A Novel Hyaluronidase from Brown Spider (*Loxosceles intermedia*) Venom (Dietrich’s Hyaluronidase): from Cloning to Functional Characterization

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Loxoscelism is the designation given to clinical symptoms evoked by *Loxosceles* spider’s bites. Clinical manifestations include skin necrosis with gravitational spreading and systemic disturbs. The venom contains several enzymatic toxins. Herein, we describe the cloning, expression, refolding and biological evaluation of a novel brown spider toxin characterized as a hyaluronidase. Employing a venom gland cDNA library, we cloned a toxin (1200bp cDNA) that encodes for a signal peptide and a mature protein. Amino acid alignment revealed a structural relationship with members of hyaluronidase family, such as scorpion and snake species. Recombinant toxin was expressed as N-terminal His-tag fusion protein (~45 kDa) in inclusion bodies and activity was achieved using refolding. Immunoblot analysis showed that antibodies cross-reacted with hyaluronidase from whole venom as well as an anti-venom serum reacted with recombinant toxin. Recombinant toxin was able to degrade purified hyaluronic acid (HA) and chondroitin sulfate (CS), while dermatan sulfate (DS) and heparan sulfate (HS) were not affected. Zymograph experiments resulted in ~45 kDa lytic zones in hyaluronic acid (HA) and chondroitin sulfate (CS) substrates. Through in vivo experiments of dermonecrosis using rabbit skin, the recombinant hyaluronidase was shown to increase the dermonecrotic effect produced by recombinant dermonecrotic toxin from *L. intermedia* venom (LiRecDT1). These data support the hypothesis that hyaluronidase is a “spreading factor”. Recombinant hyaluronidase provides a useful tool for biotechnological ends. We propose the name Dietrich’s Hyaluronidase for this toxin, in honor of Professor Carl Peter von Dietrich, who dedicated his life to studying natural proteoglycans and glycosaminoglycans.

**Key words**: Brown spider, venom, recombinant hyaluronidase, spreading factor.

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