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Review

Monthly Focus: Anti-infectives

Cationic antimicrobial peptides: towards clinical applications

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Cationic antimicrobial peptides are important components of the innate immune defences of all species of life. Variants of these natural molecules have a broad range of antibiotic, antifungal, antiviral and anti-endotoxic activity. Two of these cationic peptides have shown signs of efficacy in early clinical trials of oral mucositis and the sterilisation of central venous catheters, respectively and are currently proceeding through Phase III clinical trials. Thus, cationic antimicrobial peptides are currently being investigated as topical agents. In addition, the cationic protein rBPI 21 has recently completed Phase III clinical trials of parenteral use for meningococcaemia.

Keywords: *antibiotics, anti-endotoxic, antifungal, antimicrobial peptides, catheter-associated infections, oral mucositis, protegrins, topical*

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1. Introduction

Hundreds of peptide antibiotics have been described in the past half-century [1]. These fall into two classes, non-ribosomally synthesised peptides, such as the gramicidins, polymyxins, bacitracins and glycopeptides and ribosomally synthesised (natural) peptides, including the defensins, cecropins and magainins. The former are largely produced by bacteria and are synthesised on enzyme complexes known as peptide synthetases and thus are often drastically modified. In contrast, the latter are gene encoded and produced by all species of life (including bacteria).

Cationic antimicrobial peptides are nature's antibiotics. They are produced by virtually all organisms ranging from bacteria through insects and plants to mammals, including man, as a component of the non-specific immune defences of these organisms [2,3]. They have a major role in the immediate defences against microorganisms, with significant activities against Gram-negative and Gram-positive bacteria, fungi, enveloped viruses and parasites [2,3]. As such, they provide templates for the design of a completely novel class of antimicrobials, with an enormous chemical and structural diversity. It is generally believed that they act by a physical rather than enzyme-inhibiting mechanism, allowing very little opportunity for pathogens to develop resistance.

In a previous article in this journal, the origins, activities, animal model studies, clinical experience and concerns for these molecules and their variants developed for therapy were discussed [4]. In this article, an update

Table 1: Cationic antimicrobial peptides being developed commercially.

Peptide	Structure[a]
MSI-78	GIG**K**FL**KK**A**KK**FG**K**AFV**K**IL**KK**-NH_2
Indolicidin	ILPW**K**WPWWPW**RR**-NH_2
IB-367	**R**GGLC_1YC_2**R**G**R**FC_1VC_2VG**R**-NH_2
Bacterial nisin	IXA_1IUL$A_2$$Z_2PGA_2$**K**$Z_3$GLAMG$A_3$NM**K**$Z_4AZ_5$
Gramicidin S	Cyclic (L**O**VPF^{d}L**O**VPF^d)
Polymyxin B	Cyclised isooctanoyl **B**T**BB**(**B**F^{d}L**BB**T)

[a]One-letter amino acid code with the following additions. Positively charged residues at neutral pH are boldfaced. Parentheses indicate amino acids that are cycled. [d] D-enantiomer; all other amino acids are L-form. The subscript numbers represent amino acids that are joined by either cysteine disulphides or (for nisin) thioether bridges.
B: Diaminobutyrate; O: Ornithone; U: 2,3-Didehydroalanine; X: 2,3-Didehydrobutyrine; Z: α-Aminobutyrate.

will be presented, describing the progress made towards clinical use of antimicrobial peptides.

2. Design of improved antimicrobial peptides

Natural cationic antimicrobial peptides range from 12 to 50 amino acids and have a charge of +2 to +9 due to an excess of basic lysine, arginine and histidine residues over acidic amino acids. In addition, they usually have around 50% hydrophobic amino acids, a feature that is critical to the activity of these peptides since their action on, for example bacteria involves insertion into (and, we have suggested, passage across) bacterial membranes [5]. The peptides that are being tested in the clinic are from 12 - 22 amino acids in length and have net charges of +3 to +8 (**Table 1**). These peptides have a variety of secondary structures, including β-sheet, α-helix, extended and β-turn structures when they interact with membranes (they may be random- or β-structured in free solution). However, despite their variety of secondary structures, only two types of three-dimensional shapes have been recorded. These are an amphipathic structure with a hydrophobic face and a hydrophilic, charged face and a cationic double wing structure with two pockets of positive charge bracketing a hydrophobic core region [2,6]. The design of novel and improved peptides is more likely to be successful if peptides conform to one of these two shapes. However, the fact that these shapes are often only adopted by peptides upon contact with membranes is an added complication in the design of such peptides. There are, therefore, no absolute rules for peptide design, although several studies have indicated that peptides that are longer, more highly charged and more hydrophobic tend to be more active. Nevertheless, although there is usually an optimal size, charge and hydrophobicity, these parameters are overridden if they result in peptides that adopt the wrong shape in membranes. Other key features include the positioning of shape-modifying amino acids such as proline and glycine, the presence of amino acids such as tryptophan with affinity for membrane interfaces and in peptides with cysteine disulphides, the presence of these covalent bonds.

3. Activities of antimicrobial peptides

Table 2 describes the broad spectrum of activities that can be ascribed to cationic peptides, with some noted examples. As an illustration of the intrinsic potential of single antimicrobial peptides, the activities demonstrated for the 13 amino acid extended peptide, indolicidin, are mentioned. Thus, a single agent can have activity against a range of Gram-negative and Gram-positive bacteria, fungi, enveloped viruses such as HIV, parasites and cancer cells, while showing synergy with conventional antifungals and antibiotics. Generally speaking, such broad spectrum compounds tend to have modest activities against most of these agents. Indeed, designed peptides with low toxicity often have improved activities against a subset of these agents, at the sacrifice of their broad spectrum of activities. Regarding their antibiotic activity, the cationic peptides kill bacteria rapidly, with a three or more logarithms decrease in 5 min at four-fold the minimum inhibitory concentration (MIC) [7,8], work well against most important clinically resistant mutants [8,9] and do not easily select resistant mutants [8]. They have been shown to be active in both topical and systemic animal models, even with

Table 2: Activities of cationic antimicrobial peptides and some examples of peptides with those activities.

Activities of antimicrobial peptides	Example peptides[a]
Broad spectrum antibacterial	Protegrin, IB-367, MSI-78, indolicidin, CEMA, gramicidin S, Magainin II, polyphemusin
Anti-Gram-negative bacterial	Polymyxin B, colistin
Anti-Gram-positive bacterial	Nisin
Synergy with conventional antibiotics	CEMA, magainin II, MSI-78, IB-367
Antifungal	Protegrin, CEMA, indolicidin, gramicidin S, polyphemusin
Synergy with conventional antifungals	Indolicidin
Anti-endotoxin	CEMA, polyphemusin
Antiviral (HIV, HSV)	Indolicidin, polyphemusin, protegrin
Anticancer	CEMA, indolicidin
Synergy with conventional anticancer agents	Indolicidin
Wound healing	Magainins, PR39
Antiparasite	Magainin II, indolicidin

[a]In addition to the peptides described in **Table 1**, CEMA (previously termed CP28 or MBI-28) and magainin II are α-helical peptides [2,5]. Protegrin and polyphemusin (sequence related to tachyplesin) are β-hairpin peptides [2,7] and PR39 is an extended peptide [2].

single dose therapy [4]. In conformation of this, production of the human peptide LL-37 in transgenic mice resulted in resistance to bacterial diseases [10].

A concern implicit in this broad spectrum of activity is the issue of toxicity [4]. Virtually no data on systemic toxicity has been published to date, although there are reports that topical formulations of antimicrobial peptides are quite safe even for peptides like the protegrin IB-367, which has a therapeutic index (haemolytic concentration/minimal inhibitory concentration) of around 16 - 128 (R Hancock, unpublished data). To achieve their full potential, the acute and subtle toxicities of parenteral formulations of cationic antimicrobial peptides must be documented and methods of mitigating any toxicities defined. In this regard, two procedures have proven to reduce acute toxicity, liposomal formulation in the case of indolicidin [11] and chemical modification (methane sulphonation of positively charged residues) in the case of colistin [12].

To date, the reported clinical experience indicates that topical administration of these peptides does not result in an immune response; indeed this is commonly observed even for systemically administered peptide. Presumably, this reflects the poor antigenicity of such peptides due perhaps to clonal deletion of T-cells during development (the designed peptides mimic normal human antimicrobial peptides) and/or the fact that the hydrophilic residues are dispersed in the primary sequence (i.e., are discontinuous epitopes). No data on the pharmacokinetics of these peptides has been presented. The stability of these peptides in topical formulation is indicated by a Micrologix Biotech, Inc. report suggested that a single dose of MBI-226 could keep a skin site virtually sterile for up to three days.

4. Clinical trials

Both positive and negative experiences have been observed in clinical trials. The negative experiences with MSI-78 (pexiganan acetate) have provided a small setback for the field, possibly due to a poor choice of indications. A large Phase III trial against the polymicrobic skin infection, impetigo, was abandoned when it was observed that simply washing the infection site tended to resolve the impetigo in 75% of cases. This was followed by Phase III trials of MSI-78 cream *versus* diabetic foot ulcers, a polymicrobic, necrotic disease of diabetics [13]. At the end of this trial, it was determined that MSI-78 showed efficacy equivalent to oral ofloxacin therapy. In particular, between 18 - 30% of foot ulcer wounds closed after six weeks of therapy with both drugs. However, in July 1999, the FDA notified Magainin that their NDA had been deemed not approvable. Since this time there have been no significant announcements by Maganinin and it is uncertain whether MSI-78 can be resuscitated.

Another possible negative result involves Nisalpin™, the trade name for bacterial nisin (a common food additive) prepared by AMBI, Inc. and subjected to Phase I clinical trials of *Helicobacter pylori* infections in collaboration with Astra. Despite completion of this Phase I clinical trial in June 1996 and reports that a formulation of nisin was discovered that was able to deliver nisin directly to the colon, no further reports indicating the initiation of Phase II clinical trials have been made and given that these were originally due to start in 1997, we must assume at this time that they have been abandoned. Also, despite approval in November 1997 for AMBI/Astra to commence Phase I clinical trials to treat *Clostridium difficile* and vancomycin-resistant *Enterococcus* infections, no further word has been received.

Despite these problems, there is ample cause for optimism regarding the cationic peptides, at least as topical agents. The information revealed to date, largely from unpublished company press releases and conference presentations, is described below.

4.1 Polymyxins and gramicidin S

Polymyxins (polymyxin B and colistin) are cyclic peptides with a fatty acyl tail (**Table 1**) and carry a net charge of +5. Gramicidin S is a dibasic cyclic hexapeptide. Both are produced by bacteria by non-ribosomal means involving complex peptide synthetases and although they have some differences from each other, they act fundamentally like other cationic antimicrobial peptides [1]. Both the polymyxins and gramicidin S are considered too toxic for systemic use (although colistin is used by aerosol [14]). However, colomycin, a preparation where the amino groups of colistin (polymyxin E1, E2, E3) are neutralised by methane sulphonation [12] can be used systemically, e.g., for iv. therapy of lung infections in cystic fibrosis patients [15]. The combination of polymyxin B and gramicidin S (or neomycin), often together with bacitracin (neosporin, polysporin, mycitracin, AK-spore), is utilised in many topical medicines, including generic wound creams, eye and ear drops. Several attempts have been made to modify polymyxins [16] and gramicidins [17] to decrease toxicity, with mixed success. However, no second generation commercial product has arisen as yet.

4.2 Protegrin IB-367

IB-367 is a variant of the porcine peptide protegrin 1 and like protegrin, is comprised 18 amino acids and forms a β-hairpin structure stabilised by two disulphide bonds [18]. As such, it is related to tachyplesins and polyphemusins from horseshoe crabs, which appear frequently in the patent literature as antibacterial and anti-HIV peptides. The first clinical indication to be tested for IB-367 efficacy is oral mucositis, a painful and frequent side effect of anticancer therapies, involving ulceration and polymicrobic infections of the mouth. Results of Phase I trials demonstrated safety and provided the first indications of efficacy in the form of the reduction of the numbers of microorganisms in the mouth over the entire 10-day treatment period. Phase II trials in 134 patients in bone marrow transplantation centres indicated that IB-367 reduced post-transplant mucositis by 22%, but reduced mucositis severity by 40% when therapy was initiated four days prior to bone marrow transplant. These studies also indicated a lack of systemic absorption and antibody formation. Intrabiotics have announced that they are about to begin Phase III clinical trials. In addition to these studies, Intrabiotics is also conducting a Phase I clinical trial of aerosol administration of IB-367 for the treatment of lung infections in patients with cystic fibrosis and a Phase I clinical trial for oral decontamination in ventilator-associated pneumonia.

4.3 Micrologix peptides

Micrologix Biotech Inc. has introduced three separate antimicrobial peptides into clinical trials. The nature and sequence of these peptides has not been revealed. However, it seems likely, based on an examination of published PCT patent applications and of issued US patents licensed to Micrologix, that one or more of these peptides are indolicidins or α-helical peptides derived from cecropin-melittin hybrids.

Their most advanced peptide is MBI-226, which completed Phase II clinical trails in January 2000 and is now entering Phase III clinical trails for the 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 at reducing skin colonisation 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 randomised, 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) colonisation, while five of six catheters in control individuals became colonised. As CVC colonisation is a common cause of serious, life-threatening infections in hospitalised patients, causing 90% (180,000 each 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. Phase II clinical trials in 200 patients confirmed the lack of side effects and indicated that there was no evidence of skin irritation, immunogenicity and/or systemic adsorption of MBI-226.

The company has since initiated two further Phase I clinical trials. In January 2000, a two-part Phase I clinical trial of treatment with MBI-594AN of the disfiguring effects of acute acne (caused by *Propionobacterum acnes*) was initiated. This trial is aimed at testing safety, tolerability and efficacy. In addition, the company has recently entered a third peptide, MBI-853NL, into a randomised, double-blind, placebo-controlled Phase I clinical trial to eliminate and prevent nasal carriage of *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA). There is evidence that this is one of the major reservoirs for seeding MRSA infections in hospitalised patients, with carriers having a 3.5- to 14-fold increased risk of *S. aureus* infection.

4.4 Histatin P-113

Periodonitix is developing a 12 amino acid histatin variant, P-113, for oral use in preventing gingivitis and periodontal disease [19]. Phase I and II clinical trials have indicated that P-113, administered as a mouthwash (Histawash™) or gel formulation (Histat gel™), is safe for daily oral use and will prevent the development of gingivitis. The company has initiated a Phase IIb study of its Histawash™ mouth rinse to optimise the dosing. In addition to this, Periodontix has reported plans to initiate a Phase I/II trial against oral candidiasis and is performing preclinical studies for therapy against *Pseudomonas aeruginosa* lung infections in patients with chronic lung infection.

4.5 Neuprex™ and Mycoprex™

Neuprex™ is an injectable recombinant protein fragment (rBPI 21, a 21kDa N-terminal fragment) of the naturally occurring human neutrophil protein bactericidal/permeability increasing protein. It is being developed by Xoma Ltd. for a variety of indications and is included here because BPI is a cationic protein with many of the properties of the cationic antimicrobial peptides, i.e., antibacterial, antifungal, anti-endotoxin activity and synergy with conventional antibiotics and appears to act in a similar manner to the cationic antimicrobial peptides. Furthermore, cationic peptides derived from amino acids 148 - 161 of BPI have been reported to have antifungal activity.

Since data on rBPI 21 was last presented [4], some of the details of clinical trials have been published [20]. In 1996, a pilot open-label study of efficacy in 26 severely ill meningococcaemia patients indicated only a single, apparently unconnected death from heart failure (i.e., 3.8%). In contrast, historical rates of 22% deaths (11 out of 55 patients) had been recorded in the same medical centres in which these trials were performed. Due to these exciting results, rBPI 21 received Orphan Drug status from the FDA and has now completed a pivotal double-blind, placebo-controlled Phase III trial in the UK and North America in more than 2200 paediatric meningococcaemia patients. The data show an improvement in survival and reduction in the indicators of mortality. These have now been submitted to European authorities and the FDA for possible licensure and marketing by Baxter in late 2000.

Data from a Phase III study of the use of rBPI 21 as a treatment to prevent infectious complications in patients suffering from haemorrhagic trauma, have not been as successful. Indeed, Xoma, advised by an independent data safety monitoring board, have discontinued clinical trials, since although there were no safety concerns, the efficacy of rBPI 21 did not meet the predetermined efficacy criteria in the first 842 patients. It is worth mentioning that rBPI 21 has very impressive anti-endotoxic activity, but lesser antibiotic activity than the most potent cationic antimicrobial peptides and may be more suited for indications where endotoxin is a major player (e.g., in meningococcaemia).

Another indication being pursued is the prevention of the infectious complications in patients who have undergone a partial hepatectomy. Despite evidence of reduced hospitalisation from an interim safety analysis of 12 patients treated with rBPI 21, this Phase II clinical trial has been discontinued due to the slow rate of enrolment. Other trials, testing synergy with conventional antimicrobials for severe intrabdominal infections and in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* infections, have not yet resolved the value of rBPI 21 in these circumstances,

although some evidence of a dose-related improvement in patient outcome was observed in the former trial. Nevertheless, animal model data in the rabbit septicaemia model clearly indicated the synergistic effects of rBPI 21 and cefamandole.

As described previously [4], XMP366 (Mycoprex™) has shown activity in a systemic model of candidiasis. A related peptide, XMP391, has also demonstrated *in vivo* synergy with fluconazole against *Candida albicans* infections and activity in a murine *Aspergillus fumigatus* infection. In May 1999, Xoma announced that it was seeking partners for further development of these peptides.

5. Expert opinion

Cationic antimicrobial peptides have clear and obvious therapeutic potential. However, they are at a fragile point of the development cycle. Current indications seem to suggest that they will be successful as topical agents and may partially displace mupirocin (bactroban) and polymyxin B sulphate-neomycin-bacitracin (polysporin) as the current topical agents of choice. In addition, given their assets, novel cationic antimicrobial peptide antibiotics may be used in novel indications for which no current therapy exists. However, their development has been entirely restricted to the Biotech industry and will require the resources of large Pharma to open other markets, such as the larger, injectable drug market. The two issues of safety and stability and the potentially related issues of semisynthetic modifications and formulation must be thoroughly investigated, or the use of these peptides will become limited to topical treatments (e.g., lung aerosol delivery) that might displace parenteral antibiotics. Nevertheless, the apparent success of rBPI 21 as an injectable drug against meningococcaemia indicates that such potential does exist.

It has been widely stated that we are running out of new antibiotics and the paucity of effective, novel drugs in the pharmaceutical pipeline has been decried. While the application of genomics has been suggested as a solution to this concern, I feel that the antimicrobial cationic peptides should be thoroughly investigated. We know that antimicrobial cationic peptides have a desirable broad spectrum, rapidly-bactericidal activity and that they are biologically utilised as antimicrobial substances. The major challenge is to work out how to apply them in clinical situations.

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