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Comparison of in vitro immunogenicity between δ-ctenitoxin-Pn2a and epitope-based immunogens

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Introduction

Currently, the production of antivenom against the bite of *Phoneutria nigriventer* spider requires its capture and venom extraction by electrostimulation, a cumbersome, dangerous, and low-yielding method. Afterwards, the venom is injected into large mammalians to obtain immunoglobulins, which are then purified from plasma to make antiserum. Otherwise, those neurotoxins responsible for envenomation as well as epitope-based immunogens can be synthesized and used to optimize the production of antivenoms for meeting their demands. The neurotoxin δ-ctenitoxin-Pn2a (UniPort: P29425) is the main responsible for the symptoms of *P. nigriventer* envenoming in humans. In previous studies we identified epitope $G^{34}YFWIAWYKLANCKK^{48}$ from δ-ctenitoxin-Pn2a and used it to design immunogenic peptides. In this work δ-ctenitoxin-Pn2a was synthesized and its *in vitro* immunogenicity was compared with those immunogenic peptides.

Materials and Methods

δ-ctenitoxin-Pn2a was produced by automated microwave solid-phase peptide synthesis (SPPS) using Fmoc/tBu chemistry. Also, the epitope $G^{34}YFWIAWYKLANCKK^{48}$ was synthesized manually by SPPS using Fmoc/tBu chemistry. The Cys was replaced by α-aminobutyric acid to avoid disulfide bonds formation resulting the peptide Ac-GYFWIAWYKLAN-Abu-KKG-NH_2 (a). The branched peptide (Ac-GYFWIAWYKLAN-Abu-KK)$_2$-KG-NH_2 (b) and the lipopeptide Palm-GYFWIAWYKLAN-Abu-KKG-NH_2 (c) were also manually synthesized to increase the immunogenicity. Cytotoxicity of δ-ctenitoxin-Pn2a and the epitope-based immunogens were evaluated on murine macrophage cell line RAW 264.7 using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay in the presence of increasing doses of each peptide (0.25–10.0 μM). The cellular distribution of the transcriptional factor NF-κB was examined by immunofluorescence after exposing macrophages to 0.5 μM of each peptide or δ-ctenitoxin-Pn2a.

Results and Discussion

δ-ctenitoxin-Pn2a did not show cytotoxicity between 0.25 and 10.0 μM (Fig. 1). On the other hand, Ac-GYFWIAWYKLAN-Abu-KKG-NH_2 (a) did not show cytotoxicity between the evaluated concentrations, while peptides Palm-GYFWIAWYKLAN-Abu-KKG-NH_2 (b) and (Ac-GYFWIAWYKLANAbuKK)$_2$-KG-NH_2 (c) did at concentrations equal or over 0.5 and 10.0 μM, respectively (Fig. 2).

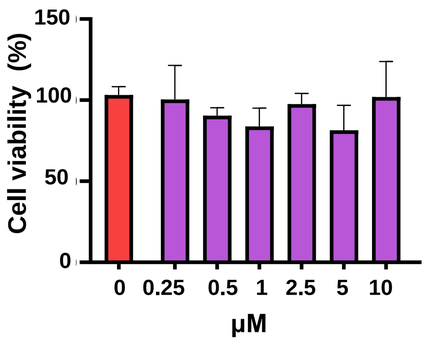

Fig. 1. Cell viability MTT assay histograms expressed as percentage of the control after their exposure to increasing concentrations of δ-ctenitoxin-Pn2a.

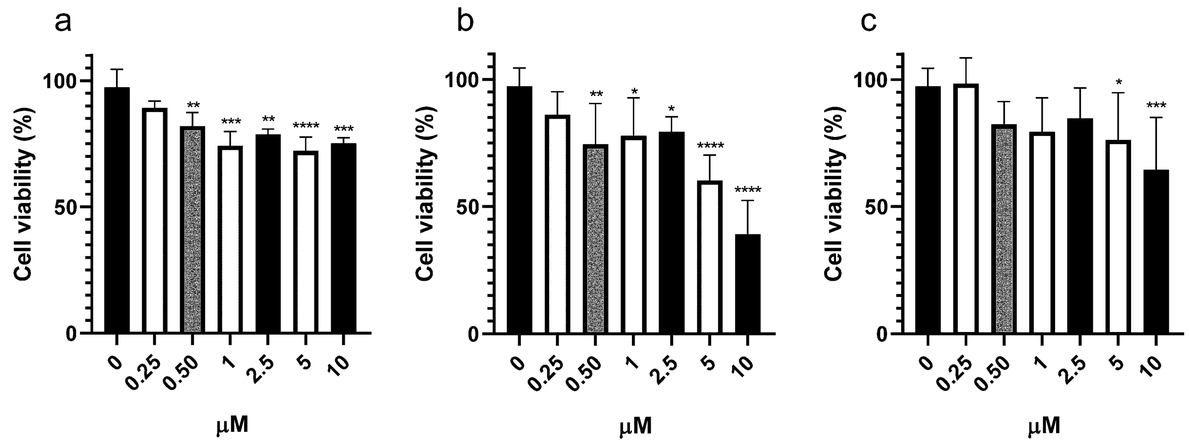

Fig. 2. Cell viability MTT assay histograms expressed as percentage of the control after their exposure to increasing concentrations of peptide.

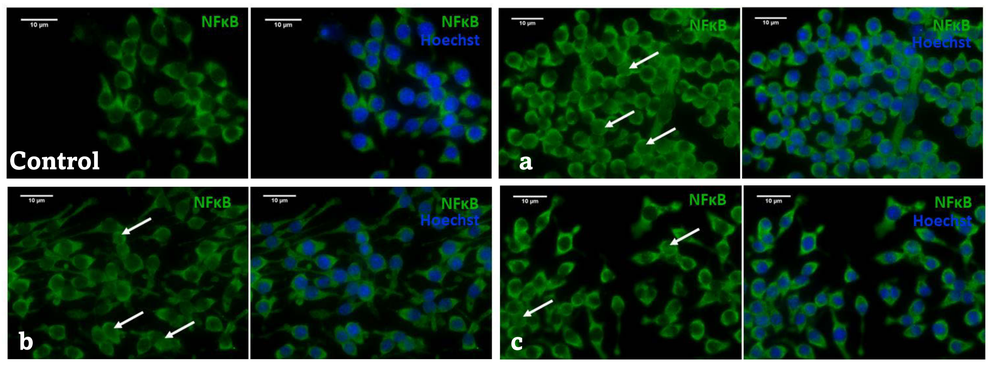

Fig. 3. NF-κB cellular distribution. Control cells, and cells treated with 0.5 μM of Ac-GYFWIAWYKLAN-Abu-KKG-NH_2 (a), Palm-GYFWIAWYKLAN-Abu-KKG-NH_2 (b) and (Ac-GYFWIAWYKLAN-Abu-KK)$_2$-KG-NH_2 (c).

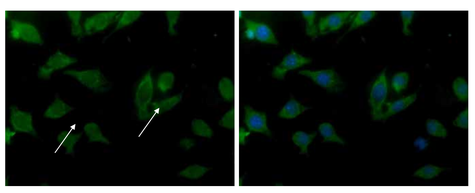

Fig. 4. NF-κB cellular distribution of cells treated with 10 μM δ-ctenitoxin-Pn2a.

NF-κB cellular distribution was detected by green immunofluorescence. Also, the nuclei were stained with Hoechst (blue). When immunogens activate cells, NF-κB migrates from the cytosol to the nucleus, where it upregulates cytokine gene expression. In resting macrophages (control), the transcription factor was restricted to the cytosol. In contrast, immunofluorescence was observed in the nucleus of the cells in the presence of 0.5 μM of each peptide thereby suggesting that they promote an immune response (Fig. 3). However, as shown in Fig. 4, δ-ctenitoxin-Pn2a only promote nuclear translocation at higher concentrations (10 μM). Probably, the cystine-knot motif of δ-ctenitoxin-Pn2a (Fig. 5) provides exceptional stability, thereby hampering immune response activation. Nevertheless, *in vivo* tests are still required to study the immunogenic capacity of the neurotoxin and the epitope-based immunogens for their use in the antivenom production.

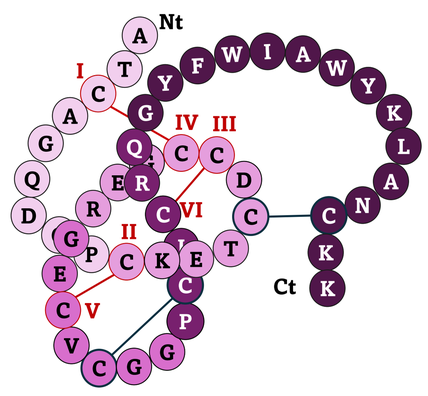

Fig. 5. Scheme of δ-ctenitoxin-Pn2a with the cystine-knot motif.

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