Naja nigricollis Venom Exosomes Biological Functions and Interactions

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Abstract

Therapeutic properties have been characterised for a variety of animal venom components, largely proteins with a role in envenomation effects. Extracellular vesicles (exosomes) have been shown to participate in cell signalling and, in snake venoms, may contribute to the effects of envenomation. The properties and mechanism of action of venom proteins and exosomes were analysed using SDS-PAGE, cell proliferation, haemolysis and Phospholipase A_2 assays as well as fluorescence staining to visualise interactions against controls of whole venom. This contributes to the understanding of *Naja nigricollis* venom and the profile of exosome action in cell signalling and metabolic influence.

Background & Aims

Features, characteristics and mechanisms of snake venom exosomes are largely unknown. Discovery of therapeutic properties of venom compounds have made characterising the venom proteome an important field of research. Exosomes have been described in a wide range of biological systems facilitating intercellular signalling. It is hypothesised that snake venom exosomes may be participate in the effects of envenomation. To investigate this, research questions were:

- do venom and exosome proteins differ
- do exosomes inhibit cell proliferation similarly to venom
- are exosomes taken up by cells to interact with internal structures and affect protein expression
- do exosomes contribute to effects of envenomation
- do exosomes contain known therapeutic compounds

Methods

Isolation of Exosomes: 100mg/ml venom samples were diluted 9:1 with pH7.5 PBS. Samples underwent differential ultracentrifugation at 2,000g for 10 minutes, 10,000g for 30 minutes and 110,000g for 90 minutes. Exosomes were resuspended in PBS and 50µl of each step supernatant retained. Thermofisher Bradford Protein assay kits were used according to manufacturers instructions.

SDS-PAGE: Samples were run through 12% resolving gels. EZ-run protein ladders were used. 5µl of supernatant and 10µl exosome samples were loaded.

CALU Protein Expression: Two sets of cells were incubated with exosomes for 2 hours, at 4 and 37°C. Cells were sonicated and proteins precipitated with TCA solution and acetone. Samples were centrifuged and pellets dried and resuspended in media prior to SDS-PAGE.

Resazurin Assay: Cell proliferation under whole venom, supernatant and exosome treatments was assessed by fluorescence at 544nm excitation and 590nm emission from 15-240 minutes.

Haemolysis Assay: Sheep blood was centrifuged and washed with PBS three times before adding SDS/venom. RBCs were incubated with samples for 2 hours at 37°C and 300rpm before centrifuging and aspirating the supernatant, diluting 1:20 with PBS and reading absorbance at 595nm.

PLA_2 Activity Assay: Phospholipase A_2 activity was assessed with an EnzChek PLA_2 assay kit (E10217) to manufacturers instructions.

Cellular Uptake of Exosomes: PKH67 staining was done according to manufacturers instructions. CALU cells were incubated with exosomes for 2 hours at 4° and 37°C. Cells were fixed with methanol and nuclei stained with DAPI. Images were obtained with an Olympus IX83 inverted microscope.

Results

SDS-PAGE CALU Protein Expression

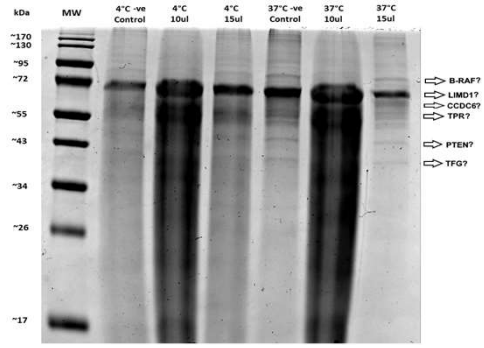

Figure 1. CALU cell protein expression under varying temperature and exosome concentration. Controls are included from cells incubated without exosomes.

Venom Protein SDS-PAGE

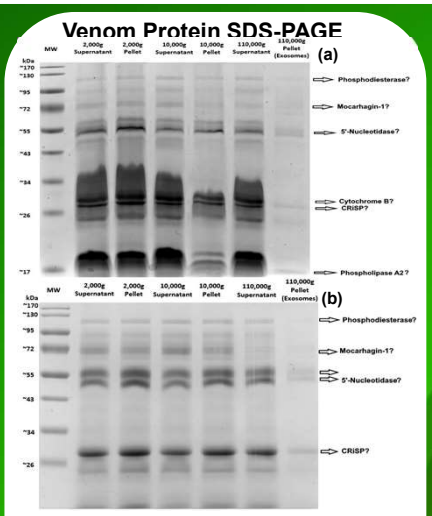

Figure 2. Venom sample 1 (**a**) and 2 (**b**) supernatant, pellet and exosome isolate protein contents. Protein identification is estimated based on those previously described in venom of other *Naja* species.

Haemolysis Assay

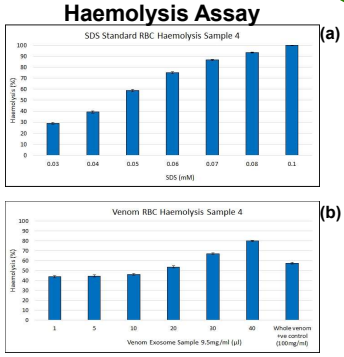

Figure 3. Haemolysis levels by SDS standards (**a**) and exosome concentrations including whole venom control (**b**).

Resazurin Assay

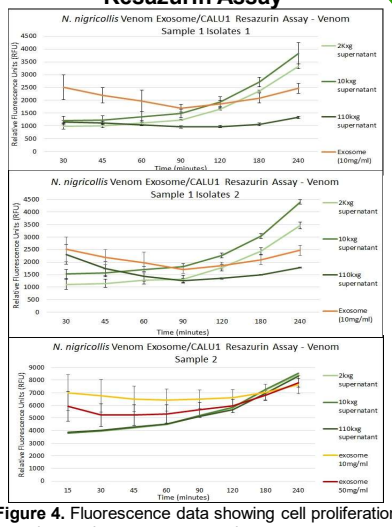

Figure 4. Fluorescence data showing cell proliferation over time under supernatant and exosome treatments. Values correlate with cell growth.

Phospholipase A_2 Assay

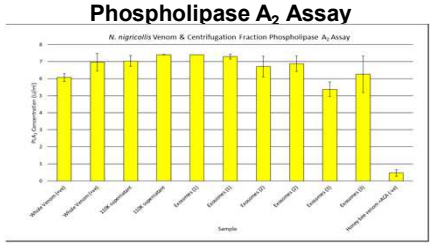

Figure 5. Levels of PLA2 activity in venom, exosome and supernatant replicates.

Cellular Uptake of Exosomes

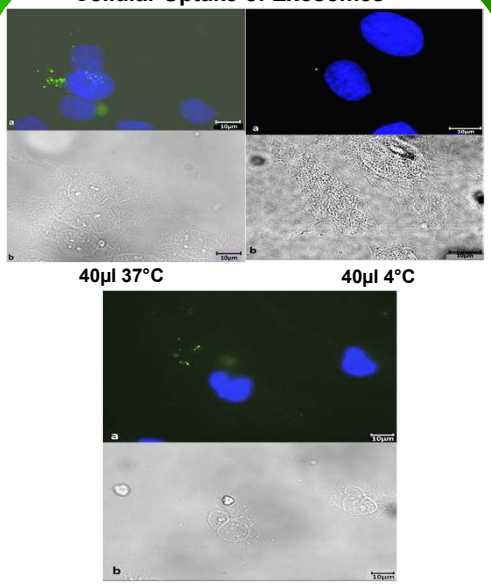

Figure 6. Fluorescence staining of exosomes and cell nuclei. Merged fluorescence (**a**) and bright field (**b**) images are shown.

Conclusions

The results suggest the following conclusions relating to the research questions:

- Exosome and venom protein groups appear to be identical
- CALU protein expression does not change with temperature or exosome concentration
- Exosomes contain higher concentration of PLA_2 than whole venom
- Exosomes may contribute to haemolysis effects in envenomation
- Cell growth is inhibited by exosomes consistently over time
- Exosomes localise to cell nuclei to induce apoptosis and uptake is blocked by low temperatures
- Further work needed to ascertain exosome contents and mechanisms of action

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