Discovery of Peptide-Based Antitoxins against Neurotoxins from Green and Black Mamba (Dendroaspis Family)

Jappe, Emma Christine; Munk, Andreas; Laustsen, Andreas Hougaard; Engmark, Mikael

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Snakebite – A neglected threat to public health

Globally, more than 5.5 million people are bitten by venomous snakes every year, leading to an estimated 125,000 deaths and 3 times as many amputations [1,2,3]. The problem is most prevalent in Sub-Saharan Africa where affordability of antivenom is low, resulting in only 2% of snakebite victims receiving treatment [1,4]. Since the introduction of antivenoms in the 1890’s, only modest advances in antivenom technology and production have been made. Current antivenoms are, therefore, still being produced by immunisation of large ruminants, typically horses, with snake venoms and subsequently bleeding them to collect blood comprising venom-specific antibodies [4]. The incompatibility of these antivenoms with the human immune system can lead to serious adverse effects [1,5]. A novel approach is needed in order to introduce safer, cheaper and more efficacious antivenoms that are compatible with the human immune system to the market.

Results – Cross-reactivity based on similarity

Based on ELISA, strong binding to Dtx I was observed for the polyclonal phage library after the third round of panning (Figure 4), yet randomly selected monodonal phages did not show strong binding to Dtx I (Figure 5). It was observed that the polyclonal phage library also bound to α-Dtx (data not shown), indicating a high degree of cross-reactivity. This was anticipated by the bioinformatics modelling of the dendrotoxins, illustrating a high degree of similarity in both their primary, secondary, and tertiary structures (Figure 3).

Method – Identification of binders with phage display

Initially, sequence alignment using the protein Needleman-Wunsch algorithm from EMBL-EBI was performed (Figure 3). Additionally, 3D structural models of the two toxins were constructed and compared. The structure of α-Dtx was based upon the available X-ray crystallographic structure with PDB entry 1DTX whilst the structure of Dtx I was estimated based on a model of the Kunitz-type serine protease inhibitor (PDB entry 3BYY), isolated from Pseudonaja textilis (brown snake) using the Bioinformatics Toolkit developed by the Max-Planck Institute, Tübingen (Figure 3).

Outlook – Discovery of antitoxins for mamba toxins

Polymodal phages with strong binding affinity, high specificity, yet displaying cross-reactivity, were discovered using phage display. However, due to time limitations, no individual monoclonal phage was found to have both high affinity and show selectivity towards the toxins.

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Based on ELISA, strong binding to Dtx I was observed for the polyclonal phage library after the third round of panning. However, randomly selected monodonal phages did not show strong binding to Dtx I. It was observed that the polyclonal phage library also bound to α-Dtx, indicating a high degree of cross-reactivity. This was anticipated by the bioinformatics modelling of the dendrotoxins, illustrating a high degree of similarity in both their primary, secondary, and tertiary structures.

The method of identification of binders with phage display involves sequence alignment using the protein Needleman-Wunsch algorithm from EMBL-EBI. Additionally, 3D structural models of the two toxins were constructed and compared. The structure of α-Dtx was based on the available X-ray crystallographic structure with PDB entry 1DTX, whilst the structure of Dtx I was estimated based on a model of the Kunitz-type serine protease inhibitor (PDB entry 3BYY), isolated from Pseudonaja textilis (brown snake) using the Bioinformatics Toolkit developed by the Max-Planck Institute, Tübingen.

Outlook – Discovery of antitoxins for mamba toxins

Polymodal phages with strong binding affinity, high specificity, yet displaying cross-reactivity, were discovered using phage display. However, due to time limitations, no individual monoclonal phage was found to have both high affinity and show selectivity towards the toxins. Subsequent steps could include further analysis of other monoclonal phages or repetition of the fourth round of panning in order to attempt to amplify phages with high affinity and specificity. If a high-affinity toxin binder was to be identified, this binder could 1) be applied as a peptide-based antitoxin, 2) be used to create a novel antidendrotoxin or 3) be grafted onto an antibody as a CDR region, paving the way for safer and more efficacious antivenoms.

References


Contact information

emj@bio.dtu.dk / (45) 5561 6775, anmunk@hotmail.com / (45) 2227 0444

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Collaborators: Jonas Johansen (KU), Grete Sørgensen (KU), Maiken Ravne (KU), Alexandra Bak Jakobsen (CBS).

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