Characterization of muscle damage and exudate produced by different metalloproteinases from Bothrops atrox venom

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Background: In Brazil, 25,000 snakebites occur per year and *Bothrops atrox* is responsible for the majority of accidents. The morbidity from local effects is high and they are mainly resulted by the action of snake venom metalloproteinases (SVMPs). The major SVMPs in *B. atrox* venom are Atroxlysin-Ia and Bathroxragin. Both are highly efficient in hydrolyzing ECM proteins, inducing rapid haemorrhage/dermonecrosis. Peptides generated after *in vitro* hydrolysis of ECM induces inflammation in mice. **Objectives:** Characterization of the exudate produced after SVMPs injection into the mice gastrocnemius muscle and its ability to activate/stimulate inflammatory responses in cells cultures. **Methods:** Muscle damage was evaluated by CK levels and histological analysis. The composition of the exudate was analysed by mass spectrometry and inflammatory mediators were quantified using CBA kit. Mediators from cell culture supernatant (C2C12/J774) treated with exudate were measured by CBA and gene expression by real-time PCR. **Results:** The SVMPs induced disorganization of muscle fibers and migration of inflammatory cells, in addition to increasing CK levels in the plasma. The proteomic characterization showed presence of collagen, fibrinogen, plasminogen, vitronectin and fibronectin. The main DAMPs/immunomodulators identified on exudate fluids were proteins from heat shock family (HSP), protein S-100 and annexin. The exudate presented moderate levels of IL-10, TNF-alpha, IL-2, IL-4 and a high concentration of IL-6. The exudate from both toxins induced production of inflammatory mediators by macrophages and muscle cells (myoblasts and myotubules). The cytokines with the highest levels detected were TNF-alpha, mainly in J774 and myoblasts. IL-6 was very high for the three cultures evaluated. The increase in gene expression of pro-inflammatory molecules also was observed for both exudates and again IL-6 was very high for all cells. **Conclusion:** The hydrolysis products of SVMPs triggers an exacerbated inflammatory response and this may be one of the reasons why anti-venom is not able to neutralize local effects.

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