Antimicrobial activity, mechanism of action, and methods for stabilisation of defensins as new therapeutic agents

Meri Amerikova, Ivanka Pencheva El-Tibi, Vania Maslarska, Stanislav Bozhanov and Konstantin Tachkov

Department of Pharmaceutical Chemistry Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria; Department of Chemistry Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria; Department of Social Pharmacy Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria

ABSTRACT
Bioactive compounds, such as antimicrobial peptides (AMPs), have increasingly been used recently to counteract the rapidly increasing incidence of bacterial resistance to usual antibiotics and chemotherapeutics. In humans, endogenous AMPs are part of the immune system and act against pathogens. Defensins compose a class of AMPs that have activity against gram-positive and -negative bacteria, viruses, and fungi. For some, antitumour activity has also been reported. Such characteristics indicate that they represent a potential new class of therapeutic agents against microorganisms, including multidrug resistant pathogens. However, pH and enzymatic degradation and variable tissue distribution of these compounds limit their clinical application. New technologies and different methods have been developed to overcome these limitations and increase their half-life, such as cyclization, lipidation, design of peptidomimetics, synthesis of hybrid peptides, and use of nanocarriers. The objective of this review was to analyse current applications of defensins as antimicrobial agents and their mechanism of action. Moreover, new technologies and methods for stabilizing defensins are discussed.

Introduction

Antimicrobial peptides (AMPs) are a class of antimicrobial drugs that can be especially effective in the treatment of infections caused by multidrug resistant pathogens. They are found in diverse organisms, from prokaryotes to humans. Different classifications of these peptides exist, but the most common classification divides them according to their secondary structure into β-sheet, α-helical, loop, and extended peptides. The first two groups are the most frequently found in nature and, thus, the most studied. The structure of these AMPs is usually less than 100 amino acid residues long and contains a positive net charge (from +2 to +9), high lysine and arginine content, and a significant number of hydrophobic residues [1–4] (Figure 1 and Table 1).

The history of AMPs dates back to 1922 with the discovery of lysozyme by Alexander Fleming [5]. Lysozyme acts by destroying the peptidoglycan sugar chains in the cell wall of bacteria [6]. The discoveries of lysozyme and penicillin by Fleming are key events in the history of antibiotics research. Not long after lysozyme was isolated from human saliva, magainins were isolated from the African clawed frog Xenopus laevis [5]. Since then, many other peptides with antimicrobial activities have been described.

Defensins are cationic AMPs with a molecular weight of 3.5–4.5 kDa. Their structure typically contains three intramolecular disulphide bridges between six cysteine residues. As mentioned for AMP in general, the classification of defensins is based on their structure, which divides them into three groups: α-defensins, β-defensins, and θ-defensins [7,8]. Differences between α-defensins and β-defensins are found at the location of disulphide bridges. In α-defensins, intramolecular bonding occurs between cysteines 1-6, 2-4, and 3-5, whereas in β-defensins it is between 1-5, 2-4, and 3-6 [9]. Human defensins (HDs) are produced in leukocytes and are also secreted by different epithelial cells and mucosal tissues. These human peptides have...
antimicrobial activity against a large number of gram-positive and -negative bacteria, fungi, and viruses [10].

**Subclasses and antimicrobial activity of HDs**

**α-Defensins**

The first subclass, α-defensin, is found in rabbit macrophages, as well as in rat, human, and guinea pig neutrophils [11]. In humans, six genes code the expression of four peptides by neutrophils, which are known as human neutrophil peptides 1-4 (HNP-1-4), and two types of enteric defensins (HD-5 and HD-6), which are secreted by the small intestine, colon and female genital epithelial cells [12,13]. These six peptides are human α-defensins, whose typical structure consists of 29-35 amino acids [14]. Figure 2 demonstrates the difference in structure between α- and β-defensins [15].

The first isolation of human α-defensins was performed in 1985 by the Lehrer’s research group [16]. Neutrophils produce HNP-1, -2, -3, and -4, which confer them their antimicrobial activity. When compared to the sequence of HNP-2 (29 amino acids), HNP-1 and HNP-3 have one more amino acid (30 amino acids).
acids). Moreover, the difference between HNP-1 and HNP-3 lies in their N-terminal amino acid residue: it is an alanine in HNP-1, and an aspartate in HNP-3. This single nucleotide change leads to a reduced anti-microbial activity of HNP-3 against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*.

HNP-4 consists of 33 amino acid residues, and it was first analysed in 1989. The antibiotic activity of HNP-4 was reported for *E. coli*, *Streptococcus faecalis*, and *Candida albicans* [16]. HNP-1 is reported to kill *Mycobacterium tuberculosis* by forming pores and destroying the bacteria’s cell membrane [17]. The assessment of antimicrobial activity showed that human α-defensins can be arranged in the following order with regard to the activity against gram-positive *S. aureus*: HNP-2 > HNP-1 > HNP-3 > HNP-4. In the case of activity against gram-negative *E. coli* the order in changed to HNP-4 > HNP-2 > HNP-1 = HNP-3. The N-terminal amino acid, acidic aspartate, endows HNP-3 with a low antibacterial activity. HD-5 has an equivalent activity to HNP-2 against *S. aureus* and HNP-4 against *E. coli*. The activity of HNP-4 and HD-5 against gram-negative *E. coli* is associated with the high net charge +4 [16,18].

The first isolation of HD-5 and HD-6 was performed using Paneth cells, located in the small intestine, as the source. These peptides are directly secreted from the intestinal crypts into the lumen. They protect intestinal stem cells and regulate the composition of the intestinal microbiota [19,20]. Increased concentration of HD-5 in male urethral tract is a marker for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections [21]. HD-5 and HD-6 are found in the cervicovaginal and cervical epithelia. Some studies reported that HD-5 and HD-6 inhibit HIV infections at the point of viral entry [22,23]. Antimicrobial activity of HD-5 against *E. coli*, *Listeria monocytogenes*, and *Salmonella typhimurium*, as well as *C. albicans*, depends on the concentration; its minimum inhibitory concentration (MIC) lies within the nanomolar range [14]. Noteworthy, it was recently reported that HD-5 also possesses antimicrobial activity against multidrug resistant *Acinetobacter baumannii* [24,25] (Table 2).

**β-Defensins**

The first description of a β-defensin was made after isolating it from bovine tracheal epithelium, where its expression was induced by infection [26]. Human β-defensins (hBDs) are expressed in epithelial cells throughout the body. Currently, more than 30 hBDs have been reported, but only four of them (hBD-1 to hBD-4) have been analysed in detail [19]. In general, the expression of hBDs is constitutive, but the expression of hBD-2 and hBD-3 are inducible by infections [27]. These peptides are differentially expressed among tissues. For example, hBD-2 is abundant in the lungs, hBD-3 is found in skin and tonsils, and hBD-4 is highly expressed in testicles and stomach [28]. High levels of hBD-2 are expressed in the cervical epithelium during pregnancy [29]. The fact that defensins are also expressed in male and female genital tracts indicates a preventive function against sexual disease and control of fertility.

hBD-1 has antimicrobial activity against *E. coli* and *P. aeruginosa*; it can be especially useful in patients with cystic fibrosis whose lungs might have been compromised. Moreover, hBD-1 also has a potent antimicrobial effect against *C. albicans* after the reduction of its disulphide bridges. hBD-2 is extremely effective against gram-negative bacteria such as enteric *E. coli* and *P. aeruginosa*. It is also effective against *C. albicans*, *S. marcescens*, *P. aeruginosa*, and *A. baumannii*.
Thus, hBD-2 can be effective in treating colon infections. hBD-2 is also effective against H. pylori induced gastritis [31] and it has antitubercular activity. It is present in human breast milk and has a protective function against respiratory infections, diarrhoea, and necrotising enterocolitis in infants. One limitation of hBD-1 and hBD-2, however, is that their activity is sensitive to salt concentration, whereas for hBD-3 and hBD-4 no such osmotic sensitivity has been reported. Indeed, the activity of β-defensins depends on surrounding conditions. For example, the activity of hBD-2 against E. coli, P. aeruginosa, Enterococcus faecalis, and S. aureus increases in the presence of lactoferrin and lysozyme [32,33].

hBD-3 presents antimicrobial activity on S. aureus and vancomycin-resistant Enterococcus faecium at physiological pH [16]. Furthermore, it possesses bactericidal effects against gram-positive enteric S. aureus and Streptococcus pyogenes, and gram-negative P. aeruginosa, E. coli, and C. albicans. Antimicrobial activity of hBD-3 has also been reported against multidrug resistant clinical isolates of S. aureus, Enterococcus faecium, and P. aeruginosa, as well as against Stenotrophomonas maltophilia and Acinetobacter baumannii [34]. For β-defensins, anti-HIV activity is reported only for hBD-2 and hBD-3, which block the virus’s replication [35,36].

Expression of hBD-4 is particularly high in stomach, uterus, testis, kidney, lung, thyroid, and neutrophils and it has been found to have antibacterial activity against P. aeruginosa [37]. Currently, hBD-4 is being investigated to determine whether it has the same antitubercular activity as hBD-2 [17]. The most recently discovered hBDs, hBD-5 and hBD-6, are expressed in the epithidymis and have antibacterial activity against E. coli, but not S. aureus. The activity of these peptides is limited by NaCl, especially against E. coli [38].

hBD-19, 23, 27, and 29 are expressed in the male reproductive tract, where they protect spermatozoa from bacteria. Analysis of hBD-19, hBD-23, hBD-27, and hBD-29 against E. coli, S. aureus and P. aeruginosa, revealed that hBD-23 is the most effective in inhibiting the growth of these gram-negative and positive bacteria, with very low toxicity on normal human cells [39].

### θ-Defensins

The third group of defensins is the θ-defensins. At first, θ-defensins were identified in rhesus macaque (Macaca mulatta) leukocytes. Genes for θ-defensins exist in human genome as pseudogenes. They have a typical structure of 18 amino acids with three disulphide bonds between cysteines 1-4, 2-5, and 3-6. They are cyclic peptides in nature and their synthesis is achieved by linking two nine-residue peptides head-to-tail [40]. Their cyclic structure confers them high stability, resistance to proteases, and antibacterial effect. Their antimicrobial effect is higher than that of acyclic peptides and does not depend on their disulphide bonds. In contrast to the other two groups, θ-defensins are stable in physiological salt concentration. This salt insensitivity might be due to their cyclic backbone, since acyclic peptides have three times less activity versus E. coli and S. aureus at physiological osmotic conditions. Retrocycline-1 has bactericidal effect against L. monocytogenes and antifungal activity against C. albicans, C. neoformans, Verticillium dahliae, and Fusarium oxysporum [41]. Rhesus θ-defensin-1 (RTD-1) has antibacterial activity against antibiotic resistant strains of S. aureus and P. aeruginosa, where it acts on the cell membrane, rather than on specific enzymes. At low concentrations, θ-defensins have antiviral activity against HIV-1 before they attach to the cell, probably blocking their entry [41]. The deficit of θ-defensins has made humans more susceptible to HIV-1 infections [16]. Other documented effects of θ-defensins are anti-influenza and anti-herpes activities. Activity against influenza A viruses stems from the prevention of viral entry by binding to protein D.

### Table 2. Distribution of human antimicrobial peptides. Reused with permission, originally given by Cole and Ganz [25].

| Antimicrobial peptide | Size (kDa) | Distribution                        |
|-----------------------|-----------|-------------------------------------|
| α-defensins           |           |                                     |
| HNP1-4                | 4         | Neutrophils                         |
| HD-5 and HD-6         | 4         | Paneth cells of the small intestine |
| β-defensins           |           |                                     |
| HBD-1                 | 5         | Urogenital tract, pancreas, oral mucosa, plasma, respiratory tract |
| HBD-2                 | 5         | epidermis, lung, respiratory secretions, oral mucosa |
| Cathelicidin (LL-37)  | 5         | Neutrophils, epithelia              |
| NK lysin              | 9         | Cytotoxic T cells                   |
| Lipophilins           | 6–8       | Tears                               |
| Histatins             | 3–5       | Saliva                              |

[30]. However, it possesses only bacteriostatic properties against gram-positive bacteria (e.g. S. aureus). Thus, hBD-2 can be effective in treating colon infections. hBD-2 is also effective against H. pylori induced gastritis [31] and it has antitubercular activity. It is present in human breast milk and has a protective function against respiratory infections, diarrhoea, and necrotising enterocolitis in infants. One limitation of hBD-1 and hBD-2, however, is that their activity is sensitive to salt concentration, whereas for hBD-3 and hBD-4 no such osmotic sensitivity has been reported. Indeed, the activity of β-defensins depends on surrounding conditions. For example, the activity of hBD-2 against E. coli, P. aeruginosa, Enterococcus faecalis, and S. aureus increases in the presence of lactoferrin and lysozyme [32,33].

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[41]. Antiviral activity against HIV-1 is not only reported for β-defensins, but also for HNP-1. It also prevents the entry of BK virus by blocking its attachment to host cells.

It is worth mentioning that in vitro assays showed that HNPs 1-3 and RTD-1 have bactericidal effects against methicillin-resistant S. aureus (MRSA) and P. aeruginosa [42]. This is particularly relevant when one considers that MRSA is totally resistant to β-lactam antibiotics and P. aeruginosa can only be treated with fluoroquinolone antibiotics. Moreover, more than 30% of the isolated strains have multidrug resistance.

Other biological properties of defensins

Defensins have the potential to be used as therapeutic agents in the treatment of cancer, either as biomarkers or as chemotherapeutics. Several studies have reported that defensins are found in the tumour microenvironment. Higher levels of HNP-1, -2, and -3 have been detected in the serum of patients with colorectal cancer than in that of healthy patients [43]. Other cases of overexpression include patients with lung cancer, in which hBD-1 and hBD-2 were detected at high concentrations, renal cell carcinoma, in which high levels of hBD-1 were documented, and oral squamous cell carcinoma (OSCC), in which overexpression of hBD-3 was reported [44]. HD-5 is also a biomarker for inflammation of the human nasal mucosa, and the levels of this peptide have also been reported to increase in patients with Crohn’s disease [45]. Defensins could be used as biomarkers in the early diagnosis of inflammatory diseases and cancer.

Defensins also have cytotoxic effects on cancer cells. For example, HNP-1 presents toxicity against OSCC cells at 100 μg/mL. Their cytotoxic properties could be attributed to DNA damage and inhibition of the neovascularisation of tumours. The viability of renal carcinoma cells could be inhibited by high concentrations (up to 25 μg/mL) of HNP-1, -2, and -3 [46]. In addition to its cytotoxicity in vitro, HNP-1 has an inhibitory effect on pathologic neovascularisation in vivo [47]. Unfortunately, the cytotoxicity of HNPs is not limited to tumour cells, it is toxic to normal epithelial cells and leukocytes; their activity is also suppressed by serum constituents [48,49].

hBDs have chemo-attractive properties for leucocytes and dendritic cells, which can induce phagocytosis and destruction of harmful microorganisms. These effects are potentiated by the production of chemokines, cytokines and other AMPs, causing the death of pathogens [50]. HDs also regulate the way microorganisms ‘present’ themselves to the immune systems by binding with the CC chemokine receptor [51], and their chemo-attractive activity mobilizes neutrophils and immunocompetent T cells. Some chemokine activity on monocytes, T cells, and dendritic cells was reported for HNPs 1-3, but no specific receptor has been reported [22,52]. Furthermore, the immunomodulatory properties of HD-5 on human cells have been reported [53].

The existence of a specific kind of defensins named corticostatins has been reported. Their activity seems to be connected to the action of the corticotrophin hormone and the way they attach to the adrenocorticotropic hormone receptor without activating it. This could prevent the production of cortisol, which is useful when fighting infections [21]. Another documented activity of defensins is their ability to activate nifedipine-sensitive calcium channels in mammalian cells. Defensins present such activity at nanomolar concentrations [22] and could play a role in healing human skin [54]. Moreover, HNP-1, -2, and -3 can be used as alternative medicines in patients with hypercholesterolemia and vascular dysfunction [55].

Mechanism of action

The classical mechanism of action of cationic AMPs, such as defensins, is the disruption of the anionic bacterial membrane [16]. This way, bacterial destruction occurs by the interaction between the electrostatic forces of positively charged amino acids and the negatively charged cell surface [56].

Bacterial membranes are rich in negatively charged phospholipids, such as phosphatidyl-glycerol (PG), cardiolipin (CL), and phosphatidylserine (PS); these are stabilized by bivalent cations such as Mg²⁺ and Ca²⁺. Gram-negative bacteria have an additional lipopolysaccharide-rich outer membrane, which stands as a barrier to the cytoplasmic membrane. On the other hand, human cells are rich in neutrally charged phospholipids, such as phosphatidylethanolamine (PE), phosphatidylcholine (PC), and sphingomyelin (SM). This difference in membrane composition between humans and bacteria makes AMPs highly selective against bacteria. In humans, the interaction with AMPs is even less likely because of the presence of cholesterol, which affects the fluidity of phospholipids in the membrane and increases its stability [49].

There are three electrostatic models that explain the activity of AMPs on bacterial membranes: (a) the barrel stave model, in which peptides would be introduced perpendicularly in the bilayers, coalescing
together to produce a pore; (b) the carpet model, which suggests that peptides are absorbed parallelly in the bilayers and, after achieving a sufficient coverage, generate a detergent effect and destroy the membrane; and (c) the toroidal pore model, which suggests that peptides are introduced perpendicularly in the lipid bilayer and generate a regional membrane curvature, where a pore is formed by both peptides and phospholipid head groups [49,57].

Although the existence of distinct models helps understanding bacteria membrane disruption, different mechanisms take place depending on the defensin and target. For example, HNP-1 mechanism of action against E. coli differs from that against S. aureus (destruction of cell membrane vs restriction of bacterial wall precursor lipid II). HD-5 is effective in killing both gram-positive and -negative bacteria by increasing bacterial membrane permeability. It has also been reported that HD-5 can bind to DNA and inhibit cell replication. One mechanism of action of HD-6 is the formation of nanonets and the arrest of bacteria before they make physiological contact with epithelial cells, which prevents bacterial invasion.

Reduction of hBD-1 disulphide bridges increases its activity against pathogens. Newly reported mechanism of action suggests that it forms extracellular traps (web-like structures containing AMPs) with neutrophils that capture and destroy bacteria. hBD-2 acts by binding to negatively charged membrane phospholipids, inducing efflux of intracellular components and leading to cell death. Two mechanisms have been reported for hBD-3, destruction of bacterial membrane and interaction with lipid II precursor. hBD-4 causes bacterial death by impairing the integrity of the membrane [58].

Lastly, intracellular targeting and inhibition of protein synthesis is also a mechanism by which some AMPs express their activity. This is the case of E. coli destruction by HNP-1 [56]. Indolicidin acts by targeting DNA and inhibiting DNA replication, thus killing bacteria [49]. Figure 3 illustrates the different mechanisms of action of AMPs [59].

**Figure 3.** The proposed diverse mechanistic modes of action for antimicrobial peptides in microbial cells. (A) Disruption of cell membrane integrity: (1) random insertion into the membrane, (2) alignment of hydrophobic sequences, and (3) removal of membrane sections and formation of pores. (B) Inhibition of DNA synthesis. (C) Blocking of RNA synthesis. (D) Inhibition of enzymes necessary for linking of cell wall structural proteins. (E) Inhibition of ribosomal function and protein synthesis. (F) Blocking of chaperone proteins necessary for proper folding of proteins. (G) Targeting of mitochondria: (1) inhibition of cellular respiration and induction of ROS formation and (2) disruption of mitochondrial cell membrane integrity and efflux of ATP and NADH, given by Peters et al. [59].

**New technological approaches**

The multitude of mentioned mechanisms makes defensins good candidates for future medicines, but there are certain disadvantages that delay their development as actual pharmaceuticals. These problems include the nonspecific haemolytic activity on human cells, osmotic sensitivity, rapid turnover in the human body, and high price of production; the last affects directly clinical trials, which requires large quantities of material [60]. These physicochemical properties have
delayed the introduction of defensins and other AMPs as medication. Before they can be used in clinical applications, stability in the human body, transportation, targeted delivery, controlled release, and immunogenicity of these peptides must be improved. The challenge lies in developing a suitable formulation for oral delivery. Many enzymes in the gastrointestinal tract (exopeptidases, aminopeptidases, and carboxypeptidases) rapidly break down amino acid’s backbones [61].

In order to overcome these challenges, much effort has been put into improving the peptides’ half-life using difference approaches, including cyclization, lipidation, design of peptidomimetics, synthesis of hybrid peptides, or use of nanocarriers. Different approaches to cyclization can increase the chemical stability of peptides and reduce their vulnerability to proteases. The interest in cyclic peptides dates back to the 1940s, when bacitracin was first used in the treatment of pneumonia caused by Staphylococci in infants in 1945. To this date, many methods of peptide cyclization exist, including: head-to-tail, head/tail-to-sidechain, or side-chain-to-side-chain. These methods depend on the structure and functional groups of peptides and were shown to have different effects. Noteworthy, some cyclised peptides have conformational restrictions.

Cyclization may lead to increased selective toxicity. For example, the analysis of the AMP ‘CKLLLKWLLKLLKC’ in its linear and cyclised forms by Oren and Shai showed that the linear form had antibacterial effect against gram-positive bacteria and high haemolytic activity. The cyclic form, however, presented bactericidal effect against gram-negative bacteria and low haemolytic activity. Another example is the cyclization of herpes simplex virus glycoprotein D derived epitope peptide, which increases its stability, while the introduction of a thioether link makes the peptide more stable in human serum. The combination of cyclization with D-amino acid substitution increases the therapeutic potential and reduces the haemolytic effect. Unfortunately, effects of cyclization are not always predictable; cyclization of the hexapeptide ‘RRWWRF’ raises both the antimicrobial and haemolytic effect, while the opposite occurs in magainin analogues, whose antimicrobial and haemolytic activities decrease after cyclization [62].

Cyclic peptides have been developed by Kirshenbaum and coworkers via head-to-tail macrocyclisation reactions (reviewed in [63]). Some of their experiments demonstrated that linear and cyclic peptoids (6-10 residues) have antimicrobial effect but cyclic peptides have higher antimicrobial activity and lower haemolytic activities than linear peptides. New research in this field indicates that cyclic peptoids can kill MRSA by forming pores in the bacterial membrane with low MIC and no significant haemolytic activity. In summary, cyclization is a promising method for developing peptoids as candidates for antimicrobial drugs [63]. In that regard, somatostatin, insulin, oxytocin, and some animal-derived neurotoxins are cyclic peptides that have disulphide bridges and could be used in medicine. Natural conotoxin was shown to be effective as a drug for neuropathic pain. Some cyclic plant peptides with three disulphide bonds have anti-HIV activity [61].

Attaching a lipid to the structure of peptides is also a method to improve its stability or give a peptide a desired property. It can be used to create a prodrug, which would improve the pharmacological distribution of the peptide, without reducing its activity or selectivity. Moreover, it can also make a peptide less soluble in water and improve its delivery. In the case of lipidation of hydrophilic compounds, this could improve the absorption by passive diffusion through epithelial cells. Increasing the lipophilic nature of peptides gives them important characteristics for intestinal delivery. Attaching a lipid to the structure of LHRH increased the half-life approximately 30-fold in Caco-2 cell homogenates. However, in the case of enkephalin, permeability and solubility did not improve after increasing it hydrophobicity [61].

Another new strategy to improve peptide stability is the construction of so-called mimetics, which are non-peptide molecules that resemble the properties and exert the activities of natural AMPs. These mimetics include peptoids, β-peptides, arylamides, oligomers, or phenylene ethynlenes, which bear physicochemical properties similar to those of natural AMPs, resulting in comparable activity and function [64].

These compounds, while acting as natural AMPs, are less vulnerable to enzymatic degradation and have a better pharmacological profile. They are active against multidrug resistant strains and appear less prone to induce bacterial resistance. Peptidomimetic design methods include the coupling of stable unnatural amino acids. Other modifications are amine alkylation, side chain substitution, structural bond extension, and isosteric replacement within the amino acid backbone. Pharmacokinetic properties are improved by isosteric replacements within a peptide backbone. The different methods of amino acids modification can be grouped as: (1) changing the
functionality of amino acids; (2) substitution of \( \alpha \)-carbon; (3) chemically extension of the backbone by one or two atoms, and (4) atom modification of the carbonyl function. Peptidomimetics can be classified as \( \beta \)-peptides, peptoids, amylamide oligomers, \( \beta \)-turn mimetics, and AA peptides (which contain N-acylated-N-aminoethyl amino acids). The high variety of the peptidomimetic molecules stems from more possible dihedral angles compared to those of canonical peptides [65].

For the past 15 years various researchers have focussed on peptoid synthesis with one goal in mind – their potential as therapeutic agents. The structure of peptoids may include a short amino acid backbone with hydrophobic tails such as fatty acids that are not normally acylated. The synthesis includes connecting the N-terminal alkyl tail with the alkylamine of the peptoid. These tails can be constructed out of 5 to 13 carbons. The varied composition and size of peptoids give them different antibacterial, antifungal, and haemolytic activities. So far, reported effects of such peptides include: antituberculotic activity of tetra-haemolytic activities. So far, reported effects of such peptides include: antituberculotic activity of tetra-haemolytic activities. So far, reported effects of such peptides include: antituberculotic activity of tetra-haemolytic activities. So far, reported effects of such peptides include: antituberculotic activity of tetra-haemolytic activities. So far, reported effects of such peptides include: antituberculotic activity of tetra-haemolytic activities.
microspheres, nanoparticles, polyethylene glycol and polyvinylpyrrolidone polymerization, and liposomes. All these systems affect the pharmacokinetic properties, altering bioadhesion, biodegradation, and biocompatibility [61]. Another advantage of this approach is the prevention of AMPs dissemination to the environment of other cells. For example, peptide carriers can be formed between silica microparticles and the AMP magainin-I [67].

Gold nanoparticles (AuNPs) have been used to counteract multidrug resistant bacteria. These nanoparticles have a great potential to kill both gram-positive and -negative bacteria, including multidrug resistant pathogens. Other benefits of these AuNPs are their low toxic activity against mammalian cells, and the fact that they do not induce bacterial resistance [68]. A study tested the activity of AuNP-conjugated AMP, through a maleimide linker to the thiol group of a cecropin-melittin AMP and the Au-particles, against E. coli and S. aureus. The results of the research showed a higher antibacterial activity against these strains, while it presented no toxicity against human cells [67]. Additionally, AuNPs can be used as non-viral gene vectors; however, they are not widely used in medicine because of their inability to transfect primary cells or stem cells. Researchers have also reported the synthesis of AMP-conjugated cationic AuNPs (AuNPs@PEP) for gene delivery with antimicrobial activity to stem cells [69]. Such AuNPs conjugated with hBD-3 have been used to enhance osteogenic differentiation of human periodontal ligament cells in inflammatory microenvironments [70].

Silver nanoparticles (AgNPs) are an alternative to AuNPs with high activity against gram-positive and -negative bacteria [71], and fungi [72]. Moreover, AgNPs are effective against multidrug resistant pathogens such as: P. aeruginosa, ampicillin resistant E. coli, erythromycin-resistant S. pyogenes, MRSA, and vancomycin-resistant S. aureus [73]. Silver is much more toxic to microorganisms than any other element. It has low toxicity potential against human cells and can prevent the induction of bacterial resistance. Current studies have tested the antimicrobial activity of plant peptide MBP-1 alone and in combination with AgNPs against S. aureus. The findings indicated that both were active against S. aureus at low concentrations, but in combination they had a strong synergistic effect [71].

Conversely, researchers have documented that the activity of AMP ‘LL37’ was not increased when conjugated to AgNP. The function of LL-37 is to stimulate the skin cells. The compound LL73@AgNP inherited the antimicrobial activity of ionic silver, already used in medicine, but not the inhibitory activity on cell proliferation. It had an antibiofilm activity, which is excellent for wound healing and preventing potential infections in burn victims [74].

The analysis of synthetic AMP pexiganan and its nanoparticles (PNPs) has shown that they have antibacterial activity against H. pylori. Pexiganan nanoparticles present strong adhesion to gastric mucosa and could remain there for prolonged periods. Researchers concluded that pexiganan could be used as anti-H. pylori drug when it is in the form of PNPs, rather than in pexiganan suspension [75].

Peptide nanoparticles could be used as specific drug delivery and target systems. They have a promising future in the treatment of different bacterial infections, as well as in cancer treatment. For example, antitumour activity of dermaceptin-chitosan nanoparticles has been reported. Functionalizing lactoferrin to the surface of polyethylene glycol – poly(lactic acid) nanoparticles is used to cross the blood-brain and blood-brain tumour barriers and reach glioma cells. Antimicrobial peptide LL-37 encapsulated in poly(lactic-co-glycolic acid) nanoparticles could be used as a drug to promote wound healing [76]. Table 3 presents some examples of AMP formulations, near here [77].
Synthesis of organometallic complexes with peptides may influence several parameters such as lipophilicity, water solubility, and other interactions of the complex with microenvironment. An example of such a synthesis is the combination of ruthenium-complex to a tryptophan residue of the peptide sequence of the AMP melittin. Melittin has both antimicrobial and anticancer activity, whereas the synthetic product with the organometallic complex ruthenium has shown the same anticancer activity without its haemolytic effect [78]. In recent years, researchers also reported a multifunctional non-covalent complex with antimicrobial and wound healing activities. The structure of this complex consists of a bivalent silver polidiguanide complex with histatin-1 [67].

Conclusions

HDs are part of a family of AMPs with a broad spectrum of activity against gram-positive and gram-negative bacteria, viruses, and fungi. Defensins also exert other functions in microorganisms – i.e. immunomodulatory, controlling spread of infections and inflammatory processes, presenting chemotactic signals, and promoting wound healing. Some defensins are overexpressed in diseases and can be used as biomarkers. Overall, they show great potential to be used as modern therapeutic agents, as long as their inherent problems are overcome. Such problems include their stability, sensitivity to proteolysis, and insignificant level of action under physiological conditions. Modern chemical and technological approaches to design drugs offer a way around these problems through binding them to nanocarriers for drug delivery, synthesis of peptidomimetics, construction of hybrid peptides, or formation of peptide-metal complexes. These methods provide a way to increase stability and half-life of peptides and open the door for their potential application in medicine.

Disclosure statement

No potential conflict of interest was reported by the authors.

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