Thesis Title

Novel membrane active peptides derived from marine organisms and their mechanisms of action

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ABSTRACT

In the current era of multi-drug resistance, the conventional antibiotics are proving inefficient in the treatment of serious life-threatening infections along with their debilitating side-effects and development of resistance. To tackle this major challenge, alternative therapeutic compounds are required and membrane active peptides (MAPs) are one such group of therapeutics that have gained attention due to their ability to interact with various biological membranes in both disruptive and non-disruptive manner. Concomitantly, the inability of pathogens to mutate their membrane components as frequently in response to MAPs, keeps the spread of antimicrobial resistance (AMR) under check. Two major classes of MAPs, namely cell penetrating peptides (CPPs) and antimicrobial peptides (AMPs) have been extensively studied and employed in therapeutics as drug delivery vectors and antimicrobial agents respectively. MAPs from marine organisms were chosen for this study because of their uniqueness and biochemical diversity that can be utilized for development of better peptide therapeutics with multi-functional properties.

The present thesis addresses the antimicrobial, anti-biofilm, cell penetrating and other functions of marine and toxin derived CPPs and AMPs along with elucidation of their mechanisms of action. It is hypothesized that the peptides used in this study will prove to be

effective and multi-functional in their bioactivity with distinct mechanisms of action that can be utilized for the development of better peptide therapeutics.

The research questions targeted in the first objective of the study were: How the marine derived peptide Tachyplesin and snake-toxin derived peptide CyLoP-1 will fare as an anti-mycobacterial peptide? What will be the role of cysteine and arginine residues in imparting antimicrobial and cell penetrating activity to these peptides? What will be their mechanisms to inhibit or kill mycobacterium cells? Will they be able to enter macrophage cells infected with mycobacterium and kill the intracellular pathogen without disturbing the host cell? Will these peptides would also be able to inhibit or eradicate mycobacterium biofilms? To answer these questions, antimicrobial and cell culture based studies and assays were performed. For determination of anti-mycobacterial and anti-biofilm activity of peptides, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), time-kill kinetics, co-culture and crystal violet assays were employed. Further, the mechanistic insights were gained by utilizing various microscopy and membrane-based assays such as transmission electron microscopy (TEM), membrane integrity and membrane depolarization assays. Intracellular reactive oxygen species (ROS) production was determined using fluorescent dye. The major outcomes of this objective were potent anti-mycobacterial and anti-biofilm activity of both the peptides with the ability to kill intracellular mycobacteria as well. Cysteine and arginine residues proved to be crucial for cell penetrating and anti-mycobacterial activity of both the peptides. Tachyplesin mainly followed membranolytic pathway whereas, CyLoP-1 employed intracellular ROS production as the mechanism to kill mycobacterium cells.

The research questions to be addressed in the second objective were: How to design a novel CPP from marine antimicrobial peptide and improve its functionality? What will be the overall bioactivity and toxicity profile of the novel peptides and will they be suitable candidates for further drug development pipeline? These research questions were answered using various *in silico* and *in vitro* techniques. In this direction, a novel peptide from marine AMP clavanin was designed *in silico* and an immunomodulatory sequence was added to its C-terminal. The peptides were named clavanin derived peptides (CDPs) with 3 variants - CDP-1 (with

immunomodulatory sequence), CDP-2 (without immunomodulatory sequence) and CDP-3 (mutated version of CDP-2). The most significant results were excellent cell penetrating, antimicrobial and anti-biofilm activity without considerable cytotoxicity demonstrated by CDP-1 as compared to the other two variants. However, cargo delivery abilities and cytotoxicity profile of all the three peptides were similar.

In conclusion, novel AMPs and CPPs from marine sources displayed multi-functionality that can be translated into viable therapeutics. This study highlights the importance of understanding structure-activity relationship and mechanistic pathways of peptides against a host of cells and microorganisms so as to develop antimicrobial peptide therapeutics for better management of diseases.