IPD Project Details

Project ID: IPD3987

Project Title: The preclinical evaluation of a second-generation antivenom for treating snake envenoming in India

Description: In this study, by employing chromatographic purification of the bulk, we have significantly enhanced the preclinical performance of the conventional Indian antivenom product. The effectiveness of the test batches of this â€˜second-generationâ€™ antivenom was evaluated using a variety of in vitro and in vivo preclinical assays, including venom recognition and toxicity and pathology neutralisation. The outcomes of these experiments demonstrated the significantly superior performance of the purified product over all other major commercial Indian antivenoms.

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Sample Preparation: Indian antivenoms were dissolved in 25 mM ammonium bicarbonate and subjected to mass spectrometric analyses. A final concentration of 10 mM was achieved by adding 100 mM of DTT (dithiothreitol), and the mixture was incubated at 37o C for 30 mins. Following the incubation period, iodoacetamide (100 mM) was added to the solution, and the mixture was subjected to another round of incubation in the dark (37o C for 30 minutes). The pH of the solution was adjusted to 8 using 25 mM ammonium bicarbonate.

Peptide Separation: The overnight digestion of the sample with trypsin (0.2 µg/ml; 1:50) at 37o C. The reaction was stopped using 0.1% formic acid, and the sample was desalted with ZipTip containing acetonitrile and 0.1% formic acid. Peptides were then separated 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 at a flow rate of 300 nL/min. Here, the mobile phase was constituted by 0.1% formic acid in HPLC grade water (buffer A) and elution buffer of 0.1% formic acid in 80% Acetonitrile (buffer B). The concentration of the buffer was altered as follows: 10-45% over 98 min, 45-95% over 4 min and 95% over 18 min.

Protein Characterization: A Thermo Orbitrap Fusion Mass Spectrometer (Thermo

Fisher Scientific, MA, USA) was used for mass spectrometric analyses of the antivenom samples. Following parameters were used for MS scans: scan range (m/z) of 375-1700 with a resolution of 120000 and maximum injection time of 50 ms. An ion trap detector with high collision energy fragmentation (30%), scan range (m/z) of 100-2000, and maximum injection time of 35 ms was employed for fragment scans (MS/MS). Using PEAKS Studio X Plus software (Bioinformatics Solutions Inc., ON, Canada), raw MS/MS spectra were searched against the National Center for Biotechnology Information's (NCBI) non-redundant (nr) database (Equus caballus: 9796; September 2021) to identify antibodies and other proteins present in the antivenom samples. The tolerances for parent and fragment mass errors were set at 10 parts per million (ppm) and 0.6 Da, respectively. Cysteine carbamidomethylation was set as a fixed modification, whereas methionine oxidation and asparagine or glutamine deamidation were designated as variable modifications. In the semispecific mode, a maximum of two missed cleavages by trypsin was permitted. The match acceptance filtering settings were: FDR of 0.1%; minimum number of unique peptides: 1; and -10lgP protein score of 40.

Experiment Type: Shotgun proteomics

Species: Equus caballus-9796

Tissue: Blood serum (bto:0000133)

Cell Type:

Disease: Unknown

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: monohydroxylated residue, iodoacetamide derivatized residue

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