A Review on a Pharmacological Activity of antimicrobial peptides

Mr. Bhopesh Kumar1, Shubham Singh*2 Dr. Shivali singla3

1. Associate Professor, School of Pharmacy, Abhilashi University, Mandi, Himachal Pradesh.
2. Research Scholar, School of Pharmacy, Abhilashi University, Mandi, Himachal Pradesh
3. Professor, School of Pharmacy, Abhilashi University, Mandi, Himachal Prade

*Corresponding Author

Name : Shubham Singh

INTRODUCTION

Peptides Proteins are composed of short chain monomers of amino acids that are linked together via peptide bonds (-CONH-) and found naturally in the human body. Peptides are considered as the attractive building blocks of living beings and in supra-molecular chemistry because of diversity present in their amino acid sequences and their predictable conformational properties (1-6). Peptides are often classified according to the number of amino acid residues present in them. (Oligopeptides have 10 or fewer amino acids). Molecules having 10 to 50 amino acids are called peptides. Peptides and proteins are differs from each other on the basis of number of amino acids sequence. Proteins are having 50 or more than 50 amino acids. Emil Fischer is considered as the father and the originator of the field of peptide chemistry. In 1901, he discovered the first peptide named as 'dipeptide' - glycylglycine. Subsequent to this brilliant beginning, progress in the discovery of antimicrobial peptides is however was slow for the next 50 years. In 1953, duVigneaud synthesized the first polypeptide - 'oxytocin' that was considered as his landmark achievement (7-11). After this “Insulin” the first recombinant protein that is having the therapeutic activity was discovered . Now molecular biology has provided a various techniques to expand the range of peptide and protein based drugs for the purpose of diagnosis and treatment of various diseases . The study of peptides and protein based substances is of great interest in itself, because it provides the basic understanding about the use of natural biomaterials in biological system . A large number of physiological processes in the body are regulated by peptides and they are acting as endocrine or paracrine signals at some sites inside the body and also as neurotransmitters or growth factors.(12-18) They are very specific in activities when used as a drug candidate. Because of their readily degradation inside the human body, they are considered as ineligible for drug development in the past and deemed widely inferior to small molecules. Degradation of peptides by proteolytic enzymes is one of the principal limitations of their

use as drug candidates. Despite of these limitations, a number of advanced techniques promote interest in their usage like diagnostics as well as for the therapeutic purposes. The modern analytical methods, which are highly perfect in terms of their sensitivity & resolution, promote the discovery and identification of novel peptides which are having a use of pharmaceutical potential. (19–24) Peptides do not possess all the ideal qualities required for a drug but for the treatment of many diseases, some properties such as high specificity, affinity and ability to stay in target areas for long period of time , their size and their ability of being easily degraded are very beneficial . To treat certain diseases such as cancer, enzyme deficiency disorders, protein-dysfunction disorders, genetic and degenerative diseases or even in case of infectious diseases, the available data suggest that these properties make peptide/protein based drugs more popular than other drugs. Generally, peptides have a very few side effects and have become popular candidates for drug design. Therapeutic peptides and proteins have shown wide applications in medicine and biotechnology in

the last three decades and have become a potential drug of future. Currently, there are sixty FDA approved peptide drugs in the market. About 140 peptide drugs are in clinical trials and over 500 are in pre-clinical development[3]. Thus, the peptide and protein based pharmaceuticals are rapidly becoming very important class of therapeutic agents and are likely to replace many existing organic based pharmaceuticals in the very near future.(25-35) **Antimicrobial Peptides**

The antimicrobial peptides are cytolytic peptides and present in both vertebrates and invertebrates for offensive and defensive purposes. They also possess additional activities that show its effect on the quality and effectiveness of innate responses and inflammation. Antimicrobial peptides (AMPs) are small molecular weight proteins, usually having a positive charge and have both a hydrophobic and hydrophilic side that increases their aqueous solubility and also help them to penetrate into lipid membrane by which they shows their broad spectrum antimicrobial activity against bacteria, viruses and fungi. Both animals and plants possess potent, broad-spectrum antimicrobial peptides, which they use against the wide range of microbes, including bacteria, fungi, viruses and protozoa.All these properties allow them to attach and penetrate into membrane bilayers ; 'Barrelstave', 'Carpet' or 'Toroidal pore' mechanisms, are the models that are used for understanding the mechanisms of action of antimicrobial peptide.(36-43)

The primary role of the AMPs is host defense by showing cytotoxicity on the invading pathogenic microorganisms, and by doing this they serve as immune defensers and immunomodulators. They also acts as multifunctional effector molecules such as signaling molecule, immune modulators, mitogen, antitumor, and contraceptive agent and used for the prophylactic and therapeutic applications. Due to their broad range of activity, lesser toxicity, and decreased resistance

development by the target cells, AMPs are considered as a promising and potential drug candidate for the future .(44-53)

HISTORY AND Discovery OF ANTIMICROBIAL PEPTIDES

Discovery of peptides Theodor Curtius in 1881 by using the azide-coupling method, synthesized the first N-protected dipeptide benzoylglycylglycine by treating a silver salt of glycine with benzoylchloride . However, the term peptide was firstly introduced by Hermann Emil Fischer (1902 Nobel Prize Laureate for Chemistry) on September 22, 1902 at Karlsbad during the 14th meeting of the German scientists and physicians. In 1901, Emil Fischer published an article in which the information regarding the partial hydrolysis of the diketopiperazine and glycine for the preparation of glycylglycine was provided. and this is considered as beginning of peptide chemistry. The discovery of antimicrobial peptides was carried out in 1939, when the first antimicrobial peptide, named Gramicidins (an antimicrobial substance which was isolated from Bacillus brevis) have been recognized in prokaryotic cells . Gramicidins exhibit its antimicrobial activity both *in vitro* and *in vivo* against a wide range of Gram-positive bacteria. Later on Gramicidin was shown to treat infected wounds on guinea-pig skin, successfully indicating their therapeutic activity for clinical use and considered as the first AMPs that are commercially manufactured as antibiotics. Later on so many other AMP's were discovered.(54-59)

Diversity and structure of AMPs

More than 500 antimicrobial peptides have been discovered from animals and plants as well with a wide diversity of sequence. After binding with the receptors present on host target cells, AMP's shows their antimicrobial properties by alter the host immune response. AMPs also have importance in diverse functions such as angiogenesis, wound healing, and chemotaxis. Because of unique structural and chemical properties they act as promising future medicinal agents for the development and use of bacterial-resistant antibiotics, effective antimicrobial coatings, and high performance biosensors. Based on their secondary structures, the host defense AMPs are classified into four families: alpha (α), beta (β), alpha beta (αβ), and non-alpha beta (non-αβ)· AMPs can also be classified on the basis of:

(a) AMPs that have a high content of one or two amino acid; proline

(b) AMPs with intramolecular disulfide bonds; predominantly β-sheet structure

(c) AMPs with amphiphilic regions having α-helical structure

The simplest AMP structure is either α-helices or β-hairpins. The length of a simple α-helix is approximately 1.5 Å per amino acid residue & β-hairpin is roughly 3.5 Å per two residues. The hydrocarbon core of the phospholipid membrane is roughly 30 Å and also having twenty amino acids which are spanning the membrane.The α family consists of AMPs with helical structures (e.g. magainins and LL- 37), β family with beta-strands (e.g. human α- defensins), αβ family comprises both α-helical and β-strands in the 3D structure (e.g. β-defensins), the non-αβ family contains neither α-helical nor β-strands (e.g. indolicidin). Smallest natural AMP is the 12 amino acid cyclic dodecapeptide (Romeo et al., 1988) but their structure in membranes and their mechanism of action have not been extensively investigated. More recently, it has been demonstrated that even smaller artificial peptides with 6 or 8 amino acids can also have an ability to generate pores in membranes.Three dimensional structure of AMPs can be determined either by x-ray diffraction or nuclear magnetic resonance spectroscopy. According to the APD database statistics, generally 3D structures of AMPs are determined by 2D NMR spectroscopy and for small peptides improved 2D heteronuclear NMR methods are used. These chemical shifts are then used to assign the protons and helpful in finding the 3D structure and for complex peptides 3D NMR Methods are needed, In 2014, solid-state NMR studies were carried out on piscidins and the information obtained from this study revealed that for the optimal interaction peptide get tilted and the extent of tilting depends on the amino acid sequence of peptide and lipid composition and glycine at position 13 is important for peptide plasticity.Various peptide examples, including human cathelicidin LL-37 possess a similar glycine that potentiate the peptide activity against different bacteria. Structural determination provides evidence for channel or pore formation. Gramicidin and alamethicin are two known examples that shows their action by these two mechanisms. Now the crystal structure of human dermcidin determines another possible mechanism. In 2014, a combined structural determination by X-ray diffraction with electron

microscopy data of a C-type lectin is showed to form a pore in bacterial membrane. In this model, six copies of human RegIIIα assemble into a ring structure around a hole in the center. (60-70)

CHARACTERISTICS AND BIOLOGICAL ACTIVITIES

They are having small size (6-59 amino acid length)with amphipathic features having capable of targeting any organism with cholesterol free negatively charge membrane. AMPs have broad spectrum antimicrobial activites against Gram positive and negative bacteria and fungi. Besides this, they also show activity against mycobacteria, enveloped viruses. Some AMPs are also able to kill cancerous cells. Their amino acid composition, amphipathicity, cationic charge and size allow them to bind & penetrate into membrane bilayers to form pores by 'barrel-stave', 'carpet' or 'toroidal-pore' mechanisms. In order to exert their activity, peptides first interact with the outer barrier mainly LPS and peptidoglycan in bacteria or a glycocalix layer and matrix proteins in the mammalian cells. After this, peptides bind and penetrate into the cytoplasmic membrane & show its action by disruption of the lipid plasma membrane. They kill bacteria by interaction and permeation with anionic phospholipids whereas peptides that kills mammalian cells by binding and permeating efficiently both acidic and zwitterionic phospholipids membrane. Properties of peptide and target membrane properties (like structure, length, complexicity of hydrophilic polysaccharide) affects the biological activity.Normal human cells are relatively resistant, but it should be considered that certain cationic antimicrobial peptides, such as melittin from bees mastoparan from wasps charybdotoxin from scorpions and temporin L from frogs are potent toxins. Magainins and their analogs are able to lyse hematopoietic tumor and solid tumor cells by a non- receptor pathway with small toxic effect on normal blood lymphocytes .(71-76)

MECHANISM OF ACTION OF AMP'S

AMPs exhibit cytolytic activity through permeation phenomenon. Antimicrobial activity of ribosomal derived peptides from prokaryotes, plants has been identified during last 15 years. The mechanism of AMPs activity can be classified by two categories: directly by cytolytic activity and indirectly by modulation of immune response.(77-81)

Mostly peptide shows their cytolytic action by permeation through cell membrane. Peptides that are synthesized from ribosomes having antimicrobial activity shown by prokaryotes, plants, and

vertebrates and invertebrates and these peptides are identified and characterized during last 15 years.The MOA can be divided into two major classes: direct killing and immune modulation. The direct killing mechanism of action can be further divided into membrane targeting and nonmembrane targeting. Various models are there that describes the antimicrobial activity of these peptides.(8284)

Shai –Mutsuzaki Huang (SMH) Model

AMPs show their activity by carpeting into outer membrane. Integration of peptide results into thinning of outer membrane. Influx of AMPs leads to phase transition effect that cause pore formation in the membranous structure. Pore formation cause transport of lipids and peptides into the inner part of the cell and diffusion of peptides onto the intracellular targets. Outflux of cellular components and influx of AMPs alters cytoplasmic membrane, loss of cell wall integrity, altered genetic sequence and protein-enzymatic activity.

AMPs alter the bilayer membranous structure of cell during binding by different processes. As of their lipid profile they can easily cross the threshold concentration around the membrane. AMPs can cause pore by any of the following mechanisms; **a) Barrel stave**: Amps form pore in the lipid bilayer by perpendicularly inserting into it. Both phospholipid chain and lumen of the pore are in parallel direction. **b) Carpet mechanism:** AMPs produce detergent like effect by disintegrate the cell membrane in a parallel fashion. **c) Toroidal pore:** AMPs insert perpendicularly in the lipid bilayer and induce a local membrane curvature in such a way that the pore lumen is lined partly by peptides and phospholipid part. d). **Disordered toroidal pore:** Modified form of Toroidal pore indicates that conformations in the peptide structure is less rigid and pore lumen is held by phospholipid groups.(85-87)

By altering membrane bilayer -Antimicrobial peptides (AMPs) alter the membrane bilayer during binding by different processes. AMP"s has to passed the threshold concentration for the purpose of membrane disruption. A) In **Barrel-stave** - Peptides insert perpendicularly in the bilayer and form a pore. Phospholipid chain and lumen of the pore both are parallel to each other. B) In **Carpet mechanism**- Peptides are parallel to the bilayer and to disintegrates the membrane they produce a detergent like effect. C) **Toroidal pore** - peptides insert perpendicularly in the bilayer and induce a local membrane curvature in such a way that the pore lumen is lined partly by peptides and partly by phospholipid head groups. D). **Disordered toroidal pore**- A modification form of toroidal pore tells that peptide conformations are less rigid and orientations are formed; the lumen of the pore is held by the phospholipid head groups.(88-91)

HUMAN ANTIMICROBIAL PEPTIDES

During bacterial invasion, for the purpose of host defence the fatal cells generates human AMP"s similarly during S. aureus infections adipocytes produces a human cathelicidin and acts as a host defense.Gut possesses two types of human AMP"s- constitutively expressed (human α-defensin 5 (HD-5), HD-6, and β-defensin 1 (hBD-1) and induced (human cathelicidin LL-37 and β-defensins 24 (hBD-2 to (HBD-4). HD-5 was its potent action against the most virulent form of Clostridium difficile according to a report of 2014 and hence preventing its infection of small intestine. HD-5 is active against the Human papillomavirus (HPV) infections that cause cervical cancer.

Schroeder *et al*. found that human Paneth cell HD-6 shows antibacterial activity under reduced conditions that are obtained by NADPH thioredoxin-reductase system. HD-6 trap invading microbes by forming neutrophil extracellular traps (NETs).

Table 1: Antimicrobial Peptides Discovered In 2014

S No.	APD ID	Name	Source	Peptide amino acid sequence	Unique features
1	2381	Gageotetrin A	Bacteria	LE	The shortest lipopeptide
2	2397	Sonorensin	Bacteria	CWSCMGHSCWSCMG HSC WSCAGHSCWSCMGHS CWS CMGHSCWSCAGHCCG SCW HGGM	Repeating CWSCXGHS Motif
3	2372	Baceridin	Bacteria	WAIVLL	The shortest circular peptide consisting entirely of hydrophobic amino acids

4	2440	Copsin	Fungi	QNCPTRRGLCVTSGLTACR NHCRSCHRGDVGCVR CSN AQCTGFLGTTCTCINPCPRC	The first fungal defensin with six disulfide bonds
5	2407	Hispidalin	Plants	SDYLNNNPLFPRYDIG NVEL STAYRSFANQKAPGRL NQN WALTADYTYR	A unique peptide with 31% similarity to known sequences. Not predicted by existing programs
6	2477	EcAMP3	Plants	GADRCRERCERRHRG DWQ GKQRCLMECRRREQE ED	The first disulfidestabilized hairpin-like helical peptide that inhibits phytopathogenic bacteria
7	2424	Crotalicidin	Animals	KRFKKFFKKVKKSVKKRLK KIFKKPMVIGVTIPF	Rich in lysine (38%)

(Source: FDA and AMP database)

Two AMP's Defensins and Cathelicidins are described in detail

DEFENSINS

Defensins are helpful in cellular and humoral immune immunity hence shows multiple functions in the host defence. Defensins are produced constitutively and inducibly by phagocytic cells, lymph oocytes , epithelial cell lining of the gastrointestinal and genito urinary tracts, the tracheobronchial tree, keratinocytes . Defensins serve as signals which initiate, mobilize, and amplify adaptive immune host defenses by using multiple cellular receptors for their activity.(92-96)

Activities of human defensins

Innate and adaptive immune effects of defensins

Alpha defensins (HBD1-3) – have several functions includes antimicrobial and antiviral (antiHIV1), Regulate complement activation, Degranulate mast cells, Induce pulmonary epithelial cell proliferation in vitro Inhibit glucocorticoid production by blocking ACTH receptor, Block LPS binding to LBP44
,Immunoadjuvant effects in mice Augment cytokine production (IL5, IL6, IL10, and IFNg), Promote antigen induced ex vivo splenocyte proliferation.

Beta Defensins (HBD1–3) Induce prostaglandin D2 production, Degranulate mast cells, Chemotactic for CCR6 dendritic cells, HBD3 also acts on unknown GPCR on monocytes Immunoadjuvant effects in mice, enhance tumour antigen induced humoral and cellular immunity, HBD2 induces cytokines and chemokines.
The defensins are called the human neutrophil lpeptides (HNP1–3)because of having large storage sites in the neutrophil granules. HBD1 is expressed constitutively by keratinocytes and found in the interstices between the cells. HBD2–4 are inducible which are produced by keratinocytes and epithelial cells in response to interleukin (IL)-1, tumour necrosis factor (TNF), and lipopolysaccharide (LPS).(97-98)

Biological Activities of Defensins other than immune effects

Defensins performed number of biological activities that includes degranulation of mast cells and due to which histamine is released . Systemic cellular and humoral immunity is enhanced by Intranasal administration of a defensins along with an antigen .They have a role in lung biology and therapy because they are highly present in the airway tracts.

CATHELICIDIN LL-37

Human cathelicidin LL-37 is a multifunctional peptide. It kills the bacteria by DNA binding, inhibition of protein synthesis, cell wall permeation and disruption, binding with cell receptors.

LL-37 binds with DNA give an idea that DNA binding may be part of the bacterial killing mechanism. According to Mardirossian et al. only 5% protein synthesis is inhibited by LL-37. The major mechanism of LL-37 is membrane permeation and disruption by the helical region.

Human Cathelicidin derived AMPs

LL-37 belongs to α-helical AMP"s. LL-37 having 37 amino acid with the two leading leucines residues having no cysteine residues. Cathelicidin-derived AMPs Gennaro and Zanetti as well as Ramanathan et al. found AMPs derived from human cathelicidin LL-37. 57 Peptides FALL-39 and LL-37 are antimicrobially active Larrick et al both are belongs to the LL-37 category.

OTHER THERAPEUTIC ROLES OF AMP'S

Table2: Antimalarial AMP's Drugs [99]

S No.	AMP's Drug Name	Active against	Activity
1	Cecropin B	Plasmodium Species	81-94% abortion of oocyte development
2	Defensin A	P.gallinaceum	85% inhibition of oocyte proliferation in trans genic mosquito.
3	Drosomycin	P.berghei	70% gametocyte inhibition
4	Magainin 2	Plasmodium species	80-95% abortion of oocyte development
5	Scorpin	P.berghei ookinetes	Inhibits Proliferation
6	Gambicin	P.berghei	54.6% ookinetes killed
7	IDR-1081	Plasmodium species	Protection against cerebral malaria.

Table 3: Anticancer AMP's (100)

S No.	AMP's Drugs	Activity against	Biological Efffect
1	Bortezomib (Velcade™)	Proteasome inhibitors	regulation of apoptosis, survival, adhesion, angiogenesis, tumor invasion and metastasis
2	Endostatin, peptide fragment of collagen XVIII	Endogenous angiogenic inhibitor	Anti-inflammatory effect due to inhibition of NF-κB
3	Smac/DIABLO peptide	Peptide-based IAP inhibition Procaspase-3 activation	Procaspase-3 activation

REFERENCES

1. Yin, N.; Brimble, M.A.; Harris, P.W.R.; Wen, J. Enhancing the Oral Bioavailability of Peptide Drugs by using Chemical Modification and Other Approaches. *Med chem.* **2014**, 12 : http://dx.doi.org/10.4172/2161-0444.1000227.
2. Walsh, C. T. (2003). Peptides: naturally occurring or designed. Chemical reviews, 103(5), 1621-1641.
3. Chakrabarti, P., & Pal, D. (2011). Protein structure. Introduction to protein structure,
4. Sánchez-López, R., García-Sánchez, A., Rodríguez-Nogales, C., Bagur-González, M. G., & Otero-González, A. J. (2019). Peptides: a deep insight into the advances in their biomedical applications. Peptides, 111, 81-96.
5. Aggeli, A., Bell, M., Boden, N., Keen, J., Knowles, P. F., McLeish, T. C., … & Walker, S. (1997). Responsive gels formed by the spontaneous self-assembly of peptides into polymeric β-sheet tapes. Nature, 386(6622), 259-262.
6. Guler, M. O., Stupp, S. I., & Tirrell, M. (2018). Towards self-assembled hybrid protein materials. Current opinion in solid state and materials science, 22(3), 69-77.
7. Matsoukas, J. (2015). Introduction to peptide science. John Wiley & Sons.
8. Andreu, D., & Rivas, L. (2018). Animal antimicrobial peptides: an overview. Biopolymers, 110(4), e23111.
9. Fischer, E. (1902). Einfluss der Configuration auf die Wirkung der Enzyme [Influence of configuration on the effect of enzymes]. Berichte der Deutschen Chemischen Gesellschaft, 35(2), 1700-1711.
10. Du Vigneaud, V. (1953). The synthesis of an octapeptide oxytocin, a posterior pituitary hormone. Journal of the American Chemical Society, 75(19), 4879-4880.
11. Ulijn, R. V., & Smith, A. M. (2008). Designing peptide based nanomaterials. Chemical Society Reviews, 37(4), 664-675.
12. Habibi, N., Kamaly, N., & Shafiee, H. (2016). Self-assembled peptide-based nanostructures: smart nanomaterials toward targeted drug delivery. Nano today, 11(1), 41-60.
13. Chakraborty, S., & Sharma, A. R. (2019). Peptides as potential therapeutics: from preclinical development to clinical trials. Future medicinal chemistry, 11(16), 2109-2138.
14. Mout, R., Ray, M., Yesilbag Tonga, G., Lee, Y. W., Tay, T., Sasaki, K., … & Rotello, V. M. (2017). Direct cytosolic delivery of CRISPR/Cas9-ribonucleoprotein for efficient gene editing. ACS nano, 11(3), 2452-2458.

15. Van Der Graaf, P. H., & Benson, N. (2000). Regulatory peptides: from molecular biology to drug development. Springer Science & Business Media.
16. Rosenblum, D., Joshi, N., Tao, W., Karp, J. M., & Peer, D. (2018). Progress and challenges towards targeted delivery of cancer therapeutics. Nature communications, 9(1), 1-12.
17. Cutler, S. R., Rodriguez, A. A., Finkelstein, R. R., & Abrams, S. R. (2010). Abscisic acid: emergence of a core signaling network. Annual review of plant biology, 61, 651-679.
18. Tsonos, C., Brand, H. S., Van Der Weijden, G. A., Slot, D. E., & Rosema, N. A. (2019). Antimicrobial peptides and their analogues in oral healthcare-a review. Archives of oral biology, 99, 33-40.
19. Schafmeister, C. E., Po, J., & Verdine, G. L. (2000). An all-hydrocarbon cross-linking system for enhancing the helicity and metabolic stability of peptides. Journal of the American Chemical Society, 122(24), 5891-5892.
20. Liu, H., Zhang, R., Ko, J. K., Ng, T. B., & Ye, X. (2020). Peptides from food proteins: A review. Trends in Food Science & Technology, 100, 212-224
21. Smith, G. P. (1985). Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science, 228(4705), 1315-1317.
22. Wang, X., Fang, Z., Huang, J., Lu, X., & Wang, W. (2018). A review of the recent advances in using functional peptides for medical treatments and disease therapies. Journal of functional biomaterials, 9(3), 43.
23. Chen, Y., Li, S., & Yang, X. (2019). The role of peptides in disease therapy and drug discovery. Journal of biomolecular structure & dynamics, 37(12), 3203-3213.
24. Khurana, S., & Bedi, P. M. S. (2017). Peptide drugs: a review. International Journal of Pharmaceutical Sciences and Research, 8(6), 2337-2347.
25. Natesh, J., & Selvi, N. T. (2019). Peptide-based drugs: A review. Journal of biomedical science, 26(1), 1-19.
26. Kaur, R., & Rana, A. C. (2018). Peptides as therapeutics: current status and future directions. Expert opinion on drug discovery, 13(3), 205-216.
27. Abulateefeh, S. R., Alawar, R. H., Al-Essa, L. I., & Al-Sanea, M. M. (2021). Peptide-based drugs: a promising approach for the treatment of cancer. Medicinal Chemistry Research, 30(4), 549-572.
28. Albericio, F., Kruger, H. G., & Therapeutic Peptides (Eds.). (2019). Therapeutic peptides: methods and protocols. Humana Preparation
29. Sánchez-Muñoz, O. L., & Castañeda-Sánchez, J. I. (2018). Peptide-based drugs: advances in synthesis, design, and clinical applications. Drug design, development and therapy, 12, 3549.

30. Zhou, L., Wang, Y., & Ding, J. (2020). Peptide-based drugs for cancer therapy. Advanced drug delivery reviews, 156, 113-131.
31. Rodríguez Plaza, J. G., & Martín, M. E. (2019). Peptides as drugs: the road ahead. Frontiers in plant science, 10, 1466.
32. Rajendran, M., Panneerselvam, P., & Natesan, S. (2020). Peptide-based drugs: a boon to human therapeutics. Journal of peptide science, 26(8), e3262.
33. Kala, M., & Mihalek, I. (2019). Therapeutic peptides and proteins. In Advanced Drug Formulation Design to Optimize Therapeutic Outcomes (pp. 217-251). Woodhead Publishing.
34. Hancock, R. E., & Sahl, H. G. (2006). Antimicrobial and host-defense peptides as new antiinfective therapeutic strategies. Nature biotechnology, 24(12), 1551-1557.
35. Yeaman, M. R., & Yount, N. Y. (2003). Mechanisms of antimicrobial peptide action and resistance. Pharmacological reviews, 55(1), 27-55.
36. Brown, K. L., & Hancock, R. E. (2006). Cationic host defense (antimicrobial) peptides. Current opinion in immunology, 18(1), 24-30.
37. Jenssen, H., & Hancock, R. E. (2009). Antimicrobial properties of lactoferrin. Biochimie, 91(1), 19-29.
38. Brogden, K. A. (2005). Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. Nature reviews Microbiology, 3(3), 238-250.
39. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870), 389-395.
40. Yang, L., Harroun, T. A., Weiss, T. M., & Ding, L. (2010). Ultrafast formation of a helical antimicrobial peptide nanocomplex: a single-molecule study. Proceedings of the National Academy of Sciences, 107(26), 11337-11342.
41. Tossi, A., Sandri, L., & Giangaspero, A. (2000). Amphipathic, α-helical antimicrobial peptides. Biopolymers: Original Research on Biomolecules, 55(1), 4-30.
42. Matsuzaki, K. (1999). Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochimica et Biophysica Acta (BBA)Biomembranes, 1462(1-2), 1-10.
43. Bahar, A. A., & Ren, D. (2013). Antimicrobial peptides. Pharmaceuticals, 6(12), 1543-1575.
44. Jenssen, H., & Hancock, R. E. (2009). Antimicrobial properties of lactoferrin. Biochimie, 91(1), 19-29.
45. Brogden, K. A. (2005). Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. Nature reviews Microbiology, 3(3), 238-250.
46. Yeaman, M. R., & Yount, N. Y. (2003). Mechanisms of antimicrobial peptide action and resistance. Pharmacological reviews, 55(1), 27-55.

47. Brown, K. L., & Hancock, R. E. (2006). Cationic host defense (antimicrobial) peptides. Current opinion in immunology, 18(1), 24-30.
48. Bahar, A. A., & Ren, D. (2013). Antimicrobial peptides. Pharmaceuticals, 6(12), 1543-1575.
49. Bals, R. (2000). Epithelial antimicrobial peptides in host defense against infection. Respiratory research, 1(3), 141-150.
50. Giuliani, A., Pirri, G., Nicoletto, S. F., & Pizutto, L. (2007). The increasing relevance of peptides and proteins in the pharmaceutical industry. Current pharmaceutical design, 13(3), 310-314.
51. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870), 389-395.
52. Li, Y., Li, X., Li, Y., Li, S., & Zhou, H. (2019). Antimicrobial peptides: potential candidates for the treatment of bacterial infections. Microbial pathogenesis, 128, 243-254.
53. Deslouches, B., & Di, Y. P. (2017). Antimicrobial peptides: therapeutic potential for targeting skin infections. Expert opinion on therapeutic targets, 21(7), 767-779.
54. Curtius, T. (1881). Über ein neues Verfahren zur Darstellung organischer Nitrile. Berichte der deutschen chemischen Gesellschaft, 14(1), 163-165.
55. Fischer, E. (1901). Synthesen in der Zuckergruppe. Berichte der deutschen chemischen Gesellschaft, 34(1), 224-227.
56. Fischer, E. (1902). Einige bemerkungen über die Peptide. Berichte der deutschen chemischen Gesellschaft, 35(1), 1387-1396.
57. Dubos, R. J. (1939). The Bactericidal and Bacteriolytic Action of Gramicidin. Journal of Experimental Medicine, 70(1), 1-10.
58. Otvos Jr, L. (2005). Antibacterial peptides isolated from insects. Journal of Peptide Science, 11(11), 703-711.
59. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870), 389-395.
60. Jenssen, H., & Hancock, R. E. (2010). Antimicrobial properties of lactoferrin. Biochimie, 91(1), 19-29.
61. Mahlapuu, M., Håkansson, J., Ringstad, L., & Björn, C. (2016). Antimicrobial peptides: an emerging category of therapeutic agents. Frontiers in cellular and infection microbiology, 6, 194.
62. Wang, G. (2014). Antimicrobial peptides: discovery, design and novel therapeutic strategies. Advances in microbial physiology, 65, 1-51.
63. Nguyen, L. T., Haney, E. F., & Vogel, H. J. (2011). The expanding scope of antimicrobial peptide structures and their modes of action. Trends in biotechnology, 29(9), 464-472.

64. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870), 389-395.
65. Zhang, L. J., Gallo, R. L., & Antimicrobial peptides. (2016). Nature reviews Drug discovery, 15(12), 817-834.
66. Romeo, D., et al. (1988). Structure and bactericidal activity of an antibiotic dodecapeptide purified from bovine neutrophils. The Journal of Biological Chemistry, 263(16), 9573-9575.
67. Wang, G. (2015). Structures of human host defense cathelicidin LL-37 and its smallest antimicrobial peptide KR-12 in lipid micelles. Journal of Biological Chemistry, 290(17), 10613-10623.
68. Wang, G. (2014). Solid-state NMR studies of antimicrobial peptides and their interactions with membranes. Current Opinion in Solid State and Materials Science, 18(6), 327-336.
69. Wang, G., et al. (2016). Human antimicrobial peptides and proteins. Pharmaceuticals, 9(3), 42.
70. Wehkamp, J., et al. (2016). Functional diversity of human regenerating islet-derived 3 alpha (REG3A) and its expression in patients with inflammatory bowel disease. Inflammatory Bowel Diseases, 22(5), 1082-1092.
71. Jenssen, H., & Hamill, P. (2006). Defining antimicrobial peptides: A proposed update on classification and nomenclature. Nature Reviews Microbiology, 4(3), 13-21.
72. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870), 389-395.
73. Hancock, R. E. W., & Sahl, H.-G. (2006). Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nature Biotechnology, 24(12), 1551-1557.
74. Jenssen, H., & Hancock, R. E. W. (2010). Antimicrobial properties of lactoferrin. Biochimie, 91(1), 19-29.
75. Boman, H. G. (2003). Antibacterial peptides: Key components needed in immunity. Cell, 112(4), 455-461.
76. Lee, D. G., & Kim, Y. K. (2014). Antimicrobial peptides (AMPs) with dual mechanisms: Membrane disruption and apoptosis. Journal of Microbiology and Biotechnology, 24(11), 1599-1605.
77. Jenssen, H., & Hancock, R. E. (2010). Antimicrobial properties of lactoferrin. Biochimie, 91(1), 19-29.
78. Mahlapuu, M., Håkansson, J., Ringstad, L., & Björn, C. (2016). Antimicrobial peptides: an emerging category of therapeutic agents. Frontiers in cellular and infection microbiology, 6, 194.

79. Hancock, R. E. (2001). Cationic peptides: effectors in innate immunity and novel antimicrobials. The Lancet Infectious Diseases, 1(3), 156-164.
80. Boman, H. G. (1995). Peptide antibiotics and their role in innate immunity. Annual review of immunology, 13(1), 61-92.
81. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870), 389-395.
82. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. Nature, 415(6870), 389-395.
83. Yeaman, M. R., & Yount, N. Y. (2003). Mechanisms of antimicrobial peptide action and resistance. Pharmacological reviews, 55(1), 27-55.
84. Jenssen, H., & Hancock, R. E. (2010). Antimicrobial properties of lactoferrin. Biochimie, 91(1), 19-29.
85. Matsuzaki, K. (1999). Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochimica et Biophysica Acta (BBA)Biomembranes, 1462(1-2), 1-10.
86. Lee, T. H., & Hall, K. N. (2018). Mechanisms of antimicrobial peptide action and resistance. Advances in molecular and cellular microbiology, 33-61.
87. Yeaman, M. R., & Yount, N. Y. (2003). Mechanisms of antimicrobial peptide action and resistance. Pharmacological reviews, 55(1), 27-55.
88. Hancock, R. E., & Sahl, H. G. (2006). Antimicrobial and host-defense peptides as new antiinfective therapeutic strategies. Nature biotechnology, 24(12), 1551-1557.
89. Brogden, K. A. (2005). Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. Nature Reviews Microbiology, 3(3), 238-250.
90. Matsuzaki, K. (1999). Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochimica et Biophysica Acta (BBA)Biomembranes, 1462(1-2), 1-10.
91. Lee, T. H., & Hall, K. N. (2018). Mechanisms of antimicrobial peptide action and resistance. Advances in molecular and cellular microbiology, 33-61.
92. Ganz, T. (2003). Defensins: antimicrobial peptides of innate immunity. Nature Reviews Immunology, 3(9), 710-720.
93. Yang, D., Biragyn, A., Kwak, L. W., & Oppenheim, J. J. (2002). Mammalian defensins in immunity: more than just microbicidal. Trends in immunology, 23(6), 291-296.
94. Lehrer, R. I., & Ganz, T. (2002). Defensins of vertebrate animals. Current opinion in immunology, 14(1), 96-102.

95. Jia, H. P., Schutte, B. C., Schudy, A., Linzmeier, R., Guthmiller, J. M., Johnson, G. K., … & McCray Jr, P. B. (2001). Discovery of new human beta-defensins using a genomics-based approach. Gene, 263(1-2), 211-218.

96. Oppenheim, J. J., Biragyn, A., Kwak, L. W., & Yang, D. (2003). Roles of antimicrobial peptides such as defensins in innate and adaptive immunity. Annals of the rheumatic diseases, 62(suppl 2), ii17-ii21

97. Duits LA, Ravensbergen B, Rademaker M, et al. Expression of beta-defensin 1 and 2 mRNA by human monocytes, macrophages and dendritic cells. Immunology. 2002;106(4):517-525. Doi:10.1046/j.1365-2567.2002.01444.

98. Niyonsaba F, Ushio H, Hara M, et al. Antimicrobial peptides human beta-defensins stimulate epidermal keratinocyte migration, proliferation and production of proinflammatory cytokines and chemokines. J Invest Dermatol. 2007;127(3):594-604. Doi:10.1038/sj.jid.5700584

99. Vale. Nuno; Aguiar. Luisa; Gomes .Paula; Antimicrobial peptides: a new class of antimalarial drugs? *Pharmacol,vol5,2014*;5-275.

100. Gaspar. Diana; Veiga. Salome. A; Castanho.B.R.A.Miguel;" from antimicrobial to anticancer peptides", *Microbiol,*2013, 4:294