Development and preclinical testing of a novel antimicrobial peptide to treat Pseudomonas aeruginosa-induced lung infections

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Keywords: antimicrobial peptides; esculentin; diastereomer; lung infections; anti-endotoxin;

Background. Pseudomonas aeruginosa is the most predominant lung pathogen in patients with cystic fibrosis (CF). It is quite difficult to eradicate because of its acquired resistance to most available antibiotics and ability to form sessile communities or biofilms, in a protective extracellular matrix. Cationic antimicrobial peptides (AMPs) hold promise as future anti-infective agents.

Hypothesis and objectives. Our objective was to develop a short-sized AMP from frog skin, Esc(1-21) and/or its diastereomer containing two D-amino acids, for treatment of P. aeruginosa lung infections. To this aim: (i) an in-vitro analysis of the anti-pseudomonal, anti-inflammatory, wound healing properties of the two peptides, and (ii) preclinical testing to establish both the peptides' safety profiles and therapeutic dosages were performed.

Methods. A multidisciplinary approach combining biochemical, cell biology, microbiological techniques, as well as murine models of P. aeruginosa acute lung infections were used to attest our purposes.

Results. Esc(1-21) rapidly kills planktonic P. aeruginosa cells and eradicates its biofilms with a membrane-perturbing activity as a plausible mode of action. In addition, the peptide limits the induction of bacterial resistance and neutralizes the toxic effect of P. aeruginosa lipopolysaccharide. However, by replacing only two L-amino acids of Esc(1-21) with the corresponding D-enantiomers, the resulting diastereomer is significantly: (i) less susceptible to host/bacterial proteases; (ii) more active in killing Pseudomonas internalized into bronchial epithelial cells; (iii) more efficient in stimulating bronchial cells migration (also in infectious-mimicking conditions) than the wild-type molecule, and harmless on airway epithelial cells. Furthermore, the diastereomer shows a better efficacy than Esc(1-21) in reducing the lung bacterial burden in murine models of acute P. aeruginosa lung infections upon a single intratracheal instillation at 0.1 mg/kg, 2h after infection, without causing any pulmonary inflammation or lung epithelial injury.

Spin-off for research and clinical purposes. Our findings indicate high potential to develop an AMP-based pharmaceutical formulation against P. aeruginosa lung infections in CF, by local administration. This would not only eliminate bacteria and attenuate inflammatory response but would also promote re-epithelialization of the injured tissue after infection. Further studies will be necessary to identify the best strategy for pulmonary release of the peptide at effective concentrations.

Acknowledgment. FFC#11/2014: funded by FFC, supported by Delegazione FFC di Siena, Delegazione FFC di Sondrio Valchiavenna, Delegazione FFC il Sorriso di Jenny, Delegazione FFC di Pavia.

References

1.Bjarnsholt, T. et al. Pediatric Pulmonol. 2009, 44: 547: 558

2.Zhang, L. et al. Antimicr Agents and Chemother. 2005, 49: 2921-2927 https://doi.org/10.1128/AAC.49.7.2921-2927.2005 PMid:15980369 PMCid:PMC1168697

3.Luca, V. et al. Cell Mol Life Sci. 2013, 70: 2773-2786 https://doi.org/10.1007/s00018-013-1291-7 PMid:23503622

4.Mangoni, ML. et al. Peptides 2015, 71:286-95 https://doi.org/10.1016/j.peptides.2015.04.018 PMid:25959536

5.Di Grazia et al. Amino Acids. 2015, 47:2505-19 https://doi.org/10.1007/s00726-015-2041-y PMid:26162435

6.Ghosh A et al. Biochim Biophys Acta 2016, 1858:800-12.