studying derivatives of penicillanic acid capable of inhibiting human neutrophil elastase. Whereas 6-aminopenicillanic acid has only very weak anti-elastase properties, preliminary results indicate that chemical modifications of the penicillin nucleus markedly enhance the anti-neutrophil elastase properties.

Finally, if serine protease inhibitors are to be studied in CF clinical trials, it will be necessary to have appropriate markers of the lung serine protease burden. Although bronchoalveolar lavage is a useful tool to study the protease-antiprotease balance in the lung, the relative invasiveness of such a procedure makes it impractical for large scale clinical trials. Sputum markers such as elastase activity, IL-8 and protein degradation products may be helpful, but will likely give highly variable results since sputum is not a homogeneous sample. It is therefore probable that serum and plasma markers of the lung protease burden will be needed to follow patients in clinical trials. One such marker is the elastase-α1AT complex measured by ELISA, however additional indicators of the lung serine protease burden in CF may be needed in the near future.

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S14.3

Small Peptide Antibiotics

Robert E.W. Hancock, Shaffique Fidai, Monisha Gough
Manhong Wu and Kevin Piers

Canadian Bacterial Diseases Network
and
Department of Microbiology and Immunology
University of British Columbia
300 - 6174 University Blvd.
Vancouver, B.C., Canada
V6T 1Z3
Tel (604) 822-2682; Fax (604) 822-6938; E-mail: Bob@CBDN.ca.

Patients with cystic fibrosis suffer from chronic lung infections. The most common organism associated with terminal lung disease is *Pseudomonas aeruginosa*. This bacterium demonstrates high intrinsic resistance to antibiotics, primarily due to low outer membrane permeability (1) combined with a secondary mechanism such as β-lactamase (2) or active efflux (3). Over time *Pseudomonas* becomes progressively resistant to one antibiotic after another until eventually antibiotic treatment becomes ineffective at reducing the bacterial population in the lung.

These difficulties in treating *Pseudomonas aeruginosa* infections are not limited to cystic fibrosis patients but are observed in many nosocomial (hospital acquired) infections, of which *P. aeruginosa* causes approximately 200,000 per year in North America. For this reason, discovering novel anti-*Pseudomonas* antibiotics has been one of the holy grails of the Pharmaceutical Industry for more than a decade. Unfortunately, in the past 20 years, this multi-billion dollar industry has not identified a single fundamentally new antibiotic class in the past 25 years, and all of the recently introduced anti-Pseudomonal antibiotics including the third and fourth generation cephalosporins, imipenem and the fluoroquinolones have rapidly suffered from resistance problems.

In the course of studying the mechanisms of antibiotic uptake and resistance in *P. aeruginosa* (4), we described a novel trans-outer membrane uptake pathway termed the self promoted pathway (4,5). Self promoted uptake, which is utilized by polycationic antibiotics such as aminoglycosides, polymyxins, and the dibasic macrolide azithromycin, is initiated when these polycations attack the divalent cation binding sites of outer membrane surface lipopolysaccharides. Since they have affinity for such binding sites that is 2 - 3 orders of magnitude higher than the divalent cations which normally occupy these sites, they displace these divalent cations, and being more bulky, cause localized perturbations of the outer membrane. This enhances uptake of various probe molecules, including antibiotics, and also enhances uptake of the permeabilizing polycation. The ability of these polycations to promote their own uptake, as judged by mutant studies (4), led to the name self promoted uptake.

Examination of the literature revealed that such polycations were ubiquitous in nature, with more than 100 small cationic antibacterial peptides observed in organisms that included bacteria, plants, insects, amphibians, crustaceans, mammals and humans (6,7). In plants and insects, such peptides represent the major inducible defence against bacteria and other microorganisms whereas in mammals and humans, they are known to be important factors in the arsenal of neutrophils [defensins being the major proteinaceous species in neutrophils] and are suspected as having a major role in defence at mucosal surfaces. To investigate the therapeutic potential of these peptides, we devised methods of producing them by recombinant DNA

procedures in bacteria (8). The method of choice involves production of these peptides by fusion protein technology wherein a four part fusion protein is encoded by a plasmid in *E. coli* or *S. aureus*. This fusion protein contains (from N- to C-terminus) an affinity binding region (for ease of purification), an anionic stabilizing fragment (to neutralize the cationic peptide portion and prevent its bactericidal action and cleavage by bacterial proteases), a methionine (to permit release of the cationic peptide by cyanogen bromide) and a cationic antimicrobial peptide. The latter three regions are encoded by synthetic DNA and their sequence can be changed rather simply permitting a large range of cationic peptides to be made by this technology. After purification, a peptide produced by recombinant DNA technology was indistinguishable from one made by protein chemical means, except that it cost only 5% as much to produce.

Using this technology, we have produced a variety of different peptides including a human defensin, alpha helical hybrids of moth cecropin and bee venom melittin (CEME and a variant MBI.28), indolicidin, bactenicin and apidaecin. The a helical class was studied in detail. These peptides were shown to access the self promoted uptake pathway in *P. aeruginosa* (and *Enterobacter cloacae*) (9). They demonstrated very rapid killing even at the MIC (cf. all other known classes of antibiotics), were equally effective against parental strains and antibiotic resistant mutants and engendered no resistance themselves in *in vitro* experiments. They also breached the outer membrane permeability barrier of *Pseudomonas aeruginosa* and enhanced the uptake of lysozyme and certain antibiotics. In addition, they bound tightly to endotoxin and neutralized it. We propose that cationic peptides offer potential as an alternative to classical antimicrobial therapy against Pseudomonal lung infections in patients with cystic fibrosis.

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S14.4 Effect of rhDNase Administration on Sputum Bacterial Density

A. Smith, M. Pepe, G. Morlin, J. Williams-Warren and B. Ramsey
Department of Pediatrics
University of Washington School of Medicine
Seattle, Washington

rhDNase decreases sputum viscosity *in vitro* and aerosol administration to patients with Cystic Fibrosis improves pulmonary function. Assuming that less viscous sputum permits facile clearance of respiratory secretions by the mucociliary elevator and cough, we questioned whether sputum bacterial density would decrease with aerosol administration of rhDNase. We enrolled 74 patients from 5 CF Centers, quantitatively culturing their sputum prior to and after 29 and 169 days of administration of placebo (24 patients) 2.5mg., or rhDNase once (22 patients) or twice (28 patients) daily.

All initial sputum cultures grew organisms commonly isolated from CF patients. Comparing the mean enrollment density of *P. aeruginosa* to that observed on day 29 (in 54 patients) and day 169 (in 45 patients) we found no significant difference among the three treatment groups (all p values >0.20). This lack of difference remained when those patients who received parenteral, aerosol, or oral anti-pseudomonal antibiotics in the two weeks prior to each culturing were excluded. Similarly, when *Staphylococcus aureus* densities were compared there were no significant differences among treatment groups before or after adjustment for prior antibiotic administration. Patients yielding *H. influenzae, C. albicans,* and *B. cepacia* were distributed equally among the treatment groups at all sampling times, but were too few to permit statistical analysis. We conclude that aerosol administration of rhDNase (at 2.5mg once or twice daily for 29 or 169 days) does not produce a significant change in sputum density of *S. aureus* and *P. aeruginosa.*

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