Exploration of the biological effects of a basic Phospholipase A_2 from *Agkistrodon piscivorus piscivorus* venom

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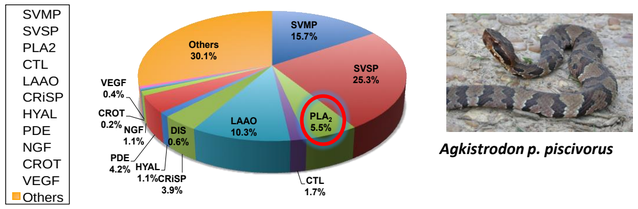
Background

Phospholipases A_2 (PLA_2s) are found in abundance in the venom of many North American snake species, making up 1.8% to 7%. They are responsible for a wide array of pharmacological effects such as anticoagulation, edema, and inflammatory response[1,2]. In *A. p. piscivorus* (*A.p.p.*) venom, these toxins make up a significant portion of venom constituents (Fig. 1).

A basic PLA_2 was recently isolated through reverse phase HPLC and identified as a D-49 PLA_2 (*A.p.p.* PLA_2). After testing activities on an *in vivo* model, the release of pro-inflammatory mediators, systemic myotoxicity, and hemolytic effects were observed. This study aims to explore the hematological, myotoxic and pro-inflammatory activities of this toxin using *in vitro* models.

Using Human Umbilical Vein Endothelial Cells (HUVEC) and a myoblast cell line (C2C12) as experimental models, we tested cell viability, cell activation molecule expression, and cell damage markers. These assays are conducted in order to better understand if this PLA_2 is able to trigger an exasperated response in muscle and endothelial cells, thus contributing to the pathophysiology of snake bite envenomation.



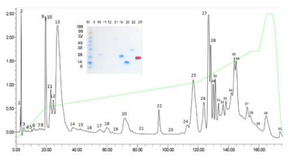
Agkistrodon p. piscivorus

Figure 1. Snake venom protein composition for *A. p. piscivorus*

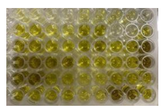
Methods

1) Isolation of *A. p. p.* PLA_2

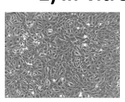
Protein purification Reverse phase C18 HPLC → N-Terminal sequencing → PLA_2 activity



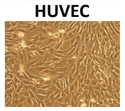
Peak 20: Basic PLA_2 (D-49) NLFQFEKL



2) *In-vitro* assays



HUVEC



C2C12

Cell Viability (MTT Assay)

Release of pro-inflammatory mediators

Human whole blood

SONOCLOT Analysis

Conclusions

- Significant PLA_2 activity was detected in crude *A. p. piscivorus* venom and its purified toxin, *A.p.p.* PLA_2 (Fig. 2).
- *A.p.p.* PLA_2 altered cell viability in C2C12 cells but not HUVEC (Fig. 3).
- At 3 h, the release of IL-8 was detected in all treatments except for TNF-α (Fig. 4A).
- Release of IL-6 was detected, but there were no significant differences compared to the negative control (Fig. 4B).
- There was an increase of pro-inflammatory mediators in cells treated with 6.25 µg/mL of PLA_2 and the positive controls after 24 h incubation, but not with the crude venom or with those treated with 25 µg/mL of PLA_2 (Fig. 4).
- PLA_2 induced the release of cytokines at 3 and 24 hr (Fig. 4).
- The *A.p.p.* PLA_2 from the venom of the *A. p. piscivorus* appears to activate endothelial cells.
- Significant activities were detected in the activated clot time, clot rate and platelet function of human whole blood by crude venom and the PLA_2 (Fig. 5).

Results

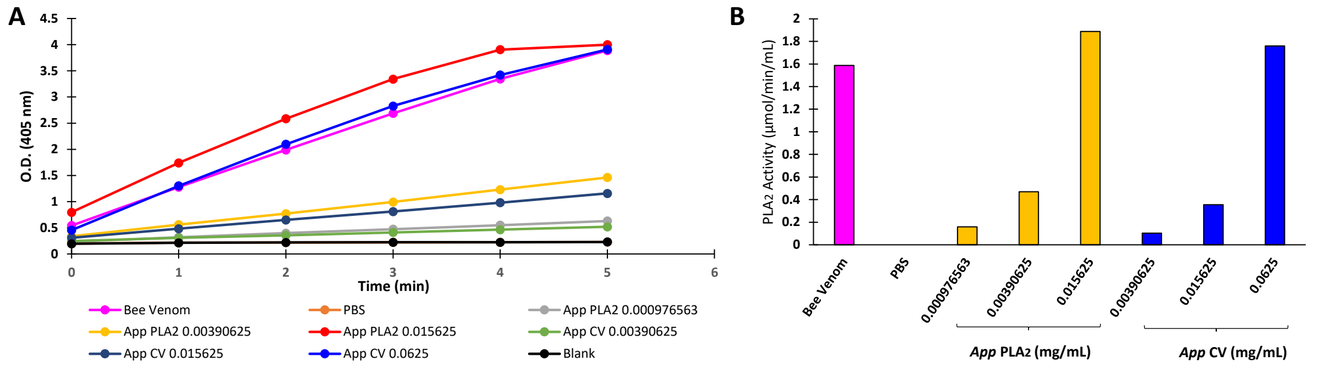


Figure 2. **PLA_2 Activity employing Ellmans' reagent (DTNB).** PLA_2 activity was determined using a commercial kit (Cayman Chemicals, USA). The plate was read at 450 nm every minute for five minutes (A). There was a remarkable activity for both the isolated PLA_2 and *A.p.p.* crude venom at multiple concentrations, being the *A.p.p.* PLA_2 more potent, activity at lower concentrations compared to the crude venom (B). Blank: PBS, Positive control: Bee venom.

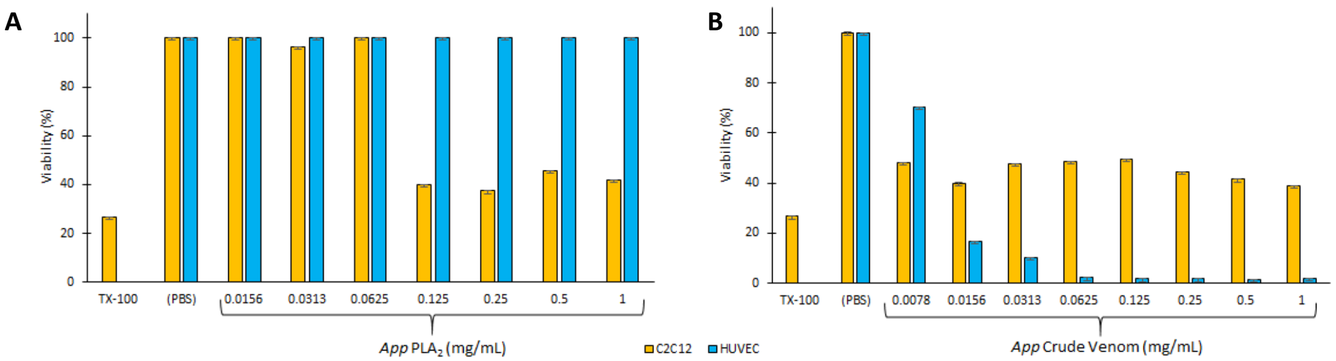


Figure 3. **Cell Viability in HUVEC and C2C12.** Cells were incubated with *A.p.p.* PLA_2 (A) and *A.p.p.* crude venom (B) for 24 h, and cell viability was detected using the MTT assay. Cell viability of C2C12 (orange) was reduced when treated with both the toxin and the crude venom. Meanwhile, HUVEC viability (blue) was only affected by the crude venom. PBS: Negative control. TX-100: Positive control

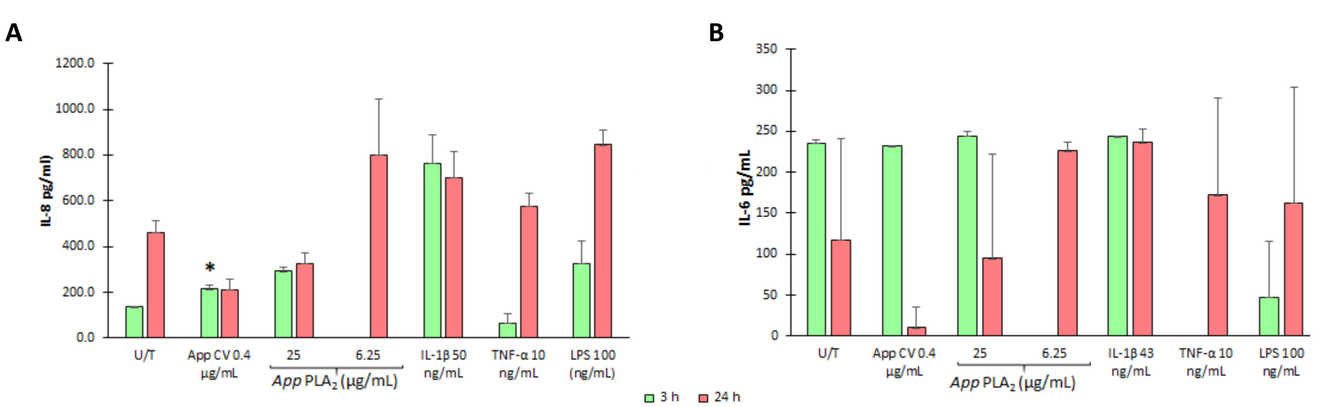


Figure 4. **Detection of pro inflammatory mediators using ELISA.** We tested the release of the pro-inflammatory mediators IL-8 (A) and IL-6 (B). Cells were incubated at 3 h (green) and 24 h (red) with the *A.p.p.* PLA_2 and controls. Supernatants were collected and the release of IL-8 (A) and IL-6 (B) were measured using commercial kits (ThermoFisher Scientific, USA). Negative controls: untreated cells (U/T). Positive controls: *A. p. piscivorus* crude venom (App CV); Interleukin 1-beta (IL-1β); Tumor necrosis factor alpha (TNF-α); Lipopolysaccharide (LPS). * p< 0.05 vs. U/T.

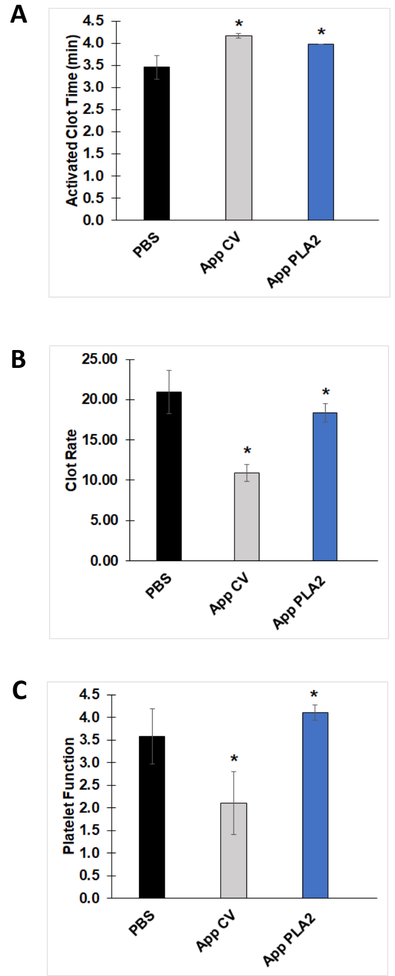


Figure 5. **Blood signature profiles generated by SONOCLOT**. Significant changes were observed for activated clot time (ACT) (A), clot rate (B) and platelet function (C). These tests were conducted on whole blood samples. *p<0.05 vs. PBS control.

Acknowledgements

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