Antimicrobial peptides and their potential as therapeutic agents

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Introduction

The rapid and continuous increase of antibiotic resistance has become a global public health problem, conventional antibiotics are becoming ineffective as a result of resistance, so there is a need to find new antibacterial strategies and develop a new class of antibiotics.

Antimicrobial peptides (AMPs) are an evolutionarily conserved component of the innate immune defense system that have raised interest for their **ability to kill multidrug-resistant microorganisms** and represent a promising alternative approach in the treatment of microbial-related diseases.

Antimicrobial peptides (AMPs) have served as natural first-line of defense system encoded by genes from the majority of living organisms. In mammals are expressed in a variety of cell types including monocytes, macrophages, neutrophils, epithelial cells, keratinocyts and mast cells. They may be constitutively expressed or be inducible depending on the specific peptide, specie, tissue or cell type.

AMPs are recognized for their **potent antimicrobial activity** to direct kill, disrupting the membrane of a wide range of microorganisms, such as bacteria, fungi, parasites and virus, as well as for their **immunomodulatory properties** (**Fig.1**).

At present, more than 1000 different antimicrobial peptides have been reported from numerous natural biological sources and thousands of synthetic variants have been produced. Several AMPs peptides have already entered pre-clinical and clinical trials for the treatment of host disease conditions including microbial infections, wound healing or acne.

Characteristics that affect antimicrobial activity

AMPs are relatively short polypeptides with **fewer than 60 amino acid residues**, **cationics** with an overall positive charge (generally **+2 to +9**) and with a substantial proportion ($\geq$**30%**) of **hydrophobic residues** due to an excess of basic lysine, arginine and histidine residues. These properties permit the peptide to fold into **an amphipathic structure** in three dimensions.

Bases of their selectivity: electrostatic interaction

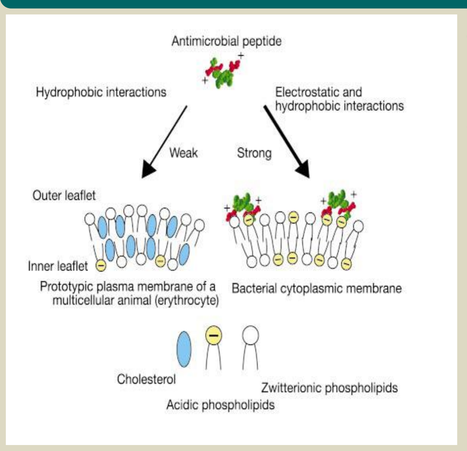

Figure 2. The membrane target of antimicrobial peptides of multicelular organisms and the basis of specificity. Figure from ref. 6.

PG: phosphatidylglycerol PE: phosphatidylethanolamine
CL: cardiolipin PC: phosphatidylcholine
PS: phosphatidylserine SM: sphingomyelin

The antimicrobial activity involves an **electrostatic attraction** between the AMP and the outer microbial membrane (**Fig.2**).

Bacterial membranes are **negatively charged** with lipids bearing phospholipids head-groups such as PG, CL or SP. In contrast, the outer layer of the mammalian membranes are enriched in zwitterionic phospholipids (**neutral in net charge**) as PE, PC, SM. Moreover, the presence of cholesterol, a major constituent of mammalian cellular membranes, can reduce the activity of AMPs stabilizing the lipid bilayer or by directly interacting and neutralizing them.

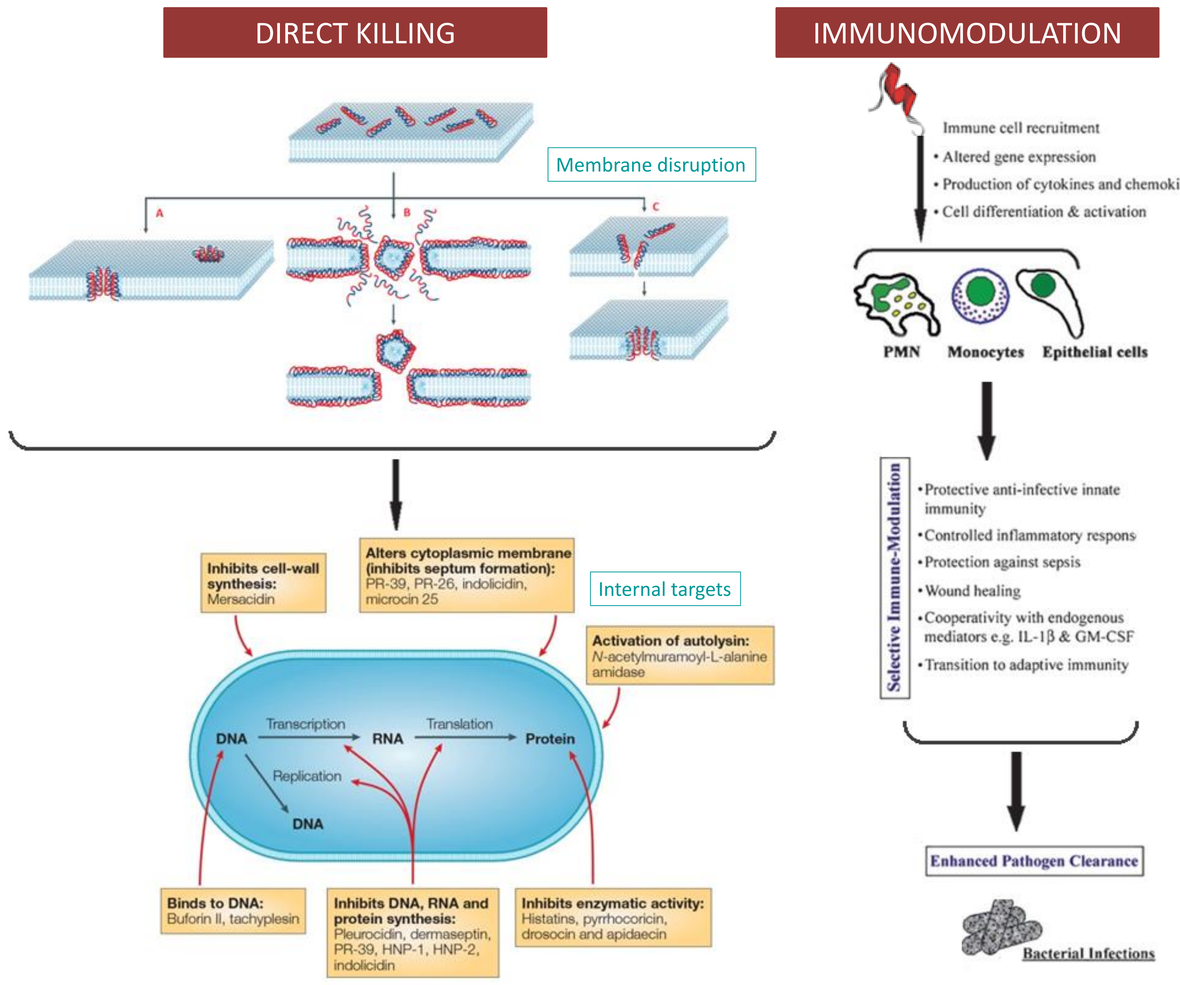

Figure1. Biological roles of AMPs: immunomodulatory properties and direct killing by membrane disruptive mechanisms (barrel-stave (A), carpet (B) and toroidal (C) models) and internal targets. Figure modified from ref. 3 and 4.

AMPs Vs Conventional antibiotics

Property	Conventionals antibiotics	AMPs
Spectrum of activity	Bacterial infections	Bacterial, fungal and viral infections; sepsis; and/or inflammation
Mechanism and Targets	Specific mechanisms. Usually one dominating target or class of targets	Relatively non-specific mechanism (based on charge). Relatively less specific (possibly multiple targets in any given cell)
Resistance rate	Resistance development after a few passages at sub-MIC.	Needs multiple passages on sub-MIC concentrations to induce resistance.
Pharmacokinetics	Generally, good bioavailibity and biodistribution	Short systemic half-life owning to proteolytic degradation
Toxicology	Antibiotics tend to be one of the safest group of pharmaceuticals	No known topical toxicities; systemic toxicity issues remain undefined
Manufacturing costs	Can be inexpensive (e.g. $0,8 for aminoglycosides)	Expensive ($50-400 per gram)

Table 1. Comparison of conventional antibiotic and cationic antimicrobial peptides.
MIC: Minimal inhibitory concentration

Advantages

✓ Their ability to kill multi-drug resistant bacteria and their low propensity for resistance development.

✓ Their broad spectrum of activity: Gram-negative and gram positive bacteria, virus, yeast, protozoa and funghi.

✓ Their rapid killing mechanism (minutes *in vitro*).

✓ Their diverse potential applications: can be used as single antimicrobials, in combination with other antibiotics for a synergistic effect or as immunomodulatory and endotoxin-neutralizing compound.

Limitations

× The high manufacturing cost of synthetic peptides.

× Systemic and local toxicity.

× Reduced activity based on salt, serum and pH sensibility.

× Pharmacokinetic and Pharmacodynamic issues.

× Low stability *in vivo* due to AMPs are sensitive toward proteolytic degradation, especially their susceptibility to mammalian proteases.

× There are some reports that resistance can be developed in response to the use of certain AMPs.

Conclusions

Although AMPs possess considerable qualities as new generation antibiotics, their clinical and commercial development still have some limitations, but there are several strategies to overcome those obstacles. Peptide design can address lability to proteases by using D-amino acids rather than L-amino acids, employing different backbones (peptidomimetics), chemically modifying protease-sensitive sites or delivering the peptides in protective vehicles such as liposomes. Regarding their high cost, one cheaper alternative is to synthesize them using recombinant expression methods or use conventional or solution-phase peptide synthesis but decreasing the size of the peptides.

There is a need of more peptide structure-activity studies to increase their tolerability and specificity. Once pharmacodynamics will be better understood, dosing regimens can be designed rationally to optimize disease outcomes and minimize toxicity problems.

At industrial level several companies worldwide are focused on the development of AMPs and there are some molecules at the preclinical and clinical stage. **Their introduction as therapeutics is limited yet** and will require significant improvements and innovations, but there is still a general optimism for their use in future clinical practice.

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