IPD Project Details

Project ID: IPD3551

Project Title: Hiss of death - Biogeographical venom variation in the Indian spectacled cobra (Naja naja) underscores the pressing need for pan-India efficacious snakebite therapy

Description: The project aims at investigating the snake venom variability of Naja naja populations from six different biogeographic zones across India.

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Sample Preparation: Crude venoms were fractionated using a Shimadzu LC-20AD series HPLC system (Kyoto, Japan). Electrospray ionization tandem mass spectrometry (ESI-MS/MS) was used to characterize the proteomic profiles of the collected HPLC fractions (40 ?g each). Samples were reduced with Dithiothreitol (DTT; 10 mM), alkylated using iodoacetamide (IAA; 30 mM).

Peptide Separation: The whole process ollowed by an overnight trypsin (0.2 µg/µL) digestion. Liquid chromatography of these processed samples was performed using Thermo EASY nLC 1200 series system (Thermo Fisher Scientific, MA, USA) with a 50 cm x 75 µm, C18 (3 µm, 100 Å) nano-LC column. The sample (injection volume of 2 µL) was run at a flow rate of 300 nL/min in buffer A (0.1% formic acid in HPLC grade water) and Buffer B (0.1% formic acid in 80% Acetonitrile) solutions. The gradient of buffer B used for the elution was 10-45% over the first 98 minutes, 45-95% over the next 4 minutes, followed by 95% over the last 18 minutes. Mass spectrometric analyses of the samples were performed using the Thermo Orbitrap FusionTM Mass Spectrometer (Thermo Fisher Scientific, MA, USA).

Protein Characterization: For the MS scan, the following parameters were used: scan range (m/z) of 375-1700 with a resolution of 120000 and maximum injection time of 50 ms. For the fragment scans, an ion trap detector was used with high collision energy fragmentation (30%), scan range (m/z) of 100-2000, and maximum injection time of 35 ms. The raw MS/MS spectra were searched against the SwissProt database (www.uniprot.org) using PEAKS Studio X (Bioinformatics Solutions Inc., ON, Canada)

with the following parameters: parent and fragment mass error tolerance limits of 10 ppm and 0.6 Da, respectively; ‘monoisotopic’ precursor ion search type; and ‘semispecific’ trypsin digestion. Carbamidomethylation and oxidation were specified as fixed and variable modifications, respectively. Error in identification of peptides was minimised by fixing the False Discovery Rate (FDR) for peptide-spectrum matching at 0.1% and the corresponding -10lgP cutoff value was automatically determined by PEAKS Studio. Only hits with one or more unique peptides were considered for downstream analyses.

Experiment Type: Shotgun proteomics

Species: Naja naja-35670

Tissue: Venom (bto:0001439)

Cell Type:

Disease: Unknown

Instrument Details: Orbitrap Fusion (MS:1002416) Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

PubMed ID: