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Comparative study of biochemical composition and enzymatic activity of *Bothrops atrox* venom

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Abstract

In Ecuadorian Amazon, *Bothrops atrox* is the main snake associated with ophidic accidents. The only approved treatment for snakebite envenomation are antivenoms and their efficiency depends on the reference venom used in their production. It is important to mention that *B. atrox* is a species with wide geographic distribution, which can generate differences in the composition and phenotype of the venom between its different populations. This variability can be responsible for changes in the toxicological effects of snakebite among populations of the same species. With this in mind, the present study analyzed two venom samples from individuals from two Amazonian areas. Based on the results, differences in the abundance of the main families of toxins and their activities between both venoms were identified.

Introduction

Bothrops atrox is a medically important venomous snake responsible for the highest incidence of envenoming events in the Ecuadorian Amazon. According to data from the Ministry of Health of Ecuador, species of the genus *Bothrops* triggered the most bites and fatalities in our territory [1]. The only validated and efficient treatment for ophidic envenoming are heterologous immunoglobulins. Nevertheless, our country does not have a national production of these pharmaceutical product. Their efficiency depends mainly on the reference immunogens used in their production [2]. In this context, it is important to mention that *B. atrox* is a species with wide geographic distribution, which can generate differences in the composition and phenotype of the venom among its different individuals and populations [3,4]. Various studies in countries such as Brazil and Colombia have characterized some differences, mainly in the abundance of the most important venom toxins. This molecular variability is responsible for changes in the toxicological effects of envenoming among populations of the same species [5,6]. For this reason, the study of protein and toxin diversity and the enzymatic and biological activities of venoms has great clinical relevance in tropical countries such as Ecuador, where snakebite accidents are a public health issue[7]. For these reasons, the present work aimed to characterize enzymatically and biochemically the venom of *Bothrops atrox* from different areas of the Ecuadorian Amazon.

Materials and Methods

Two samples were obtained from Orellana and Pastaza. The venoms were dried and transferred to the laboratory. Initially, the chromatographic profile of these samples was characterized according to the methodology described by Almeida, *et. al* [8]. At this stage, the samples were fractionated by reverse-phase high efficiency liquid chromatography in order to know the elution profile of each venom. Next, enzymatic assays were performed with synthetic substrates. The phospholipase A_2 and serine protease activities of each of the samples were evaluated by spectrophotometric assays according to the methodology of Holzer *et. al* [9], and Lomonte and Gutierrez [10], respectively.

Results and Discussion

The results showed remarkable differences in the chromatographic profile of the analyzed samples. There are variations in the absorbance of the peaks associated with the main families of toxins of *B. atrox* venom. Such changes reflect the different protein abundance of the analyzed venoms. Enzymatic assays revealed significant differences in the phospholipase A_2 and serine protease activity of venoms, corroborating the variation of the chromatographic profile observed. These variations may involve changes in the toxicological effects induced by venoms, as previously demonstrated in other studies [6,11].

Conclusions

The results are key to the design of efficient therapeutic strategies and can justify clinical variations reported in ophidic accidents. The variation between individuals from different areas underscores the relevance of more detailed studies of the phenotype of venoms from Ecuadorian Amazon and the neutralization efficiency of imported antivenoms against these variations.

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