A closer look at the functional diversity of cytotoxins from Southeast Asian cobras: Biomedical and clinical significance

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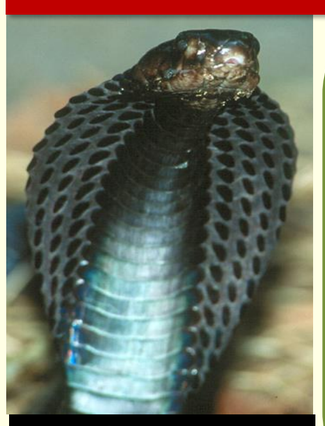
Introduction



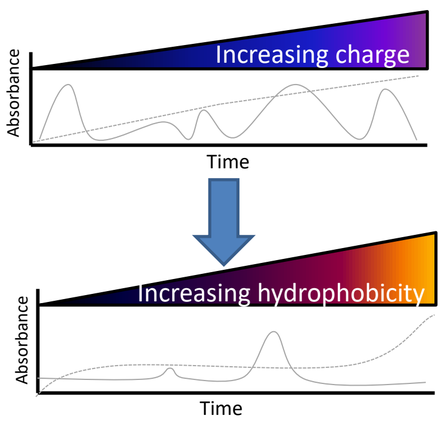
Naja sumatrana
Photo credit: Nick Baker

Cobra venoms contain cytotoxic components that can be further explored for anticancer potential. Cobra venom cytotoxins are polypeptides of 60–70 amino acid residues held by four disulfide crosslinks, forming a three protruding fingers-like structure and is grouped under the three-finger toxin superfamily. Its unique structure gives rise to its diverse pharmacological actions such as pore formation and disruption of cell membranes. Its cytotoxic properties, nevertheless, imply the possibility to harness cobra cytotoxins in the development of new anticancer drugs. The present study explores the two most common cobra species in Southeast Asia, *Naja sumatrana* (equatorial spitting cobra) and *Naja kaouthia* (monocled cobra) to elucidate the prospect of cytotoxin-derived anticancer therapeutics, through investigating the anticancer properties of the purified cytotoxins of *N. sumatrana* and *N. kaouthia* in breast, prostate, and lung cancer cell lines. The study further delved into the specificity and selectivity of the cytotoxins and verified the cell death mechanisms underlying their cytotoxic activities.

Naja kaouthia
Photo credit: Wolfgang Wuster

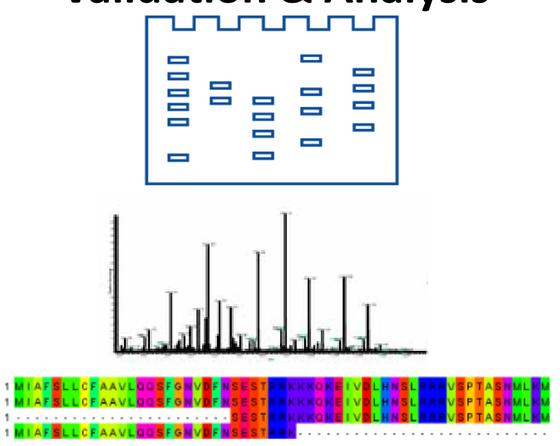
Methods

Fractionation & Purification



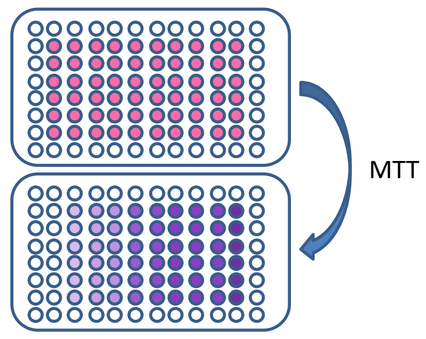
Sequential fractionation with cation exchange chromatography followed by reverse-phase high performance liquid chromatography

Validation & Analysis



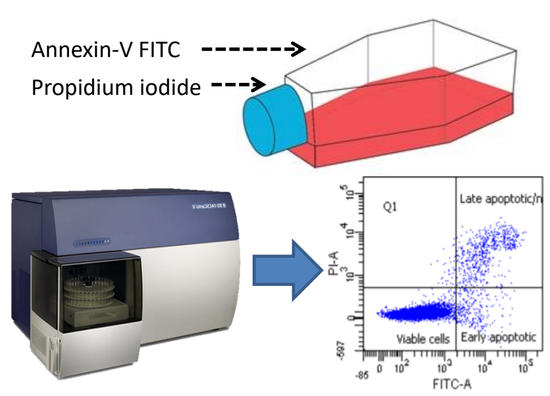
Validate and identity with 15 % reducing SDS-PAGE and LCMS/MS. Sequence analysis with Multiple Sequence Alignment

Cytotoxicity of cytotoxin



Cytotoxicity tested with MTT on A549, PC-3 and MCF-7 cell lines. 5-fluorouracil used as positive control.

Phosphatidylserine exposure



Flow cytometry with Annexin-V fluorescein isothiocyanate (FITC) conjugated / propidium iodide stains

Results

Purification of cytotoxins

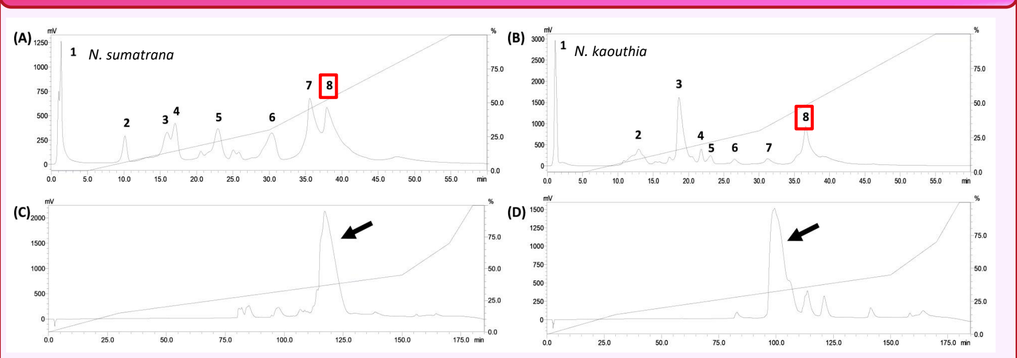


Figure 1. Purification of cytotoxins. Resource S cation-exchange HPLC of *N. sumatrana* **(A)** and *N. kaouthia* **(B)** venoms. C_{18} reverse phase-HPLC of CTX-containing fraction 8 of *N. sumatrana* **(C)** and *N. kaouthia* **(D)** venoms. Arrows indicate the major basic cytotoxins purified.

Both cobra species were resolved into 8 fractions with cytotoxins present at fraction 8. *N. sumatrana* and *N. kaouthia* cytotoxins eluted at 110-125 min and 90-115 min respectively.

Validation and sequence analysis of cytotoxins

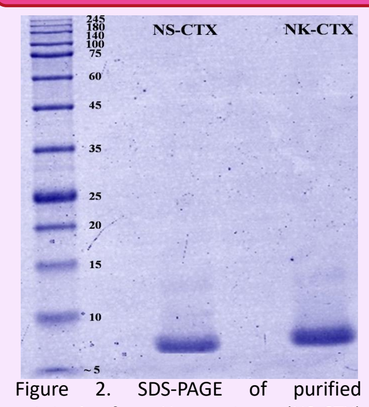


Figure 2. SDS-PAGE of purified cytotoxins from *N. sumatrana* (NS-CTX) and *N. kaouthia* (NK-CTX) venoms

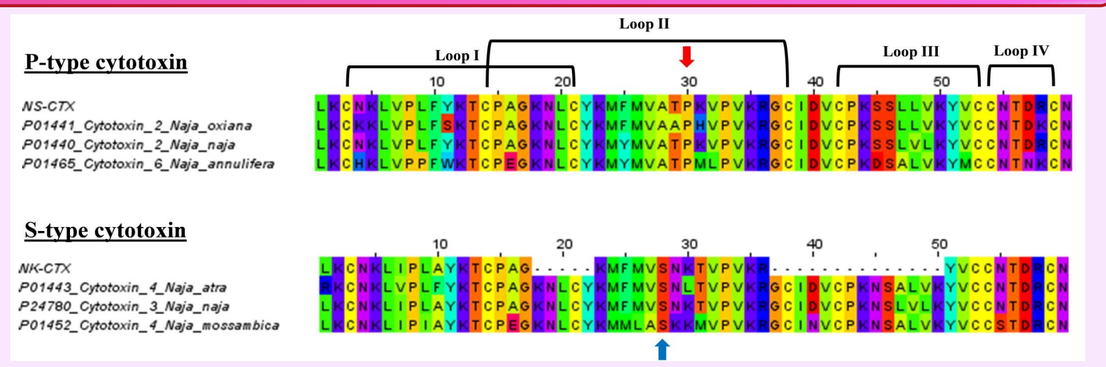


Figure 3. Multiple sequence alignments of NS-CTX and NK-CTX, showing P-type and S-type cytotoxins. Arrow in red indicated Proline[30] of P-type cytotoxins; blue arrow indicates Serine[28] of S-type cytotoxin.

SDS-PAGE showed homogenous protein band of approximately 7 kDa for both purified cytotoxins. Multiple sequence alignment showed the presence of Pro[30] in NS-CTX whilst NK-CTX possessing Ser[28], respectively categorizing the cytotoxins are P-type and S-type cytotoxins.

Cell-type specificity, selectivity and mechanism of action of cytotoxins

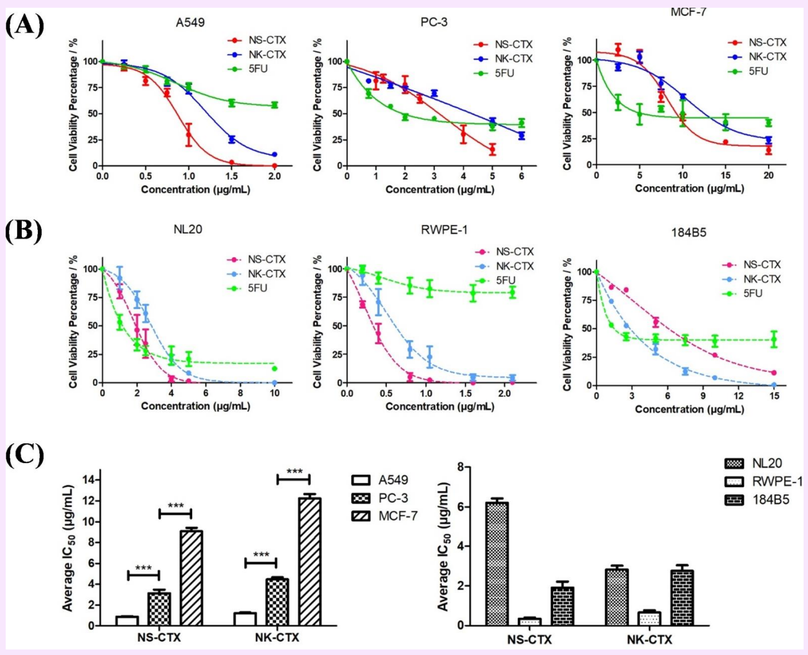


Figure 4. Cytotoxicity of NS-CTX, NK-CTX and 5FU on cancer and normal cell lines. Cell viability plot for cancer (**A**; A549, PC-3, and MCF-7) and normal (**B**; NL20, RWPE-1, and 184B5) cell lines. IC_{50} of cell lines treated with NS-CTX and NK-CTX **(C)**. One-way ANOVA with Bonferroni post hoc test was used to determine statistical significance across three cancer cell lines (*** indicates $p < 0.001$).

Table 1. Half maximal inhibitory concentrations (IC_{50}) and selectivity index of NS-CTX and NK-CTX in cancer and normal lung, prostate and breast cell lines.

Tissue origin	Cell line	NS-CTX		NK-CTX	
		IC_{50} (μg/ml)	Selectivity index	IC_{50} (μg/ml)	Selectivity index
Lung	A549	0.88 ± 0.06	2.17	1.22 ± 0.09	2.26
	NL20	1.91 ± 0.52		2.76 ± 0.49	
Prostate	PC-3	3.13 ± 0.58	0.11	4.46 ± 0.36	0.15
	RWPE-1	0.35 ± 0.08		0.65 ± 0.20	
Breast	MCF-7	9.10 ± 0.56	0.68	12.23 ± 0.76	0.23
	184B5	6.21 ± 0.37		2.83 ± 0.34	

Values were expressed in mean ± S.E.M.

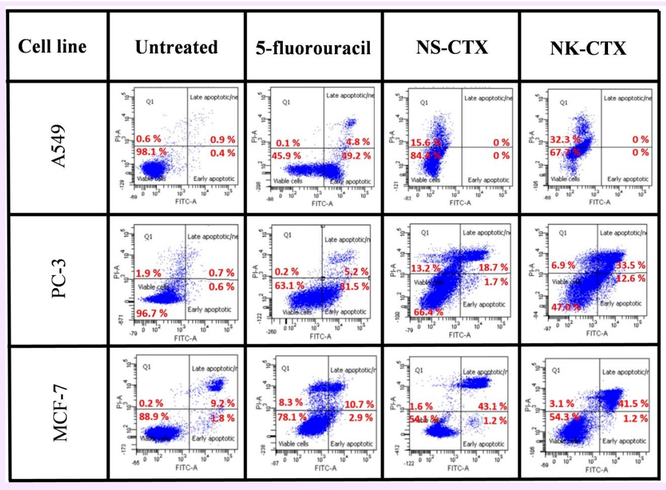


Figure 5. Dot plots showing flow cytometric cell death mechanism of cancer cell lines induced by NS-CTX and NK-CTX treatment.

NS-CTX, NK-CTX, and 5FU showed dose-dependent cytotoxic activities in cancer cell lines and normal cell lines. Increasing cytotoxicity in the following order: MCF-7 < PC-3 < A549. Normal breast and prostate cells were more susceptible to NS-CTX and NK-CTX, whilst selectivity was seen in lung cell lines. Flow cytometry demonstrated varying cell death mechanisms, Predominantly necrosis and late apoptosis in the cancer cell lines. Necrosis in the A549 and mainly late apoptosis in the MCF-7 cell line.

Conclusion and future work

NS-CTX and NK-CTX were highly potent in inhibiting the growth of the lung, prostate and breast cancer cell lines but lack selectivity for the latter two cell lines. Although A549 showed good selectivity, the cells mainly underwent necrosis. The study underscores the need for a comprehensive, fundamental investigation to address the selectivity and cell death mechanism of venom proteins. The anticancer potential of the toxins, however, may be further improved through structural modification to deliver a more specific, molecularly targeted cancer therapy in the future.

Reference

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