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Variations in *Loxosceles* spider venom composition and toxicity contribute to the severity of envenomation

Kátia C. de Oliveira[a], Rute M. Gonçalves de Andrade[a], Roxane M.F. Piazza[b], Jorge M.C. Ferreira Jr[a], C.W. van den Berg[c], Denise V. Tambourgi[a,*]

[a]*Laboratório de Imunoquímica, Instituto Butantan, Av. Prof. Vital Brazil, 1500, CEP 05503-900 São Paulo, Brazil*
[b]*Laboratório Especial de Microbiologia, Instituto Butantan, SP, Brazil*
[c]*Department of Pharmacology, Therapeutics and Toxicology, Wales College of Medicine, Cardiff University, Cardiff, UK*

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Abstract

Envenomation by *Loxosceles* spiders causes two main clinical manifestations: cutaneous and systemic loxoscelism. The factors contributing to the severity of loxoscelism are not fully understood. We have analysed biochemical and toxicity variations in venom of *L. laeta* and *L. intermedia*, with the aim to find a correlation with the seriousness of loxoscelism. Differences in expression of proteins, glycoproteins and sphingomyelinase activity were observed between venom from male and female spiders and between venom from the two species. These differences were reflected in the toxicity of the venoms including the capacity to induce complement-dependent haemolysis, dermonecrosis and lethality. Comparative analysis of gender and species, showed that these biological activities were more prominent in venom from female spiders, especially from *L. laeta*. Antiserum raised against venom from females *L. laeta* spiders had the highest efficacy in neutralizing venoms of males and females of both species. These results indicate that the severity of loxoscelism depends, at least partially, on the species and sex of the spider and suggest that for accidents involving *L. laeta* an specific serum therapy is necessary. Furthermore, it emphasizes the efficacy of the antiserum produced against *L. laeta* female venom in neutralizing *Loxosceles* venoms from different species and gender.

Keywords: Loxosceles; Venoms; Species; Genders; Toxicity

1. Introduction

Loxosceles spiders (Araneae, Sicariidae) are found in temperate and tropical regions of Africa, Europe and North, Central and South America (Platnick, 2003). At least three different species of *Loxosceles* of medical importance are known in Brazil (*L. intermedia*, *L. gaucho*, *L. laeta*) and more than 3000 cases of envenomation by *L. intermedia* alone are reported each year.

Loxoscelism can be observed as two well-defined clinical variants: cutaneous loxoscelism (CL) and systemic or viscerocutaneous loxoscelism (VCL) which occur in around 83.3 and 16.7% of cases, respectively (Schenone et al., 1989). Pain, oedema and a livedoid plaque, which develops later into a necrotic scar, are the predominant local manifestations in CL. In VCL, hematuria and hemoglobinuria are always observed, while jaundice and fever occur in most cases. Although systemic loxoscelism is less common than the cutaneous form, it is the main cause of death associated with *Loxosceles* envenomation.

* Corresponding author. Tel.: +55 11 3726 7222x2231; fax: +55 11 3726 1505.
E-mail address: dvtambourgi@butantan.gov.br (D.V. Tambourgi).

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Most of the deaths occur in children and are related to the South American species, *L. laeta* (Futrell, 1992).

We recently have purified, cloned and characterized the toxins from *L. intermedia* and *L. laeta* venom that are responsible for all the local and systemic effects induced by whole venom (Tambourgi et al., 1995, 1998a). Sphingomyelinases D with M_r 35 kDa, purified from whole venom or expressed as recombinant proteins were able to induce dermonecrosis in rabbits and render human erythrocytes susceptible to lysis by complement (Tambourgi et al., 1998a; Fernandes Pedrosa et al., 2002; Tambourgi et al., 2004). In a mouse model of *Loxosceles* envenomation, we showed that the toxins also induced intravascular haemolysis and provoked a cytokine response, which resembles that seen in endotoxic shock (Tambourgi et al., 1998b). We subsequently elucidated the mechanism of complement susceptibility and showed that the toxins facilitate activation of the alternative pathway of complement on human erythrocytes by removal of glycophorins as a consequence of activation of an endogenous metalloproteinase (Tambourgi et al., 2000), and activation of the classical pathway of complement, possibly by alteration of the membrane asymmetry with exposure of phosphatidylserine (Tambourgi et al., 2002).

The fact that viscerocutaneous loxoscelism develops only in 16% of cases of envenomation, led to the assumption of a possible intrinsic susceptibility of these individuals (Barreto et al., 1985; Schenone et al., 1989). In accordance with this idea, we demonstrated in a murine model that susceptibility to the systemic effects of *L. intermedia* venom varied between animals of different haplotypes (Tambourgi et al., 1998b). We also demonstrated that variations in *L. intermedia* venom composition were associated with the ontogenetic stage of development and sex of the spiders, which were important factors contributing to the severity of loxoscelism (Gonçalves de Andrade et al., 1999; de Oliveira et al., 1999).

To further investigate the factors contributing to the range of loxoscelic reactions we analysed biochemical and functional inter- and intra-species variations of venoms of *L. intermedia* and *L. laeta*, the two main *Loxosceles* spiders of medical importance in Brazil and elsewhere in South America.

2. Materials and methods

2.1. Chemicals, reagents and buffers

Tween 20, bovine serum albumin (BSA), trinitrophenylaminolauroyl-sphingomyelin (TNPAL-sphingomyelin), Concanavalin A (ConA) labelled with horseradish peroxidase (ConA-HRPO), Wheat Germ Agglutinin (WGA) lectin labelled with horseradish peroxidase (WGA-HRPO), paraformaldehyde and diaminobenzidine (DAB) were purchased from Sigma (St Louis, MO, USA). Buffers were Veronal Buffered Saline (VBS^{++}), pH 7.4, containing (in mM) 10 Na barbitone, 0.15 $CaCl_2$ and 0.5 $MgCl_2$; PBS, pH 7.2, containing (in mM) 10 Na phosphate and 150 NaCl; FACS buffer (PBS, 1% BSA, 0.01% sodium azide).

2.2. Antibodies

Monoclonal antibodies against glycophorin A/B (anti-GPA/GPB) were from Sigma (St Louis, MO, USA) and anti-Glycophorin C (anti-GPC: Bric 4) was from the International Blood Group Reference Laboratory (IBGRL, Bristol, UK) (Reid et al., 1997). Rabbit anti-mouse IgG labelled with fluorescein isothiocyanate (FITC), was from Amersham Pharmacia Biotech (Buckinghamshire, England, UK).

2.3. Venom

L. intermedia Mello-Leitão and *L. laeta* spiders were provided by Laboratório de Imunoquímica, Instituto Butantan, SP, Brazil. Venom from adult male and female specimens were obtained by electrostimulation by the method of Bucherl (1969), with slight modifications. Briefly, 15–20 V electrical stimuli were repeatedly applied to the spider sternum and the venom drops were collected with a micropipette, vacuum dried and stored at −20 °C. In some extractions, the venom was collected in the presence of a mixture of proteases inhibitors: 1,10-phenantroline 1 mM (Sigma); phenylmethylsulfonyl fluoride (PMSF) 0.1 mM (Boehringer Mannheim Corporation, IN, USA) and ethylenediamine tetra-acetic acid (EDTA) 0.5 mM (Sigma). Stock solutions were prepared in PBS at 1.0 mg/ml. The protein content of the samples was evaluated by the Lowry method (1951).

2.4. Animals

BALB/c mice aged 2 months and weighing 18–20 g and adult rabbits weighing approximately 3 kg were obtained from Biotério de Criação de Animais Isogênicos do Instituto Butantan, SP, Brazil. All the procedures involving animals were in accordance with the ethical principles in animal research adopted by the Brazilian College of Animal Experimentation.

2.5. Preparation of antisera

Adult rabbits were injected intradermally with 2 μg of *L. intermedia* or *L. laeta* female or male venom absorbed with $Al(OH)_3$. The injections were repeated six times at 15-day intervals. Blood samples were collected 1 week after the last injection and sera stored at −20 °C.