

IPD Project Details

Project ID: IPD6520

Project Title: Elusive elapid: Biogeographic venom variation in Indian kraits and its repercussion on snakebite therapy

Description: Snakebite is a major public health concern in many parts of the world, including India, where over 58,000 deaths occur annually due to this highly neglected tropical disease. The common krait (*Bungarus caeruleus*), one of the 'big four' Indian snakes, is responsible for the second-highest number of snakebite-related deaths in the country. The venom protein composition of common krait venoms from different locations were investigated. Venoms were pre-fractionated using RP-HPLC and SDS-PAGE, and subjected to mass spectrometry analysis. The relative abundance of each toxin family was estimated and compared across different locations.

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Sample Preparation: The venoms were fractionated on a Shimadzu LC-20AD series HPLC system (Kyoto, Japan). A total of 1 mg of venoms were reconstituted in water and injected into a reversed-phase C18 Shim-Pack GIST column [4.6 x 250 mm, 5 μ m particle size and 100 Å pore size (Shimadzu, Japan)]. The fractions were eluted at a flow rate of 1 mL/min under following gradient of buffer A (0.1% TFA in HPLC grade water), buffer B (0.1% TFA in acetonitrile) : 5% B for 5 min, followed by 5–15% B for 10 min, 15–45% B for 60 min, and 45–70% B for 10 min and held at 70% r B for 9 min, followed by 70-98% B for 6 min and again held at 98% B for 5 min and completed with 5% B for 5 min. Fractions were collected separately by monitoring the absorbance peaks at 215 nm. A combined analysis strategy was adopted for mass spectrometry, wherein RP-HPLC fractions with higher peak area were subjected to reducing SDS-PAGE and smaller peaks that could not be visualised on the gel were directly subjected to in-solution digestion. The excised gel bands were destained using 30% acetonitrile in 50 mM ammonium bicarbonate buffer. The gel pieces were dehydrated using 100% acetonitrile, followed by reduction using 10 mM dithiothreitol (DTT) at 56 °C for 45 min and alkylation using 55 mM iodoacetamide for 35 min at room temperature.

Peptide Separation: The samples were then digested using sequencing-grade trypsin

and incubated overnight at 37 °C. The following day, the supernatant was collected and desalted in a Pierce C18 spin column (ThermoFisher Scientific, USA) following the manufacturer's protocol before being subjected to mass spectrometric analyses. Similarly, for in-solution digestion, fractions were reduced and alkylated using DTT (100 mM) and iodoacetamide (100 mM), respectively, followed by tryptic digestion. The digested peptides were subjected to nano-Liquid Chromatography (nano-LC) and electrospray ionisation tandem mass spectrometry (ESI-MS/MS). The samples were injected into a PepMap C18 nano-LC column (50 cm x 75 µm, 2 µm particle size and 100 Å pore size) mounted on the Thermo EASY nLC Ultimate 3000 series system (Thermo Fisher Scientific, MA, USA), following a gradient elution of buffer A (0.1% formic acid in MS grade water) and buffer B (0.1% formic acid in 80% acetonitrile) at a constant flow rate of 250 nL/min for 90 min. An 8-35% gradient of buffer B was used over the first 70 min for elution, followed by 35 - 95% over the next 5 min, and finally 95% for the last 15 min. The following protocol was adopted to perform the MS scans: scan range (m/z) of 300 - 2000 with a resolution of 120000 and maximum injection time of 100 ms. To perform the precursor (MS) and fragment (MS/MS) scans, an orbitrap detector with high collision dissociation (HCD) fragmentation (30%) was used with the following parameters: scan range (m/z) of 110 –2000 and maximum injection time of 50 ms.

Protein Characterization: Raw MS/MS spectra were searched against the National Center for Biotechnology Information non-redundant (NCBI-NR) Serpentes database (taxid: 8570) to identify toxins that constitute the venom fractions. The search was performed in PEAKS Studio X Plus software (Bioinformatics Solutions Inc., ON, Canada) by setting parent and fragment mass error tolerance limits to 10 ppm and 0.6 Da, respectively. A 'monoisotopic' precursor ion search type and semispecific trypsin digestion were specified as parameters. Fixed and variable modifications were set as carbamidomethylation (+57.02) and oxidation (+14.99), respectively. The False Discovery Rate (FDR) was set to 0.1%, and PEAKS Studio automatically determined the corresponding -10lgP cutoff value. Hits with at least one unique matching peptide were considered for protein identification, and redundant hits from each protein family were manually removed.

Experiment Type: Bottom-up

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Unknown No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

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