

CATIONIC ANTIMICROBIAL PEPTIDES — A FUTURE THERAPEUTIC OPTION?

PÉPTIDOS CATIONICOS ANTIMICROBIANOS. ¿UNA FUTURA OPCIÓN TERAPÉUTICA?

McDERMOTT AM¹

Cationic antimicrobial peptides (CAPs,) such as defensins and cathelicidins, are small endogenously expressed molecules that have been shown to have antimicrobial activity against Gram positive and negative bacteria, fungi and some viruses. In addition, these peptides also have the ability to modulate the behaviour of a variety of mammalian cells, in particular epithelial cells and cells of the innate and adaptive immune responses. These observations have led to the concept that many CAPs are multifunctional molecules that may provide direct protection against invading pathogens and that can promote wound healing and regulate the adaptive immune response.

A number of studies have addressed the localization of CAPs in the human eye, with particular emphasis being placed on the ocular surface epithelia. Here, the expression of three beta-defensins (hBD)-1, hBD-2 and hBD-3 and of the cathelicidin LL-37, has been confirmed at the mRNA and protein level for epithelia of both the cornea and conjunctiva (1,2). Defensins hBD-1 and hBD-3 are constitutively expressed, whereas the expression of hBD-2 and LL-37 is upregulated in response to pro-inflammatory cytokines, infection and injury (1). Alpha-defensins, which chiefly come from neutrophils have been detected in the tear film (1). The precise roles of the CAPs at the ocular surface are, at present, largely speculative as the majority of studies have focused on localization rather than function and have been performed in vitro or ex vivo. In vitro studies have shown some of the CAPs to be active against common ocular pathogens such as *Pseudomonas aeruginosa* (PA) and *Staphylococcus aureus* thus leading to speculation that they are

involved in protecting the ocular surface from infection (1). A recent study from my laboratory in which we observed that genetically modified mice missing the cathelicidin gene showed enhanced susceptibility to PA keratitis, is the first to show the importance of cathelicidin in protection from ocular surface infection in vivo (3). Other studies show that in vitro CAPs can modulate corneal and conjunctival cell functions, such as proliferation, migration, gene expression and cytokine secretion. Thus, in addition to extending antimicrobial protection to the ocular surface CAPs may also help regulate processes such as corneal wound healing (1,4). It is likely that for some CAPs modulation of cell function is their primary role in vivo, whilst others may be both antimicrobial and modulatory.

CAPs were initially recognized for their antimicrobial properties with the observation that they manipulate mammalian cell function following on a few years later. Their antimicrobial properties alone make them highly attractive for clinical applications, particularly in the face of ever increasing microbial resistance to traditional antibiotics. Advantages put forth for CAPs include their mechanism of action, in which the positively charged peptide disrupts the negatively charged microbial membrane ultimately leading to cessation of replication/death of the organism. Such a mechanism makes it difficult, although not impossible, for an organism to develop resistance. However, although a number of naturally occurring peptides and their derivatives have been investigated and a variety are in various stages of clinical trials, none has yet been brought to market successfully. Factors confounding the development of the peptides for therapeutic use include inherent pro-

¹ Associate Professor of Optometry and Vision Sciences. University of Houston. College of Optometry.
E-mail: AMcDermott@popmail.opt.uh.edu

blems of working with peptides e.g. degradation and cost of manufacture and also problems of efficacy versus toxicity. Ongoing studies of ways to circumvent some of these issues, for example using D rather than L amino acids in the manufacturing process to improve in vivo half-life, are likely to bear fruit in the next few years. A recent excellent review by Hancock and Sahl is recommended for further reading on the future of CAPs in a variety of clinical applications (5).

Thus, it is conceivable that in the not too distant future a CAP or its derivative/mimetic may have a place in the armamentarium of the eye care professional. There are two approaches to capitalizing on the multi-functional properties of CAPs for the treatment and prevention of ocular surface disease. The first is simply to deliver the peptide to the ocular surface for example for the purpose of treating an infection or for promoting wound healing. Topical drop-wise application of a CAP in solution is likely to have minimal effect, certainly in terms of antimicrobial activity, due to inactivation of the peptide by components in the tear film, such as salt and negatively charged entities that would interact electrostatically. Rather, more creative delivery systems need to be considered such as incorporating a CAP in to nanoparticles or attaching it to a contact lens. The second approach, rather than to add a CAP exogenously, is to boost the expression/activity of the endogenously expressed CAPs to provide additional antimicrobial protection and/or modulate epithelial and immune cell behaviour. This is not a trivial issue as several factors, such as interleukin-1, known to up-regulate defensin expression for example may themselves cause inflammation. Interestingly, at the annual meeting of the Association for Research in Vision and Ophthalmology earlier this year it was reported by Dr. Yu's laboratory from the Department of Ophthalmology, Wayne State University in Detroit, USA, that mice pre-tre-

ated with bacterial flagellin, a toll-like receptor 5 agonist, showed vastly improved clinical outcome of PA keratitis, including substantially reduced inflammation, and that this in part was due to enhanced expression of CAPs. This study indicates that CAPs can be upregulated without causing inflammation by appropriate choice of stimulatory agent, so opening up the possibility of pharmaceutical enhancement of endogenous CAP action.

In summary, although CAPs were identified more than two decades ago, their role(s) at the ocular surface is only beginning to be elucidated. Understanding their function is essential to our understanding of ocular surface immunity and will reveal routes by which CAP activity and expression can be modulated so opening up the possibility of boosting immunity. Whilst there is still some way to go in the development of a commercial product, due to their multi-functionality, CAPs do present an attractive possibility for future use in the prevention and treatment of ocular surface disease.

REFERENCES

1. McDermott AM. *Defensins and other antimicrobial peptides at the ocular surface. Ocul Surf* 2004; 2: 229-247.
2. Gordon YJ, Huang LC, Romanowski EG, Yates KA, Proseke RJ, McDermott AM. *Human cathelicidin (LL-37), a multifunctional peptide, is expressed by ocular surface epithelia and has potent antibacterial and antiviral activity. Curr Eye Res* 2005; 30: 385-394.
3. Huang LC, Reins RY, Gallo RL, McDermott AM. *Cathelicin-deficient (Cnlp^{-/-}) mice show increased susceptibility to Pseudomonas aeruginosa keratitis. Invest Ophthalmol Vis Sci* 2007 (in press).
4. Li J, Raghunath M, Tan D, Lareu RR, Chen Z, Beuerman RW. *Defensins HNP1 and HBD2 stimulation of wound-associated responses in human conjunctival fibroblasts. Invest Ophthalmol Vis Sci* 2006; 47: 3811-3819.
5. Hancock RE, Sahl H-G. *Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol* 2006; 24: 1551-1557.