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

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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 1890s, 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 venom 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.

We attempt to discover cross-reactive, peptide-based antitoxins against the structurally similar dendrotoxins α-dendrotoxin (α-Dtx, UniProtKB P09980), isolated from Dendroaspis angusticeps (Green mamba), and dendrotoxin I (Dtx I, UniProtKB P09979) from Dendroaspis polylepis (Black mamba) by phage display [5,6]. Cross-reactive antitoxins with the ability to neutralise several toxins are of interest to antivenom development, since only a few cross-reactive antitoxins would be needed to neutralise a complete snake venom [4].

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 1OTX whilst the structure of Dtx I was estimated based on a model of the Kunitz-type serine protease inhibitor (PDB entry 3B3Y), isolated from Pseudonaja textilis (brown snake) using the Bioinformatics Toolkit developed by the Max-Planck Institute, Tübingen (Figure 3).

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 monosonal 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).

Figure 4: ELISA results (Absorbance at 490 nm). Iterative rounds of panning of the phages lead to the amplification of good binders. The polyclonal phage library from the third round of panning yields a strong ELISA signal as well as a signal ratio of 8.6 between Dtx I and PBS + skim milk control, indicating the presence of strong peptide binders to Dtx I.

Outlook – Discovery of antitoxins for mamba toxins

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

References


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