A comparative proteomic and protein stability analysis of Crotalus durissus Venom Pools Used for the Production of crotalic Antivenom

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In the past decade, the Brazilian official surveillance system (SINAN) reported that over 23,000 accidents involving the *Crotalus durissus* snake have occurred. Currently, the most effective treatment for these accidents is antivenom immunoglobulins or immunoglobulin fragments obtained from the plasma of animals hyperimmunized with venoms. Therefore, it is essential to characterize these venoms to ensure the quality of the material used in the antivenom production. *Crotalus durissus* venom contains mainly the enzymes giroxin, convulxin, crotamin, and crotoxin. These combined toxins produce the systemic effects characteristic of envenomation, including paralysis, ptosis, blurred vision, blood coagulation alterations and muscle pain. However, previous works have shown that *Crolatus durissus* venom composition shows marked individual and populational variation. In this context, the aim of this study was a comparative analysis of venom pools from Crotalus durissus used in equine immunization for the production of crotalic antivenom. Additionally, we aimed to evaluate the PLA2 stability of the samples, considering the storage time of the previously prepared stock solution. Three different, lyophilized venom pools from Fundação Ezequiel Dias (FUNED), obtained at different extraction times were used in this work. Samples were subjected to enzymatic/immunochemical activities, and proteomic analysis. The PLA2 activity demonstrated a similar profile among the pools and allowed confirmation of the stability of PLA2, the main component of crotaline venom, in all samples, even for those stored in aqueous solutions for over 60 days. Total ion chromatogram profiles of the three pools, obtained by nanoHPLC coupled to mass spectrometry, suggested compositional uniformity of the samples with subtle variations in the venom pools that may correspond to the number and the intensity of the components. It was noticed that most of the peaks in pool 1 have a slightly higher intensity compared to those in pool 2 and 3. Furthermore, it was found that pool 2 and 3 are more similar to each other, as evidenced by the number of \"spectral countings\" in each sample. Proteomic results identified 137 proteins, allowing for the evaluation of similarities and differences between the pools. Of these, 105 are common to all three pools (76.6%), including PLA2, serine proteases, type C lectins, metalloproteases, and myotoxins, which are the main toxins involved in the production of the antivenom as they are responsible for the neurotoxic, myotoxic, and coagulant effects of crotalic venom. Despite differences in the composition of the pools, the samples were equally recognized by the anticrotalic antivenom in the Western blot test. This suggests that the lyophilized venom pools from the three samples share comparable capabilities to induce antibody production. Our findings highlighted minor differences in the composition of the raw materials used in the production of FUNED\'s crotalic antivenom. These results are instrumental in advancing standardized protocols for the characterization of essential materials. The insights gained from this study could significantly enhance the production of effective, high-quality antivenoms, thereby addressing the urgent and often neglected issue of snakebite envenomation.

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