Combinatorial screening of antimicrobial and anti-inflammatory peptides for the development of novel mastitis therapeutics

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Abstract

Mastitis, an inflammatory response of the mammary tissue to invading pathogenic bacteria, is a common disease in breast-feeding women and dairy animals. Bovine mastitis is a worldwide problem and causes multibillion-dollar economic losses due to reduce milk production and quality. The disease is commonly caused by bacterial infection ascending through the teat canal and frequent pathogens are *Escherichia coli* ,*staphylococc*us aureus, *streptococcus uberis* and *agalactiae*, and *mycoplasma*. Currently, the administration of antibiotics is the most common method for its treatment and prevention, but this strategy has several disadvantages including the emergence of bacterial resistance, low cure rate and presence of antibiotic residues in milk and meat products exposing consumers to public health hazards. Therefore, there is an urgent need to develop novel approaches for managing bovine mastitis more efficiently. In recent years, anti-microbial peptides (AMPs) have emerged as potential biocompatible candidates to tackle bacterial antibiotic resistance crisis and filling the current void in novel antibiotic discovery. In our lab, we have developed a novel approach to synthesize AMPs mixtures. An important advantage of the AMPs mixtures is their low cost, simple synthesis, broad spectrum, and low probability for bacterial resistance occurrence due to the repertoire of AMPs sequences received throughout the synthesis.

Here we aimed to develop and evaluate the anti-microbial and anti-inflammatory roles of AMPs mixtures, random antimicrobial peptide mixture (RPMs) as well as their feasibility to treat acute coliform mastitis, using *in vitro* and *in vivo* model systems. We have discovered that random peptide mixture displayed strong and broad antimicrobial activity towards mammary pathogenic *Escherichia coli* (MPEC) bacteria and in addition showed strong antimicrobial activity towards rough mutants of MPEC bacteria in bovine raw milk. In addition, we have demonstrated that RPMs abrogated mouse mammary epithelial cell line (EpH4) inflammatory response elicited by lipopolysaccharide (LPS), the most significant pathogen-associated molecular pattern

(PAMP) in coliform mastitis. We have demonstrated that treatment of RPMs did not affect the morphology of mammary epithelial cells (MAECs) indicating on the safety of RPMs. In addition, RPMs partially succeeded to reduce the MPEC microbial load in the mammary gland. These results demonstrate the potential of RPMs to treat mastitis but for future studies are needed to improve and optimize RPMs activity.