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## A Review of Antimicrobial Peptides and Their Therapeutic Potential as Anti-Infective Drugs

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### Abstract

**Purpose.** Antimicrobial peptides (AMPs) are an essential part of innate immunity that evolved in most living organisms over 2.6 billion years to combat microbial challenge. These small cationic peptides are multifunctional as effectors of innate immunity on skin and mucosal surfaces and have demonstrated direct antimicrobial activity against various bacteria, viruses, fungi, and parasites. This review summarizes their progress to date as commercial antimicrobial drugs for topical and systemic indications.

**Methods.** Literature review.

**Results.** Despite numerous clinical trials, no modified AMP has obtained Food & Drug Administration approval yet for any topical or systemic medical indications.

**Conclusions.** While AMPs are recognized as essential components of natural host innate immunity against microbial challenge, their usefulness as a new class of antimicrobial drugs still remains to be proven.

### Keywords

antibiotics; antimicrobial peptides; cathelicidin; cationic peptides; defensins

### INNATE IMMUNITY AND ANTIMICROBIAL PEPTIDES (AMPs)

Before the evolution of adaptive immunity in higher vertebrates added complexity, specificity, and memory to fight microbial challenge, a simpler, non-specific ancient system of innate immunity evolved 2.6 billion years ago and continues to function as the principal defense for almost all living organisms.<sup>1</sup> Innate immunity is necessarily rapid, clonal, redundant, and multifunctional.<sup>2–4</sup> The antimicrobial function of innate immunity is mediated, in part, by small cationic peptides with potent antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, parasites, and some viruses.<sup>5–7</sup> The principal mechanism of rapid killing of microbial pathogens is attributed to perturbation of the microbial cell membrane,<sup>8,9</sup> but our understanding is incomplete and other mechanisms may also be operative.<sup>10–14</sup> Human antimicrobial peptides (AMPs) such as defensins and cathelicidin (LL-37) are present in leukocytes and are also secreted by various epithelia in skin and mucosal surfaces including the ocular surface.<sup>2,15–20</sup> In addition to their antimicrobial role, AMPs also serve as important effector molecules in inflammation, immune activation, and wound healing.<sup>21–24</sup>

## DRUG DEVELOPMENT OF ANTIMICROBIAL PEPTIDES

The driving force for the development of newer anti-infectives is almost always the inevitable emergence of bacterial resistance to antibiotics following widespread clinical, veterinary, and animal agriculture (growth promoter in chickens, pigs, and feedlot cattle) usage.<sup>25,26</sup> The pharmaceutical industry has continuously met this need by modifying existing antibiotics and developing newer antibiotics in a timely fashion. These successful efforts have produced the wide variety of currently available drug classes of antibiotics [beta lactams (penicillins, carbapenems, cephalosporins), glycopeptides, macrolides, ketolides, aminoglycosides, fluoroquinolones, oxazolidinones, and others]. Similarly, there have been dramatic successes in developing effective antivirals to kill important clinical viral pathogens (e.g., HIV, herpesviruses, and influenza). However, the rapid emergence of resistance is even a greater problem for life-threatening viral infections. The best example remains HIV, where the rapid emergence of resistance to single drugs posed daunting clinical problems. The only effective solution to this problem was to develop combination therapy involving several antivirals with different mechanisms of inhibitory action. Currently, there are 19 different approved drugs for anti-HIV therapy in use as components of combination therapy. They include (1) nucleoside reverse transcriptase inhibitors, (2) nucleotide reverse transcriptase inhibitors, (3) non-nucleoside reverse transcriptase inhibitors, (4) proteases, and (5) viral entry blockers inhibitors.<sup>27</sup>

Despite the success to date in antimicrobial development, the inexorable, ongoing emergence of resistance worldwide continues to spur the search for novel anti-infectives to replace and/or supplement conventional antibiotics. Human defensins, cathelicidin, and a significant number of diverse AMPs from bacteria, viruses, plants, vertebrates, and invertebrates all appear to have a universal multidimensional signature (i.e., a common three-dimensional structure) that defines antimicrobial activity.<sup>28</sup> Manipulation of this chemical structure to create designer synthetic peptides represents a promising strategy for the development of AMPs as a new class of drugs to prevent and treat systemic and topical infections.

For antimicrobial peptides, there are several different potential strategies for their general therapeutic application: (1) as single anti-infective agents, (2) in combination with conventional antibiotics or antivirals to promote any additive or synergistic effects, (3) as immunostimulatory agents that enhance natural innate immunity, and (4) as endotoxin-neutralizing agents to prevent the potentially fatal complications associated with bacterial virulence factors that cause septic shock.

To date, the use of AMPs as single therapeutic antibiotic agents has received the most attention. However, the use of endotoxin-neutralizing agents as adjuncts to conventional antibiotic therapy has also been explored. As broad spectrum antimicrobials, AMPs may also have a role in treating important viral infections.<sup>14,29-31</sup> Table 1 summarizes the theoretical advantages and disadvantages of developing antimicrobial peptides as single therapeutic antimicrobial agents.<sup>21,32,33</sup> Despite many attractive attributes, the challenges of bringing a successful AMP to market remain formidable. There have been no published reports of commercial success in developing AMPs as topical ophthalmic agents (or any kind of therapeutic agent), and we will explore in this review the unique challenges and issues that the eye presents as a site of action for these novel anti-infective agents.

## HISTORY AND CURRENT STATUS OF CLINICAL DEVELOPMENT

Of the potential pool of thousands of natural peptides and millions of synthetic peptide possibilities, relatively few have actually proceeded into clinical trials based on promising data from *in vitro* and animal studies. Despite a continuing optimism,<sup>34</sup> a critical review of the

literature to date suggests that commercial success remains elusive. In 2004, a list of antimicrobial peptides in clinical trials was published,<sup>34</sup> and to date, none of the peptides described has obtained FDA approval for their various clinical indications. A more detailed review of some of these peptides is instructive of the challenges and difficulties encountered in the development of a new class of therapeutic drugs (Table 2).

## TOPICAL SKIN AND LOCAL APPLICATIONS (NONOCULAR)

### Pexiganan (MSI-78)

Pexiganan (MSI-78) (Magainin Pharmaceutical Inc, since renamed Genaera, Plymouth Meeting, PA, USA) was the first antimicrobial peptide to undergo commercial development. In 1987, Zasloff discovered that a cationic peptide in the skin of the African clawed frog *Xenopus laevis* had broad-spectrum antibacterial activity based on a “pore-formation” mechanism.<sup>6,35</sup> He called it magainin. Pexiganan, a synthetic 22-amino-acid analogue of magainin 2, demonstrated excellent *in vitro* broad-spectrum activity against 3109 bacterial clinical isolates. Resistant mutants could not be generated following repeated passage with subinhibitory concentrations.<sup>36</sup> In two Phase III clinical trials involving 835 patients with infected diabetic foot ulcers, both topical pexiganan acetate 1% and oral ofloxacin 800 mg/day achieved clinical cure or improvement in 90% of patients. Eradication of pathogens was achieved in 82% of the ofloxacin recipients compared to 66% of pexiganan recipients at the end of therapy.<sup>37</sup> In 1999, FDA approval was denied because pexiganan was deemed to be no more effective than other antibiotics used to treat foot ulcers.<sup>38</sup> Following this somewhat controversial decision, Genaera ([www.genaera.com](http://www.genaera.com)) discontinued its development.

### Iseganan (IB-367)

Iseganan (IB-367) (Intrabiotics Pharmaceuticals, Inc, Mountainview, CA, USA; [www.intrabiotics.com](http://www.intrabiotics.com)) is a synthetic protegrin 1 derived from the naturally occurring protegrins in pig leucocytes.<sup>39</sup> As a cationic antimicrobial peptide, it has broad-spectrum *in vitro* antibacterial and antifungal inhibitory activity. A Phase IIa trial will explore the safety and efficacy of aerosolized iseganan HCl for decreasing the bacterial burden in the lungs and improving pulmonary function in cystic fibrosis patients with chronic respiratory infections. Iseganan was initially developed as a local mouthwash to prevent high-risk patients from developing ulcerative oral mucositis. In 502 patients receiving stomatotoxic chemotherapy in a Phase III clinical trial, treatment with oral iseganan mouthrinse (9 mg/dose) six times a day failed to prevent or reduce stomatitis, ulcerative oral mucositis, or its clinical sequelae relative to a placebo.<sup>40</sup> In another Phase III clinical trial involving 545 patients receiving radiotherapy for head-and-neck malignancy, iseganan combined with standard-of-care oral hygiene (SOC) demonstrated no benefit in reducing the severity of ulcerative oral mucositis compared to placebo + SOC, and SOC alone.<sup>41</sup> Based on these results, Intrabiotics abandoned development for this particular indication.<sup>42</sup> More recently, the data monitoring committee of a Phase III clinical trial stopped the use of aerosolized iseganan for the prevention of ventilator-associated pneumonia because of a higher rate of pneumonia and mortality in the iseganan treatment group compared to the control group. ([www.intrabiotics.com](http://www.intrabiotics.com), Press Release June 23, 2004)

### Omiganan (MBI-226)

Omiganan (MBI-226) (Microbiologix Biotech, Vancouver, BC, Canada) is a synthetic analogue of indolicidin, a cationic peptide originally purified from cytoplasmic granules of bovine neutrophils, that has broad antibacterial and antifungal activity *in vitro*.<sup>43</sup> In a Phase III clinical trial involving more than 1400 patients in 29 U.S. centers, topical treatment with omiganan failed to demonstrate a statistically significant reduction in venous catheter-related bloodstream infections, the primary efficacy end-point, compared to povidone iodine as a control ([www.drugs.com/nda\\_mbi-226.html](http://www.drugs.com/nda_mbi-226.html), Press Release January 21, 2004). Nevertheless,

in the same study, iseganan did achieve two statistically significant secondary efficacy endpoints: (1) a reduction in catheter colonization ( $p = 0.002$ ) and (2) a reduction in catheter-related local infections ( $p = 0.004$ ). In consultation with the FDA, the company is considering options of how to proceed.

### MBI 594AN

MBI 594AN (Microbiologix Biotech, Vancouver, BC, Canada) is another cathelicidin- based, indolicidin-like novel, topical antibiotic under development for the treatment of acne. The most important bacterium associated with acne is *Propionibacterium acnes* (*P. acnes*) for which antibiotic resistance has increased dramatically. The majority of isolates have a constitutive resistance to erythromycin, clindamycin, and to all other macrolide-lincosamide-streptogramin type B (MLS) antibacterials.<sup>44</sup> Preclinical studies demonstrated excellent *in vitro* efficacy against sensitive and resistant *P. acnes* strains, and topical MBI 594AN was found to be nontoxic and nonirritating in animal models. A Phase IIb trial (255 patients at 9 centers) of topical 2.5% MBI 594AN administered twice a day demonstrated a statistically significant reduction in inflammatory ( $p < 0.004$ ), noninflammatory ( $p < 0.037$ ), and total lesions ( $p < 0.001$ ) at 6 weeks compared to a vehicle control. (www.mbiotix.com, Press Release November 17, 2003).

### Histatin Variants

P113 (developed by Periodontix, Watertown, MA, USA, then acquired by Demegen, Pittsburgh, PA, USA; *Pittsburgh Business Times* July 16, 2001) is a 12-amino-acid cationic peptide based on histatins, naturally occurring antimicrobial peptides in the saliva.<sup>45</sup> P113 demonstrated excellent *in vitro* activity against *Candida albicans* and common Gram-positive and Gram-negative pathogens. The target indication for P113 is for treatment as a mouthrinse for oral candidiasis in HIV patients (Phase I/II clinical study completed), and P113D is to be used as an inhalation treatment for *Pseudomonas aeruginosa* lung infections in cystic fibrosis patients. Another peptide, D2A21, has shown promise as a topical antibiotic against *Pseudomonas aeruginosa* in rat models of skin infection<sup>46</sup> and burn wounds.<sup>47</sup>

### XMP.629

XMP.629 [Xoma (US) LLC, Berkeley, CA, USA] is a 9-amino-acid peptide derived from bactericidal/permeability-increasing protein (BPI) that was created by reverse synthesis of the native 9mer peptide using 9 amino acids and substitution of two residues with naphthyl-ala. XMP.629 has demonstrated potent antimicrobial activity against *P. acnes* with minimal bactericidal concentrations (MBC) ranging from 0.5 to 4.0  $\mu\text{g/ml}$ . A Phase I clinical trial established the safety of topical dermal therapy, but a Phase II clinical trial in 253 patients failed to demonstrate a benefit in the topical therapy of acne. (www.xoma.com, Press Release August 16, 2004).

## SYSTEMIC (PARENTERAL) APPLICATION

### Neuprex (rBPI21)

Neuprex (rBPI<sub>21</sub>) [Xoma (US) LLC, Berkeley, CA, USA] bactericidal permeability-increasing protein (BPI) and N-terminal fragments of BPI (rBPI<sub>21</sub>) bind and neutralize endotoxin and are potently bactericidal against *Neisseria meningitidis*. A Phase III clinical trial, carried out at 22 sites in the U.K. and the U.S.A., enrolled 393 children with severe meningococemia. In addition to conventional antibiotic therapy, adjunctive parenteral therapy with rBPI<sub>21</sub> (2 mg/kg over 30 min followed by 2 mg/kg over 24 hr) failed to significantly reduce mortality [(7.4% versus 9.9% (odds ratio 1.31 [95% CI 0.62–2.74,  $p = 0.48$ ]) compared to the placebo control (0.2 mg/ml human albumin solution).<sup>48</sup> An open-label, single-center, dose-escalation,

investigator-sponsored Phase I/II clinical trial of Nuprex (rBPI<sub>21</sub>) to reduce inflammatory complications associated with pediatric open heart surgery patients is planned. (www.xoma.com, Press Release October 2, 2003).

## ANTIMICROBIAL PEPTIDES IN PRECLINICAL DEVELOPMENT

Several peptides have shown promise for possible drug development in preclinical studies. Helix Biomedix Inc, (Bothell, WA, USA) is developing a broad-spectrum microbicide to be used against bacteria (*Garnerella*, *Prevotella*, *Peptostreptococcus*, and *Bacteroids*), fungi (*Candida* sp.), and viruses (HSV-2, HSV-1) that cause sexually transmitted diseases (STDs).<sup>31</sup> Plectasin, (Novozymes, Bagsvaerd, Denmark) is a recently discovered fungal defensin from *Pseudoplectania nigrella* that is effective against Gram-positive bacteria *in vitro* and *Streptococcus pneumoniae* in mouse peritonitis and pneumonia models.<sup>49</sup> Finally, Inimex Pharmaceuticals (Vancouver, BC, Canada) is developing immunoenhancement AMPs with no intrinsic antimicrobial inhibitory activity but that act by selectively upregulating innate immunity without overstimulation of proinflammatory mediators. Preliminary data demonstrated that combination therapy of these immunostimulating peptides with subtherapeutic doses of conventional antibiotics significantly lowered bacterial load *in vivo* in a murine *S. aureus* infection model.<sup>50</sup> Additional studies are ongoing to explore this new approach to treat topical and systemic infections.<sup>51</sup>

## DEVELOPMENT OF AMPs FOR TOPICAL OPHTHALMIC USE

### Issues and Challenges

As previously stated, there are no ongoing trials at the present time to evaluate antimicrobial peptides for topical ocular infections. Furthermore, there are no published preclinical studies suggesting promising candidates. Because interest in commercial development of AMPs has been ongoing since the 1987 discovery of magainins, why has there been no apparent progress over the past 17 years in the ophthalmic field? Ophthalmic pharmaceutical companies have expressed intermittent interest in this potential new class of drugs and have supported contract research for exploratory *in vitro* and animal studies. However, a major disincentive has been the continuous pipeline of newer, highly successful, conventional antibiotics to replace older antibiotics with diminished efficacy due to increasing bacterial resistance. The recent introduction of topical gatifloxacin (Zymar, Allergan Inc., Irvine, CA, USA) and moxifloxacin (Vigamox, Alcon Laboratories Inc., Ft. Worth, TX, USA) for prophylaxis and treatment of ocular infections has again provided for another smooth transition away from the older drugs, ciprofloxacin (Ciloxan, Alcon Laboratories Inc.), and ofloxacin (Ocuflox, Allergan Inc.), to newer ones.<sup>52–54</sup> Specifically, the emergence of increasing resistance among important ocular pathogens (e.g., *Staphylococcus aureus*, *Pseudomonas aeruginosa*)<sup>55–60</sup> taken together with the expiration of patent protection on the older fluoroquinolones have led ophthalmic pharmaceutical companies to place marketing emphasis on the newer, more effective fluoroquinolones.

The potential advantages and disadvantages of antimicrobial peptides as single therapeutic agents for general use have already been summarized in Table 1. Although AMPs have many attractive qualities, the challenges for successful development for nonocular applications are considerable. The development of AMPs as topical agents for ophthalmic infections has several attractive features: (1) direct delivery to the infected site, (2) achievement of high local tissue levels through aggressive dosing, (3) possible combination therapy with conventional antibiotics<sup>61–63</sup> to achieve a synergistic or additive antibacterial effect at nontoxic AMP concentrations. Furthermore, a broad-spectrum antimicrobial effective against both bacterial and viral ocular pathogens (HSV-1, adenoviruses<sup>14,29</sup>) may allow for better empirical treatment of superficial ocular infections by generalists.

However, there are also potential drawbacks to putting AMPs in the eye. A candidate AMP that is non-toxic to skin (an epithelial surface protected by keratin) may turn out to be much more toxic to the epithelia of a delicate mucosal surface (e.g., conjunctiva) and the richly innervated, exquisitely sensitive corneal epithelium. The differences in context between the natural function of AMPs as part of innate immunity versus their repeated application in higher concentrations as topical ophthalmic drops or ointments raise a number of important theoretical issues regarding toxicity. As part of innate immunity, toxicity is controlled *in vivo* by several important mechanisms: (1) protected storage in granules (leucocyte defensins, cathelicidin), (2) epithelial synthesis as nontoxic, inactive propeptides requiring enzymatic activation for biological activity (cathelicidin), (3) minimal concentration because of the additive and synergistic effects achieved in combination with other effector molecules with overlapping antimicrobial functions, and (4) programmed, on site deactivating mechanisms to limit damage.<sup>61</sup> Topical AMPs as drugs can have their potential toxicity reduced by manipulating concentration, dose frequency, route of application, formulation, and possibly the concurrent administration of additional protective drugs. At worst, failure to successfully address the issues of ocular toxicity will inevitably lead to failure in clinical trials and denial of FDA approval. At best, limited but unavoidable toxicity can lead to poor patient compliance, reduced clinical efficacy and smaller profits if there are less toxic alternatives.

In addition to the major issue of ocular toxicity, the antimicrobial activity of AMPs may be reduced following interaction with tear film components.<sup>64,65</sup> Furthermore, efficacy of AMPs on the ocular surface may be adversely affected by increased proteolysis, serum protein binding, ionic and pH changes, immune clearance from prior sensitization, and decrease residence time due to increased lacrimation, faster lacrimal drainage, and poor tissue penetration. The multieffector functions of AMPs may also prove confounding.<sup>22,32</sup> For example, AMPs can have either or both pro- and anti-inflammatory functions.<sup>21</sup> If the antimicrobial efficacy of a specific peptide is also associated with proinflammatory effects, enhanced neutrophil recruitment may lead to a greater likelihood of corneal melting and perforation. If the antimicrobial efficacy of a specific peptide is also associated with enhanced cell migration and proliferation, fibroblasts may promote corneal scarring, and angiogenesis promoters may facilitate corneal neovascularization. The outcome of treating a corneal infection with an antimicrobial peptide could well be the successful killing of the invading pathogen, but the price paid may be a scarred, vascularized cornea. Not only will the patient have decreased vision if there is significant central scarring, but he will also have a poorer prognosis for successful vision restoration by corneal transplantation due to the presence of preexisting corneal vessels. Therefore, any peptide designed for topical ocular use must be carefully engineered to optimize its antimicrobial function and to reduce any other confounding biological functions.

Finally, before a pharmaceutical company will invest millions to develop a topical AMP ophthalmic product, the peptide must offer more than the promise of efficacy and safety in clinical trials as required for FDA approval. The AMP product must also be economical to manufacture by chemical or biological means, inexpensive to package, have an appropriate shelf life based on its chemical stability and formulation, and offer the likelihood of significant profits given the anticipated market and the available competing products. Taken together, these issues and challenges make it clear that the reason we do not yet have an ophthalmic antimicrobial peptide for the treatment of ocular infections is that these issues have not all been satisfactorily resolved for any peptide candidate, and the perceived need has not made it a program priority for any pharmaceutical company.

## HISTORY OF *IN VITRO* AND ANIMAL STUDIES

Next, we will review the sparse published literature and the experience of The Charles T. Campbell Laboratory and other leading investigators in the field to determine what progress has been made over the past 17 years toward the development of an AMP as a commercial ophthalmic anti-infective agent. The seminal work on the application of antimicrobial peptides to ophthalmology was begun in 1988 by Mark Mannis and colleagues at the University of California, Davis, and is summarized in his AOS thesis, which is highly recommended as an excellent introduction to the subject.<sup>61</sup> Between 1989 and 2002, Mannis, Schwab, and others presented numerous studies at ARVO and published data on several different AMPs (rabbit defensin NP-1, various cecropins, and synthetic peptides created by computational drug design software) that they had tested *in vitro* and in an animal ocular model. Their focus was to determine the *in vitro* antimicrobial activity of various AMPs against pathogenic bacterial, fungal, and protozoal ocular isolates with the practical goals of developing effective preservatives for contact lens solutions and corneal storage media as well as a topical antimicrobial drug. Their research group reported that rabbit alpha defensin (NP-1) was effective against various bacterial ocular pathogens in phosphate buffer<sup>66,67</sup> and in modified corneal storage media<sup>68</sup> and also stimulated epithelial cell growth<sup>69,70</sup>. Among the cecropin analogs tested, Shiva-11 was similarly active against a panel of ocular isolates<sup>71</sup> including gentamicin-resistant bacteria,<sup>72</sup> showed potential as a preservative in timolol and contact lens solutions, and stimulated epithelial and fibroblast growth.<sup>73</sup> Hecate, another cecropin analogue, inhibited various *Acanthamoeba* species *in vitro*.<sup>74</sup> DeSousa reported that D<sub>5</sub>C, another cecropin analogue, augmented contact lens disinfecting solutions against *Pseudomonas aeruginosa*<sup>75</sup> and was active against common Gram-positive and Gram-negative ocular pathogens and *Candida* in contact lens sterilization media and cornea storage media.<sup>75</sup> In contrast, Schwab<sup>76</sup> failed to demonstrate inhibitory activity for D<sub>5</sub>C or Nisin, a natural polypeptide derived from *Streptococcus lactis*, in modified corneal storage media (Optisol without antibiotics) at 4°C or 27°C, although the addition of EDTA did augment the killing of *Pseudomonas in vitro*.

More recently, Mannis<sup>61</sup> reported several synthetic peptides (CCI A, B, C and COL-1) that had been selected by “in virtuo” screening using a proprietary computer software program that optimized for activity against ocular bacterial pathogens (CyberChemics, Inc, Huntsville, AL, USA). As predicted, all of the synthetic peptides demonstrated significant inhibitory activity against a broad panel of bacterial and fungal ocular pathogens in a controlled system of 10 mM sodium phosphate buffer. However in modified Optisol, only *Staphylococcus epidermidis* was inhibited at all temperatures by the peptides. *Staphylococcus aureus* was inhibited at 23°C, but not at 4°C, and *Pseudomonas aeruginosa* was not inhibited at all. The addition of EDTA augmented the killing of *Pseudomonas* but had no effect on *Staphylococcus aureus*. Finally, COL-1, which had been specifically designed to have anti-*Pseudomonas* activity, was tested in a robust rabbit keratitis model involving aggressive topical dosing for 4–6 days. Topical administration of COL-1 produced significant ocular toxicity *in vivo* and failed to demonstrate efficacy in the *Pseudomonas* rabbit keratitis model compared to an effective conventional antibiotic, tobramycin, using clinical signs and microbiological outcome measures. In the only other published study using topical AMPs in an ocular model, Nos-Barbera *et al.*<sup>77</sup> reported in 1997 that a synthetic hybrid peptide from cecropin A and mellitin (bee venom) was equivalent to gentamicin in reducing clinical signs of infection in a *Pseudomonas aeruginosa* rabbit keratitis model. However, enthusiasm for this study is limited by the lack of any microbiological confirmation of antibacterial activity by the cecropin-mellitin hybrid in this model.

## THE CAMPBELL LABORATORY EXPERIENCE (1996–2004)

The Campbell Laboratory tested a variety of antimicrobial peptides over an 8-year period as part of contract research for several different companies. Binding confidentiality agreements preclude any detailed discussion of specific AMPs and the companies involved. Nevertheless, we believe that presentation of the results in general terms and insights gained still provide valuable information worth sharing in the ophthalmic literature.

The various antimicrobial peptides tested included synthetic analogues derived from various natural sources. These AMPs were tested for efficacy and toxicity *in vitro* and in rabbit ocular models of bacterial and viral infections (HSV-1, adenoviruses). Our general results can be summarized as follows: (1) Most AMPs, but not all, failed to demonstrate significant efficacy in animal models despite promising *in vitro* data. (2) Many AMPs demonstrated significant ocular toxicity following repeated topical administration. (3) Both efficacy (when present) and toxicity were dose-dependent and tended to occur at higher concentrations. (4) AMPs were generally disappointing as stand-alone therapy but could enhance the effectiveness of conventional antibiotics in some cases *in vivo*.

Richard O'Callaghan, Ph.D., Professor of Microbiology and Immunology, LSU Health Sciences Center, New Orleans, has tested numerous AMPs *in vitro* and some in ocular keratitis models. He confirms that his overall experience with AMPs was similar to our currently reported observations; namely, that AMPs usually demonstrated limited efficacy compared to conventional anti-infective therapies in ocular infection and were usually associated with significant ocular toxicity (personal communication). James M. Hill, Ph.D., Professor of Ophthalmology, Neuroscience, Pharmacology, & Microbiology, LSU Eye Center, New Orleans, is another widely recognized authority on antimicrobial testing in animal ocular models. He reports, "My experience with most polypeptide antimicrobial agents is that they do not have sufficient efficacy compared to any currently accepted ophthalmic drugs, as measured in rabbit models of ocular infection. In general, these compounds have been safe and well tolerated when applied as topical drops to rabbit eyes. They do not inhibit corneal healing, nor do they reduce cell migration in animal models of wound healing" (personal communication).

In general, we found that a number of AMPs were effective *in vitro* against Gram-positive and Gram-negative ocular pathogens under carefully controlled experimental conditions. This observation supports the previous findings of Mannis<sup>61</sup> who also observed reduced inhibitory activity of some AMPs under less stringent conditions (e.g., when corneal storage media replaced phosphate buffer; when the temperature was reduced from room temperature (23°C) to a lower storage temperature (4°C)). In general, we also found that the more stringent the conditions required for optimized drug inhibition *in vitro*, the less the likelihood of success *in vivo* where inhibitory factors (binding proteins, lytic proteases, ionic changes, excess lacrimation, etc.) were expressed during acute inflammation on the infected ocular surface.

We believe that the issue of ocular toxicity is a major problem associated with the administration of topical AMPs as single therapeutic agents. We have observed repeatedly in our *in vivo* ocular models that AMPs are far more toxic in inflamed, infected eyes than in uninfected rabbit eyes. Therefore, the common practice of evaluating ocular toxicity of AMPs in a dose-escalation study in normal rabbit eyes is inadequate to accurately assess potential clinical toxicity of an anti-infective agent. Although it is true that toxicity demonstrated in normal animal eyes would likely predict a clinical toxicity issue, the absence of significant toxicity in normal eyes cannot provide the desired reassurance of clinical safety. Our observations and those of others show that normal eyes are *not* predictive of AMP toxicity in infected eyes. For example, Nos-Barbera *et al.*<sup>77</sup> reported in 1997 that topical administration



of cecropin-mellitin hybrids in a both high- and low-treatment regimens produced more signs of ocular toxicity than either PBS or gentamicin controls at 6 hr. Despite the obvious toxicity induced by the AMPs in infected PBS-treated control eyes, contralateral normal (uninfected) eyes showed no toxicity!

Another published example of this toxicity phenomenon was reported by Mannis in the *Pseudomonas* rabbit keratitis model.<sup>61</sup> Similar to our data and that of Nos-Barbera,<sup>77</sup> he found that the COL-1 peptide treated eyes were again more inflamed than the vehicle control eyes in the infection model at a peptide concentration (50 µg/ml) that was nontoxic in normal rabbit eyes. Critical review of his data also suggested that toxicity was a major issue based on (1) an enhanced perforation rate in the COL-1 group [8 of 17 (47%)] versus [2 of 9 (23%)] the vehicle control group, and (2) a reduced rate of surface sterilization in the COL-1 treated eyes 1 of 17 (6%) versus 3 of 9 (33%) for the vehicle control. This data is consistent with the interpretation that the toxic effects of the peptide may have interfered with the normal defense mechanisms responsible for wound healing and bacterial clearance on the ocular surface. Taken together, most animal studies indicate that the issue of ocular toxicity remains a major challenge for the successful development of AMPs as therapeutic ocular agents.

## PERSPECTIVES ON THE FUTURE OF A TOPICAL OPHTHALMIC AMP DRUG

Of the thousands of potential synthetic peptides with antimicrobial activity, only a small fraction have been systematically studied and tested. The field is young, and it would be premature to conclude that AMPs have no apparent clinical role as therapeutic agents despite their evolutionary success as essential components of innate immunity in most species. The experience to date suggests that *in vitro* efficacy reproducibly demonstrated by many AMPs under controlled experimental conditions has not translated easily to success as single therapeutic agents in clinical trials when compared to conventional antibiotics. Furthermore, the unique challenges of toxicity for ophthalmic development will need to be overcome by custom peptide design, imaginative formulation, and strategies for reducing frequency of administration. The experience of The Campbell Laboratory with *in vivo* testing in animal models suggests that AMPs in combination with conventional antibiotics and/or antivirals (to promote any additive or synergistic effects) may prove to be a more successful developmental strategy for a topical ocular application. Finally, the therapeutic potential of AMPs lacking direct antimicrobial activity but having immunostimulatory properties that enhance natural innate immunity requires further assessment. Although some early preclinical studies were encouraging, much more data needs to be obtained before the value of this intriguing proposition can be properly evaluated. In summary, although antimicrobial peptides are generally recognized as essential components of natural host innate immunity against microbial challenge, their promise as a new class of drugs and their value as external therapeutic agents still remain to be proven.

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**TABLE 1**  
Development of Antimicrobial Peptides as Anti-Infective Drugs<sup>a</sup>

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Advantages
Broad-spectrum activity (antibacterial, antiviral, antifungal)
Rapid onset of killing
Cidal activity
Potentially low levels of induced resistance
Concomitant broad anti-inflammatory activities
Disadvantages
Discovery costs of synthesis and screening
Patent exclusivity for economic viability
Systemic and local toxicity
Reduced activity based on salt, serum, and pH sensitivity
Susceptibility to proteolysis
Pharmacokinetic (PK) and pharmacodynamic (PD) issues
Sensitization and allergy after repeated application
Natural resistance (e.g., <i>Serratia marcescens</i> )
Confounding biological functions (e.g., angiogenesis)
High manufacturing costs

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<sup>a</sup>Refs. 21, 32, 33.

TABLE 2

## Summary of Antimicrobial Peptides in Clinical Development for Nonocular Applications

Synthetic compound	Company	AMP type (Species)	Clinical trial outcomes	Planned clinical trials
Pexiganan (MSI-78)	Genaera Plymouth Meeting, PA (formerly Magainin Pharmaceutical Inc.)	Magainin 2 ( <i>Xenopus</i> frog)	No advantage over conventional antibiotics in a Phase III trial for topical Rx of diabetic foot ulcers. Failed to gain FDA approval.	None.
Iseganan (IB-367)	Intrabiotics Pharmaceuticals, Inc. Mountainview, CA	Protegrin (Pig)	Failed two Phase III trials as mouthrinse for stomatitis in highrisk patients. Failed Phase III trial as aerosolized Rx in ventilator-associated pneumonia.	Phase IIa trial as aerosolized Rx for CF patients with chronic respiratory infections.
Omiganan (MBI-226)	Microbiologix Biotech Vancouver, BC	Indolicidin (Bovine)	Failed Phase III trial as topical Rx to prevent or reduce venous catheter-related bloodstream infections.	Repeat Phase III trial to confirm reduction in catheter colonization and local infections under consideration.
MBI 594AN	Microbiologix Biotech Vancouver, BC, Canada	Indolicidin (Bovine)	Phase IIb trial showed efficacy as topical Rx for acne.	Phase III trial.
P113 P113D	Demegen, Pittsburgh, PA Dow Pharmaceutical Sciences, Patuloma, CA	Histatins (Human)	Completed Phase II trial as mouthrinse for oral candidiasis in HIV patients.	Inhalation Rx for <i>Pseudomonas</i> infections in CF patients under consideration.
XMP, 629 Neuprex (rBPI21)	Xoma (US) Berkeley, CA Xoma (US) Berkeley, CA	BPI (Human) BPI (Human)	Failed Phase III trial as topical Rx for acne. Failed Phase III trial as adjunctive parenteral Rx to reduce mortality in pediatric meningococemia.	None at present time. Planned Phase I/II trial to reduce inflammatory complications in pediatric open heart-surgery patients.