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Biochemical and immunological characteristics of Peruvian *Loxosceles laeta* spider venom: Neutralization of its toxic effects by anti-loxoscelic antivenoms

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ABSTRACT

This manuscript describes the general biochemical properties and immunological characteristics of Peruvian spider *Loxosceles laeta* venom (P*Llv*), which is responsible for the largest number of accidents involving venomous animals in Peru. In this work, we observed that the venom of this spider is more lethal to mice when compared with *L. laeta* venom from Brazil (B*Llv*). The LD_{50} of P*Llv* was 1.213 mg/kg when the venom was intradermally injected. The venom displayed sphingomyelinase activity and produced dermonecrotic, hemorrhagic and edema effects in rabbits. 2-D SDS-PAGE separation of the soluble venoms resulted in protein a profile ranging from 20 to 205 kDa. Anti-P*Llv* and anti-B*Llv* sera produced in rabbits and assayed by ELISA showed that rabbit antibodies cross-reacted with P*Llv* and B*Llv* and also with other Brazilian *Loxosceles* venoms. Western blotting analysis showed that bands corresponding to 25–35 kDa are the proteins best recognized in every *Loxosceles* spp venoms analyzed. The immunized rabbits displayed protective effect after challenge with P*Llv* and B*Llv*. *In vitro* assays with horse anti-loxoscelic antivenoms produced in Brazil and Peru demonstrated that these commercial antivenoms were efficient to inhibit the sphingomyelinase activity of P*Llv* and B*Llv*.

1. Introduction

Loxoscelism is the most important clinical syndrome resulting from *Loxosceles* spp spider bite and follows two well-defined clinical variants: the cutaneous form which manifests as erythema and edema that may develop into necrotic ulcer, whilst systemic loxoscelism is characterized by intravascular hemolysis and occasional renal failure (da Silva et al., 2004; Ministério da Saúde, 2011).

Loxosceles laeta (Nicolet, 1849) (Araneae, Sicariidae), known as "brown spider", "corner spider" and "spider violin", is an endemic species of South America, which has been introduced into the East of this continent and also into both North and Central America (Gerstch, 1967). *L. laeta*

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species is found throughout Argentina (de Roodt et al., 2002), frequently reported in the South region of Brazil (Malaque et al., 2002), widely distributed in Chile (Manriquez and Silva, 2009) and also found throughout the Peruvian territory, where it is also named "killer spider", due to the association of this spider with many fatal cases of loxoscelism (Maguiña-Vargas et al., 2004). Loxoscelism is a serious public health problem in Peru, the number of human accidents caused by spiders of *Loxosceles* genus attains 2500 per year (Panaftosa, 2007). *L. Laeta* and a lesser extent *Loxosceles rufipes* are the most medically relevant species in Peru (Sanabria and Zavaleta, 1997). The highest incidence of envenomations is recorded in cities along the Peruvian Coast (Sanabria and Zavaleta, 1997).

In Peru, although early work on loxoscelism dates from 1953 (Yzu, 1953), hardly anything is known about the structural and functional characteristics of this Peruvian spider venom, while significant progress has been made in other regions where *Loxosceles* spp spider live. *Loxosceles* venoms contain several protein toxins including alkaline phosphatases, hyaluronidases, metalloproteases, sphingomyelinases, and insecticidal peptides (da Silva et al., 2004). Among venom toxins, sphingomyelinases, also called dermonecrotic toxins, are the major toxic components and play an essential role on the pathogenesis of loxoscelism (Tambourgi et al., 2010). By using molecular biology tools, dermonecrotic toxins have been identified, the crystal structure determined, the cDNAs encoding toxins isolated, characterized and the recombinant proteins expressed, providing new insight for this group of toxins (Kalapothakis et al., 2002; Murakami et al., 2006; de Santi Ferrara et al., 2009; Catalán et al., 2011; da Silveira et al., 2006). Immunization strategies using crude *Loxosceles* venoms, recombinant toxins or synthetic epitopes derived from these toxins support the notion of using these immunogens as therapeutics via anti-sera development or vaccine strategy (Olvera et al., 2006; de Almeida et al., 2008; Dias-Lopes et al., 2010; de Moura et al., 2011). Antivenoms prepared from horse sera immunized with crude *Loxosceles* venoms are an important tool for treatment of human envenomation by spider and its use recommended by the Public Health Organizations (Pauli et al., 2009).

In view of absence of information about the properties of P*Ll*v toxin, the main goal of this work is to report some biochemical, immunological and toxic properties of this venom. In this paper, the sphingomyelinase, dermonecrotic, hemorrhagic, edematogenic and lethal activities of crude venom were investigated. This manuscript also describes the separation of soluble venoms proteins by 2-D SDS-PAGE, highlighting the differences between P*Ll*v and B*Ll*v protein pattern. In addition, this study shows the capacity of rabbit polyclonal anti-P*Ll*v, anti-B*Ll*v and, also horse anti-loxoscelic sera to neutralize Brazilian and Peruvian *Loxosceles laeta* venoms toxic effects.

2. Materials and methods

2.1. Animals, venoms and antivenoms

Adult male Swiss mice (weighing 18–22 g) were maintained at the Centro de Bioterismo of the Instituto de Ciências Biológicas of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil. All animals received water and food *ad libitum*. The experimental protocols conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) (A5452-01). Eight- to nine-week-old New Zealand rabbits were used to produce the sera anti-P*Ll*v and anti-B*Ll*v. Animals were maintained and handled as described above.

L. laeta (Peru) mature spiders were collected in the region of Cañete (Lima, Peru) and maintained in the herpetarium of the Centro Nacional de Producción de Biologicos of Instituto Nacional de Salud (INS), in Lima, Peru. Spiders were maintained in plastic boxes with water *ad libitum* and were fed weekly with cockroaches. The venom from mature spiders was obtained from dissected venom glands of ten spiders. Venom was collected according to da Silveira et al. (2002), pooled and stored at −20 °C until use. Protein concentration was determined by Bradford method (Bradford, 1976). *L. laeta, Loxosceles intermedia and Loxosceles gaucho* Brazilian mature spiders were collected in the region of Curitiba, PR, Brazil and maintained at the Centro de Produção e Pesquisa de Imunobiológicos (CPPI) of the State of Paraná, Brazil. The venoms from mature spiders were obtained as described before. *Phoneutria nigriventer* spiders and *Tityus serrulatus* scorpions were collected in the region of Belo Horizonte and maintained at the "Seção de Animais Peçonhentos" of Ezequiel Dias Foundation (FUNED) of Belo Horizonte, Brazil. The crude venoms were obtained by electric stimulation, lyophilized and stored at −20 °C in the dark until use.

Two commercial antivenoms were used for the neutralization assay, the antivenom produced in CPPI, Brazil (Lot. S02100) against B*Ll*v, *L. intermedia* and *L. gaucho* venoms and an antivenom produced by Instituto Nacional de Salud del Perú (INS) (Lot. 0300069), containing antibodies against P*Ll*v.

2.2. Toxic activities of L. laeta venom

2.2.1. Determination of median lethal dose (LD_{50})

The lethality was assessed via intradermal (i.d.) route. Groups of four mice were injected with different doses of venoms (0.4, 0.56, 0.784, 1.098, 1.537, 2.152 mg per kg of body weight) dissolved in 0.1 mL of PBS-BSA 0.5%. Seventy-two hours later deaths were recorded and the LD_{50} was then calculated by Probit analysis (Finney, 1971).

2.2.2. Determination of dermonecrotic, hemorrhagic and edematogenic activities

The dermonecrotic, hemorrhagic and edematogenic activities of P*Ll*v and B*Ll*v were determined by intradermal injection of 10 μg of crude venom in 100 μL of PBS pH 7.2 into a shaved back of five rabbits for each venom, as described by Furlanetto (1962). Injection of PBS alone was used as negative control. The diameters of dermonecrotic, hemorrhagic and edematogenic lesions were measured in the skin areas with a scale meter and caliper rule, 72 h after injection. Three measures of each lesion were made and their arithmetic mean was considered the mean diameter of the lesion.

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