

REVIEW: HUMAN CATHELICIDIN ANTIMICROBIAL PEPTIDE LL-37 AS AN ANTIBACTERIA, ANTIFUNGAL, ANTIVIRAL AND WOUND HEALING AGENT

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ABSTRACT

LL-37 is a cathelicidin-derived peptide in human. This protein contains two helical regions, an unstructured C-terminal tail and a cationic amphipathic charge. This review described the functions of human cathelicidin antimicrobial peptide LL-37. Cathelicidin peptides were shown to have some functions like to kill bacteria by disrupting the bacterial membranes of *Staphylococcus aureus*, *Micrococcus luteus* and *Salmonella gastroenteritis*, fungicidal against *Candida albicans* and *Aspergillus* sp, and work as antiviral agent against vaccinia virus by direct disruptive action on the viral envelope. Besides its antimicrobial effects, cathelicidins also play a role in wound healing through direct interaction or stimulation of the immune system, stimulating angiogenesis and re-epithelialization. From the studies, it showed that LL-37 is a potential protein for the development of new drugs in the future.

Keywords : *antibacterial, antifungal, antiviral, cathelicidin, LL-37, wound healing*

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Introduction

Cathelicidin is a gene family that is preserved in vertebrates from fishes to mammals. CAMP (Cathelicidin Antimicrobial Peptide) is the only cathelicidin gene in humans, and it encodes the 18-kDa precursor protein (1). Cathelicidin was discovered in 1995 in humans and was later referred to as hCAP18 (2). This protein called as LL-37 because it begins with two leucine. The discovery of LL-37 marked the beginning of more than a decade of research on this exciting antimicrobial peptide family (3).

LL-37 has a cationic amphipathic charge (4) it composed of two helical and

an unstructured C-terminal regions (5,6). hCAP18 becomes active after cleavage by proteinase 3 at the C-terminal 37 residue peptide. The cathelicidin precursor protein hCAP18 is expressed in immune cells (8)

Cathelicidin is expressed in the small intestine (9), respiratory tract (10), genitals (11), skin (12), ocular (13), in eccrine glands (14) and the innate immune cells (15). The expression of LL-37 in the epithelium is constitutive, although expression in keratinocytes is induced, where its precursor is deposited in granules and lamellar bodies (16).

Cathelicidin peptides were shown to have some functions as an antibacterial (17), antifungal (18) and even antiviral

agents (19). Besides its antimicrobial effects, cathelicidins also play a role in wound healing through direct interaction or stimulation of the immune system, by stimulating angiogenesis (20) and re-epithelialization (21).

This review described the human cathelicidin antimicrobial peptide LL-37. The focus of this review lies in this protein function.

Methods

Cochrane Collaboration study is used to write this review. It investigate the effects of LL-37 for treatment in a healthcare setting.

Results and Discussion

Antimicrobial peptide LL-37

The LL-37 peptide is expressed by exon 1-4 located on chromosome 3p21 and then transcribed as a single gene encoding 18-kDa which is also called as hCAP18 pre-pro-protein (22). It has 37 residue peptide

LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES (23), a hydrophobic N-terminal domain, a +6 charge at physiological pH, and a clear alpha-helical conformation (24).

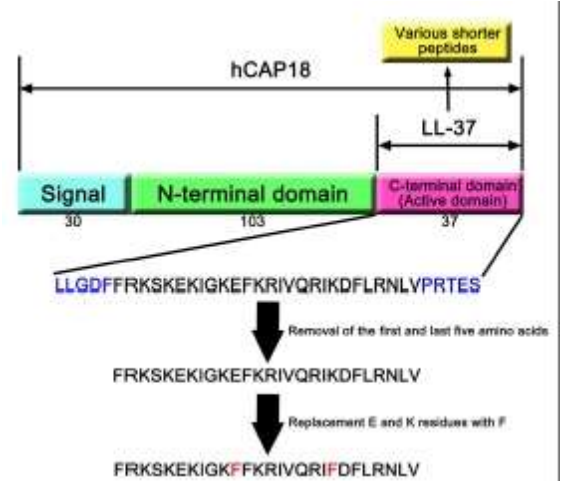


Figure 1. hCAP18 and LL-37

LL-37 is produced from the hCAP18 pro-protein from its C-terminal domain by proteolytic cleavage. hCAP18 is activated to be LL-37 by serine proteases, especially proteinase 3 from azurophil granules after exocytosis. This enzyme detruncate hCAP18 among leucyl and alanyl amino acid residues (24).

Antibacterial Activity

LL-37 has a wide spectrum activity toward both positive and negative bacteria (25). Variance of LL-37 transformants showed antibacterial activities against *Staphylococcus aureus*, *Micrococcus luteus* and *Salmonella gastroenteritis* in agar diffusion method (26). The secondary structure of this protein is a structure that has antibacterial activity (27).

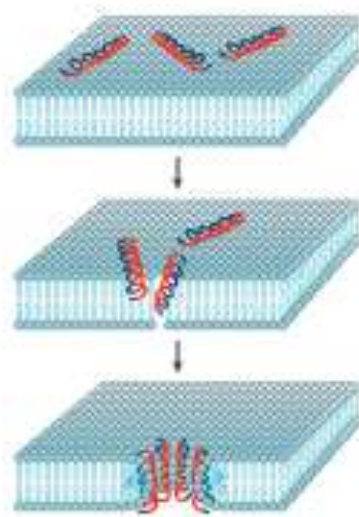


Figure 2. Simplified mechanism of the toroidal model

LL-37 mechanism of action is toroidal pore carpet-like mechanism (3). This protein reaches the outer membrane as an oligomer and / or monomer which then cover up the membrane surface. Positive amino acids on the protein will interact with the phospholipid head on the membrane (28). In addition, the transport electrons and the production of ATP are disturbed which lead to the membrane homeostasis disruption (2).

LL-37 has low specificity to differentiate between prokaryotic and eukaryotic cells.

It binds to the bacterial membrane at lower concentration because the bacterial membrane contains negatively charged lipopolysaccharides (gram -) or teichoic acid (gram +) which opposed to the zwitterionic eukaryotic membrane. However, this protein can binds to human

erythrocyte membranes which contains sialic acid. Therefore, hemolysis will occur when LL-37 concentrations in the blood is too high (3)

Antifungal Activity

Antifungal activity showed by LL-37 and its fragments (LL13-37 and LL17-32) against *Candida albicans* (29) and *Aspergillus* sp (30). In other studies, hyphae thickness becomes increasingly lean with budding becoming less normal and cell death could be detected after treatment with LL-37 (31). Its main mechanism of action is by disrupting the membrane permeabilization shown by strong membrane staining during killing process (32).

Antiviral Activity

LL-37 is found to have activity against vaccinia viral through direct disruption mechanism on the viral envelope (33). The occurrence of bonding with HDL, pre-incubation with human serum or HDL may reduce antiviral activity of this protein, whereas in combination with other innate inhibitors such as SP-D, HNPs and surfactant protein A can increase its antiviral activity (34).

Wound Healing Activity

Wound healing happens by four distinct but overlapping phases. These phases are interrelated and play a key role for a complete healing of the wounded tissue (35). LL-37 heals wounds by stimulating

angiogenesis (20) and re-epithelization (21).

Although not fully understood, several cell receptors such as FPRL-1 (36) P2X7 (37) and epidermal growth factor receptor (EGFR) have been associated with these activities (38). LL-37 binds directly to FPRL-1 (36) and inducing cellular signaling and Ca^{2+} flux (39). LL-37 also stimulates the proliferation, migration and formation of tubule-like structures by endothelial cells. This proves that LL-37 stimulates angiogenesis. Other study showed that the use of LL-37 antibody inhibited re-epithelization (40) while increased re-epithelialization and granulation tissue formation occurred after intradermal injection in mice with adenoviral vector containing LL-37 (41).

Conclusion

LL-37 peptide is expressed by exon 1-4 located on chromosome 3p21. It expressed the small intestine, respiratory tract, genitals, skin, ocular, in eccrine glands and the innate immune cells. It shown to have antibacterial function by disrupting bacterial membranes but it has low specificity to differentiate between prokaryotic or bacterial and eukaryotic cells especially human erythrocytes. Therefore, hemolysis will occur when LL-37 concentrations in the blood is too high. This protein is also kill fungi and work as antiviral agent against vaccinia virus by

direct disruptive action on the viral envelope. Besides its antimicrobial effects, cathelicidins also play a role in wound healing through direct interaction or stimulation of the immune system, stimulating angiogenesis and re-epithelialization. From the studies, it showed that LL-37 is a potential protein for the development of new drugs in the future.

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