Inflammatory effect of a PLA_2 isolated from *Bothrops diporus* venom

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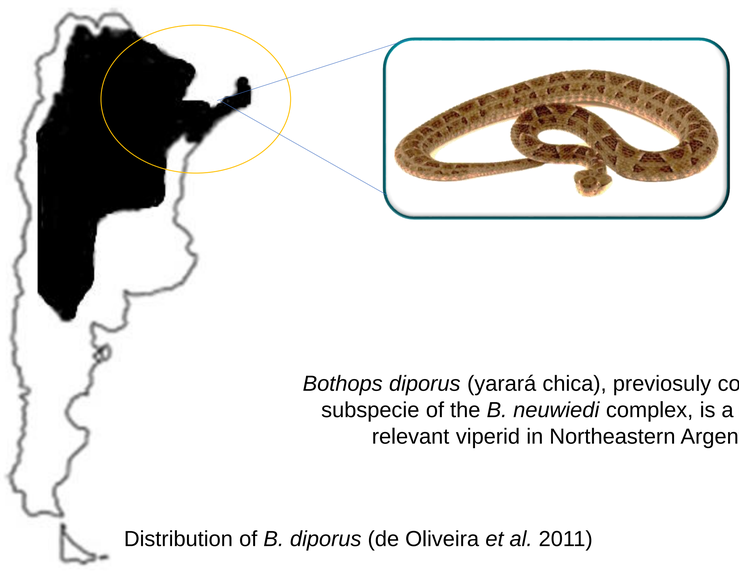
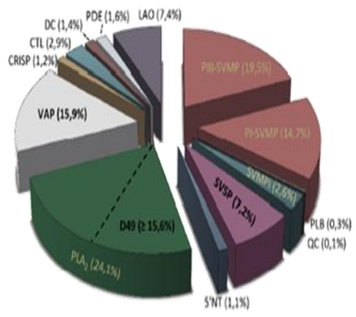
1 INTRODUCTION

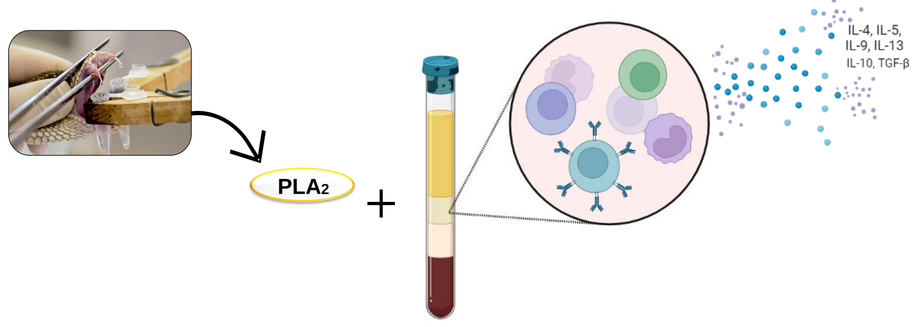


Distribution of *B. diporus* (de Oliveira *et al.* 2011)

Bothops diporus (yarará chica), previosuly considered a subspecie of the *B. neuwiedi* complex, is a medically relevant viperid in Northeastern Argentina.



A proteomic study found that the venom of this species contains a high relative abundance of PLA_2S (24%) that induce inflammatory events.

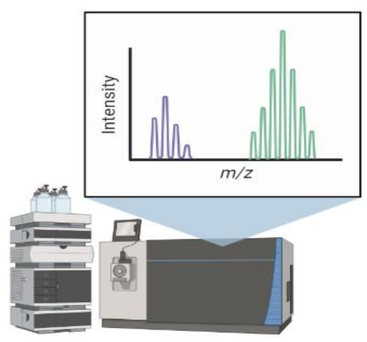


In this work we quantified a panel of cytokines on peripheral blood mononuclear cells (PBMC) previously incubated with a PLA_2 isoform from *B. diporus* venom.

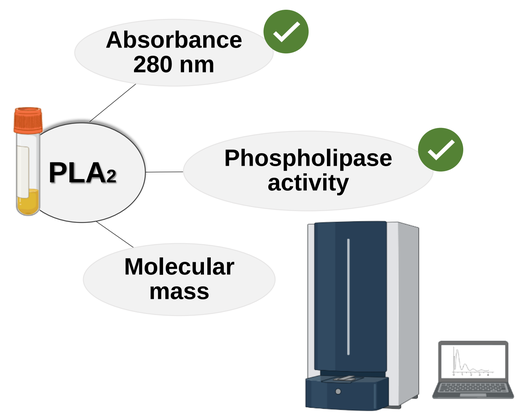
2 MATERIALS & METHODS

A. Isolation and Purity Control of the PLA_2

B. diporus venom was vacuum dried, pooled and stored at -20 °C

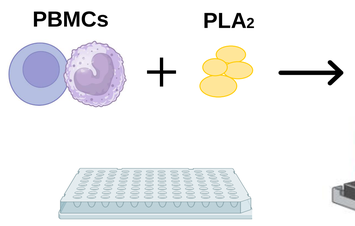


PLA_2 was isolated by reverse phase chromatography (RP-HPLC) on a C18 column. *B. diporus* venom (2 mg) was dissolved in 200 µL of 0.1% trifluoroacetic acid (TFA) and elution was performed at 1 mL/min in acetonitrile gradient with 0.1% TFA

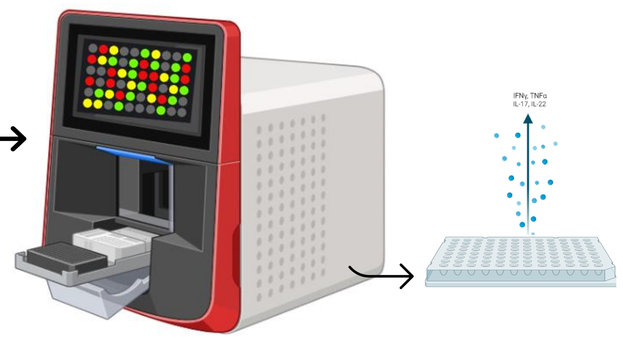


Concentration at 280 nm, specific phospholipase A_2 activity by a colorimetric assay using phenol red and molecular mass by MALDI-TOF MS were determined

B. Analysis of the inflammatory response



10h incubation of $1x10^6$ PBMCs (three different donors) with PLA_2 (25 µg/mL) or positive control (PMA/ionomycin)



Human Panel Th17 using luminex multiplex technology. Experiments were performed in duplicate and statistically analyzed using two-way ANOVA and the FDR Benjamini-Hochberg method

3 RESULTS

A. PLA_2 isoform isolated from *B. diporus* venom

The HPLC profile of *B. diporus* venom from Argentina presented a protein peak eluting at 56 min (Fig. 1), a chromatographic region where PLA_2s are commonly found. The peak was collected and subsequently identified as PLA_2. PLA_2 was assayed for enzymatic activity using micellar phosphatidylcholine and phenol red indicator (Fig. 1, inset) which was rapidly acidified to yellow by the fatty acids released by PLA_2. For a more precise molecular mass determination a MALDI-TOF MS analysis was performed. Protein PLA_2 was deposited on the MALDI plate. The purified fraction gave a main peak with a molecular mass of 14,048 Da. (Fig. 2).

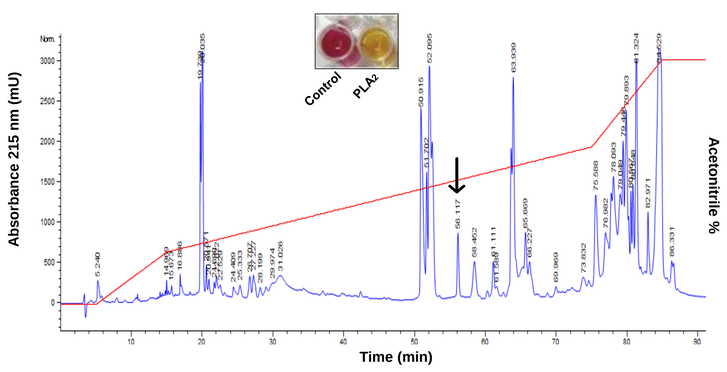


Fig. 1. Reverse-phase HPLC separation of *Bothrops diporus* venom (2 mg). Elution was done with an acetonitrile gradient (red line). The peak eluted at 56 minutes was identified as PLA_2. The inset shows the activity of PLA_2, a shift from red to yellow indicates positive activity.

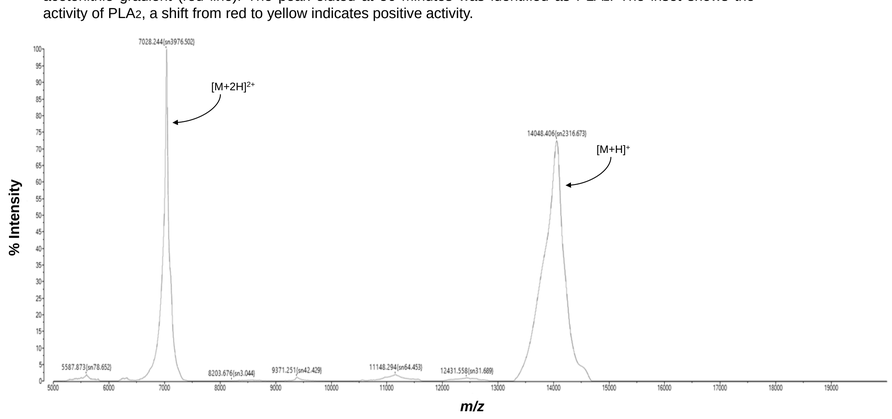


Fig. 2. MALDI-TOF mass spectra of the PLA_2 isoform isolated from *B. diporus* venom. Major protein detected [M+H]+ = 14,048 Da.

B. Inflammatory response

The PLA_2 isoform isolated from *B. diporus* venom, induced a significant increase in the release of the pro-inflammatory cytokines IL-6 and TNFα and the macrophage inflammatory chemokine MIP3a, after 10h incubation. Even the IL-10, an anti-inflammatory cytokine, was also over expressed; the predominantly inflammatory effect induced by PLA_2 was confirmed.

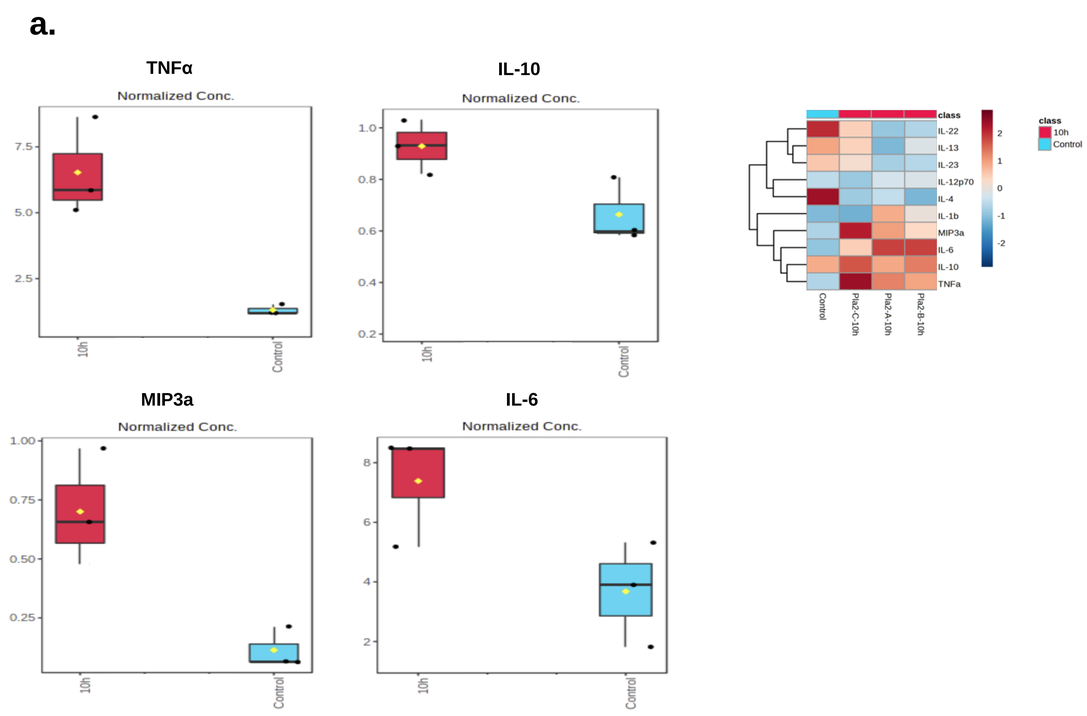


Fig. 3. (a) Cytokine levels in PBMCs culture. The secretion of the proinflammatory cytokines TNF-α, IL-6, the macrophage inflammatory chemokine MIP3a, and the anti-inflammatory cytokine IL-10 by PBMCs were determined in the supernatants incubated for 10h with 25 µg/mL of the PLA_2 isoform isolated from *B. diporus* venom or a positive control (PMA/ionomycin). (b) Heatmap and dendrogram for the same data as in (a). Colors indicate log-transformed secretion intensities in a range from blue (low) to red (high), numerically ranging from -2 to 2, respectively. Three different donors were used for the experiment (PLA_2 A-B-C).

4 CONCLUSION

In this work, we confirmed the predominantly inflammatory effect of a phospholipase A_2 (PLA_2) isoform isolated from *Bothrops diporus* snake venom on human peripheral blood mononuclear cells (PBMCs). Further investigations are needed to complete this information that could be useful to develop new strategies in anti-venom therapy.

5 ACKNOWLEDGMENTES

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REFERENCES