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ANTIMICROBIAL PEPTIDES: AN EFFECTIVE ALTERNATIVE FOR ANTIBIOTIC THERAPY

JB PERAVALI[1*], SR KOTRA[2], K SOBHA[2], R NELSON[3], K.V.RAJESH[1], KK PULICHERLA[2]

[1] Department of Biotechnology, Bapatla Engineering College, Bapatla, Guntur, Andhra Pradesh, India.[2] Department of Biotechnology, R.V.R. & J.C.C.E, Guntur, Andhra Pradesh, India,[3] Department of Botany, Govt. Arts College, Ariyalur, Tamilnadu, India. Email - jawaharbiotech@gmail.com

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ABSTRACT

Extensive use of classical antibiotics has led to the growing emergence of many resistant strains of pathogenic bacteria. Evidence has suggested that cationic antimicrobial peptides (AMP's) are of greatest potential to represent a new class of antibiotics. These peptides have a good scope in current antibiotic research. During the past two decades several AMPs have been isolated from a wide variety of animals (both vertebrates and invertebrates), and plants as well as from bacteria and fungi. These are relatively small (<10kDa), cationic and amphipathic peptides of variable length, sequence and structure. These peptides exhibit broad-spectrum activity against a wide range of microorganisms including gram-positive and gram-negative bacteria, protozoa, yeast, fungi and viruses. Most of these peptides are believed to act by disrupting the plasma membrane leading to the lysis of the cell. Antimicrobial peptides encompass a wide variety of structural motifs such as α -helical peptides, β -sheet peptides, looped peptides and extended peptides. Preparations enriched by a specific protein are rarely easily obtained from natural host cells. Hence, recombinant protein production is frequently the sole applicable procedure. Several fusion strategies have been developed for the expression and purification of small antimicrobial peptides (AMPs) in recombinant bacterial expression systems which were produced by cloning. This article aims to review in brief the sources of antimicrobial peptides, diversity in structural features, mode of action, production strategies and insight into the current data on their antimicrobial activity followed by a brief comment on the peptides that have entered clinical trials.

Key words: Antibiotics, Antimicrobial peptides, Anionic peptides, Cationic peptides, Membrane permeability, Production.

INTRODUCTION

Most living organisms are constantly exposed to potentially harmful pathogens. When antibiotics were first identified they were called wonder drugs, and doctors and patients alike considered them appropriate for just about everything (Barra *et al.,* 1995 & Nguyen *et al.,* 2011). Though antibiotics have saved the life for a long time, the extensive usage and human errors like misuse in medicine, agriculture and household purposes are thought to be the causes of an alarming increase in antibiotic and multidrug resistant pathogens (Witte *et al.,* 2000). So there is an immediate demand to develop an efficient molecule for life-threatening infectious diseases. Antimicrobial peptides are the upcoming therapeutic molecules as alternative drugs to the antibiotics. The assets of the AMPs over antibiotics are mainly due to their potential for broad spectrum activity, rapid bactericidal activity and low propensity for resistance development. Consisting not more than a dozen amino acids, rapidly produced and diffusable they seem ideal for fast and efficient defense against microbes (Nissen-Meyer and Nes, 1997). Their usefulness is also evident from their persistence throughout evolution.

Antimicrobial peptides are ubiquitous among all eukaryotes, including mammals, amphibians, insects, plants and protozoa (Gabay, 1994). In vertebrates, they act as the first line of defense, inhibiting pathogen growth in the earliest stages of invasion in advance of the mobilisation of specific immunity (Hancock *et al.,* 2006). Currently, more than 500 cationic antimicrobial peptides have been isolated from a wide range of organisms and can be found in the Antimicrobial Sequences Database. Natural cationic peptides show considerable sequence diversity, but share certain common structural features, including a high of basic amino acid content and the dispersion of hydrophobic and hydrophilic residues, which gives the peptides their amphipathic character under hydrophobic conditions (Merrifield *et al.,* 1994). A number of interaction mechanisms between cationic peptides and the cell envelope has been proposed, including the formation of membrane-spanning pores that disrupt the ionic homeostasis of the bacteria; the ''barrel stave'' mechanism in which individual monomeric peptides from the staves of the barrel-like pores; and the ''carpet'' model, in which the peptides saturate the surface of the membrane before disrupting the membrane permeability barrier (Hancock and Lehrer, 1998). They have low MICs and broad-spectrum activity in both low and high ionic strength conditions (Travis *et al.,* 2000 & Porciatti *et al.*, 2010), neutralize LPS (Hirata *et al.,* 1994), promote wound healing properties and the fact that they have potential to overcome bacterial resistance makes them promising candidates for therapeutic drugs (Gallo *et al.,* 1997). This article aims to review in brief the sources of such peptides and their classification based on structure and composition, their mechanism, cloning, expression, purification strategies and insight into the current data on their antimicrobial activity followed by a brief comment on the peptides that have entered clinical trials (Min-Duke Seo *et al.,* 2012).

SOURCES

AMPs are widely distributed in nature, being produced by mammals, birds, amphibians, insects, plants, and microorganisms (Cammue *et al.,* 1994, Velden *et al*., 2009). Most of these peptides are synthesized as a prepropeptide consisting of an N-terminal signal sequence (which aids in targeting of endoplasmic reticulum), a pro segment and a C-terminal cationic peptide that demonstrates antimicrobial activity after it is cleaved from the rest of the protein (Bals, 2000).

AMP'S FROM BACTERIA

Bacterial ribosomes synthesize antimicrobial peptides which are generally called as bacteriocins. About 50 of them have been isolated from various gram-positive bacteria especially lacticacid producing organisms (Bohaychuk *et al.,* 1999) eg., colicins produced by *E.coli*. A receptor domain in the colicin protein that binds a specific cell surface receptor determines target recognition. This mode of targeting results in the relatively narrow phylogenetic killing range often cited for bacteriocins.

AMP'S FROM INSECTS

Since the discovery of inducible AMPs in the moth *Hyalophora cecropia* more than 150 such peptides have been identified in various insects (Steiner *et al.,* 1981). These peptides called cecropins are 3, 4 K.Da linear amphipathic peptides and demonstrate activity against protozoa and metazoan parasites in addition to bacteria and fungi (Zasloff, 2002). Drosophila has served as an ideal model for the analysis of innate immune mechanisms. Septic injury in this insect rapidly induces the AMP genes in the fat body cells to produce a lineage of peptides namely drosomycin, cecropins, diptericin, drosocin, attacin and metchnikowin. Drosomycin and metchnikowin are potent

antifungal while others exhibit antibacterial properties (Hoffman *et al.,* 1999 & Japelj *et al.,* 2007). In certain species such as the ant, Pachycondyla goeldii, about 15 different peptides demonstrating antibacterial and insecticidal properties have been isolated from its venom. Named ponericidins, these peptides range from 1.8 to 3.3 K.Da and share sequence similarities with cecropins, mellitins and dermaseptins (Orivel *et al.,* 2001).

AMP'S FROM AMPHIBIANS

Amphibian AMPs are synthesized in the skin of a single species as structurally related members of a family. The first AMP was found in the skin of the European frog *Bombina variegata* some 30 years ago. The subsequent discovery of the potent magainins (from the Hebrew ''magain'', shield) in the skin secretions of the African clawed frog *Xenopus laevis* was a new, decisive spur to further research (Fjell *et al.,* 2012). Acting as wide-spectrum microbicides against a variety of bacteria, protozoa and fungi, amphibian peptides have stimulated increasing interest because of their unique characteristics and potential therapeutic usefulness (Maria Papagianni, 2003). The AMPs from amphibian skin isolated so far (about 500) share some main characteristics, as that of bearing a net cationic charge at physiological pH, due to the presence of Lys and/or Arg residues.

AMP'S FROM VERTEBRATES

In mammals, AMPs are expressed in phagocytes and mucosal epithelial cells (Lehler *et al.,* 1993) and represent crucial components of the innate immune system. The defensins belong to the largest group of AMPs, which are widely distributed in animals and plants. Invertebrate (Bulet P *et al.,* 1999; Andreu and Rivas, 1998; Dimarcq, 1998) and plant (Garcia-Olmedo, 1998) defensins are characterized by three and four disulfide bridges, respectively, and show a common structure comprising of a α-helix linked to a β-sheet by two disulfide bridges.

TYPES OF ANTIMICROBIAL PEPTIDES

Antimicrobial peptides have been a popular topic of research and over 750 eukaryotic antimicrobial peptides have been reported. These peptides are grouped according to similarities in charge, sequence homology, functional similarity and 3-dimensional structure (Brogden *et al.,* 2003).

ANIONIC PEPTIDES

These are small (721.6 – 823.8 Da) peptides present in surfactant extracts, bronchoalveolar lavage fluid and airway epithelial cells (Brogden *et al.,* 2003) They are produced in mM concentrations, require zinc as a cofactor for antimicrobial activity and are active against both Gram- positive and Gram-negative bacteria. They are similar to the charge-neutralizing pro-peptides of larger zymogens, which also have antimicrobial activity when synthesized alone (Brogden *et al.,* 1997). In addition to their innate antimicrobial activity, anionic antimicrobial peptides may also have a regulatory role in pulmonary metabolism. Their structure is similar to the charge neutralizing propeptides of Group I serine proteases, and they may be capable of regulating, via negative feedback inhibition, the activity of pulmonary enzyme systems. Anionic peptides have been shown to be trypsin inhibitors. The mechanism of bacterial killing by anionic pep- tides is not known. Anionic peptides require zinc for maximal activity (LaForce F.M and Boose, 1984; Caverly *et al.,* 2001) and form a complex with it (Bottari, 1990 & Marr *et al.,* 2006). Therefore, it is attractive to speculate that zinc may form a cationic salt bridge that allows the peptide to overcome the net negative charge on the microbial surface. The peptide then penetrates the outer membrane without inducing any morphological changes (Brogden *et al.,* 1996). Once in the cytoplasm, anionic peptides may then attach to ribosomes and inhibit ribonuclease activity similar to that seen with polymers of aspartic acid (Vandendriessche, 1956). Ultimately, the cytoplasmic protein precipitates and settles out, suggesting an internal mechanism of protein inactivation. Killing occurs within 30 minutes (Brogden *et al.,* 1996).

CATIONIC PEPTIDES

Cationic peptides are found in all living species. They contain 12-50 amino acids with net positive charge of +2 to +7 owing to an excess of basic amino acid residues (argine, lysine and histidine) over acidic amino acids. cationic antimicrobial peptides have a diverse range of targets (Huang *et al.,* 2010). The only defining characteristic of these targets is their possession of a membrane. Cationic peptides have been found to have activity against both Gram negative and Gram positive bacteria as well as fungi, eukaryotic parasites, and viruses. Certain cationic peptides have been shown to inhibit the replication of enveloped viruses such as Influenza A (Powers and Hancock, 2003).Vesicular stomatitis virus and human immunodeficiency virus. . Most importantly, cationic peptides are effective against strains of antibiotic resistant bacteria. There are four major classes of cationic peptides: β-sheet, α-helices, extended molecules and loops (Hancock, 2001).

Cationic peptides are amphipathic meaning they possess both a hydrophobic region that interacts with lipids and a positively charged hydrophilic region that interacts with water or negatively charged residues. This feature allows the peptides to interact well with membranes that are composed of amphipathic molecules, especially negatively charged bacterial membranes. For the most part, animal cells tend to have membranes with no net charge so they are unaffected by cationic peptides.

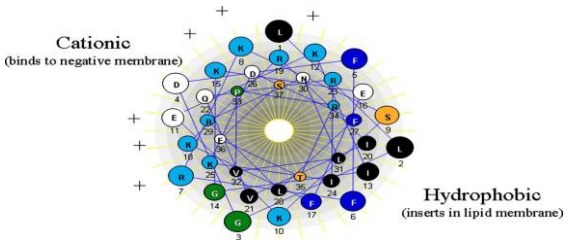


Figure 1: Schematic representation of cationic peptide

CLASSIFICATION

Nuclear magnetic resonance (NMR) has emerged as a useful technique for studying the details of structures of most of the known antimicrobial peptides. Analysis of the three dimensional structure of these peptides has led to the better under- standing of their function. Based on the NMR structures of known peptides along with sequence analysis AMPs are broadly classified into four groups (Wim van, *et al.,* 2001).

(a) α-helices (b) β-sheet molecules (c) Extended molecules (d) Loops due to a single disulphide bond.

α-HELICAL PEPTIDES

Peptides of the α-helical class is characterized by their α-helical conformation, and often contain a slight bend in the center of the molecule. In one study, this bending was critical for selectivity by suppressing the hemolytic activity (Zhang *et al.,* 1999). The α-helical magainins are representative of this structural class Isolated from the skin of the African clawed frog *Xenopus laevis*, magainin 1 and 2 are 23 residues in length and possess modest antimicrobial activities (Ex. MIC of 50 g/ml versus *E. coli*) (Zasloff *et al.,* 1988). The structure of magainin 2 has been determined by NMR in the presence of DPC and SDS micelles. The peptide adopts an amphipathic α-helical conformation with a slight bend centered at residues 12 and 13 (Gesell *et al.,* 1997). The antimicrobial mechanism of magainin has been proposed to involve selective permeabilisation of bacterial membranes leading to disruption of the membrane potential (Matsuzaki *et al.,* 1993).

β – SHEET PEPTIDES

This class of peptides is characterized by the presence of an antiparallel β-sheet, generally stabilized by disulfide bonds. Larger peptides within this family may also contain minor helical segments. Perhaps the best characterized β-sheet peptides are

the small 17–18 residue tachyplesins. Although the structure and in vitro activity of the tachyplesins are well characterized, the exact mechanism of antimicrobial activity remains poorly understood. Additional studies involving the related β-sheet peptide, polyphemusin I, demonstrate that these peptides are effective at inducing lipid flip-flop and undergoing membrane translocation but do not cause substantial calcein release from model membrane systems (Zhang *et al.,* 2001). This suggests these peptides disrupt lipid organization leading to the translocation of peptide molecules across the bilayer but do not form long-lived pores or channels. At present several β-sheets AMP's are identified like tachyplesin, Thanatin whose structure was studied by NMR.

A) β-sheet peptide (Alain *et al.,* 2007) (B) α-helical peptide (Gesell *et al.,* 1997) (C) extended peptide (Rozek *et al.,* 1986) (D) looped peptide (Mandard *et al.,* 1998)

EXTENDED PEPTIDES

The extended class of peptides lacks classical secondary structures, generally due to their high proline and/or glycine contents. Indeed, these peptides form their final structures not through inter residue hydrogen bonds but by hydrogen bond and Van der Waals interactions with membrane lipids. These peptides are generally rich in regular amino acids like proline and tryptophan. Histatin, a peptide isolated from human saliva is rich in histidine residues and is active against *C. albicans* (Xu *et al.,* 1991 & Oyston *et al.,* 2009). While cathelicidins are proline rich peptides and have irregular structures, indolicidins and tritripticin (Lawyer *et al.,* 1996) are rich in tryptophan. Bactenecins Bac-5 and Bac-7, like cathelicidins, are proline rich (Gennaro *et al.,* 1989 & Rotem *et al.,* 2009) while the peptide PR-39, is rich in arginine residues.The antimicrobial mechanism of indolicidin has yet to be unambiguously identified. Indolicidin possesses reasonable antimicrobial activity (MIC of 10 g/ml against *E. coli*) but does not have a high affinity for LPS when compared to other peptides such as the β-hairpin tachyplesins. It was first hypothesized that indolicidin acts by disrupting the cytoplasmic membrane by voltage-induced channel formation driven by membrane potential (Falla and Karunaratne, 1996).

LOOP PEPTIDES

This class of peptides is characterized by their loop structure imparted by the presence of a single bond. The only member of the loop family of peptides with an available high resolution structure is thanatin. Thanatin is a 21-residue, loop peptide isolated from the spined soldier bug, *Podisus maculiventris* (Fehlbaum *et al.,* 1996). The solution structure of thanatin has been determined by ^{1}H NMR and is that of an anti-parallel β-sheet, formed by residues 8–21, stabilized by the single disulfide bond between residues 11 and 18.Thanatin possesses reasonable antimicrobial activity against Gram-negative and positive bacteria as well as fungi (Won *et al.,* 2006) and is comparable in activity to members of the β-sheet family of peptides.

MODE OF ACTION OF ANTIMICROBIAL PEPTIDES

The membrane-active properties of such peptides, predicted by their physicochemical characteristics, have been corroborated by model studies demonstrating that antimicrobial peptides induce leakage of artificial liposomes (Matsuzaki, 1999; Wu *et al.,* 1999). Positively charged antimicrobial peptide binds to the negatively charged bacterial phospholipid membrane by means of an electrostatic force until a threshold concentration has been reached. Upon binding AMPs adapt antipathies structure followed by membrane permeation/ degradation.

It should be noted that structural transitions occur in peptides on passing from aqueous medium to the lipid medium of the membrane. In the case of gram-negative bacteria, it has been suggested that the peptides interact with and cross both cell envelope membranes killing cells by a multihit mechanism that involves action on more than one anionic target (Eisenberg, 1984). Several models have been proposed to describe the molecular events taking place during the peptide induced leakage of the target cell, but direct experimental evidence is still lacking. Below the most common models are treated in more detail (Capsoni *et al.,* 2007).

THE BARREL-STAVE MODEL

The peptide helices form a bundle in the membrane with a central lumen, much like a barrel composed of helical peptides as the staves (Yang *et al.,* 2001). The nonpolar side chains face the hydrophobic fatty acid tails at the inside of the phospholipid bilayer and the hydrophilic side-chains are pointed inward into the water filled pore. Progressive recruitment of additional peptide monomers leads to a steadily increasing pore size. Leakage of intracellular components through these pores subsequently leads to cell death. Peptides that act via this mechanism should presumably kill bacteria below the experimentally observed micromolar concentrations, becoming lethal once they penetrate into the phospholipid membrane of the target cell. This mechanism is well explained by analyzing a lantibiotic peptide nisin.

CARPET MODEL

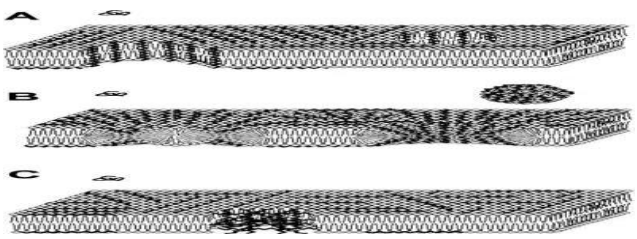
Membrane interaction of more amphipahtic peptides would rather occur according to the so-called carpet model (Shai, 1999). In the 'carpet model' peptides accumulates on the bilayer surface (Pouny and Shai, 1992). This model explains the activity of antimicrobial peptides such as ovispirin that orientate parallel ('in-plane') to the membrane surface (Bechinger, 1999). In this model, the microbial cell membrane is fully covered by a carpet-like cluster of peptides. When a critical concentration is reached, the membrane collapses, and in a short span of time, worm holes are formed all over the membrane, leading to lysis of the microbial cell. The carpet model has been proposed as the mechanism of action of magainins.

TOROIDAL-PORE MODEL

In the 'toroidal-pore model' antimicrobial peptide helices insert in to the membrane and induce the lipid monolayers to bend continuously through the pore so that the water core is lined by both the inserted peptides and the lipid head groups (Matsuzaki *et al.,* 1996). This type of transmembrane pore is induced by magainins, protegrins and melittin. In forming a toroidal pore, the polar faces of the peptides associate with the polar head groups of the lipids62, melittin, LL-37and MSI-78 65 9062.The lipids in these openings then tilt from the lamellar normal and connect the two leaflets of the membrane, forming a continuous bend from the top to the bottom in the fashion of a toroidal hole; the pore is lined by both the peptides and the lipid head groups, which are likely to screen and mask cationic peptide charges (Won *et al.,* 2009).

ION CHANNEL FORMATION

Besides membrane perturbing activities, AMPs also possess the ability to form ion-channels. Linear polycationic helical peptides (dermaseptins, cecropins, magainins and alamethicin) form pores or channels that can be assayed by conductance studies in planar lipid bilayers (Winans *et al.,* 1999). This ability to form transbilayer ion channels is correlated to the helical hydrophilic and hydrophobic components of the peptide. Alamethicin is one of the best-studied models with regard to its channel forming properties. Alamethicin when incorporated into planar lipid bilayers under applied voltage displays unique conductance properties characterized by high voltage dependence of microscopic current voltage curves and multistate single channel behaviour.



A.Barrel stave model B. Carpet model C. Ion channel model

INTRACELLULAR KILLING MECHANISM OF PEPTIDES

Although these models are helpful for defining mechanisms of antimicrobial peptide activity, their relevance to how peptides damage and kill microorganisms still need to be clarified. Recently, there has been speculation that transmembrane pore formation is not the only mechanism of microbial killing. In fact several observations suggest that translocated peptides can alter cytoplasmic membrane septum formation, inhibit cell-wall synthesis, inhibit nucleic-acid synthesis, inhibit protein synthesis or inhibit enzymatic activity (Tossi *et al.,* 2000). In the following figure different models of antimicrobial-peptide-induced pore formation and cell killing are presented.

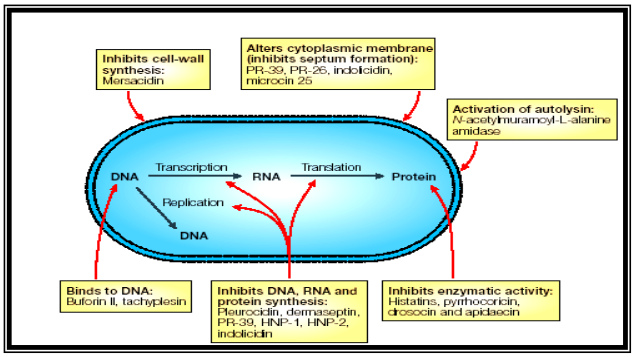


Figure: The mode of action for antimicrobial peptide activity, in this fig. Escherichia coli is shown as target microorganism (Brogden, 2011)

PRODUCTION STRATEGIES

There are several antimicrobial peptides which are naturally produced by many organisms like Indolicidin, α, β- defensins, cecropins maganins cathelicidin etc, (Teixeira *et al.,* 2012). Generally natural antimicrobial peptides are not cost effective but these natural peptides possess less broad spectrum activity against microorganisms than compared with synthetic peptides. Production of synthetic peptides is an expensive thing, hence it is important to develop effective production methods with less cost (Porcelli *et al.,* 2006). Peptide synthesis by chemical procedure is quite costly compared with the traditional solid phase synthesis method (Merrifield *et al.,* 1994). Intensive industrial research utilizing solution phase chemistry has reduced the costs remarkably, but the current production cost is still high. An alternative method for this is the production of peptides by recombinant DNA technology. In rDNA technology various procedures have been developed but the most broadly effect is produced as fusion proteins in bacterial cultures (Piers *et al.,* 1993).

For production of synthetic peptides by fusion protein technology, a fusion protein comprises of a carrier region which may contain an affinity purification tag, an anionic segment to stabilize the cationic peptide by binding to it and preventing both antibiotic activity of the cationic peptide segment against the host bacterium and proteolysis of this segment during recombinant production, a cleavage region and the cationic peptide region by using this we can produce a novel antimicrobial peptide to control the pathogenic activity of the microorganisms (Lamberty *et al.,* 2001). Purification of a fusion protein is easy compared with the other type of proteins because a wide range of protein fusion partners has been developed in order to simplify the purification and expression of recombinant proteins (Stevens, 2000). Fusion proteins or chimeric proteins usually include a partner or "tag" linked to the passenger or target protein by a recognition site for a specific protease which acts as the cleavage site to separate and to purify the peptide. There are several tags like glutathione *S*-transferase (GST) (Smith and Johnson, 1988) FLAG-tag and polyhistidine (His6) tags (Hochuli *et al.,* 1987) which are used in production of peptides by means of recombinant DNA technology. The hexahistidine tag enables the uses of immobilized metal affinity chromatography (Porath *et al.,* 1975) for the purification of the recombinant peptides.

VECTOR SYSTEM

A variety of vectors for the expression of antimicrobial peptide gene by rDNA technology are in use A plasmid expression vector pH6EX3 is used in synthesizing a novel fusion protein (Berthold *et al.,* 1992). pET28a+ vector is widely used in rDNA technology for the expression of novel antimicrobial peptide. Tachycitin was cloned by using pET22b (Kawabata *et al.,* 1996). A plant AMP MiAMP1 was cloned into a modified pET vector (Stuart J Harrison *et al.,* 1999). The purified ctxB fragment was cloned into the pea vector.

HOST SYSTEM

It is obvious that for any cloning strategy, it is necessary to express the recombinant protein in a suitable host system. Cecropin A has been produced in two different baculovirus expression systems (Andersons *et al.,* 1991), and insect defensin A from *Phormia terranovae* has been expressed in yeast and purified .The only example of an antimicrobial cationic peptide to be expressed in bacteria is a scorpion insectotoxin. *E.coli* BL21 (DE3) is the most common host and has proven outstanding in standard recombinant expression applications. BL21 (DE3) is a robust *E. coli* B strain, able to grow vigorously in minimal media but however non-pathogenic and unlikely to survive in host tissues and cause disease (Chart *et al.,* 2000).

Several antimicrobial peptides has been produced using the rDNA technology among them the well known antimicrobial peptides are MiAMP1 which is a low molecular weight says rich antimicrobial peptide isolated from *Macadamia integrifolia* using a pET and pSB161 vectors, cholera toxin B subunit was isolated from *E.coli* BL21 using pGEMT vector, His-P68 (A'-B') Fusion protein was isolated from *E.coli* strain using pH6EX3 re-FHL-1 His proteins produced by using baculo virus expression system using pBSV-8His vector production of Streptolysin O using *E. coli* by pBAD (Hancock *et al.,* 2000). All these peptides are in clinical trails.

ANTIMICROBIAL PEPTIDES IN IN CLINICAL TRIALS

Antimicrobial peptides tend to be involved in a local response to infections and the first clinical trials thus have been directed towards topical infections. Magainin Pharmaceuticals have taken the α-helical magainin variant peptide MSI-78 into phase-III clinical trials in studies of efficacy against polymicrobic foot-ulcer infections in diabetes. It was announced that these trials demonstrated equivalence to orally administered ofloxacin, but with less side effect. Iseganan (IB-367, Intrabiotics, Mountain View, CA, USA), a protegrin derivative, has passed phase II clinical trials for application against oral mucositis successfully and the company has announced plans to launch Phase II/III clinical study to investigate iseganan HCl in the prevention of ventilator-associated pneumonia (VAP) (Giles *et al.,* 2002 & Brouwer *et al.,* 2011) Demegen (Pittsburgh) has successfully completed animal studies with peptide D2A21 as therapeutic for several types of cancer and has been developing this peptide gel formulation as a wound healing product to treat infected burns and wounds. (Laederach *et al.,* 2002). Another product of Demegen, P113D derived from histatins, had been granted orphan drug status for the treatment of cystic fibrosis infections. (Sajjan *et al.,* 2001). Periodontix Inc. (Watertown,) has entered phase I clinical trials for the application of a histatin-derived peptide against oral candidiasis. Trimmers (Durham) had successfully completed a phase II clinical trial, in which peptide T-20 reduced the viral load of HIV-infected patients with up to 97% (Wieprecht *et al.,* 1997). Neuprex™, (Xoma Corp., Berkeley) a systemic formulation of the recombinant BPI-derived peptide rBPI 21 has proven to be very effective in treatment of meningococcal sepsis in phase II/III clinical trials and more than 1000 patients have received NEUPREX in clinical studies without any safety concerns. (Horwitz *et al.,* 1996).

CONCLUSION

From the foregoing discussion it may be concluded that AMPs are an important component of innate host defense in a wide range of organisms, from bacteria to humans. Many AMPs act in a manner entirely different from antibiotics and preservatives, they can

complement or, in selected cases, substitute for antibiotics and chemical preservatives. It is encouraging to know that a few peptides have shown potential and desirable therapeutic properties like antimicrobial, antiviral, anticancer and contraceptive activities. There are a wide variety of peptides with different chemical structures and different peptide conformations which all exhibit antimicrobial activity. These peptides however, have certain properties in common. They all have an affinity for membrane lipids and their specificity for microbial membranes in many cases has been shown to be related to the positive charge on the peptide favoring interaction with the exposed anionic lipids of microorganisms. The peptides may form pores in the membrane allowing for leakage of ions and other materials from the cell. The activity of the peptide is explained by mechanisms like carpet, barrel stave, toroidal along with these mechanisms, it shows an intracellular killing activity which affects the nucleic acid of the microorganism.

A wide range of therapeutic application explains the need of AMPs in the clinical field. Production methods of synthetic antimicrobial peptides proved that rDNA technology is the best way to produce a novel antimicrobial peptide. At present several antimicrobial peptides are produced by means of a cloning technology which are in clinical trials for effective treatment of microbial infections. Recent advances have led to new methods of cloning genes for the over expression and purification of proteins. These technologies are faster, easier to use and more flexible. In the future we are likely to witness further improvements, as interest moves from the antibiotics to the antimicrobial peptides and the need to obtain purified antimicrobial peptides to treat several pathogenic infections.

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