Proteomics as an investigative approach in the enrichment of snake antivenoms.

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Snakebite accidents are a potentially fatal medical emergency resulting from snakebite envenomation. Considered by the World Health Organization (WHO) as a neglected tropical disease, snakebites affect approximately 28,000 Brazilians annually. In Brazil, *Bothrops* genus snakes are the most epidemiologically significant, and the pathophysiology of these incidents includes proteolytic, coagulant, and hemorrhagic mechanisms, with local and/or systemic manifestations. The unique effective treatment is serotherapy, which can lead to adverse effects such as anaphylactic reactions and serum sickness, related to the manufacturing practices and purity of the antivenom. The aim of this study was to evaluate the efficacy of coupling the *Bothrops jararaca* snake venom to an affinity chromatography matrix and to identify the proteins of a commercial *Bothrops* antivenom that specifically interact with the toxins of this snake's venom. As this study is part of a public-private research and development agreement, the methodology cannot be detailed. In summary, low-pressure liquid chromatography using the AKTA Pure equipment (Cytiva) and shotgun mass spectrometry using the Q-Exactive mass spectrometry equipment (Thermo Fisher) were employed in this study. As a result, the affinity chromatography matrix prepared was able to purify the antivenom fraction that has greater specificity with the toxins of the *Bothrops* venom. The purified proteins were characterized and 68% of them were classified as therapeutically relevant for the treatment of a snakebite (immunoglobulins), and 10% of albumin. On the other hand, analyses of the crude antivenom showed 45% immunoglobulins and 27% albumin. Additionally, the characterization of the snake venom profile coupled to the chromatography matrix revealed 100% of the immunogenic protein classes of *Bothrops* venom, compared to the previously characterized crude venom. However, the only class of non-coupled toxins were bradykinin-potentiating peptides, which do not have immunological potential. This proposal demonstrates that this affinity chromatography approach is effective in obtaining specific antibodies present in bothropic antivenoms, and these findings can support further studies by production centers regarding the enrichment of commercial snake antivenoms.

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