LEADING ARTICLE

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Host Defence (Cationic) Peptides

What Is Their Future Clinical Potential?

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Abstract

Host defence, cationic antimicrobial peptides are now recognised as an important component, in most species, of the early innate and induced defences against invading microbes. They are small (12 to 35 amino acids), cationic due to the presence of an excess of arginine and lysine over acidic amino acids, and able to fold into a variety of different secondary structures. They have highly desirable properties, such as the ability to kill rapidly a broad spectrum of microorganisms including drug resistant bacteria and often fungi at around the minimal inhibitory concentration, a low level of resistance development *in vitro*, the ability to protect animals against both topical and systemic infections and the capability to neutralise endotoxin and demonstrated synergy with conventional antibiotics. In addition, given the 20 building blocks (amino acids) for these peptides, even a small peptide offers enormous diversity and potential for design of improved variants. For this reason such peptides have entered clinical trials, largely as agents for topical therapy of polymicrobial infections and are considered to have excellent potential for being a novel antibiotic class.

1. Role of Cationic Peptides in Host Defence

A revolution has occurred in the past decade in our thinking about how organisms defend themselves against infectious agents. The role of various nonspecific defences such as phagocytes, certain circulating enzymes and fatty acids, and complement, as well as specific humoral and cellular defences has been well recognised. However, only recently has evidence accumulated for a new and potent nonspecific defence agent, the antimicrobial (cationic) peptide.[1-4]

There are now compelling data to suggest that an inducible peptide response serves as a surrogate for the immune system in more primitive species of life, including insects and plants. Indeed, the peptide induction pathway involves signalling cascades and transcriptional factors (including an NF Kappa B homolog) that mirror the immune response pathway.[5] Cationic peptides are, however, far more general in their action than antibodies or primed T cells, and the inducibility occurs over hours rather than days.

In animals, including birds, fish, amphibians, crustaceans, and mammals (including humans), there exist both noninducible and inducible peptides.[1,2,4] These peptides appear to be important agents of local defences. They are found on virtually every mucosal surface, within the granules of phagocytes, and on the surface of the body. Those peptides which are inducible have similar induction pathways to the insect and plant peptides. Both intact bacteria and lipopolysaccharide (LPS) tend to result in induction of these peptides.

Clearly, cationic peptides are not a perfect defence

mechanism since all species of life can develop infections. Indeed, it seems likely that their role in most species is in rapid defence against moderate numbers of bacteria. Generally speaking, each natural peptide has a broad but incomplete spectrum of activity. The host compensates for this by producing an array of different peptides that together have a broader spectrum of activity, and often work in synergy with one another. A single individual may produce dozens of different peptides and more than 500 natural cationic peptides are now known. This provides, to those interested in the therapeutic potential of such compounds, many different templates for design of single peptides with very broad activity spectra.

Cationic host defence peptides can be defined as proteins with less than 100 amino acids (more typically less than 35), and a net positive charge (usually more than +4) by virtue of their having an excess of lysine and arginine residues over acidic residues.[3] Such peptides fold into at least 4 classes of structures, namely β-sheets and β-loops stabilised by disulphide bridges, amphipathic α-helices, and extended structures.[6] The latter 2 classes fold into these structures when they interact with lipidic (membrane) environments.

2. Antibacterial Efficacy

Natural peptides have a wide variety of activities ranging from modest to reasonably broad spectra and from very potent[7-9] to weakly active. It is possible to design reasonably nontoxic variants which have good minimal inhibitory concentrations (MICs) against a broad range of bacteria. In our experience, the best designed peptides can have either potent anti–Gram-negative activities with little anti–Gram-positive activity, or Gram-positive selectivity with little or no activity *vs* Gram-negative bacteria, or reasonably broad spectrum effective against most major Gram-negative and Gram-positive pathogens (some of these peptides can be quite toxic, e.g. gramicidin S).

MICs are usually in the range of 1 to 8 mg/L, which is quite reasonable for the more antibiotic-resistant pathogens. However, peptides have several strong assets.[6-9] First, they are bactericidal at concentrations around the MIC. Second, they kill rapidly with a 4 to 6 log reduction in survival within 5 minutes at 4 times the MIC. Third, peptide activities are unaffected by the common resistance mechanisms observed in the clinic (e.g. methicillin resistance in *Staphylococcus aureus*, multiple antibiotic resistance in *Pseudomonas*, and antibiotic resistance plasmids in the Enterobacteriaceae). Fourth, it is extremely difficult to select mutants resistant to antimicrobial peptides. Fifth, peptides show the ability to protect in animal models of both local and systemic infections.

Thus, the antibacterial activities of peptides indicate these are very promising antibiotics especially against highly resistant pathogens. Most experts subscribe to the view that peptides kill by entering and permeabilising bacterial cytoplasmic membranes, although there are some indications that other mechanisms are important in some cases.[10]

3. Synergy with Antibiotics and Anti-Endotoxin Activities

Cationic peptides are taken up across the outer membrane by a process termed self-promoted uptake.[3,6] In this process, the cationic peptides interact initially with divalent cation binding sites on surface LPS, displace these divalent cations (because they have 10^3-10^4–fold higher affinity for these sites), and being bulkier than the divalent cations they displace, cause distortion of the outer membrane structure. It is through these distortions (observed by electron microscopy as surface blebs) that the cationic peptides pass across the membrane (i.e. self promote their uptake).

The above mechanism has two consequences. First, antimicrobial cationic peptides can promote the uptake of other agents, e.g. antibiotics and lysozyme, and thus show synergy with conventional antibiotics, especially against antibiotic resistant mutants. This has been shown in an animal model by Darveau et al.,[11] who showed that the peptide magainin was synergistic with the β-lactam cefepime. Antimicrobial peptides can also show synergy with conventional antibiotics against Gram-positive bac-

teria, (e.g. they can reverse vancomycin resistance in vancomycin-resistant enterococci, VRE) and show synergy with antifungals against fungi. However, the mechanisms of synergy in these latter cases have not been studied.

As discussed, cationic peptides have a high affinity for LPS,[9] a molecule which also bears the name endotoxin, and is a major player in Gram-negative sepsis (about 300 000 cases per year in the US) and endotoxaemia. As a consequence of their high LPS binding capacity, cationic peptides (and cationic proteins like bactericidal permeability increasing protein; BPI) neutralise LPS and can protect galactosamine-sensitised mice against lethal endotoxaemia.[9,12]

4. Antifungal, Antiviral, Wound Healing and Anticancer Activities

Some natural cationic peptides (e.g. certain ones from plants) have preferential activity against fungi[13] compared with bacteria. This has not been studied in detail, but peptides with antifungal activity (with or without antibacterial activity) seem to kill a very broad range of fungi, presumably by acting on the fungal membranes.

The antiviral activity of specific cationic peptides has been demonstrated, generally with a single viral type.[14] However, although these cationic peptides seem to act only against enveloped viruses (e.g. hepatitis, HIV, etc.), implying a possible action on viral membranes, nothing is known about how they work. Similarly, the action of antimicrobial peptides against malaria and nematodes is poorly understood.[15] Cationic peptides have a general ability to promote non-opsonic phagocytosis.[2,16]

Cationic peptides also appear to promote wound healing,[10] although this is not well documented in the literature. They presumably do this by weakening the contacts between epithelial cells, thus promoting epithelial cell migration into damaged sites. Of considerable concern is the potential of certain cationic peptides, (e.g. melittin from bee venom and charybdotoxin from scorpions) to kill mammalian cells.[2,10] Still other cationic peptides seem to attack cancerous cells preferentially,[17] although such peptides probably do not have a high enough therapeutic index to be considered as primary anticancer drugs. Another potential use for antimicrobial peptides is as a spermicidal agent since some peptides can immobilise sperm.[18]

5. Clinical Studies

Despite the growing array of impressive preclinical data on the host defence peptides,[10] they have as yet failed to capture the imagination of large pharmaceutical companies. Thus, most preclinical and clinical studies to date have been performed by small biotechnology companies. Since there are no published data on such studies, I have had to rely on company press releases for information.[6,10] The greatest amount of information concerns the cationic protein $rBPI_{21}$ (Neuprex®, Xoma Corp, CA). Although it is a cationic protein (more than 200 amino acids) rather than a peptide, it is included here because small cationic peptide portions of this agent have the same activities as the intact molecule. In a phase II/III clinical trial of therapy against meningococcaemia, Neuprex® given as a bolus followed by intravenous infusion resulted in a dramatic decrease in deaths. Neuprex® has excellent anti-endotoxic activity and lesser antibacterial activity and thus it is undergoing a range of clinical trials in which endotoxin is indicated as an important factor.

Another well-studied peptide is pexiganan (MSI-78, Cytolex®; Magainan Sciences Inc.). Topical pexiganan has been announced to show equivalence to oral ofloxacin against polymicrobic diabetic foot ulcers in phase III trials of 926 patients. A distribution agreement has been signed with SmithKline Beecham, and other phase III trials have been initiated.

Colimycin, the methosulphate derivative of the cationic lipopeptide colistin, has been utilised quite successfully in aerosol formulation against *Pseudomonas aeruginosa* lung infections.[19] Pathogenesis Inc. is currently planning clinical trials for this agent in patients with cystic fibrosis. The related polymyxin B cationic lipopeptide, together with another cationic cyclic decapeptide, gramicidin S,

and bacitracin, is a very highly utilised topical preparation.

Both ambicin (nisin; a lantibiotic cationic peptide produced by AMBI) and IB-367 (a protegrin-like cationic peptide from Intrabiotics) have undergone phase I (safety) clinical trials successfully. They are being considered for stomach ulcers due to *Helicobacter pylori* (ambicin) and oral mucositis (IB-367) respectively, although other indications are being considered. Another company, Micrologix Biotech Inc., has entered phase I clinical trials against catheter-associated infections and acute acne.

Thus there is a considerable drive to try to examine clinical situations in which the assets of antimicrobial peptides will be efficacious. However, it is very difficult to assess the success of such ventures because of a dearth of information available.

6. Concerns

Most antibiotics are small (molecular weight 500), relatively stable, nontoxic, and inexpensive chemicals. Unfortunately, cationic peptides do not fit into this simple mould. Therefore, despite their admirable *in vitro* properties and apparent importance in the resistance of most species of life to antimicrobial infection, they have not apparently caught the attention of large pharmaceutical companies. This is due in part to anticipation of unknown toxicities, concerns about stability *in vivo* and worries about the cost of production. Unfortunately, there are few data about any of these issues. However, in this author's opinion, these concerns should not be overriding since solutions to all of these seem apparent.

There are cationic peptides with rather high toxicity, including bee venom melittin, scorpion charybdotoxin and fish pardoxin.[3] This together with the dearth of published toxicological studies creates anxieties over the potential toxicity of these peptides. However, in my experience it is not difficult to design peptides which show no acute toxicities (e.g. red blood cell lysis). Moreover, an alternative method for creating nontoxic peptides would be to create a prodrug. For example, for colistin, the amino groups were detoxified by creating the methosulphate derivative colimycin; *in vivo* the methosulphates are slowly removed.

The issue of stability is hinged around the potential *in vivo* proteolysis of antimicrobial peptides, especially proteolysis by trypsin/chymotrypsin-like proteases which cleave adjacent to arginine and lysine residues. Prospective solutions would include co-administration with a protease inhibitor, chemical modification of the amino group (either to make a prodrug as above or to change the chemical nature of the lysine and arginine residues so they are not recognised by proteases), changing the backbone to create a peptidomimetic, and liposomal formulation.[20]

The issue of production is critical. Natural sources do not yield large enough amounts of peptides and chemical synthesis is too expensive. Thus, recombinant synthesis seems likely to be the production method of choice and at least one practical procedure is available and is likely to be cost effective.[21]

7. Conclusions

In this author's opinion, cationic peptides have proven to have an important role in host defences and have many desirable features. While they have some features that cause concern, it is worth considering that they represent an entirely novel class of antimicrobials. Although this invites questions such as the concerns raised above, it also offers a prospectively exciting new tool in the physicians' armamentarium. I believe the clinical success of antimicrobial peptides in topical therapy is assured. Whether these peptides will also succeed in parenteral therapy is as yet an open question.

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