Synthesizing Human Antimicrobial Peptides: Harmful or Helpful?

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With the emergence of antibiotic resistance to bacteria, researchers are investigating the potential impact of Anti-Microbial Peptides (AMPs) as a counter-measure. The isolation of these peptides, mechanisms of bacterial resistance to these agents, and how AMPs fight against bacteria must be understood before the use of human AMPs to treat diseases can occur. Although there is potential risk for bacteria to develop resistance against AMPs, research on these peptides looks to be a promising endeavour in increasing the number of available strategies to treat infectious disease.

Human Antimicrobial Peptides

With the introduction of antibiotics in the 1940's, bacterial resistance to these new drugs was originally dismissed because it was thought to require an unusually high rate of advantageous adaptive mutations (Bel & Gouyon, 2003). This premise turned out to be inaccurate, as antibiotic resistance is currently one of the largest problems confronting the health care sector. Scientists looking for new ways to fight bacteria have identified AMPs, a new class of self-protection proteins. AMPs act in conjunction with other factors as part of the innate immune system, which is the first line of defence against pathogenic attack (Bals & Wilson, 2003).

AMP Classifications

Although there are different types of AMPs, they are generally small (less than 10 kDa), cationic, and hydrophobic, with activity at cell membranes (Hultmark, 2003). There are two main types of AMPs produced in the human body: cathalicidins and defensins. The latter are composed of β-sheets and three disulphide bridges, and can be separated further into two groups based on patterns of disulfide bridging and amino acid sequence motifs (Lehree & Ganz, 2002). α-Defensins are produced by neutrophils and β-defensins are found in epithelia and skin. Humans produce only one type of cathalicidins, termed α-helical LL-37. This AMP is found in various neutrophils and epithelia (Pescel, 2002). AMPs also exhibit fungicidal, tumouricidal, and virucidal properties along with activity against Gram positive and Gram negative bacteria, including antibiotic resistant strains, which make them good candidates for therapeutic drugs (Bals, 2000).

Mechanisms of Action of Human Antimicrobial Peptides

Research into the functional mechanisms of AMPs has been an important area of study because it provides the basis for examining their pharmacological potential. There are three general models to explain the mechanisms of action of human cathalicidins and defensins (Figure 1). The first model is termed

barrel stave. In this model the bacterial membrane is perpendicular to the amphipathic peptides that align themselves so their hydrophobic side chains face outward into the lipid environment, while transmembrane pores are formed by the polar side chains that are aligned inward. These pores allow leakage of cytoplasmic components that disrupt the membrane and kill the bacteria (Ehrenstein & Lecar, 1977).

In the second model, termed the carpet, peptides are not inserted into the membrane, but are aligned in parallel to the bilayer while remaining in contact with the lipid bilayer and lipid head groups, thus coating the surrounding area. This model causes membrane cracks, leakage of cytoplasmic contents, membrane potential disruption, and eventually the disintegration of the membrane (Pouny et al., 1992).

The third model is the micellar aggregate model. This model suggests that an informal membrane-spanning micellar arrangement is formed by the peptide's orientation and association with the bilayer and can be used to explain translocation into the cytoplasm by the collapse of the micellar aggregates (Powers & Hancock, 2003).

Any three of these models may be used by varying cathelicidins and defensins. However, the net result is the same in all cases - the disruption of bacterial membrane integrity causing rapid depolarization of the cell membrane, leading to cell death (Powers & Hancock, 2003).

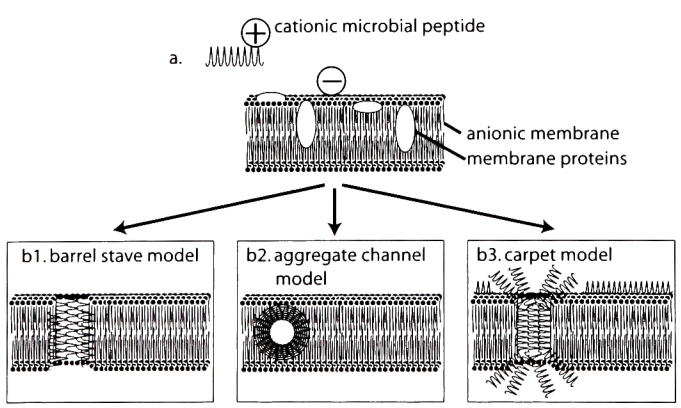

Figure 1: Mechanisms of action of AMPs. After electrostatic interactions between the negatively charged bacterial wall and the positively charged peptides (a), the peptides associate with the membranes, leading to a destabilization of the membrane and subsequent cell death (b). b1, barrel stave model; b2, aggregate channel model and b3, carpet model (Bals & Wilson, 2003).

Mechanisms of Bacterial Resistance to AMPs

When AMPs are used, bacteria retaliate using their own defence mechanisms.

One mechanism involves the disruption of AMP aggregation on the bacterial cell membrane, which causes the incorporation of components with reduced anionic charge and leads to obstruction of antimicrobial activity (Bel & Gouyon, 2003). Gram positive bacteria use this mechanism to substitute positively charged residues of the AMPs with the cell wall teichoic acids (Bel & Gouyon, 2003). The cell membrane can also be altered to counter the effects of AMPs. This is accomplished by lowering the concentration of anioic phospholipids, which in turn, generates resistance. The negative surface charge of the bacterium *Staphylococcus aureus* is reduced by substitution of lysine into membrane lipids, which minimizes loading of AMPs (Bel & Gouyon, 2003; Figure 2). Other mechanisms include the use of an efflux pump system to remove AMPs from the bacterial cytoplasm and the cleavage of α-helical AMPs by proteases (Guina, 2000).

Pharmaceutical Considerations

Human immune cells and microbial organisms are in a constant battle for supremacy, however, the lytic properties of AMPs have tended to out-compete bacteria (Reddy et al., 2004). AMPs possess the ability to rapidly kill, within 1 to 2 minutes, a broad spectrum of microorganisms and pathogens, including bacteria that have been deemed multi-drug resistant (Reddy et al., 2004). Some cathelicidins have already been found to prevent oral bacteria and yeasts from replicating and causing sickness (Guthmiller et al., 2001). Defensins have also shown a 99% reduction in the formation of colonies of *Mycobacterium tuberculosis* (Miyakawa et al., 1996). These, and other human AMPs, are undergoing laboratory tests and clinical trials (Reddy et al., 2004). There are many different strategies for AMPs therapeutic application: as single anti-infective agents, in combination with antivirals or antibiotics to induce any additive effects, as agents that enhance innate immunity, and as endotoxin-neutralizing

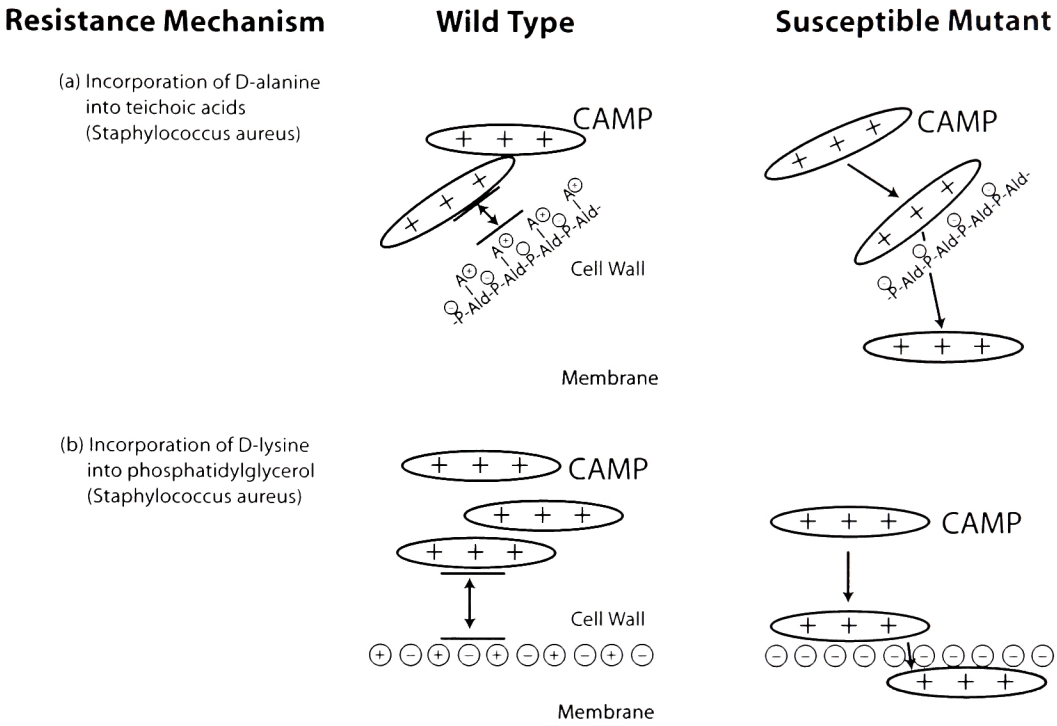

Figure 2: Proposed mechanisms of antibiotic resistance in Staphylococcus aureus. The left panel is wild type bacteria and the right panel is cationic AMP-susceptible mutants. Anionic molecules such as (a) teichoic acids or (b) phosphatidylglycerol are substituted with positively charged residues causing repulsion of cationic AMPs by wild-type cell envelopes (Peschel, 2002).

agents (Gordon et al., 2005). A salivary AMP used to treat oral candidaisis affecting immunocompromised patients and an AMP found in neutrophils for treatment of severe pediatric meningococcaemia and Crohn's disease are also among the AMP treatments in clinical trials (Paquette et al., 2002, Reddy et al., 2004). Inimex Pharmaceuticals, a Vancouver based pharmaceutical company, is developing immunoenhancement AMPs that selectively upregulate innate immunity without overstimulation of proinflammatory mediators (Gordon et al., 2005).

To date there have been no published reports of commercial success in developing AMPs as therapeutic agents, but lab tests and clinical trials are being conducted. The major concern is whether bacteria will be able to develop resistance to the synthesized drugs and *in vivo* human AMPs. If this were to occur, many of the AMP responses to bacteria may exhibit anergy. The neutrophil system could experience a higher rate and greater severity of diseases due to chronic infection (Bel & Gouyon, 2003). Although some resistance to AMPs has been found, the impact on health depends on the management of the problem.

The Future of AMPs

Bacterial resistance has been linked to the over-prescription of antibiotics. With careful control of AMP use, this novel discovery will be a promising method for treating infections and diseases. Some disadvantages of synthesizing AMPs include high costs, patent exclusivity, sensitization and allergy after repeated application, confounding biological functions, and most importantly, natural resistance by bacteria (Gordon et al., 2005). On the other hand, AMPs possess broad-spectrum activity, rapid onset of killing, potential low levels of induced resistance, and connection with broad anti-inflammatory activities (Gordon et al., 2005). The next step for researchers is to overcome the difficulties in synthesizing AMPs. A thorough understanding of AMP selectivity and potential development of bacterial resistance to the peptides is necessary. Hopefully, continuing research in this area will provide us with a new therapeutic tool to fight antibiotic resistant bacteria. M