Venomics of Bothrops spp. venoms from regions in Brazil and Colombia

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Snake envenoming is a globally neglected public health problem. Venoms produced by snakes of the Viperidae family are complex mixtures of active molecules that have a diverse array of effects on both prey and human victims. The genus *Bothrops* is responsible for most of the snake envenoming in Central and South America, leading to high morbidity and mortality rates. This study aimed to identify and compare the compositions of three different snake venoms, including those from two specimens of *Bothrops atrox* (one from Manaus and one from Colombia) and one from *Bothrops jararacussu*, which are endemic to the southern and southeastern regions of Brazil. Lyophilized crude venom (1,5-2 mg) was solubilized in 50 mM ammonium bicarbonate pH 8.5 (NH_4HCO_3). Initially, 50 µL of venom were incubated with 0.2% of RapiGest SF reduced by adding dithiothreitol and alkylation with iodoacetamide. Tryptic digestion was performed at 37 °C for 16h. Peptides were separated by reverse-phase chromatography, using an analytical column EASY-Spray™ PepMap RSLC (C18, 2 µm, 100A°, 75 µm x 50 cm). 1 ?L samples were injected with a constant flow of 400 nL.min-1 and elution gradient was performed within the range 5-98 % of solvent B (ACN+0.1% AF) in solvent A (0.1% AF). The NSI spray voltage was set to 2 kV. Precursor mass was measured by an orbitrap mass analyzer at the resolution of 70,000, mass range of 375-1500 m/z, AGC target was set to be 3×10^6, maximum injection time of 100 ms, in positive mode. Data dependent acquisition was performed in which the 5 most abundant precursor ions were selected for a high energy collision-induced dissociation fragmentation with the isolation window of 4.0 m/z and a normalized collision energy of 30 was applied. The product ions were scanned at a resolution of 17,500, AGC was set to be 1×10^5 and IT of 50 ms. For the MS/MS ion search, mass spectra were searched against the snake venom protein database available on the UniProt KnowledgeBase, using PatternLab 5.0 software. Carbamidomethylation was specified as a fixed modification, while the variable modifications included oxidation of methionine, deamidation of asparagine and glutamine, and pyroglutamate formation from glutamic acid (E) and glutamine (Q). The results from the MS study demonstrated that we confidently identified a significant number of proteins in each of the three venoms, with high confidence (FDR <1%). Specifically, we identified 505 proteins in *Bothrops atrox* from Manaus, 501 proteins in *B. atrox* from Colombia, and 497 proteins in *Bothrops jararacussu*. Of these, 425 proteins were common across all the venoms analyzed. On average, most of the venom proteins were phospholipase A2 (PLA2), snake venom metalloproteases (SVMPs) of both low and high molecular weight, as well as other proteins such as C-type lectins, L-amino acid oxidases, bradykinin-potentiating peptides, and thrombin-like enzymes, respectively. Thus, a proteomic study of snake venoms endemic to the region is important for supporting the development of new treatments, such as antivenom nanobodies, diagnostic strategies, and a better understanding of the pathological effects of snakebites.

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