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Review

Antimicrobial peptides: premises and promises

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Abstract

Antimicrobial peptides (AMPs) are an important component of the natural defences of most living organisms against invading pathogens. These are relatively small (<10 kDa), cationic and amphipathic peptides of variable length, sequence and structure. 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. Most of these peptides are obtained from different sources like macrophages, neutrophils, epithelial cells, haemocytes, fat body, reproductive tract, etc. These peptides exhibit broad-spectrum activity against a wide range of microorganisms including Gram-positive and Gram-negative bacteria, protozoa, yeast, fungi and viruses. A few peptides have also been found to be cytotoxic to sperm and tumour cells. AMPs are classified based on the three dimensional structural studies carried out with the help of NMR. The peptides are broadly classified into five major groups namely (a) peptides that form α-helical structures, (b) peptides rich in cysteine residues, (c) peptides that form β-sheet, (d) peptides rich in regular amino acids namely histatin, arginine and proline and (e) peptides composed of rare and modified amino acids. Most of these peptides are believed to act by disrupting the plasma membrane leading to the lysis of the cell. AMPs have been found to be excellent candidates for developing novel antimicrobial agents and a few of these peptides show antimicrobial activity against pathogens causing sexually transmitted infection (STI), including HIV/HSV. Peptides, namely magainin and nisin have been shown to demonstrate contraceptive properties in vitro and in vivo. A few peptides have already entered clinical trials for the treatment of impetigo, diabetic foot ulcers and gastric helicobacter infections. In this review, we discuss the source, structures and mode of action with special reference to therapeutic considerations of various AMPs.

Keywords: Antimicrobial peptides; Microbicides; Sexually transmitted infections

1. Introduction

Most living organisms are constantly exposed to potentially harmful pathogens through contact, ingestion and inhalation [1,2]. The survival of such organisms in a microbe-thriving environment depends on a network of host defence mechanisms involving various components [3]. Often during pathogenic invasion, the first line of defence involves the innate mechanisms of immunity which in turn is followed by acquired immune responses involving the activation of T and B cells against specific antigens [4,5]. In contrast to these acquired immune mechanisms, endogenous peptides, which are constitutively expressed or induced (in a few cases), provide a fast and effective means of defence against the pathogen. This group of molecules termed 'antimicrobial peptides' (AMPs) constitutes a primitive immune defence mechanism and is found in a wide range of eukaryotic organisms, from humans to plants to insects [6]. Most of these gene-encoded peptides are mobilized shortly after microbial infection and act rapidly to neutralize a broad range of microbes and share several common properties.

For instance, most of these peptides are less than 10 kDa, have an overall net positive charge, they are hydrophobic and are membrane active [1]. The AMPs discovered so far have been divided into several groups based on their length, secondary and tertiary structure and presence or absence of disulfide bridges. These peptides exhibit bactericidal, fungicidal, virucidal and tumouricidal properties and the fact that they have potential to overcome bacterial resistance makes them promising candidates for therapeutic drugs [3]. This article aims to review in brief the sources of such peptides

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and their classification based on structure and composition, their mode of action and insight into the current data on their antimicrobial activity followed by a brief comment on the peptides that have entered clinical trials.

2. Source of AMPs

So far more than 750 different AMPs have been identified in various organisms ranging from insects to plants to animals including humans [7–15]. Besides these, bacteria themselves produce AMPs and about 50 of them have been isolated from various Gram-positive bacteria especially lactic acid-producing organisms [16]. 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 [3]. These peptides have been grouped based on their primary structure, amino acid composition and their size. Discussed below are the peptides obtained from various species, both invertebrates and vertebrates including humans.

2.1. AMPs from insects

Since the discovery of inducible AMPs in the moth *Hyalophora cecropia* more than 150 such peptides have been identified in various insects [17,18]. These peptides called cecropins are 3, 4 kDa linear amphipathic peptides and demonstrate activity against protozoa and metazoan parasites in addition to bacteria and fungi [19–21]. A better understanding of the peptide production in response to pathogenic invasion came from the fruit fly, *Drosophila melanogaster*. 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 potently antifungal while others exhibit antibacterial properties [19]. Diptericins are expressed in the midgut of the drosophila and reach concentrations up to 0.5 μM during systemic infections. 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 kDa and share sequence similarities with cecropins, mellitins and dermaseptins [22].

Insect defensins were initially isolated from cell cultures of the flesh fly, *Sarcophaga peregrina* [23] and from challenged larvae of the black brown fly, *Phormia terranovae* [24]. Since then, a large number of insect defensins have been characterized from various species (Table 1). These peptides mostly consist of 34–46 residues with exception of the 51-residue defensins characterized in bees [25]. All insect defensins share a consensus motif of six cysteines which form intramolecular disulfide bonds. Thanatin is a 21 amino acid peptide isolated from the hemipteran insect, *Podisus maculiventris*. The peptide shows up to 50% homology with brevinin peptides isolated from frog skin secretions [26]. Yet in another study, Imamura et al. [27] have purified three structurally related AMPs with molecular masses of 8 kDa named acaloleptin A1, A2 and A3 from the haemolymph of the beetle, *Acalolepta luxuriosa*. Acaloleptin A1 showed significant sequence similarity with coleoptemicin and holotricin-2. Another group of peptides, lycotoxins and cupiennin-1 were isolated from the venom of spiders, *Lycosa carolinensis* and *Cupiennius salei*, respectively [28]. These peptides have a charge of about +8 under physiological conditions and have the potential to form helical structures. The peptides exhibit antimicrobial, insecticidal and slight haemolytic activity. Compared with lycotoxins, melittin, a 26-residue peptide isolated from the venom of European honeybee, *Apis mellifera*, exhibits higher haemolytic activity in addition to its antimicrobial effect. Hybrid peptides composed of various segments of cecropin and melittin have been synthesized and showed broad-spectrum antimicrobial activity with low haemolytic activity [29].

2.2. AMPs from other invertebrates

Apart from insects, other invertebrates from various phylla provide rich sources of antimicrobial peptides. Tachyplesins

Table 1
A comprehensive list of antimicrobial peptides from insects

Source	Peptide	Number of amino acids	Antibacterial activity
Stomoxys calcitrans	Smd 1	46	G^+, G^-
	Smd 2	40	G^+, G^-
Drosophila melanogaster	Drosomycin	44	F
	Drosocin	19	G^-
	Diptericin	82	G^-
	Metchnikowin	26	F
Phormia terranovae	Defensin-α	40	G^+, G^-
Sarcophaga peregrina	Sapecin-α	40	G^+, G^-
	Sapecin-β	34	G^+, G^-
	Sapecin-c	40	G^+, G^-
Aedes aegypti	Defensin-α	40	G^+, G^-
	Defensin-β	40	G^+, G^-
Apis mellifera	Mellitin	26	G^+, G^-, H
	Defensin	51	G^+, G^-
	Royalisin	51	G^+, G
Holotrichia diomphalia	Holotricin	43	G^+, G^-
Acalolepta luxuriosa	Acaloleptin	71	G^+, G^-
Hyalophora cecropia	Cecropin	37	G^-
Lycosa carolinensis	Lycotoxin	27	G^+, G^-
Cupiennius salei	Cupiennin	35	G^+, G^-
Podisus maculiventris	Thanatin	21	G^+, G^-
Tenebrio molitor	Tenicin 1	43	G^+, G^-
Mytilus edulis	Defensin	35	G^+, G^-
Androctonus australis	Defensin	37	G^+, G^-

G^+: gram-positive; G^-: gram-negative; F: antifungal; H: haemolytic.

are 17,18 amino acid long peptides isolated from the haemocytes of the Japanese horseshoe crab, *Tachypleus tridentatus* [30]. These β-sheet forming peptides are synthesized as a large 77-residue precursor that contains an acidic amino acid cluster in its C-terminal portion [31]. Polyphemusin, an isoform of tachyplesin has been purified from the haemocytes of *Limulus polyphemus*, a related horse shoe crab [32]. Both types of molecules have in common similar sizes, cysteine contents, intramolecular disulfide-pairing patterns and C-terminal amidation (change sequence). Another peptide, big defensin, was purified from the haemocyte granules of *T. tridentatus* [33]. The C-terminal of this 8 kDa peptide shows significant homology with mammalian β-defensin. A notable feature of this peptide is that the N-terminal of the molecule displays activity against Gram-positive bacteria, while the C-terminal portion is more active against Gram-negative bacteria. *Tachypleus tridentatus* also produces in addition to tachyplesins and big defensin, another peptide named tachycitin. The 73-residue peptide shows a specific binding activity for chitin, an essential component of fungal cell wall [34].

Ehret-Sabatier et al. [35] isolated a 25-residue AMP, androctonin, from the scorpion, *Androctonus australis*. This highly cationic peptide has four cysteine residues involved in the formation of two intramolecular disulfide bridges. The peptide is active against Gram-positive and Gram-negative bacteria and fungi. While in another study Destoumieux and colleagues [36] purified three peptides from the haemolymph of the shrimp, *Penaeus vannamei*. Named penaeidins, these peptides range from 5.4 to 6.6 kDa and exhibit activity only against filamentous fungi but not against *Candida albicans* or *Sacchromyces cerevisiae* [37]. The mussel, *Mytilus edulis*, also has been shown to produce two cysteine-rich antimicrobial peptides viz. 3.7 kDa mytilin and 6.2 kDa mytimycin [38]. While mytilin shows activity against Gram-positive bacteria, mytimycin is active against filamentous fungi.

2.3. AMPs from vertebrates

Defensins were first discovered in rabbit and guinea pig granulocytes as small cationic antimicrobial peptides [39]. Now known as α-defensins, these are small peptides with 29–35 residues with three intramolecular disulfide linkages. Humans are known to possess six α defensins viz. four human neutrophil peptides (HNP 1–4) expressed by granulocytes and certain lymphocytes, while other two (HD-5 and 6) are expressed by intestinal paneth cells [40]. The β-defensins present in humans are of four types viz. human β-defensins (HBD 1–4). These peptides are composed of up to 45 residues and are expressed by the epithelial cells of skin and psoriatic scales [41]. It has been noted that the synthesis of these peptides can be induced by cytokines (e.g. TNF- α and IL-1 β) [42].

Cathelicidins are yet another group of AMPs located at the C-terminal of a 15–18 kDa precursor that contains a highly conserved domain called cathelin (acronym for cathepsin L inhibitor) [43]. Initially isolated from pig leucocytes, these peptides are stored as inactive propeptides in the secretory granules of neutrophils and are released upon cellular stimulation by proteolytic processing [44]. In humans, cathelicidins CAP-18 and LL-37 have been identified in testis [45], squamous epithelia and different lymphocyte/monocyte population [46]. SMAP-29 from sheep, BMAP-28 from cattle and bactenecins Bac-5 and Bac-7 from bovine are cathelicidin-derived peptides exhibiting differential antimicrobial activities [47,48].

Protegrins, isolated from porcine neutrophils are 2 kDa peptides composed of 16–18 amino acids [49]. These membrane-disrupting peptides retain activity even in the presence of physiological concentrations of sodium chloride. Another family of peptides are histatins which are histidine-rich proteins isolated from human saliva displaying a moderate activity against *C. albicans* [50].

3. Classification of AMPs

Nuclear magnetic resonance (NMR) has emerged as a useful technique for studying details of structures of most of the known antimicrobial peptides. Analysis of the three dimensional structure of these peptides has led to the better understanding of their function. Since a majority of these peptides are small in length, their three dimensional structures can be obtained using conventional two dimensional NMR methods [51]. Based on the NMR structures of known peptides along with sequence analysis AMPs are broadly classified into five groups.

3.1. α-Helical AMPs

Much of the structural and biochemical work has been focused on cecropins, which were the first to be identified and characterized [52]. All cecropins have helix-forming tendencies in certain organic cosolvents like trifluoroethanol [53]. Initial studies with NMR showed that cecropin-A from *H. cecropia* exhibited an α-helical pattern in 15% hexafluoroisopropyl alcohol [54]. The results suggested a highly amphipathic helix with hydrophobic and cationic charged surfaces, a motif observed in many other AMPs.

Magainins are another group of well characterized peptides composed of 23 residues isolated from the skin of the African clawed frog, *Xenopus laevis* [55]. NMR studies showed that like cecropins, magainins also form amphipathic α-helical structures in 25% trifluoroethanol [56].

3.2. Cysteine rich AMPs

The human neutrophil peptides HNP-1, -2 and -3 were first of the cysteine-rich peptides isolated from the human granules [57]. These α-defensins are 30 amino acid peptides rich in cysteine residues and are present in a wide variety of organisms. Most of these defensin molecules harbour a consensus motif of six cysteine residues forming three in-

tramolecular disulfide bonds. The positions of the disulfide bridges are mostly between C1–C4, C2–C5 and C3–C6. X-ray crystallography studies with HNP-3, in combination with sedimentation equilibrium centrifugation, suggest that the peptide exists as a dimer [58]. The NMR structure of α-defensin shows the presence of three-stranded antiparallel β-sheets [59]. Drosomycin, isolated from drosophila contain four disulfide bonds and are made up of three antiparallel β strands with an α helix in between the first two strands [60].

3.3. β-Sheet AMPs

A few of the known AMPs form a single β-hairpin structure and are approximately 20 residues long containing one or two disulfide linkages. Horseshoe crab peptides, tachyplesins and polyphemusin II, both share a β-hairpin motif stabilized by two disulfide bonds [61,62]. NMR studies along with 3D structures indicate that tachyplesin shows strong resemblance to protegrins, peptides isolated from porcine leukocytes. Both these molecules forms antiparallel β-sheet connected to a β-turn and are composed of two disulfide bridges [62].

NMR studies with thanatin isolated from the hemipteran insect *P. maculiventris* showed results similar to that of tachyplesin, including an antiparallel β-sheet maintained by a single disulfide bridge. Lactoferricin B, a 25 amino acid proteolytic derivative of lactoferrin in solution adopts a β-sheet structure stabilized by a single disulfide bond, as shown by NMR studies [63].

3.4. AMPs rich in regular amino acids

Some AMPs are composed of high numbers of regular amino acids. The structural conformations of such peptides are different from the regular α-helical or β-sheet peptides. Histatin, a peptide isolated from human saliva is rich in histidine residues and is active against *C. albicans* [64]. While cathelicidins are proline rich peptides and have irregular structures, indolicidins [65] and tritripticin [66] are rich in tryptophan. Bactenecins Bac-5 and Bac-7, like cathelicidins, are proline-rich [67] while the peptide PR-39, is rich in arginine residues [68].

3.5. AMPs with rare modified amino acids

Few peptides are unusual as they are composed of rare modified amino acids. Best examples of such peptides are those produced by the bacteria themselves. Nisin, a lantibiotic, is one such peptide produced by *Lactococcus lactis* and is composed of rare amino acids like lanthionine, 3-methyllanthionine, dehydroalanine and dehydrobutyrine [69]. The peptide is active against Gram-positive bacteria and shows no defined structural conformation in water, while it reveals several β-turn structures when bound to dodecylphosphocholine [70]. Another peptide leucocin A, a 37-residue AMP isolated from *Leuconostoc gelidum*, is shown to form an amphiphilic conformation well suited for interacting with membranes [71]. Such peptides undergo post-translational modification that result in conformations not seen in other classes of antimicrobial peptides. The gramicidins are composed of several DH-amino acids that allow them to form an unusual cyclic β-hairpin [72].

4. Mode of action of AMPs

Although the exact mechanism of action of AMPs remains a matter of controversy, there is a consensus that these peptides selectively disrupt the cell membranes and the amphipathic structural arrangement of the peptides is believed to play an important role in this mechanism. The phospholipids head group charge on cell membranes and peptide charge distribution appears to play an important role in the peptide membrane interactions [73–75]. There is accumulating evidence suggesting that the antibacterial or self defence peptides which are usually highly basic, recognize the acidic phospholipids exposed on the surface of the bacterial membrane [76]. In the case of microbes, the anionic lipids are present on the outer surface of the membrane whereas for mammalian cells, anionic lipids are present along the cytoplasmic side of the membrane. This feature might account for their preferential activity against bacteria but not against mammalian cells. Studies on magainins have shown that liposomal leakage induced by the peptide is related to the nature of the anionic lipid [77]. It has been shown that in liposomes of phosphatidylglycerol, present in bacterial cell membranes with high abundance, the peptide induces effective leakage. Whereas in liposomes of phosphatidylserine present in mammalian membranes, the peptide is less effective in inducing leakage [78]. Melittin, paradaxin, dermaseptins and cecropins are mostly lytic to both bacterial cells and mammalian erythrocytes.

Several structure function studies on AMPs have been published [79–81]. It is well documented that biophysical properties such as secondary structure, overall charge and hydrophobicity influence the interaction of AMPs with model membranes and biological cells. Based on the available data, two models explaining the mode of action of AMPs have been proposed.

4.1. Barrel stave model

The barrel stave mechanism describes the formation of transmembrane channel/pores by bundles of amphipathic α-helices, such that their hydrophobic surfaces interact with the lipid core of the membrane and their hydrophilic surfaces point inward producing an aqueous pore [82]. This pore formation can be confirmed by stepwise conductivity increases in channel measurement. The transmembrane pore formation involves the following steps, namely binding of peptide monomers to the membrane in a helical fashion followed by insertion of the helices into the hydrophobic core of the membrane. Progressive recruitment of additional monomers

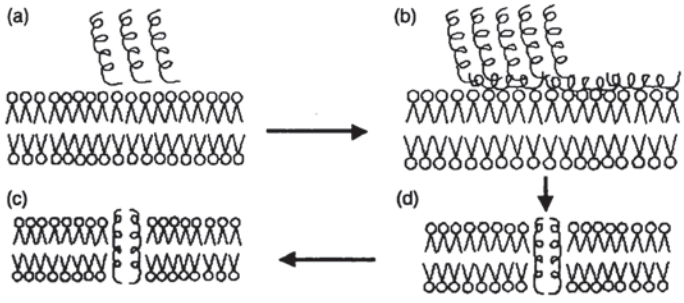

Fig. 1. Pictorial description of barrel stave model for transmembrane pore formation. The peptide monomers bind to the cell membrane in a α-helical confirmation (a), this is followed by the localization of more peptide molecules on the cell membrane (b) after which the peptide helices insert themselves into the hydrophobic core of the membrane (c). Progressive recruitment of additional monomers increase the pore size causing leakage of cytoplasmic material (d) and hence death of the cell.

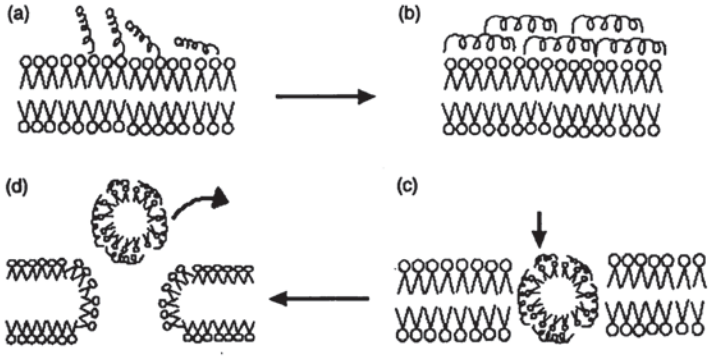

Fig. 2. Pictorial description of carpet model for membrane disruption. According to the model the peptides are in contact with the phospholipid head group throughout the entire process of membrane permeation. The steps involved are as follows: firstly preferential binding of the peptide monomers to the phospholipid head groups occurs (a), this is followed by the alignment of the peptide monomers on the membrane surface such that the hydrophilic residues face the phospholipid head groups (b). Later the peptides reorient themselves towards the hydrophobic core of the membrane (c) followed by the disintegration of the membrane due to disruption of bilayer curvature (d).

increases the pore size leading to leakage of cell contents and thereby death of the cell (Fig. 1). Pore formation accompanies the reorientation of the helix from the parallel state to the perpendicular membrane spanning state. Alamethicin, a member of the peptabiol family, behaves in a similar manner. This peptide forms an open pore consisting of 3–11 helical rods arranged around a water filled pore that forms transmembrane helical bundles or barrel staves.

4.2. Carpet model

This model was proposed for the first time to describe the mode of action of dermaseptin [83]. In this model, the peptides at high concentration are in contact with the phospholipid head group on the outer leaflet of the membrane and cause membrane permeation. The peptides first bind to the surface of the target membrane and cover it in a carpet like manner. In this model, the peptides are not inserted into the hydrophobic core of the membrane nor do they assemble with their hydrophilic surfaces facing each other. The following are the stepwise mechanisms involved in this model. (1) There is preferential binding of the peptide monomers to the phospholipid head groups. (2) This is followed by the alignment of peptide monomers on the surface of the membrane such that their hydrophilic surface is facing the phospholipid head groups or water molecules leading to reorientation of the hydrophilic residues towards the hydrophobic core of the membrane. (3) The peptide disintegrates the membrane by disrupting the bilayer curvature [82] (Fig. 2).

5. Ion channel formation

Besides membrane perturbing activities, AMPs also possess the ability to form ion-channels [84]. Most of the well-studied linear polycationic helical peptides (dermaseptins, cecropins, magainins and alamethicin) form pores or channels that can be assayed by conductance studies in planar lipid bilayers [84,85]. This ability to form transbilayer ion channels is correlated to the helical hydrophilic and hydrophobic components of the peptide. Membrane permeabilization is best studied through electrical measurements on planar

lipid bilayers exposed to the peptide [86]. Single channel and microscopic conductance resulting from the formation of hundreds or thousands of channels are recorded to yield such parameters as voltage dependence, concentration dependence, oligomerization state of the conducting aggregates, channel duration and ion selectivity. 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. These peptides also induce leakage of K+ and other cellular components [86].

6. Other modes of action of AMPs

Apart from ion channel formation, AMPs also have other mechanisms for killing pathogens. Seminalplasmin, isolated from bovine seminal plasma has been hypothesized to lyse bacteria by activating the molecules in the autolysis cascade within bacterial cells [78]. Peptides PP-39 and the mammalian relative of apidaecin have been shown to kill bacteria by a non lytic mechanism. They inhibit protein synthesis and also induce degradation of proteins required for DNA replication. While peptides bac-5 and bac-7 share amino acid profiles similar to PP-39, their mechanism of action is different in that they interfere with the transport and energy metabolism of bacterial cells. Both thrombin induced platelet microbicidal protein-1 and human neutrophil defensin-1 are small cationic peptides that have been shown to affect the structure and function of the cytoplasmic membrane and are involved in the inhibition of protein and DNA synthesis [87]. Another peptide, attacin inhibits the synthesis of outer membrane proteins without actually entering the bacterial cells [88], whereas diptericin increases the permeability of outer and inner membranes of *Escherichia coli* [89].

7. Therapeutic considerations of AMPs

In the early 1970s, it was believed that virtually any bacterial infection could be treated, as a wide range of antimicrobial agents were available. These agents generally known as antibiotics were products isolated from a few fungal and bacterial species. The belief was soon proved false when pathogens emerged resistant to the conventional antibiotics routinely used to treat bacterial infections. This event has resulted in the identification of novel molecules which can resist pathogens developing resistance. One such group of compounds explored for such novel properties are the AMPs.

The therapeutic potential of AMPs are attributed to their membrane lytic properties. The peptides have demonstrated their ability to kill rapidly a broad spectrum of microorganisms including multidrug resistant bacteria, fungi and viruses. They also combat other pathogens including protozoa. Briefly discussed below are antimicrobial properties of known peptides followed by a note on their contraceptive potential and ongoing clinical trials.

7.1. AMPs as anti-microbials

Most of these membrane active peptides have exhibited antimicrobial effects against a wide range of Gram-positive and Gram-negative bacteria [90,91]. Tables 2 and 3 summarize a list of AMPs exhibiting activity against various bacterial species. Haverson et al. [92] have demonstrated that oral administration of human lactoferrin B and its derivative are effective in reducing infection and inflammation of theurinary tract of mice infected with *E. coli*. The possibility of peptide transfer of the lactoferrin or peptides to the site of infection via renal secretion is suggested to be of therapeutic importance in treating urinary tract infections.

In another study, Giacometti et al. [93] have reported that the activity of cationic peptides (e.g. cecropin PI, indolicidin and nisin) against *Pseudomonas aeruginosa* and have observed that the activity of these peptides is enhanced when they are used in combination with clinically used antibiotics like polymyxin E and clarithromycin. Giacometti et al. [94] have also shown that combinations of ranalexin and buforins-1 (coated grafts with cefazolin) treatment was more effective against methicillin-resistant strains of *S. epidermidis* than rifampicin-coated grafts and cefazolin treatment.

Susceptibility of oral bacteria and yeasts (e.g. *Fusobacterium* species and *Candida* species) to mammalian cathelicidins like SMAP-29 and CAP-18, highlight the therapeutic potentials of cathelicidin peptides [95]. Also in vitro studies with human and rabbit defensins and porcine leukocyte protegrins have shown to cause up to 99% reduction in colony forming units of *Mycobacterium tuberculosis* [96].

AMPs have also been tested against various protozoa. Using existing antimicrobial peptide sequences as templates, Arrighi et al. [97] have designed three short novel hybrid peptides, Vida-1, 2 and 3 which showed activity against the sporogenic-stage of the rodent malarial parasites *Plasmod-*

Table 2
Antimicrobial peptides demonstrating activity against fungi

Peptide	Source	Mode of action	Antifungal activity
Gallinacin-1	Chicken	Lysis	*C. albicans*
Lactoferricin-B	Human, bovine	Lysis	*C. albicans*
Defensin NP-1	Rabbit granulocyte	Lysis	*C. neoformans*
Defensin NP-2	Rabbit granulocyte	Lysis	*A. fumigatus*
Defensin HNP-1	Human neutrophil	Lysis	*C. albicans*
Defensin HNP-3	Human neutrophil	Lysis	*C. neoformans*
Protegrin	Human, porcine	Lysis	*C. albicans*
Tripticin	Human, porcine	Lysis	*A. flavus*
Thanatin	*Podisus maculiventris*	Unknown	*A. fumigatus*
Magainin-2	*Xenopus laevis*	Lysis	*C. albicans*
Metchnikowin	*Drosophila melanogaster*	Lysis	*F. oxysporum*
Drosomycin	*Drosophila melanogaster*	Lysis	*F. oxysporum*
Dermaseptin	*Phyllomedusa sauvagii*	Lysis	*C. neoformans*

Table 3
Activity of antimicrobial peptides against sexually transmitted infection causing pathogens

Peptide	Anti-STI/HIV activities	Mode of action
Rabbit α-defensin mcp-1/mcp-2	Herpes simplex virus	Prevents entry and intracellular spread of virus
Human α-defensin-1, -2 and -3	HIV	Coordinated with CD8 antiviral factor (CAF) secreted by CD8 T cells
Human α-defensin	*T. pallidum*	Partially inhibits infection and entry of the pathogen
Rabbit defensin NP-1	*C. trachomatis*	Reduces the number of inclusion bodies
Protegrin	*C. trachomatis*	Prevents uptake of elementary bodies by target cells
	N. gonorrhoeae	Membrane disruption of bacteria
Cathelicidins	*T. pallidum*	Inhibits multiplication of pathogen
Cecropin (D2A21/D4E1)	*C. trachomatis*	Cytotoxic to the pathogen
	HIV	Suppression of viral transcription
Mellitin	HIV	Suppression of viral transcription
Polyphemusin	HIV	Prevents entry of virus

ium berghei and *P. yoelii nigeriensis*. The propionyl and isobutyryl derivatives of K4S4 (1–13), a derivative of dermaseptin peptide, is reported to kill the intraerythrocytic parasite *P. falciparum* without lysing the erythrocyte [98]. DS-01, a 29 amino acid long dermaseptin peptide isolated from *Phyllomedusa oreades* and *P. distincta* displayed activity against *Trypanosoma cruzi* in its trypomatigotic and epimastigotic forms in both cell culture and blood media indicating its therapeutic value to prevent infections during blood transfusion.

AMPs also exhibit a wide range of activity against various fungal species. Cecropins have been shown to be fungicidal against pathogenic *Asperigillus* species and *Fusarium* species at concentrations of 25–100 μg/ml [99]. While non haemolytic antifungal peptides, dermaseptins, are active against *Cryptococcus neoformans* [100]. Table 2 summarizes AMPs having activity against various pathogenic fungal species.

7.2. Activity of AMPs against STI causing pathogens

Few studies have concentrated on the action of AMPs on sexually transmitted pathogens. Studies in a rabbit model have indicated that defensins interact locally with cell surfaces and partially inhibit the entry of *Treponema pallidum* [101]. The human α-defensins 1–3 have been shown to contribute to the anti-HIV-1 activity of CD8 antiviral factor secreted by the CD8 T cells in the HIV infected long term precursors [102]. Recently, Sinha et al. [103] have shown that NP-1, a rabbit α-defensin, prevents the entry and intracellular spread of HSV-2, in vitro.

The activity of protegrins against *Neisseria gonorrhoeae* has been studied. Protegrins PG-1, -2, -3 and -5 are highly active against the pathogen at low micromolar concentrations [104]. The peptides exhibit activity against serum-resistant, serum-sensitive and antibiotic-resistant strains. Yasin et al. [105] have demonstrated the susceptibility of *Chlamydia trachomatis* to protegrins and defensins; while defensins are inactivated in presence of serum, protegrins retain their activity. A list of peptides showing activity against sexually transmitted infection (STI) causing pathogens is summarized in Table 3.

Cathelicidins, originally isolated from porcine neutrophils, have shown activity against syphilis. Sambri et al. [106] have studied the activity of five cathelicidin-derived peptides against the spirochaete *T. pallidum*. While PG-1 (porcine leukocytes) and SMAP-29 (sheep) showed strong antibacterial properties, LL-37 (human testis), CRAMP (mouse) and BMAP-28 (cattle) were less effective.

Recently, Ballweber et al. [107] have studied the activity of two synthetic cecropin peptides D2A21 and D4E1 against *C. trachomatis*. The group also have tested the activity of gel formulations containing 2, 0.5, 0.1 and 0% of the D2A21 peptide against *C. trachomatis*. The formulations were active up to 90% causing complete killing of the organisms. Recently our group has reported that magainin peptides inhibit the growth of various STI causing pathogens [108]. The MIC for various standard and clinical isolates (*E. coli*, *S. aureus*, *C. albicans*, *P. aeruginosa* and *Trichomonas vaginalis*) was found in the range of 200–300 μg/10^6 cfu/ml (Fig. 5).

7.3. Contraceptive potential of AMPs

Apart from antimicrobial activities few peptides have been explored for their contraceptive potential. Our group

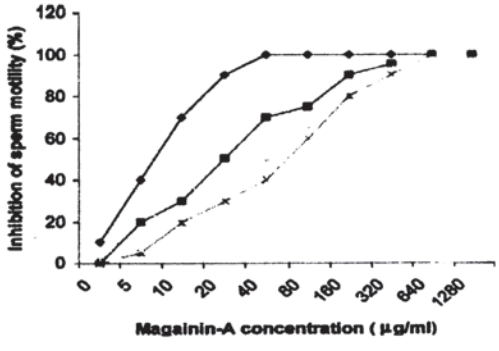

Fig. 3. In vitro inhibition of sperm motility in rats (♦), rabbits (■), monkey (▲) and human (×) by magainin-A. Fresh aliquots of sample were incubated in the presence of different concentrations of Magainin-A and sperm motility was evaluated by sander cramer assay. Sperm immobilization was found to be time and dose dependant.

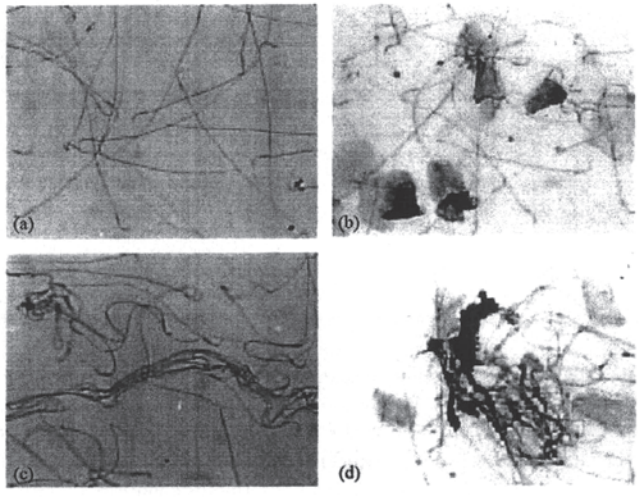

Fig. 4. Spermicidal activity of magainin-A in rat in vitro (a and c) and in vivo (b and d) before (a and b) and after (c and d) treatment. Magainin showed complete sperm immobilization within 20 s at a concentration of 50 μg/ml of sperm obtained from cauda epididymis. Maginin introduced intravaginally at a concentration of 200 μg/100 μl of saline caused complete immobilization of sperm preventing pregnancy (b and d). Samples were stained with papanicolaou (original magnification ×40).

[109,110] has studied the spermicidal activity of magainins in both in vitro and in vivo. Magainin-A caused 100% sperm immobilization at a concentration of 50 μg (rat), 400 μg (rabbit) and 800 μg (monkey and human) (Figs. 3 and 4a and c). The in vivo studies in rats indicated that Magainin-A, when applied intravaginally at the dose level of 200 μg (rat) (Fig. 4b and d) and 1 mg (rabbit and monkey), inhibited conception.

Magainins also exhibit embryotoxic properties as shown by Sawicki and Mystkowska [111]. Initially they showed that magainin demonstrated high toxicity against mouse preimplantation embryos. At a concentration of 250 μg/ml magainin brought about complete killing of mouse oocytes, 1 and 2 cell embryos and blastocysts in 1 h. Recently, Mystkowska et al. [112] have reported that the embryotoxicity of magainin is enhanced in the presence of cyclodextrin, albumin and hydrogen peroxide.

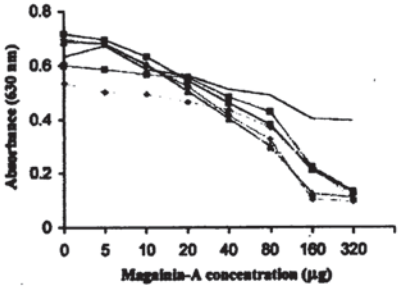

Fig. 5. Dose dependent growth inhibition of pathogens by magainin-A treatment. Standard strains and clinical isolates of various pathogens were incubated with twofold serial dilution of magainin-A. The MIC of magainin-A required to inhibit the growth of pathogens was measured by ELISA. *S. aureus* (■) and *E. coli* (♦) followed by *Candida albicans* (▲) and *P. aeruginosa* (—). The clinical isolates of *E. coli* (♦) and *S. aureus* (■) were less susceptible than their standard counterparts.

Our group was the first to report the contraceptive efficacy of nisin both in vitro and in vivo. Nisin showed a time and dose dependent effect on sperm motility. A concentration of 300–400 μg/ml was found to be sufficient to inhibit human sperm motility with in 20 s in vitro [113]. In vivo studies in rabbits indicated that vaginal administration of 1 mg of nisin stopped sperm motility completely and none of the treated animals became pregnant.

7.4. AMPs in clinical trials

Several AMPs are currently undergoing laboratory testing but few have already reached clinical trials. For instance P-113, a derivative of histatin, a human salivary peptide, is undergoing phase I/II trials to treat oral candidiasis affecting immunocompromised patients [114]. Another study with an indolicidin analogue, MBI-549 is in Phase II trials for treatment of acne infections [115]. Pexiganan, derived from magainin, is being developed for treatment of infected foot ulcers in diabetics [114]. The study is undergoing Phase III clinical trials. While Intrabiotics claim that the peptide protegin PG-1 confers up to 100% systemic protection against intraperitoneal infections caused by *P. aeruginosa*, *S. aureus* and methicillin-resistant *S. aureus*, another derivative of protegrin-1, iseganan is undergoing Phase II/III clinical testing for ventilator associated pneumonia. rBPI-21 derived from a human neutrophil peptide, BPI, is undergoing Phase II/III trials for treatment of severe paediatric meningococcaemia and Crohn's disease. Mycoprex peptides (BPI-derived) are being tested for activity against systemic candidiasis in rat models.

Gough et al. [116] have demonstrated anti-endotoxin activities of α-helical peptides MBI-27 and MBI-28 and have shown that the peptides confer protection against peritoneal infections caused by *P. aeruginosa* in mouse models. Indolicidin is expected to protect mice against systemic fungal infections [117]. The lantibiotic nisin has been developed commercially by Astra and Merck for treatment of gastric helicobacter infections and ulcers, while other nisin variants (nisin-A and Z) have entered preclinical trials for treating vancomycin-resistant enterococci.

8. Conclusions

From the above studies it is understood that AMPs are an important component of innate host defence in a wide range of organisms, from bacteria to humans. It is encouraging to know that a few peptides have shown potential and desirable therapeutic properties like antimicrobial, antiviral, anticancer and contraceptive activities. Also they have been shown to protect against topical and systemic infections in combination with conventional antibiotics. Another encouraging prospect has been the entry of a few such peptide based formulations into phase I/II clinical trials [117].

In spite of all the positive facts associate with AMPs, there have been a few problems. Firstly, there are fewer data available on the unknown in vitro/in vivo toxicities of the peptide [118]. Secondly, the stability of the peptide/peptide-formulations in vivo has not been studied in detail. And lastly the cost of production of these peptides on a large scale has been a major obstacle for quite some time.

Subsequently, these issues have been sorted out one by one. The problem of stability has been removed by using the peptide formulations in combination with a protease-inhibitor or by modifying the amino acid composition enabling them to be recognized by the proteases. The problem of large scale production has been taken care by the growing advent of recombinant synthesis technology which is effective and cheaper. Hence, the present foci would be to identify more of such novel peptides, re-design the existing peptides to get rid of their toxicity and develop novel recombinant protocols to obtain greater yield of peptides at a lower cost.

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