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Antimicrobial peptides-a promising novel antimicrobial agent

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Abstract. Antibiotic resistance has become one of the most critical public health problems in the 21st century, and infections caused by drug-resistant bacteria severely threaten human health. Otherwise, the development rate of conventional antibiotics has been unable to keep up with the speed at which bacteria develop resistance. Therefore, it is urgent to develop antimicrobial agents with novel mechanisms of action. Antimicrobial peptides (AMPs) are naturally occurring polypeptides with antibacterial activity, which have different mechanisms of action from existing antibiotics and thus have incomparable advantages over traditional antibiotics in treating infections caused by drug-resistant bacteria. Up to now, AMPs have been used in several clinical studies to destroy drug-resistant bacteria. The review introduces the basic properties of AMPs and their therapeutic mechanisms, summarizes some advances in preclinical studies and clinical applications, and analyzes the factors limiting their application in clinical treatment. In addition, some new strategies to overcome the shortcomings of AMPs in clinical applications are also introduced. Efficient and diverse synthesis technologies and optimization strategies keep coming to overcome the difficulties of clinical applications for AMPs, which may become an important weapon against drug-resistant bacterial infections in the future.

Keywords: antimicrobial peptides, bacterial infection, drug resistance, clinical applications

1. Introduction

The discovery and application of antibiotics is one of medicine's greatest achievements in the 20th century. The application of antibiotics in clinical treatment has greatly increased human life expectancy and improved human life quality. However, due to the widespread use of antibiotics in clinical practice, especially its irrational use and abuse, a variety of drug-resistant strains have appeared. The emergence of drug-resistant strains is a major threat to human health and survival now and in the future [1], so there is an urgent need to develop antibacterial drugs with new structures and new mechanisms to overcome the bacterial resistance.

Antimicrobial peptides (AMPs) are peptides or small proteins with a net positive charge composed of various amino acids and exist widely in multicellular organ organisms. Naturally occurring AMPs protect the body from various pathogenic microorganisms. Even though they have different structures, they all share a common characteristic, that is, the hydrophilic positive charge and lipophilic side chain are separated into completely different regions or different sides of the molecule, in which the positive charge is mainly used to ensure the interaction between AMPs and bacterial membranes. At the same time, the hydrophobic groups are responsible for penetrating and destroying the structure of bacterial membranes [2]. AMPs acts on the bacterial cell membrane, which reduces the probability of bacterial

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resistance due to the lack of specific targets. Therefore, AMPs are expected to develop into a new class of complementary drugs that can overcome bacterial resistance and may even replace traditional antimicrobial drugs for the treatment of bacterial infections.

In the paper, we review the structure, synthesis, and therapeutic mechanism of AMPs and the research progress in the treatment of bacterial infections, combining the current situation of bacterial drug resistance. At the same time, we also discuss some challenges faced by AMPs in clinical application and countermeasures.

2. Drug resistance in bacteria

There are four main antibacterial mechanisms [3] of antimicrobial agents for clinical application, including inhibiting the formation of bacterial cell walls, destroying the structure of cell membranes, interfering with the replication, transcription and translation of DNA in bacteria, and disrupting protein synthesis. It is worth noting that antibacterial drugs can exert their antibacterial effect not only through one of these mechanisms, but also through a combination of multiple mechanisms.

Bacterial drug resistance is divided into two types: inherent and acquired. Inherent resistance refers to bacteria that are not sensitive to certain antibiotics mediated by resistance genes and usually don't change due to external environment and other factors. Acquired drug resistance is a defense mechanism caused by the adverse environmental conditions when bacteria are under the continuous action of antibacterial drugs. Bacteria can develop antibiotic resistance through changes in cell membrane permeability, overexpression of active efflux pump system, changes in the structure of antibiotic targets, destruction and modification of antibacterial drug structure, and carrying exogenous resistance genes. Drug resistance can be acquired through gene mutation, and the transmission, transfer and mutation of drug resistance genes between cells will lead to the emergence of multi-drug resistant bacteria [4].

3. Sources and structures of AMPs

Natural AMPs can be divided into five categories according to their sources [5], which are insect-derived, amphibious, plant-derived, aquatic, and microbial, while those derived from viruses are less. AMPs from different sources have different effects on microorganisms, which may be related to the molecular structures and amino acid composition of AMPs. Natural AMPs are small multi-functional peptides synthesized in ribosomes. Their relative molecular weight is about 4 k Da, and most AMPs have isoelectric points greater than 7.0, which can show cationic solid properties. With the development of technology, the AMPs and their simulants synthesized by genetic engineering or chemical synthesis are also the focus of current research [6]. Therefore, according to the sources and approaches of acquisition for AMPs, it can be divided into natural or synthetic AMPs.

AMPs are generally divided into four classes based on their signature secondary structure: α-helix, β-sheet, extended, and cyclic [7]. Some AMPs consist entirely of a single spiral or sheet fold. Extension peptides are characterized by their lack of recognizable structural motifs. However, they contain large amounts of specific amino acids such as arginine, tryptophan, glycine, and histidine. In addition, several cyclic peptides with more complex topologies, including lasso peptides and thioether bridges, exist. The structure of AMPs will change with the environmental conditions, which is mainly related to the change of hydrophobicity and net charge of the cell membrane. In nature, the α-helix structure is the most abundant natural peptide. These α-helical peptides interact with the target cell membrane and fold into amphiphilic structural features. "Amphiphilicity" refers to the separation of hydrophobic and hydrophilic regions in space, dominated by the helical side, which contains hydrophobic amino acids, and the opposite side, which contains charged amino acids. β-folded peptides are inverted parallel β-lamellae, which usually have one or more disulfide bonds to maintain their structural stability. Peptides with extended structures mainly include indolicidin, which contains a high proportion of amino acids such as tryptophan, histidine, and proline. Cyclic peptides are characterized in their ring structure formed by single bonds (i.e., disulfide and amide).

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4. Antimicrobial mechanisms of AMPs

According to the different sequences and structures of AMPs, there are various possible mechanisms of action. Its antibacterial mechanisms are generally divided into two types: direct killing and regulation of host defense and immunity, and direct killing of microorganisms can be further divided into membrane-target and non-membrane-target [8]. In membrane targeting mechanism, cationic peptides bind to bacteria with negative electric components (teichoic acid of gram-positive bacteria and lipopolysaccharides of gram-negative bacteria) through electrostatic interactions, and AMPs subsequently act through different membrane destruction mechanisms. At present, there are three main mechanism models, namely: (1) the barrel wall model (in which peptides form bundles across the pores of the membrane and are inserted into the membrane); (2) ring model (peptide polarity residues facing inward, hydrophobic residues facing outward, causing local membrane bending, thus resulting in membrane rupture); (3) carpet model (peptides cover the surface of the membrane, causing the membrane to disperse into particles isolated by the peptides). The above model can cause the membrane to break or form ion channels so as to leak bacterial contents, resulting in bacterial death and achieving antibacterial effect.

Membrane targeting is a common mechanism for all AMPs, while non-membrane targeting is limited to some AMPs with different peptides and different targets. Some AMPs bind to the cell wall to form a precursor, inhibit cell wall synthesis, and even lead to cell membrane perforation and rupture. AMPs can also degrade DNA/RNA through inhibiting the enzymes which are responsible for the synthesis of DNA/RNA, such as topoisomerase. AMPs can also induce proteins degradation by altering the transcription and translation of them, or prevent them from folding properly. In addition to the above roles, there are many newly discovered mechanisms, such as preventing the formation of bacterial biofilms and destroying current biofilms [9].

In addition, since the cell membrane of bacteria is mainly composed of oligosaccharide or teichoic acid with a net negatively charge, while which of mammalian is mainly composed of sphingomyelin and lecithin in the form of positive and negative ion pairs, probably resulting in the force strength between them and AMPs is different, so that AMPs show certain selectivity in the membrane of mammalian cells and bacterial.

5. Acquisition of AMP

The synthesis of AMPs for antimicrobial applications can be roughly divided into ribosome synthesis and non-ribosome synthesis, and the latter is further divided into enzyme-catalyzed synthesis and chemical synthesis [10].

Ribosome-synthesized AMPs are usually derived from relatively short precursor peptide sequences, and the peptides synthesized by ribosomes and modified after translation are considered to be an untapped source of antimicrobial agents. Several ribosome-synthesized AMPs are currently undergoing clinical trials and have a small number of applications in agriculture and the food industry.

Enzyme-catalyzed synthesis is synthesized by non-ribosomal peptide synthase without the help of ribosomes and mRNA. Non-ribosome-synthesized AMPs are commonly used as systemic and local antimicrobial agents and also have effects to protect from tumors and fungi. Early discovery of AMPs relies on isolation from natural sources, and usually requires large quantities of biological feedstock from which only small amounts of pure peptides can be extracted. Currently, AMPs can be obtained on a large scale through chemical synthesis. Solid phase peptide synthesis of AMPs has the advantages of shorter production cycles and higher automation and scalability. Microwave-assisted peptide synthesis technology can significantly shorten the reaction time of coupling and deprotection steps by microwave heating. It can be used to improve the purity of crude peptide extracts.

In addition to the sequence optimization and novel design of AMPs based on the structure of natural peptides, the researchers began to design and synthesize AMPs mimics according to the function and amphiphilic structural characteristics of AMPs through chemical modification strategy (including introducing natural amino acids or non-natural amino acids for point mutation, lipidation modification, glycosylation modification, hybridization, cyclization, etc.) [11].

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6. Progress of clinical research on AMPs for the treatment of bacterial infections

AMPs did not enter clinical trials in the decades after its initial discovery despite its extensive activity against a wide range of pathogenic microorganisms, and it gradually enters the pharmaceutical market until the problem of bacterial resistance soared. The current progress of AMPs in the treatment of bacterial infections is divided into two categories: already in use and in the development process [12-13].

Polymyxin is a classic AMPs drug that targets bacterial cell membranes to play an antibacterial role and has a protective effectiveness on most gram-negative bacteria. Vancomycin is a non-ribosome-synthesized tricyclic glycopeptide, consisting of a 7-membered tricyclic peptide structure linked to vancomycin-glucose disaccharide. Vancomycin inhibits bacterial cell wall formation and is therefore most effective against Gram-positive bacilli. It is used as a first-line drug to treat MRSA infections.

Other AMPs entering the clinical stages are briefly described as follows [13]: pexiganan (MSI-78), the first commercially developed AMP in the world, has a better effect on diabetic foot patients than ofloxacin and has successfully passed the phase III trial. PL-5 is the world's first AMPs with secondary wound infection as an indication. At present, the Phase III trial on secondary wound infection has been completed in China, and the Phase II trial has also been successfully applied for in the USA. Compared with traditional antibiotics and even partial AMPs, it has a stronger and wider effect on both gram-positive and gram-negative bacteria, and is more secure. Friulimicin B, produced by a special Actinoplanes friuliensis, has a strong effect on gram-positive bacteria and exerts its antibacterial activity mainly by inhibiting the synthesis of bacterial cell walls through the cell wall-targeting mechanism. LL37 is an important part of human innate immunity and can fight off a variety of pathogens. In addition to antibacterial activity, it can also regulate the immune system. LTX-109 is a chemical synthesis peptide mimic with broad-spectrum antimicrobial activity, especially against drug-resistant strains. It is a promising drug for the treatment of infections, benefiting greatly from the high safety in vivo and the reduced possibility of drug resistance due to its membrane-targeting effect. DPK-060 is a class of chemosynthetic peptides derived from human kininogen, showing strong and broad-spectrum activity against both gram-positive and gram-negative bacteria, and it's mainly used in the treatment of local microbial infections such as atopic dermatitis.

7. Clinical translational limitations of AMPs

With the increase of antibiotic resistance, AMPs have attracted extensive attention due to their broad-spectrum antibacterial properties. At present, more than 3,000 natural AMPs have been discovered, but there are still less than 100 AMPs drugs approved by FDA or entered the clinical trials. The most obvious challenges in developing AMPs are as follows [10,14]: (1) As a polypeptide drug, AMPs have attracted much attention for their stability, and many AMPs have short half-lives, which has become a major obstacle and limitation for their clinical application. (2) Although most AMPs exhibit strong activity in vitro, their activity in vivo may be weakened due to the complexity of the microenvironment, such as the role of proteases and changes in pH, which is another main challenge for AMP research and development. (3) AMPs can be divided into receptor-binding peptides and membrane-active peptides. off-target effects of receptor-binding peptides may occur and may trigger other chronic inflammatory diseases with increasing concentrations of them, and membrane-active peptides have been shown to be hemolytic and cytotoxic. (4) The dose-response curve for AMPs is steeper, suggesting that AMPs are less likely to evolve drug tolerance. However, it has been suggested that evolutionary resistance to AMPs may create potential cross-resistance to endogenous host AMPs. At this stage, researchers tend to improve the amino acid sequence of natural AMPs or directly design and synthesize novel ones from scratch to improve their therapeutic efficacy.

8. Other optimization strategies for AMPs

In addition to modifying and changing the structure of AMPs, some researchers have focused on the use of joint modification with other structures to overcome the defects of AMPs. Several research focus directions are as follows:(1) conjugated with small-molecule antibiotics[15]: The covalent coupling of

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AMPs and small-molecule antibiotics through linkers can simultaneously combine the intracellular bactericidal function of small molecules with the broad-spectrum bactericidal and high activity against multi-drug resistant bacteria of AMPs to obtain better antibacterial effect than the above two drugs alone. (2) Polymerization with other materials [16]: polymerizing AMPs with other materials to form composite materials, can highlight the advantages of the broad-spectrum bactericidal of AMPs, made up the defects of expensive and potentially toxic of it, and realize the functions of other materials at the same time. (3) Introduction of nanocarriers [17]: using nanocarriers to load AMPs for delivery in vivo can overcome the shortcomings of AMPs, such as enzymatic instability and off-target toxicity, thereby improve the pharmacokinetic and pharmacodynamic data of it.

9. Conclusion

In recent decades, the proliferation of microbial infections worldwide and the overuse or misuse of antibiotics have led to increasingly severe microbial resistance to existing antibiotics. The non-specific interaction between AMPs and pathogens enables it to have unique inhibitory activities against bacteria, fungi, viruses and parasites, thus it has wide application potential in different industries such as medicine, food and agriculture. AMPs will be a promising drug against microbial infections, especially in eliminating resistant and multi-resistant pathogenic microorganisms. However, the difficult screening process, low bioavailability, potential cytotoxicity, and high production cost of AMPs have limited the conversion of it from the laboratory to clinical. At the same time, specificity and high synergistic ability of AMPs need to be further improved [18].

In order to deal with the situation, more and more researchers synthesize new AMPs by optimizing the structure in order to eliminate its toxic or unstable factors, enhance its antimicrobial activity, and make AMPs a new antibacterial agent. Future research on antimicrobial peptides will focus on the interactions between AMPs and the complex biological environment in vivo, which will help evaluate the true potential of AMPs as drugs. Clinical trials of AMPs should focus on the unmet clinical needs to gain lasting momentum for research and development. At present, the research and development of AMPs is still in the early stage, and in vitro and in vivo trials are still needed to elucidate the mechanisms of AMPs further, and fully characterize its structure and physicochemical properties, so that it can eventually be applied to clinical therapy. Despite these shortcomings, advances in design methods will open up more possibilities for designing new AMPs in the future, resulting in more secure and effective drug candidates with higher clinical application potential.

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