Antimicrobial peptides, a promising alternative for the treatment of infectious diseases

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Abstract

Microorganisms that cause diseases in humans are constantly evolving, which represents a challenge in the search for effective treatments against them. Even when currently there are several pharmacological alternatives available, sometimes they are inefficient for the control of infectious diseases, especially because pathogens have generated multiple resistance mechanisms against them. Antimicrobial peptides have been described in many species of organisms, from fungi, plants and insects to humans; currently, there are molecules that appear as a solution that can be effective in modern therapeutics. The advantage of these natural peptides lies in that they have been evolving almost the same amount of time than the species that produce them and their effect on the control of microorganisms is highly significant; some of these molecules are isolated from living organisms, others are starting to be produced by synthetic methods, which allows having access to an endless number of peptides with diverse therapeutic activities.

KEY WORDS: Antimicrobial peptides. Infectious diseases. Drug resistance.

Human antimicrobial peptides

Antimicrobial peptides (AMP) are relatively small molecules that contain between 12 and 60 amino acids, which are part of the defense response of various species of organisms and participate by mediating the primary response to several pathogens such as bacteria, parasites, and to viral and fungal infections. In mammals, they are synthesized in the skin, mucous membranes or sites prone to infections.¹

The first AMP of animal origin was isolated from rabbit leukocytes in 1956.² Currently, more than 5000 AMPs of natural origin, both from eukaryotic (animals, plants, fungi, insects) and prokaryote cells (bacteria), have been discovered or synthesized.^{3,4}

AMPs in humans are capable of activating different immune response mechanisms such as phagocytosis, releasing prostaglandins, neutralizing lipopolysaccharide effects, increasing the number of immune cells at

inflammation sites and repairing wounds by activating healing systems.⁵ These molecules usually have positive charge and are classified in two large groups: defensins and cathelicidins.⁶

According to their molecular structure, defensins are subdivided into α and β .⁷ They are produced by most somatic cells and by immune response cells; they occur at high concentrations at the site of inflammation, in infectious processes, and some are detected in plasma at basal concentrations.⁸

α defensins contain 6 cysteine residues and 3 disulfide bridges, and are produced by leukocytes and epithelial cells. There are 6 α defensins described in humans; the first 4 were identified in neutrophils (HNP 1-4) and the other two in Paneth intestinal cells.⁹

β -defensins have structures similar to those of the α type. There are four types of human β defensins (hBD 1-4); they are produced by epithelial cells, cells of the respiratory and urinary tract, as well as by keratinocytes.⁸

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Date of reception: 05-05-2017

Date of acceptance: 25-03-2018

DOI: 10.24875/GMM.M19000210

Gac Med Mex. 2018;154:582-588

Contents available at PubMed

www.gacetamedicademexico.com

Cathelicidins are short peptides that contain an amino-terminal group and at the other end a carboxyl-terminal group; the charge of this molecule is positive, and it is therefore considered a cationic peptide. Cathelicidins show rapid action against pathogens, are potent and contain a broad spectrum of antimicrobial activity. The cathelicidin with the highest relevance described in humans is LL-37, made up by a helical α structure; the cells that produce it secrete it in its inactive form and its activation is carried out by proteases, leaving a peptide of 37 amino acid residues with two leucines next to each other in one extreme.⁷

General mechanism of action

The general mechanism by means of which peptides exert their action consists in damaging the pathogen cell membrane. AMPs interaction of with the microorganism is by means electrostatic forces between their amino-positive residue and the negative charge of the pathogen cell membrane. AMPs interaction with the pathogenic cell depends on the pathogen membrane composition, as well as on the concentration of the available antimicrobial peptide. If there is AMP affinity for the pathogen membrane, intramembrane pores will be formed through the bilayer lipid membrane, which causes its lysis and, therefore, its death.¹⁰

Almost all AMPs interact with lipids of the cell membrane, but other require receptors to exert their activity.¹¹ Possibly, cell death caused by AMPs involves membrane synthesis interruption followed by cell lysis. Not always does the mechanism focus on the pathogen membrane; interactions of AMPs with other targets have been described, including the cell wall, DNA, RNA and molecules involved with the synthesis of proteins.¹²

AMPs potential and applications are almost unlimited, since thousands of these molecules been characterized in nature and, in addition, millions of possible peptides with pharmacological activity can be synthetically obtained. According to the results of clinical studies, in animal models and in vitro studies, it has been suggested that AMPs may be highly promising molecules for the treatment of infectious diseases.¹³ Even when human AMPs are effective, most research has focused on obtaining and purifying of molecules from insects, plants and other mammals.

AMPs in the treatment of infectious diseases

AMPs are being used in the treatment of diseases caused by pathogens, with some being at experimental phases and other already being marketed; for example, AMPs are used against fungal agents such as *Candida albicans*, *Cryptococcus neoformans* and *Fusarium oxysporum*;¹⁴ some topically-applied antiviral peptides that fight infection with human immunodeficiency virus and human papilloma virus have been developed,¹⁵ as well as AMPs that fight protozoa and nematodes¹⁶ and against gram-negative bacteria;¹⁷ some others are used for their neuroprotective and anti-tumor effect.^{18,19}

Antifungal AMPs

Fungi are an extensive group of microorganisms that are present in almost any environment; they are part of the human and animal microbiota, and some have the ability to cause dangerous diseases for humans, most of them non-lethal but chronic.²⁰

Antifungal AMPs properties have been under investigation for more than 40 years. Recently, interest in antifungal compounds has grown owing to the resistance mechanisms developed by pathogens of this type.²¹ Approximately 100 peptides with antifungal properties have been isolated and are at different stages of research. Most are of natural origin, although the number of semi-synthetic and synthetic peptides is increasing. Antifungal AMPs are usually linear and cyclical molecules, with amphipathic or hydrophobic properties, exhibit lysis, binding and external membrane rupture activity.²²

Antifungal AMPs mechanism of action is poorly known. Some peptides bind to nuclear envelope proteins of certain species of fungi, producing reactive oxygen species, ion output flow and ATP; this series of events results in cell death. Some AMPs contain mitochondrial regulatory proteins that are involved in fungal energy metabolism, which positively regulates their synthesis and decreases mitochondrial ATP synthesis.²³ Another mechanism is membrane surface tension alteration, which elicits the formation of pores and the release of K^+ and other vital ions.^{24,25}

The anti-fungal AMPs with greater importance and that, in addition, are structurally related are defensins isolated from plants, animals and insects.²⁶

Table 1. Antifungal peptides

Compound	Company	Structure	Origin	Use	Evaluation phase
Isegran. ²⁸ (IB-367)	Intrabiotics Pharmaceuticals	Peptides with disulfide bridges	Pig leukocytes	Anticancer therapy-induced mucositis	Phase III
Omiganan. ²⁸ (MBI-226)	Microbiologix Biotech	Cationic peptide	Bovine neutrophils	Catheter infection reduction	Phase III
Cm-p1. ²⁹	Havana University	Hydrophobic α -helix	<i>Cenchrutis muricatus</i>	Inhibitor of several filamentous fungi <i>in vitro</i> activity	Phase I
Arenicine-1. ³⁰	Adenium Biotech	β -helix	<i>Arenicola marina</i>	Against <i>Candida</i> spp.	Preclinical
ApoEdpL-W. ³¹		Lipoprotein	Synthetic	Against <i>Candida</i> spp.	Phase II
HM-1 killer ³²	Nigata University & Applied Life Sciences	Toxin	<i>Williopsis mrakii</i> toxin	Against <i>Candida</i> spp. and <i>Cryptococcus</i> spp.	Phase I
PAF-26 ³³	Instituto de Agroquímica y Tecnología de Alimentos-CSIC	Hexapeptide	Synthetic	<i>Neurospora crassa</i> <i>Penicillium digitatum</i> <i>Saccharomyces cerevisiae</i>	Phase I
Echinocandin B ³⁴	Pharmatech	Lipopeptide	<i>Zalerion arboicola</i> fermentation	<i>Pneumocystis carinii</i> <i>Aspergillus aculeatus</i>	Phase II
Pneumocandin ³⁵	Pharmatech	Ether phosphate	Semisynthetic	<i>Candida albicans</i>	Clinical
Mulundocandin ³⁵	Aventis Pharma Deutschland	Lipopeptide	<i>Aspergillus syndowi</i>	<i>Candida albicans</i> <i>Aspergillus niger</i>	Phase II

The commercialization of peptides with antifungal activity is relatively recent. The United States Food and Drug Administration has approved antifungal peptides that have been shown to be viable in the treatment of common pathogens in humans; many of these molecules are marketed in the form of topical application²⁷⁻³⁵ (Table 1).

Antiviral AMPs

Some diseases in humans and animals are caused by viruses such as influenza, dengue, hepatitis C, herpes simplex, just to name a few. Against them, antiviral therapies are limited, even for some a cure has not yet been found; in addition, the formation of resistance of these viruses to drugs has been increased, and antiviral therapy is therefore currently a challenge. The innovations to meet this challenge include antiviral peptides (AVPs), which promise to be excellent options.³⁶

AVPs are a part of antimicrobial peptides that can act directly or through the immune response by inhibiting one or more stages of the viruses' life cycle, either at entry, replication, transcription, etc., showing a clear antiviral effect.³⁷

In 1985, one of the first studies of these AVPs was revealed, where herpes simplex virus replication inhibition was demonstrated.³⁷ Subsequently, other researchers took this as a basis to continue working with antiviral development.

Some advantages of AVP-based drugs in relation to the conventional ones include their low molecular weight, higher efficiency, lower toxicity and fewer side effects. These AVPs can be easily modified with the addition of chemical groups or amino acids to improve their activity and stability. The first AVP that approved the clinical trials was enfuvirtide (T20), an HIV fusion inhibitor that is currently marketed under the name Fuzeon.^{38,39}

Cecropin and melittin

Cecropin and melittin are α -helix antimicrobial peptides isolated from insects with antiviral and antimicrobial (gram-negative) activity. The cecropin family includes sarcotoxins, hyphancin, enboncin and spodopsin.

Melittin is a cationic peptide of two α -helices bound by a flexible segment and amphipathic structure; it has 26 amino acid residues and its action consists in

inhibiting murine retroviruses, tobacco mosaic viruses and herpes simplex virus replication, as well as promoting viral membranes direct lysis. At low concentrations, it also shows antiviral activity for chronically HIV-1-infected T cells.^{40,41}

Alloferons

Alloferons are peptides isolated from *Calliphora vicina* fly larvae hemolymph, which are produced as 13-residue (alloferon 1) and 12-residue molecules (alloferon 2); the name was given to demonstrate functional similarity to the cytokine known as interferon. Their activity was demonstrated with a synthetic peptide in a model of mice with lethal pulmonary infection with human influenza virus A and B. When alloferon was administered, mortality was prevented in most animals infected with type A influenza virus, in addition to stimulating resistance to influenza B virus infection. The results were almost equivalent to those observed with the administration of ribavirin, and alloferon was thus shown to have antiviral activity.⁴²

N-myristoylated peptide

It is an antiviral compound with low molecular weight of 916 kDa extracted from the tobacco caterpillar larvae *Heliothis viresces* hemolymph that has six amino acids; the N-terminus portion of this peptide contains the fatty acid myristoyl, while the C-terminus, histidine with two methyl groups, which confers permanent positive charge. The antiviral action of this compound corresponds to the inhibition of the binding of viruses to the host membrane cytoplasmic side, which prevents the assembly of viral particles. Thanks to its low molecular weight and non-polar nature, it can improve its ability to cross the plasma membrane, where it can inactivate viruses such as HIV-1 and HSV-1.⁴²

AVPs mechanisms of action

Peptides such as alloferon stimulate natural killer (NK) cells and the production of type 1 interferons, which collaborate together by activating NK cells cytotoxicity, which actively participate in the control viral infections.⁴³

Another mechanism of action of peptides is the influence on the intracellular steps of viral infection,

interaction with DNA and viral messenger RNA. Examples of this are melittin and cecropin A, which inhibit the production of HIV-1-associated cells by gene expression suppression. Cecropin A also inhibits the multiplication of Sendai virus by reducing the synthesis of viral proteins without affecting the host cell protein synthesis.⁴⁰

Being a membrane protein, melittin is able to interfere with cell signal transduction pathways such as phospholipase A2 activation, and decrease carmodulin and protein C kinase activities; this is how it can change the balance and stimulating or inhibitory transcription activity in HIV-1-infected cells. It can also be involved in post-transcriptional regulation of human immunodeficiency virus gene expression and influence on the regulation of large and intermediate messenger RNAs; preliminary studies reveal that it also inhibits the replication of retroviruses such as that which causes murine leukemia, as well as the feline immunodeficiency virus.⁴²

Another AVP example is the N-myristoylated peptide, which acts on HIV viral control; its probable mechanism is the inhibition of assembly or budding of the virus in host cells.⁴⁴

Cell-penetrating peptides are molecules that increase cell uptake and traffic towards the cytoplasm of peptides that might have antiviral effects; basically, they insert into membranes, allowing the passage of really effector molecules.^{45,46}

Antibacterial AMPs

Antibacterial AMPs have started being used as an alternative to the use of antibiotics, which currently are not enough and some are no longer efficient in bacterial control. Most bacterial pathogens have resistance mechanisms to current drugs, which allows them to survive and even spread in their hosts. The use of AMPs could be a good alternative, especially those that are of synthetic origin and that are already in clinical phases of research⁴⁷⁻⁵⁰ (Table 2) or those that are directly isolated from organisms; most are still on laboratory experimentation phase⁵¹⁻⁵⁴ (Table 3).

Multiple mechanisms are used by AMPs for the control of bacteria, depending on each species; generally, they act on membrane permeabilization, metabolism disruption and DNA and protein function interruption.⁵⁵

Table 2. Peptides with antibacterial effect

Compound	Company	Structure	Origin	Inhibited microorganism	Evaluation phase
BmKn2 ⁴⁸	Thammasat University	Alpha-helical, basic peptide without disulfide bridge	Scorpion	<i>Miseria gonorrheae</i>	Phase II
GL 13K ⁴⁹	University of Minnesota	13-amino acid antimicrobial peptide	Human salivary glands	<i>Pseudomonas aeruginosa</i> biofilms reduction	Clinical
Alysterin-1 ⁵⁰	Bio-X	β -helix	Synthetic	<i>Escherichia coli</i>	Clinical
A3APO ⁵⁰	University of Minnesota	Lipopeptide	Synthetic	<i>Escherichia coli</i> <i>Salmonella</i> spp.	Preclinical
XMP. 629 ¹³	Xoma technology	9-amino acid peptide	Synthetic	<i>Propionibacterium acnes</i>	Phase I
CEME ⁵¹	British Columbia University	α -helix	Synthetic	<i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i> <i>Staphylococcus pneumoniae</i>	Clinical
CEMA ⁵¹	University of British Columbia	Modified CEME with 2 extra peptides	Produced by transgenic plants	<i>Porphyromonas gingivalis</i> <i>Streptococcus mutans</i>	Clinical
MBI 594AN ¹³	Microbiologix Biotech	Lipopeptide	Synthetic	<i>Propionibacterium acnes</i>	Phase II
Neuprex ¹³	Xoma technology	β -helix	Synthetic	<i>Neisseria meningitidis</i>	Phase II

Table 3. Human-isolated peptides with antibacterial effect

Antimicrobial peptide	Structure	Production/synthesis	Action	Inhibited microorganisms
hBD3 defensins ^{52,53}	Linear peptides with alpha helical structure	Inflammatory stimuli	Innate response activation	<i>Staphylococcus aureus</i> <i>Mycobacterium tuberculosis</i>
Lactoferrins ^{54,55}	Single polypeptide chain, folded in two symmetric globular lobes	Produced by mucosal epithelial cells	Bacteriostatic and bactericidal functions	<i>Listeria monocytogenes</i> <i>Streptococcus mutans</i> <i>Staphylococcus aureus</i> <i>Bacillus stearothermophilus</i>

Antiparasitic AMPs

Protozoa are simple eukaryotic cells (organisms whose cells have a nuclear membrane) with characteristics of the animal kingdom, since they are mobile and heterotroph; many of these species are cosmopolitan, while others are of limited distribution.⁵⁶

Parasitic infections in humans are increasing due to the emergence of strains that are resistant to antiparasitic compounds, and thus the search and development of new compounds has been undertaken, focusing on extraction and purification of active ingredients of natural or synthetic origin. AMPs could be used in the control of these infections.⁵⁷

The skin of amphibians has been studied, where around 400 peptides with therapeutic potential have been described; most are synthesized in the granular glands that are located on the skin, and play an important role in these species innate immunity. These peptides are involved in the defense of bare skin against pathogenic bacteria and fungi, and also

participate in wound repair.^{58,59} They are grouped in 3 families:

- Linear amphipathic helical peptides (magainins and dermaseptins).⁶⁰
- Peptides that contain two cysteine residues on the terminal carboxyl group (brevinins, gaegurins, esculentins, ranatuerins and ranalexin).⁶¹⁻⁶³
- Temporins.^{64,65}

Dermaseptins are part of an 8-peptide family related to the skin of a South American frog (*Phyllomedusa sauvagei*). These are linear peptides, of 28 to 34 amino acids, with an α -helix structure and are amphipathic in apolar solvents.⁶⁶ They are highly efficacious against protozoa such as *Leishmania Mexicana* and *Plasmodium falciparum*.^{67,68}

Magainins are molecules isolated from secretions of the skin of the frog *Xenopus laevis*;⁶⁹ different studies have reported that they have a spectrum with rather broad antimicrobial activity against bacteria, fungi and parasites, in addition to participating in the activation of mechanisms that reduce inflammation.⁷⁰

Table 4. Peptides deriving from *Hadruroides mauryi* and *Centruroides margaritatus*

Organism	Active fractions	Inhibited microorganisms
<i>Hadruroides mauryi</i> ⁷²	IV	<i>Escherichia coli</i> <i>Pseudomonas aeruginosa</i> <i>Bacillus cereus</i>
	VI	<i>Bacillus cereus</i>
<i>Centruroides margaritatus</i> ⁷²	II	<i>Staphylococcus aureus</i>
	III, IV, V and VI	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i> <i>Bacillus cereus</i>
	VII	<i>Pseudomonas aeruginosa</i>

Other AMPs. Scorpion venom antibacterial properties

Although the existence of some antibacterial peptides in scorpion venoms suggests that the venom gland could be protected by these effector molecules, the antibacterial activity of the venom itself has not been extensively assessed.⁷¹

Some antibacterial peptides of the *Hadruroides mauryi* and *Centruroides margaritatus* scorpions' venom have been isolated by ion exchange chromatography (Table 4); although they can be used in the control of bacteria, their activity has to be specifically assessed.⁷²

Conclusion

Due to the drug resistance presented by microorganisms, implementing new treatment mechanisms is necessary using as models substances of nature that are able to directly act in the control of pathogens or that indirectly activate the host immune system. AMPs can be an excellent option for the treatment of infectious diseases, since there is a large diversity of these molecules, and their uses are not limited to infections caused by common microorganisms (bacteria, viruses, fungi and parasites). Attention has also been paid to those that may be an option against cancer.

Antimicrobial peptides for therapeutic use can be obtained by chemical synthesis or can be directly isolated from some natural source, and hence their number can be rather wide. AMPs by themselves show a broad spectrum, although the possibility of combining them in the treatment of infections, with drugs that are commercially available and conventionally used remains to be explored, in order to assess whether combined therapy can enhance their effect.

The use of these molecules is increasingly common and successful, and even though certain peptides are still at early clinical stages, their use in the medium-term can be a promising option, especially if we consider the drug resistance that many pathogens that generate diseases in humans have developed.

Acknowledgements

The authors thank Universidad Autónoma Metropolitana Unidad Xochimilco for the support received. The presented work is part of the CB-2015-1-258735 project.

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