Chapter 10
Antimicrobial Peptides: Do They Have a Future as Therapeutics?

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Abstract Antimicrobial peptides of higher organisms have been studied for the past 25 years and their importance as components of innate immunity is now well established. The basic simplicity of the chemical structure of antimicrobial peptides along with the lower likelihood of the emergence of resistance compared with conventional antibiotics have made them attractive candidates for development as therapeutics. In this chapter, I describe the stories behind three drug candidates currently in clinical trials: Pexiganan, Plectasin, and Brilacidin. Each of these compounds has faced specific challenges in development and has a high likelihood of reaching commercialization. Antimicrobial peptides appear to be coming of age as therapeutics.

10.1 Introduction

I have been asked to address the question as to whether antimicrobial peptides have a future as therapeutics. Antimicrobial peptides of multicellular organisms were characterized in the 1980s from insects, mammals, and frogs (Zasloff 2002). As yet, antimicrobial agents of this class have not yet been approved as drugs. It is reasonable to ask what the problems have been that have interfered with their commercialization. In this piece I will focus on three examples of antimicrobial peptides that have advanced deeply into development but faced specific challenges that interrupted their progress: Pexiganan developed by Magainin Pharmaceuticals; plectasin, developed by Novozymes; and brilacidin, developed by Polymedix. Although there are other compounds that could be included, I have selected these because I am personally most familiar with these three, and I believe the lessons learned apply broadly to all compounds of this class destined for therapeutic development.

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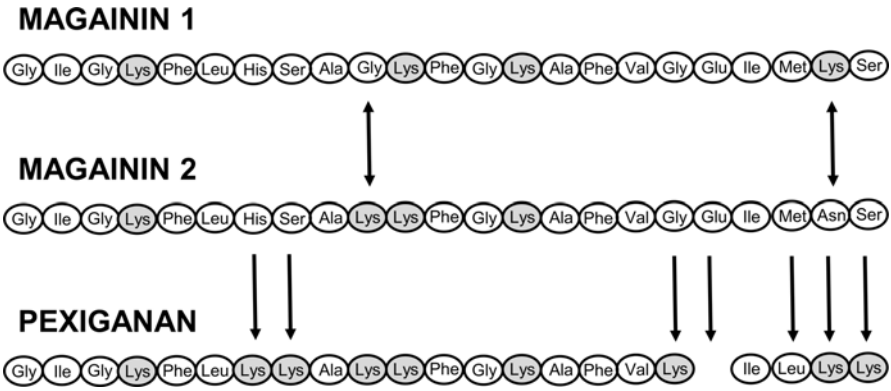


Fig. 10.1 The amino acid sequences of pexiganan, magainin 1 and magainin 2, are compared. Each peptide has an amide at the carboxyl terminus. Lysine residues are highlighted in *gray*

10.2 Pexiganan

Pexiganan was developed as a topical anti-infective. It is a 22 amino acid analogue of magainin 2, the natural peptide produced by Xenopus laevis (Zasloff 1987; Ge et al. 1999a, b). Pexiganan differs from magainin 2 by the substitution of 5 lysines and the deletion of a glutamic acid (Fig. 10.1), which dramatically extends the antimicrobial spectrum of the peptide while only minimally increasing its lytic activity against mammalian cells. These substitutions were conceived using the principle that linear antimicrobial peptides capable of forming alpha helical structures in membranes would likely exhibit greater antimicrobial potency by increasing the density of positively charged amino acids on the hydrophilic face of the alpha helix.

The decision to develop this peptide as a locally applied therapeutic was based on the results of extensive *in vivo* assessments of the anti-infective properties of magainin 2 in systemic infections. It was evident that the therapeutic index of this peptide was too narrow to be advanced as a systemically administered drug. Pexiganan, although more active *in vitro* than magainin 2, did not exhibit a larger therapeutic window. Initially we chose to develop pexiganan against the superficial infection, impetigo, but subsequently focused on its use for the treatment of infected diabetic foot ulcers.

Infected diabetic ulcers remain a major medical problem. About 50,000 amputations are performed annually on the lower limbs of diabetics due to the progressive and irreversible destruction of soft tissue and bone that often follows the appearance of a minor infection on the foot. Although the etiology of these infections is not fully understood, patients are advised to attend to even the most minor signs of injury on the foot, including debridement of open wounds and the administration of broad-spectrum antibiotics if any indication of infection is evident (swelling, redness, purulent discharge). The infections are complex polymicrobial, and no specific microorganism is implicated in the destructive outcome (Ge et al. 2002). The antibiotics must be administered until control of the infection is clinically apparent, generally about 1 month of treatment. Unfortunately, many individuals find these broad-spectrum antibiotics

intolerable, due to side effects such a diarrhea, and frequently stop taking them despite being fully aware of the dire consequences of progressive infection.

Pexiganan was developed as a locally applied alternative to a systemically administered broad-spectrum antibiotic. The antibiotic spectrum was broad enough to cover the range of microbes known to be present on the diabetic ulcer. Furthermore, as a topical agent, high local concentrations in the superficial soft tissues could be achieved easily, surpassing the concentrations that could be reached systemically. We chose as our intended diabetic patients those with the most superficial of infected ulcers, excluding those cases where deep penetration of a topically applied ointment could not occur, including those with soft tissue infections in close proximity to bone. We argued that high concentrations of pexiganan would also reduce the likelihood of selection of resistant organisms and thus could be used repeatedly over the course of a patient's lifetime.

The choice of developing pexiganan as a topical agent was also influenced by several practical and strategic considerations. The primary structure of the peptide makes it susceptible to proteolytic cleavage, especially by trypsin-like enzymes. As a consequence, the intact peptide does not pass from the soft tissue into the systemic circulation. This property permitted us to drastically reduce very costly preclinical toxicology prior to clinical evaluation, since chronic systemic exposure to an intact peptide would not occur following long-term local application to an open wound. In addition, a therapeutic course of local administration would require relatively small amounts of peptide compared with systemic administration. Based on the minimal inhibitory concentrations of pexiganan, one could estimate that intravenous dosing would require about 1 g daily, not very different from the dosing of most antibiotics currently in use. At the time we began the development process, the chemical synthesis of pexiganan cost about $1000 per gram, using our proprietary solution-phase process. Only if this peptide were dramatically more effective than the less expensive agents in use would it make sense to develop it for systemic use, and we did not believe that this was the case. As a topical agent, however, much lesser amounts of peptide could be applied directly to a site of infection, and the cost of goods relative to the anticipated price of the commercial product made sense. Eventually, we were able to bring the cost of production of pharmaceutical grade pexiganan down to about $100 per gram.

We conducted two extensive pivotal phase III clinical trials in the late 1990s involving about 1000 subjects (Lipsky et al. 2008). Topically applied pexiganan was evaluated against orally administered ofloxacin in what is a called an "inferiority" study. The hypothesis being tested was whether topically applied pexiganan exhibited was less effective than orally administered ofloxacin, an antibiotic approved for deep soft tissue infections (no specific agent had been approved for diabetic foot infections, so the FDA permitted us to use this antibiotic as the comparator). In both treatment arms, the wounds were debrided of dead tissue as part of the study. Ideally, it would have been scientifically more exciting to have conducted a "superiority" trial, where pexiganan treatment was compared to simple wound debridement (a placebo-controlled trial), but all parties, including the FDA and participating physicians and patients, felt this to be unethical.

Pexiganan proved itself to be as effective as oral ofloxacin in the treatment of infected diabetic foot ulcers over about a month of treatment. Of particular interest

was that pexiganan-resistant bacteria did not appear on the treated wounds. In contrast, the microbes cultured from the wounds of those subjects treated with ofloxacin shifted to a more resistant concentration range following the month of therapy. The rates and extent of wound healing were comparable as were the impact on the clinical appearance of the treated ulcers. As anticipated, those receiving topical treatment reported fewer side effects than those taking the oral therapeutics.

Despite the positive outcome, the FDA Advisory Panel voted 7–5 against approval of pexiganan. Their primary concern was the uncertainty over the "placebo" outcome. Suppose these individuals had not been treated with any antibiotic, just surgical curettage, what would the outcome have been? Despite being presented with the historical data then available, the panel was not convinced. Indeed, Dr. Julie Parsonnet of Stanford University suggested to the panel that all a doctor had to do to treat an infected ulcer was "to cut out the infected tissue and drop it into a trash can and forget the antibiotics!" (She herself had never treated such a patient, nor had most members of that FDA panel). Several panelists could not believe that it was possible for a topical agent to be as effective as we had claimed. In response to the Advisory Panel, the FDA requested that we repeat a trial using a placebo arm. We did not believe this to be practical, and the pexiganan program was halted.

Over the past few years, pexiganan's development as a topical agent for the treatment of infected diabetic foot ulcers has been taken up by Dipexium Pharmaceuticals, Inc. Working closely with the FDA and many of the American experts in the treatment of infected diabetic foot ulcers, the company has designed a pivotal phase III placebo-controlled trial which should both fulfill the requirements of a superiority study and also satisfy any concerns regarding the placing of untreated patients at risk. Within the next few years, we will learn of this study's outcome.

10.3 Plectasin

The discovery and characterization of plectasin was first reported in 2005 (Mygind et al. 2005) (Fig. 10.2). A team of scientists at Novozymes, headed by Dr. Hans Henrik Kristensen, had exploited a novel method for the discovery of antimicrobial peptides from natural sources. The team had created a cDNA library from a wild mushroom like fungus (Pseudoplectania nigrella). By reengineering the library, the team searched for the expressed gene products that would be secreted (i.e., that contained "signal" sequences). Of the cDNAs identified, one exhibited the primary sequence of a defensin antimicrobial peptide. Using several fungal- and yeast-based expression systems, the Novozymes team biosynthetically produced the peptide in sufficient amounts to begin characterization. The defensin, named "plectasin," was found to have a primary, secondary, and tertiary structure very similar (surprisingly!) to defensins from insects and mussels. Of particular interest was its antibacterial spectrum. Of the bacteria studied, plectasin exhibited its greatest potency against Streptococcus pneumonia (Mygind et al. 2005). *In vivo*, plectasin demonstrated potency when administered systemically that equaled or exceeded conventional antibiotics such as penicillin. In addition, the antibiotic, following parenteral

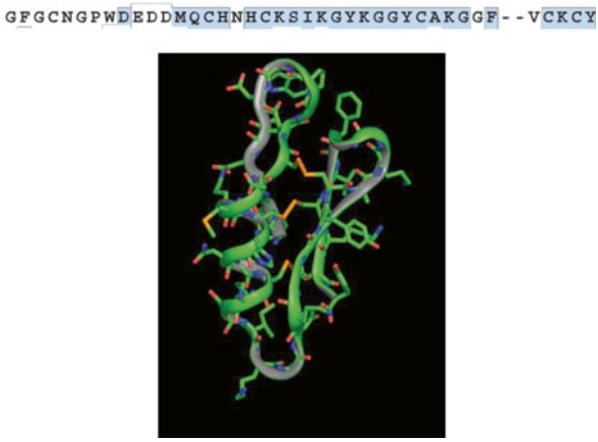


Fig. 10.2 A representative tertiary structure of plectasin as determined by solution NMR presented beneath the primary structure (Mygind et al. 2005)

administration to the mouse, could be recovered intact from its urine. Plectasin was essentially nontoxic in early preclinical experiments. Its mechanism of action was established and shown to involve interaction with lipid II (Schneider et al. 2010). Almost all of the clinical isolates of Pneumococcus studied exhibited a minimal inhibitory concentration at sub-micromolar values, suggesting that most infections would be effectively treated initially with this antibiotic. Furthermore, because plectasin was of fungal origin, it could be expressed robustly in several recombinant vectors used for the industrial production of commodity substances, such as enzymes used in the food industry. Hence, it could potentially be produced inexpensively and in large amounts.

From one perspective plectasin was an ideal antibiotic. Here was a narrow-spectrum antibiotic that a physician could call upon to treat an infection caused by Pneumococcus. In a setting where a specific bacterial organism is involved, a specific antibiotic should be used. Since many strains of Pneumococcus have developed resistance to beta-lactams, and plectasin retained activity against penicillin-resistant Pneumococcus, plectasin could become the antibiotic of choice for the treatment of pneumococcal infections.

Unfortunately, the economics of drug development intervened. The market for a pneumococcal-specific antibiotic was too small to justify the expensive investment required to bring the antibiotic through development into clinical use. The Novozymes team began a long and highly creative series of structure-activity studies to extend the spectrum of plectasin so as to include human pathogens such as *Staphylococcus aureus*. The Kristensen team succeeded creating the plectasin derivative NZ2114 which was effective against systemic S. aureus infections in several animal models (Andes et al. 2009; Xiong et al. 2011). Similar to plectasin, NZ2114 could be produced at commercial viable expression levels through recombinant expression in a yeast system (Zhang et al. 2014).

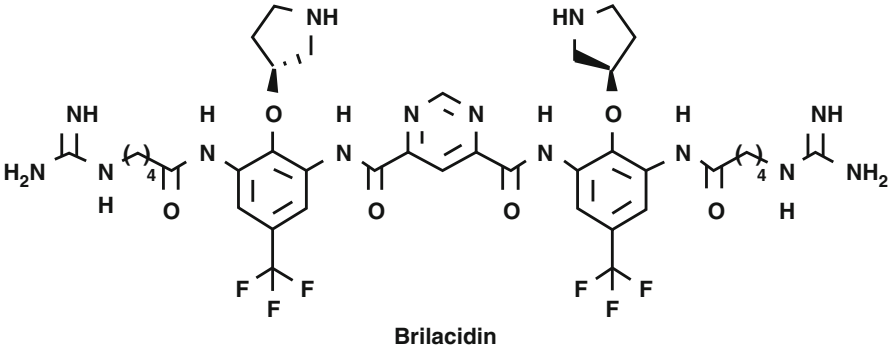
In late 2009 Sanofi-Aventis entered a partnership with Novozymes to lead the clinical development of NZ2114 and in early 2010 announced that planning for a phase 1 clinical program had begun. Very little public information has been disclosed since then.

10.4 Brilacidin

Linear amphipathic alpha helical cationic antimicrobial peptides target the external membranes of microbes. In some cases they damage the permeability of the membrane; in others they translocate into to the cytoplasm and cause functional disturbance. Molecules that adopt comparable secondary structures can exhibit antimicrobial activity, and many synthetic mimetics of the naturally occurring linear peptides have been synthesized. In 2002 DeGrado reported the synthesis and characterization of a novel family of arylamide amphipathic anionic polymers that exhibited many of the physical and biological properties of antimicrobial peptides, such as magainin (Tew et al. 2002). These mimetics could be synthesized from inexpensive monomers via classical polymerization methods and were resistant to proteolytic hydrolysis. Through structure-activity refinements, potency against Gram-positive bacteria was optimized while maintaining minimal lytic activity against human erythrocytes, resulting in the creation of brilacidin (Fig. 10.3) (Choi et al. 2009). This molecule adopts a planar secondary structure, with guanidinyl and pyridinyl groups positioned on one edge of the scaffold and trifluoromethane groups on another. As reported recently, brilacidin acts by selectively damaging the microbial membrane, similar to linear antimicrobial peptides and the lipopeptide in commercial use, daptomycin (Mensa et al. 2014).

Brilacidin was initially licensed to Polymedix, Inc., and subsequently to Cellceutix, Inc. It has been advanced in development as a parenteral drug for the treatment of acute skin and skin structure infections caused by Gram-positive bacteria, positioned to compete with drugs such as daptomycin.

Brilacidin advanced successfully through phase 1 and phase 2 clinical trials. On October 23, 2014, Cellceutix reported unpublished results of a phase 2b randomized double-blind study comparing brilacidin to daptomycin for the treatment of Gram-positive acute skin and skin structure infections (http://cellceutix.com/cellceutix-releases-confidence-interval-statistics-showing-clinical-success-rates-for-brilacidin-in-treatment-of-absssi/#sthash.UpDWgko4.dpbs). Two-hundred and fifteen subjects were enrolled. The primary end point was clinical success defined as reduction of at least 20 % in area of the infected site when evaluated 48–72 h after the first dose of the



Brilacidin

Fig. 10.3 The chemical structure of brilacidin is shown

study drug. As reported by Cellceutix: "In treated patients assessed at 48–72 h, 47/51 (92.2 %), 46/48 (95.8 %), 51/52 (98.1 %), and 45/48 (93.8 %) achieved clinical success in the brilacidin 0.6 mg/kg single-dose group, brilacidin 0.8 mg/kg single-dose group, brilacidin 1.2 mg/kg 3-day group, and daptomycin 7-day group, respectively. All three brilacidin treatment arms (two single-dose regimens and one 3-day dose regimen) reached the primary endpoint, with the clinical success rate for each dosing regimen statistically comparable to the clinical success rate of the FDA-approved 7-day dosing regimen of daptomycin. All brilacidin treatment regimens were well tolerated. There were six severe adverse events (SAE) reported across the study, none of which were considered related to brilacidin by the principal investigator." Brilacidin will be advanced into a phase 3 clinical trial.

10.5 Conclusions

The three compounds that I have discussed are advancing through the therapeutic development process. Unless they face clinical failure, due either to a lack of efficacy or an unacceptable toxicity, they will represent the first "graduating class" of antimicrobial peptides.

Each of the three highlights several attractive characteristics of antimicrobial peptides:

1. Antimicrobial resistance does not readily occur.
2. The mechanism of action can be reproduced with molecules as structurally distant from a linear peptide as brilacidin.
3. Certain naturally occurring peptides from fungal sources with very narrow antibiotic spectra, such as plectasin, could be developed for the treatment of specific bacterial infections. Many uncharacterized fungal defensins have already been identified (Zhu 2008).
4. Naturally occurring antimicrobial peptides, such as plectasin, exhibit acceptable therapeutic indices and can serve as the models upon which the structural principles that govern bio-distribution of therapeutically effective antimicrobial peptides can be deciphered.
5. Antimicrobial peptides and mimetics can be commercially synthesized cost-effectively.
6. As yet, no class specific toxicity has become apparent.

Although it seems like an eternity that we have been awaiting the introduction of antimicrobial peptides into the clinic, we are now on the threshold of seeing this happen. We are now facing a medical crisis some have called the "End of the Antibiotic Era." Resistance of both Gram-positive and Gram-negative human pathogens to our conventional antibiotics has spread throughout the world. Infections that could be treated routinely several years ago now present as life-threatening medical challenges. The introduction of antimicrobial peptides as therapeutic drugs could not be happening at a more critical moment in time than now.

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