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Strategy of Human Defence Peptide LI-37 in Wound Healing

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Abstract

The single human cathelicidin peptide LL-37 has been shown to have antimicrobial and anti-biofilm activity against the gram negative and gram positive human pathogen and have wound healing effect. In diabetic patients often have ulcers on their lower limbs that are infected by multiple bio-film forming genera of bacteria, and the elimination of the bio-film has proven highly successful in resolving such wounds in patients. On developing LL-37 for healing of venous leg ulcers but also sees a strong potential to develop LL-37 for use in the treatment of diabetic foot ulcers. LL-37 modulates the inflammatory section of wound healing through unleash of proteins and peptides that govern the inflammatory sequence and this to lead to re-epithelization and closure of the wound. These review summaries known the mechanism of interaction in healing of chronic wounds and with the microbial membrane.

Keywords

anti-biofilm, diabetic foot ulcers, re-epithelization, immunomodulatory properties, wound healing.

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INTRODUCTION:

The goal of this critique is to explore and analyze in-depth the recent revealed literature and in progress clinical trials that have centered on the potential of the antimicrobial peptide(AMP) LL-37 to be used in infected wound treatment, prevents the formation of bacterial biofilm formation especially as a potential topical treatment.

ABOUT HUMAN CATHELICIDIN LL-37:

LL-37 is a gene encoding for the only membrane of human cathelicidin family. Cathelicidin connected antimicrobial peptides area unit a family of polypeptides found in lysosomes of macrophages and polymorphonuclear leucocytes such as neutrophils, monocytes, NK cells, T-cells, B-cells, epithelial cells of testis, keratinocytes, GIT and respiratory tract cathelicidins serve a crucial role in

mammalian bacterial, fungi and viral infections. LL-37 is a37 amino acids in length and is amphipatic (it has water infatuated and water avoiding components) alpha helix. It has broad activity against a number of pathogens and is also thought to play a central role in inflammatory process. The LL-37 can also activate other immune system components such as mast cells (associated with allergies), monocyets, T-lymphocytes and neutrophills. It also promotes healing, growth of blood vessels and blood capillaries. Since their discovery in the late 80`s cathelicidins have demonstrated a remarkably wide functional repertorie. They can act as `alarmins`, helping to orchestrate the immune response to infection. They can modulate inflammation, both enhancing it to aid in combating infection and limiting it to prevent damage to the host. While speaking the term cathelicidin indicates the proform, when the active Human Defence Peptide (HDP)is variously indicated by size and sequence features (Eg.Human LL-37 starts with two Leu residues and is 37 residues long),by size and provenance(Eg. its bovine orthologve BMAP-34 stands for Bovine Myeloid Antimicrobial Peptide of 34 residues), by provenance and proform (Eg. Mouse CRAMP stands for Cathelin Related AMP) or by various other features. However, it has become customary to refer to the active HDP`s as cathelicidins as well. hCAP18 is the only human cathelicidin. The HDP it releases, LL-37, has been extensively studied since its discovery in 1995 and is a paradigm for the multiple roles of cathelicidin peptides in host defence. Nibbering and a team of Dutch collaborators try to combat these biofilms associated infections by rising on a individual's amide called LL-37, which helps regulate the body`s immune response. LL37 already has some natural killing abilities and the researchers previously short end the peptide to make a powerful variant, consisting of 24 of the 37 original amino acids. In new work they optimized this peptide by making a series of random replacements to its building blocks without disturbing its overall structure.

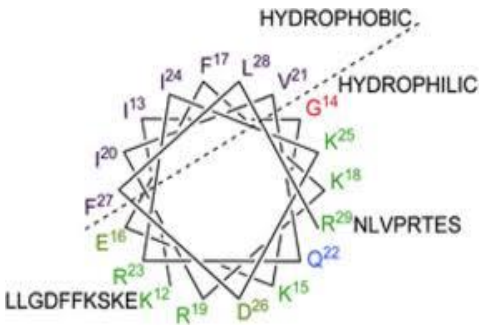
PRODUCT SPECIFICATION

Molecular weight: 4493.3daltons.
Purity : >95% by HPLC.
Appearance: Freeze dried solid.
Formula:$C_{205}H_{341}N_{61}O_{52}$
Solubility: soluble in dilute acid and physiological buffer. Soluble in DMSO as stock.
Long term Storage: Store in dry, dark and frozen.

Miscellaneous/General: LL-37 is a 37 amino acid host defence peptide derived from the C-terminus of the only human cathelicidin, hCAP18. This amide has been found in many alternative cell varieties and body fluids and is linked with antimicrobial, antitumor, antiviral and immunomodulatory properties. LL-37 has also been investigated as a wound-healing agent and is known to inhibit LPS-induced aspects of septic shock. It is known to be involved in transcriptional regulation of melanoma cells.

LL-37 Sequence: LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES

Helicle wheel diagram for LL-37 showing the region 12-29 as an amphipathic helix. N-(Residues 1-11) and C-(Residues 30-37) terminal residues are unstructured.



The treatment of difficult to heal wounds is a major clinical problem worldwide. In particular, chronic nonhealing skin ulcers are one of the major obstacles for diabetic patients. Skin and soft tissue infections are the most common manifestations for antimicrobial therapy but the emergence of bacterial resistances against clinically used antibiotics complicates the treatment and underscores the need for new therapeutic options. Antimicrobial peptides, also called host defense peptides, are effector molecules of innate immunity known to play a major role in nonspecific host defense against pathogens. They serve as first line of defense and display both direct killing of microbes and immunomodulatory

possessions. The only antimicrobial peptide of the cathelicidin family identified in humans is hCAP-18/LL-37 and is produced as a prepropeptide. The extracellular cleavage of hCAP-18 by proteinase 3 releases a cathelin-like domain and the 37-aminoacid- long C-terminus LL-37 bioactive peptide.5 In addition to its broad range of antimicrobial activities against bacteria, fungi, and viruses, human LL-37 appears particularly interesting for wound healing because it also promotes neurovascularization. It is involved in reepithelialization and granulation tissue formation for skin wound repair. Earlier studies with rat deficient for the murine cathelicidin CRAMP, an ortholog of human LL-37, also uncovered a direct role in the maintenance of a first line of defense against bacterial skin infections. In accordance with these findings, chronic wounds of elderly patients who fail to express LL-37 in the epidermis but still express LL-37 in polymorphonuclear neutrophils were colonized with S.

MECHANISM OF ACTION:

IN HEALING OF CHRONIC WOUNDS:

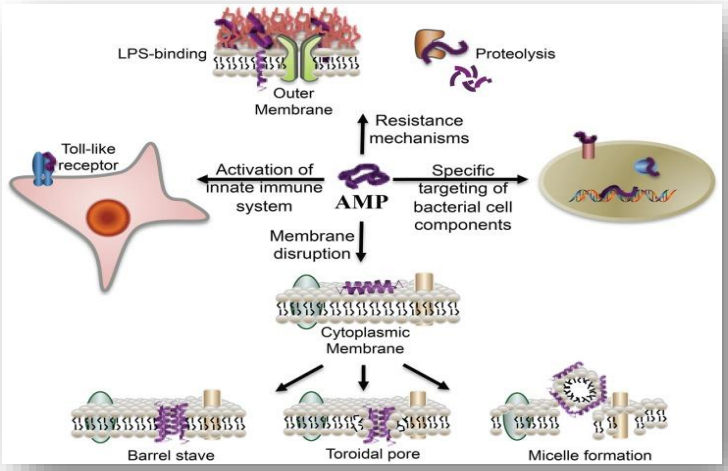
LL-37 associate degree element of someone's antimicrobial macromolecule (human cathelicidin) that is an important constituent in the natural wound healing process. In acute wounds, LL-37 is present in the wound edge, and therefore the quantity of the peptide is usually augmented at time intervals many hours once an injury. In contrast, in venous leg ulcers, the expression of LL-37 is absent in the wound edge. LL-37 impacts many biological mechanisms of connectedness in wound healing. The peptide attracts inflammatory cells, including monocytes and granulocytes. It is postulated that LL-37 modulates the inflammatory section of wound healing through unleash of proteins and peptides that govern the inflammatory sequence (cytokines). Keratinocytes (skin epithelial cells) are activated by LL-37, which in turn results in activation of growth factors in outer skin layer with a consequent cell migration. This is assumed to lead to re-epithelization and closure of the wound. Additionally, the production of vascular growth factors such as VEGF and the stimulates of epithelial cells in blood vessels also are vital for the formation of latest blood vessels that is observed after treatment with LL-37.

LL-37 MECHANISM OF INTERACTION WITH MICROBIAL MEMBRANES

LL-37 has broad-spectrum antimicrobial activity against each gram-negative and gram-positive bacterium (Durr et al., 2006) including drug-resistant strains (Saiman et al., 2001; Zaiou et al., 2003; Schittek et al., 2008). AMPs, and LL-37 specifically, have a different mode of action compared to traditional antibiotics as its size, shape, lipophilicity and cationic nature interacts with the lipophilic and anionic nature of LPS, a component of the outer membrane of most Gram-negative bacteria. Using fluorescence microscopy, the antimicrobial activity of LL-37 attacking the Gram-negative bacteria E. coli was recently compound into stages (Sochacki et al., 2011). The first stage was binding to the outer membrane and its LPS and O-antigen layers, which quickly saturate. At 8 μM, LL-37 binding saturated the outer membrane within 1 min. Translocation across the outer membrane and access to the periplasmic house related to in time (5–25 min later) with the halting of growth, which may occur because of LL-37 interference with cell wall biogenesis. After membrane association there are several proposed models of how the AMP might induce microbial killing (for an summary see Brogden, 2005). In the barrel-stave model, the LL-37 peptides would attach, aggregate, and insert into the inner membrane bilayer with the hydrophobic facet of the amide orientating with the lipoid core region and therefore the deliquescent facet forming the interior region of the pore. In the Toroidal model, the attached LL-37 peptides would combination and bend the lipoid monolayers unceasingly through the pore so the core would become lined by the pinnacle teams of each the inserted peptides and therefore the lipoid monolayer. In the carpet model (micelle formation), the LL-37 peptides would disrupt the membrane by orienting parallel to the surface of the lipoid bilayer and kind an in depth layer or carpet. In all cases, the interaction would lead to pores being fashioned at intervals the inner membrane followed by bacterial lysis.

LL-37 and its LL-31 truncated analog (lacking the half-dozen C-terminus residues) exhibited a strong killing result against Burkholderia pseudomallei (Kanthawong et al., 2012). The percentage of α-helical structure as determined by circular pleochroism was similar for LL-37 vs. LL-31. In this study, the killing of B. pseudomallei (as well as B. thailandensis) was shown to be caused by disruption of membrane as measured by freeze-fracture microscopy of microorganism cells. Both peptides exhibited stronger antimicrobial activity against B. pseudomallei in biofilm compared to ceftazidime, a cephalosporin antibiotic that's used clinically for initial melioidosis treatment. This results in line with LL-37's ability to permeabilize and/or to form pores within the cytoplasmic membrane. In recent studies, LL-37 has also been shown to have specific binding

interactions with the outer membrane conjugated protein Lpp in Enterobacteriaceae (Chang et al., 2012). Lpp is composed of trimeric α-helices (in aqueous solution) (Shu et al., 2000), and although proposed to act as a barrier against antibiotics, there is evidence that LL-37 binds and internalizes Lpp. The crystal structure of Lpp provides an explanation for assembly and insertion of the conjugated protein molecules into the outer membrane of Gram-negative bacteria. The authors recommended that the susceptibleness of bacterium to associate AMP isn't strictly related to with the presence of Lpp on bacterium, as the bactericidal activities were blocked by anti-Lpp antibodies. As specific receptors and mechanisms that LL-37 interacts with bacteria are gradually becoming understood, it is clear that further research is warranted in the area. Finally, for few AMPs, there is a mechanism by which the peptide can penetrate the bacterial cell and potentially act directly on intracellular targets, though primarily to be nucleic acids (DNA or RNA) (Takeshima et al., 2003; Madani et al., 2011). Recent studies suggest that LL-37 is also ready to be a cell-penetrating amide furthermore (Zhang et al., 2010; Hoyer et al., 2012).



Overview of the broad-spectrum of cellular interactions related to antimicrobial peptides.

ANTIMICROBIAL PEPTIDES AS DRUGS

The use of protein-biochemical methods and r-DNA technology, the structures of naturally occurring peptides serve as initiating points for the development of new drugs. Many number of derivatives of antimicrobial peptides are through the pharmaceutical method, including human phase I-III studies. The utilization of human antimicrobial peptides as medication is restricted to date by the still unknown biological perform of those molecules and therefore the high prices of the generation of decent amounts. On the basis of their functions not serve only as antibiotics, but also as modulators of inflammation.

CLINICAL SUDY RESULTS:

LL-37 has been explored in a Phase IIa clinical trial (also referred to as a Phase I/IIa trial) including thirty-four patients with blood vessel leg ulcers; a study wherever safety and tolerability were the first endpoint. The patients were randomized in four different groups; three dose strengths of LL-37 and placebo. The trial additionally enclosed secondary endpoints such a wound space size estimations and wound healing rate. The two lower doses of LL-37 demonstrated high tolerability and were associated with a pronounced healing; the healing rate being nearly six-fold beyond for the placebo cluster. Treatment for one month with the foremost efficacious dose resulted during a relative wound space reduction of seventy-five percent. In distinction, the highest dose was associated with local reactions (redness, pain and effusion). The highest dose didn't produce a wound healing result as compared with the placebo cluster, but was not worse than placebo. These findings are important, since it indicates that the Company has succeeded in identifying the efficacious dose interval.

ONGOING CLINICAL TRIALS:

The Company has initiated a Phase IIb trial (HEAL) of LL-37 in Sweden and Poland. The study is aimed toward as well as around a hundred and twenty

patients with venous leg ulcers which will be irregular into 3 teams (two doses of LL-37 and placebo) and have a treatment time of 13 weeks. The primary endpoint is the proportion of patients that reach complete wound closure, which is considered to be the pivotal endpoint for market authorization (later to be tested in a Phase III program).

OTHER POTENTIAL INDICATIONS

Diabetic Foot Ulcers:

There is scientific proof suggesting that LL-37 additionally is also efficacious in diabetic foot ulcers. Diabetic foot ulcers, even as venous leg ulcers, lack LL-37 in the wound edge (6). The Company so assumes that diabetic foot ulcers might gift a chance for indication broadening. A trial to explore the preliminary effectualness of LL-37 within the treatment of diabetic foot ulcers will in all probability be accomplished as a tiny low well-controlled Part IIa study. The company is assuming that the dose range defined in the Phase IIb trial completed in venous leg ulcers can be used to reduce the amount of arms of active drug administered, in order to minimize the number of patients required to reach statistically relevant data.

CONCLUSION:

Novel treatments for chronic wounds, pneumonia and medical implant associated infections are critically needed. These infections are often characterized by polymicrobial infections mediated by biofilm forming bacteria, including P.aereginosa. Desired characteristics of a novel therapeutics for these wounds would come with a broad spectrum, anti-biofilm treatment that is capable of withstanding the host environment, including protease and wound-exudate secreations. It is clear that the date to date for LL-37 as a possible treatment for infected wounds is encouraging. The use of multiple concurrent strategies to treat these wounds is probably going to be only, combining physical surgery, systemic antibiotics, and topical treatments such as peptides the are able to reduce biofilm.

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