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Mode-of-Action of Antimicrobial Peptides

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ABSTRACT

Antimicrobial peptides (AMPs) are natural antibiotics, synthesized by ribosomes, with an effect on the natural immunity of animal organisms. AMPs differ in composition [sequence and type of amino acids (AAs)] and structure, which contribute to rapid lysis and have a varied spectrum of antimicrobial activity. AMPs are: peptides (Pps) with alpha-helix structure (i.e., cecropins, magainins, mellitin, etc.); cyclic Pps and ring with several cysteine residues (i.e., defensins, protegrins, etc.); Pps rich in one or another AA (i.e., proline arginine-rich PR39, histidine, glycine, etc.). Most AMPs are characterized by hydrophobic and cationic properties, adopt an amphipathic structure (alpha-helix, spiral beta or alpha-helix / spiral beta), which is essential for antimicrobial activity. AMPs have the potential for therapeutic use in medicine. One of the major concerns of life sciences research is finding new ways to enhance the body's defense against pathogens. One way is to produce drugs based on AMPs. The AMPs are a class of small Pps that have the ability to destroy pathogens of microbial and viral origin. The mechanisms of action are known only partially and for a small number of AMPs, and the toxic action of AMPs is generally considered to be based on the induction by these molecules in the outer membrane of the pathogen of aqueous pores that facilitate nonspecific ion transfer, which ultimately leads to lysis of the target cell.

Keywords: Antimicrobial Activity; Hydrophobic; Cationic Properties; Innate Immunity; Antibiotics; Microbiomes; Natural Products

Background

The mechanism of action of the preparation consists of: stimulation of recognition of viral antigens and affected cells by natural killers (NK), neutrophils and other effector systems of natural (innate) immunity; and stimulation of interferon synthesis. Insects show a specific resistance to infectious agents either through its natural structures (the cuticle made of rigid chitin) or through a mechanism still incompletely determined, but which seems to be mediated by receptors that recognize structural elements: proteins, glycoproteins and polysaccharides. One such receptor, called GNBP (Gram negative binding protein) has been identified in the silkworm. It turned out to be part of the family of scavenger receptors, which in humans achieves the clearance of oxidized lipoproteins in the blood. Non-shelf recognition stimulates the phagocytic activity of hemocytes and triggers two proteolytic cascades: one responsible for hemolymph coagulation, and the second leading to prophenoloxidase activation [1]. This enzyme produces melanin deposits on the surface of the microbe, being also responsible for the formation of free radicals and other cytotoxic compounds, such as: quinones, semiquinones, reactive oxygen species, etc. At the same time, the synthesis of Pps with antimicrobial activity is induced in the body of the insect, which is the functional counterpart of the cat, and in certain hemocytes. The kinetics of this process is similar to the acute phase reaction observed in humans in case of inflammation.

The AMPs exhibit antibacterial, antifungal, antiviral and antiprotozoal action, including against strains resistant to antibiotics and chemotherapy. Some Pps are characterized by selective action on gram-positive or gram-negative flora. Others fight bacteria, fungi, viruses or protozoa [2]. In the process of evolution, organisms acquired the property of producing substances that change the permeability of the membrane of other organisms, which were called poroformers–proteins capable of incorporating into the foreign membrane with the formation of pores. A common primary effect of these proteins, different in chemical structure, is reduced to the coupling with the cell membrane and the formation in it of a hydrophilic channel that facilitates, depending on the size of the pores, the transport through

the hydrophobic part of the membrane of various substances (ions, carbohydrates and even proteins). The formation of additional pores initiates various mechanisms of cell death [3]. The cytoplasmic membrane is not directly accessible to PAMs. Thus, in gram-positive bacteria it is protected by the peptidoglycan layer, and in gram-negative ones by the outer membrane and peptidoglycan. In this context, some PAMs can be effective only against gram-positive flora, others against gram-negative flora, a fact that is explained by their specific recognition. Thus, gram-negative bacteria are identified by their lipopolysaccharide content. The AMPs pass through external structures substituting calcium and magnesium ions due to cationic properties with increased permeability that facilitate the penetration of other molecules [1]. After penetrating the outer membrane, PAMs get the opportunity to be absorbed on the surface of the cytoplasmic membrane, and subsequently their behavior can be determined by several mechanisms:

- The formation of permanent barrel-type channels (barrel-staves), the walls of which are formed by the aggregation of several Pps. This type is characteristic of the few Pps (melitins, pardoxins, alamethicins) that are not strong cations and have the role of forming hydrophobic bonds that reduce the selectivity towards bacteria acting on macroorganism cells (erythrocytes);

- The formation of toroid-type pores (toroidal pore), the walls of which are made up of the polar parts of PAMs and hydrophilic lipids. This type of pore is characteristic for some PAMs with an alpha-helix structure (mageinins).

- The formation of carpet-type pores (carpet model), when the interaction between PAMs and the membrane does not lead to the formation of permanent or temporary channels, but to the substitution of lipids with the amphipathic molecules of PAMs on a limited sector with the destruction of membranes, similar to the action of detergents. The respective mechanisms are not self-exclusive, but can complement–1 and 2 with 3 [4,5].

Target Cell Death Can Occur According to the Following Hypotheses:

- Deregulation of the membrane state by: changing the fluidity of the lipid layer; deregulation of the stability of intercalated protein complexes; stop breathing; disruption of membrane potential and energy balance; affecting membrane barrier functions; uncontrolled influx of water into the cell; loss of essential metabolites;

- Penetration of PAMs into the cytoplasm and coupling with intracellular structures (less elucidated mechanism), for example, with cellular polyanions (such as DNA and RNA) resulting in the arrest of protein biosynthesis and cell death [6]. It is also believed that PAMs, due to their positive charge, would form a film covering the membrane with negative charges, which subsequently leads to the destruction of the bacteria. In experimental studies, although a clear correlation between the structure and the mechanism of action of PAMs was not determined, some trends can still be stipulated: the positive correlation between the ability to act aggressively on the membrane (structure and functional parameters) and low selectivity (action on bacteria and eukaryotic cells) [7];

- The repeat of the segment of 3–6 AAs for PAMs with alpha-helix is important for the interaction with the membrane, especially if the PAMs are amphipathic and the hydrophobic domain is larger. The smaller the polar angle in PAMs, the more actively they form pores and the more stable they are [8];

- Presence or substitution with proline contributes to change the mechanism–from pore formation–to intracellular targets. Linear Pps with alpha helix (spirals) also bear the generic name of cecropins, since the first compounds of this type were identified through experimental infections of the pupae of the butterfly Hyalophora cecropia. Currently, 21 such Pps are known both from diptera: Drosophilla melanogaster (contains 3 isoforms), Sarcophaga peregrina (flesh weevil), Aedes albopictus (a species of American mosquito), and from lepidoptera: Hyalophora cecropia, Manduca sexta, Bombyx mori. Cecropins have been determined in the hemolymph of various insects (sarcotoxin A in flies, cecropin in butterflies, spinigerin in termites) and are eliminated as toxins with the function of cytolysins. It has been found that they exhibit antimicrobial action, weak hemolytic and are a component of the immune system of insects that ensures their protection from microorganisms [9]. Cecropin Pps are secreted in an inactive form, having a signal Pps and a sequence at the N-terminal end which are then removed by proteases, and at the C-terminal end they always present a glycine residue which under the action of a specific monooxidase (starch enzyme) is removed, the second amino acid remaining with an amido-terminal group [10]. The biologically active part comprises 36–40 AAs that adopt a secondary structure consisting of an amphipathic alpha helix, followed by a linker region and a shorter hydrophobic alpha helix towards the C-terminus.

Initially, both helices are oriented parallel to the cell membrane, so that the N-terminal helix, positively charged, interacts electrostatically with the negative groups of the membrane lipids, and the C-terminal helix infiltrates the membrane with the formation of pores. It was found that binding to lipid membranes seems to be favored by anionic phospholipids (phosphatidylserine) and hindered by cholesterol, an effect that is not seen in the case of other types of sterols. This would justify the selectivity with which cecropins lyse prokaryote membranes, their fluid composition permissive for their mechanism of action, unlike eukaryotic membranes. Tests on liposomes loaded with fluorescent dyes and in which a difference in transmembrane potential was achieved, showed that cecropins cause membrane potential deregulation in just a few minutes, and only after about an hour the

lysis of the membrane and the release of the dye are observed. In vitro cecropins have been shown to be active especially on gram-negative germs, less on gram-positive and almost non-toxic on mammalian cells [11]. The PAMs represent a class of antimicrobial preparations, the further development of which is justified by the multiple advantages: they act on numerous germs, including polyresistant ones; the mechanism of action makes resistance unlikely; show minimal toxicity to mammals [12]. The AMPs have a role in the immune defense against infections, being present both in plants and insects as well as in higher vertebrates. Each species synthesizes, following antigenic stimulation, specific AMPs, different from each other.

In humans and mammals, there is a large number of AMPs, which intervene in non-specific defense, both through the antibacterial, antifungal, antiviral microbicidal effect, as well as through the modulation of immunity. Natural AMPs generally have a small molecular mass, that is, a relatively small number of AAs, being the simplest weapons of cellular defense, belonging to the non-specific immune system. Their structure is amphipathic, presenting two regions, one hydrophilic and one hydrophobic on one side and the other of the Pps structure, and the net electric charge is intensely positive [13]. The hydrophobic region can interact with lipids in the microbial membrane, and the hydrophilic region interacts with water or negatively charged structures in the target cell membrane, but does not typically affect normal cells that have a membrane rich in cholesterol and neutral lipids. The cationic and amphipathic structure allows the creation of strong electrostatic bonds with the cell membranes of bacteria, fungi or with the envelope of enveloped viruses [14]. In addition to the antibacterial activity and modulation of the immune response that these Pps have, recent studies have shown that some of these cationic Pps have an important cytotoxic activity on cancer cells and do not act on normal mammalian cells. Most of the available anticancer drugs allow tumor growth to be controlled only at concentrations that also affect healthy cells, resulting in undesirable side effects. Thus, it is imperative to find new products with innovative mechanisms of action, and one of the current research directions is represented by the use of cytotoxic AMPs [15]. Several hypotheses could be made regarding the biological effect of the Pps that did not influence the viability and proliferation of the studied tumor cell lines:

- Membrane pore formation: probably the membrane pores formed are small in size or the density of membrane pores is low, so that cell death by necrosis or apoptosis cannot be induced as a result of caspase activation. We can assume that cells sensed the presence of Pps on the cell membrane, which led to the stimulation of some cellular activation pathways with the appearance of secondary messengers with the final effect of stimulating cell proliferation. Therefore, the activation of a certain pathway will lead to the stimulation of proliferation with the probable tendency to strengthen the ability of the cells to expel the Pps from the cell membrane [16,17].
- The transformation of an antagonist into an agonist: it was found that 24 hours after incubating the tumor cells with the Pps, there was a transient inhibition that was removed after 48 and 72 hours, respectively, and cell proliferation was stimulated. It is likely that the Pps could be processed (coupled) by the tumor cell so that its insertion into the cell membrane does not lead to cell death [18,19]. Since the molecular mechanisms of interaction between proteins and membranes are at an important frontier of cell biology, investigations on these Pps could bring useful information on the mechanism of interaction between small Pps and cell membranes as well as on the possibilities in which this process can be modulated [20]. Although the exact mechanism of action of AMPs remains a matter of controversy, there is a consensus that these Pps selectively disrupt cell membranes, and the amphiphatic structural arrangement of the Pps is believed to play an important role in this mechanism.

Concluding Remarks and Future Perspectives

Considering all these aspects from the specialized literature, in these experimental studies the hypothesis was verified according to which cytotoxic Pps known as antimicrobials have tumoricidal potential whose intensity depends both on the nature of the Pps used and on its concentration in the living environment of the cells, but also the type of cell line used experimentally, in vitro. It can be stated that the information is relevant for medical practice because, in addition to cytostatics commonly used in the therapy of various types of cancer, other compounds could be used, as is the case with these cytotoxic Pps that have been shown to have tumoricidal potential. The cytotoxic effect on tumor cells of these Pps must also be evaluated in vivo on experimental animal models because it is important for oncological medical practice, to test and permanently improve therapeutic pathways, by finding new compounds with tumoricidal potential such as those from the category of AMPs. These compounds could lead to new therapeutic possibilities, that is, to provide significant tumoricidal effects with minimal side effects, in the sense of minimal cytotoxicity for normal cells.

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Conflicts of Interest/Competing Interests

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Authors' Contribution

Conceptualization, M.D.C.; I.G. and M.B.; data curation, M.D.C. and M.B.; writing—original draft preparation, M.B.; writing—review and editing, M.D.C. I.G. and M.B.; visualization, M.B.; supervision, I.G and M.B. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Consent to Participate

Not applicable.

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Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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