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Review Article

Antimicrobial Peptides Past, Present and Future

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Abstract: Uncontrolled antibiotic use lead to increasing the level of resistant bacterial species. Antimicrobial peptides (AMPs) are one of the few alternatives in the fight with everyday growing bacterial resistance to commonly used antibiotics. AMPs general application could be also as endotoxin neutralizing agents or as an adjuvants to regular antimicrobial therapy. In this review we were focused on the most recent data that concern the diverse use of AMPs, their activities and modes of actions.

Keywords: Antimicrobial Peptides, Bacterial Resistance, Antibiotics

1. Introduction

Bacteria exist with and within us for thousands of years. They inhabit just about every part of the human body, living on the skin, in the gut, and up the nose. Sometimes they cause sickness, but most of the time, microorganisms live in harmony with their human hosts, providing vital functions essential for human survival. Even those friendly bacteria could “betray us” by passing plasmid DNA that encode genes for antibiotic resistance to pathogenic strains. Uncontrolled antibiotic use lead to increasing the level of resistant bacterial species, but not only health care system all over the world should take the blame use of antibiotics in food industry also contributes to situation today. The damaging effects of antimicrobial resistance (AMR) are already manifesting themselves across the world. Antimicrobial-resistant infections currently claim at least 50,000 lives each year across Europe and the US alone, with many hundreds of thousands more dying in other areas of the world [1].

But reliable estimates of the true burden are scarce. There is considerable variation globally in the patterns of AMR, with different countries often experiencing different major problems. Despite this and in contrast to some health issues, AMR is a problem that should concern every country irrespective of its level of income. For instance, in 15 European countries more than 10% of bloodstream Staphylococcus aureus infections are caused by methicillin-resistant strains (MRSA), with several of these countries seeing resistance rates closer to 50%. These data have to be the driving force for the development of newer alternatives to antibiotics. The pharmaceutical industry has continuously met this need by modifying existing antibiotics and developing newer antibiotics in a timely fashion. These successful efforts have produced the wide variety of currently available drug classes of antibiotics [beta lactams (penicillins, carbapenems, cepahalosporins), glycopeptides, macrolides, ketolides, aminoglycosides, fluoroquinolones, oxazolidinones, and others] [2]. Despite the success to date in antimicrobial development, the inexorable, ongoing emergence of resistance worldwide continues to spur the search for novel anti-infectives to replace and/or supplement conventional antibiotics. General therapeutic application for antimicrobial peptides could be: (1) as single anti-infective agents, (2) in combination with conventional antibiotics or antivirals to promote any additive or synergistic effects, (3) as immunostimulatory agents that enhance natural innate

immunity, and (4) as endotoxin-neutralizing agents to prevent the potentially fatal complications associated with bacterial virulence factors that cause septic shock [3].

2. Antimicrobial Peptides: Past and Present

Pexiganan (MSI-78) (Genaera, Plymouth Meeting, PA, USA) was the first antimicrobial peptide to undergo commercial development. In 1987, Zasloff discovered that a cationic peptide in the skin of the African clawed frog Xenopus laevis had broad-spectrum antibacterial activity based on a "pore-formation" mechanism. He called it magainin. Pexiganan, a synthetic 22-amino-acid analogue of magainin 2, demonstrated excellent in vitro broad-spectrum activity against 3109 bacterial clinical isolates. Resistant mutants could not be generated following repeated passage with subinhibitory concentrations [4]. In two Phase III clinical trials involving 835 patients with infected diabetic foot ulcers, both topical pexiganan actetate 1% and oral ofloxacin 800 mg/day achieved clinical cure or improvement in 90% of patients. Eradication of pathogens was achieved in 82% of the ofloxacin recipients compared to 66% of pexiganan recipients at the end of therapy [5]. Due to this inconclusive results from clinical trials FDA stop the approval of Pexiganan.

Nowadays Depexium pharmaceuticals conducting two pivotal phase 3 clinical trials called "One step one and One step two, "to established safety and efficacy of Pexiganan cream 0.8% twice daily for 14 days in patients with diabetic foot ulcers suffering from mild to moderate skin infections [6]. Iseganan is a cationic antimicrobial peptide, it has broad-spectrum in vitro antibacterial and antifungal inhibitory activity. Iseganan was initially developed as a local mouthwash to prevent high-risk patients from developing ulcerative oral mucositis. In 504 patients receiving stomatotoxic chemotherapy in a Phase III clinical they use mouthwash solution six times a day as long as radiotherapy regiment is applied. The trial is still ongoing last updated October 2014 [7]. Most recent information about its efficiency is inconclusive.

Arenicin-1, a 21-mer antimicrobial peptide, has been known to exert broad antibacterial activity. Hyemin Choi and colleagues demonstrated that the combination between Arenicin-1 and conventional antibiotics exert synergistic effects against gram negative bacteria *in vitro*. They used FIC concentration of arenicin-1 around 0.75 micromoles with ampicilin. This synergistic activity the show is due to free radical formation and therefore increasing the activity of the beta – lactam antibiotic, also arenicin- 1 increase the entry in the bacterial cells of the macrolide erythromycin [8]. In addition to their traditional antibiotic roles (e. g. disrupting bacterial membranes, inhibiting DNA replication/transcription) several papers also provide strong evidence suggesting that many antimicrobial peptides act as immunomodulators, rapidly stimulating a "ramped-up" adaptive immunological response [9]. In adition to immunological action antimicrobial peptides could be modified to look alike lipopolysaccharide binding protein (LBP) and CD14, they are key molecules in the cellular response to endotoxin. Much interest has focused on developing effective anti-endotoxin treatments to abrogate the inflammatory consequences of Gram-negative infection. The therapeutic options can be divided into those aimed at neutralizing or clearing circulating endotoxin, including anti-endotoxin antibodies and endotoxin neutralizing proteins, and those that antagonize the effects of endotoxin on human cells: for example, lipid A analogues. Initial experiences with anti-lipopolysaccharide antibodies have been disappointing but a new generation of anti-endotoxin agents is about to enter clinical trials. Whether these will prove sufficiently effective to reduce the morbidity and mortality of Gram-negative sepsis remains to be seen [10]. Nowadays despite the extensive research in this area of application of AMP the results from existing clinical trials are confusing. A series of paradoxical observations have emerged from these studies which are of interest to the questions are the AMP the best way to clear endotoxins? Despite those results further research in that area is for sure needed to clear all the pharmacological questions [11].

Lin et al. describe AMPs with amphipathic β-hairpin structures which possess potent antimicrobial activities and also cell selectivity. They developed model of β-hairpin AMPs with small size and substantial stability, based on the tryptophan zipper motif. The AMPs showed activity against gram-positive and gram-negative bacteria. The antibacterial potency of the peptides initially increased and then decreased with increasing chain length [12].

Recently four AMPs from the skin of Paa spinosa were tested for their antimicrobial, antioxidative, cyotoxic and haemolytic activities. Only one of them showed weak but broad-spectrum antimicrobial activities against Gram-positive and Gram-negative bacteria. All peptides were weakly haemolytic towards rabbit erythrocytes, had a strong antioxidative activity, and a low cytotoxic activity against HeLa cells [13].

Trying to understand the molecular mechanisms of anti-infective milk effects, Liu et al. tested 23 potential peptides from milk for antimicrobial activity. In the tested concentration range (<2 mM), of 14 peptides showed bacteriostatic activity 9 of which inhibiting both Gram-positive and Gram-negative bacteria. The most effective fragment was TKLTEEEKNRLNFLKKISQRYQKFALPQYLK corresponding to αS2 -casein151 -181, with minimum inhibitory concentration (MIC) of 4.0 μM against Bacillus subtilis ATCC6051, and MICs of 16.2 μM against both Escherichia coli strains [14].

3. Mechanisms of Action of Antimicrobial Peptides

Christine Cézard, Viviane Silva-Pires, Catherine Mullié and Pascal Sonnet [15] classified the mode of action of antimicrobial peptides as AMPs-membrane attraction,

attachment of the AMPs onto the membrane and insertion of the AMPs into the membrane causing its disruption, leading to the leakage of ions and metabolites.

Attraction: Numerous studies demonstrated that the net charge of Aps was directly correlated with their attar tion/interaction with the bacterial membrane. Indeed, cationic APs possess a positive net charge, ranging from +2 to +9 at physiological pH, while bacteria present a highly negatively charged outer membrane due to the presence of phosphate groups within lipopolysaccharides (LPS) for Gram negative bacteria and within lipoteichoic acids for Gram positive bacteria, respectively. Attractive electrostatic interactions between APs and the outer membrane of the bacteria then occur bringing the two moieties together. It has been demonstrated that, up to a certain threshold value, the more positively charged the AP, the better the antibacterial activity and selectivity [15].

Attachment – insertion: AMPs are now in close vicinity to the bacterial surface. The initial electrostatic interactions lead to an actual and nonspecific association of the AMPs with the bacterial membrane. In the case of Gram negative bacteria, cationic AMPs will bind the outer membrane *via* the anionic phosphate groups. Gram positive bacteria neither have an outer membrane nor LPS moieties at their surface for the AMP to bind to. Instead, their surface presents peptidoglycan featuring anionic teichoic acid groups, on which the AMPs will be able to attach. It has to be acknowledged that it is because of the negative charge borne by the membrane that AMPs are able to differentiate bacteria from a host cell, thus preventing APs from being toxic [15].

Permeabilization mechanism 1: AMPs accumulate monomers on the bacterial surface, but forming circle patterns. Upon binding, AMPs adopt an orientation parallel to the lipid bilayer. They then reorient perpendicularly and insert into the lipid core of the membrane resulting in a shape resembling a barrel. During this process, AMPs undergo conformational phase transition: the hydrophobic surfaces of the AMPs face outward, towards the acyl chains of the membrane thus aligning with the lipid core of the bilayer, while the hydrophilic regions face each other and form the interior of the pore. Progressively, new AMPs are recruited and through a process of self-aggregation, the pore size increases as more and more APs adopt a trans-membrane configuration. In this model, the membrane is neither deformed nor bent during the insertion process. Indeed, the AP inserts itself in the bilayer by "drilling" the membrane [15].

Permeabilization mechanism 2: The carpet model. A new mechanism, namely the carpet model was then proposed. Like for the barrel-stave model, AMPs aggregate onto the bilayer surface but keep a parallel alignment to the membrane surface during the process. The peptides are bound to the bacterial surface on their hydrophobic side while their hydrophilic side faces the exterior. For this mode of action to be efficient, the concentration of APs must be high as they must cover the whole bacterial membrane. It has to be noted that in contrast to the barrel-stave and the toroidal pore model, AMPs do not need to adopt a specific structure (*e. g.* α-helical) to permeabilize the membrane [15].

Permeabilization mechanism 3: The toroidal pore model. This third model combines the actions of the barrel-stave and carpet models [15].

Intracellular targets: Many studies indicate that membrane disruption is often not sufficient to lead to bacteria death. In some cases, the killing of bacteria may occur with very little to no membrane disruption. Some evidence suggests that there are intracellular targets as well. There is no general scheme to describe these mechanisms as they are specific to one AMPs. Once translocated in the cytoplasm AMPs are able to act on many levels: inhibition of cell-wall synthesis, inhibition of enzymatic activity, inhibition of DNA, RNA and protein synthesis, binding to DNA, altering cytoplasmic membrane (inhibition of septum formation) and/or activation of autolysin [15].

4. Antimicrobial Peptides: Future Applications

Gomes et al. researched for new antimicrobial wound-dressings based on the incorporation of antimicrobial peptides into polyelectrolyte multilayer films built by the alternate deposition of polycation (chitosan) and polyanion (alginic acid sodium salt) over cotton gauzes. They performed antimicrobial assays Gram-positive and Gram-negative bacterium, and the results showed that all antimicrobial peptides used in their work had a higher antimicrobial effect (in the range of 4log-6log reduction) for both microorganisms, in comparison with the controls, and are non-cytotoxic to normal human dermal fibroblasts at the tested concentrations [16].

Burkholderia pseudomallei is a classified category B priority pathogen and therefore could be used as a biological weapon. Burkholderia species are "highly resistant" to antimicrobial agents, including cyclic peptide antibiotics. Blower et al. report that the Chinese cobra (Naja atra) cathelicidin NA-CATH was found significantly antimicrobial against B. thailandensis used as a model for B. pseudomallei [16]. To develop new AMP with improved antiinflammatory activity and antimicrobial selectivity, Rajasekaran et al. designed and synthesized a series of Temporin-1Tl analogs (frog antimicrobial peptide) by substituting Trp, Arg and Lys at selected positions. Except for Escherichia coli and Staphylococcus epidermidis, all analogs exhibited retained or increased antimicrobial activity against seven bacterial strains including three methicillin-resistant Staphylococcus aureus strains compared with TL, also greatly increased anti-inflammatory activity and no significant increase in hemolysis. This makes the TL analogs promising candidates for the development of peptide therapeutics for gram-negative bacterial infection [17].

A series of hybrids (DM1-DM5) from two unrelated classes of AMPs showed antipneumococcal activity higher than Penicillin. The hybrids were also broad spectrum against multiple common clinical bacteria, and combination treatment with both hybrids and penicillin produced synergism.

Researchers also found that specific residues has a major role in affecting the antimicrobial and cell toxicity of the hybrids than physicochemical properties [18].

Hemocyanins present in invertebrate hemolymph are multifunctional proteins identified as a source of broad-spectrum antimicrobial peptides during infection. A series of synthetic peptides based on a putative antimicrobial region, termed haliotisin, located within the linking sequence between the α-helical domain and β-sheet domain of abalone (Haliotis tuberculata) hemocyanin functional unit E, were tested over Gram-positive and Gram-negative bacteria. Results showed damages to the microbial cell wall, thus suggesting that molluscan hemocyanin may act as a source of anti-infective peptides [19].

5. Conclusion

Uncontrolled use of antibiotics in health care and in the farm industry leads to everyday growing bacterial resistance. Lack of newly discovered antibiotics, open the need of different opportunities such as antimicrobial peptides. Several AMPs has reached stages II and III in clinical trial proceeding as topical antimicrobials. The data up to now suggest that AMPs lead us to the conclusion that AMPs has promising activity as immunomodulators and adjuvant to antimicrobial therapy. AMPs are very promising agents in the field of antimicrobial chemotherapy, therefore further research is needed in the aspect to broaden their applications.

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