Proteomic characterization of murine plasma by LC-MS/MS to assess the systemic effects of Bothrops jararaca venom

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INTRODUCTION: Snake envenomation by viperid species is a significant public health problem in Brazil and it is acknowledged by the World Health Organization as a Neglected Tropical Disease. More than 100,000 people die each year from snakebites worldwide, and another 400,000 are left with permanent disability. In Brazil, *Bothrops* snakes are responsible for about 20,000 snakebites each year. While is well known that systemic effects of *Bothrops* envenomation comprise coagulopathy, hemorrhage and renal failure, the molecular mechanisms involved in the disturbances of plasma proteins are only partially understood. Proteomic analysis based on mass spectrometry has proven to be a powerful tool to assess the systemic effects associated with snakebite envenomation.

OBJECTIVES: i) using mass spectrometry, to characterize changes in plasma protein abundance in animals injected with venom in the thigh muscle; ii) to assess the impact of bothropic antivenom on the plasma proteome.

METHODS: The *in vivo* experiments were conducted after approval by the animal research ethics committee of Butantan Institute under no. CEUA 9991131219. Swiss mice were injected with *B. jararaca* venom (1.6 mg/kg) in the gastrocnemius muscle or saline, and anti-bothropic antivenom was injected 1 h later (1.6 mg/kg; i.v. tail). After 3 h, 6 h, and 24 h, citrated blood samples were collected and centrifuged to obtain plasma, which was further processed to deplete albumin, IgG, and transferrin by affinity chromatography. Afterwards, proteins were submitted to reduction, alkylation, and the single-pot, solid-phase-enhanced sample preparation (sp3) protocol was performed followed by trypsin digestion and analysis by LC-MS/MS using data-independent acquisition in a QExactive mass spectrometer. Data were searched in Spectronaut 18, and MSstats R package was used for statistical analysis.

RESULTS: A total of 759 unique proteins were identified in the dataset. The number of significantly differentially abundant proteins increased over time in response to venom injection. After 3 h, 6 h and 24 h an increase of acute phase proteins such as apolipoproteins and serpins was observed, whereas the abundance of fibrinogen was decreased. In addition, several inflammatory protein markers, such as Apcs, Lcn2, Itih4 and Ckm were detected as increased.

CONCLUSIONS: Murine envenomation by *B. jararaca* resulted in clear changes in the plasma protein profile compatible with inflammation in addition to alterations in hemostasis, which were similar in animals that received antivenom.

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