At the interface of innate and adaptive immunity, antimicrobial (host defence) peptides have been shown to enhance the overall immune response, where peptide expression and activity map onto aspects of the response to infection. This includes the ability to chemoattract phagocytic and antigen-presenting cells, and regulate the host cytokine response. After two decades of basic research, the effects of peptides on B and T lymphocyte function, including B cell activation and antibody production, cytotoxic T cell and natural killer cell killing, and T helper cell function, are starting to demonstrate that some of these peptides are capable of directing a prolonged cellular and humoral response to a pathogen. As a spin-off from such fundamental studies, the commercial properties of some antimicrobial (host defence) peptides have been recognised. From these, attempts to characterise and exploit natural peptides, and design new analogues have identified a number of potentially valuable antimicrobial molecules. There is a huge world-wide demand for compounds with novel properties, capable of addressing emerging antibacterial resistance in the clinic. These will be addressed in this event.

Meeting chairs:
Dr Rob Allaker. Reader in Mucocutaneous Microbiology & London Technology Network Business Fellow Barts and The London School of Medicine & Dentistry and Dr Ron Dixon, Dept of Forensic & Biomedical Sciences, University of Lincoln

This meeting has CPD accreditation

9:00 – 9:45  Registration

9:45 – 10:00 Introduction by the Chairs: Dr Rob Allaker, Reader in Mucocutaneous Microbiology and London Technology Network Business Fellow Barts and The London School of Medicine & Dentistry and Dr Ron Dixon, Dept of Forensic & Biomedical Sciences, University of Lincoln

10:00 – 10:30 Host defence peptides and selection/control of oral biofilms
Professor Deirdre Devine, Leeds Dental Institute, Leeds
The mouth is colonised by diverse microorganisms in biofilm communities; disease occurs when an ecological imbalance causes selection of pathogenic consortia from healthy communities. Oral biofilms are exposed to a range of antimicrobial host defence peptides (HDPs) but we know little about how they influence the bacterial composition of plaque. We have shown that LL37, HBD1 and histatin penetrate in vivo and in vitro oral biofilms. Low concentrations of HDPs, commensurate with health, altered the balance of species within plaque biofilms and thereby may influence host-microbe homeostasis and the emergence of consortia associated with disease or maintenance of health.

10:30 – 11:00 Innate defence at mucosal surfaces: an update
Dr Mona Bajaj-Elliott, Infectious Disease and Microbiology, Institute of Child Health, London
Although the direct bactericidal function of antimicrobial peptides is well established I will highlight some novel interactions between microbes and beta-defensins, interactions that may also contribute to host-microbe homeostasis at the mucosal surface.

11:00- 11:05 Speakers photo
11:05 – 11:30 Mid-morning break

11:30 – 12:00 Antimicrobial host defense and mechanisms of defensin deficincy in chronic intestinal inflammation
Professor Eduard F. Stange, Robert Bosch Krankenhaus Stuttgart, Germany
Defensins are endogenous antibiotics with broad microbicidal activity. A disturbed antimicrobial defense, as provided by Paneth and other epithelial defensins, seems to be a critical factor in the pathogenesis of inflammatory bowel diseases. Conspicuously, there is a relative lack of Paneth-cell alpha-defensins in ileal Crohn's disease (CD), both in the absence of a pattern recognition receptor nucleotide-binding oligomerization domain 2 (NOD2) frameshift mutation and, even more pronounced, in its presence. This deficit is independent of concurrent active inflammation and cannot be seen in active small intestinal ulcerative colitis (UC; pouchitis) as well as NOD2 wild-type graft vs. host ileitis. After intestinal transplantation, in case of NOD2 mutation, defensins are decreased before the onset of inflammation. In the majority of patients, the Paneth-cell deficiency is mediated by Wnt-TCF4, which suggests a disturbed Paneth-cell differentiation. In contrast, colonic CD is characterized by an impaired induction of mucosal beta-defensins, partly because of a low copy number of the beta-defensin gene cluster. In both ileal and colonic CD,
the lack in defensins results in a broadly diminished antibacterial killing by the mucosa, which can also be found independent of inflammation. In summary, the main disease locations can be linked to distinct mechanisms of epithelial barrier dysfunction.

12:00 – 12:10 Optimising antimicrobial peptides as broad spectrum countermeasures
Dr Marc Fox, Dstl, Porton Down., UK.
A range of antimicrobial peptides (AMPs) have been evaluated for their efficacy against Gram negative and Gram positive bacterial species. In order to optimise the antimicrobial effect, novel synthetic peptides have been created from fragments of the previously tested peptides. Peptides were analysed and combined in silico to determine which hybrids would form the best amphipathic α-helices. Four hybrids were synthesised and tested alongside their parents against the bacterial agents. The hybrid peptides were found to show a greater antimicrobial effect than their parents, demonstrated by time kill and minimal inhibition concentration assays.

12:10 – 12:20 A novel antimicrobial peptide from the skin secretion of the waxy monkey frog, Phyllomedusa sauvagei
Ruowen Zhang, Queen's University, Northern Ireland, UK

12:20 - 12:30 Differential activity of antimicrobial peptides against vaccinia virus outer and inner membranes
Dr. David Ulaeto, Dstl, Porton Down, UK.
In this study magainin-II amide and cathelicidin derived LL37 are shown to have differential activity against the single- and double-membraned forms of vaccinia virus. The peptides synergize with the membrane stripping action of a non-ionic detergent and density gradient analysis of the treated virions indicates the entire outer membrane of individual virions is removed in a single event.

12:30 – 12:40 Secondary Necrosis of Apoptotic Neutrophils Induced by LL-37 Has Anti-inflammatory Effects on Macrophages
Hsin-Ni Li, Queen's Medical Research Institute, Edinburgh, Scotland.
Cathelicidins are cationic host defence peptides (CHDP) with microbicidal, anti-endotoxic, and multiple immunomodulatory properties. Neutrophils (PMN) are the main reservoir of cathelicidins and play key roles in first line defence against infection. We demonstrate that the human cathelicidin LL-37 rapidly induced secondary necrosis of apoptotic human PMNs and identify the essential C-terminal region of LL-37 required this activity. LL-37-induced secondary necrosis did not affect PMN ingestion by human monocyte-derived macrophages and was not proinflammatory. However, LL-37 did promote PMN granule contents release which may mediate host damage under chronic or dysregulated conditions.

12:40–13:40 Lunch and Poster Viewing

13:40 – 13:50 Characterization of antimicrobial peptides from the skin secretions of the Chinese Black-spotted Pond frog, Pelophylax nigromaculatus
Min Wang, Molecular Queen's University, Northern Ireland, UK
The main peptidic components of the defensive skin secretions of Rana frogs are broad-spectrum antimicrobial peptides. Two novel cationic antimicrobial peptides were isolated from skin secretions of the Pelophylax nigromaculatus. Antimicrobial assays indicated a potent activity against Gram-positive and Gram-negative bacteria and a moderate haemolytic activity. These data further substantiate the roles played by net positive charge and associated amphipathic conformation in determining the potency of antimicrobial peptide—factors important for the design of effective therapeutics by medicinal chemists.

13:50 – 14:00 Identification and molecular cloning of a novel antimicrobial peptide from the venom of the scorpion, Androctonus Austraus Hector
Zhihao Zhou, Queen's University Belfast, Northern Ireland, UK
Here we report the isolation, primary structure and antimicrobial activity of a novel 18 amino acid antimicrobial peptide from the venom of the scorpion, Androctonus australis Hector. In addition, the structure of its biosynthetic precursor was deduced from a cDNA cloned from a library made from lyophilized venom. By means of reverse-phase HPLC fractionation, a single active peptide was isolated that was active against the Gram positive bacterium, Staphylococcus aureus, the Gram negative bacterium, Escherichia coli and the pathogenic yeast, Candida albicans. Minimal inhibitory concentrations (MICs) for each of these microorganisms was determined using a synthetic replicate of the 18 amino acid residue, C-terminally amidated peptide. The structure of the peptide implied that it could interact with negatively-charged groups on the microbial cell surface, where it could adopt an α-helical conformation and accumulate on the membrane. This was the most probable mechanism of action through formation of transient pores with resulting membrane perturbation effecting cell lysis.
Identification and functional characterization of novel antimicrobial peptides from the venom of the scorpion *Androctonus amoreuxi*

Ammar Almaaytah, Queen’s University, Northern Ireland, UK

Ileal conduit urinary diversion and cationic AMPs

Dr Judith Hall, Institute for Cell & Molecular Biosciences, Newcastle University

The construction of an ileal conduit for urinary diversion after bladder removal brings together bacterial tolerant gut epithelium and urothelium that seeks to maintain a sterile environment creating an interesting functional paradox. Following reconstructive surgery it is possible that an individual's ability to adapt and balance innate epithelial defence mechanisms may determine whether or not they suffer symptomatic recurrent infection. A previous study has demonstrated the presence of HD5 in urine from patients immediately following urinary diversion we therefore sought extend this work to investigate whether alterations in tissue CAMP profiles and urine cationic anti-microbial activities are associated with the susceptibility of patients with urinary diversion to urinary infection. This talk will discuss data from this study.

Using biology to engineer better Lantibiotics

Dr Paul Cotter, Moorepark Food Research Centre, Cork

Lantibiotics are antimicrobial peptides that frequently function at nanomolar concentrations by targeting the essential cell wall precursor, lipid II. Being gene-encoded, the lantibiotics can serve as biological templates for novel bioengineered antimicrobials. In recent years there have been a number of significant developments which have highlighted the benefits of taking such an approach and these will be the focus of this presentation.

Causes and consequences of beta-defensin gene copy number variation

Dr Ed Hollox, Lecturer in Genetics, University of Leicester, UK

This talk will introduce copy number variation as an important aspect of human genetic variation, and discuss medical and evolutionary implications of this variation.

The human cathelicidin LL-37: a modulator of inflammation and immunity

Dr Donald J. Davidson, MRC / University of Edinburgh Centre for Inflammation Research, Queen's Medical Research Institute, Scotland, UK

Cationic host defence peptides (CHDP) are conserved components of innate host defences. In addition to direct microbicidal potential, many CHDP have multiple immunomodulatory properties, raising questions about their primary physiological roles and potential as novel microbicidal therapeutics with immunomodulatory activities. The human cathelicidin LL-37 is an important CHDP produced primarily by neutrophils and epithelial cells, with the capacity to modulate inflammation and immunity by altering the differentiation, function and death of key innate immune effector cells. This talk will discuss the roles of LL-37 in innate host defence and disease pathogenesis, and the implications for the development of novel therapeutics.

Chairman’s summing up and close

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About the chairs

Dr Ron Dixon, University of Lincoln, UK
Ron Dixon is Head of the School of Natural and Applied Sciences at the University of Lincoln. He obtained a PhD in Microbiology from the University of Bristol and was recently elected FIBiol. Before Lincoln, he was at the University of Bradford for 10 years. He heads the Centre for Antimicrobial Research and Education (CARE) at Lincoln and the research group works on aspects of traditional antimicrobial interaction with cell membranes, isolation and characterisation of bacteriocins and the investigation of bacteriophage and larval therapy.

Dr Rob Allaker, Queen Mary, University of London, UK
Dr Rob Allaker is Reader in Mucocutaneous Microbiology at Queen Mary, University of London. He also acts as a London Technology Network Business Fellow to help facilitate the transfer of technology-enabled innovations from Academia to Industry. He obtained a PhD in Skin & Oral Microbiology from the University of the West of England, Bristol. He then joined the Royal Veterinary College as a Wellcome Trust Post Doctoral Research Fellow before moving to Queen Mary. Host - microbial interactions have provided the focus for Rob’s research career. In relation to antimicrobial peptides, studies have included investigations of adrenomedullin and calcitonin gene related peptide with respect to expression, post-secretory processing, mechanisms of antimicrobial action and microbial resistance.

About The Speakers

Dr Donald J. Davidson is a medical graduate of the Edinburgh University, who pursued a research career after completing a PhD at the MRC Human Genetics Unit, studying the pathogenesis of cystic fibrosis lung disease. He undertook post-doctoral training in innate immunity research at the University of British Columbia, Vancouver; studying antimicrobial peptides and pattern recognition receptor signalling. Donald is now a Wellcome Trust Fellow and Senior Lecturer at the MRC / University of Edinburgh Centre for Inflammation Research, with a research group focused on the roles of cationic host defence peptides in cell death and inflammation, and pulmonary innate defence mechanisms.

Following a PhD at the University of Reading, Dr Marc Fox joined the Defence Science and Technology Laboratory where he is working on medical countermeasures to bacterial pathogens, specifically with antimicrobial peptides. Marc is investigating the optimisation of peptides that have been previously reported with the ultimate aim of developing a therapeutic treatment for infection. Currently, research is focussed on hybrid peptides based on the sequences of peptides with demonstrated antimicrobial activity, lowering haemolytic activity and increasing proteolytic stability.

After gaining at Ph.D. at the Institute for Cancer Research at Birmingham University Dr David Ulaeto did his post-doctoral fellowship in the Department of Pathology at Washington University in St.Louis. From there he moved to Oregon State University as an assistant professor studying morphogenesis in poxviruses. He moved to Dstl in 1995 to run diagnostics and medical countermeasures programmes for orthopoxvirus infections. He is an advisor to the World Health Organisation and has also advised the UN Food and Agriculture Organisation.

Dr Judith Hall graduated from Warwick University with BSc Hons Biochemistry & worked two years for the NHS as a Research Scientific Officer before embarking on a PhD in Physiology at Newcastle University. After graduation she worked as a post-doctoral Research Associate & was appointed to a lectureship in the Dept of Biological & Nutritional Sciences, Faculty of Agriculture, Newcastle University, focusing on the manipulation of the GI tract of simple-stomached animals for which she was awarded a British Nutrition Foundation Young Scientist Award. Following the controversies in the late 1990s concerning genetically modified foods Dr Judith Hall refocused research direction, concentrating on the epithelial synthesis of host cationic antimicrobial peptides (CAMPs). Initially the research was focused on the avian and encompassed the roles of these peptides in relation to food safety. In 2002 she relocated to ICaMB in the Faculty of Medical Sciences and in collaboration with Prof RS Pickard, Institute for Cellular Medicine has focussed on investigating the potential roles of such peptides in urinary tract disease.

Professor Eduard F. Stange - 1992 Chief of Gastroenterology University of Lübeck, 2000 Chief of Gastroenterology RBK Stuttgart Since 1997 work on intestinal and gastric defensins with respect to inflammatory bowel diseases and helicobacter, together with Jan Wehkamp and Klaus Fellermann

Following his first degree at the University of Cambridge and a PhD at University College London, Dr Hollox spent six years as postdoctoral researcher in Nottingham developing a research interest in copy number variation in humans. He is now Lecturer in Genetics at the University of Leicester, running a small research group funded by a MRC New Investigator Award and the Wellcome Trust.
Dr Paul Cotter is a Principal Research Officer at the Teagasc Moorepark Food Research Centre in Ireland and previously served as a Principal Investigator and lecturer at University College Cork. His research focuses on the metagenomic analysis of the human gut microbiome and the investigation of ribosomally-synthesized peptide antibiotics and toxins. Dr Cotter is the author of 38 peer-reviewed publications and has presented his data at multiple international conferences. He was appointed as Faculty member of F1000 Biology in 2006 and was awarded the title of ESCMID and ESCMID/FEMS Research Fellow in 2007 and the SFAM Pierce Prize in 2008.

Dr Mona Bajaj-Elliott was a research fellow in Tom MacDonald lab at Barts and The London before taking up her lecturership with Prof Mike Farthing. Since 2002 she has been at ICH where her main interest is in studying mucosal infections and food allergy.

Professor Deirdre Devine (Professor of Oral Microbiology, Director of Research, Leeds Dental Institute) joined the University of Leeds in 1995. Having graduated from the Universities of Reading (BSc) and London (PhD), she has >20 years experience researching antimicrobial host defence peptides, host responses to commensal and probiotic bacteria, and oral biofilms. Professor Devine is a member of the Medical Research Council College of Experts, and has been elected to national committees of the Society for Applied Microbiology and Society for General Microbiology. She has published numerous research papers and reviews, and edited two books on biofilms and host defence peptides.

POSTER PRESENTATIONS

CHARACTERIZATION OF ANTIMICROBIAL PEPTIDES FROM THE SKIN SECRETIONS OF THE CHINESE BLACK-SPOTTED POND FROG, PELOPHYLAX NIGROMACULATUS
Min Wang, Mei Zhou, Tianbao Chen, Brian Walker and Chris Shaw
Molecular Therapeutics Research, School of Pharmacy, Queen’s University, Belfast BT9 7BL

The main peptidic components of the defensive skin secretions of Rana frogs are broad-spectrum antimicrobial peptides that are representative of several distinct structurally-related families. Two novel cationic antimicrobial peptides were isolated from skin secretions of the Chinese Black-spotted Pond frog, Pelophylax nigromaculatus, and were named Brevinin-2PNa, and Brevinin-2PNb, due to their high sequence homology with members of the Brevinin-2 family. Antimicrobial assays indicated a potent activity against Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacteria and a moderate haemolytic activity. The net positive charges displayed by each peptide at physiological pH (+5 for Brevinin-2PNb and +3 for Brevinin-2PNa) reflected their relative bacterial growth inhibitory activities against both E.coli and S.aureus. These data further substantiate the roles played by net positive charge and associated amphipathic conformation in determining the potency of antimicrobial peptide – factors important for the design of effective therapeutics by medicinal chemists.

IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF NOVEL ANTIMICROBIAL PEPTIDES FROM THE VENOM OF THE SCORPION ANDROCOTONUS AMOREUXI
A.Almaaytah, T.Chen, B.Walker, C.Shaw
School of pharmacy, Queen’s University, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, UK

Scorpion venoms are rich sources of biologically-active peptides. Most of the peptides identified from scorpion venoms are disulfide-bridged and function as ion channel modulators. Until recently, little interest has been shown in the non disulfide-bridged peptides yet more than 10 antimicrobial peptides lacking this modification have been identified from scorpion venoms. Here we report the identification, structural characterization and precursor cDNA cloning of four novel antimicrobial peptides from the venom of the scorpion, Androctonus amoreuxi. These peptides were found to be structural homologs of a known antimicrobial peptide named BmKb1 originally identified in the venom of the scorpion, Buthus martensi Karsch. Synthetic replicates of two of the four sequences identified displayed inhibitory activity against Staphylococcus aureus, Escherichia coli and Candida albicans, and they also displayed haemolytic activity against horse erythrocytes. The two synthetic replicates of the natural peptides differed by only two amino acid residues but they exhibited significant differences in activity by a factor of two against S.aureus. Secondary structure prediction analysis indicated that this was probably due to conformational changes in the secondary structure of these peptides. At the high concentrations which were required to inhibit both E.coli and C.albicans, no significant differences in potency was observed between the two peptides, however, their haemolytic activity was dramatically increased at higher concentrations. These data indicate the importance of identifying the major structure/activity determinants within these peptides as a prerequisite for optimisation as potential therapeutics.
CATHELICIDIN-INDUCED CELL DEATH AS AN INNATE DEFENCE MECHANISM: LL-37 PROMOTES APOPTOSIS OF PSEUDOMONAS-INFECTED AIRWAY EPITHELIUM.


1MRC / University of Edinburgh Centre for Inflammation Research, Queen’s Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, UK

The cationic host defence peptide LL-37 is a multifunctional peptide resulting from proteolytic cleavage of the human cathelicidin, hCAP-18. Produced by neutrophils and epithelial cells and upregulated in the lung during inflammation, LL-37 is known to exert a multitude of immunomodulatory functions in addition to being directly microbicidal. We have previously shown that high concentrations of LL-37 induce apoptosis of airway epithelial cells in vitro and in vivo. Now, using an in vitro model of human airway epithelium (bronchial epithelial cell line 16HBE14o-, or primary airway epithelial cells) infected with Pseudomonas aeruginosa, we demonstrate induction of apoptosis at lower, physiological concentrations of LL-37, only in the presence of epithelial cell infection. This induction of apoptosis is characterised by mitochondrial depolarisation and release of cytochrome c, with activation of caspase -9 and -3 occurring only in the presence of both bacteria and peptide. The synergistic effect is primarily caspase-dependent, occurs with PA01 or a clinical lung isolate of P. aeruginosa, and requires epithelial cell invasion by live bacteria. Recent results indicate that that a soluble factor from PA01-infected cell supernatant can rescue this phenotype when using a non-invasive mutant. We propose that LL-37 may promote the removal of compromised, infected epithelial cells as a component of innate defence, to protect against the initial stages of infection with this opportunistic pathogen.

SECONDARY NECROSIS OF APOPTOTIC NEUTROPHILS INDUCED BY LL-37 HAS ANTI-INFLAMMATORY EFFECTS ON MACROPHAGES

Hsin-Ni Li 1, Peter G. Barlow 1, Johan Bylund 2, Annie Mackellar 1, Åse Björstad 2, James Conlon 1, Pieter S. Hiemstra 3, Chris Haslett 1, Mohini Gray 1, A. John Simpson 1, Adriano G. Rossi 1 and Donald J. Davidson 1

1 MRC / University of Edinburgh Centre for Inflammation Research, Queen’s Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland. 2 Rheumatology Dept., University of Gothenburg, Sweden. 3 Department of Pulmonology, Leiden University Medical Center, The Netherlands.

Cathelicidins are cationic host defence peptides (CHDP) with essential roles in the innate defence system. These peptides have antimicrobial potential and the capacity to modulate innate immunity and inflammatory processes. Despite the importance of these peptides in host defence, they are also recently found to be more associated with the pathogenesis of certain chronic diseases. Neutrophils (PMN) are the main reservoir of cathelicidins and play key roles in first line defence against infection. The appropriate regulation of PMN function, death, and clearance is critical to innate immunity, and the efferocytosis of apoptotic PMN, in contrast to necrotic cells, is proposed to promote the resolution of inflammation. We demonstrate that the human cathelicidin LL-37 rapidly induced secondary necrosis of apoptotic human PMNs and identify the essential C-terminal region of LL-37 required this activity. LL-37-induced secondary necrosis did not affect PMN ingestion by human monocyte-derived macrophages and, in contrast to expectation, was not proinflammatory. The anti-inflammatory effects of apoptotic PMN on activated macrophages were retained and -3 occurring only in the presence of both bacteria and peptide. The synergistic effect is primarily caspase-dependent, occurs with PA01 or a clinical lung isolate of P. aeruginosa, and requires epithelial cell invasion by live bacteria. Recent results indicate that that a soluble factor from PA01-infected cell supernatant can rescue this phenotype when using a non-invasive mutant. We propose that LL-37 may promote the removal of compromised, infected epithelial cells as a component of innate defence, to protect against the initial stages of infection with this opportunistic pathogen.

IDENTIFICATION AND MOLECULAR CLONING OF A NOVEL ANTIMICROBIAL PEPTIDE FROM THE VENOM OF THE SCORPION, ANDROCTONUS AUSTRALIS HECTOR

Zhihao Zhou, Lei Wang, Mei Zhou, Tianbao Chen, Brian Walker, Chris Shaw, Shaw Gorman, Stef McGrath

School of Pharmacy, Queen’s University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast, Northern Ireland, United Kingdom BT9 7BL

Scorpion venom is known to contain many biologically-active peptides, most notably ion-channel blockers, but recent evidence has alluded to the presence of broad-spectrum antimicrobial peptides. Here we report the isolation, primary structure and antimicrobial activity of a novel 18 amino acid antimicrobial peptide from the venom of the scorpion, Androctonus australis Hector. In addition, the structure of its biosynthetic precursor was deduced from a cDNA cloned from a library made from lyophilized venom. By means of reverse-phase HPLC fractionation, a single active peptide was isolated that was active against the Gram positive bacterium, Staphylococcus aureus, the Gram negative bacterium, Escherichia coli and the pathogenic yeast, Candida albicans. Minimal inhibitory concentrations (MICs) for each of these microorganisms was determined using a synthetic replicate of the 18 amino acid residue, C-terminally amidated peptide. The structure of the peptide implied that it could interact with negatively-charged groups.
on the microbial cell surface, where it could adopt an α-helical conformation and accumulate on the membrane. This was the most probable mechanism of action through formation of transient pores with resulting membrane perturbation effecting cell lysis.

OPTIMISING ANTIMICROBIAL PEPTIDES AS BROAD SPECTRUM COUNTERMEASURES

Dstl, Porton Down, Salisbury, Wiltshire, SP4 0JQ, UK.

A range of antimicrobial peptides (AMPs), originally isolated as part of the host’s innate immune response, have been evaluated for their efficacy against Gram negative and Gram positive bacterial species. In order to optimise the antimicrobial effect of these AMPs, novel synthetic peptides have been created from fragments of the previously tested peptides. Parent peptides were selected based on their predicted α-helical secondary structure and fragments of these parents were analysed and combined in silico to determine which hybrids would form the best amphipathic α-helices. Four hybrids were synthesised and tested alongside their parents against the bacterial agents. The hybrid peptides were found to show a greater antimicrobial effect then their parents, demonstrated by time kill and minimal inhibition concentration assays. Furthermore, some hybrids showed a greater range of antimicrobial treatment and proteolytic stability when compared to the parent peptides. Overall, this work highlights the potential for rational design and synthesis of improved AMPs that may be used therapeutically.

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A NOVEL ANTIMICROBIAL PEPTIDE FROM THE SKIN SECRETION OF THE WAXY MONKEY FROG, PHYLLOMEDUSA SAUVAGEI

Ruowen Zhang, Chris Shaw, Tianbao chen, Mei Zhou
School of pharmacy, Queen’s University, 97 Lisburn Road, Belfast BT9 7BL Northern Ireland, UK

The skins of anurans (frogs and toads) are an important source of biologically-active peptides, particularly broad-spectrum antimicrobials, which are regarded as having potential to resolve the crisis of multiple drug–resistant bacteria and to develop into therapeutically-useful pharmaceuticals. Here, we report the primary structure of phylloseptin from the skin secretion of the waxy monkey frog, *Phyllomedusa sauvagei*, that was determined by “shotgun” cloning of precursor cDNA from a library constructed from lyophilized skin secretion. Subsequent deduction of molecular mass was determined by LC/MS and the primary structure was confirmed by either automated Edman degradation or MS/MS fragmentation sequencing. After confirmation of structure, a synthetic replicate was synthesised and minimal inhibitory concentrations (MICs) were titred for several microorganisms and haemolytic potential assessed. The peptide was found to be broad-spectrum with activity against *Escherichia coli* (80μM/L) and *Candida albicans* (100 μM/L) but with high potency against *Staphylococcus aureus* (5μM/L) and little haemolytic activity. In order to speculate about the antimicrobial mechanism and to determine the functional region of the peptide, we synthesised an analogue which maintained the same net positive charge and basic secondary structure (long α-helical domain) but removed one amino acid which was predicted to be of significance to the transmembrane domain. The antimicrobial activity of the analogue was reduced significantly with MICs for *S.aureus*, *E.coli* and *C.albicans* of greater than 160 μM/L, 200μM/L and 200μM/L, respectively. Thus we can conclude that this novel peptide causes a high degree of disruption to prokaryotic membranes with a low effect on the membranes of erythrocytes.

DIFFERENTIAL ACTIVITY OF ANTIMICROBIAL PEPTIDES AGAINST VACCINIA VIRUS OUTER AND INNER MEMBRANES

Dr. David Ulaeto
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Antimicrobial peptides have activity against a wide variety of biological membranes and are an important component of innate immunity in vertebrate as well as invertebrate systems. The mechanisms of action of these peptides are not well understood and a number of competing but not necessarily mutually exclusive models exist. In this study we examine the virucidal activity of two peptides, the human cathelicidin derived LL37, and Xenopus alanine substituted magainin-II amide against vaccinia virus EEV and IMV particles. The two peptides are shown to have differential activity against the single- and double-membraned forms of vaccinia virus. Density gradient analysis of the treated virions indicates the outer membrane of EEV particles is efficiently removed by peptide action and suggests a mechanism that is consistent with the carpet model for peptide-mediated membrane disruption.

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