Insights into brown spider and loxoscelism

MH Appel¹, R Bertoni da Silveira¹³, W Gremski¹², SS Veiga¹

¹Department of Cell Biology, Federal University of Paraná, Jardim das Américas, Curitiba, Paraná, Brazil
²Catholic University of Paraná, Health and Biological Sciences Institute, Curitiba, Paraná, Brazil
³Department of Biochemistry, Federal University of São Paulo, São Paulo, Brazil

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Abstract

Loxosceles is a genus of cosmopolitan spiders comprising several species, and popularly known as brown spiders or brown recluses. Brown spider bites can cause dermonecrotic lesions and systemic reactions known as loxoscelism. Systemic effects are less common but may be severe or even fatal in some patients. Systemic manifestations include intravascular hemolysis, disseminated intravascular coagulation and acute renal failure. A rapid diagnosis and an understanding of the venom’s molecular activity are crucial for satisfactory treatment. Mechanisms by which venoms exert their deleterious effects are under investigation, and searches are underway for diagnostic envenomation assays. Molecular biology is being used to produce quantities of several of the most important venom molecules and has contributed to the study and understanding of their mechanisms of action.

Key words: brown spider; loxoscelism; venom; recombinant toxins; dermonecrosis

Introduction

More than 40,000 spider species exist, with probably 100,000 to be described, but only 3 taxa are recognized as dangerous, namely Theridiidae, Loxoscelidae and Ctenidae. Moreover, only the genera Atrax, Lactrodectus and Loxosceles are associated with human deaths (Escoubas et al., 2000; Rash and Hodgson, 2002). Early European tales during the Middle Ages linked injuries or illness to spider bites (Schienele et al., 2005). For example the tarantula bite was associated with a disease (tarantism) for which the cure was a frenetic dancing for 3-4 days. This energetic dance, called tarantella, is now a typical Italian dance (Isbister, 2004). Today, as a consequence of mistaken diagnoses of spider bites, scientists are looking for methods to characterize and identify spider bites and their manifestations as well as to better understand the biological and molecular mechanisms of envenomation.

The genus Loxosceles (variously known as the brown spider, brown recluse, fiddleback, or gaucho spiders) is important in these studies because of its commonness in and around human dwellings. Their bite is characterized by dermonecrosis and systemic effects known as loxoscelism (Hogan et al., 2004).

The first case of documented loxoscelism occurred in 1879 in Tennessee. However, consistent data traced back about 50 years ago and were collected in Chile, then other observations were made in Brazil followed by the United States. These reports linked brown spider bite with necrotic skin lesions (Macchiavello, 1947; Atkin et al., 1958; Sams et al., 2001). Spiders' habits have caused a close association with humans, and the number of bites is increasing and has become a public health problem in Brazil, Chile and the United States (da Silva et al., 2004). Most bites occur during sleep or dressing, and women are bitten more often than men. Thighs, trunk, hands and arms are more often bitten (Hogan et al., 2004).

Loxosceles spiders

Loxosceles spiders are known as violin (fiddleback) spiders due to a characteristic violin
shape on their cephalothorax (Futrell, 1992). They are also known as brown spiders because their colour varies from a pale (L. laeta) to a dark brown (L. gaucho). *Loxosceles* body length ranges from 8 to 15 mm with legs measuring from 8 to 30 mm (da Silva et al., 2004). They are sedentary and nocturnal (Andrade et al., 1999) with a lifetime of 3 – 7 years (Andrade et al., 2000). Brown spiders have three pairs of eyes (an important characteristic useful to identify the genus) (Vetter and Visscher, 1998). They build irregular, cottony webs (Futrell, 1992) and normally prefer dead scavenged prey rather than live preys (Sandidge, 2003). They can survive months without food or water and withstand temperatures ranging from 8 °C to 43 °C. They are not aggressive and prefer dark dry places (Futrell, 1992; Málaque et al., 2002; Vetter and Barger, 2002; da Silva et al., 2004). The sexes produce venom with differences in volume, toxicity and compounds proportion (Oliveira et al., 1999). Comparative analysis of sex and species in *L. laeta* and *L. intermedia* venom showed some biological activities (complement-dependent hemolysis and dermonecrosis) more prominent in venom from female spiders, especially from *L. laeta* (Oliveira et al., 2005).

**Epidemiology**

*Loxosceles* spiders can be found distributed all over the world. In North America, the most important species are *L. reclusa*, *L. deserta*, *L. arizonica*, *L. rufences* (United States and Mexico) and *L. laeta* (Canada) (Sams et al., 2001; Vetter and Bush, 2002a). Europe, Africa, Middle East, some parts of Asia, Israel, and Australia are hosts to some *Loxosceles* species (Futrell, 1992; Borkkan et al., 1995; Young and Pincus, 2001; Nicholson and Graudins, 2003).

In Brazil, seven species have been described but three are the most implicated in human bites *L. intermedia*, *L. gaucho* and *L. laeta* (Sezerino et al., 1998). From 1990 to 1993, the Brazilian Ministry of Health received 17,781 reports of spiders’ bites, of which 36 % were due to *Loxosceles* (Sezerino et al., 1998). In the metropolitan area of Curitiba, in the state of Parana (southern Brazil) about 3,000 brown spider bites are reported annually (Málaque et al., 2002). In a retrospective study in Florianopolis, in the state of Santa Catarina, Brazil, 487 suspected cases of brown spider bites were found, 267 of which fulfilled the criteria for inclusion in the study (Sezerino et al., 1998). In 359 cases of loxoscelism between January 1985 and December 1996 at Butantan Intitute, São Paulo, Brazil, 14 % of patients captured the spiders so that 28 were classified as *L. gaucho*, 5 as *L. laeta* and 18 as non-classified *Loxosceles* (Málaque et al., 2002). More bites occur in warmer months (Schenone, 1996). In Curitiba, from 1998 to 2001 the incidence of *Loxosceles* bites was 1.4 cases per 1,000 habitants. Of these, 23 % were in the thigh, 16.7 % in the trunk, 14 % in the arm and 13 % in the lower leg. Only 1 % of cases were severe (Health Secretary, Curitiba, Paraná, Brazil, 2002).

**Pathophysiology of Loxosceles**

Dermonecrosis is the hallmark of loxoscelism (Fig. 1). Histopathology and clinical data are obtained from biopsies of human patients after brown spider bites. Rabbit skin artificially injected with *Loxosceles* venom is used for more controlled investigation since this animal model reproduces human skin lesions that follow envenomation (Ospedal et al., 2002). Systemic effects, such as renal failure, are less common and are usually reproduced in mouse (Luciano et al., 2004). Observation of human skin biopsies showed an inflammatory infiltrate, thrombosis, hemorrhage, dermatis, erytethma, induration of affected area and liquefactive necrosis of the epidermis and dermis consistent with pyoderma grangrenosum (Futrell, 1992; Yannias and Winkelmann, 1992). Symptoms in an experimental study in rabbits showed that after 4 h oedema, hemorrhage, degeneration of blood vessel walls, plasma exudation, thrombosis, neutrophil accumulation in and around blood vessels with an intensive diapedesis, a diffuse collection of inflammatory cells (polymorphonuclear leucocytes) in the dermis, and subcutaneous muscular oedema all occur. Over the following hours and up to 5 days after envenomation, the changes progressed to a massive neutrophil infiltration into the dermis and even into subcutaneous muscle tissue, destruction of blood vessels, thrombosis, hemorrhage, myonecrosis, and coagulative necrosis on the 5th day (Ospedal et al., 2002). Neutrophil participation and the inflammatory response seem to be dependent on an endothelial cell agonist effect triggered by the venom that leads to an indirect and dysregulated neutrophil activation involved in dermonecrosis (Patel, 1994). Envenomation of rabbit skin with *L. reclusa* venom after 14 days results in a mixed inflammatory cell infiltrate, coagulative tissue necrosis, vasculitis and a dense band of neutrophils bordering the zone of necrosis (Elston et al., 2000). *L. intermedia* venom damaged vessel endothelia, as shown by vessel instability, endothelium cell vaculization in biopsies of rabbit skin (Veiga et al., 2001a; Zanetti et al., 2002). In vitro experiments on rabbit aorta endothelium cell cultures showed cytotoxicity of *L. intermedia* venom associated with loss of cell adhesion to the culture substrate and the shedding of proteoglycans from the extracellular matrix and cell surface into the medium (Veiga et al., 2001a). In human umbilical vein endothelial cell (HUVEC) cultures treated with *L. reclusa* venom, agonist activity ensued, inducing endothelial cell expression of E-selectin and the release of interleukin (IL)-8 and granulocyte macrophage colony-stimulating factor, resulting in dysregulated inflammatory response (Patel et al., 1994). HUVEC exposed to *L. deserta* venom produced IL-8, growth-related oncogene-α and monocyte chemoattractant protein-1 via an NF-κB-dependent pathway (Desai et al., 1999; Gomez et al., 1999). *L. desserta* venom induces the expression of vascular endothelial growth factor (VEGF) in human keratinoocytes, suggesting that keratinoocyte-derived VEGF may contribute to vasodilatation, oedema and erytethma in brown spider envenomation (Desai et al., 2000). Primary cultures of keratinoocytes exposed to 100 ng/ml of *L. gaucho* venom release tumour necrosis factor (TNF)-α into the medium after 6 h (Málaque et al., 1999).

Mice injected with *L. reclusa* venom developed local hemorrhage after 6 h accompanied by blistering of the ear skin (Sunderkötter et al., 2001).
Fig. 1 Cellular and molecular aspects of brown spider and loxoscelism. (A) Loxosceles intermedia (brown spider) male. (B) L. intermedia (brown spider) female. (C) SDS-PAGE 3-20 % venom profile stained by Coomassie blue dye. (D) Dermonecrotic lesion on rabbit skin after 24 h post-L. intermedia venom (10 µg) exposure. Arrowhead indicates the site of venom injection with characteristic black and white eschar named marble plate. Black arrow points an erythema surrounding the lesion and white arrow shows the gravitational spreading of lesion (a hallmark of dermonecrotic loxoscelism). (E) Microscopical view of dermonecrotic lesion showing inflammatory leukocytes accumulated in the connective tissue (arrowhead) and disorganization of collagen fiber and oedema (black arrow) (magnification 400X). The inset shows inflammatory cells of the infiltrate represented by neutrophils (white arrow) (magnification 1.000X).

Histopathology showed a vasculitis reaction 2 h after exposure. The microscopical analysis of some mouse organs injected with different doses of L. intermedia venom revealed remarkable kidney alterations. Acute tubular necrosis accompanied by deposition of eosinophilic material inside the proximal and distal renal tubules was seen in several nephrons (Tambourgi et al., 1998). Mouse kidneys, treated with L. intermedia venom showed hyalination and erythrocytes in the Bowman’s space, glomerular collapse, tubular epithelial cell cytotoxicity and deposition of eosinophilic material within the tubular lumen (Luciano et al., 2004). Confocal microscopy observations of double staining immunofluorescence against type IV collagen or laminin and L. intermedia venom showed that toxins deposit and bind along the tubular and glomerular basement membrane of mice kidneys. Ultrastructural observations showed glomerular epithelial and endothelial cell cytotoxicity, the collapse and destruction of glomerular basement membrane and tubular epithelial cell degeneration. The basement membrane is a target for brown spider venom, as shown administrating L. intermedia venom to murine tumor Engelbreth-Holm-Swarm (EHS), which is rich in basement membrane molecules. L. intermedia venom degraded and fragmented the basement membrane (Veiga et al., 2000a). Venom
displays hydrolytic activity for entactin and heparan sulphate proteoglycan, two important constituents of basement membranes, while having no apparent activity on purified type IV collagen and laminin (Veiga et al., 2000a, 2001a,b).

In the bone marrow and peripheral blood cells, L. intermedia initially causes a decrease in the number of nucleated red cells, bone-marrow depression of megakaryocytes with thrombocytopenia in peripheral blood and decrease of platelet count (da Silva et al., 2003). Neutropenia in peripheral blood and low neutrophil counts were observed as consequence of bone-marrow depletion, which may reflect an extensive neutrophil influx to the tissues. Eosinophils are apparently unaffected.

Brown spider venom toxins

L. intermedia and L. laeta have different protein patterns of glycosylation and the same is between sexes of the same species (Oliveira et al., 2005). Hemolytic and dermonecrotic activities have been described for L. similes venom. Sphingomyelinase D molecules, with molecular mass ranging from 30 to 35 kDa and having hemolytic, necrotic and platelet aggregation activity were found in L. reclusa, L. rufescens, L. gaucho, L. laeta and L. intermedia venoms (Futrell 1992; Barboro et al., 1994; Mota and Barboro, 1995; Tambourgi et al., 1995; Barboro et al., 1996a,b, 1997). Three sphingomyelinase D isoforms were purified from L. boreli venom (Lb1, Lb2 and Lb3). Only Lb1 and Lb2 had dermonecrotic activity (Ramos-Cerrillo et al., 2004). An alkaline phosphatase was described in L. reclusa venom (Futrell, 1992). Hyaluronidase (32.5 kDa) was found in L. rufescens and L. reclusa (Futrell, 1992; Young and Pincus, 2001). L. deserta, L. gaucho, L. intermedia, L. laeta and L. reclusa venoms contained an enzyme of similar molecular size (44 kDa), which digested hyaluronic acid (Barboro et al., 2005). A 5'-ribonucleotide phosphohydrolase was found in L. reclusa venom (Futrell, 1992). Loxncerogin A (31.4 kDa) and Loxncerogin B (31.6 kDa) with necrotic activity on rabbit skin were found in L. gaucho venom (da Silva et al., 2000). L. gaucho has a broad molecular range of caseinolytic, gelatinolytic and fibrinogenolytic metalloproteases (Young and Pincus, 2001). To test whether proteases in L. intermedia venom were due to natural constitution and not a digest fluid contamination, da Silveira et al., (2002) compared the proteolytic activity of the venom obtained directly from venom glands with that obtained by electroshock. Both protein profiles showed very similar electrophoretic and enzymatic characteristics.

At present, a new generation of molecules developed through cloning techniques is under study. L. intermedia LiD1 recombinant protein (31.4 kDa) is a sphingomyelinase D family molecule without dermonecrotic activity but with antigenic activity (Kalapothakis et al., 2002). L. laeta recombinant protein (33 kDa) is a sphingomyelinase isoform able to degrade s-phingomyelin (Pedrosa et al., 2002). This recombinant protein induced complement susceptibility, release of glycoprophins and had dermonecrotic activity. L. intermedia recombinant protein (LiRecDT, 34 kDa) has dermonecrotic activity and was able to directly induce nephrotoxicity in mice (Chaim et al., 2005). L. laeta recombinant phospholipase D generated lysophosphatidic acid and was hemolytic (Lee and Lynch, 2005).

Clinical features, diagnosis and treatment of brown spider bites

Diagnosis of loxoscelism is rarely based on spider identification and therefore clinical features, epidemiological and historical findings must be well known (Wright et al., 1997; Vetter, 1999; Málaque et al., 2004). Lesion recovery improves once the patient is treated. However, brown recluse bites have been misdiagnosed in North America because they occurred in regions of non-endemicity (Vetter, 1999; Nishioka, 2001; Vetter and Barger, 2002; Vetter and Bush, 2002a,b; Vetter et al., 2003). A typical necrotic skin lesion begins soon after the spider bites the victim, followed by gravitational spreading (da Silva et al., 2004). The bite is painless, hence the patient is often unaware that he has been bitten (Futrell, 1992), and the delay between the bite and when the victim pursues help makes the treatment less effective. From mild to severe pain begins 2-8 h after the bite. At the bite a small puncture wound may appear, associated with transient erythema with itching and swelling and mild to severe tenderness (Futrell, 1992; da Silva et al., 2004). Blebs or blisters appear (12-24 h), may become hemorrhagic, and surrounded by a halo of ischemic tissue. In the following days, necrotized lesions become a dull blue-violet, the area of the gravitational spread turns blue, and the size of the blue area increases. Within three to seven days an eschar may form. The eschar may drop off leaving an ulcer that may require a skin graft (Schenone, 1996; Sezerino et al., 1998; Málaque et al., 2002; da Silva et al., 2004). Success of therapy depends upon a correct and rapid diagnosis, the volume of the venom injected, and the patient susceptibility to the venom (Futrell, 1992; da Silva et al., 2004). Phentolamine, heparin, topical nitro-glycerine, cyproheptadine and hyperbaric oxygenation have been used for therapy, but the efficacy of these therapies is inconclusive and their use is not recommended (Futrell, 1992; Wendell, 2003; da Silva et al., 2004). The established therapy is dapsone, acetylsalicylic acid (aspirin), antibiotics (erythromycin and cephalosporin), ice and elevation, avoidance of strenuous activity and heat and, when necessary, surgery. Early surgical excision has not been shown to be effective and often delays healing (Futrell, 1992; Merigian and Blaho, 1996; Goddard, 1998, Monteiro et al., 2002; da Silva et al., 2004). Serum anti-Loxosceles venom is used only in severe cases and effectiveness is doubtful especially against local manifestation. Systemic envenomation studies in
animals and humans have demonstrated that antivenom neutralizes the deleterious effects of the venom and reduces paediatric mortality (lsbister et al., 2003). Effectiveness of antivenom to prevent dermonecrotic lesions seems to be time dependent and usually patient looks for medical help 4 h after the bite when lesions is already established (ospedal et al., 2002; nicholson and Graudins, 2003). Some local and systemic noxious activities of the venom are attributed to proteolytic toxins that degrade fibrinogen, fibronectin, entactin and heparan sulphate proteoglycan and disrupt basement membrane structures, thereby causing local hemorrhage, gravitational spreading, disseminated intravascular structures, thereby causing local hemorrhage, gravitational spreading, disseminated intravascular coagulation and renal failure (feitosa, et al., 1998; veiga et al., 1999, 2000b, 2001a,b; luciano et al., 2004; chaim et al., 2005).

biotechnological products from brown spider venom

Recently developed technologies are being used to produce biotechnological products from l. gauch. venom. ARACHnase (hemostasis diagnosis international co., denver, CO, USA), normal plasma containing L. reclusa venom, mimics a lupus anticoagulant and may provide a positive control for anticoagulant testing (McGlasson et al., 1993). An antisierum against venoms of L. gauch., Phoneutria nigriventer, Tityus serrulatus, and Tityus bahiensis that reacts with L. intermedia and L. laeta toxins is produced by the Butantan Institute, são paulo, brazil. The CCPI (production center of immunobiological products, Parana, Brazil) has also produced antisierum using L. intermedia venom that is able to neutralize some activities of Loxosceles venom. L. laeta antisierum is produced by the national institute of health (Peru) (da silva et al., 2004). These antisieras have all been used as bioproducts for serum therapy (Roodt et al., 2002; barbaro et al., 1994; 1996a; health secretary, curitiba, Parana, Brazil). Guilherme et al. (2001) produced monoclonal antibodies recognising L. gauch. venom toxins, which were able to neutralize the dermonecrotic effect and lethal activities of this species venom but not those of heterologous venoms.

Monoclonal and polyclonal antibodies are not only powerful tools for neutralizing the effects of venom; they are also useful for research. They can be used to purify toxins from venom by affinity chromatography. They can be used on location of specific toxins on cell and tissue treated with venom toxins. Immunofluorescence techniques such as confocal microscopy and flow cytomtery are modern techniques based on antibody specific binding.

In contrast to the collection of snake venom, spiders provide very little venom, which limits the ability to study spider venom toxins. Protein cloning techniques are helping to solve this problem. After cloning it is possible to have milligrams of the same protein thereby improving the quality of research work and allowing more controlled experimental studies. Today several spider venom recombinant proteins are under investigation most of which are in the sphenogomyelinae protein family (Kalapothakis et al., 2002; pedroza et al., 2002; chaim et al., 2005).

future perspectives

Toxins from Loxosceles spiders are a group of proteins with a great range of different activities. Each toxin may be used to investigate molecular and cellular effects of venom. Also each of these proteins is a putative molecular model for drug design and to develop knowledge on some effects not yet fully understood such as the inflammatory reaction of dermonecrosis and platelet aggregation.

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References


