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Review

Recent advances in the understanding of brown spider venoms: From the biology of spiders to the molecular mechanisms of toxins

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ABSTRACT

The *Loxosceles* genus spiders (the brown spiders) are encountered in all the continents, and the clinical manifestations following spider bites include skin necrosis with gravitational lesion spreading and occasional systemic manifestations, such as intravascular hemolysis, thrombocytopenia and acute renal failure. Brown spider venoms are complex mixtures of toxins especially enriched in three molecular families: the phospholipases D, astacin-like metalloproteases and Inhibitor Cystine Knot (ICK) peptides. Other toxins with low level of expression also present in the venom include the serine proteases, serine protease inhibitors, hyaluronidases, allergen factors and translationally controlled tumor protein (TCTP). The mechanisms by which the *Loxosceles* venoms act and exert their noxious effects are not fully understood. Except for the brown spider venom phospholipase D, which causes dermonecrosis, hemolysis, thrombocytopenia and renal failure, the pathological activities of the other venom toxins remain unclear. The objective of the present review is to provide insights into the brown spider venoms and loxoscelism based on recent results. These insights include the biology of brown spiders, the clinical features of loxoscelism and the diagnosis and therapy of brown spider bites. Regarding the brown spider venom, this review includes a description of the novel toxins **revealed by molecular biology and proteomics techniques**, the data regarding three-dimensional toxin structures, and the mechanism of action of these molecules. Finally, the biotechnological applications of the

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venom components, especially for those toxins reported as recombinant molecules, and the challenges for future study are discussed.

1. Introduction

Spider bites of the genus *Loxosceles* have been associated with clinical manifestations characterized by dermonecrosis with gravitational spreading near the lesion site and, to a lesser extent, with systemic toxicity, such as the hematological disturbances of intravascular hemolysis, thrombocytopenia, disseminated intravascular coagulation and acute renal failure. The spiders of the genus *Loxosceles* are encountered in all continents and different species have been reported in North America, Central America, South America, Europe, Africa, the Middle East, Oceania and Asia. Five species (*Loxosceles rufescens*, *Loxosceles laeta*, *Loxosceles intermedia*, *Loxosceles gaucho* and *Loxosceles reclusa*) are responsible for most cases of human envenomation by the *Loxosceles* genus, and the pathology and clinical features of these spider bites are termed loxoscelism. Nevertheless, sporadic accidents caused by others *Loxosceles* species (*Loxosceles deserta*, *Loxosceles arizonica*, *Loxosceles anomala*, *Loxosceles similis*, for instance) have been described around the world (da Silva et al., 2004; Hogan et al., 2004; Swanson and Vetter, 2006; Bucaretchi et al., 2010; Isbister and Fan, 2011; Chatzaki et al., 2012).

The venom of the brown spider is a colorless and crystalline liquid, formed by a complex mixture of toxins enriched in proteins, glycoproteins and low molecular mass peptides with a predominance of toxins in the range of 5–40 kDa (Sams et al., 2001; da Silveira et al., 2002; da Silva et al., 2004; Machado et al., 2005; Swanson and Vetter, 2006). Previously published data have described highly expressed molecules, such as phospholipases D, astacin-like metalloproteases and low molecular mass insecticidal peptides (characterized as ICK peptides) (da Silva et al., 2004; de Castro et al., 2004; da Silveira et al., 2007a; Gremski et al., 2010; Matsubara et al., 2013). Together, these three toxin classes comprise the majority of the toxin-encoding transcripts in the venom gland of *L. intermedia* (approximately 95%) (Gremski et al., 2010). Other toxins with low level of expression, such as hyaluronidase, serine proteases, serine protease inhibitors, venom allergens and a TCTP family member, have been identified in the venom (de Castro et al., 2004; Barbaro et al., 2005; Gremski et al., 2010; Sade et al., 2012; Ferrer et al., 2013).

Regarding the hemolymph of brown spiders, no current description of its molecular composition, biological activities or even physical properties exists. Nevertheless, the potential of the hemolymph to contain natural inhibitors, antifungals or antibiotics is significant and is based on the environment in which the spiders live and the toxins that the brown spiders produce.

In recent years, knowledge of brown spider venoms has advanced significantly through the use of molecular biology techniques. The transcriptomes of the *L. laeta* and *L. intermedia* venom glands were analyzed for the first time, and this analysis confirmed the complexity of *Loxosceles* venoms (Fernandes-Pedrosa et al., 2008; Gremski et al., 2010). Additionally, by using recombinant DNA technology, heterologous toxins have been expressed and purified. These advances obtained with the recombinant *Loxosceles* venom toxins helped to overcome the obstacles to studying spider toxins, such as the low venom volumes and the difficulty in the purification of native toxins from crude venom. Moreover, these recent advances have enabled researchers to utilize cell biology, biochemistry, immunology, pharmacology and crystallography to clarify the general characteristics of *Loxosceles* toxins.

By using proteomics approaches, such as **two-dimensional gel** electrophoresis, N-terminal amino acid sequencing and mass spectrometry, the venoms of *L. gaucho and L. intermedia* have been investigated (Machado et al., 2005; dos Santos et al., 2009).

Recent advances in protein purification techniques, the application of different models for the synthesis of recombinant toxins, the modeling of domains, the knowledge of the binding or catalytic sites of the toxins of interest and, finally, the availability of the varied cellular and animal models for assessing the products obtained have created possibilities for a broad putative biotechnological use of brown spider venom toxins as important tools (Senff-Ribeiro et al., 2008; Gremski et al., 2010; Chaim et al., 2011a; Wille et al., 2013).

This review focuses on the most recent literature examining brown spider venom and loxoscelism. It discusses the molecular biology techniques used for the characterization of the molecules in brown spider venom, such as transcriptome projects, as well as the production and evaluation of recombinant toxins. Furthermore, it also describes the recent advances in the molecular complexity of venom toxins, and finally, it lists the putative biotechnological applications of several brown spider venom components.

2. Biology of brown spiders

The spiders of the *Loxosceles* genus belong to the *Sicariidae* family, sub-order *Labidognatha*, order *Araneida*, class *Arachnida*, and phylum *Arthropoda* (Platnick, 2013) (Fig. 1). In North America, this genus is popularly referred as recluse spiders, brown recluse spiders and violin spiders (fiddle back), due to a characteristic violin shape on the dorsal surface of the spider's cephalothorax. In South America, they are called brown spiders (da Silva et al., 2004; Vetter, 2008). The name *Loxosceles* means "*slanted legs*" because of the way the spider positions its legs at rest (Vetter, 2008). **Approximately 130 species** of the *Loxosceles* genus have been described and are extensively distributed worldwide (Platnick, 2013). The majority of these spiders are present in the Americas, West Indies and Africa, and some species