Modulation of cell membrane lipids and cytosolic Calcium influx followed by treatment of B16-F10 cells with recombinant phospholipase-D from Loxosceles intermedia (Brown spider) venom

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The mechanism by which the venom of brown spiders (Loxosceles genus) causes dermonecrosis, inflammatory response and hematological disorders is currently attributed to toxins characterized as phospholipases-D. Literature data have described the cloning and expression of recombinant brown spider venom phospholipases-D from different species. In the present investigation, we used two-dimensional gel electrophoresis and immunoblotting (2D-immunoblotting) with antibodies that recognize a recombinant phospholipase-D of L. intermedia (LiRecDT1) to identify proteins in the crude venom. We reported the immunological cross-reactivity for at least twenty spots, supporting an idea of high expression level for members of this toxin family. Kinetic experiments of Phospholipid hydrolysis showed that LiRecDT1 degrades mainly sphingomyelin, but also lysophosphatidylcholine, generating respectively C1P and LPA; and has low activity upon phosphatidylcholine. Immunofluorescence assays using antibodies that recognize LiRecDT1 and a recombinant fusion GFP-phospholipase-D demonstrated the direct binding of this toxin to the membrane of B16-F10 cells. We also showed that phospholipase-D degrades phospholipids in detergent extract and B16-F10 ghost cells, supporting the accessibility and activity of this toxin on cell membrane phospholipids. Even at higher concentrations, treatment with phospholipase-D did not change viability or morphology of B16-F10 cells. Nevertheless, it has induced an increase in cytoplasmic calcium concentration detected by a calcium-sensitive fluorescent probe (Fluo-4) at spectrofluorimetry, inverted light microscopy for differential interference contrast (DIC) and fluorescence microscope. The ability of an exogenous phospholipase-D to generate bioactive phospholipids and Calcium influx in cytoplasm qualify this molecule as a tool for experimental biochemistry and cell biology protocols.

Keywords: brown spider, calcium influx, membrane phospholipids, phospholipase-D, venom.
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