IPD Project Details

Project ID: IPD3822

Project Title: Fangs in the ghats: preclinical insights into the medical importance of endemic pit vipers from the Western Ghats

Description: The socioeconomic burden of snakebite in India is largely attributed to the â€˜big fourâ€™ snakes, completely neglecting the considerable impact of envenoming by many other snake species. Bites from the so-called â€˜neglected manyâ€™ are often treated with a polyvalent antivenom that is manufactured against the â€˜big fourâ€™ snakes - a strategy that has been widely documented to fail. Yet, specific antivenoms are not commercially manufactured against these snakes. While the medical importance of various species of cobras, saw-scaled vipers, and kraits is very well-known, the clinical impact of pit vipers from the rainforests of the Western Ghats, northeastern India, and Andaman and Nicobar islands has remained elusive. Amongst the 90+ species of snakes found in the Western Ghats, the hump-nosed (Hypnale hypnale), Malabar (Craspedocephalus malabaricus) and bamboo (Craspedocephalus gramineus) pit vipers can potentially inflict clinically severe envenoming in humans. To evaluate the severity of toxicity inflicted by these snakes, we characterised their venom composition, biochemical and pharmacological activities, and toxicity- and morbidity-inducing potentials. Our findings highlight the therapeutic inadequacies of the generic Indian and Hypnale-specific Sri Lankan polyvalent antivenoms in neutralising morbidity and mortality resulting from pit viper envenomings and underscore the need for a regional antivenom therapy in India.

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Sample Preparation: Individual venom bands were excised and subjected to in-gel digestion, followed by tandem mass spectrometry. Briefly, the isolated gel bands were destained and then dehydrated with 50% acetonitrile. Following dehydration, the samples were reduced by treatment with 10 mM dithiothreitol (DTT) at 56 °C for 1 hour. This was followed by a 30 mM iodoacetamide treatment for alkylation at room temperature for 45 min under dark conditions. A mixture of 25 mM ammonium bicarbonate in water and acetonitrile solution (1:1, v/v) was used to wash the bands. The excess solvent was removed using a vacuum concentrator (Thermo Fisher Scientific,

MA, USA).

Peptide Separation: Samples were then digested with trypsin (0.2 µg/µl) overnight at 37° C, and the peptides were extracted the next day into 50 ?l of 50% acetonitrile solution. The relative abundance of various toxins present in pit viper venoms was determined by subjecting the individually excised venom bands to tandem mass spectrometry {Senji Laxme, 2022 #343}. Briefly, the individual bands were reduced, alkylated, trypsinised and injected into the liquid chromatographic system programmed at a constant flow rate of 300 nl/min for a total run time of 120 mins with varying concentrations of buffer A (0.1% formic acid in HPLC grade water) and buffer B (0.1% formic acid in 80% acetonitrile). The buffer gradient was set as 10-45% over 98 minutes, 45-95% over 4 minutes and 95% over 18 minutes. The eluted fractions were then introduced into a Thermo Orbitrap Fusion Mass Spectrometer (Thermo Fisher Scientific, MA, USA) for tandem mass spectrometry. The MS scans were performed with the following parameters range (m/z) of 375-1700 with a resolution of 120000 and a maximum injection time of 50 ms. Furthermore, an ion trap detector with high collision energy fragmentation (30%) was used to perform fragment scans (MS/MS) with a range (m/z) of 100-2000 and a maximum injection time of 35 ms.

Protein Characterization: The mass spectrometry data were acquired with an online Thermo Orbitrap FusionTM Mass Spectrometer (Thermo Fisher Scientific, MA, USA) that was coupled with the Thermo EASY nLC 1200 series system. MS scans were performed in an Orbitrap detector using the following parameters: positive ion polarity, resolution 120000, scan range (m/z) of 375-1700, and maximum injection time of 50 ms. The ion trap detector was used for MS/MS scans with successive parameters: high collision energy fragmentation (30%), scan range (m/z) of 100-2000 and maximum injection time of 35 ms. PEAKS Studio X+ (Bioinformatics Solutions Inc., ON, Canada) was used to search the MS/MS spectra against the UniProt/SwissProt and NCBI-NR Serpentes databases (taxid: 8570; November 2020) with peptide mass tolerance of 10 ppm, fragment mass tolerance of 0.6 Da, and False Discovery Rate (FDR) of 0.1. Hits with at least one unique matching peptide were considered for downstream analyses.

Experiment Type: Shotgun proteomics

Species: Hypnale hypnale-44720, Craspedocephalus malabaricus-109784, Craspedocephalus gramineus-8767

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: 37298463