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Antimicrobial peptides in the treatment of infectious and inflammatory conditions

Preclinical studies of mechanism of action, efficacy, and safety

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer att offentligen försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, tisdagen den 22 november, klockan 09:00

av

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Avhandlingen baseras på följande delarbeten:

I. Nilsson E.*, Björn C.*, Sjöstrand V., Lindgren K., Münnich M., Mattsby-Baltzer I., Ivarsson ML., Olmarker K., Mahlapuu M. **A novel polypeptide derived from human lactoferrin in sodium hyaluronate prevents postsurgical adhesion formation in the rat.** *Annals of Surgery* 2009; 250: 1021–1028. * Equal contribution.

II. Björn C., Håkansson J., Myhrman E., Sjöstrand V., Haug T., Lindgren K., Blencke HM., Stensvåg K., Mahlapuu M. **Anti-infectious and anti-inflammatory effects of peptide fragments sequentially derived from the antimicrobial peptide centrocin 1 isolated from the green sea urchin, Strongylocentrotus droebachiensis.** *AMB Express* 2012; 2: 67.

III. Myhrman E., Håkansson J., Lindgren K., Björn C., Sjöstrand V., Mahlapuu M. **The novel antimicrobial peptide PXL150 in the local treatment of skin and soft tissue infections.** *Applied Microbiology and Biotechnology* 2013; 97: 3085–3096.

IV. Håkansson J., Björn C., Lindgren K., Sjöström E., Sjöstrand V., Mahlapuu M. **Efficacy of the novel topical antimicrobial agent PXL150 in a mouse model of surgical site infections.** *Antimicrobial Agents and Chemotherapy* 2014; 58: 2982–2984.

V. Björn C., Noppa L., Näslund Salomonsson E., Johansson AL., Nilsson E., Mahlapuu M., Håkansson J. **Efficacy and safety profile of the novel antimicrobial peptide PXL150 in a mouse model of infected burn wounds.** *International Journal of Antimicrobial Agents* 2015; 45: 519–524.

VI. Björn C., Mahlapuu M., Mattsby-Baltzer I., Håkansson J. **Anti-infective efficacy of the lactoferrin-derived antimicrobial peptide HLR1r.** *Peptides* 2016; 81: 21–28.

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Avdelningen för molekylär och klinisk medicin, Institutionen för medicin, Sahlgrenska akademin, Göteborgs universitet, Sverige, 2016.

Abstract

The rapid emergence of antibiotic-resistant microbes worldwide and the urgent need of new antimicrobial agents have stimulated interest in antimicrobial peptides (AMPs) as new therapeutics for treatment of infectious diseases. AMPs are present in all living species and constitute an important part of the innate immune system in multicellular organisms, including humans. AMPs display a remarkably broad spectrum of antimicrobial activity covering both Gram-positive and Gram-negative bacteria, including many antibiotic-resistant strains, as well as fungi, viruses, and protozoa. Further, in contrast to many conventional antibiotics, AMPs rapidly kill bacteria instead of just inhibiting bacterial growth. In addition, AMPs act as modulators of the innate immune system and, importantly, bacteria seem less efficient in developing resistance towards AMPs than towards conventional antibiotics. Together these properties make AMPs highly attractive as a new class of antimicrobials, with clinical potential also extending to diseases where inflammation is part of the pathology.

The aim of this thesis was to study novel AMPs with respect to their mechanism of action (MOA), antimicrobial spectrum, propensity to select for resistance, and *in vivo* efficacy and safety. To achieve this, we used a number of *in vitro* and *in vivo* assays, together generating a comprehensive preclinical evaluation of the peptides. The hypothesis was that the AMPs in this thesis have potential to be developed as therapeutic agents for several infectious and inflammatory conditions, including treatment of skin and soft tissue infections and prevention of postsurgical adhesion formation.

The results showed that all AMPs tested (i.e. PXL03, PXL150, HLR1r, and five variants of CEN1 HC-Br) had broad antimicrobial spectra *in vitro* with varying sensitivity to salt and serum. Furthermore, PXL150 caused a rapid permeabilization of bacterial membrane *in vitro*, indicating that this is at least one part of the MOA of this peptide. Under selection pressure *in vitro*, bacteria did not develop resistance to the peptides tested, i.e. PXL150 and CEN1 HC. Interestingly, all peptides showed anti-inflammatory activity by inhibiting the secretion of proinflammatory mediators from stimulated human cell lines. In addition, PXL01, PXL150, and HLR1r demonstrated fibrinolytic ability *in vitro* by suppressing the release of plasminogen activator inhibitor-1 (PAI-1). In *ex vivo* and *in vivo* skin/wound infection models, the peptides reduced the number of viable bacteria and yeast cells. Further, PXL01 decreased postsurgical adhesion formation *in vivo*. Notably, nonclinical safety studies showed that PXL150 was safe and well tolerated.

In conclusion, several of the peptides evaluated in this thesis demonstrated a promising preclinical efficacy and safety profile motivating further development as drug candidates for local treatment of infectious and inflammatory conditions.

Keywords: Antimicrobial peptides, AMPs, innate immunity, infection, inflammation, mechanism of action, efficacy, safety, antimicrobial resistance, antibiotic resistance

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