Proteomic profile of the effects of Bothrops jararaca venom and antibothropic antivenom on the mouse brain.

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BACKGROUND: In Brazil, more than 90% of snakebites are caused by snakes of the genus Bothrops. The envenomation is characterized by local and systemic events, including edema, hemorrhage, coagulopathy, cardiovascular shock, and renal failure. Additionally, complications such as cerebral infarction and hemorrhagic or ischemic stroke may occur, often resulting in death. These clinical manifestations are caused, directly or indirectly by abundant venom toxins, such as metalloproteases, serine proteases, C-type lectins, and phospholipases A2. Although there are reports in the literature of stroke cases associated with Bothrops envenomation, studies on the venom effects in the brain and the mechanisms by which toxins reach different brain structures are still poorly understood. In this context, mass spectrometry-based proteomic analysis has been applied as a robust tool to characterize snake venoms and to understand the pathogenesis of envenomation. AIMS: By using mass spectrometry approaches, this study aims to characterize and compare the systemic effects of B. jararaca venom on the mouse brain tissue and brainstem, in the presence or not to anti-bothropic antivenom. METHODS: After approval by the Butantan Institute Animal Ethics Committee (CEUA 9991131219), 1.6 mg.kg-1 of B. jararaca venom or saline solution was injected into the gastrocnemius muscle of mice. After 1 h, the animals either received or not 1.6 mg.kg-1 of antibothropic antivenom in the tail vein. At 3 h and 24 h after the first injection, the brains and brainstem were collected and cryopreserved. For proteomic analysis, after tissue lysis, the soluble proteins were subjected to reduction, alkylation, desalting, and trypsin digestion using the single-pot, solid-phase-enhanced sample preparation (sp3) protocol. The samples were analyzed by LC-MS/MS, and data were acquired by Data Independent Acquisition (DIA) using a Vanquish Neo/Orbitrap Exploris 480 system. Searches were performed using the DIANN software, and statistical analyses were performed using R language and scripts developed specifically for the project. RESULTS: Proteomic analysis of the brain tissue identified 8,596 unique proteins, including 5,119 common proteins, which were quantified. On the other hand, analysis of the brainstem resulted in the identification of 7,543 unique proteins, from which 5,583 common proteins were quantified. In both analyses, an increase in the abundance of some common proteins involved in autophagy (CISD2) and immune system (B2m) was observed. In addition, the administration of the antivenom mitigated the effects of B. jararaca venom injection after 24 h. CONCLUSION: The results of this study contribute to the understanding of brain effects of Bothrops venom by the identification of proteins and pathways potentially involved in the pathogenesis of envenomation in this model.

Agradecimentos: