Clinical Development of Cationic Antimicrobial Peptides: From Natural to Novel Antibiotics

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Abstract: Over the past decade, levels of bacterial resistance to antibiotics have risen dramatically and "superbugs" resistant to most or all available agents have appeared in the clinic. Thus there is a growing need to discover and introduce new drugs. One potential source of novel antibiotics is the cationic antimicrobial peptides, which have been isolated from most living entities as components of their non-specific defenses against infectious organisms. Based on these natural templates, scores of structurally diverse antimicrobial cationic peptides have been designed, manufactured both chemically and biologically, and tested for activity against specific pathogens. A few of these peptide antibiotics have entered clinical trials to date, with mixed success. However, their diverse portfolio of structures, activity spectra, biological activities, and modes of action, provide substantial potential.

Keywords: Cationic peptides, Antimicrobial peptides, Antibiotics, Peptides, Diversity, Mode of Action, Membranes, Sepsis, Clinical Studies

INTRODUCTION

With the dramatic rise of antibiotic resistance, including the emergence of untreatable infections by multi-resistant tuberculosis and vancomycin-resistant *Enterococcus* strains there is no doubt we need novel antimicrobials [1]. Prior to 1999, no new structural class of antibiotic had been introduced into medical practice in 30 years. At present there are four structurally novel classes of antibiotics entering the clinic, three of these being the lipopeptide daptomycin, the oxazolidinone, linezolid, and the streptogramins. With the increasing recognition of the central role of cationic antimicrobial peptides in preventing the onset of infection in many organisms [2, 3], it seems likely that these peptides will provide the basis for a fourth new class of antibiotics. Furthermore, the antimicrobial peptides have the advantage of being broad-spectrum and bactericidal. The best of these peptides have good activities vs. a wide range of human pathogens, including antibiotic resistant isolates, kill very rapidly, are markedly salt resistant, do not easily select resistant mutants, and are synergistic with conventional antibiotics. Certain α-helical peptides neutralize the ability of both Gram negative and positive sepsis molecules, endotoxin and lipoteichoic acid, to induce cytokines in macrophage cell lines and prevent endotoxaemia in animal models. Selected peptides are protective against infections in animal models [2].

BIOLOGICAL DIVERSITY

Cationic peptides in nature can be synthesized by multienzyme complexes (gramicidins, polymyxins), or on the ribosome, with or without post-translational modifications [4-6]. While the former have furnished antibiotics that are used in current medical practice (polymyxin B, gramicidin S), the latter provide extraordinary opportunity for peptide variation by mutation. In 1966, several small arginine-rich microbicidal peptides were found in the granules of polymorphonuclear leukocytes by Zeya and Spitznagel [7]. Subsequently these were identified as β-sheet-structured defensins [8]. In the meantime, purothionins were identified in wheat plants [9], cecropins in silk moths [10] and magainins in frogs [11]. There are now known to be more than 500 antimicrobial peptides that derive from all species in nature.

Cationic peptides exhibit a range of secondary structures including, (A) β-sheets stabilized by two to three disulphide bridges, (B) amphipathic α-helices formed upon membrane contact, (C) extended structures (again formed upon membrane contact) which are Trp, Pro and/or His rich, and (D) peptides with loops formed by a disulphide bridge. A number of peptides from those classes have amidated C-termini, which enhances the activity of these peptides. However, there are many variants amongst these basic classes, with at least eight sub-classes of β-sheet peptides having been described in plants alone [12]. Two main peptide folds have been recognized: amphipathic structures comprising a hydrophilic, positively charged face and a hydrophobic face, and a cationic double wing structure with two pockets of positive charge bracketing a hydrophobic core.

In the past decade, it has become clear that cationic antimicrobial peptides represent a ubiquitous response in nature for overcoming microbial infections. They are produced by bacteria, fungi, plants, insects, amphibians, crustaceans, fish, birds and mammals, including man, either constitutively or in response to the presence of a microbe [2, 13]. In more primitive species they represent the major response to infection, and their induction appears to be the primitive equivalent of the immune response. In higher

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animals, these peptides tend to be induced as a local response to infection [14]. Most natural antimicrobial peptides have a limited spectrum of activity, and are usually present in modest (sub-MIC) amounts. Hosts generally compensate for this by producing a variety of peptides with overlapping activities, and by up-regulating them in the response to the presence of microbes. However, although this strategy usually works when pathogens are present in low amounts (e.g. in air or ingested food or water), we feel it would be less successful against large pathogen loads, or against pathogens that are less susceptible to some of the peptides. Thus, the inability of the natural peptides to protect against the onset of infections should not be seen as an argument against using novel improved synthetic peptides as a therapeutic.

Microbicidal peptides can successfully kill Gram-negative and Gram-positive bacteria, fungi, enveloped viruses and even cancer cells *in vitro*, but these activities can come at the cost of toxicity to healthy host cells. In such cases, rational modifications of existing peptides and customized delivery methods can reduce peptide toxicity [15, 16], as well as enhance the desired activities [17], and increase peptide stability [18]. Along with their antimicrobial activities, selected peptides have been described as agents in wound healing [19-21], or as chemoattractants for immune cells [22-24]. Another activity involves an ability to protect against endotoxic shock [25]. A so-called "enhancer activity," manifesting as synergy effect with classical antibiotics has also been described [35].

MODE OF ANTIBACTERIAL ACTION

With the multitude of cationic peptide sources, structures, and spectra of activity come a number of complex and controversial structure-function theories attempting to describe and explain peptide modes of action. Generally, the theories differ as to the nature and impact of peptide-membrane interactions.

One of the earliest and still popular propositions is the *barrel-stave model*, in which clusters of amphipathic peptides (or toroidal mixtures of peptides and lipids) are proposed to form hydrophilic pores embedded in the hydrophobic core of the cell membrane [26]. The channels thus formed are believed to lead to cell leakage and hence cell death. While there is little doubt that selected peptides at specific concentrations conform to this model, pore formation does not always accompany antimicrobial activity of cationic peptides [27]. To account for the lack of pore formation Shai developed the so-called *carpet model* [28]. In this model it was proposed that loss of cell membrane integrity occurs upon the membrane being covered by a "carpet" of peptides resulting in collapse of structural integrity. This model is favored by many, but does not fully explain the way peptides kill bacteria since in certain cases killing is not accompanied by a complete loss of cytoplasmic membrane potential, and in others the loss of membrane potential occurs gradually over a 4-8 fold range of concentrations rather than catastrophically as indicated by the model. It has also been proposed [29] that cationic peptides reversibly and randomly cluster prior to, or upon, entering cell membranes, and form transient variable-sized, water-containing aggregates, comprising peptide and lipids (*aggregate channel model*). This leads to either depolarization of the cytoplasmic membrane, or with kinetics ranging from microseconds to seconds, the dissociation of peptide aggregates leads to translocation through the bacterial cytoplasmic membrane, to access internal targets of peptide action, such as disruption of macromolecular synthesis [30, 31]. Consistent with this picture some peptides kill cells or inhibit macromolecular synthesis at concentrations at which no substantative cell depolarization occurs. The "leakiness" of peptide-treated bacterial cells would thus be a consequence of the membrane disturbance and of ions being carried by the interstitial water. An advantage of the aggregate model is its ability to account both for membrane "leakiness" and for peptide activity inside the bacterial cell. Multimodal models, where both membrane and intracellular targets are involved have also been proposed [32].

As a consequence of their physical mechanisms of action, the peptides are rapidly bactericidal, killing bacteria within minutes of contact [33, 34]. While natural peptides are seldom active at concentrations less than 1 μg/ml, when expressed in molar terms these activities are equivalent to many of the better conventional antibiotics. The best peptides have very broad activities against Gram negative and Gram positive bacteria and fungi, with MICs of 0.25 to 4 μg/ml [35-37]. In our experience, the only clinical pathogen that is universally resistant to peptides is *Burkholderia cepacia*, although *Serratia* and *Proteus* sp. tend to be quite resistant.

As outlined above, while the mode of action of cationic peptides is not well understood, it is generally agreed that peptides need to interact with cell membranes as part of their action against microbes. This has been the assumption behind many of the rational modifications to the existing peptide structures. In addition, a wide variety of studies have been performed looking at structure/activity relationships [13]. These studies have generally indicated that the following properties can be important: overall charge, amphipathicity and formation of a hydrophobic face when folded into the final membrane-inserted conformation. Although peptide sequences vary greatly in nature, for any given peptide the permitted substitutions for any given amino acid in the peptide can be quite moderate.

SYNERGY AND ANTI SEPSIS

Cationic peptides are taken up across the outer membrane of Gram-negative bacteria by a process termed self-promoted uptake [2]. In this process, the cationic peptides interact initially with divalent cation binding sites on surface lipopolysaccharide (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

Table 1. Sequences and Properties of Selected Natural and Synthetic Cationic Peptides

Peptide	Class[a]	Derivation	Sequence[b]
HNP1	β3(α)	Human neutrophils	AC_1YC_2**R**IPAC_3IAGE**RR**YGTC_3IYQG**R**LWAFC_2C_1
HBD-3	β3(β)	Human skin	GIINTLQ**K**YYC$_1$**R**V**R**GG**R**C_2AVLSC_3LP**K**EEQIG**K**C_2ST**R**G **RK**C_1C_3**RRKK**
Protegrin	β2	Pig	**R**GG**R**LC_1YC_2**RRR**FC_2VC_1VG**R**-NH_2
IB-367	β2	Synthetic	**R**GGLC$_1$YC_2**R**G**R**FC_1VC_2VG**R**-NH_2
Magainin II	α	Frog	GIG**K**FLHSA**KK**FG**K**AFVGEIMNS
MSI-78	α	Synthetic	GIG**K**FL**KK**A**KK**FG**K**AFV**K**IL**KK**-NH_2
Indolicidin	E	Cattle neutrophils	ILPW**K**WPWWPW**RR**-NH_2
Bactenecin	C	Cattle neutrophils	**R**LC$_1$**R**IVVI**R**VC$_1$**R**
Gramicidin S	βC	Bacteria	Cyclic (L**O**VPF^{d}L**O**VPF^d)
Polymyxin B	CL	Bacteria	Isooctanoyl **BTBB(B**F^{d}L**BB**T) Cyclized

[a] Classes are: β, beta-structured (number refers to the number of disulphide bridges); (α) or (β) after the number refer to the family of mammalian α or β defensins from which the peptides come); α , amphipathic α-helical; E, extended structure; C, cyclic; L (polymyxin only), lipopeptide.

[b] One-letter amino acid code with the following additions: Positively charged residues at neutral pH are boldfaced. Parentheses indicate amino acids that are cyclized. Superscript d represents the D-enantiomer; all other amino acids are L-form. The subscript numbers represent amino acids that are joined by either cysteine disulfides or (for nisin) thioether bridges. O, ornithine; B, diaminobutyrate; X, 2,3-didehydrobutyrine; U, 2,3-didehydroalanine; Z, α-aminobutyrate.

cationic peptides can promote the uptake of other agents, e.g. antibiotics and lysozyme, and thus show synergy with conventional antibiotics [35], especially against antibiotic resistant mutants. Synergy of magainin and a β-lactam, cefepime, has also been shown in an animal model. Antimicrobial peptides can also show synergy with conventional antibiotics against Gram positive bacteria (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.

Cationic peptides have a high affinity for LPS, a molecule that also bears the name endotoxin, and is a major player in Gram negative sepsis (about 300,000 cases per year in the U.S.A.) and endotoxaemia. As a consequence of their high LPS binding capacity, cationic peptides (and cationic proteins like bactericidal permeability increasing protein, BPI) neutralize LPS and can protect galactosamine-sensitized mice against lethal endotoxaemia [25]. Antimicrobial peptides were also able to bind to lipoteichoic acid, which is the major molecule from Gram positive bacteria that has been implicated in sepsis by this group of bacteria. Thus peptides seem to have considerable potential against sepsis syndromes which affect up to 500,000 patients per year in North America, provided that structural aspects responsible for LPS and lipoteichoic acid binding are explored further.

FROM NATURAL PEPTIDES TO COMBINATORIAL LIBRARIES

Remarkably only three structural aspects are common to most cationic peptide antimicrobials: they are cationic, with three or more lysines or arginines; they are small, being generally 6-40 amino acids in length; and they tend to contain at least 50% hydrophobic amino acid and their hydrophobic and hydrophilic residues are separated in the folded structure. The latter characteristic supports the contention that interactions with the amphipathic membranes of microbes are critical for peptide action on microbes. The amphipathic α-helical peptides have received the most attention in the literature as prototypes for building improved peptides, since the design of α-helices is relatively straightforward.

Primary sequence modifications of natural peptides are commonly employed to increase the overall charge or amphipathicity of the peptides, improve their predicted folding patterns, or facilitate production. Some of the most successful alterations include amidation of the C-terminus, and amino acid replacements, insertions or deletions [34, 38]. While the rational approach has shown some success, one very effective peptide CEME was produced by empirically combining the N-terminus of cecropin and the C-terminus of melittin [39]. In addition to rationally exploiting the structural patterns among existing cationic peptides, great potential thus exists for the employment of random techniques such as random combinatorial peptide libraries [40-43] or mutagenesis of DNA sequences encoding such peptides.

One must realize that in the case of completely random combinatorial libraries the number of prospective variants is enormous since there are more than 10^{26} possible 20-residue peptides when only the natural amino acids are considered. While advances in constructing and screening chemical and phage-display libraries are considerable, the technical and financial constraints of using combinatorial chemistry to find an optimal 20 amino acid peptide through a solely random approach are still prohibitive. One way to overcome this

problem is to introduce significant constraints on the structure of peptides to be produced and screened. The presence of cationic residues, prolines, or disulfide bonds could restrict the number of candidates. With the advent of computer modeling technology, more sophisticated restraints such as specified hydrophobic properties, or perhaps even aspects of a 3-dimensional folded structure could be defined.

We indeed contend that approaches to constructing new peptide antimicrobials will be more successful if changes are introduced based on rational consideration of the 3-dimensional folded structure of the peptides. With that in mind it is inevitable that more effective and less toxic peptides be discovered all along the spectrum ranging from improved natural structures to artificial constructs in the coming years.

CLINICAL EXPERIENCE

Only a few peptides have entered clinical trials, with mixed success (for more information the reader can visit the web sites *www.mbiotech.com*, *www.intrabiotics.com*, *www.xoma.com*, *www.magainin.com/home.htm*). The most prominent failure to date was MSI-78 (pexiganan acetate) that, in a phase III trial of therapy of diabetic foot ulcer infections, showed efficacy equivalent to oral ofloxacin therapy, but in July 1999, the FDA notified Magainin that based on inadequate trial design, their NDA had been deemed not approvable. There were also issues with the protegrin IB-367 (iseganan HCl oral solution) wherein Intrabiotics reported preliminary results from a phase III clinical trial in chemotherapy patients. The trial achieved its secondary endpoint for reduction of pain but did not meet the primary endpoint for presence of ulceration. Nevertheless this trial continues to enroll patients for a second phase III trial.

Micrologix Biotech Inc. has introduced 3 separate antimicrobial peptides related to indolicidin into clinical trials. The most advanced peptide is MBI-226, which is in phase III clinical trails for prevention of catheter-related bloodstream infections. According to company press releases and conference presentations, preclinical studies demonstrated that MBI-226 was effective in animal models in reducing skin colonization by a variety of bacteria known to cause catheter-related infections, and also demonstrated good antifungal activity against *Candida albicans* in guinea pig skin. A randomized, double-blind phase I trial in 18 healthy volunteers demonstrated that MBI-226 was safe and well tolerated and eliminated 99.9% of common skin bacteria for prolonged periods. Furthermore it completely prevented short-term central venous catheter (CVC) colonization, while 5 out of 6 catheters in control individuals became colonized. Because CVC colonization is a common cause of serious, life-threatening infections in hospitalized patients, causing 90% (180,000/year) of bloodstream infections resulting in an average of 6.5 additional days of intensive care and up to 50,000 deaths annually, Micrologix have received fast track status from the FDA. Micrologix has since initiated two further clinical trials using other indolicidin-like peptides for therapy of acute acne (in phase II clinical trials) and killing of MRSA in the nares (in phase Ib trials).

The research investment required to bring more peptide antibiotics to the clinic will likely remain substantial in the foreseeable future, since any novel class of antibiotics will inevitably raise unique questions. However, the incontestable need for new ways to manage infections, and the proven importance of peptides in innate immunity, should render the investment worthwhile for human medicine.

The authors believe that most of the existing concerns regarding cationic peptide antibiotics can be addressed by the extensive pool of peptide structural motifs and activities available to the researcher. One of the major advantages of antimicrobial peptides is that nature has taught us that many different types of structures are acceptable. Given the major problems we are experiencing with resistance amongst the classical antibiotics, and the very desirable properties of antimicrobial peptides, we should make every effort to develop this novel class through clinical trials.

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LIST OF ABBREVIATIONS

BPI	=	Bactericidal permeability increasing protein
CVC	=	Central venous catheter
FDA	=	Food and Drug Administration
LPS	=	Lipopolysaccharide
MIC	=	Minimal inhibitory concentration
MRSA	=	Methicillin resistant *Staphylococcus aureus*
NDA	=	New Drug Application
VRE	=	Vancomycin resistant *Entrococcus*

REFERENCES

[1] Travis, J. *Science*, **1994**, *264*, 360.

[2] Hancock, R. E. W.; Lehrer, R. *Trends Biotechnol.*, **1998,** *16*, 82.

[3] Boman, H. G. *Annu. Rev. Immunol.*, **1995,** *13*, 61.

[4] Bayer, A.; Freund, S.; Jung, G. *Eur. J. Biochem.*, **1995,** *234*, 414.

[5] Metz-Boutigue, M. H.; Goumon, Y. ; Lugardon, K. ; Strub, J. M.; Aunis, D. *Cell Mol. Neurobiol.*, **1998,** *18*, 249.

[6] Yorgey, P.; Lee, J.; Kordel, J.; Vivas, E.; Warner, P.; Jebaratnam, D.; Kolter, R. *Proc. Natl. Acad. Sci. USA*, **1994,** *91*, 4519.

[7] Zeya. A.; Sptznagel, J.K. *J.Bact.*, **1966,** *91*, 755.

[8] Ganz, T.; Selsted, M.E.; Szklarek, D.; Harwig, S.S.L.; Dahert, K.; Bainton, D.F.; Lehrer, R.I. *J. Clin. Invest.*, **1985,** *76*, 1427.

[9] Fernandez de Caleya, C.; Gonzales-Pasqual, B.; Garcia-Olmeido, F., Carbonero, P. *Appl. Microbiol.*, **1972,** *23*, 998.

[10] Steiner, H.; Hultmark, D.; Engstrom, A.; Bennich, H.; Boman, H.G. *Nature*, **1981,** *292*, 246.

[11] Zasloff, M. *Proc. Natl. Acad. Sci. USA*, **1987,** *84*, 5449.

[12] Garcia-Olmeido, F.; Molina, A.; Alamillo, J. M.; Rodriguez-Palenzuela, P. *Biopolymers*, **1998,** *47*, 479.

[13] Hancock, R. E.W.; Falla, T.; Brown, M. *Adv. Microb. Physiol.*, **1995,** *37*, 135.

[14] Ganz, T.; Lehrer, R. I. *Curr. Opin. Hematol.*, **1997,** *4*, 53.

[15] Moestrup, S. K.; Cui, S.; Vorum, H.; Bregengard, C.; Bjorn, S. E.; Norris, K.; Gliemann, J.; Christensen, E. I. *J. Clin. Invest.*, **1995,** *96*, 1404.

[16] Ahmad, I.; Perkins, W. R.; Lupan, D. M.; Selsted, M. E.; Janoff, A. S. *Biochim. Biophys. Acta*, **1995,** *1237*, 109.

[17] Dykes, G. A.; Aimoto, S.; Hastings, J. W. *Biochem. Biophys. Res. Commun.*, **1998,** *248*, 268.

[18] Rollema, H. S.; Kuipers, O. P.; Both, P.; de Vos, W. M. ; Siezen, R. J. *Appl. Environ. Microbiol.*, **1995,** *61*, 2873.

[19] Gallo, R. L.; Huttner, K. M. *J. Invest. Dermatol.*, **1998,** *111*, 739.

[20] Gallo, R. L.; Ono, M.; Povsic, T.; Page, C.; Eriksson, E.; Klagsbrun, M.; Bernfield, M. *Proc. Natl. Acad. Sci. USA*, **1994,** *91*, 11035.

[21] Cannon, M. *Nature*, **1987,** *328*, 478.

[22] Chertov, O.; Michiel, D. F.; Xu, L.; Wang, J. M.; Tani, K.; Murphy, W. J.; Longo, D. L.; Taub, D. D.; Oppenheim, J. J. *J. Biol. Chem.*, **1996,** *271*, 2935.

[23] Huang, H. J.; Ross, C. R.; Blecha, F. *J. Leukoc. Biol.*, **1997,** *61*, 624.

[24] Verbanac, D.; Zanetti, M.; Romeo, D. *FEBS Lett.*, **1993,** *317*, 255.

[25] Gough, M.; Hancock, R. E.W.; Kelly, N. M. *Infect. Immun.*, **1996,** *64*, 4922.

[26] Perez-Paya, E.; Houghten, R. A.; Blondelle, S. E. *J. Biol. Chem.*, **1995,** *270*, 1048.

[27] Wu, M.; Maier, E.; Benz, R.; Hancock, R. E. W. *Biochemistry*, **1999,** *38*, 7235.

[28] Shai, Y. *Trends. Biochem. Sci.*, **1995,** *20*, 460.

[29] Hancock, R. E.; Chapple, D. S. *Antimicrob. Agents Chemother.*, **1999,** *43*, 1317.

[30] Boman, H. G.; Agerberth, B.; Boman, A. *Infect. Immun.*, **1993,** *61*, 2978.

[31] Subbalakshmi, C.; Sitaram, N. *FEMS Microbiol. Lett.*, **1998,** *160*, 91.

[32] Xiong, Y. Q.; Yeaman, M. R.; Bayer, A. S. *Antimicrob. Agents Chemother.*, **1999,** *43*, 1111.

[33] Friedrich, C.; Scott, M. G.; Karunaratne, N.; Yan, H.; Hancock, R. E. W. *Antimicrob. Agents Chemother.*, **1999,** *43*, 1542.

[34] Zhang, L.; Benz, R.; Hancock, R. E. W. *Biochemistry*, **1999,** *38*, 8102.

[35] Scott, M. G.; Yan, H.; Hancock, R. E. W. *Infect. Immun.*, **1999,** *67*, 2005.

[36] Wu, M.; Hancock, R. E. W. *Antimicrob. Agents Chemother.*, **1999,** *43*, 1274.

[37] Falla, T. J.; Hancock, R. E. W. *Antimicrob. Agents Chemother.*, **1997,** *41*, 771.

[38] Shafer, W. M.; Hubalek, F.; Huang, M.; Pohl, J. *Infect. Immun.*, **1996,** *64*, 4842.

[39] Wade, D.; Andreu, D.; Mitchell, S.; Boman, H.; Merrifield, R. *Int. J. Peptide Protein Res.*, **1992,** *40*, 429.

[40] Houghten, R. A. *Gene*, **1993,** *137*, 7.

[41] Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C. T.; Cuervo, J. H. *Nature*, **1991,** *354*, 84.

[42] Goodson, B.; Ehrhardt, A.; Ng, S.; Nuss, J.; Johnson, K.; Giedlin, M.; Yamamoto, R.; Moos, W. H.; Krebber, A.; Ladner, M.; Giacona, M. B.; Vitt, C.; Winter, J. *Antimicrob. Agents. Chemother.*, **1999,** *43*, 1429.

[43] Oh, J. E.; Hong, S. Y.; Lee, K. H. *J. Pept. Res.*, **1999,** *53*, 41.