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THESIS

Functional and structural characterization of phospholipases A_2 isolated from *Bothrops asper* snake venom in Panamá

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Abstract: Envenoming by *Bothrops* snakes is the most serious type of envenoming from the medical and economic point of view in Central America. *Bothrops asper* is responsible for 90% of the snakebites registered in Panamá every year. Despite its medical and economic relevance, only the venom of Costa Rican and Guatemalan populations of this species has been studied to some detail, and there is very little information on intraspecies variability in venom composition and toxicity. In this study the crude venom of *B. asper* from Panamá was characterized and its pharmacological and biochemistry activities were investigated with standard laboratory assays. Furthermore, we described the isolation, functional and structural characterization of four basic phospholipases A_2, namely MTX-I, MTX-II, MTX-III, MTX-IV, and a new acid phospholipase A_2 called Basp-I-PLA_2. The proteins were isolated from the crude venom by a combination of two chromatographic steps, using ion-exchange chromatography on CM-Sepharose (0.05 M NH_4HCO_3 pH 8.1 buffer), and hydrophobic chromatography on Phenyl-Sepharose (0.05 M Tris-HCl pH 7.4), followed by concentration gradient from 4 to 0 M NaCl at 25°C in the same buffer. Analyses of phospholipids hydrolyzed by these enzymes have shown that all phospholipases belong to type A_2. The acidic isoform demonstrated more catalytic activity than basic PLA_2s. This enzyme was more active on substrates such as phosphotidylcholine and phosphatidylglycerol. The isoelectric focusing evidenced pIs between 8.1 to 8.3 for MTXs and 4.6 for the isoform Basp-I-PLA_2. The molecular weight was estimated by mass spectrometry to be: MTX-1 = 14,156.5; MTX-2 = 14,249.5 and MTX-3 = 14,253.0 and Basp-I-PLA_2 = 14,246.0.8 Da. The PLA_2s (MTX-I, II, III and IV) induced myotoxic activity, inflammatory reaction (mainly leukocyte migration to the muscle) and activation of macrophages to exert phagocytic activity and production of superoxide. MTX-II, the most abundant one, showed to be cytotoxic against JURKAT tumor cell line, *C. albicans* and *E. coli*. The acidic phospholipases A_2, when tested in platelet rich plasma, showed a potent inhibitory effect on aggregation induced by ADP and collagen. The analysis of the N-terminal sequence demonstrated that MTX-I, MTX-III and BASP-I-PLA_2 belong to the subclass of Asp49 phospholipases A_2 catalytically active whereas MTX-II and MTX-IV belong to proteins of the subclass of the enzymatically inactive Lys49 PLA_2s-like. In addition, a sequence of the N-terminal region of the basic PLA_2 isolated demonstrated clearly that isolated myotoxins in this work are similar to previously isolated myotoxins of *Bothrops asper* snake venom from Costa Rica. The Basp-I-PLA_2 is a new acidic PLA_2 and its N-terminal sequence revealed a high homology with other Asp49 acidic PLA_2s from snake venoms.

Key words: *Bothrops asper*, Panamá, snake venom characterization, phospholipases A_2, myotoxicity, inhibition of platelet aggregation, inflammation.

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