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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 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.

We attempt to discover cross-reactive, peptide-based antitoxins against the structurally similar dendrotoxins α- and β-dendrotoxin (α-Dtx, UniProtKB P01998), isolated from Dendroaspis angusticeps (Green mamba), and dendrotoxin I (Dtx I, UniProtKB P00979) 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 1DTX whilst the structure of Dtx I was estimated based on a model of the Kunitz-type serine protease inhibitor (PDB entry 3BYB), isolated from Pseudonaja textilis (brown snake) using the Bioinformatics Toolkit developed by the Max-Planck Institute, Tübingen (Figure 3).

Figure 1: Dendroaspis polylepis (black mamba). Photo: Larsa D. 2011

Figure 3: Surface models and overlapping cartoon models of α-dendrotoxin (α-Dtx) and dendrotoxin I (Dtx I) from D. angusticeps and D. polylepis, respectively, illustrating the largely similar secondary and tertiary structures of the two toxins. Models are drawn in PyMOL. Sequence similarity of 95% is observed when performing sequence alignment using the protein Needleman-Wunsch algorithm from EMBL-EBI.

Figure 4: ELISA results (Absorbance at 490 nm). Iterative rounds of panning of the phages lead to 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

Polyvalent 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 to 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 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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