detectable levels of TNF-α. We demonstrated that the edematogenic activity induced by *S. viridicornis* venom was of rapid onset, and the venom stimulated secretion of pro-inflammatory mediators which contribute to the inflammatory reaction induced by *S. viridicornis* venom in an experimental model.

1. Introduction

Centipedes (Class: Chilopoda) constitute an important group of terrestrial arthropods (Barnes, 1984; Edgecombe and Giribet, 2007). These animals are distributed worldwide except in Antarctica, with some specimens inhabiting arid, open fields or caves. They have a greater diversity in the tropics and warm regions, and can be found among

Abbreviations: IL-1β, interleukin 1 beta; IL-6, interleukin 6; IL-8, interleukin 8; TNF-α, tumor necrosis factor alpha; MCP-1, monocyte chemoattractant protein-1; KC, keratinocyte chemoattractant.

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litter, under rocks and fallen logs, and moist soils. These animals are generalist predators, feeding themselves with a great variety of prey, including other arthropods, amphibians, and small vertebrates (Edgecombe and Giribet, 2007). To capture their prey and/or to defend themselves, centipedes use the first pair of legs, specialized and modified into a pair of forcipules, also known as maxillipeds. Inside of each forcipule, there is a venom gland, involved by a muscular layer, which ends in an orifice. These features allow centipedes to capture, arrest, and inject venom into the victim (Antoniazzi et al., 2009). The scolopendromorphs (Order: Scolopendromorpha) are the more aggressive and voracious, reaching 30 cm in length (e.g. *Scolopendra gigantea*) (Edgecombe and Giribet, 2007).

Since centipedes are found worldwide, their contact to humans is frequent and it may cause accidents. The clinical symptoms presented by patients bitten by centipedes are usually local, including manly pain, erythema and edema. The proposed treatment is mainly the administration of analgesics, local anesthetics, antihistaminic drugs, among others (Chaou et al., 2009; Knysak et al., 1998; Medeiros et al., 2008). Their venom is generally not lethal to humans, although complications such as rhabdomyolysis, acute renal failure, proteinuria, myocardial ischemia and infarction, hemoglobinuria and hematuria, hemorrhage, pruritus, fever and chills, general rash, eosinophilic cellulitis, anaphylaxis, and cases of death related to *Scolopendra* spp. have been reported in the literature (Harada et al., 1999; Undheim and King, 2011).

Given the fact that centipede venoms can cause a great diversity of symptoms, it can be implied that they constitute a mixture of different toxins with distinct functions. Although there are few studies published about centipede venoms, some bioactive compounds have already being identified. Gomes et al. (1983) purified a toxin from *Scolopendra subspinipes dehaani* venom (Toxin – S) that seems to play a role in the cardiovascular system from toad, rat, guinea pig, and rabbit. Mohamed et al. (1983) and Stankiewicz et al. (1999) characterized some substances, such as cholesterol esters, phospholipids, histamine, polysaccharides, cholesterol and triglycerides from *Scolopendra* spp. venoms. Using a proteomic approach to compare *Scolopendra viridicornis nigra* and *Scolopendra angulata* venoms, Rates et al. (2007) detected more than 60 proteins/peptides, and some of them were responsible for toxic effects in insects like house flies. Thus, centipede venoms became a source of interesting compounds in the last years, so that some authors consider them as a putative neglected source of bioactive substances, such as antimicrobial peptides (Peng et al., 2010) and neurotoxins (Yang et al., 2012). In addition, recently, the construction of a cDNA library from *S. subspinipes mutilans* venom gland was accomplished, indicating molecular advances on centipede venoms studying (Liu et al., 2012).

Concerning the venom from the Brazilian centipede *S. viridicornis*, a specie commonly found in many regions of the country, venom has been reported to show phospholipasic, direct hemolytic, gelatinolytic, caseinolytic, and fibrinogenolytic activities *in vitro* (Malta et al., 2008). Besides, a relatively small amount of venom induced an intense hyaluronidasic activity. In relation to studies *in vivo*, the venom caused nociception, edema, and myotoxicity in mice (Malta et al., 2008).

Since the reports of mechanisms of action from Brazilian centipede venoms are scarce in literature and therefore the pathogenesis of envenomation are mostly unknown, the aim of this work was to study some aspects of the local inflammatory response induced by *S. viridicornis* venom in murine model.

2. Materials and methods

2.1. Animals and venom

Swiss mice (male, 18–20 g) were provided by the Butantan Institute animal house. All experimental procedures involving animals were conducted according to with the Guide for the Care and Use of Laboratory Animals and were approved by Ethical Committee for the Use of Animals of Butantan Institute (certificate 782/10). Specimens of *S. viridicornis* were collected in several locations in Tocantins State, Brazil and transferred to the Laboratory of Arthropods (Butantan Institute), where they were kept in captivity. ICMBio provided animal collection permission no. 15702-3 and CGEN provided the license for genetic patrimony Access (02001005111/2008). Venom was milked every month by electric stimulation, and protein content of venom pool was determined by bicinchoninic acid method (Smith et al., 1985), using bovine serum albumin (BSA – Sigma–Aldrich, MO, USA) as a standard.

2.2. Evaluation of paw edema

Mice ($n = 6$) were injected (30 μL) with *S. viridicornis* venom (3.8, 15 or 60 μg) or PBS (negative control) in the right hind paw. After 0.5, 1, 4, 6, 24 and 48 h of injection, edema was measured by plethysmometry (7140 Plethysmometer, Ugo Basile, IT). Results expressed the difference in paw volume (μL) prior to (control) and after (experimental) injection (mean ± SEM).

2.3. Leukocyte count in footpad pellets

Mice ($n = 4$–6) were injected (30 μL) with *S. viridicornis* venom (3.8, 15 or 60 μg) or PBS (negative control) in the right hind paw. After 0.5, 1, 4, 6, 24 and 48 h of injection, the right paws were removed at the level of the tibiotarsal joint and tissue was disrupted and homogenized in 2 mL in a solution containing PBS pH 7.4, EDTA 2 mM, 5% $NaHCO_3$ and indomethacin 10 μM. Following centrifugation at 4 °C for 10 min, supernatants were recovered to assay the release of cytokines and chemokines by enzymatic immunoassay. Cell pellets were recovered in PBS and 0.1% BSA solution to perform cell counts. Total leukocyte counts from footpad homogenates were performed by 0.1% Trypan blue exclusion (Sigma–Aldrich, MO, USA) using a hemocytometer chamber; differential counts were accomplished using cytocentrifuge slides stained with HEMA-3 (Fischer Scientific Company, MI, USA). For differential cell counts, 100 leukocytes were counted and identified as monocytes/macrophages, lymphocytes, neutrophils or eosinophils, based on staining and morphologic