International Journal for Pharmaceutical Research Scholars (IJPRS)

V-5, I-2, 2016 ISSN No: 2277 - 7873

REVIEW ARTICLE

Antimicrobial Peptides: A Review on its Types, Mechanism of Action, Synthesis and Therapeutic Applications

Niranjan Ramesh Mondal*, Deepali Maruti Jagdale

Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai-400614, Maharashtra, India.

Manuscript No: IJPRS/V5/I2/00069, Received On: 26/04/2016, Accepted On: 30/04/2016

ABSTRACT

Over last two decade, conventional antimicrobial drugs are losing their effectiveness due to drug resistance which may lead to serious problems. As a result there is a need for continuous research on other treatment approaches, one of which is antimicrobial peptides. Antimicrobial peptides (AMPs) are small (6 to 100 amino acid) amphipathic molecules with wide range activity against bacteria, protozoa, yeast, fungi, viruses and tumor cells. A large numbers of antimicrobial peptides are under the pre-clinical and clinical trials for treatment of lethal infections. In this review, we have discussed the different types of AMPs, its mechanism of action, synthesis and therapeutic applications.

KEYWORDS

Antimicrobial Peptides, α-Helical, Antibiotic Resistance

INTRODUCTION

Antibiotics resistance has become a worldwide human health problem. So it is necessary that new antibiotics continue to develop. In 2002, 57.1% (as estimated 10,000 cases) of the staphylococcus bacteria found in U.S. hospitals were methicillin resistant. According to Centre of Disease Control and Prevention (CDC), about 2 million people acquire bacterial infection in U.S. hospitals each year and 90,000 die as a result and of that nearly 70% are resistant to at least one drug[1]. This problem can be overcome by artificial intelligence in the design of small peptides which are effective against a broad spectrum of highly antibiotics-resistant superbugs. This field is now moving rapidly. These peptides are termed as antimicrobial because they have usually broad spectra of activity.

***Address for Correspondence:**
Niranjan Ramesh Mondal,
Bharati Vidyapeeth's College of Pharmacy, Sector-8, C.B.D. Belapur, Navi Mumbai-400614, Maharashtra, India.
E-Mail Id: niranjan.mondal2005@gmail.com

These have ability to kill or neutralize gram-negative and gram-positive bacteria, fungi (including yeast), parasite (planaria and nematodes) and even enveloped virus like HIV and herpes simplex virus[2].

In 1922s, Alexander Fleming identified human lysozyme (130 amino acids) which is considered by some author to be the first reported peptide with antimicrobial activity[3]. In 1939s, Dubos extracted an antimicrobial agent from soil of *Bacillus* strain and this extract was used to protect mice from Pneumococcal infection. Hotchkiss and Dubos fractionated this extract and named as Gramicidin. In 1942s, the antimicrobial agent which was previously detected in wheat flour, was isolated from wheat endosperm of plant *Triticum aestivum* showing antimicrobial activity that inhibited the growth of variety of phytopathogens (*Pseudomonas solanacerarum).* The diversity of antimicrobial peptides is so great that it is difficult to classify them except on the basis of their secondary structure. Mainly antimicrobial peptides are

classified in four classes: viz., α-helical, β-sheet, loop and extended peptides[4].

Antimicrobial peptides are found on all species of life ranging from plant and insect to animals including amphibians, bird, fish, mammals and human. In human, these antimicrobial peptides are found in skin, ear, and eye epithelial cell surface including tongue, trachea, lungs, gut and bone marrow[2]. Peptides are synthesized vary rapidly within living cell but unfortunately artificial synthesis gave poor yield and impure product and it is also a very slow and long process. Recently a new technique known as solid phase synthesis has been developed which produces large number of peptides. In cellular aqueous environmental, antimicrobial peptides show different conformational changes and these characteristics of antimicrobial peptides measures the cell specificity and cell toxicity toward the host cell. Cell specificity and toxicity depends upon various factors of antimicrobial peptides like conformation change, charge, polar angle, amphiphathicity and hydrophobicity and this is elaborated later in this article.

The purpose of this review article is to

- Describe the role of antimicrobial peptides in human innate immune system.
- Discuss the broad spectrum activity against the infectious pathogenic micro-organism.
- Create awareness about mechanism of action employed by the antimicrobial peptides.
- Improve the understanding of how these molecules defend against the infectious disease.
- Facilitate new idea and approach to develop and optimize of these antimicrobial peptides.

Classification of Antimicrobial Peptides

Antimicrobial peptides are classified into four types.

1. α-helix peptide.
2. β-sheet peptides.
3. Extended peptides.
4. Loop peptides.

α-Helix Peptides

The structural group of α-helix and β-sheet are similar. In α-helix, the distance between two amino acid is about 0.15nm and the angle between this amino acid is around 100 degree[5]. The overall stability, half-life and the ability to create membrane pore by peptides are dependent upon the polar angle of amino acids in peptide i.e. it produces toroid or pore will be greater if the polar angle is smaller. The α-helix class includes magainin, cecropin and pexiganan[2]. This group of peptides is usually unstructured in aqueous solution and forms amphipathic helices in membrane or membrane-mimicking environment. The α- helical amphipathic peptides form barrel-like bundle in the bacterial membrane and these trans-membrane cluster lines amphipathic pores (barrel-stave model).

β-sheet Antimicrobial Peptides

The β-sheet antimicrobial peptides class includes β-defensin and protegrinare. These peptides contain minimum two β-stands that are stabilized by one to five disulfide bridges, there by forming a rigid structure. They are perpendicularly inserted into the phospholipids bi-layer to form a toroid pore[1, 6].

Extended Antimicrobial Peptides

The extended antimicrobial peptides are produced by porcine neurtrophils rich in proline and arginine or proline and phenylalanine amino acids. Many extended antimicrobial peptides are inactive against the micro-organism membrane. In other hand, extended peptides such as indolithicin are membrane active because indolithicin (13-amino acid) contains five tryptophan and three proline residue. So, this peptide undergoes ply-L-II helical structural modification in the presence of liposome and there is accumulation of high amount of tryptophan residue which is responsible for interaction with membrane[2].

Synthesis of Peptides

Solid phase technique is the most common method for synthesis of peptides initially developed by Robert Bruce Merrifield. Merrifield did not isolate and characterize the

product at each step but he later improved his original condition through the use of N-terminal t-butoxy carbonyl protecting group and benzyl derivatives. In addition, hydrogen fluoride (HF) used for cleavage of the peptides and removed of side chain and Trifluoroacetic acid was used for the N-terminal deprotection. In 1972, Carpino suggested the use of 9-fluorenylmethoxycarbonyl (FmoC) as N-terminal protecting group and use of 20% solution of piperiden in dimethyl formamide (DMF) for de-protected of resin-bound peptides. Once the chain was completed, Trifluoroacetic acid was used to cleave the peptides from the resin and remove amino acid side chain protecting group[7].

In solid phase synthesis, first step is selection of resin which builds the peptide chain. Resin selection greatly affects the outcome of the synthesis. Generally cross-linked polystyrene i.e. polymer of polyethylene glycol and polystyrene (particle size 90 micrometer) was used as resin in solid phase synthesis. PEG-PS resin is quite expensive. Literature search showed that a cheaper alternative is cross-linked ethoxylated Acrylate resin[8]. Next step is selection of linker. Linker is bi-functional molecule which bound both resin and amino acid of peptide. Some important example of linker used in solid phase synthesis is Rink, PAC, Nonb, and HMFA.

Initially coupling was performed by adding 100 micromole excess of 0.60M solution of 9-fluorenyl methlycarbonyl (FmoC) amino acid in DMF and transferred to disposable fitted reaction vessel placed in the symphony peptide synthesizer. Some amino acid require side chain protecting group i.e FmoC-Asp(otBu)-OH, FmoC-Gln(Trt)-OH, FmoC-Glu(OtBu)-OH, FmoC-Lys(Boc)-OH, FmoC-Arg(Pbf)-OH, FmoC-Ser(tBu)-OH.

The symphony peptides synthesizers have 6 pressurized solvent bottles as follows.

1. Bottle 1- DMF solvent for washing.
2. Bottle 2- 20% pipperidine in DMF for deprotection
3. Bottle 3- 100 Mm O-Benzotriazole-N,N,N,N-tetramethyl-uranium-hexafluoro phosphate (HBTU) and N-Hydroxybenzotriazole(HOBt)/400 Mm N-Methylmorpholine (NNM) in DMF.
4. Bottle 4- empty
5. Bottle 5- dichloromethane
6. Bottle 6- cleavage cocktail (reagent B)

After protecting with Fmoc, each peptide is bound to the resin in reaction vessel. Then this resin is de-protected for five minutes followed by a second ten minutes deportation. The de-protected peptides are washed six times with dimethyl formamide followed by washing with dichloromethane and drying with a steam of nitrogen for 30 minutes. Then 4 ml of reagent B is add to the reaction vessel and nitrogen gas is passed for two hours. An additional 2.5 ml of reagent B is added to the reaction vessels containing cleavage cocktail then this solution is transferred to the collection tube. The collection tube is removed from the symphony synthesizer and 40 ml of -20 ^{0}C cold methyl tert-butyl ether is added to a 50 ml of centrifuge tube. The ether solution is stored overnight in deep freezer (-20^{0}C). Next day, this ether solution is centrifuged and the resulting precipitated was collected from the bottom of the 50 ml centrifuge tube. The ether solution is poured off; then fresh cold ether is added and the ether solution is centrifuged again. This procedure is repeated for 3 times. After the final wash, produce peptides are dried for 2 hours in a hood. The resulting peptide is dissolved in 25 ml mixture of acetonitrile (50%) and water (50%) and lyophilization is done by freezone liquid nitrogen to produce white powder of crude peptides.

Physical Properties that Affect the Antimicrobial Activity and Cell Specificity of Antimicrobial Peptides

Conformation

Except α-helical and β-sheet, the most of remaining peptides are classified on the basis that enriches in one or more amino acid residue e.g. proline, arginine or tryptophan rich[9]. In cellular aqueous medium, antimicrobial peptides conformed to secondary structure which is more active than peptide with less-defined secondary

structure. For example, proline-arginine rich peptide and tryptophan rich indolicidin, conform to polyproline helical type-II structure which is greatly enhance the antimicrobial activity[9, 10].

Charge

The most interesting characteristic of antimicrobial peptides is cell specificity and antimicrobial activity. This depends upon the net positive charge of antimicrobial peptide accounted for preferential binding to negative charged phospholipids membrane of bacteria which is different from the zwitterionic surface of normal mammalian cell. Most of antimicrobial peptides have net positive charge from +2 to +9 and cationic domains which are important for interaction of positive charge AMP to negative charge phospholipids membrane of bacteria or micro-organism[2,10].

Amphipathicity

Amphipathic properties are found in nearly all antimicrobial peptides. This property exerted antimicrobial activity not only toward the negative charged cell membrane but also to the neutral charged cell membrane. Amphipathic property of antimicrobial peptides can be measure by its hydrophobic moment which is calculated by the vectorial sum of the individual amino acid hydrophobicities, normalized to an ideal helix[11].

Polar angle

Polar angle of antimicrobial peptides defines the relative proportion of polar and nonpolar facet of peptide conforms to amphipathic helix. Polar angle of helix peptide is 180 because one face of helix peptides composed of polar amino acid residue and other face composed of non-polar amino acid residue[9].

Hydrophobicity

The hydrophobicity of peptides may refer the percentage of hydrophobic amino acids residue present in a primary structure of antimicrobial peptide, which is approximately 50%. Most of antimicrobial peptides need certain amount of hydrophobicity for their function. But increased the hydrophobicity may cause the destroying of the host cell and reduce cell specificity toward the microbial cell[3].

4.6. Amino acid of antimicrobial peptides

Some antimicrobial peptides contain basic amino acid like lysine or arginine. Some peptides contain hydrophobic amino acid e.g. alanine, leucine and phenylalanine. The ratio of hydrophobic and charged residue in antimicrobial peptide can vary from 1:1 to 1:2[12]. Cationic peptides are enriching in specific amino acid such as proline, arginine, phenylalanine, glycine, tryptophan.

Cell Specificity, Cell Toxicity and Drug Synergetic Effect of Antimicrobial Peptides

The most interesting characteristic of antimicrobial peptides is cell specificity. This property determined the killing activity of peptides towards microbes and non-toxic to eukaryotic or mammalian cell. The cell specificity of antimicrobial peptides is due to the difference between cell membrane composition of prokaryotic cell and eukaryotic cell. All bio-membrane consist phospholipids bi-layer as the core components. This bi-layer has amphipathic behavior i.e. they have both hydrophilic and hydrophobic region. The elementary components of both eukaryotic and prokaryotic cell membrane are different. In physiological conditions, phosphatidylcholine (PC), phosphatidylethanolamine (PE) have no net charge and sphingomyelin (SP) which is close analog of PC is also neutrally charged. Sterols such as ergosterol and cholesterol are mainly presented in eukaryotic but rarely found in prokaryotic membrane. Many hydroxylated phospholipids such as sulphatidylserine (PS), phosphatidylglycerol(PG) and cardiolipin(PL) contain net negative charge under physiological condition[10]. Cell membrane of pathogenic micro-organism mostly comprises PG, PL and PS, which are very electronegative, whereas the eukaryotic/mammalian membrane that are rich in PC, PE, or SP tend to have a neutral charge. Thus antimicrobial peptides are more active towards the pathogenic microbe's cell membrane than eukaryotic membrane[9].

In aqueous cellular environment, it is believed that some antimicrobial peptides undergo significant conformational change such as α-helix and binds to the cell membrane of pathogenic microbes. Antimicrobial peptides undergo significant conformational (or oligomerization) change within the target microbes but not on the host cell membrane which increases the cell specific toxicity. Zhang and coworker performed an experiment in which they tested antimicrobial peptides including α-helix, β-sheet and extended peptides. This experiment concluded that all the peptides interact with lipid monolayer which consist of negatively charged phosphatidylglycerol (PG) but the α-helix only penetrates neutrally charged phosphatidylcholine (PC) lipid cell membrane and β-sheet peptides translocation in the cell membrane at a the concentration lower than the "MIC" (minimum inhibition concentration).

This study concluded that the antimicrobial peptides not only interact with specific lipid membrane but also remodels the cell membrane of specific cell. Welling and colleagues conducted an in-vivo experiment by using radio labeled fragment of antimicrobial peptides Tc-UBI 29-41 to compare the binding affinity of antimicrobial peptides between host and micro-organism cell membrane[9]. In this study, animals were infected with *Candida albicans, Klebsiella pneumonia* and sterile inflammation were induced by injection of heat-killed micro-organism into the thigh of animal. The radio labeled peptides were more significantly accumulated on the infected tissue than normal or sterile inflamed tissue. This study demonstrated that peptides can distinguish between the host cell and microbe's cell. These features of antimicrobial peptides can increase the use of synergic effect of peptides with convectional antibiotics. The combined administration of antibiotics and antimicrobial peptides causes increase in the synergestic antibacterial effect which enables to prevent the development of drug-resistance for an individual drug. Several study are repotted such as synergies of magainin II and cecropin A administration with rifampicin against the MDR *pseudomonas aeruginosa* both *in-vitro* and *in-vivo* causing the reduction in bacterial multiplication.

Mechanism of Action of Antimicrobial Peptides on the Basis of Membrane Model

Major antimicrobial peptides bind on negatively charged microbial cell membrane and permeate through membranes to form a pathway for ions and solute. Before reaching the phospholipids membrane, peptides transverse through negative outer cell wall of bacteria containing LPs. In this mechanism, peptides interact with LPs and competitively displace the divalent polyanionic, cationic and neutral LPs.

This displacement of cations cause the disputation of outer membrane and peptides reach to the negatively charge phospholipids cytoplasmic membrane. These membrane active properties of peptides have been extensively studied by using model membrane. The interaction of antimicrobial peptides and membrane can be studied either by an *in vivo* or by *in vitro* experiment with model membranes[1].

Besides the above bi-layer model system, phospholipids monolayer is of interest due to their homogeneity and their stability. The interaction of peptides with phospholipids at the air or water interface provides a unique model in understanding the insertion mechanism of antimicrobial peptides into cell membrane[2].

Barrel-stave Model for Trans-Membrane Channel Pore Formation

In "barrel-stave" mechanism, the hydrophobic surface of peptides interact with the lipid core membrane to form a channel or pore by bundle of α-helices and their hydrophilic surface point inward to produce an aqueous pore[10].

The following criteria must be fulfilled in order that the molecules form a trans-membrane pore.

1. Binding of monomer to the membrane in alpha-helical structure.
2. Insertion of helices into the hydrophobic core of the membrane.
3. Progressive recruitment of additional monomer to increase the pore size.

A single α-helix monomer is energetically unfavorable to transverse the membrane. So, the assembly of monomer on the surface of the membrane before the peptides inserted is an important step. Based on those criteria, it is that the helix will be highly homogeneously charged because their hydrophilic surfaces are facing to each other while forming trans-membrane pore[13].

"Carpet" Model for Membrane Disruption

Dermaseptin S (AMP) mode of action was first time described by the carpet model and later on was used to describe the mode of action of other antimicrobial peptides such as dermaseptin S natural analogues, LL-37, caerin 1.1 and cecropins[1]. In this model antimicrobial peptides initially bind to the surface of the cell membrane and cover it in a carpet-like manner[10]. Membrane permeation occurs only after high threshold concentration of the membrane-bound peptides.

High local concentration of peptides on the membrane can be achieving either by the complete covering of membrane with peptides monomer or after their association with membrane-bound peptides, forming localized "carpet". Unlike "barrel-stave model", in "carpet" peptides monomer neither inserts itself to the hydrophobic core of membrane nor do assemble on the hydrophilic surface[13].

The four steps to involve in this model are as follow.

1. Binding of peptides monomer to the phospholipid layer.
2. Alignment of the peptides monomer on the surface of the membrane so that their hydrophilic surface is facing the phospholipid heads or water molecules.
3. Rotation of the molecule leading to reorientation of the hydrophobic residue towards hydrophobic core of the membrane.
4. Disintegration of the membrane by disrupting the belayed curvature.

Toroid Pore and Worm Hole Mechanism

One of the well characterized mechanisms of peptides interactions is that of the toroid pore. A primary difference between the toroid pore and barrel-stave model is that in the former, lipid are intercalated with peptides in the trans-membrane channel[10].

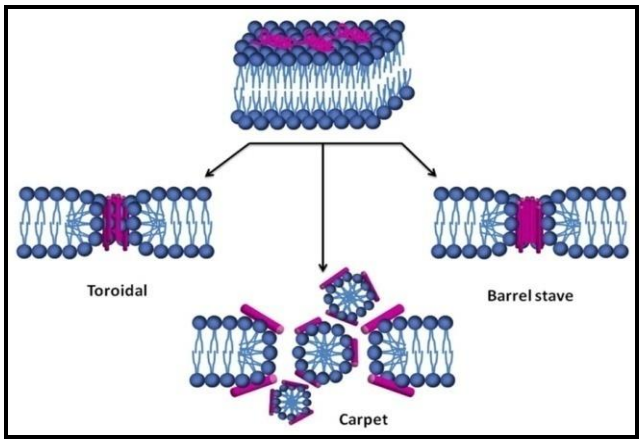

Figure 1: Different mechanism of action of antimicrobial peptides

The toroid pore model has been derived principally from the experiment using alpha-helical peptides, including magainins and PGLs. In this model, peptides in the extracellular environment take on α-helical structure as they interact with charged hydrophobic bacterial membrane. The hydrophobic residue of the bound peptides displaces the polar head group, creating a breach in the hydrophobic region and inducing positive curvature strain in the membrane there by destabilizing the membrane. Estimated threshold peptide to lipid ratio is 1:30 for magainin[9].

Biological Activity of Antimicrobial Peptides

Antimicrobial peptides show a board spectrum of activity. Many antimicrobial peptides not only kill bacteria, but also are cytotoxic to fungi, protozoa, malignant cell and even enveloped viruses like HIV, herpes simplex and vascular stomatitis[2].

Recent research shows that vitamin D up-regulates the ability to fight against the infection by helping production over 200 antimicrobial peptides, the most important of which is *cathelicidin*[14]. The antimicrobial peptide (magainins) and their analogs have found to able lyse hematopoietic tumor and solid tumor cell with little toxicity[2]. Several cationic amphipathic

peptides have displayed antiviral activity in vitro. Defensins are able to neutralize herpes simplex virus (HSV), and vesicular stomatitis virus.

Activities of cationic antimicrobial peptides and some example of peptides with those activities are given in table 1[15].

Table 1: Examples of peptides and their activity

Activity	Example
Broad- spectrum antibiotics	Defensin, indolicin, protegrin and LL-37
Synergy with other peptides	Defensin, NP-1 and NP-5
Antifungal	Protegrin, indolicidin and histadine
Anti-endotoxin	LL-37
Anti-enveloped virus (HIV,HSV,VSV)	Lindolicin, protegrin and defensins
Anticancer	Lindocidin and defensins
Wound healing	PR39 and defensins

According to antimicrobial peptide database (APD), about 97% of antimicrobial peptides contain 500 residues having average length 27.97 and average net charge 4.56. The antimicrobial peptides are categorized on the basis of their targets and amino acid. Based upon their biological activity, antimicrobial peptides are classified in following types.

Antibacterial Peptides

These peptides contain 498 peptides residue, length of 27.82 and average net charge is 4.56. Antibacterial peptides are the most studied antimicrobial peptides to date. These peptides act on cell membrane and disintegrate the lipid bi-layer of cell membrane. Recently some researchers demonstrated that some antibacterial peptides kill micro-organism in very small concentration by membrane-disrupting action and inhibiting DNA replication and protein synthesis. In some cases, antimicrobial peptides kill the antibiotic-resistance micro-organism. Nisin (AMPs) and vincomycin both inhibit cell wall synthesis. However, methicillin resistant *Staphylococcus aureus* gain resistance to vincomycin but it sensitive to nisin[5]. In addition, ten human AMPs are inhibitory mainly to gram-negative bacteria: these include hBD-26, hBD-27, and human calcitermin, psoriasin/S100A7, CCL8, CCL13, CCL19, alarin, HMGN2, and KDAMP peptides[16].

Antifungal Peptides

According to APD (antimicrobial peptides data base), 58 human antifungal peptides have been identified which includes α-defensin, cathelicidin, LL-37 and histatins. Antifungal peptides contain 155 peptides residue with length 29.72 and net charge 5.01. Similar to Antibacterial peptides, antifungal peptides also act on the cell wall. But the bacterial cell wall components are different from the fungal cell wall. The main components of fungal cell wall are chitin which causes the binding of peptides to target fungal cell. In case of LL-37, protease causes the fragmentation of LL-37 into KS-30 and RK-31 which essential for inhibiting *Candida albicans*[16].

Antiviral Peptides

In APD, there are 16 human antiviral peptides which includes six well characterized human α defensins e.g. HNP-1, HNP-2, HMP-3, HNP-4, HD-5 and HD-6 and three β-defensins e.g. hBD-1, hBD-2 and hBD-3[3]. Mainly antiviral peptides are short peptides with 28 peptides residue, length 25.68 and average net charge 4.11. Antiviral peptide act by integrating either with viral envelope or host cell membrane. Besides these, some antiviral peptides prevent entering into host cell by binding with receptor on the cell. Example, lactoferrin (AMP) prevent HSV infection by binding with negatively charge (glycosaminoglycan molecule) of heparan[16, 17].

Human Application of Antimicrobial Peptides

Now a day, use of antimicrobial peptides has increased very rapidly. Antimicrobial peptide has many more advanced features over the conventional antibiotics therapy, due to increase in the resistance of conventional antibiotics causing the development of new antimicrobial peptides for the human therapeutic use. Although synthesis of peptides are more expensive than antibiotics but many studies found that the activity of peptides are more affective. The solid state NMR spectroscopic study demonstrated secondary structure, orientation, and penetration of antimicrobial peptides into phospholipids bi-layer.

Following are the example of antimicrobial peptides used in human use.

- During the screening of LL-37 library peptides, Wang found that chymotrypsin-resistant template that contains partial active D-amino acid which is novel but it is not active against the methicillin-resistant staphylococcus *aureus* (MRSA). In addition according to 3D structure, activity is increased by introducing a bulky hydrophobic group into their structural cavity[17].
- In 2014, LL-37 containing vector was used for the healing of wounds. The active form of 1, 25-Dihydroxyvitamin D3 is also used to induce the LL-37 and Hbd-2 for wound healing in diabetic foot ulcer[18].
- Many human AMPs were closely examined for detection and diagnosis of human diseases. For example of this application is association of Technetium-99m labeled ubiquicidin with bacteria which enable to differentiate infection from aseptic loosening of hip prostheses in 30 minutes[5].
- Multi-drug resistance in malignant tumor cell are most commonly arising from the over expression of p-glycoprotein (a membrane bound afflux pump) that can remove easily many convectional drugs. A few antimicrobial peptides (e.g. doxorubicin, etoposite, cisplatin) having cytotoxic activity against the MDRs tumour cell[6]. Some antimicrobial peptides independent on the cell proliferation, so clinically these peptides are used to combine high and specific anticancer activity with serum.
- In respiratory airways, α-and-β-defensins and the cathelicidin LL-37/hCAP-18 are produced by epithelium cell and macrophage which is helpful to protect the respiratory tract[19].
- Now a day, bio-film is the major problem in hospital and healthcare organization. Bio-film is the extended cultivation of bacterial cell formed by adherence to animal tissue. Bio-film is the multi-layered community of sessile bacterial cell which provides survival advantages over the planktonic or free-floating bacteria by enhancing the nutrients and colonization[20]. Clinically, antimicrobial peptides are used to prevent the bio-film formation. Patient life is largely affected due to the medical-device contamination or infections which are often by the contamination of bacteria on the surface of device can cause the formation of bio-film. Generally, in case of convectional antibiotics inhibition or lethal concentration are increases 1000 fold against the bio-film formation because they are not translocated into extracellular polymeric substance. But due to different mechanisms of antimicrobial peptides like membrane-disrupting action, functional inhibition of proteins, binding with DNA, and detoxification of polysaccharides (lipopolysaccharide and lipoteichoic acid) used as anti-biofilm agent[21].

Limitation of Antimicrobial Peptides

Antimicrobial peptides have broad antibacterial activity, multidrug resistance activity which is not hindered by the bacterial resistance. But there is some limitation of antimicrobial peptides like cost of production, proteolysis of peptides, antimicrobial peptides resistance. In the view of antimicrobial peptides production, 400 US$ cost to prepare one gram of antimicrobial peptides. But for preparation of convectional antibiotics the price can be under 1 US $[1]. Next drawback is proteolysis of AMPs. Mainly antimicrobial

peptides are highly sensitive towards the proteolytic degradation. Due to proteolytic degradation of antimicrobial peptides, the rapid clearance and unfavourable pharmacokinetics may create various limitations for their application. So clinically uses of peptides focus on the topical application[22].

Third drawback is antimicrobial peptide resistance, although antimicrobial peptides retained their effectiveness against pathogens infection for at least 108 years. But due to their stable structural and functional properties some pathogens get resistance against antimicrobial peptides for example resistant species of genera such as *Morganella* and *Serratia* express an outer membrane that lacks the appropriate density of acidic lipids to provide peptide-binding sites and some other peptides resistance pathogen species like, *Porphyromonas gingivalis* secrete digestive proteases enzyme that degraded the peptides[14].

CONCLUSION

Due to various drawbacks of conventional antibiotics, there is urgent need to obtained new antimicrobial agents. Antimicrobial peptide therapy is the most effective strategy in defending the micro-organisms. Nowadays, AMPs have application in medical fields such as in diagnosis of diseases, deportation of medical devices and in treatment of various diseases. There are certain limitations of AMPs as discussed above. But these can be overcome by certain strategies or chemical modification such as introduction of D-amino acid, amidation at N-terminus and cyclization of peptides. These are most common strategies to increase the stability of antimicrobial peptides. In the present article, a though discussion on the various mechanism of action of AMP on bacterial cell wall, physical and biological properties of AMP, specificity of AMPs is done.

REFERENCES

1. Cézard, C., Silva-Pires, V., Mullié, C., & Sonnet, P. (2011). Antibacterial peptides: a review. Science against microbial pathogens: communicating current research and technological advances. A. Méndez-Vilas. *Formatex Research Center*, 2, 926-937.
2. Zhao, H. (2003). *Mode of action of antimicrobial peptides*. University of Helsinki.
3. Wang, G. (2014). Human antimicrobial peptides and proteins. *Pharmaceuticals*, *7*(5), 545-594.
4. Phoenix, D. A., Dennison, S. R., & Harris, F. (2013). Antimicrobial peptides: their history, evolution, and functional promiscuity. *Antimicrobial Peptides*, 1-37.
5. Bahar, A. A., & Ren, D. (2013). Antimicrobial peptides. *Pharmaceuticals*, 6(12), 1543-1575.
6. Díaz i Cirac, A. (2011). Mechanism of action of cyclic antimicrobial peptides.
7. Snyder, C. R. (2012). Synthesis and Purification of Potential Antimicrobial Peptides.
8. R. F. Malagon. (2010). Design and Synthesis of Short Antimicrobial peptides for Plant Protection. Study of their Mode of action. *University de Girona*
9. Ebenhan, T., Gheysens, O., Kruger, H. G., Zeevaart, J. R., & Sathekge, M. M. (2014). Antimicrobial peptides: their role as infection-selective tracers for molecular imaging. *BioMed Research International*, *2014*.
10. Yeaman, M. R., & Yount, N. Y. (2003). Mechanisms of antimicrobial peptide action and resistance. *Pharmacological Reviews*, *55*(1), 27-55.
11. Pirtskhalava, M., Vishnepolsky, B., & Grigolava, M. (2013). Transmembrane and antimicrobial peptides. Hydrophobicity, amphiphilicity and propensity to aggregation. *arXiv preprint arXiv: 1307.6160*.
12. Brogden, K. A. (2005). Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria?. *Nature Reviews Microbiology*, *3*(3), 238-250.
13. Cheng-Hua, L., Jian-Min, Z., & Lin-Sheng, S. (2009). A review of advances in research on marine molluscan antimicrobial peptides and their potential application in aquaculture. *Molluscan Research*, 29(1), 17.

14. J. Hardy. Small but potent killer. *Antimicrobial Peptides.*
15. Hancock, R. E., & Diamond, G. (2000). The role of cationic antimicrobial peptides in innate host defences. *Trends in Microbiology*, 8(9), 402-410.
16. Schuerholz, T., Brandenburg, K., & Marx, G. (2012). Antimicrobial peptides and their potential application in inflammation and sepsis. *Critical Care*, 16(2), 207.
17. Brandenburg, L. O., Merres, J., Albrecht, L. J., Varoga, D., & Pufe, T. (2012). Antimicrobial peptides: multifunctional drugs for different applications. *Polymers*, 4(1), 539-560.
18. Wang, G., Mishra, B., Lau, K., Lushnikova, T., Golla, R., & Wang, X. (2015). Antimicrobial peptides in 2014. *Pharmaceuticals*, 8(1), 123-150.
19. Marshall, S. H., & Arenas, G. (2003). Antimicrobial peptides: A natural alternative to chemical antibiotics and a potential for applied biotechnology. *Electronic Journal of Biotechnology*, 6(3), 271-284.
20. Jacobsen, A. S. (2013). *The Effect of Antimicrobial Peptides on Bacterial Biofilms* (Doctoral dissertation).
21. Park, S. C., Park, Y., & Hahm, K. S. (2011). The role of antimicrobial peptides in preventing multidrug-resistant bacterial infections and biofilm formation. *International Journal of Molecular Sciences*, 12(9), 5971-5992.
22. Yu, J. Z., Wen, C. M., & Tsao, P. H. (2013). Antimicrobial peptides characteristics and their applications as feed additives in domestic animals. *Journal of the Chinese Society of Animal Science*, 42(1), 1-13.