Antibacterial peptides for therapeutic use: obstacles and realistic outlook

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Cationic antimicrobial peptides are produced by almost all species of life as a component of their immediate non-specific defense against infections. The assets of these peptides in clinical application include their potential for broad-spectrum activity, rapid bactericidal activity and low propensity for resistance development, whereas possible disadvantages include their high cost, limited stability (especially when composed of L-amino acids), and unknown toxicology and pharmacokinetics. Initial barriers to their success are being increasingly overcome with the development of stable, more cost-effective and potent broad-spectrum synthetic peptides. Thus, there is hope that they will spawn a new generation of antimicrobials with a broad range of topical and systemic applications against infections.

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Introduction

Since antibiotics became widely available more than half a century ago, they have had an enormous impact on our ability to treat bacterial diseases worldwide. However, the past 40 years have seen only three new classes of antibiotics enter medicine (lipopeptides, oxazolidinones and streptogramins), all geared towards Gram-positive bacterial infections. A lack of new antibiotics for treatment of Gram-negative infections combined with emerging multi-drug resistance issues (Table 1) demands that new antimicrobial strategies be explored for treating these infections. With an understanding of the pivotal role that cationic host defense (antimicrobial) peptides play in preventing infections by microbial pathogens in many organisms, it has been proposed that these peptides might form the foundation for a new class of clinically useful antimicrobials. To date, more than 600 peptides (in virtually all species of life) have been described that not only kill pathogenic microorganisms, including Gram-positive and Gram-negative bacteria, viruses, protozoa and fungi (Table 1), but also play a central role in recruiting and promoting elements of the innate immune system [1–4]. This enormous peptide diversity is achieved through several structural classes, whereby all peptides, regardless of class, share a net positive charge and approximately 50% hydrophobic residues, which confers the ability to fold into an amphiphilic conformation upon interaction with bacterial membranes [5]. The development of peptides for clinical use is accompanied by challenges now being resolved owing to an increased understanding of how peptide structure influences mechanism of action. This review focuses primarily on the advantages and disadvantages of cationic antimicrobial peptides when compared with conventional antibiotics, and summarizes recent clinical developments with these peptides.

Advantages of peptides over conventional antibiotics

A major motivation for therapeutic peptide use is their diverse potential applications: they can be used as single antimicrobials, in combination with other antibiotics for a synergistic effect, or as immunomodulatory and/or endotoxin-neutralizing compounds [4]. In particular, the most potent agents have unusually broad spectra of activity against most Gram-negative and Gram-positive bacteria, and this spectrum can extend further to fungi and even a variety of viruses (Table 1). Although the potency of these antimicrobial peptides against the more susceptible pathogens is normally not as strong as certain conventional antibiotics, one of their major strengths is their ability to kill multi-drug-resistant bacteria at similar concentrations. Compared with conventional antibiotics, the killing of bacteria by peptides is extremely rapid and can involve multiple bacterial cellular targets [6••] (Table 1). Although interaction with the cytoplasmic membrane is obligatory and some peptides are able to perforate membranes at their minimal inhibition concentration (MIC), a number of peptides have been shown to translocate across the membrane and have an effect on cytoplasmic processes, including inhibition of macromolecular synthesis, particular enzymes or cell division, or the stimulation of autolysis. Minimal inhibitory concentrations and minimal bactericidal concentrations often coincide (less than a two-fold difference), indicating that killing is generally bactericidal, a highly desirable mode of action. Furthermore, peptides are not hindered by the resistance mechanisms that are placing currently used antibiotics in jeopardy, as excellent activity is seen, for example,

Table 1

A comparison of conventional antibiotics with cationic antimicrobial peptides.

Property	Conventional antibiotics	Cationic antimicrobial peptides
Spectrum of activity	Bacterial infections (often selective)	Bacterial, fungal and viral infections; septicaemia; and/or inflammation
Uptake	Specific mechanisms	Relatively non-specific: based on charge. Self-promoted uptake
Targets	Usually one dominating target or class of targets (e.g. penicillin-binding proteins, topoisomerases, ribosomes)	Relatively less specific (possibly multiple targets in any given cell)
Resistance rate and mechanism	Resistance development at frequencies of 10^{-7} to 10^{-10}, or after a few passages at sub-MIC. Resistance caused by mechanisms such as reduced uptake or increased efflux, chemical modification or degradation of antibiotic, or altered target	Resistance generally cannot be directly selected. Needs multiple passages on sub-MIC concentrations to induce resistance. Resistance caused by mechanisms such as an impermeable outer membrane or specific proteases (can be overcome by incorporating D-amino acids or backbone alterations)
Additional activities	No	Include anti-endotoxic and/or boosting of innate immunity
Pharmacokinetics	Varies but once per week antimicrobials under development	Short systemic half-life owing to proteolytic degradation
Toxicology	Antibiotics tend to be one of the safest groups of pharmaceuticals	No known topical toxicities; systemic toxicity issues remain undefined
Manufacturing costs	Can be inexpensive (e.g. $0.8 per gram for aminoglycosides)	Expensive ($50–400 per gram)

against methicillin-resistant *Staphylococcus aureus* and multi-drug resistant *Pseudomonas aeruginosa* [7]. Indeed, killing can occur synergistically with other peptides and conventional antibiotics, which might help overcome some of the barriers that resistant bacteria have created against currently used antibiotics.

As with any new class of antimicrobial therapeutics, a central issue is whether or not resistance can be provoked. The development of complete cationic peptide resistance has been proposed to be unlikely because of the obligatory interaction of these peptides with the bacterial cytoplasmic membrane and the consequent necessity to reconfigure this membrane, and/or the possibility that peptides have multiple targets, making elimination of any one target of lesser consequence. Indeed, although some resistance mechanisms have been described [8], these result in only a modest two- to four-fold increase in resistance. To illustrate the diversity of these peptides, there appears to be no general peptide cross-resistance mechanism whereby bacteria are resistant to every single peptide. It takes 30 passages of *P. aeruginosa* in sub-MIC peptide to increase its resistance by two- to four-fold [7] whereas, under the same conditions, resistance to the aminoglycoside gentamicin can increase by 190-fold [9]; conversely, direct selection does not generally lead to resistance (Table 1). One scenario that has been proposed is that therapeutically administered peptides might potentially promote resistance to peptides of the innate immune system, rendering ourselves more vulnerable to peptide-resistant bacterial infections [10]. This view is severe, as widespread bacterial peptide resistance is rare; despite the fact that several peptides have been used in over-the-counter products (polymyxin B, gramicidin S) and in foods (nisin), this has not impacted on the level of peptide susceptibility of organisms or on our immune systems' ability to ward off bacterial infections [11]. Indeed, there are very few naturally peptide-resistant organisms such as *Burkholderia*, *Proteus* and *Serratia* sp.

Severe bacterial infections require systemic antibacterial drug administration to quickly halt and limit the spread of infection; in such cases, endotoxaemia/sepsis is a common and dangerous complication of systemic therapy in individuals with bacteremia. One substantial advantage of peptides over conventional antibiotics is that they have the ability to neutralize sepsis/endotoxemia. In addition, some peptides have been demonstrated to have diverse roles in mammalian innate immunity [12] (Table 1). One of the most important roles described is an ability to stimulate the innate immune response while simultaneously dampening the potentially harmful inflammatory response [2]. For example, IMX00C1, a synthetic peptide with no antibacterial activity *in vitro*, has been shown to be protective in bacterial infections in animals [13].

Impediments to therapeutic peptide use

The high cost of manufacturing peptides is arguably the principal problem preventing the widespread clinical use of this class of antibacterial therapeutics (Table 1). As a result, there is a growing need for a commercial-scale peptide production platform. Novozyme Inc (http://www.novozymes.com/en) reported, using a proprietary fungal-based system [14•], recombinant production of the peptide plectasin at the scale and purity required for therapeutic administration. Plectasin is a promising new antimicrobial peptide, as it is tolerated in high doses and has demonstrated usefulness in animal models of bacterial peritonitis and pneumonia [14•]. Another approach is to use conventional or solution-phase peptide

synthesis but to decrease the size of peptides; in this regard, there has been a noticeable transition among peptide companies towards smaller, less expensive peptides rather than developing larger, more expensive natural peptides [15••,16].

Although peptides exhibit significant *in vitro* activity against bacteria, for many peptides this activity appears to be lost under physiological salt and serum conditions. For example, salt-dependent inactivation of human β-defensins in high-salt cystic fibrosis bronchopulmonary fluids abrogates the ability of these peptides to kill *P. aeruginosa* colonizing lungs of these patients, leading to deadly chronic infections [17,18], whereas another human peptide, LL-37, is strongly antagonized by physiological concentrations of mono- and di-valent cations [13]. For such peptides, the term 'host defence peptides' might be more accurate, as antimicrobial activity is probably a result of immunomodulatory effects. However, not all peptides are salt sensitive, and some peptides show potent salt-insensitive antimicrobial activities (e.g. tachyplesins and polyphemusins) [19]. It is possible to develop synthetic α-helical peptides that vary substantially in activity and salt resistance by changing peptide hydrophobicity, amphipathicity, charge and degree of α-helicity [20].

As few studies of peptide-mediated toxicity (long- or short-term) have been published, future focus must be placed on understanding the nature of any prospective toxicity problems. Mechanistically, peptide action centres on membrane interaction, so toxicity problems have not been unexpected, although selectivity can indeed be achieved through lipid charge, membrane potential and the presence of cholesterol. Single amino acid substitutions in α-helical peptides demonstrate that hemolytic activity of peptides correlates with high hydrophobicity, high amphipathicity and high helicity [21]. Conversely, antimicrobial activity is less dependent upon high hydrophobicity, high amphipathicity and high helicity [22,23]. These and other studies are aimed at the rational design of peptides with high specificity towards prokaryotic membranes but minimal toxicity towards eukaryotic membranes. Other potential toxicities that are not *per se* a result of membrane interactions have not been addressed. Despite these toxicity issues, which are now being more thoroughly elucidated, cationic lipopeptide polymyxins are being used as last resort therapeutic for multi-drug-resistant *Pseudomonas* [24].

There is a shortage of studies thoroughly examining systemic peptide pharmacodynamic and pharmacokinetic issues. Issues yet to be resolved include peptide aggregation problems, the *in vivo* half-life of peptides (and particularly their susceptibility to mammalian proteases), and the required dosing frequency. Several studies have employed mouse models of bacterial infections; for example, the efficacy of a *de novo* engineered peptide in a *P. aeruginosa* mouse intraperitoneal bacteremia model indicates that peptides can be constructed to resist systemic factors while displaying systemic antibacterial properties [25]. Likewise, intravenously administered cyclic D,L-α-peptides were shown to be both highly stable in serum and protease resistant, while retaining antibacterial action against *S. aureus* for a prolonged time period [26]. In order to widen their therapeutic window, further peptide structure–activity studies must be conducted to increase tolerability and specificity. Once the pharmacodynamics of peptides are more thoroughly understood, dosing regimes can be designed rationally to optimize disease outcomes and to minimize toxicity concerns.

Commercialization and clinical development of antimicrobial peptides

The starting point for drug development is the identification of natural antimicrobial peptides followed by their modification and optimization. Companies such as Magainin Pharmaceuticals (http://www.genaera.com), Micrologix (Migenix; http://www.migenix.com) and IntraBiotics (http://www.intrabiotics.com) designed therapeutic peptides that differed from their natural progenitor antimicrobial peptide by only a few amino acids. One of these 'first generation' antimicrobial peptides was pexiganan (MSI-78), a synthetic 22-amino-acid variant of the amphibian peptide magainin 2 (Magainin Pharmaceutical Inc, since renamed Genaera, PA, USA). Even though Phase III clinical studies proved pexiganan to be efficient in wound healing, with few reports of toxicity [27], the FDA rejected this potential new drug in 1999 as it did not offer any great advantage over the current standard of care. Similarly, IB367 — a pig protegrin analogue — failed to achieve efficacy against polymicrobic infections in oral mucasitis [28]. Xoma (Berkley, CA; http://www.xoma.com) developed an injectable formulation of the cationic bactericidal/permeability-increasing protein fragment rBPI21 (NEUPREX®), which showed only a trend towards efficacy in a Phase III trial for endotoxin-mediated complications of meningococcal disease. Recent effort has thus focused on relatively small and cost-effective molecules that contain only the biologically active core region of the natural antimicrobial peptide.

Currently, only one anti-infective with topical application has shown efficacy in Phase III clinical studies (MX-226; also known as CPI-226). MX-226 (omiganan pentahydrochloride 1% gel; Migenix), a bovine indolicidin-based peptide, was developed for the prevention of contamination of central venous catheters. In a completed Phase III study, MX-226 demonstrated a statistically significant 49% reduction of local catheter site infections, as well as a 21% reduction of catheter colonization (http://www.migenix.com/prod_226.html). Cadence (http://www.cadencepharm.com) is currently conducting a confirmatory

Phase IIIb study of MX-226 for the prevention of local catheter site infections. Other current trials involve successfully completed Phase II clinical trials against mild-to-moderate acne (indolicidin-based MX594AN; Migenix) and completed Phase I trials for the prevention of infections in patients undergoing allogeneic stem cell transplantation (human lactoferricin-based hLF1-11; AM Pharma [http://www.am-pharma.com]).

Antimicrobial peptides are currently used clinically in two topical and two systemically applied formulations for the treatment of several diseases, as well as prophylactically to prevent infections in neutropenic or cystic fibrosis patients [29]. Topical applications of polymyxins (polymyxin B and polymyxin E) and gramicidin S in the treatment of infections caused by *P. aeruginosa* and *Acinetobacter baumannii* are clinically safe and effective, with little development of resistance. Polymyxin B is a cyclic 10-amino-acid cationic antimicrobial lipopeptide that also binds and neutralizes endotoxin. Unfortunately, both polymyxins and gramicidin S are too toxic at clinically used doses to be utilized systemically as anti-bactericidal or anti-endotoxic reagents. A topical combination of polymyxin B and gramicidin S (or neomycin) are routinely used clinically, often together with bacitracin for generic wound creams, eye drops and ear drops. Even though substantial effort was put into the modification of polymyxins and gramicidin to decrease their toxicity, no 'second generation' drug has yet arisen from these studies. However, a pro-drug, colomycin, in which the amino groups of colistin (polymyxin E1, E2, E3) are neutralized by methane sulphonation, is used systemically in intravenous therapy of lung infections in cystic fibrosis patients [30]. Conversely, daptomycin is an anionic lipopeptide antibiotic with bactericidal activity against Gram-positive microorganisms. In September 2003, the FDA approved daptomycin for the treatment of complicated skin and skin-structure infections caused by susceptible strains of *S. aureus* (including methicillin-resistant strains), *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* subspecies *equisimilis* and *Enterococcus faecalis* (vancomycin-susceptible strains only) (Cubicin: package insert; Cubist Pharmaceuticals, Lexington MA; 2003). Intriguingly, it is Ca^{2+}-dependent and, in the presence of Ca^{2+}, has a similar action on membranes to the antimicrobial peptides [31]. It was shown that daptomycin is highly efficacious against experimental pneumococcal meningitis [32].

Conclusions

Several attempts have been made over recent years to advance novel broad-spectrum cationic antimicrobial peptides into clinical use, with limited success. Reasons for this failure are certainly diverse, but key unresolved issues regarding toxicity and stability are major causes of the lack of systemic application, towards which peptide therapeutic application holds the most potential. Without question, the size of peptides is an important issue for reducing manufacturing costs. Given that the average clinically utilized drug reflects the analysis of thousands of compounds and decades of scientific research in reaching the market, all of the above-mentioned hurdles for antimicrobial peptides are common to virtually every drug. Cationic antimicrobial peptides possess qualities that make them excellent candidates for antibacterial therapeutics, including a broad spectrum of antibacterial activity, ease of synthesis, and a novel mechanism of action. Low levels of peptide resistance are observed, and only after a large number of repeated passages in sub-inhibitory concentrations. At a time when resistance to commercially available antibiotics has been steadily increasing, there is an urgent need for novel, effective and safe antimicrobial therapeutics. The rapid emergence of antibiotic-resistant bacterial infections is one of the greatest challenges facing modern medicine. With the successful development of peptides and a sensible strategy for therapeutic implementation, mankind might remain one step ahead of our antibiotic-resistant bacterial adversaries.

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