Use of data-independent acquisition (DIA) mass spectrometry to identify the cytotoxic effects of Bothrops jararaca venom on mouse renal mesangial cells.

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Background & aims: Snake venoms are composed of several families of protein toxins, in addition to bioactive peptides. In Brazil, Bothrops snakes are responsible for ~90% of human accidents, in which the clinical outcomes after the bite involve local and systemic effects, including kidney injury, which may progress to renal failure. To better understand the mechanisms of venom-induced renal toxicity, the aim of the present study is to evaluate the effects of B. jararacavenom on murine glomerular cells in culture with a data-independent acquisition (DIA)-mode proteomics approach. Methods: 5x106 SV40 MES 13 cells, derived from the murine renal glomerulus, were incubated with increasing concentrations of B. jararaca venom (0.25; 0.5; 1.0; 2.0 and 4.0 ug/mL) for 2 h. After cell viability assays, proteins of the cell content and secretome were analyzed by LC-MS/MS using a Vanquish Neo/Orbitrap Exploris 480 system. Results: 8,157 unique proteins were identified in all samples of the cell content, of which 92% (7,489 proteins) were quantified in terms of their relative abundance in label-free analysis. The data showed a decrease in cell viability and abundance of proteins related to cell adhesion, cytoskeleton structure, DNA repair and metabolism. Furthermore, at venom concentrations of 2.0 and 4.0 ug/mL, proteins related to apoptosis, inflammation and oxidative stress were observed with increased abundance. In the secretome, 5,866 unique proteins were identified, and 4,580 proteins were quantified. At venom concentrations of 2.0 and 4.0 ug/mL, proteins involved in several cellular pathways were identified in higher abundance, such as the inflammatory response. Conclusions: Taken together, these results indicate important changes in kidney glomerular cells upon treatment with B. jararaca venom in vitro and also demonstrate the potential of LC-MS/MS-based proteomics in studying the cellular response to a complex mixture of toxins

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