ASTACIN CONTENT AND ACTIVITY IN LOXOSCELES (BROWN SPIDER) VENOM

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Loxosceles spider bites generate characteristic dermonecrotic lesions with gravitacional spreading and/or systemic manifestations. The venom is composed of a mixture of peptides and proteins (5-40 kDa) with multiple and distinct biological activities. Metalloproteases have been described in venom of many different species of Loxosceles. The characterization of an astacin-like protease in Loxosceles intermedia venom (LALP1) was the first report of a member of astacin family as a component of animal venoms. The aim of this study was to evaluate the content and activity of astacin proteases in the venom of three Loxosceles species: L. intermedia, L. laeta and L. gaucho. Biochemical and molecular biology techniques were applied, such as SDS-electrophoresis, enzymatic degradation of distinct substrates, qualitative PCR, molecular cloning and cDNA sequencing. Also, the subproteome of astacin-like protease were explored by 2-DE immunostaining revealed by anti-LALP1 antibodies and 2-DE gelatin zymography. The results obtained showed the existence of a family of astacin-like protease toxins in L. intermedia venom, composed of three astacin protease isoforms (LAP1, LALP2 and LALP3). In addition, the identification of partial sequences of astacin proteases in L. laeta (LAP4) and L. gaucho (LALP5) indicates the existence of an interspecies gene family of astacin protease and suggests that astacins probably will be found throughout the Loxosceles genus. Subproteome of astacin-like proteases revealed the existence of greater number of biologically active astacin-like protease isoforms. 2-DE immunoblotting results showed positive reactions for the three analyzed Loxosceles venom. Besides, 2-DE zymography results showed 6 spots with gelatinolytic activity in L. intermedia venom, 8 spots in L. gaucho and at least 24 spots in L. laeta venom. The content and differential protease activity of Loxosceles astacins reinforces the relevance of this toxin family in loxoscelism and highlights Loxosceles venom as a great source of bioactive astacins for use in distinct biotechnological applications.

Keywords: Astacin, Loxosceles, Venom.

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