

Exploring the biological properties and therapeutic potential of antimicrobial peptides

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Abstract—The researchers involved in the Trans2Care project at the Department of Medical and Biological Sciences of the University of Udine investigate the biological properties of the antimicrobial peptides (AMPs) of the immune system and their therapeutic potential for human and veterinary application. In addition to potent and broad-spectrum antimicrobial activities, some AMPs display anti-inflammatory and immunomodulatory effects and hold promise as novel anti-infective agents combining antibiotic and immunostimulating properties. A detailed knowledge of their physicochemical, biological and pharmacological properties and of their impact on clinical settings is an important prerequisite to this end. The Trans2Care project offers an invaluable opportunity to share knowledge, technical expertise and laboratory facilities to achieve a better understanding of the biological features and therapeutic potential of AMPs.

Index Terms — antimicrobial peptides, anti-infective drugs, antimicrobial activity, cytokine release, immunomodulatory activity

1 UNIVERSITY OF UDINE

The University of Udine is a public institution founded in 1978 as part of the reconstruction plan of Friuli after the earthquake in 1976. It is devoted to higher education, research and technology transfer. The University is organized into 10 faculties and 14 research departments with approx. 16000 enrolled students (a. y. 2009-2010). The University is actively involved in student and staff exchange projects with universities within EU and other non-EU countries and participates in many national and international research projects.

2 WORKING UNIT

The Udine working unit includes researchers interested in the characterization and development of plant-based models of drug transport (Department of Agricultural and Environmental Sciences), and others involved in the study of antimicrobial peptides (AMPs) as templates for the development of novel anti-infective drugs (Department of Medical and Biological Sciences).

3 ACTIVITIES AND ROLES IN TRANS2CARE PROJECT

3.1 Antimicrobial peptides (AMPs)

AMPs are small, evolutionarily conserved protein components of the innate immune system involved in first-line host defence against infection [1,2]. In mammals, two distinct families of such peptides, the defensins [3] and the cathelicidins [4], have been detected on epithelial surfaces and in circulating phagocytes. These peptides are either constitutively expressed or readily inducible upon infection, inflammation or injury [5]. AMPs are quite diverse by length, sequence and secondary structure. Common features of these peptides are a small size (12-50 amino acid residues), a net positive charge and an amphipathic character [1]. These features enable AMPs to interact with negatively-charged microbial membranes and affect the membrane integrity, leading to microbial killing [1,5]. Besides exerting direct antimicrobial activities, several AMPs have shown the ability to interact with host cells and influence cellular processes relevant to inflammation and immunity such as chemotaxis and cytokine and chemokine release, and to promote angiogenesis and wound healing [2,5].

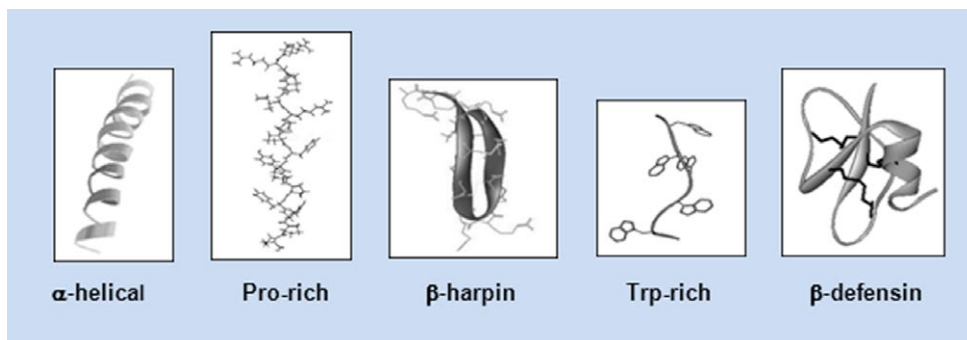


Fig. 1. Molecular diversity of mammalian AMPs.

3.2 AMPs as templates for the development of novel antiinfective drugs

Various AMPs have received attention as candidates for the development of novel anti-infective drugs [2,6] to be used in human and veterinary medicine. They show several attractive properties in this regard, including i) a potent antimicrobial

activity against a wide range of Gram-negative and -positive bacteria and fungi, also including multi-drug resistant clinical isolates [6,7]; ii) in vitro antimicrobial efficacy in the submicromolar range [6]; iii) low propensity to select resistant mutants [5,6]; iv) ability to neutralize proinflammatory microbial components such as the lipopolysaccharide and lipoteichoic acid [6,8]; v) ability to modulate host cell functions [2,5,7]. Our increasing awareness of the multiple activities of AMPs has encouraged studies aimed to evaluate the impact of these molecules on pathophysiological processes taking place at specific clinical settings, to define their anti-infective role and achieve a safe clinical application.

3.3 Ongoing research activities

The AMPs under study in our group are examined for i) antimicrobial activity against clinical isolates from epithelial infections; ii) immunomodulatory potential; iii) cell proliferation or cytotoxicity-inducing effects.

i) We are currently investigating the activities of selected AMPs against clinical isolates of *Candida albicans* from human vaginal infections and opportunistic yeast pathogens from other sources. The antifungal activity is determined as the minimum inhibitory and the minimum fungicidal concentration (MIC and MFC), according to the CLSI guidelines. The efficacy of AMPs is also examined using medically relevant fungal biofilms, implicated in increased fungal pathogenesis and resistance to drugs [9], and adhesion to biotic and abiotic surfaces [10]. Yeast viability is assessed by optical density, enumeration of colony forming units (CFU) and XTT tetrazolium salt-based assay. The effect of AMPs on yeast membrane integrity is quantified by flow cytometric and spectrofluorimetric analysis of the cellular uptake of the fluorescent dye propidium iodide (PI).

ii) The ability of AMPs to induce cytokine and chemokine gene expression in epithelial and macrophagic cells is assessed by qPCR and ELISA.

iii) Cell proliferation is assayed by a tetrazolium salt-based colorimetric assay. The cell membrane integrity is assessed by measuring the cellular uptake of PI and the extracellular release of the cytoplasmic enzyme lactate dehydrogenase. Apoptotic effects are evaluated by flow cytometry using the annexin V/PI assay.

4 CONCLUSION

A detailed knowledge of their physicochemical, biological and pharmacological properties is a basic prerequisite for translating native peptide molecules into innovative products for prevention of infectious/inflammatory diseases. The Trans2Care project offers an opportunity to share knowledge, technical expertise and laboratory facilities, that can be invaluable to increase our understanding of the biological features and therapeutic potential of AMPs. Critically important in this regard is the collaboration with Trans2Care partners involved in clinical activities, to set up experimental models of infection and inflammation and evaluate the role and efficacy of AMPs in specific clinical settings.

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