EXPERT REVIEWS

Antimicrobial peptides: therapeutic potentials

Expert Rev. Anti Infect. Ther. 12(12), 1477–1486 (2014)

Su-Jin Kang[1], Sung Jean Park[2], Tsogbadrakh Mishig-Ochir[3] and Bong-Jin Lee*[1]

[1]*Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul, 151-742, Korea*
[2]*College of Pharmacy, Gachon University, 534-2 Yeonsu-dong, Yeonsu-gu, Incheon, Korea*
[3]*Department of Biophysics, Faculty of Biology, National University of Mongolia, Ullanbaatar, Box-377, Mongolia*
**Author for correspondence:*
Tel.: +82 2880 7869
Fax: +82 2872 3632
lbj@nmr.snu.ac.kr.

The increasing appearance of multidrug-resistant pathogens has created an urgent need for suitable alternatives to current antibiotics. Antimicrobial peptides (AMPs), which act as defensive weapons against microbes, have received great attention because of broad-spectrum activities, unique action mechanisms and rare antibiotic-resistant variants. Despite desirable characteristics, they have shown limitations in pharmaceutical development due to toxicity, stability and manufacturing costs. Because of these drawbacks, only a few AMPs have been tested in Phase III clinical trials and no AMPs have been approved by the US FDA yet. However, these obstacles could be overcome by well-known methods such as changing physicochemical characteristics and introducing nonnatural amino acids, acetylation or amidation, as well as modern techniques like molecular targeted AMPs, liposomal formulations and drug delivery systems. Thus, the current challenge in this field is to develop therapeutic AMPs at a reasonable cost as well as to overcome the limitations.

Keywords: AMPs in drug development • antimicrobial peptides • limitations of AMPs • multidrug resistance • strategies for new therapeutic drug

For over 80 years after the discovery of the first antibiotic penicillin, antibiotics have been considered as a wonder drug against various infections, and the development of many different kinds of antibiotics has improved people's quality of life [1–3]. However, these drugs have been used so widely and for so long that antibiotics abuse has raised another concern, which is frequent emergence of multidrug-resistant microorganisms. This situation includes overuse not only in the hospital but also in industrial farming. Actually, essential human pathogens such as *Staphylococcus aureus*, *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa* have showed increased resistance against existing antibiotics, and some of them cannot be killed by any of the antibiotics at all [4,5]. Despite continuing efforts on combating multidrug resistance, spreading of resistant pathogenic bacteria to conventional antibiotics has become a major global concern. In 2014, WHO's report on global surveillance of antimicrobial resistance using data provided by 114 countries shows that the very high rate of resistant bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *S. aureus* and *M. tuberculosis* still is a threat to global public health in all WHO regions, resulting in health and economical burden. As an example, the death rate of patients with methicillin-resistant *S. aureus* is estimated to be about 64% more than that of people with infection caused by a nonresistant form. To overcome this issue, new alternatives of antibiotics, which have different mechanisms of action compared to conventional antibiotics, are definitely required [6]. In this regard, one of the promising therapeutics is the natural antimicrobial peptides (AMPs) [7–9]. AMPs are defensive weapons in the animal and plant kingdoms. They show strong antimicrobial activity against a very broad spectrum of microorganisms, such as gram-negative and gram-positive bacteria, fungi, parasites and viruses, via various mechanisms of action [10]. In addition to direct effects on microorganisms, AMPs seem to control the innate and adaptive immune responses by raising the accumulation of immune cells such as macrophages, lymphocytes and so on [11]. Moreover, they cancel out the toxicity of lipopolysaccharide endotoxin from gram-negative bacteria by stimulating angiogenesis [12]. These findings may be related to the altered immune responses by AMPs [13]. Furthermore, they are also capable of removing microorganisms with great resistance to conventional drugs because the microorganisms can be killed by rapid and direct action of AMPs [2,14–16]. Taken together, AMPs have

 10.1586/14787210.2014.976613 ISSN 1478-7210

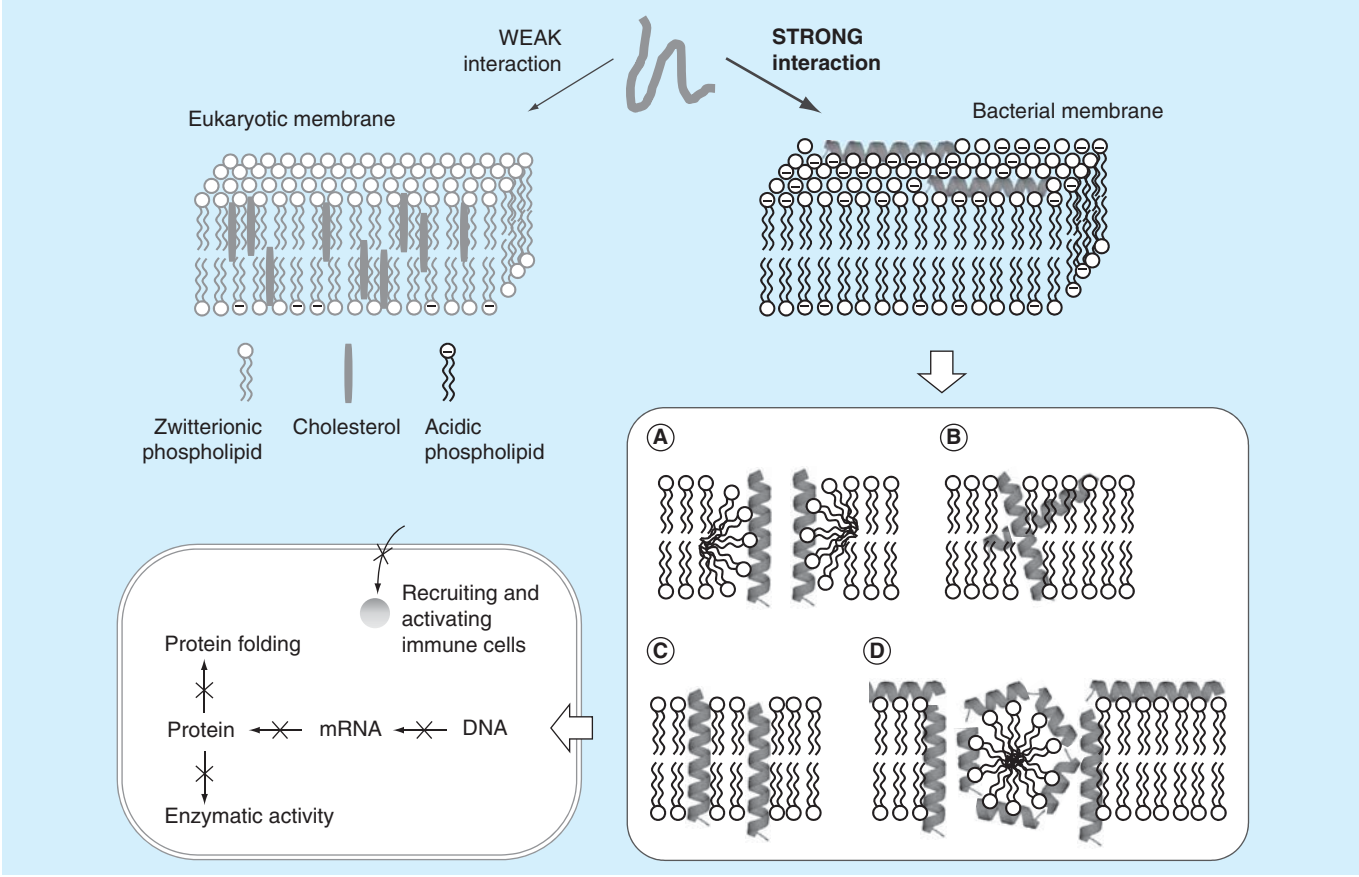

Figure 1. Mechanisms of action of antimicrobial peptides. Because the composition of the eukaryotic cell membrane and the bacterial membrane is different, cationic antimicrobial peptides strongly interact with the bacterial plasma membrane. **(A)** Toroidal pore model, **(B)** aggregate model, **(C)** barrel-stave model and **(D)** carpet model. After penetrating the bacterial membrane using one of the four mechanisms of action, antimicrobial peptides can inhibit the synthesis of DNA, mRNA and proteins and can inhibit cell wall synthesis, recruitment and activation of immune cells and enzymatic activity.

several benefits in the treatment of infections, and development of AMPs has been considered as a promising strategy for the generation of novel antibiotics.

General characteristics of AMPs

Naturally occurring AMPs are short polypeptides of which lengths in sequences are at most 60 amino acids and typically the lengths are between 15 and 40 residues. Almost all of them have a positively charged surface. The number of basic amino acids such as Arg, Lys and His is usually in excess of +2 to +9 (most commonly +4 to +6) [17]. While AMPs are generally unstructured in aqueous solution, a membrane mimetic environment causes AMPs to form a structure and induces significant amphipathicity, which means that hydrophilic residues lie on one side of the peptide and hydrophobic residues lie on the other side [14,18–21]. The selective interaction with the membrane of microorganisms generally depends on the cationic nature of AMPs, and the perturbation of membrane structures by AMPs generally depends on the amphipathic nature of AMPs. The mode of action of AMPs are related to the interaction with bacterial cell membranes [22,23]. In the first stage of their action, cationic AMPs are diffused to the negatively charged surfaces of the microbial membranes within which acidic polymers such as lipopolysaccharide and teichoic acids are found [2,20,24] In contrast, positively charged AMPs have difficulty binding to the mammalian cell membrane since the composition of the mammalian cell membrane is quite different from that of the bacterial membrane. Compared to the bacterial membrane, neutral phospholipids such as phosphatidylcholine, phosphatidylethanolamine and/or sphingomyelin are abundant in the mammalian cell membrane [25] as shown in Figure 1. Upon binding to membranes of pathogens, linear AMPs usually adopt a helical structure with significant conformational changes. Subsequently, these are attached to the interface between the hydrophilic head groups and the fatty acyl chains of the membrane phospholipids and then form membrane pores by oligomerization. The formation of membrane pores, through which the cellular components can be leaked, affects the viability of pathogenic bacteria [26].

Currently, several complex and controversial mechanisms of action exist as to how AMPs disrupt the bacterial cell membrane. The four popular models are the toroidal pore model, the aggregate model, the barrel-stave model and the carpet model [23,27–29]. The toroidal model explains the disruption of

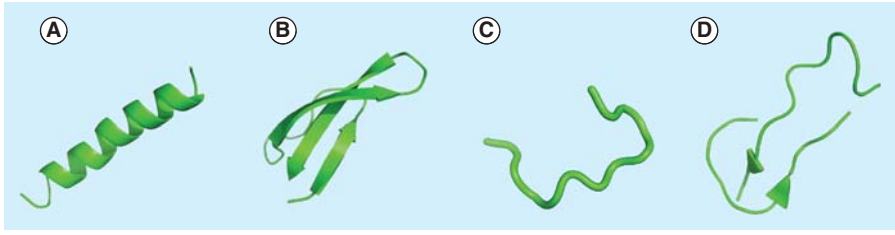

Figure 2. Four representative structural classes of antimicrobial peptides.
(A) α-helical (magainin-2, PDB ID: 2MAG), **(B)** β-sheet (defensins, PDB ID: 2LXZ), **(C)** extended (indolicidin, PDB ID: 1QXQ) and **(D)** loop (microcin, PDB ID: 1S7P) peptides.

the bacterial membrane as follows: when the peptides meet the lipid bilayer, they are perpendicularly incorporated into the cell membrane, in which hydrophobic residues of peptides interact with the hydrophobic region of the membrane forming a pore. As the peptides keep curving inward into the membrane, the pores continuously become larger and larger, resulting in irreversible membrane disruption. In the aggregate channel model, the peptides bind to the head groups of phospholipids in the lipid bilayer and are inserted into the membrane by randomly aggregating with lipids. Aggregation of peptides and lipids resembles micelles and occurs without a particular orientation. These aggregates span a wide range of membrane surfaces and provide channels for ion leakage through the membrane. The barrel-stave model emphasizes the surface electrostatic characteristic upon binding to the outer membrane in bacteria. After binding, α-helical or β-sheet amphipathic peptides assemble on the surface of the membrane forming a stave in a so-called 'barrel-shaped cluster.' These AMPs are perpendicularly incorporated into the membrane like in the toroidal model. The continued recruitment of peptide monomers makes the size of a channel or a pore larger, finally resulting in the release of cell contents from bacteria. In the carpet model, the peptides interact electrostatically with the cell membrane and orient parallel to the membrane surface covering it like a carpet. Peptide micelles complexed with membrane components act like a detergent that disrupts the structure of lipid bilayers. As a result, the formation of worm holes over the membrane causes abrupt lysis of bacteria. Whatever the mechanism of action is, AMPs directly kill the bacteria by penetrating the bacterial membrane or by affecting intracellular targets independently or synergistically with membrane disruption. After entering into the cell, AMPs show various actions such as inhibition of synthesis of DNA, mRNA and proteins and inhibition of cell wall synthesis, recruitment and activation of immune cells and enzymatic activity [30,31].

AMPs can be classified into four groups based on their secondary structures and physiochemical characteristics: linear, mostly α-helical peptides; β-sheet peptides with two or more disulfide bridges; extended linear peptides, which are rich in Trp, Pro and/or His residues; and loop (cyclic) peptides formed by a disulfide bridge [14,19–21]. The α-helical peptides, including magainin, cecropin, pexiganan and temporin, adopt random structures without rigidity in water solution, but undergo conformational changes in a hydrophobic membrane environment adopting amphipathic helices. β-sheet peptides, including defensins and protegrins, show more ordered structures for which rigidity is normally dependent on the presence of intramolecular disulfide bonds. In contrast, the extended peptides such as indolicidin and loop peptides such as microcin are relatively unstructured [32]. The structure of the representative peptides in each group is shown in Figure 2. Of these four groups, α-helical peptides are the most studied group because the largest population of AMPs discovered from the nature adopts α-helical conformation.

AMPs in drug development

Several AMPs have been successfully developed for pharmaceutical and commercial applications [33]. By now, structure and sequence information on nearly over 2000 AMPs from various sources can be found in the worldwide databases [34,35]. Representative AMPs in clinical trials are summarized in Table 1.

P-113 (Histatins)

The Periodontix Company is developing P-113 which is a derivative of histatin. Histatin is naturally found in the human saliva and consists of 12 amino acids with a few histidine residues. It can be orally applied to prevent gingivitis and periodontal disease [36,37]. P-113 showed potent *in vitro* bactericidal and also fungicidal activity against *Candida albicans* and common gram-positive and gram-negative pathogens. Phase I and II clinical trials of P-113 for oral candidiasis in HIV patients are completed. Phase I and II clinical studies have indicated that P-113 is safe for daily oral use as a mouthwash or a gel formulation [4,38]. The use of this peptide was shown to prevent gingivitis and gum bleeding without any side effects. In addition, the possibility of using these peptides in mucoviscidosis patients for the treatment of lung infections caused by *P. aeruginosa* is under consideration as a topical antibiotic [27]. The license for P-113 was transferred from Periodontix to Demegen company. Demegen had finished a Phase IIb dose-ranging clinical trial demonstrating positive results. Demegen is developing P-113 in a rinse formulation for the treatment and prevention of oral candidiasis [39].

MSI-78 (Pexiganan)

The original peptide of MSI-78 is the cationic peptide magainin. Magainin was first discovered from the skin of the African clawed frog *Xenopus laevis* in 1987. The mechanism of action is related to the 'pore-formation' model and it showed broad-spectrum antibacterial activity [4,14]. MSI-78 is an analog of magainin 2 with 22 amino acids. It showed excellent *in vitro* broad-spectrum bactericidal activity against clinically isolated 3109 bacteria [4]. Its application has been proposed for the treatment of diabetic foot ulcers caused by infections [40]. A Phase II clinical trial involving 835 patients with infected

Table 1. Antimicrobial peptides in clinical trials.

Name	Manufacturer	AMP type (species)	Structure	Application	Results of clinical trials	Planned clinical trials	Ref.
Histatins (P-113)	Demegen, Pittsburgh, PA Dow Pharmaceutical Sciences, Patuloma, CA	Histatins (human)	Nonlinear α-helical peptide	Mouthwash	Phase I and II clinical trials (mouthwash in HIV-infected patients with candidiasis) and a Phase IIb dose-ranging clinical trial were finished successfully	On developing in a rinse formulation	[4,38,39]
Pexiganan (MSI-78)	Genera Plymouth Meeting, PA (known previously as Magainin Pharmaceutical Inc.)	Magainin 2 (*Xenopus* frog skin)	α-Helix	Cream	Phase III clinical trials did not demonstrate any advantages over conventional antibiotics for the treatment of impetigo and diabetic ulcers; not approved by US FDA	Another Phase III clinical trials and on developing as a topical cream formulation named Locilex	[4,41]
Omiganan (MBI-226)	Microbiologix Biotech Vancouver, BC (Canada)	Synthetic analog of indolicidin (bovine)	Nonlinear α-helical peptide	Cream	Phase III clinical trials (topical application for the prevention of bloodstream infections caused by catheterization) were unsuccessful	Another Phase III clinical trial for the prevention of catheter-related infections	[27,95]
Iseganan (IB-367)	Intrabiotics Pharmaceuticals, Inc. Mountainview, CA	Protegrin (pig leukocytes)	Peptide containing two disulfide bonds	Mouthwash	Phase III clinical trial (mouthwash for stomatitis, used as an aerosol in pneumonia) was unsuccessful	Temporarily stopped	[49–51]
Neuprex (rBPI21)	Xoma, Berkeley, CA	BPI (bacterial permeability-increasing protein histatin (human))	Cationic 450 amino acid residues	Injections	Phase III clinical trial (treatment of meningococcemia in children) was successful	On developing as Neuprex T cream	[97,98]

AMPs: Antimicrobial peptides.

diabetic foot ulcers showed that the infection was cured or improved in 90% of the patients [4]. Furthermore, Phase II clinical studies of MSI-78 proved that it has sufficient efficacy in wound healing without significant toxicity problems. However, this promising new drug could not be approved by the FDA since it showed similar efficacy as existing antibiotics for infected diabetic foot ulcers [4]. Currently, Dipexium Pharmaceuticals has developed MSI-78 as a topical cream formulation named Locilex (pexiganan cream 0.8%). In 2014, Dipexium company has initiated Phase III clinical trials for establishing the clinical superiority and safety of Locilex [41].

MBI-226 (Omiganan)

MBI-226, Omiganan, is a synthetic analog of indolicidin and is a 12 amino acid residue, cationic AMP. Among many derivatives of indolicidin, Omiganan was selected for clinical development because it showed rapid microbial activity. The mode of action is to disrupt the cytoplasmic membrane of gram-positive and gram-negative bacteria like similar AMPs. It has several benefits such as it can be applied topically up to 5% concentration without toxicity and resistance [4,42–44]. Unfortunately, Phase III clinical trials for the prevention of infectious complications after blood vessel catheterization failed. This is because the study involving more than 1400 patients did not show statistically significant evidence of higher efficacy compared to conventional antibiotics such as povidone iodine [27]. Omiganan 1% gel, named Omigard, is now under another Phase III clinical trial for the prevention of catheter-related infections as topical treatment by Carrus Capital Corporation (formerly BioWest Therapeutics) [45]. In addition, Cutanea Life Sciences Inc. has developed a Phase II clinical trial for the treatment of rosacea [46].

IB-367 (Iseganan)

IB-367, Iseganan, is an analog of protegrin 1 which is found in pig leukocytes. IB-367 consists of 18 amino acids forming a β-hairpin structure stabilized by two disulfide bonds [47,48]. This cationic

AMP has both broad-spectrum antibacterial and antifungal activity [4]. A Phase I clinical study demonstrated its safety and provided the first evidence of efficacy by the reduction of microorganisms by mouth treatment over 10 days. Phase II clinical trials involving 134 patients indicated that IB-367 decreased the population of bacteria in infected lungs and helped in recovering pulmonary function in cystic fibrosis patients. IB-367 was intended to be developed as a local mouthwash to inhibit ulcerative oral mucositis for patients who are susceptible to infection with high risk. The company, IntraBiotics Pharmaceuticals, tried a Phase III clinical study with 502 patients receiving stomatotoxic chemotherapy. Unfortunately, this trial was not successful because of a dispensing error of a portion or the clinical supplies during the trial, resulting in consequent reduction of statistical power. In addition, another Phase III trial with 545 patients receiving radiotherapy for head-and-neck malignancies did not show any clinical improvement in ulcerative oral mucositis [49,50]. As a result, the development of IB-367 was stopped for this particular indication [51].

rBPI21 (Neuprex)

rBPI21, Neuprex, is a recombinant short peptide that originates from the N-terminal region of human bactericidal/permeability-increasing protein (BPI) [38,52]. rBPI21 is potently bactericidal against *Neisseria meningitidis* and has enhanced antibiotic activity. In addition, it binds and neutralizes endotoxins and inhibits angiogenesis [53]. It is being developed by Xoma Ltd. as an injectable formulation named Opebacan. The Xoma company launched Phase I and II clinical trials of rBPI21 for patients suffering from allogeneic hematopoietic stem cell transplant and the results showed the reduction of lipopolysaccharide-induced inflammatory sequelae [54]. Other Phase I and II clinical trials of rBPI21 were conducted by the same company, resulting in the reduction of inflammatory complications in pediatric patients who had undergone open heart surgery [54]. In 2006, a Phase III clinical trial involving 393 children with severe meningococcemia, the most prevalent bacterial infection in babies and children, was carried out in the UK and the USA, demonstrating a reduction in mortality. Neuprex was designated to Orphan Drug status for meningococcemia by the FDA and the EMA [55]. Neuprex is currently developing Neuprex T cream, a fast-acting, liposome-based, topical product.

The above-mentioned five AMPs are representative AMPs which have been most actively developed for clinical use. Although they are the most studied among around 2000 AMPs which are discovered to date, they could not get FDA approvals as authentic medicines yet. They easily passed Phase I clinical trials but could not step over the hurdles of Phase II and/or Phase III clinical studies. In addition, their administrations are limited to mainly injectable formulations or topical such as mouth rinse. It may be because they could not show better activity than conventional antibiotics and they have physicochemical problems such as toxicity and stability. Furthermore, their sizes, from 12 to 22 amino acid residues, are still too long to be developed for antibiotics with an effective administration and a reasonable production cost. These clinical application problems will be discussed in the following section.

Limitations into the market

Despite the great potential of AMPs including their wide-spectrum bactericidal activity, rapid onset of action and low resistance mechanism due to the physical action of AMPs, they still have several problems with application to clinical cases.

The first disadvantage is their possibility for toxicity. While the natural bacterial cell membrane which can be a direct target of AMPs contains negatively charged lipids (20–25%), the human cell membrane possesses zwitterionic lipids and indispensable cholesterol [23]. Usually, cationic AMPs use this difference for their selective antimicrobial activity by interacting with the negatively charged bacterial membrane. However, a number of studies have shown that mammalian cells could also be the targets of these molecules, that is, AMPs could directly bind to host cells, resulting in undesirable effects [56,57]. In addition, administration of broad-spectrum AMPs can eliminate the entire indigenous microflora found in humans which can cause other severe infections due to the absence of protective function of microflora. AMPs could also bind to various host components, such as extracellular surfaces, the extracellular matrix and the host cellular membrane [58]. Thus, all clinical trials up to date are limited to topical application for surface infections rather than parenteral and oral administration [59]. However, it should be possible to develop synthetic AMPs which can overcome the above obstacles by modifying the physicochemical features.

The second disadvantage of natural AMPs is related to their physical stability under physiological conditions. Potentially, they have lability to proteases, serum, salt, pH, etc. Because AMPs mainly use electrostatic interactions with bacterial membranes to adopt ordered structures, the structural stability can be affected by ionic strength in the drug solution. The salt can neutralize the cationic properties and, therefore, prevent the initial electrostatic interaction of AMPs with the bacterial surface.

The instability of AMPs is often a problem when they are clinically applied [60]. The short half-lives of AMPs that may or may not be caused by proteolysis make chronic administration of AMPs quite problematic with high cost. In regard to proteolysis, the linear structure of AMPs can be easily attacked by host proteases and peptidases [61–63]. Moreover, proteolytic activity of serum is strong, which can provide unfavorable circumstances to the administered AMPs into blood [64]. That is why AMPs are usually used for the treatment of skin infections associated with burns, diabetic wounds, the eyes and gum infections in practice. Thus, to overcome the limitation of application, the escaping strategy for protease degradation of AMPs is required.

There are still other limitations impeding the clinical use of AMPs as therapeutics. The principle issue hindering the development of AMPs is that the production cost is extremely high. Compared to small chemical drugs, the production costs of AMPs is considerable. It is known that the production of 1 g of peptide costs $100–600 by general chemical synthesis. During manufacturing, numerous technical difficulties in the

Table 2. Strategies for new therapeutic drug development.

Limitations	Strategies
Stability	Cyclization D- or nonnatural amino acids Acetylation, amidation Modification of amphipathic balance Reducing cationic residue content
Toxicity	Control of hydrophobicity Molecular targeted AMPs Polymeric nanoencapsulation Liposomal formulations PEGylation Drug delivery systems
Cost	Size reduction *De novo* synthesis New expression system Cost–effective purification

AMPs: Antimicrobial peptides.

synthesis and purification process additionally exist [19]. In this regard, pharmaceutical companies have tried to shift their strategies for developing AMPs from more expensive, larger natural peptides to much cheaper, smaller synthetic analogs [16]. The most successful example of cost reduction in manufacturing AMPs is plectasin. Mygind *et al.* (2005) have demonstrated that use of the fungal expression system for plectasin can efficiently reduce the high cost with high yields of plectasin [16].

Strategies for new therapeutic drug development

To develop new antibiotics from AMPs, the fundamental issues of stability, toxicity and cost are now being targeted by a number of distinct strategies (Table 2).

Stability enhancement

First of all, the cyclization of peptides by linking the C- and N-terminus is a well-known method to improve both microbicidal activity and serum stability, in comparison to the linear peptide form [25,32]. This is because the increased bulkiness by cyclization can decrease opportunity to contact with proteases due to steric hindrance [65,66]. A comparison study of linear and cyclic forms of an indolicidin analog CP-11 has demonstrated that head-to-tail cyclization improved stability and resistance against proteolysis [67]. By adding two cysteine residues at both N- and C-terminals, the linear CP-11 peptide was cyclized resulting in a more constrained structural framework. The compact structure of cyclo-CP-11 could sterically protect its basic residues which are selective targets against trypsin-like proteases in the human body.

Otherwise, substitution of vulnerable residues to proteases, use of unusual amino acids and/or change of their physiochemical properties can be other ways to protect from degradation [25,32,68–71]. Several methods for escaping degradation have been introduced. The introduction of D- or nonnatural amino acids: since host proteases can recognize and hydrolyze natural abundant L-amino acids, replacement to D- or nonnatural amino acids can protect the peptides from proteolytic enzyme degradation. A study of the EFK17 peptide, the internal segment of LL-37, demonstrated effectiveness of D-isomerization in AMPs [66]. End modification of the N-terminus and C-terminus of peptides through acetylation and/or amidation: the N-terminal acetylation of peptides enhanced stability against proteases [72,73]. The C-terminal amidation usually improves peptide activity and decreases hemolytic activity by reducing a negative charge in the peptide and stabilizing amphipathic helix formation [74]. N-terminal acetylation and/or C-terminal amidation of hexameric AMPs from lactoferricin showed enhanced proteolytic resistance against exopeptidases [74]. Modification of amphipathic balance in peptides: several model peptide studies using Lys and Leu, which have the propensity to form a helix, suggested that alteration of the amphipathic ratio in AMPs affects α-helicity, resulting in improvement of their stability [75]. Another study which adjusted acidic and basic residues in mastoparan peptides showed improved stability and biological activity of peptides [76].

Fast degradation of cationic AMPs usually results from cationic residue content such as Arg and Lys in the sequence. Kim *et al.* placed the Pro residue at the carboxyl side of the Arg and Glu residue between two Lys residues to protect cleavage by trypsin when they designed stable peptides. In addition, they substituted Arg with Lys because trypsin has a higher affinity to Arg than to Lys [77]. To avoid this degradation, Agp (α-amino-3-guanidino-propionic acid), which is a derivative of arginine, was also introduced through 9-fluorenylmethoxycarbonyl (Fmoc)-$_L$-Agp(Boc)$_2$-OH. This study had proven that the use of Agp instead of Arg could dramatically reduce the cleavage of arginine-rich short model peptides [78].

Toxicity reduction

As for the toxicity of AMPs, high hydrophobicity is related to increased hemolytic activity [79,80]. Control of hydrophobicity and charge can reduce the toxicity of AMPs and closely link the improved selectivity to bacteria. Several analogs from brevinin-1EMa, a frog AMP, were designed by amino-acid substitution to monitor the effect of hydrophobicity. An analog peptide in which Leu was substituted with Ala in the hydrophobic side of the amphipathic helix showed decreased hydrophobicity, showing lower hemolytic activity against human blood cells and a higher therapeutic index than the parent molecule [81]. Another study controlling hydrophobicity of mastoparan-X analogues was also tried in order to search for improved membrane selectivity. They designed several analogs of the mastoparan-X peptide by substitution with Ala and/or Leu at positions 1, 8 and 14 and compared their hemolytic activities. This study showed that reduction in hydrophobicity near the N-terminal was much more effective in reducing the toxicity problem than reduction near the C-terminal [82].

Likewise, the improvement of selectivity by design of molecular targeted AMPs, such as bacterium-selective AMPs, specifically targeted AMPs and environment-sensing AMPs, can be a solution for reducing toxicity [15,68]. A synthesized glycine-rich peptide, Adepantin 1, which is highly bacterium selective, is an example of this

approach. Adepantin 1 was developed through statistically screening the database of natural helical AMPs against gram-negative bacteria. Considering the amino-acid preference to gram-negative bacteria in AMPs, MIC, therapeutic index and so on, Adepantin 1 was derived as the best candidate. Resultantly, it showed good bactericidal activity with improved selectivity [83]. Another example of specifically targeted AMPs is a fusion peptide, M8G2. Generally, a fusion peptide has two domains: one is a domain for selectively targeting bacteria and the other is a domain for bactericidal action. The two domains are connected with a short linker. The targeting domain consists of species-specific monoclonal antibodies or target bacterial-specific pheromones and delivers the AMP domain by binding to the bacterial surface [15,68,84]. The M8G2 peptide is conjugated to the targeting domain, which can specifically recognize *Streptococcus mutans* because it was made from a pheromone produced by *S. mutans*. Therefore, this peptide selectively eliminates the plaque bacterium *S. mutans* from the normal microflora in humans [84]. Environment-sensing AMPs use the changes in the surrounding induced by pathogenic organisms. For example, the AAP2 peptide was designed to be activated at acidic pH but not at physiological pH 7.5. Under low pH conditions triggered by highly populated bacterium such as *S. mutans*, this AMP can show maximum activity and successfully remove infected microorganisms [85].

Recently, other techniques such as polymeric nanoencapsulation of peptides [86] and liposomal formulations [87] have been applied to solve stability and toxicity problems. Encapsulation of AMPs using nanoparticles such as nanospheres and nanovesicles could improve therapeutic activity and stability at the same time, which was demonstrated in the study of the P34 peptide encapsulated in phosphatidylcholine nanovesicles [88]. In addition, as shown in several examples such as indolicidin and polymyxin E, their liposomal formulations reduced peptide toxicity and increased plasma half-life maintaining their original activity [89]. Moreover, PEGylating peptides and usage of drug delivery systems have been newly introduced in this field [90]. The PEGylated M33 peptide showed its increased resistance to peptidase and decreased toxicity, resulting in improving peptide activity [91]. As a drug delivery system, human serum albumin is a good carrier of peptides to reduce toxicity [92].

Production cost

In terms of industry, the final goal of development is headed toward production of smaller AMPs with sufficient stability. This issue is mainly related to the production cost of AMPs. Size reduction and/or *de novo* synthesis of natural AMPs have been tried to reduce the production cost. For example, Won *et al.* tried to synthesize the shortest AMP analogs from Gaegurin 5, which showed good antimicrobial activity in their former studies. They found that 11 residues are the marginal length showing activity, and the location of Trp4 and Trp8 is important in the activity. Furthermore, to develop simple and short AMPs, they engineered a LK model of AMP based on their results [93]. Faccone *et al. de novo* designed a group of cationic peptides with different sizes and physicochemical properties. In this study, they tried to find the smallest peptide retaining biological activity, and finally proved that the P5 peptide with 18 amino acid residues showed the best antimicrobial activity and low hemolytic activity [94].

However, if the chemical synthesis of active AMPs is not possible, new production strategies can be considered to cut production costs. Development of a suitable expression system is the most common, as seen in the example of plectasin, of which the fungal expression system was helpful to obtain a high yield of peptides [16]. In addition, the improved solubility of AMPs using a fusion expression system that contains a solubility-enhancing moiety could increase efficiency in producing AMPs, leading to a reduction in cost [25]. Recent studies have shown that thioredoxin and small ubiquitin-related modifiers can be used for improving the solubility of peptides and finally producing high yields of peptides [95–97]. Moreover, a cost–effective purification method using the intein system can be introduced to reduce the production cost [98].

Expert commentary & five-year view

Recently, an increase in pathogen resistance to antibiotics has been observed worldwide. Antibiotic resistance has a great social and economical impact and is regarded as a threat to national security by developed countries. During the past 5 years, the pharmaceutical industry has spent more than 30 billion dollars in the development of new antibiotics [27]. AMPs are considered as an important class of antibiotics due to their novel mode of action, but very few peptides are extensively used in clinical practice. Academic research suggests that AMPs are a viable therapeutic alternative, and many of the original problems intrinsic to AMPs have been overcome. Moreover, recent researches and clinical trials have shown some possible solutions. In 2012, the FDA and the EMA announced to support the development of new antimicrobial agents with new guidelines and budgets. As seen in many successful trials, AMPs have proved excellent possibilities in clinical application to develop a novel class of antibiotics. We believe that these concerns and efforts regarding cationic peptide antibiotics will produce innovative technologies and create new antibiotics.

In conclusion, it is expected that much more AMPs will substitute resistance-acquired antibiotics as therapeutics in the near future, although a limited number of AMPs are currently being used clinically. Currently, numerous peptides are under preclinical or clinical trials, which are anticipated to offer more options to infected patients.

Financial & competing interests disclosure

This work was financially supported by a grant from the Korea Healthcare Technology R&D Project, Ministry for Health Welfare, Republic of Korea (No. A092006), and by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MEST) (No. 2007-0056817, 2012-R1A2A1A01003569). This work was also supported by the 2014 BK21 Plus Project for Medicine, Dentistry and Pharmacy. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict

with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Key issues

- Abuse of antibiotics leads to the emergence of multidrug-resistant microorganisms, resulting in an urgent necessity for a new class of antibiotics with different mechanisms of action from traditional antibiotics.
- Antimicrobial peptides (AMPs) have been considered as potential therapeutics because of their antibacterial, antifungal, antiviral and even anticancer activities and different mechanisms of action from traditional antibiotics.
- AMPs have some disadvantages such as toxicity, stability and cost issues for their clinical and commercial development.
- Obstacles of AMPs can be overcome through structural modifications or through prodrug formation.
- AMPs are still attractive sources of novel antibiotics to pharmaceutical companies.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1. Demain AL, Sanchez S. Microbial drug discovery: 80 years of progress. J Antibiot (Tokyo) 2009;62(1):5-16
2. Jenssen H, Hamill P, Hancock RE. Peptide antimicrobial agents. Clin Microbiol Rev 2006;19(3):491-511
3. Lopez AD, Mathers CD, Ezzati M, et al. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet 2006; 367(9524):1747-57
4. Aloush V, Navon-Venezia S, Seigman-Igra Y, et al. Multidrug-resistant Pseudomonas aeruginosa: risk factors and clinical impact. Antimicrob Agents Chemother 2005;50(1):43-8
5. Manchanda V, Sanchaita S, Singh N. Multidrug resistant acinetobacter. J Glob Infect Dis 2010;2(3):291-304
6. Gordon YJ, Romanowski EG, McDermott AM. A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. Curr Eye Res 2005; 30(7):505-15
7. van't Hof W, Veerman EC, Helmerhorst EJ, Amerongen AV. Antimicrobial peptides: properties and applicability. Biol Chem 2001; 382(4):597-619
8. Marr AK, Gooderham WJ, Hancock RE. Antibacterial peptides for therapeutic use: obstacles and realistic outlook. Curr Opin Pharmacol 2006;6(5):468-72
9. Huang Y, Huang J, Chen Y. Alpha-helical cationic antimicrobial peptides: relationships of structure and function. Protein Cell 2010;1(2):143-52
10. Andreu D, Rivas L. Animal antimicrobial peptides: an overview. Biopolymers 1998; 47(6):415-33
11. Ganz T. The role of antimicrobial peptides in innate immunity. Integr Comp Biol 2003;43(2):300-4
12. Kai-Larsen Y, Agerberth B. The role of the multifunctional peptide LL-37 in host defense. Front Biosci 2008;13:3760-7
13. Laverty G, Gorman SP, Gilmore BF. The potential of antimicrobial peptides as biocides. Int J Mol Sci 2011;12(10): 6566-96
14. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002; 415(6870):389-95

•• **Extensively describes the general characteristics of antimicrobial peptides (AMPs).**

15. Aoki W, Kuroda K, Ueda M. Next generation of antimicrobial peptides as molecular targeted medicines. J Biosci Bioeng 2012;114(4):365-70
16. Mygind PH, Fischer RL, Schnorr KM, et al. Plectasin is a peptide antibiotic with therapeutic potential from a saprophytic fungus. Nature 2005;437(7061):975-80
17. Hancock RE, Scott MG. The role of antimicrobial peptides in animal defenses. Proc Nat Acad Sci 2000;97(16):8856-61
18. Pistolesi S, Pogni R, Feix JB. Membrane insertion and bilayer perturbation by antimicrobial peptide CM15. Biophys J 2007;93(5):1651-60
19. Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 2006; 24(12):1551-7
20. Hancock RE, Lehrer R. Cationic peptides: a new source of antibiotics. Trends Biotechnol 1998;16(2):82-8
21. Hancock RE. Peptide antibiotics. Lancet 1997;349(9049):418-22
22. Shai Y. Mode of action of membrane active antimicrobial peptides. Biopolymers (Pept. Sci.) 2002;66:236-48
23. Dawson RM, Liu CQ. Properties and applications of antimicrobial peptides in biodefense against biological warfare threat agents. Crit Rev Microbiol 2008;34(2): 89-107
24. Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. Biochim Biophys Acta 2008; 1778(2):357-75
25. Giuliani A, Pirri G, Nicoletto SF. Antimicrobial peptides: an overview of a promising class of therapeutics. Cent Eur J Biol 2007;2(1):1-33

• **Shows the potential of AMPs as a promising class of therapeutics.**

26. Brogden KA, Ackermann M, McCray PB Jr, Tack BF. Antimicrobial peptides in animals and their role in host defences. Int J Antimicrob Agents 2003;22(5):465-78
27. Lazarev VN, Govorun VM. Antimicrobial peptides and their use in medicine. Appl Biochem Micro+ 2010;46(9):803-14
28. Pushpanathan M, Gunasekaran P, Rajendhran J. Antimicrobial peptides: versatile biological properties. Int J Pept 2013;1-15
29. Kang SJ, Kim DH, Mishig-Ochir T, Lee BJ. Antimicrobial peptides: their physicochemical properties and therapeutic application. Arch Pharm Res 2012;35(5):409-13

• **Describes the mechanisms of action of AMPs.**

30. Aggarwal K, Silverman N. Positive and negative regulation of the Drosophila immune response. BMB Rep 2008;41(4): 267-77
31. Cruz J, Ortiz C, Guzman F, et al. Antimicrobial peptides: promising compounds against pathogenic

microorganisms. Curr Med Chem 2014; 21(20):2299-21

32. Oyston PC, Fox MA, Richards SJ, Clark GC. Novel peptide therapeutics for treatment of infections. J Med Microbiol 2009;58(Pt 8):977-87

33. Moberg CL, Cohn ZA. Lauching the antibiotic era. Personal accounts of the discovery and use of the first antibiotics. Rockefeller University Press; New York: 1990

34. Wang Z, Wang G. APD: the antimicrobial peptide database. Nucleic Acids Res 2004;32:Database issue D590-2

35. Wang G, Li X, Wang Z. APD2: the updated antimicrobial peptide database and its application in peptide design. Nucleic Acids Res 2009;37:Database issue D933-7

• **Reports the updated AMP database.**

36. Paquette DW, Waters GS, Stefanidou VL, et al. Inhibition of experimental gingivitis in beagle dogs with topical salivary histatins. J Clin Periodontol 1997;24(4):216-22

37. Kavanagh K, Dowd S. Histatins: antimicrobial peptides with therapeutic potential. J Pharm Pharmacol 2004;56(3): 285-9

38. Hancock RE. Cationic antimicrobial peptides: towards clinical applications. Expert Opin Investig Drugs 2000;9(8): 1723-9

39. Homepage of Demegen Inc. Available from: http://www.demegen.com

40. Ge Y, MacDonald D, Hait H, et al. Microbiological profile of infected diabetic foot ulcers. Diabet Med 2002;19(12): 1032-4

41. Homepage of dipexium pharmaceuticals. Available from: http://www. dipexiumpharmaceuticals. com/locilex/ overview

42. Melo MN, Dugourd D, Castanho MA. Omiganan pentahydrochloride in the front line of clinical applications of antimicrobial peptides. Recent Pat Antiinfect Drug Discov 2006;1(2):201-7

43. Sader HS, Fedler KA, Rennie RP, et al. Omiganan pentahydrochloride (MBI 226), a topical 12-amino-acid cationic peptide: spectrum of antimicrobial activity and measurements of bactericidal activity. Antimicrob Agents Chemother 2004;48(8): 3112-18

44. Roscia G, Falciani C, Bracci L, Pini A. The development of antimicrobial peptides as new antibacterial drugs. Curr Protein Pept Sci 2013;14(8):641-9

45. Carrus capital corporation announces plan of arrangement for asset spinoff. Available from: http://www.marketwatch.com/story/ carrus-capital-corporation-announces-plan-of-arrangement-for-asset-spinoff-2014-02-12-141733845

46. Homepage of cutanea life sciences inc. Available from: http://www.cutanealife.com

47. Loury D, Embree JR, Steinberg DA, et al. Effect of local application of the antimicrobial peptide IB-367 on the incidence and severity of oral mucositis in hamsters. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;87(5):544-51

48. Panyutich A, Shi J, Boutz PL, et al. Porcine polymorphonuclear leukocytes generate extracellular microbicidal activity by elastase-mediated activation of secreted proprotegrins. Infect Immun 1997;65(3): 978-85

49. Giles FJ, Rodriguez R, Weisdorf D, et al. A phase III, randomized, double-blind, placebo-controlled, study of iseganan for the reduction of stomatitis in patients receiving stomatotoxic chemotherapy. Leuk Res 2004; 28(6):559-65

50. Trotti A, Garden A, Warde P, et al. A multinational, randomized phase III trial of iseganan HCl oral solution for reducing the severity of oral mucositis in patients receiving radiotherapy for head-and-neck malignancy. Int J Radiat Oncol Biol Phys 2004;58(3):674-81

51. Donnelly JP, Bellm LA, Epstein JB, et al. Antimicrobial therapy to prevent or treat oral mucositis. Lancet Infect Dis 2003;3(7): 405-12

52. Schultz H, Weiss JP. The bactericidal/ permeability-increasing protein (BPI) in infection and inflammatory disease. Clin Chim Acta 2007;384(1-2):12-23

53. Domingues MM, Santos NC, Castanho MA. Antimicrobial peptide rBPI21: a translational overview from bench to clinical studies. Curr Protein Pept Sci 2012;13(7):611-19

54. Xoma 2006 annual report. Available from: www.annualreports.com/HostedData/Annual Reports/PDF/xoma2006.pdf

55. Homepage of Xoma Corporation. Available from: http://investors.xoma.com

56. Zheng R, Yao B, Yu H, et al. Novel family of antimicrobial peptides from the skin of Rana shuchinae. Peptides 2010;31(9): 1674-7

57. de Sa PB, Havens WM, Ghabrial SA. Characterization of a novel broad-spectrum antifungal protein from virus-infected Helminthosporium (Cochliobolus) victoriae. Phytopathology 2010;100(9):880-9

58. Dathe M, Wieprecht T. Structural features of helical antimicrobial peptides: their potential to modulate activity on model membranes and biological cells. Biochim Biophys Acta 1999;1462(1-2):71-87

59. Mylne JS, Wang CK, van der Weerden NL. Cyclotides are a component of the innate defense of Oldenlandia affinis. Biopolymers 2010;94(5):635-46

60. Matsuzaki K, Sugishita K, Fujii N, Miyajima K. Molecular-Basis for Membrane Selectivity of an Antimicrobial Peptide, Magainin-2. Biochemistry 1995;34(10): 3423-9

61. Ganz T. Defensins and host defense. Science 1999;286(5439):420-1

62. Tossi A, Sandri L, Giangaspero A. Amphipathic, alpha-helical antimicrobial peptides. Biopolymers 2000;55(1):4-30

63. Tam JP, Lu YA, Yang JL. Correlations of cationic charges with salt sensitivity and microbial specificity of cystine-stabilized beta-strand antimicrobial peptides. J Biol Chem 2002;277(52):50450-6

64. Chan DI, Prenner EJ, Vogel HJ. Tryptophan- and arginine-rich antimicrobial peptides: structures and mechanisms of action. Biochim Biophys Acta 2006; 1758(9):1184-202

65. Pakkala M, Hekim C, Soininen P, et al. Activity and stability of human kallikrein-2-specific linear and cyclic peptide inhibitors. J Pept Sci 2007;13(5):348-53

66. Stromstedt AA, Pasupuleti M, Schmidtchen A, Malmsten M. Evaluation of strategies for improving proteolytic resistance of antimicrobial peptides by using variants of EFK17, an internal segment of LL-37. Antimicrob Agents Chemother 2009;53(2):593-602

67. Rozek A, Powers JP, Friedrich CL, Hancock RE. Structure-based design of an indolicidin peptide analogue with increased protease stability. Biochemistry 2003;42(48): 14130-8

68. Aoki W, Ueda M. Characterization of Antimicrobial Peptides toward the Development of Novel Antibiotics. Pharmaceuticals 2013;6(8):1055-81

69. Svenson J, Stensen W, Brandsdal BO, et al. Antimicrobial peptides with stability toward tryptic degradation. Biochemistry 2008; 47(12):3777-88

70. Brandenburg LO, Merres J, Albrecht LJ, et al. Antimicrobial Peptides: multifunctional Drugs for Different

Applications. Polymers-Basel 2012;4(1):539-60

•• **Suggests strategies for developing therapeutic drug of AMPs.**

71. Zhang L, Falla TJ. Antimicrobial peptides: therapeutic potential. Expert Opin Pharmacother 2006;7(6):653-63
72. Nguyen LT, Schibli DJ, Vogel HJ. Structural studies and model membrane interactions of two peptides derived from bovine lactoferricin. J Pept Sci 2005;11(7):379-89
73. Papo N, Shai Y. Effect of drastic sequence alteration and D-amino acid incorporation on the membrane binding behavior of lytic peptides. Biochemistry 2004;43(21):6393-403
74. Nguyen LT, Chau JK, Perry NA, et al. Serum stabilities of short tryptophan- and arginine-rich antimicrobial peptide analogs. PLoS ONE 2010;5(9):1-8
75. Kang SJ, Won HS, Choi WS, Lee BJ. De novo generation of antimicrobial LK peptides with a single tryptophan at the critical amphipathic interface. J Pept Sci 2009;15(9):583-8
76. Leite NB, da Costa LC, Dos Santos Alvares D, et al. The effect of acidic residues and amphipathicity on the lytic activities of mastoparan peptides studied by fluorescence and CD spectroscopy. Amino Acids 2010;40(1):91-100
77. Kim H, Jang JH, Kim SC, Cho JH. De novo generation of short antimicrobial peptides with enhanced stability and cell specificity. J Antimicrob Chemother 2013;69(1):121-32
78. Knappe D, Henklein P, Hoffmann R, Hilpert K. Easy strategy to protect antimicrobial peptides from fast degradation in serum. Antimicrob Agents Chemother 2010;54(9):4003-5
79. Chou HT, Kuo TY, Chiang JC, et al. Design and synthesis of cationic antimicrobial peptides with improved activity and selectivity against Vibrio spp. Int J Antimicrob Agents 2008;32(2):130-8
80. Yeaman MR, Yount NY. Mechanisms of antimicrobial peptide action and resistance. Pharmacol Rev 2003;55(1):27-55
81. Won HS, Kang SJ, Choi WS, Lee BJ. Activity optimization of an undecapeptide analogue derived from a frog-skin antimicrobial peptide. Mol Cells 2011;31(1):49-54
82. Henriksen JR, Etzerodt T, Gjetting T, Andresen TL. Side chain hydrophobicity modulates therapeutic activity and membrane selectivity of antimicrobial peptide mastoparan-X. PLoS One 2014;9(3):e91007
83. Juretic D, Vukicevic D, Ilic N, et al. Computational design of highly selective antimicrobial peptides. J Chem Inf Model 2009;49(12):2873-82
84. Eckert R, He J, Yarbrough DK, et al. Targeted killing of Streptococcus mutans by a pheromone-guided "smart" antimicrobial peptide. Antimicrob Agents Chemother 2006;50(11):3651-7
85. Tsuboi R, Matsuda K, Ko IJ, Ogawa H. Correlation between culture medium pH, extracellular proteinase activity, and cell growth of Candida albicans in insoluble stratum corneum-supplemented media. Arch Dermatol Res 1989;281(5):342-5
86. Brandelli A. Nanostructures as promising tools for delivery of antimicrobial peptides. Mini Rev Med Chem 2012;12(8):731-41
87. Ali AB, Dacheng R. Antimicrobial peptides. Pharmaceuticals 2014;6:1543-75
88. Brandelli A. Nanostructures as promising tools for delivery of antimicrobial peptides. Mini Rev Med Chem 2012;12(8):731-41
89. Yount NY, Yeaman MR. Emerging themes and therapeutic prospects for anti-infective peptides. Annu Rev Pharmacol Toxicol 2012;52:337-60
90. Danial M, van Dulmen TH, Aleksandrowicz J, et al. Site-specific PEGylation of HR2 peptides: effects of PEG conjugation position and chain length on HIV-1 membrane fusion inhibition and proteolytic degradation. Bioconjug Chem 2012;23(8):1648-60
91. Falciani C, Lozzi L, Scali S, et al. Site-specific pegylation of an antimicrobial peptide increases resistance to Pseudomonas aeruginosa elastase. Amino Acids 2014;46(5):1403-7
92. Hussain R, Siligardi G. Novel drug delivery system for lipophilic therapeutics of small molecule, peptide-based and protein drugs. Chirality 2010;22(Suppl 1):E44-6
93. Won HS, Seo MD, Jung SJ, et al. Structural determinants for the membrane interaction of novel bioactive undecapeptides derived from gaegurin 5. J Med Chem 2006;49(16):4887-95
94. Faccone D, Veliz O, Corso A, et al. Antimicrobial activity of de novo designed cationic peptides against multi-resistant clinical isolates. Eur J Med Chem 2013;71:31-5
95. Butt TR, Edavettal SC, Hall JP, Mattern MR. SUMO fusion technology for difficult-to-express proteins. Protein Expr Purif 2005;43(1):1-9
96. Bogomolovas J, Simon B, Sattler M, Stier G. Screening of fusion partners for high yield expression and purification of bioactive viscotoxins. Protein Expr Purif 2008;64(1):16-23
97. Bommarius B, Jenssen H, Elliott M, et al. Cost-effective expression and purification of antimicrobial and host defense peptides in Escherichia coli. Peptides 2010;31(11):1957-65
98. Seetha RK, M Mary VK, S Divya S, et al. Cost effective purification of intein based syntetic cationic antimicrobial peptide expressed in cold shock expression system using salt inducible E. coli GJ1158. J Microbiol Infect Dis 2014;4(1):13-19