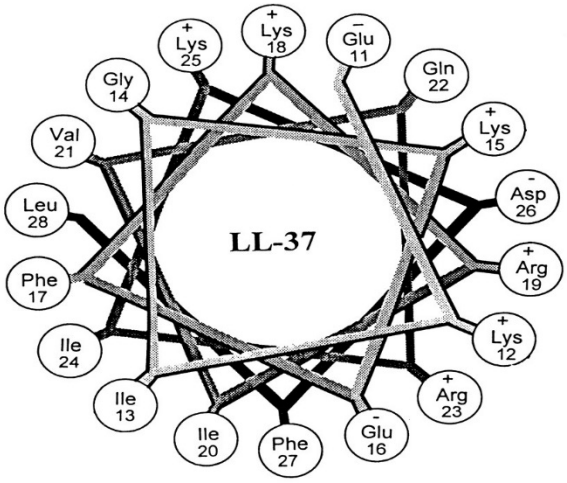
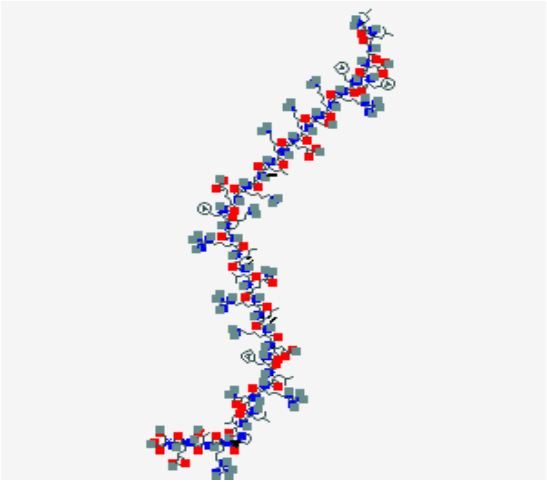
Medical Professional Monograph

LL-37





Sequence -Leu-Leu-Gly-Asp-Phe-Phe-Arg-Lys-Ser-Lys-Glu-Lys-Ile-Gly-Lys-Glu-Phe-Lys-Arg-Ile-Val-Gln-Arg-Ile-Lys-Asp-Phe-Leu-Arg-Asn-Leu-Val-Pro-Arg-Thr-Glu-Ser

Molecular Formula – C205H340N60O53

Molecular Weight – 4493.33 g/mol

Indication and Usage Summary

- Immune Defense Against Bacterial Invasion

- Antiviral and Antifungal Activity
- Increases Epithelial Stiffness and Decreases Permeability to Bacterial Invasion
- Treatment of Respiratory Syncytial Virus (RSV)
- Potential Contraceptive Therapy

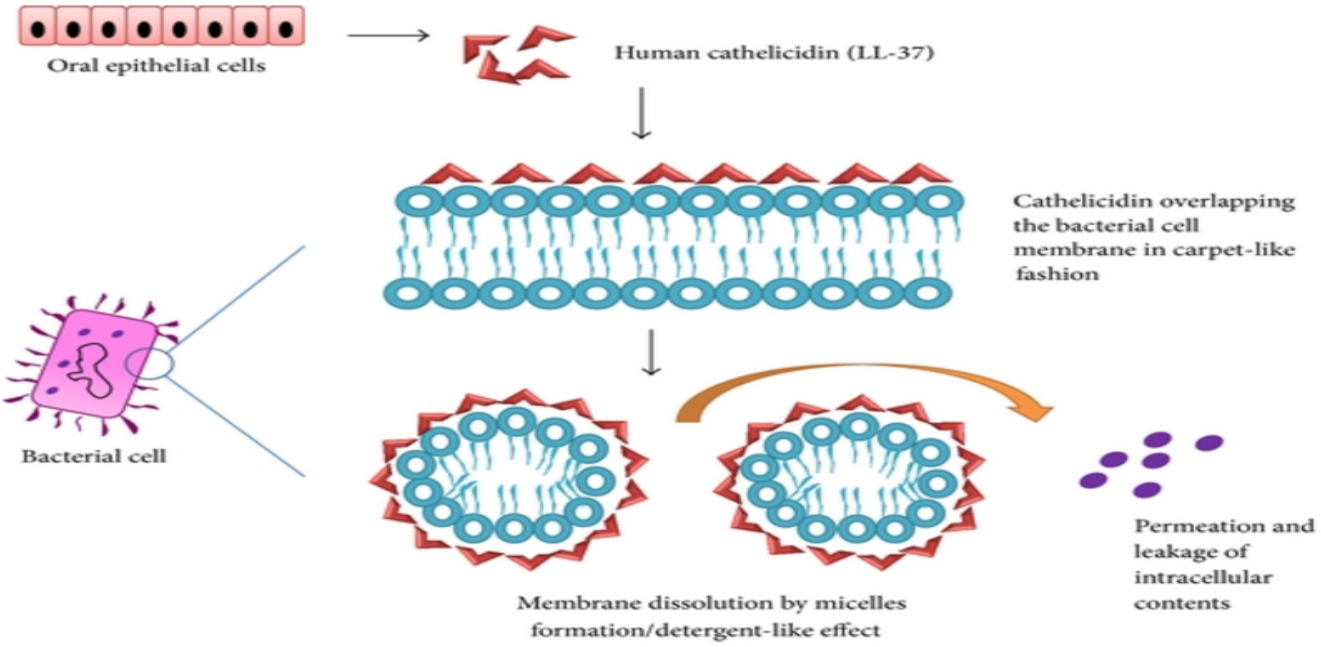
Description/Classification:

Anti-microbial peptide LL-37, belongs to the cathelicidin family of peptides, and this peptide corresponds to the sequence of the first amphipathic alpha-helical peptide isolated from human. They are small, cationic peptides found in humans and other species. Specifically, they are stored in neutrophil granules as inactive precursors and are released as mature peptides when required and cleaved by neutrophil elastase. LL-37 is expressed in various cells and tissues such as circulating neutrophils and myeloid bone marrow cells, epithelial cells of the skin, and is also expressed in the gastrointestinal tract, as well as in the epididymis and lungs. Expression was also detected in squamous epithelium of the mouth, tongue, esophagus and in the colonic and bronchial mucosal epithelium. Moreover, production of LL-37 in macrophages is stimulated by vitamin D released by sunlight through the skin.[i] LL-37 plays an important role in the first line of defense against infection and systemic invasion of pathogens at sites of inflammation and wound. It is cytotoxic to both bacterial and normal eukaryotic cells and is significantly resistant to proteolytic degradation in solution. They show a broad spectrum of antimicrobial activity against bacteria, enveloped viruses, and fungi. Along with exerting direct antimicrobial effects, they are also critical in triggering specific defense responses in the host.

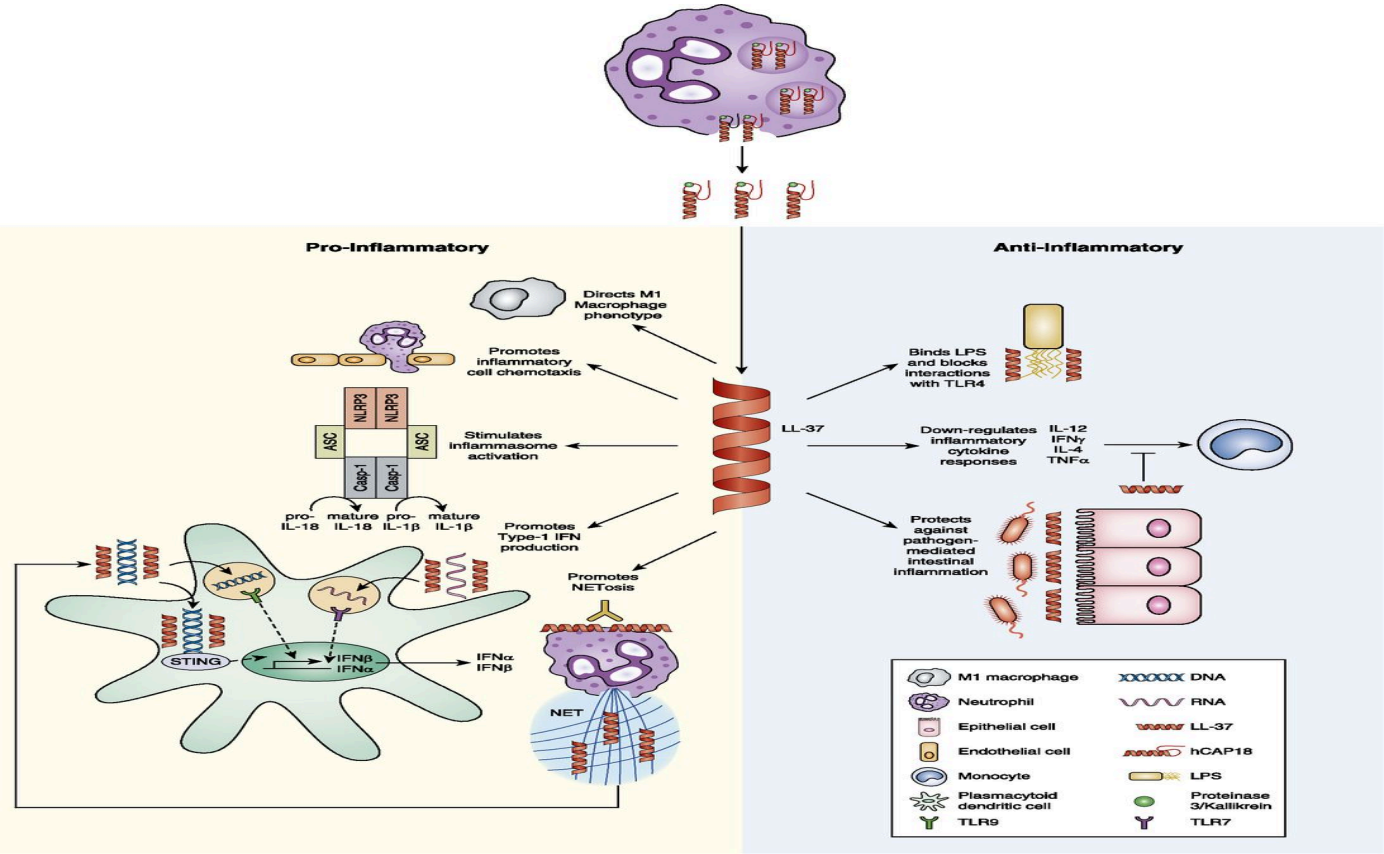
Mechanism of Action

In its direct antibacterial role, it is believed that LL-37 acts *via* disrupting the bacterial membrane.[ii] Generally membrane disrupting AMPs are assumed to act *via* one of three mechanisms of action: (i) formation of a pore with a barrel-stave conformation, where a tight bundle of amphiphilic peptides forms a hydrophilic pore across the membrane, (ii) toroidal pore formation, where a loose bundle of peptides modulates the membrane into a lipid headgroup-lined pore, and (iii) the carpet mode, where peptides remain on the surface of the membrane until a threshold is reached to facilitate a breakdown in membrane integrity. However, the mechanism of action of LL-37 does not fit into any of these categories; it remains parallel to the surface throughout its action and does not insert into the membrane[iii], and its orientation is

unaffected by peptide concentration, membrane charge, presence of ions, or temperature. Furthermore, LL-37 is not as selective as other α-helical, amphipathic AMPs; it does not exhibit a clear preference for charged membranes and while its minimum inhibitory concentration (MIC) ranges from 1 to 10 µM for a variety of Gram positive and Gram negative bacteria, it exhibits eukaryotic cytotoxicity at 13–25 µM concentrations.[iv] Hence, it was proposed that LL-37 is a nonspecific, albeit highly effective, cell killer that acts *via* the carpet mechanism.[v] However, it was shown that LL-37 disrupts the lipid bilayer without breaking the membrane into small fragments, and fluorescence measurements also suggested a pore forming mechanism.[vi] The activity against mammalian cell membranes is also ambiguous: it was proposed that LL-37 could act, at least in part, by decreasing the fluidity and hence lowering the permeability of epithelial cell membranes, making it harder for certain bacteria to attack. Hence, there are many uncertainties around the mechanism of LL-37 action and attention has shifted to developing more active variants of LL-37 using systematic mutation while the study of the actual mechanism of action has been largely neglected.



LL-37 also has an indirect role in the immune response to foreign antigens. It displays an ability to activate various immune cells thus possessing functional dualism in the human body. Following stimulation by proinflammatory signals hCAP18 is released into the extracellular environment and cleaved by proteinase 3 in neutrophils and kallikrein. Exposure to LL-37 results in recruitment of inflammatory cells, induction of M1 macrophages, and stimulation of inflammatory responses such as inflammasome activation and type 1 IFN production. LL-37 has strong anti-inflammatory effects such as neutralization of TLR4 activation by LPS, downmodulation of inflammatory cytokine responses, and preventing invasion and inflammatory responses to pathogenic bacteria.



Dosage

- SubQ General Dosage*
    - 50 mcg/kg SubQ daily

* Clinical studies have been performed using IV bolus injections rather than SubQ injection

Clinical Indications

Immune Defense Against Bacterial Invasion

A number of studies have been performed to determine the immune activity of LL-37 against a wide array of bacterial species and have been proven effective in the treatment of such pathogens. Many patients with indwelling devices obtain chronic infections which are refractory to antibiotic treatment and most of the time caused by staphylococcus epidermidis. A study was performed which indicated that the use of LL-37 is a potential candidate in prevention and treatment strategies of staph epidermidis.[vii] Specifically, the study indicated that LL37 significantly decreased both the attachment of bacteria to the surface while also inhibiting biofilm production. Thus, its potential indications for the prevention of bacterial infections post-medical device implantation are significant.

Due to the increasing resistance of bacteria to conventional antibiotics, the effectiveness of LL-37, along with other medications, have been tested to determine how well elimination of both extra- and intracellular pathogens can be done.[viii] In a recent study done, LL-37 was compared to conventional antibiotics such as lactoferricin B and doxycycline; and, it was found to be superior at killing both intra- and extracellular staph aureus in that it was more potent, faster, and concretely more effective quantitively. Thus, LL-37 could potentially be used to treat chronic and recurrent infections due to its effectiveness in eliminating pathogens.

Antiviral and Antifungal Activity

LL-37 not only contains antibacterial characteristics, but it can affect viruses and fungi as well. Specifically, it has been shown to be a potent contributor to the defense against Influenza A Virus. A recent study was done to determine how it affects viral membranes under electron microscopy.[ix] Unlike collectins and defensins, LL-37 did not induce viral aggregation, rather it appeared to cause disruption of viral membranes. Thus, confirming that is acts differently from surfactant protein D or defensins in the defense against viral infections such as influenza A.

LL-37 has also shown significant antifungal properties. Candida albicans is one of the most common fungal pathogens against humans, and at times can be difficult to treat due to the presence of moisture and warmth throughout the human body. There have been numerous studies that indicate that LL-37 has the ability to kill Candida albicans fungal species.[x] Altogether it was shown that LL-37 can affect fungal species in a variety of ways. It has the

ability to change the integrity and architecture of the cell wall, along with being able to modulate the expression of genes with a variety of functions for the development and replication of the fungi.

Treatment for Respiratory Syncytial Virus

The leading cause of respiratory tract infections in infants is Respiratory Syncytial Virus (RSV), and it causes significant morbidity and mortality. There have been many studies done to determine the effect LL-37 has on the virus and the results have been significant. Multiple studies suggest that LL-37 significantly impacts the ability of the virus to survive and replicate.[xi] It damages the viral envelope, disrupts viral particles, and inhibits binding to epithelial cells. Further, exogenously applied LL-37 is protective against RSV-mediated disease in pulmonary RSV infection in vivo. In a healthy human adult RSV infection model, nasal levels of LL-37 were significantly increased. These studies indicate LL-37 is crucial in first line of defense against pulmonary infection with RSV and are an inducible target for the prevention of severe infections by the virus.

Contraceptive Therapy

The demand for oral contraceptives with antimicrobial is increasing with the vast increase in global sexually transmitted disease. LL-37 is naturally found in the reproductive tract, specifically the vagina, in the female. LL-37 is produced in the vagina and has been shown to have bacteriastatic effects against HIV-1 infections. One study indicated that binding of LL-37 to the sperm head dose-dependently inhibited fertilizing ability in vitro. This same study showed how LL-37 can have a significant impact on the perforation of the sperm cell membrane while not causing toxicity to the human vaginal and ecto- and endocervical cell lines. Thus, there is potential therapeutic benefit for LL-37 as a vaginal contraceptive as well as prevention of reproductive infections.

Respiratory

LL-37 has been indicated in a variety of medical problems within the respiratory system. Chronic obstructive pulmonary disease (COPD) increases risk for decline in lung function, health status problems, and even death. One of the most common things that can lead to exacerbations of problems associated with COPD is lung infection. LL-37 plays an important role in fighting lung infections, and it has been shown that in patients with COPD, the risk of infection was higher due to lower levels of circulating LL-37.[xii] On the flip side of that coin, LL-37 can itself play a role in the progression of COPD due to its inflammatory response in mucosal production. It has been shown that LL-37 is associated with MUC5AC production involving the

TACE-EGFR-ERK1/2 pathway. Further, the increase collage matrix formed leads to an increase in fibroblast. Thus, it enhances airway mucus production which leads to the progression of COPD as more of the lung is obstructed and subsequent problems arise from that. So, although it seems that LL-37 is essential for the innate immune response to infection and exacerbations of problems with COPD, LL-37's innate role in the progression of COPD due to mucus overgrowth must be considered.

Gastrointestinal

LL-37 has also been shown to play a significant role in many diseases associated with the Gastrointestinal (GI) tract. It has been shown that in the inflamed mucosa of ulcerative colitis and Crohn's disease has significantly elevated expression of LL-37.[xiii] This increase in expression was elicited by TLR-3 stimulation and upregulation, and through the TLR9-ERK pathway, leads to fibrosis. LL-37 has an anti-fibrotic effect. And, its antimicrobial action in the mucosa was shown due to its action against LPS. It has also been shown that low levels of circulating LL-37 lead to progressively worse outcomes in patients with irritable bowel disease.

Skin/Wound Healing

Psoriasis is a common skin disease and is largely mediated by a T cell auto-response. It occurs in a largely unknown fashion, but it is known that it is an autoimmune disease regulated by T cells. It has been found, however, that a majority of patients contain T cells specific for LL-37 which is overexpressed in the skin of patients with psoriasis, and can trigger activation of innate immune cells. Further, pro-inflammatory signaling is triggered by T cells specific to LL-37. They act on a pathway to induce IFN-gamma and Th17 cytokines. It has been shown that circulating LL-37 significantly correlates with disease activity and contributes to pathology behind psoriasis.[xiv] On the flip side, it has been shown that topical vitamin D enhances the upregulation of LL-37 after acute skin injury. This is an indication that vitamin D(3) is a key factor in the regulation of LL-37 in the skin and is fundamental in the process of protecting the integrity of the skin upon injury.[xv] So, again, LL-37 shows its double sided properties of being fundamental for protection of the skin acting as an innate immune system member, but it can further act on the adaptive immune system to enhance inflammatory diseases such as psoriasis.

Cancer

LL-37 has also been shown to play in cancer pathophysiology in the gut. Specifically, colorectal and gastric adenomas play a role in the down regulation of cathelicidins. Thus, the effects of LL-37 tumor suppression have been investigated. One study showed that LL-37 activates a GPCR-p53-Bax/Bak/Bcl-2 signaling cascade that triggers AIF/EndoG-mediated apoptosis in colon

cancer cells. Exposure of these colon cancer cells to LL-37 induced phosphatidylserine externalization and DNA fragmentation which was independent of caspase activation.[xvi] Further, LL-37 was shown to inhibit gastric cancer cell proliferation through activation of bone morphogenetic protein (BMP) signaling via proteasome-dependent mechanism which subsequently phosphorylates Smad1/5 and induces p21.[xvii] Thus, there is potential for an anti-tumor therapy using LL-37 in the GI tract.

Conclusions

Understanding that LL-37 could be an excellent tool to improve the innate immune system we must be respectful of the potential where the over production of AMPs can lead to up regulating the adaptive immune system where AMP's are up regulated in Psoriasis, rheumatoid arthritis and SLE. Autoimmune disease may be the AMP's are dysregulating the innate system leading to changes in the adaptive immunity. Further investigation of pro inflammatory and anti-inflammatory effects are underway.

Frequently Asked Questions (FAQs)

What are the side effects or contraindications of LL-37?

Due to LL-37's pro-inflammatory response in the innate immune system, this peptide may propagate inflammatory signals, stimulate type 1 IFN production, and result in induction of autoimmune diseases.[xviii] However, at the current time there are no reported cases of exogenous LL-37 causing the onset of such diseases. Further, one study has been linked to elevated levels of LL-37 in elderly patients with depression due to a high correlation between inflammatory response pathway and depression.[xix]

Why would we use LL-37 as opposed to current anti-microbials?

Research has indicated that the level of resistance to current anti-microbial drugs is increasing heavily. LL-37 is a natural component of the innate immunity that directly and indirectly causes pathogen killing. It is a novel approach to treating infections as it utilizes the body's natural immune and inflammatory response to destroy foreign pathogens.

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