REVIEW ARTICLE

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Utilisation of peptides against microbial infections – a review

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Abstract

The emergence of resistance in microorganisms on a global scale has made it necessary to search for new antimicrobial factors. Antimicrobial peptides (AMPs) seem to meet these expectations. AMPs are produced by bacteria, viruses, plants, and animals, and may be considered as a new class of drugs intended for the prophylaxis and treatment of both systemic and topical infections. The aim of this study is to review the results of studies on the use of peptides to combat infections *in vivo*. Antimicrobial peptides may be applied topically and systemically. Among the peptides used topically, a very important area for their application is ophthalmology. AMPs in ophthalmology may be used mainly for the protection of contact lenses from ocular pathogens. Many AMPs are in clinical trials for application in the therapy of local infections. There may be mentioned such preparations as: pexiganan (magainin analogue), MX-226 (based on indolicidin), NEUPREX (isolated from human BPI (bactericidal/permeability-increasing) protein), IB-367 (variant of porcine protegrin), P113 (based on histatin), daptomycin, polymyxins, as well as peptidomimetics. In the combat against systemic infections are used such peptides as: P113D (modified P113 peptide containing D-amino acids), colistin, peptoids, and peptides containing non-typical amino acids or non-peptide elements. AMPs are also used as antiprotozoal, antifungal, antitoxic and immunostimulatory agents. The limitations in the use of peptides in the treatment of infections, such as susceptibility to proteolysis, and resistance of microorganisms to the peptides, are also discussed. AMPs are a promising strategy in the fight against microbial infections.

Key words

antimicrobial peptides, infections, drugs, therapy

INTRODUCTION

Peptides (from the Greek Πεπτίδια – 'digestible") are organic compounds arising from the linking of amino acid molecules by amide bonds. It is assumed that peptides are polyamino acids with a molecular weight of less than 5,000–10,000 D. Antimicrobial peptides (AMPs), also called peptide antibiotics, were discovered in frogs in the early 1980s, in which antimicrobial substances called magainins were detected [1]. AMPs are among the oldest defence mechanisms of plants, humans, and animals [2, 3]. AMPs include all oligo- and polypeptides that kill microbes or inhibit their growth, peptides derived from larger proteins, and non-ribosomally synthesized examples [3]. AMPs possess various mechanisms of action, such as permeabilisation of microbial membranes, destabilisation of bilayer lipid structures, formation of micelles or channels within a membrane, lipopolysaccharide (LPS) binding, inhibition of DNA replication and expression of proteins or release of ATP [2, 3, 4, 5, 6, 7, 8, 9]. A detailed review of research on peptides can be found in the work of Hancock & Lehrer [4], Zasloff [10], Brodgen et al. [11], Mirski et al. [12], Kołodziej et al. [13], Eckert [14], Brandenburg et al. [15], and Devocelle [16].

The emergence of resistance in microorganisms on a global scale has made it necessary to search for new factors against infections. Human defensins, cathelicidin, and a significant number of various AMPs derived from bacteria, viruses, plants, vertebrates and invertebrates, have a universal, multi-dimensional structure determining their antimicrobial activity [17]. Chemical modification of these structures for the preparation of synthetic peptides is a promising strategy for the development of AMPs as a new class of drugs intended for the prophylaxis and treatment of both systemic and topical infections.

There are several potential strategies for the therapeutic use of peptides, falling broadly into two groups: in the form of a single preparation or in combination with antibiotics or immunostimulatory agents [18]. Most attention is given to the use of AMPs as monotherapy [19] for the treatment of bacterial, parasitic, fungal, and severe viral infections [15, 18, 20, 21].

The aim of this study is to review the results of studies on the use of peptides to combat infections *in vivo*.

The application of peptides in ophthalmology. Much attention has been paid to the use of peptides in ophthalmology. It has focused on determining the antimicrobial activity of different AMPs against bacterial pathogens, fungi and protozoa, evaluating the possibilities for their use for the protection of contact lenses and cornea, and topical application. For example, it has been shown that rabbit alpha defensin (NP-1) was effective against a wide range of ocular bacteria in phosphate buffer and modified corneal storage media. Among the tested cecropin analogues, Shiva-11 proved to be active against a wide variety of ocular isolates, including gentamycin-resistant bacteria. Another preparation, Hecate (a cecropin analogue), inhibited the growth of many *Acanthamoeba* species *in vitro*. Sousa et al. [22] showed that the cecropin analogue D5C increases the effectiveness of contact lens disinfectant solutions against

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Pseudomonas aeruginosa, and other commonly occurring Gram-positive and Gram-negative ocular pathogens, and against *Candida* in contact lens sterilization solutions and corneal storage media. Cole et al. [23] demonstrated that coating contact lenses with melamine prevented the growth of bacteria on their surfaces, which reduced the amount of adverse inflammatory reaction in the wearer caused by Gram-positive and Gram-negative bacteria. However, Schwab et al. [24] did not show inhibitory activity of D5C or nisin, a natural polypeptide derived from *Streptococcus lactis* in modified corneal storage media. The addition of EDTA increased the bactericidal activity of the peptide against *Pseudomonas in vitro*.

Mannis [25] describes several synthetic peptides (CCI A, B and C and COL-1) selected by a suitable computer programme as showing antibacterial activity against a broad panel of ocular bacterial and fungal pathogens in the environment of 10 mM sodium phosphate buffer.

COL-1, designed against *Pseudomonas,* was tested in a rabbit keratitis model using topical administration for 4–6 days. In view of the toxicity of the preparation to ocular cells *in vivo,* it was not possible, however, to demonstrate efficacy in this model. In some of the published studies on the use of local AMPs, Nos-Barbera et al. [26] showed that a synthetic peptide which merged cecropin A and mellitin (from bee venom) was equivalent to gentamicin at inhibiting clinical symptoms of keratitis caused by *Pseudomonas aeruginosa* in rabbits.

Treatment of local infections. Pexiganan, a 22-amino acid analogue of magainin 2, was the first antimicrobial peptide for use in the form of a cream for the local treatment of diabetic foot ulcers [27]. Two indolicidin-based peptide variants deserve closer attention, namely, MX-226 (omiganan pentahydrochloride, 1% gel) and MX-594AN. The first of these is a topical antibiotic to prevent infection associated with the use of catheters. In August 2005, confirmatory phase III clinical trials were initiated that showed a reduction in the number of local catheter-related infections. The second indolicin-based peptide variant is a new antibiotic for topical treatment of acne vulgaris (phase IIb completed in 2003) [15, 28]. A third peptide, MBI-853NL, has also been evaluated in phase I studies to eliminate *Staphylococcus aureus,* including methicillin-resistant strains (MRSA), and to prevent their carriage in the nasal cavity [29]. Also, two peptides (XOMA and NEUPREX) have been isolated from human BPI (bactericidal/permeability-increasing) protein, which is a part of the early line of defence against invasive microorganisms, and is known for its bactericidal and LPS neutralization activity. XOMA 629 shows activity against *Propionibacterium acnes* and other skin flora. However, preliminary results of the phase III studies were not conclusive [28]. XOMA 629 is currently undergoing further research aimed at the design and evaluation of new or improved formulations for skin penetration. NEUPREX (rBPI21, opebacan) is an injectable preparation of rBPI21, a modified recombinant fragment of BPI that can be applied in paediatric patients requiring open heart surgery with cardiopulmonary bypass, patients with severe burns or meningococcal sepsis, and after allogeneic haematopoietic stem cell transplantation. IB-367 is a variant of porcine protegrin-1 consisting of 18 amino acids and forming a β-hairpin structure stabilized by two disulphide bonds. It is related to tachyplesins and polyphemusins from crabs antibacterial and anti-HIV peptides. The first clinical indication of the efficacy of IB-367 was in a study using it in the treatment of oral mucositis occurring as a side-effect of anticancer therapies with 'mixed' infections in the mouth. The results of the phase I studies showed the capability of the formulation as reflected by a reduction in the number of microorganisms in the mouth throughout the 10-day treatment period. Phase II studies in 134 patients in bone marrow transplantation centres showed that IB-367 reduced post-transplantation mucositis to 22% from the 40% incidence it was assessed at when treatment was initiated four days prior to bone marrow transplantation [29]. Pfeufer et al. [30] demonstrated the effectiveness of coating titanium surfaces with recombinant HBD-2 in the elimination of infections associated with the implantation of prosthetic. It has been shown that amphiphilic peptides covalently bound to a water-insoluble resin retain antimicrobial activity for a long time [31], and hence, in such a form can be used for the disinfection of medical equipment, including catheters. The possibility of clinical application of peptides derived from amphibian skin has been considered, for example, alyteserin, brevinin, ascaphin, pseudin, kassinatuerin and temporin in the treatment of local infections caused by multi-drug resistant strains of bacteria, such as the multidrug resistant *Acinetobacter baumannii* (MDRAB) strains, *Klebsiella pneumoniae* producing extended spectrum β-lactamase (ESBL), *Escherichia coli*, multi-drug resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* and fluconazole-resistant *Candida spp.* [32].

P113 is a 12-amino acid, histatin-based cationic peptide naturally occurring in saliva [28], exhibiting high *in vitro* activity against *Candida albicans,* and commonly occurring Gram-positive and Gram-negative pathogens. A license for the use of P113 in the form of a mouthwash for the treatment of oral candidiasis in HIV patients has been obtained (selected for phase I /II clinical studies in March 2006). It can also be used for the prevention of gingivitis and periodontal diseases [33]. In September 2003, daptomycin, a cyclic anionic lipopeptide antibiotic, was approved for the treatment of complicated skin and subcutaneous tissue infections caused by Gram-positive bacteria. Daptomycin is only active against Gram-positive bacteria (enterococci, including glycopeptide-resistant enterococci [GRE]), staphylococci (MRSA), streptococci and corynebacteria [28]. This effect is dependent on calcium (Ca2 +), since the binding of Ca2+ ions with daptomycin enables interaction with bacterial membranes, resulting in a similar effect to that in the case of cationic AMPs. Polymyxins (polymyxin B and polymyxin E) are cyclic cationic lipopeptides which exhibit bactericidal activity against the Gram-negative bacteria *Acinetobacter*, *P. aeruginosa*, *Klebsiella*, *Enterobacter*, *E. coli*, *Citrobacter*, *Morganella*, *Haemophilus influenzae*, and some strains of *Stenotrophomonas maltophilia* [34, 35]. Polymyxins are topically applied to skin burns, for washing of wounds after operations, in superficial eye infections, and to protect minor wounds against infections. Polymyxins are combined with neomycin sulphate and bacitracin [36] or gramicidin S, and are useful for many topical applications in the form of wound creams and eye and ear drops [29]. Unfortunately, polymyxins are too toxic (at therapeutic doses they show high nephrotoxicity, neurotoxicity, and cause neuromuscular blockage), and may not be used regularly [37]. Colistin may be used in the treatment of wound infections [38]. Due to

the potential toxicity, the use of colistin and polymyxin B should be limited to patients with serious infections caused by multi-drug resistant Gram-negative pathogens for which there are no other therapeutic options.

An AMP array is being developed for use in local infections, wound healing, and cystic fibrosis. Synthetic antimicrobial peptidomimetics (SAMPs) with a broad spectrum of action, effective against bacteria (including MRSA) and fungi are also being developed [28]. SB006 and its derivatives are synthetic peptides of four branched chains, obtained by cloning, not sensitive to proteases, and exhibiting activity against Gram-negative, multi-drug resistant clinical isolates [28]. Preclinical tests carried out on mice have shown that SB006 has a high activity while also being safe. Formulations (PTX series) are being developed which are based on the 33-mer peptides of β-sheet structure [28] obtained by the incorporation of stabilizing residues of β-sheet domains of α-chemokines as well as residues of β-sheet domains of BPI protein. These exhibit a broad spectrum of activity against Gram-negative bacteria in nanomolar concentrations, and against Gram-positive bacteria in submicromolar concentrations (PTX002), their utility is further promised by their antiendotoxic activity.

Peptides in the combat of systemic infections. Through modification of P113 by introducing D-amino acids, a preparation called P113D has been obtained, which is less susceptible to enzymatic degradation while having the same antimicrobial activity as the parent compound. It proved to be effective in inhalation therapy against lung infections caused by *Pseudomonas aeruginosa* in patients with cystic fibrosis. Studies on the use of a lactoferricin-derived peptide (an 11-mer peptide from the N-terminus of human lactoferricin, hLF-11) have been carried out in the prevention of bacterial infections in patients undergoing haematopoietic stem cell transplantation. Phase I of the study has been completed. It showed activity against Gram-positive bacteria including MRSA [15] and MDRAB [39] and Gram-negative bacteria and fungi.

Colistin and its derivatives have been rediscovered for systematic use in blood infections in patients with cystic fibrosis [37].

Peptoids differ from peptides in that their side chains are linked to the amide nitrogen of glycine, not α-carbon, which confers resistance to proteases. The *in vitro* activity of some antimicrobial peptoids is similar to many AMPs – they are antibacterial over a large range and little cytotoxic in mammals. For example, a 12-residue ingredient called 1-Pro6 has been discovered which showed strong antibacterial activity (MIC value of 3.1 and 1.6 μM against *E. coli* and *B. subtilis*), and low haemolytic activity (LC50> 110 μM against hRBC) [40].

Tew et al. [41] described a class of facially amphipathic arylamide oligomers that showed an extensive antibacterial activity range. Modifications to the basic oligomers with various hydrophobic and hydrophilic side chains, and also replacement of benzene with pyrimidine, resulted in a few effective and moderately selective components. One, component 10 (with a single pyrimidine in the backbone), was 10- times more active (MIC approx. 1 μM) than the benzene analogue (component 11) – MIC approx. 18 μM. The same authors showed selective antibacterial activity of phenylene-ethynylene oligomers with a hydrocarbon backbone. The most active ingredient composed of three phenolic rings (compound 1) was highly effective against a broad panel of pathogenic bacteria. Component 1 did not induce resistance in *S. aureus* and inhibited LPS-induced activation of macrophages in nM concentrations. It has been shown that shortening of the ethylamine group of component 1 to a single methylene group resulted in loss of its activity against both bacteria and RBCs (red blood cells). Conversely, extension of the ethylamine group to the propylamine group increases the activity against bacteria and RBCs. Moreover, component 1 induced maximal penetration of the dye from lipid membrane vesicles of *E. coli*. In contrast to component 1's propylamine analogue, its penetration was reduced by enrichment in phosphatidylcholine, which suggests that component 1 degrades the membrane via a mechanism similar to many AMPs, where selectivity is dependent on the concentration of anionic lipids [40].

Lantibiotics are a unique class of peptide antibiotics produced by Gram-positive bacteria. They are highly modified peptides rich in lanthionines, composed of nonproteinogenic amino acids and two alanine residues linked to their β-carbon atoms by thioether bonds. These compounds combine advantageous biological interactions and desirable pharmaceutical properties, e.g. stability after passing into the circulatory system. The first defensin, plectasin was discovered in 2002 in the fungus *Pseudoplectania nigrella* that grows on the shady floors of northern-European pine forests. Plectasin showed activity comparable to penicillin and vancomycin against *S. pneumoniae* bacteria responsible for pneumonia, otitis media, and sepsis, among other diseases and conditions. Plectasin represents the first of some effective new classes of antibiotics and antiviral drugs [42].

Recently, studies have been performed on HBV core protein (HBc) containing an arginine-rich domain (ARD) on its C-terminal consisting of 16 arginine residues divided into 4 groups (ARD I to IV. They showed that a peptide containing the complete ARD I–IV (HBc 147–183) acts antimicrobially at micromolar concentrations against some multidrug-resistant pathogens, including colistin-polymyxin E-resistant *Acinetobacter baumannii* [43]. It was also determined that the peptides ARD II–IV (HBc 153–176) and ARD I–III (HBc 147–167) showed activity against *P. aeruginosa* and *K. pneumoniae*. The antimicrobial activity of HBc ARD peptides may, however, be diminished in the presence of LPS (lipopolysaccharide). The HBc ARD peptides showed the ability to bind directly to the lipid A of lipopolysaccharide, which has been demonstrated *in vitro*. The ARD I–IV (HBc 147–183) peptide showed no detectable cytotoxicity in tissue cultures or in a murine model. In the latter case, an intraperitoneal inoculation of *Staphylococcus aureus* and subsequent administration of the ARD peptide caused a hundredfold reduction in the number of bacteria in the blood, liver and spleen, and gave the infected animals 100% protection from death. When the peptide was administered at the time of the peak phase of bacterial growth in blood, the protective index decreased to 40%. Similar results were obtained in the case of *K. pneumoniae*. The above-mentioned studies supported the fact that the ARD HBc peptide may be a new clinically useful antimicrobial [43].

AMPs can also enhance the effect of existing antibiotics *in vivo* by facilitating their access to bacterial cells [15, 44].

Raqib et al. [45] observed that the use of sodium butyrate was beneficial in the treatment of shigellosis, a digestive

tract infection caused by bacteria from the genus *Shigella* in artificially infected rabbits. Further studies have led to the hypothesis that this is the result of LL-37 peptide induction after the interaction of butyrate with the rectal mucosa. Cirioni et al. [46] found that LL-37 protected against sepsis induced by Gram-negative bacteria in rats, which may confirm this thesis.

Xia et al. [47] found cathelicidin-BF may be a potential therapeutic in the treatment of intestinal infections caused by *Salmonella typhimurium*.

Anti-malarial effect. Peptides based on dermaseptin proved to be active against human erythrocytes infected with the malaria parasite *Plasmodium falciparum* [48], which suggests the possibility of their use in intracellular infection cases. It has been shown that the native dermaseptins destroy intraerythrocytic malarial parasites by lysis of the host cells. The acylation of shortened derivative S4 fortifies the anti-malarial effect. It has been found that the isobutyryl and aminoheptanoyl analogues of dermaseptin gave better selectivity (i.e. were more effective anti-malarially with less haemolytic effect). Some lauryl derivatives of dermaseptin (OAKs) showed highly selective antiparasitic activity with LC50 (hemolysis) to IC50 (inhibition of parasite growth) ratio > 1,000 for the most selective OAKs composed of three subunits α12 (3α12). The most active OAK, lauryl-lysyl-3α12, had an IC50 equal to 0.08 μM and a selectivity coefficient of > 1,000. These results indicate that OAKs do not counter malaria by lysis of infected RBC as in the case of the parent dermaseptins, which opens up the possibility of producing highly selective, low-cost formulations useful in the fight against malaria [40]. Other researchers [49] have also discovered new anti-malarial peptides isolated from the non-coding regions of the yeast genome. Berrocal-Lobo et al. [7] described *in vitro* activity of the plant antimicrobial peptides thionins against *Leishmania donovani*.

Action of peptides to treat fungal infections. Promising results have been obtained in a murine model in fungal infections, wherein the potential cytotoxicity of indolicidin for the host cells was reduced by the administration of the formulation in a liposome-encapsulated form [50]. The family of defensins have given cause for great hope. Several of these cysteine-stabilized peptides show pronounced activity against fungi. Two peptides, PAF and AFP, were fungicidal to the classes *Zygomycetes*, *Ascomycetes* and *Basidiomycota*. *Penicillium brevicompactum* vesicle protein inhibited the growth of *Saccharomyces cerevisiae*. From plants, three peptides have been isolated that have shown activity against *Aspergillus spp.*, *Candida albicans*, and *Neurospora crassa*, among others. In arthropods, the two peptides koprisin and juruin have been described as being destructive to fungi of the genera *Aspergillus* and *Candida*, and to *Malassezia furfur*, *Trichosporon beigeliii* and *Trichophyton rubrum*. Crotamine has been also isolated from a reptilian organism and is a peptide effective against *Candida spp.*, *Trichosporon spp.* and *Cryptococcus neoformans*. Most of these peptides did not show haemolytic activity against human erythrocytes [51].

Antitoxic effect of peptides. An extremely important feature of AMPs is their antitoxic activity. Tsutsuki et al. [52] developed a tetravalent peptide (PPP-tet) which negates the cytotoxicity of Shiga-toxin 2 (Stx2), a major virulence factor of enterohaemorrhagic *Escherichia coli* strains, by inducing disorders of their intracellular transport. In this study, the authors used a polyvalent peptide library to identify a series of tetravalent peptides binding with high affinity to Stx1, another major Stx family member, by blocking the receptor-binding site of the subunit B of toxin. One of the peptides, MMA-tet, significantly prevented the cytotoxicity of Stx1 and Stx2, showing a stronger effect than PPP-tet. The Stx1-MMA-tet complex had no effect on vesicular transport of toxin into the endoplasmic reticulum, but endured inhibition of protein synthesis by Stx1. Oral administration of MMA-tet protected mice from a lethal dose of *E. coli* O157:H7 strain producing both toxins, which may be promising in the treatment of these serious infections. Another author [53] isolated an agent from the opossum neutralizing animal, plant and bacterial toxins. Treatment of mice with synthetic peptides LT-15 and LT-10 obtained from LTNF (lethal toxin neutralizing factor) protected them from death when administered intramuscular injections of lethal doses of various toxins from animals, plants, and bacteria. Lethality was inhibited by the administration of the peptide either before or after injection of the toxin. Synthetic LTNF can be produced in larger quantities and should become a universal tool in the treatment of poisoning. A new cyclic peptide (nostocyclopeptide M1, N CP-M1) from cyanobacteria present in the Baltic Sea has been isolated and identified [54], which was an inhibitor of apoptosis induced by microcystin (a toxin cyanotic) in hepatocytes.

Immunostimulatory effect. Studies on AMPs are also being performed which do not act directly on the bacteria, but prevent infections developing by selectively stimulating the innate immune system. Interestingly, this activity is already observed at subtherapeutic doses. It has been shown that treatment with immunostimulant peptides significantly reduced the intensity of a bacterial infection in mice infected with *Staphylococcus aureus* [28]. Additional studies have been conducted on the use of these formulations in the treatment of both local and systemic infections, such as nosocomial pneumonia. Antoni et al. [55] synthesised short peptide fragments of human and murine interleukin 1 (IL-1), one of which, nine-residue fragment of human IL-1β (163–171), exhibited high ability to activate T cells. The mechanism was stimulation of the proliferation of murine thymocytes and strong induction of the production of interleukin-2 in spleen cells. An immunomodulatory activity of peptides, e.g. the ability to reduce pro-inflammatory cytokine response by inhibiting the intracellular inflammatory pathways, has been also used [15]. It has been planned to use the peptides as regulators of innate response in various diseases, such as inflammatory bowel disease, arthritis, asthma or ulcerative colitis [56].

Susceptibility of peptides to proteolysis. Peptides are relatively susceptible to proteolytic degradation [28]. Clinical trials of peptide therapeutics were initially concentrated on their local use [28], then on systemic administration. It has been indicated recently that the combination of peptides with negatively charged or lipophilic proteins can eliminate AMPs from the circulation [28] or protect against excessive proteolytic degradation *in vivo* [57]. The introduction of D-amino acids, amidation of the N-terminus, use of unnatural amino acids and cyclisation are the most common

methods of increasing the stability of peptides. An example may be peptaibols, a family of short-chain peptides (≤ 20 residues) having C-terminal alcohol residues and a high non-standard amino acid content (especially α-aminoisobutyric acid, isovaleric acid and hydroxyproline), which gives them profound resistance to proteolysis [58]. Other non-standard amino acids may also be included to prevent binding to the active sites of proteolytic enzymes [59], or the peptide bonds may be modified, e.g. by alkylation of nitrogen atoms [60].

Microbial resistance to peptides. In contrast to conventional antibiotics, the acquisition of resistance to AMPs by a sensitive strain of microorganism is unlikely because of their great diversity and the fact that they have been effective against bacterial infections for at least 108 years. However, some pathogens are more resistant to AMPs and others are more sensitive. For example, resistant species of such types as *Serratia* and *Morganella* have an outer membrane lacking the appropriate density of the acidic lipids which are peptide binding sites. Other resistant species such as *Porphyromonas gingivalis* secrete digestive proteases that destroy the peptides [28]. In many species of Gram-negative bacteria the outer membrane charge is modulated by the PhoPQ regulon, a two-component system consisting of the sensor (PhoQ) and the intracellular effector (PhoP) [61]. Under the influence of potentially lethal stress conditions, the PhoP/PhoQ regulon affects the sensitivity of the microorganism to the peptide by PmrA regulon modulation. The modulation is of the gene pool intermediating in the formation of outer membrane structure with the positively charged groups of ethanolamine and 4-aminoarabinose [62], and the result is modification of proteins, phospholipids and lipopolysaccharides. Other pathogenic bacteria produce a capsule for easier adherence to a tissue or to prevent opsonization and phagocytosis, e.g. the highly anionic capsular exopolysaccharide produced by virulent strains of *P. aeruginosa* [63].

Until recently, attempts to induce resistance to pexiganan in *E. coli* and *S. aureus* by chemical mutagenesis have been unsuccessful. Recent studies, however, have shown the acquisition of resistance to pexiganan by extensive natural selection under the laboratory conditions. In this experiment, 22 out of 24 lines of *E. coli* and *P. fluorescens* independently developed inherited resistance mechanisms to pexiganan during cultivation in medium containing the peptide when allowed to evolve through 600–700 generations [64]. This proves that the process takes a long time, whereas the peptides are administered once or only a few times to achieve therapeutic purposes thereby disfavouring the emergence of antimicrobial resistance.

CONCLUSIONS

The presented article reviewed the application of antimicrobial peptides (AMPs) in the treatment of local and systemic infections. These compounds occur naturally in nature and may constitute a promising strategy for the development of a new class of drugs intended for the prophylaxis and treatment of bacterial, viral, fungal, and protozoal infections, as well as used as antitoxic or immunostimulatory agents.

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