21. Hunt BE, Weber A, Berger A, Ramsey B, Smith AL. Macromolecular mechanisms of sputum inhibition of tobramycin activity. Antimicrob Agents Chemother 1995; 39: 34–9.
22. Eschenbacher WL, Boushey HA, Sheppard D. Alteration in osmolarity of inhaled aerosols cause bronchoconstriction and cough, but absence of a permeant anion causes cough alone. Am Rev Respir Dis 1984; 129: 211–15.
23. Fine JM, Gordon T, Thompson JE, Sheppard D. The role of titratable acidity in acid aerosol-induced bronchoconstriction. Am Rev Respir Dis 1987; 135: 826–30.
24. Smith AL, Ramsay B, Hedges DL, et al. Safety of aerosol tobramycin administration for three months to patients with cystic fibrosis. Pediatr Pulmonol 1989; 7: 265–71.
25. Ramsey BW, Dorkin HL, Eisenberg JD, et al. Efficacy of aerosolized tobramycin in patients with cystic fibrosis. N Engl J Med 1993; 328: 1740–6.
26. Ramsey BW, Smith AL. Aerosolized tobramycin in patients with cystic fibrosis. N Engl J Med 1993; 329: 659.
27. Eisenberg J, Pepe M, Williams-Warren J, et al. A comparison of peak sputum tobramycin concentration in patients with cystic fibrosis using jet and ultrasonic nebulizer systems. Chest 1997; 111: 955–62.
28. Ramsey BW, Pepe MS, Quan JM, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic fibrosis inhaled tobramycin study group. N Engl J Med 1999; 340: 23–30.
29. Ramsey BW, Quan JM, Otto KL, et al. Interim analysis of data from an open-label follow-on trial of inhaled tobramycin (PC-TNDS-004) in patients with cystic fibrosis. Pediatra Pulmonol 1998; suppl 17: 320 [abstract 414].

Cationic peptide antibiotics for use in treatment of *Pseudomonas aeruginosa* infections of cystic fibrosis patients

Clin Microbiol Infect 1999; 5: 5S52–5S53

Robert E. W. Hancock, Monisha G. Scott, Carol Friedrich, Lijuan Zhang, Hong Yan, Pradeep Nair, Aleksander Patrzykat and Annett Rozek

Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada

Hundreds of peptide antibiotics have been described in the past half-century [1–3]. These fall into two classes, non-ribosomally synthesized peptides, such as the gramicidins, polymyxins, bacitracins and glycopeptides, and ribosomally synthesized (natural) peptides, including the defensins, cecropins and magainins. The former are often drastically modified and are largely produced by bacteria, whereas the latter are produced by all species of life (including bacteria) as a major component of the natural host defense molecules of these species. Both include peptides with a strong net positive charge and are being considered for or are already used for the therapy of *Pseudomonas aeruginosa* infections of cystic fibrosis (CF) patients.

CF is the most common, eventually fatal, autosomal-recessive genetic disease in our society. It is caused by a defect in the CF transmembrane regulator chloride channel protein. The eventual cause of death is lung function deterioration due to chronic lung infections, particularly by *P. aeruginosa*. It was recently suggested that lung epithelial cells secrete cationic antimicrobial peptides and proteins (possibly including β-defensins, lysozyme, lactoferrin, LL-37, etc.) that can normally kill *P. aeruginosa*. In contrast, in the high-salt environment created outside CF epithelial cells (due to the CFTR mutation), peptides are ineffective due to salt antagonism [4,5]. Although this finding has been disputed by others, it has indicated that one therapeutic approach against *P. aeruginosa* infections of CF patients would be to exogenously apply these agents via aerosol to the lung. Indeed, colimycin, the methosulfate derivative of the cationic lipopeptide colistin (polymyxin E), has been utilized quite successfully in aerosol formulations against *P. aeruginosa* lung infections [6]. Pathogenesis Inc. (Seattle, Wa, USA) is currently planning clinical trials for polymyxin E1 in patients with CF.

Ribosomally synthesized, cationic antimicrobial peptides are so widespread that they are likely to play an important protective role [2,3]. Although certain peptide structural groups occur (β-structures stabilized by disulfide bonds, amphipathic α-helices, extended structures, and loops) [3,7], and these structures tend

Corresponding author and reprint requests:

Robert E. W. Hancock, Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC VGT 123, Canada

Tel: +604 822 2682 Fax: +604 822 6041

to be amphipathic (with a polar and a hydrophobic face), no positional conservation of even classes of amino acids occurs. Such cationic antimicrobial peptides generally have two to nine excess positively charged amino acids (arginine or lysine), at least 50% hydrophobic amino acid residues and a low proportion of both neutral polar and negatively charged amino acids. This tremendous diversity in nature provides many potential candidates for development as antimicrobials.

Our major thrust against *P. aeruginosa* has involved the development of the α-helical peptides derived from hybrids of silk moth cecropin and honeybee melittin [8–10]. These cationic antimicrobial peptides have a variety of activities, ranging from Gram-negative selective to Gram-positive selective to broad spectrum in nature. It is important to measure MICs in the correct fashion [11,12], since these peptides tend to precipitate at high concentrations and bind to many surfaces. The best peptides have good MICs (1–4 mg/L) against *P. aeruginosa*, including all mutants resistant to conventional antibiotics. For the best peptides, these MICs are unaffected by even 200 mM NaCl [10]. They are bactericidal, with very rapid killing kinetics, even around the MIC. It is also very difficult to raise mutants resistant to these bacteria, and there are very few naturally resistant bacteria (although one of these, *Burkholderia cepacia* [9], is relevant to CF). As a result of their mechanism of action (self-promoted uptake across the outer membrane), some peptides have subsidiary activities that offer added side benefits, including an ability to neutralize endotoxin (in vitro and in animal models [8]) and synergy with conventional antibiotics, especially against resistant mutants [9]. For these reasons, they appear to have excellent potential in the fight against antibiotic-resistant bacterial pathogens, including *P. aeruginosa*.

Individual peptides have also been shown to have a variety of interesting activities, including an ability to promote wound healing, a potential advantage in dealing with the damaged lungs of CF patients [13].

Activity in animal models of both topical and systemic *P. aeruginosa* infections has been demonstrated [8,14,15]. In intraperitoneal infection models (neutropenic mice), a single dose of 8 mg/kg results in a halving of lethality. In our experience, the peptides do not offer better protection after multiple dosing, possibly due to toxicity. However, aerosol dosing in rats does protect against *P. aeruginosa* lung infections (D.E. Woods and R.E.W. Hancock, unpublished). Thus we feel that antimicrobial cationic peptides have excellent potential in CF to defeat *P. aeruginosa* lung infections. Both the cationic protein $rBPI_{21}$ (Neuprex, Xoma Corp, Ca, USA) and IB-367 (a protegrin-like cationic peptide from Intrabiotics) are reported to be undergoing phase I (safety) clinical trials for use in CF patients.

Acknowledgments

REWH is an MRC Distinguished Scientist and would like to acknowledge the Canadian Bacterial Diseases Network and the Canadian Cystic Fibrosis Foundation for funding his own peptide research.

References

1. Kleinkauf H, von Dohren H. Peptide antibiotics, β-lactams and related compounds. CRC Crit Rev Biotechnol 1988; 8: 1–32.
2. Boman HG. Peptide antibiotics and their role in innate immunity. Annu Rev Immunol 1995; 13: 61–92.
3. Hancock REW, Lehrer R. Cationic peptides: a new source of antibiotics. Trends Biotechnol 1998; 16: 82–8.
4. Goldman MJ, Anderson GM, Stoltzenberg ED, et al. Human beta-defensin-1 is a salt sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell 1997; 88: 553–60.
5. Smith JJ, Travis SM, Greeberg EP, Walsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. Cell 1996; 85: 229–36.
6. Jensen T, Pedersen SS, Garne S, et al. Colistin inhalation therapy in cystic fibrosis patients with chronic *Pseudomonas aeruginosa* lung infection. J Antimicrob Chemother 1987; 19: 831–8.
7. Hancock REW. Peptide antibiotics. Lancet 1997; 349: 418–22.
8. Gough M, Hancock REW, Kelly NM. Anti-endotoxic potential of cationic peptide antimicrobials. Infect Immun 1996; 64: 4922–49.
9. Scott M, Yan H, Hancock REW. Biological properties of structurally related cationic antimicrobial peptides. Infect Immun 1999; in press.
10. Friedrich C, Gough M, Karunaratne N, Yan H, Hancock REW. Salt resistant α-helical cationic antimicrobial peptides. Antimicrob Agents Chemother 1999; in press.
11. Steinberg DA, Hurst MA, Fujii CA, et al. Protegrin-1: a broad spectrum, rapidly microbicidal peptide with in vivo activity. Antimicrob Agents Chemother 1997; 41: 1738–42.
12. Wu M, Hancock REW. Interaction of the cyclic antimicrobial cationic peptide bactenecin with the outer and cytoplasmic membrane. J Biol Chem 1999; 274: 29–35.
13. Gallo RL, Huttner KM. Antimicrobial peptides—an emerging concept in cutaneous biology. J Invest Dermatol 1998; 111: 739–43.
14. Hancock REW. Therapeutic potential of cationic peptides. Expert Opin Invest Dis 1998; 7: 167–74.
15. Nos-Bar Bera S, Portoles M, Morilla A, et al. Effect of hybrid peptides of cecropin A and melittin in an experimental model of bacterial keratitis. Cornea 1997; 16: 101–6.