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Review

The therapeutic potential of cationic peptides

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Novel classes of antibiotics that are useful against resistant bacteria are a major need in human medicine. Cationic antimicrobial peptides are utilised as nature's antibiotics, being produced constitutively or in response to infection in virtually every type of organism from plants and insects to man. Thus, these peptides are now being considered as potential antibiotics for infections. They have the following assets: structural diversity, rapid bactericidal action, a broad spectrum of activity that includes most of the clinically important resistant pathogens, and several ancillary activities which can include antifungal, antiviral, anti-endotoxin activities, and promotion of wound healing. Cationic peptides and proteins are now proceeding through clinical trials as topical antibiotics and anti-endotoxins.

Keywords: *antibiotics, anti-endotoxin, antifungal, antimicrobial peptides, Neuprex, nisin, peptides, synergy, systemic, topical, wound healing*

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1. Introduction

With the dramatic rise in antibiotic resistance, including the emergence of untreatable infections of multi-resistant tuberculosis and vancomycin-resistant *Enterococcus* strains, there is no doubt that novel antimicrobials are urgently needed [1]. No radically new structural class of antibiotics has been introduced into medical practice over the past 30 years. However, with the increasing recognition of the central role of cationic antimicrobial peptides in preventing the onset of infection in many organisms [2,3], it is possible that these peptides will provide the basis for a novel class of antibiotics.

This article will review the current status of cationic antimicrobial peptides and will discuss their role in the future. As these peptides are in the very early stages of development, the author has had to rely on company announcements, unpublished presentations by companies at meetings, and database reports for much of the enclosed information regarding clinical trials and/or company plans.

1.1 Peptides as nature's antibiotics

Over the past decade, it has become clear that cationic antimicrobial peptides represent a ubiquitous response in nature to microbial infections. They are produced by bacteria, fungi, plants, insects, amphibians, crustaceans, fish and mammals, including man, either constitutively, or in response to the presence of a microbe [2,4]. In more primitive species they represent the major response to infection, and their induction could be

Table 1: Examples of the primary sequence of natural cationic antimicrobial peptides.

Rabbit Defensin (NP-1)	VVC$_1$AC$_2$**RR**ALC$_3$LP**RE**R**R**AGFC$_3$**RI**R**G**RIHLC$_2$C$_1$**RR**
Crab Tachyplesin	**RR**WC$_1$F**R**VC$_2$Y**R**GFC$_2$Y**RK**C$_1$**R**
Cattle Bactenecin	**R**LC$_1$**R**IVVI**R**VC$_1$**R**
Silk Moth Cecropin A	**K**W**K**F**KK**IE**K**MG**R**NI**R**DGIV**K**AGPAIEVIGSA**K**AI
Cattle Indolicidin	ILPW**K**WPWWPW**RR**
Bacterial Nisin	IXA$_1$IYLA$_1$Z$_2$PGA$_2$**K**Z$_3$GLAMGA$_3$NM**K**Z$_4$AZ$_5$A$_4$HA$_5$SIHVY**K**

X: 2,3-didehydrobutyrine; Y: 2,3-Didehydroalanine; Z: α-Amino butyrate.
Positively charged residues are emboldened.
Subscripts refer to residues that are covalently linked *via* disulfide (for cysteines) or thioether (for nisin) bonds.

considered the primitive equivalent of the immune response. For example, in plants and insects they are under the control of a conserved complex regulatory system, involving the transcription factor nuclear factor (NF)-Kappa B, a homologue of which controls the mammalian immune response [5]. In higher animals, these peptides tend to be induced as a local response, e.g., on the skin of amphibians, fish and humans [3,4,6], at mucosal sites in cows and man [2,7], or in the granules of neutrophils attracted to infection sites [8]. Generally speaking, natural peptides have a moderate spectrum of activity, and are usually present in modest amounts. Producing species generally compensate for this by generating a range of peptides with overlapping activities and by up-regulating them in the presence of microbes. However, although this strategy usually works when pathogens are present in low amounts (e.g., in the air or ingested in food or water), it tends to be less successful against large pathogen loads, or against pathogens that are less susceptible to some of the peptides. This creates a niche for improved synthetic peptides.

1.2 Physical and structural characteristics of antimicrobial peptides

Cationic antimicrobial peptides are generally small (12 - 50 amino acids) and positively charged due to the presence of excess basic residues, lysine or arginine, over acidic residues (**Table 1**) [4]. The more successful antimicrobial peptides tend to have a charge of +3 to +9. In addition to the natural peptides, several basic proteins with weak antimicrobial activity [4], e.g., bactericidal/permeability increasing protein (BPI) and lactoferrin, have activities that can be delimited to cationic peptide fragments (some of which can be produced proteolytically), and are thus considered here.

The peptides, although having similar physical properties, exhibit a range of classes of secondary structures including:

- β-sheets stabilised by 2 - 3 disulfide bridges
- amphipathic α-helices
- extended helices
- loops formed by a disulfide bridge [9]

The amino acid sequences of these peptides can vary substantially, although the β-structured peptides can fit into clear subgroups, and peptides from all families can have homologues that are present in very different species (e.g., cecropins from moths and pig intestine). Specific post-translational modifications (C-terminal amidation, glycosylation, bromylation, the formation of lanthionine, etc.) can occur with specific peptides, although their purpose is not always clear.

1.3 Cystic fibrosis as a case example

Cystic fibrosis (CF) is the most common, eventually fatal, autosomal-negative genetic disease in our society. It is caused by a defect in the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel protein. The eventual cause of death is the deterioration of lung function due to chronic lung infections, particularly by *Pseudomonas aeruginosa*. It was recently discovered that lung epithelial cells secrete cationic antimicrobial peptides (one of which is β-defensin 1) that can normally kill *P. aeruginosa*, but which, in the high salt environment created outside CF epithelial cells (due to the CFTR mutation), are ineffective due to salt antagonism [10,11]. This illustrates the importance of an effective peptide response.

2. *In vitro* activities

One problem in comparing peptide activities has been the lack of a consistent method for the measurement of antimicrobial activities. Thus, results from different laboratories have been impossible to compare. To circumvent this, we have adopted a method devised by the company Intrabiotics [12] and posted a detailed protocol on the internet at http://www.interchange.ubc.ca/bobh/methods.htm. The comments below are based on published and unpublished studies from many different sources and are not intended to reflect the relative merit of selected peptides.

2.1 Gram-negative outer membranes

Antimicrobial peptides tend to be either broad spectrum, or Gram-negative bacterium specific. In our experience, those claimed to be Gram-positive selective may also have Gram-negative activity that is unrecognised because the wrong method has been used (e.g., solid phase assay using an agar based medium) to assess activity [13]. However, the lantibiotic nisin is extremely Gram-positive selective [14]. Those peptides that affect Gram-negative bacteria must interact with and cross the outer membrane, which they do by a process termed self-promoted uptake [4,9]. In this process, the peptides initially interact with the surface of Gram-negative bacteria, at sites in the outer membrane where divalent cations (Mg^{2+} or Ca^{2+}), noncovalently cross-bridge adjacent, negatively-charged lipopolysaccharide (LPS) molecules. Since they have 100- to 1000-fold higher affinity for LPS than the native divalent cations, and are far bulkier, the peptides competitively displace these cations and cause a distortion of the outer membrane structure and barrier properties. It is through this distorted outer membrane that the peptides pass.

This mechanism has two consequences of therapeutic interest. Firstly, LPS bears the special name endotoxin, which causes substantial problems in human medicine, such as sepsis and endotoxic shock. Cationic antimicrobial peptides and proteins like BPI (Neuprex) have the potential to bind to LPS and thus prevent endotoxic shock [15,16]. Secondly, during self promoted uptake these peptides overcome the outer membrane permeability barrier, not only to the antimicrobial peptides themselves, but also to conventional antibiotics [9,17]. Thus, synergy with conventional antimicrobials is an observed property for many of these peptides. Interestingly, this property can extend to Gram-positive bacteria and fungi.

2.2 Antibacterial action

The basis for antibacterial action has been assumed to be due to the formation of channels, and/or a general collapse of membrane integrity (the so-called carpet-like effect), resulting in a general loss of the cytoplasmic membrane permeability barrier [2,4]. It is certain that these peptides tend to form such channels, and/or lesions in model systems, and that the presence of a large membrane potential (interior negative) of anionic lipids in the exterior facing lipid layer, and a lack of cholesterol, separately, or together, determine specificity for microbes compared to host cell membranes [9]. However, the exact nature of the lethal activity of these peptides is still uncertain [18]. In any event, the peptides are rapidly bactericidal, killing bacteria within minutes of contact [4,9]. Generally speaking, the peptides are seldom active at concentrations less than 1 μg/ml. This corresponds in molar terms to activities that are equivalent to many of the better conventional antibiotics. However, of greater importance is the fact that the best peptides have very broad activities against Gram-negative and Gram-positive bacteria and fungi, with MICs of less than 8 μg/ml (cf. 16 μg/ml which is the clinical cut-off for susceptibility for many β-lactams) [12,19]. In our experience, the only clinical pathogen that is universally resistant to peptides is *Burkholderia cepacia*.

A wide variety of studies have been performed which have looked at structure/activity relationships [4]. These studies have generally indicated that the following properties can be important:

- overall charge
- amphipathicity
- formation of a hydrophobic face when folded into the final membrane-inserted conformation

Although peptide sequences for any given peptide vary greatly in nature, the permitted substitutions for any given amino acid in the peptide can be quite moderate.

2.3 Antifungal activity

Very few studies showing antifungal activity have been published, although several peptides have been reported to be active against *Candida albicans*, with MICs of 8 - 16 μg/ml [19]. Generally speaking, plant-derived peptides often have better antifungal than antibacterial activities [20]. Nevertheless, synthetic peptides with antifungal activity have been described and are apparently being developed [20].

2.4 Antiviral activity

The activity of certain antimicrobial peptides against enveloped viruses, such as HIV and herpes, has been described [21]. The basis for their activity is entirely unclear, although it has been assumed that it is related to the insertion of the peptides into the membranous envelope of these viruses. Until a mechanism for such antiviral activity has been defined, one should be careful in classifying peptides as antivirals.

2.5 Other activities

Cationic peptides have been suggested to stimulate wound healing through the promotion of re-epithelialisation of damaged surfaces [22,31]. Other similar peptides appear to actually preferentially prevent the proliferation of cancer cell lines [3,23,31]. Neither of these properties has been well characterised. Antiparasite activity has also been clearly demonstrated for selected peptides [3,24].

3. Animal models

Relatively few animal model studies have been published for this group of antimicrobials (**Table 2**). However, anecdotal reports by companies and the progression of 3 peptides into clinical trials suggest that many such studies have been performed. Those studies that have been reported show clear evidence of *in vivo* efficacy, even with single dose administration. Although current clinical trials are designed to test topical efficacy, it is clear that antimicrobial peptides can also be effective when administered systemically. Other *in vitro* properties of antimicrobial peptides have also been shown to extrapolate to efficacy in animal models, including anti-endotoxin activity, synergy with conventional antibiotics, antifungal activity, antiparasitic activity and wound healing. Although these studies present a compelling case for the potential of this class of antimicrobials, there are no comparative studies to assess relative efficacy, few hints about optimal formulations, routes of administration, or dosing, only cryptic comments about toxicity and no detailed published studies of pharmacokinetics. It is hoped that such data will be published as this drug class approaches clinical acceptance.

4. Clinical experience and trials

There are as yet no published clinical trials and the following information is substantively derived from the claims and press releases of the biotechnology companies that have initiated these trials.

4.1 Polymyxins and gramicidins

Polymyxin B, polymyxin E (colistin) and gramicidins are cyclic, bacterium-produced cationic antimicrobial peptides. The polymyxins carry an acyl tail and are thus lipopeptides. All of these agents have been utilised as topical agents, and polymyxin B and gramicidin S, usually together with bacitracin, are the most common antibiotics used in generic formulations of wound creams and other topical medicines. In addition, colistin has been used in the clinic in an aerosol formulation for the suppression of *Pseudomonas* lung infection [30].

4.2 Cytolex (MSI-78)

Magainin initiated Phase I studies of its 22 amino acid magainin peptide, MSI-78, in the early 1990s [22]. An initial Phase III study of its efficacy against impetigo, a polymicrobic skin infection, largely due to Gram-positive bacteria, was abandoned, when it was found that this disease was largely self-resolving given good hygiene. Magainin then initiated two Phase III trials for the use of topical MSI-78 (Cytolex) cream, compared to the conventional oral ofloxacin therapy, against polymicrobic diabetic foot ulcers. On 25 September, 1997, it was announced that this peptide showed efficacy equivalent to that of oral ofloxacin in the 926 patients enrolled in the two Phase III studies. SmithKline Beecham has signed a distribution agreement. Other Phase III trials for the treatment of surgical wounds, decubitus ulcers, venous stasis ulcers and burn infections are apparently planned.

4.3 Neuprex (rBPI-21)

Xoma has developed a version of the cationic neutrophil-derived protein BPI, comprising the recombinantly-produced, 21 kDa N-terminal half (rBPI-21) [28]. Although Neuprex is a cationic protein, it shares many properties with the cationic peptides and can be cleaved to produce active cationic antimicrobial peptides [29]. In May 1995, Phase I trials with Neuprex, were initiated in paediatric meningococcaemia patients. To date, more than 700 patients have received BPI *via* bolus, and/or continuous infusion by iv. administration, and Neuprex has proven to be very

Table 2: Animal model studies of antimicrobial peptides.

Peptide	Model	Route of administration	Dose	Results	Reference
Nisin	Germicidal activity on teat skin of cows	Topical	16.1% nisin in 1-propanol, 1 min exposure	4 log redction in *S. aureus*, *K pneumoniae* and *E. coli*	[25]
Cecropin-melittin hybrids	*P. aeruginosa* keratitis (eye infection) in rabbits	Topical	6 - 12 x 30 doses in PBS at 1 or 2 h intervals	30% lower inflammatory scores ($p < 0.05$)	[26]
MIMS™ peptide	Wound closure in rats	No details provided		3-fold increase in rate of wound closure	[Demeter Biotech press release]
Protegrin-1	Bacteraemia models with bacteria given ip. (*S. aureus*, MRSA or *P. aeruginosa*) or iv. (MRSA or VRE) in mice	Systemic	0.5 - 5 mg/kg given ip. or iv. (same route as bacteria) as a single dose	54 - 100% protection from lethality	[12]
MBI-28 (a cecropin melittin variant)	Bacteraemia in neutropenic mice; *P. aeruginosa* given ip. in mice	Systemic	2 - 8 mg/kg given ip. as a single dose in mice	35 - 50% protection from lethality	[16]
	Endotoxaemia in galactosamine sensitised mice; LPS given ip.	Systemic	2 - 8 mg/kg given ip. as a single dose in mice	62 - 78% protection from endotoxic shock	[16]
Magainin-2	Synergy with β-lactam cefpirome *vs. E. coli* bacteraemia in neutropenic mice	Systemic	0.2 mg/kg cefepime plus 2 mg/kg magainin-2 given im. in mice at 1 h and 3.5 h post challenge	44% more animals protected than with cefepime alone	[17]
Indolicidin	Systemic *Aspergillus fumigatus* infection	Systemic	40 mg/kg of liposomal indolicidin given iv. 6 h post fungi	30% protection from an LD_{100}	[27]
BPI (Neuprex)	Endotoxaemia in galactosamine-sensitised mice	Systemic	10 mg/kg ip.	93% protection	[28]
XMP 366 (aa 148-161 of BPI)	Murine model of *Candida albicans*	Systemic	Single dose of 0.5 mg/kg	Significant decrease in cfu in kidney	[20]

safe. Phase II studies of Neuprex for the treatment of paediatric meningococcaemia were successful enough that the US FDA granted Xoma a subpart E designation in August 1996, permitting the immediate start on Phase III pivotal trials, which are now ongoing in the USA, Canada and in the UK. Phase II trials of efficacy against infectious complications in hepatectomy patients, as an adjuvant to antibiotics in severe intra-abdominal infections, and in 400 patients suffering from haemorrhagic trauma are underway. In the last group of patients, Neuprex led to a reduced incidence of infections, organ dysfunction and death compared to placebo. A trial in cystic fibrosis patients is also apparently planned.

4.4 Nisin

Nisin is a 32 amino acid, cationic peptide bacteriocin from *Lactobacillus lactis*. It falls into the class of lantibiotics, since it has several posttranslationally modified amino acids, including lanthionine [14]. Nisin has been used as a food additive, especially in soft cheeses, for more than 40 years. This peptide tends to have excellent activity against many Gram-positive bacteria, but little activity against Gram-negative bacteria (unless formulated with chelators). Interestingly, the first clinical trials were actually initiated for its efficacy against the Gram-negative bacterium, *Helicobacter pylori*, a major cause of gastritis, gastric ulcers and gastric cancers. In collaboration with Astra, Phase I studies of safety in 96 patients of oral delivery of up to 4 mg/day for 7 days, by AMBI, were successful in demonstrating safety, and a Phase II study is imminent. Other indications being investigated include tooth disease, skin infection, mouth ulcers, *Clostridium difficile* and vancomycin-resistant *Enterococcus* infections.

4.5 IB-367

Recently, Intrabiotics initiated a Phase Ia clinical trial for the safety of IB-367 in healthy volunteers, followed by a Phase Ib safety study in bone-marrow transplant patients. The objective is to test its efficacy against polymicrobic oral mucositis, a side-effect of anticancer therapies.

4.6 Other compounds

Micrologix has announced that it will perform a trial of the efficacy of one of their peptides in topical therapy in 1998, but neither the peptide nor the clinical indication has been announced. Xoma is expected to initiate trials of mycoprex, one of their BPI-derived peptides, against fungal infections within the next 12 months.

5. Concerns and perspectives

5.1 Pharmacokinetics

A major concern with peptides, especially those introduced systemically, is the susceptibility of such compounds to proteolytic degradation. Trypsin/chymotrypsin-like proteases, which abound in the body, preferentially cleave at basic residues that are the key elements of cationic antimicrobial peptides. Even for a highly modified peptide like nisin, such proteases can rapidly destroy activity *in vitro*. However, the actual *in vivo* impact of such proteases is, at present, unknown, although the existence of successful animal model data (**Table 2**) rather indicates that certain peptides must remain stable for long enough to act. There are a number of obvious strategies that can be employed in an attempt to increase *in vivo* half-life. These include the use of peptides at sites in the body with lower levels of proteases (e.g., the skin), the co-administration of a protease inhibitor (in this regard it is of note that many protease inhibitors, such as basic pancreatic trypsin inhibitor, are cationic peptides), liposomal formulation, or chemical modification of lysine or arginine residues to maintain the positive charge, but alter protease recognition.

5.2 Toxicity

Toxicity is always an issue with a new class of drugs. With cationic peptides one must be even more vigilant since related proteins, such as melittin, mastoparan and charybdotoxin, are components of bee, wasp and scorpion venom, respectively. On the other hand, humans are now known to produce a variety of cationic antimicrobial peptides that are presumably harmless. Nevertheless, several peptides have cleared preclinical and/or Phase I safety trials for oral (nisin) or topical (MSI-78, IB-367) administration, whereas the cationic protein, Neuprex, has proven safe in humans when administered intravenously. Although peptides do enter membranes in microbial cells, they do not seem to easily insert into host cell plasma membranes, due to the paucity of anionic lipids, high cholesterol content and minimal electrical potential gradient of the latter.

5.3 Immunogenicity

One issue that always arises with peptides is whether immunogenicity will limit efficacy. Unfortunately, there are virtually no published studies, except for a few in which antibodies have been made in animals using appropriate carriers or adjuvants. An immune response to antimicrobial peptides would not necessarily be expected to be harmful and it is of note that many antibiotics induce specific antibody production without major consequences. Nevertheless, it is not easy to make peptide-specific antibodies. It is possible, given the importance of these peptides in preventing the initiation of infections, that humans become tolerant to cationic peptides.

5.4 Production

Production is a critical issue in the field. Of the cationic peptides and proteins currently in clinical trials,

Neuprex and nisin are made recombinantly by processes somewhat specific to these agents, whereas MSI-78 and IB-367 were manufactured by solution phase chemistry. The latter method is unlikely to be cost-effective for peptides of 10 - 15 or more residues, and the current manufacturing cost of MSI-78 is around $100 per gram (about a daily dose). Therefore, a variety of recombinant expression systems have been, or are being developed in an attempt to reduce production costs by approximately 20-fold or more [2,4].

6. Expert opinion

The advantages of antimicrobial peptides far outweigh the concerns, and this class of antibiotics promises to be the first breakthrough class in 30 years. Two areas which will be the subject of substantial research will be production, to make these peptides economically competitive, and pharmacology/toxicology, to discover which features of these peptides will favour a good therapeutic index. However, if these problems are solved, the enormous chemical diversity implicit in peptides (a 20-mer peptide has 20^{20} possible variants) and the plethora of useful activities of cationic peptides, will guarantee the place of such peptides as part of the therapeutic armamentarium.

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