IPD Project Details

Project ID: IPD1873

Project Title: Biogeographic venom variation in medically important Indian snakes and the inadequacy of antivenom therapy in snakebite hotspots

Description: The project aims at investigating the snake venom variability of Daboia russelii populations from five different biogeographic zones across India.

Principal Investigator: Dr. Kartik Sunagar

PI Affiliation: Evolutionary Venomics Lab, Center for Ecological Sciences, Indian Institute of Science, Bangalore, India

Sample Preparation: Crude venoms were fractionated using a Shimadzu LC-20AD series HPLC system (Kyoto, Japan). HPLC fractions (40 ?g each) were subjected to electrospray ionization tandem mass spectrometry (ESI-MS/MS) for the characterisation of proteomic profiles.

Peptide Separation: Samples were reduced with Dithiothreitol (DTT; 10 mM), alkylated using iodoacetamide (IAA; 30 mM), and subsequently digested with trypsin (0.2 µg/µL) overnight and desalted. Thermo EASY nLC 1200 series system (Thermo Fisher Scientific, MA, USA) with a C18 nano-LC column (dimension 50 cm x 75 µm, 3 µm particle size and 100 Å pore size) was used for liquid chromatography of these processed samples. A sample volume of 2 µL was injected into the column and run with buffer A (0.1% formic acid in HPLC grade water) and buffer B (0.1% formic acid in 80% Acetonitrile) solutions at a constant flow rate of 300 nL/min for 120 minutes. The gradient of buffer B (10-45%) was used for the elution over the first 98 minutes, followed by 45-95% over the next 4 minutes and finally 95% for the last 18 minutes. Thermo Orbitrap Fusion Mass Spectrometer (Thermo Fisher Scientific, MA, USA) was used for mass spectrometric analyses of the samples.

Protein Characterization: MS scan was performed using the following parameters: scan range (m/z) of 375-1700 with a resolution of 120000 and maximum injection time of 50 ms. Fragment scans (MS/MS) was performed using an ion trap detector with high collision energy fragmentation (30%), scan range (m/z) of 100-2000, and maximum injection time of 35 ms. For identification of various toxin families in the proteomic profiles of venom fractions, the raw MS/MS spectra were searched against the

SwissProt database (www.uniprot.org) using PEAKS Studio X Plus (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 peptide identification 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. Hits with at least one unique matching peptide were considered for downstream analyses.

Experiment Type: Shotgun proteomics

Species: Daboia russelii-8707

Tissue: Venom (bto:0001439)

Cell Type:

Disease: Unknown

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

PubMed ID: