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Involvement of mast cells and histamine in edema induced in mice by *Scolopendra viridicornis* centipede venom

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ABSTRACT

Bites caused by *Scolopendra viridicornis* centipede are mainly characterized by burning pain, paresthesia and edema. On this regard, the aim of this work was to study the involvement of mast cells and histamine in edema induced by *Scolopendra viridicornis* (*Sv*) centipede venom. The edema was analyzed on mice paws. The mice were pretreated with cromolyn (mast cell degranulation inhibitor) and antagonists of histamine receptors, such as promethazine (H_1R), cimetidine (H_2R) and thioperamide (H_3/H_4R). The analyses were carried out at different times after the injection of *Sv* venom (15 μg) or PBS in the footpad of mice. Our results showed a significant inhibition of the edema induced by *Sv* venom injection in mice previously treated: cromolyn (38–91%), promethazine (50–59%) and thioperamide (around 30%). The treatment with cimetidine did not alter the edema induced by *Sv* venom. Histopathological analysis showed that *Sv* venom injection (15 μg) induced edema, leukocyte recruitment and mast cells degranulation, when compared with the PBS-injected mice. Direct effects of the *Sv* venom on mast cells were studied in PT-18 line (mouse mast cell) and RBL-2H3 cells (rat mast cells). The data showed that higher doses (3.8 and 7.5 μg) of *Sv* venom were cytotoxic for both cell lineages and induced morphological changes. However, lower doses of the venom induced degranulation of both mast cell lines, as well as the secretion of MCP-1, IL-6 and IL-1β. The production of PGD_2 was only observed in the RBL-2H3 line incubated with *Sv* venom. Taking our results together, we demonstrated that upon *Sv* venom exposure, mast cells and histamine are crucial for the establishment of the local inflammatory reaction.

1. Introduction

Centipedes are invertebrate animals of the Chilopoda class and are part of the Arthropoda phylum. These animals are present in every continent except Antarctica and constitute an important group of terrestrial arthropod predators (Edgecombe and Giribet, 2007). These animals are found preferentially in damp, dark environments, taking refuge under rocks, leaves, bark and caves (Jangi, 1984). However, due to the exponential growth of cities, which provides plenty of prey, the centipedes are also found in urban areas (Medeiros et al., 2008). Therefore, they have been causing accidents on humans, as the bite injects venom produced in the glands located inside their maxillipeds (Antoniazzi et al., 2009). Centipedes of the Scolopendromorpha order are the more aggressive and voracious, with species measuring about 30 cm long (*Scolopendra gigantea*) (Edgecombe and Giribet, 2007). They feed on invertebrates and also small vertebrates such as birds, rodents and snakes.

Most of the accidents in humans occur on the limbs (hands and feet) and the clinical symptoms of envenomation are mainly characterized by intense local pain, erythema and edema (Medeiros et al., 2008). Furthermore, the presence of vesicles, blisters and pustules on the bite site has been described, accompanied by fever (Veraldi et al., 2010), and urticaria (Harada et al., 2005). Systemic manifestations are uncommon, but have been observed, such as nausea, sweating, anxiety, depression and vomiting (Knysak et al., 1998; Bush et al., 2001); multiple neuropathy, dyspnoea (Friedman et al., 1998); acute myocardial infarction (Yildiz et al., 2006; Üreyen et al., 2015); rhabdomyolysis, acute renal failure (Logan and Ogden, 1985); proteinuria (Hasan and Hassan, 2005);

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acute disseminated encephalomyelitis (Yao et al., 2013) and secondary cutaneous diphtheria (Jungling et al., 2014). Centipede envenomation is usually not lethal to humans; however, there are some reports of death following the bite (Harada et al., 1999; Serinken et al., 2005).

There are few reports in the literature characterizing the venom of centipedes. However, for *Scolopendra* genus, a great toxins diversity in the venoms has been observed, such as: esterase (Rates et al., 2007; González-Morales et al., 2009), myotoxins, proteases, hyaluronidases (You et al., 2004; Malta et al., 2008), cardiotoxins (Gomes et al., 1983) and neurotoxins (Stankiewicz et al., 1999; Chen et al., 2014; Rong et al., 2015). Furthermore, the presence of cholesterol esters phospholipids, histamine, polysaccharides and triglycerides on these venoms was showed (Mohamed et al., 1983). In *Scolopendra subspinipes mutilans* peptides with diverse activities were identified, such as: antimicrobial (Peng et al., 2010; Hou et al., 2013; Kwon et al., 2013; Choi et al., 2014; Yoo et al., 2014; Lee et al., 2015), antifungal (Choi et al., 2013) and Xa factor inhibitor (Kong et al., 2013).

In this context, Liu et al. (2012) conducted transcriptomic and venomic analyses of *S. subspinipes dehaani* and verified the presence of neurotoxins, components with activity on ion channels and allergens. Furthermore, these authors also characterized at least 40 proteins/peptides with distinct activities such as: platelet aggregation, anticoagulant, phospholipase A2 and trypsin inhibitor. Recently, in another study Lee et al., (2015) identified an antimicrobial peptide on the body extract of *S. subspinipes mutilans* with necrotizing activity in cancer cells. According to these observations, some authors suggest that the venom from these animals represents a neglected source of new bioactive compounds (Rates et al., 2007; Undheim and King, 2011).

It was also verified that the venom of the Brazilian *Scolopendra viridicornis* centipede presents hyaluronidasic, nociceptive, edematogenic, myotoxic activities, as well as hemolytic action in human erythrocytes (Malta et al., 2008). In this sense, Kimura et al. (2013) showed, in mice model, that the venom of *Scolopendra viridicornis* induces an intense local inflammation, with persistent edema accompanied by cellular influx predominantly of neutrophils and macrophages. Moreover, the authors observed the production of some pro-inflammatory cytokines and chemokines, such as IL-6, MCP-1, IL-1β and KC in the site of venom injection. The inflammatory process is a complex mechanism that involves cell recruitment and secretion of a variety of cytokines, chemokines and soluble mediators. Mast cells are closely related to the pathophysiology of inflammation, and their activation causes the release of chemical mediators, which are responsible for significant tissue changes, such as: vasodilation, increased vascular permeability, chemotaxis of neutrophils, among others (Metz and Maurer, 2007).

Since the *S. viridicornis* venom induces severe local reaction with rapid formation of edema and intense cell recruitment (Kimura et al., 2013), in this study we evaluated the involvement of mast cells and histamine in the edema triggered by this venom. Our data contribute to the understanding of the immune response induced by centipede accidents, not yet completely understood.

2. Material and methods

2.1. Animals and venom

Swiss male mice (18–20 g) were provided by the animal facility of the Butantan Institute, São Paulo, Brazil. Specimens of *S. viridicornis* (*Sv*) were collected in several locations of Tocantins State, Brazil and transferred to the Arthropods Laboratory (Butantan Institute), where they were kept in captivity. Venom was milked every month by electric stimulation, and stored at −20 °C. Protein content of the venom pool was determined by the bicinchoninic acid method (Smith et al., 1985), using bovine serum albumin (BSA) as the standard. The procedures involving animals were conducted according to national laws and policies (protocol numbers: CEUAIB 782/10, CGEN 02001.005130/2008, and SISBIO 15222).

2.2. Pharmacological treatments

Pretreatments as per recommendation (Galvão-Nascimento et al., 2010) were applied in order to establish the involvement of mast cells (MC) and histamine in the edema induced by *Sv* venom. For this, four groups of mice received different pharmacological treatments. On group 1, cromolyn (CROM), an inhibitor of MC degranulation, was administered (30 mg/kg) via intraperitoneal (i.p.) procedure, for 3 consecutive days before the intraplantar venom injection. On group 2, promethazine (PRO), a histamine type 1 receptor (H_1R) antagonist (5 mg/kg) was intravenously injected (i.v.) 30 min before the venom injection. Group 3 received cimetidine (CIM), a histamine type 2 receptor (H_2R) antagonist, via i.p. (15 mg/kg) 2 h before venom injection. Group 4 was treated with thioperamide (THIO), a histamine type 3 and 4 receptor (H_3R/H_4R) antagonist (20 mg/kg) via i.p. 30 min before the venom. Drugs were purchased from Sigma − Aldrich, Brazil. Sterile PBS (phosphate buffer saline) was used as vehicle and control for all treatments.

2.3. Evaluation of paw edema

Mice (n = 6) submitted to the different pharmacological treatments above, or to PBS (negative control) were injected (30 μL) with *Sv* venom (15 μg) in the right hind paw. After 15 or 30 min; 1; 4; 6; 24 and 48 h of injection, the edema was measured by plethysmometry (7140 Plethysmometer, Ugo Basile, IT). The results were expressed as the difference in paw volume (μL) prior to (control) and after (experimental) injection (mean ± SEM).

2.4. Histopathological analysis

Mice (n = 3) were injected in the right paw with *Sv* venom (15 μg/30 μL) or PBS (negative control). At 30 min after the injection, the animals were euthanized and the right paws were collected for footpad skin removal. The samples were then fixed in 4% paraformaldehyde in PBS, pH 7.2, for 24 h. After dehydration in crescent ethanol series up to 95%, the samples were embedded in glycol methacrylate (Leica Microsystems Nussloch GmbH, Heidelberg, GR). Sections of 4 μm, were obtained in a Microm HM340 microtome and stained with toluidine blue and fuchsine, and examined under a light microscope. Photomicrographs were taken using an Olympus BX51 microscope, equipped with a digital camera and Image-Pro Express software (Media Cybernetics).

2.5. In vitro assay of mast cell degranulation

The release of the β-hexosaminidase enzyme was used as a parameter for mast cell degranulation *in vitro* assay, according to Hide et al. (1993) and Faquim-Mauro et al. (2003). For this, PT-18 (4×10^5) and RBL-2H3 (2×10^5) cell lines were incubated in Tyrode's buffer (160 μL/well) in 96-well round-bottom plates (Costar) for 2 h at 37 °C in humidified 5% CO_2 incubator. Afterwards, it was added or not 1.9 μg of *Sv* venom diluted in Tyrode's buffer (40 μL) and the cells were incubated at different periods (30 min, 1 and 2 h). After this, the cells were centrifuged and the supernatants collected and transferred to a new plate. The cells were lysed with a 0.5% Triton X-100 (200 μL) solution to evaluate the total enzyme content. Samples of all cell supernatants (10 μL/well) were