MECHANISTIC RELATIONSHIP OF PHOSPHOLIPASIC ACTIVITY AND TOXICITY OF DERMONECROTIC TOXIN FROM BROWN SPIDER VENOM

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Brown spider dermonecrotic toxins are the best well-characterized biochemically constituents of *Loxosceles* spp. venom. Recombinant forms are able to reproduce most of all cutaneous and systemic manifestations such as dermonecrotic lesions, hematological disorders and renal failure. They belong to a toxin family with 30-35 kDa characterized as phospholipase-D. In order to elucidate if phospholipasic activity is directly related with described effects, we modified a toxin isoform (LiRecDT1) by site-directed mutagenesis. The mutated toxin has a substitution of histidine residue at position 12 (conserved active site) by alanine, named as LiRecDT1H12A. LiRecDT1H12A sphingomyelinase activity was drastically reduced comparing to original LiRecDT1. Crude venom and LiRecDT1 antisera showed antigenic cross-reactivity by ELISA and Western Blotting to both toxins. Dermonecrosis *in vivo* was abolished by mutation in this toxin, but rabbit skin dermis revealed a massive inflammatory response in both forms. Hypotesis of residual phospholipasic activity was confirmed using crescent and much higher concentrations of LiRecDT1H12A by dermonecrosis *in vivo* and fluorimetric measurement. Lipid array showed both toxins have affinity for the same lipids and also colocalized with plasma membrane of treated cell culture. Detection of choline release in treated cells supernatant and detergent extract of plasma membrane in vitro corroborates with phospholipasic-dependent toxic mechanism.