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Cystic Fibrosis Microbiology:

Advances in antimicrobial therapy

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Abstract

Much of the improvement in the survival of individuals with cystic fibrosis (CF) is due to advancements in antimicrobial treatments. New aerosolized antibiotic formulations have recently been introduced (such as inhaled aztreonam), and others are in development (inhaled levofloxacin and liposomal amikacin). Licenced dry powder formulations include tobramycin inhalation powder and dry powder colistimethate (available in Europe). Although inhaled antibiotics have the advantage of being able to deliver high intrapulmonary concentrations of drug, antimicrobial resistance can still develop and is a concern in CF. Antimicrobial resistance might be mitigated by using non-antibiotic treatments, antibiotic adjuvants, which have activity against bacteria. Examples include agents such as gallium, antimicrobial peptides and anti-biofilm compounds such as alginate oligosaccharides (OligoG) and garlic. Vaccination strategies and antibody therapy (IgY) against *P. aeruginosa* have also been attempted to prevent initial infection with this organism in CF. Although aggressive and long-term use of antibiotics has been crucial in slowing lung function decline and improving survival in people with CF, it has added a significant burden of care and associated toxicities in these individuals. Careful surveillance and the use of preventative strategies for antibiotic related toxicity (such as nephrotoxicity and ototoxicity) are essential. Continued development of effective antimicrobial agents that can function in the conditions encountered in the CF lung, such as against bacterial biofilm growth and under anaerobic conditions, is needed.

Introduction

There has been significant improvement in the survival of individuals with cystic fibrosis (CF) over the last half century, from a median age of survival of 5 years in the 1970s to approximately 40 years of age as of 2011. The reasons for the improvement in clinical outcomes are multifactorial but include the intense use of antibiotic therapy in this patient population. Despite these improvements, however, the majority of CF deaths still occur in young adulthood, typically between the ages of 21-30 years (1). Continued development and optimal usage of new antimicrobial compounds (including antibiotic combinations) is essential to improve the quality and survival of people with CF.

In the last several years, there have been an increasing number of available antibiotics, of different classes and formulations, for the treatment of pulmonary infections in CF patients. This review will discuss some of the new antibiotics including novel methods of delivery, the implications of the development of antimicrobial resistance, potential non-antibiotic treatments of CF lung infections and the lifetime burden and toxicity of repeated courses of antibiotic therapy.

New antimicrobials and methods of delivery

Several new inhalational antibiotics have recently become available for the treatment *Pseudomonas aeruginosa* pulmonary infections in CF patients (Table 1). Antibiotics delivered via aerosolization have the advantage of being able to achieve very high intrapulmonary concentrations with few associated systemic side effects.

Inhaled aztreonam

Inhaled aztreonam, commercially known as AZLI or Cayston, is currently on the market for the treatment of *Pseudomonas aeruginosa* infections in CF patients. It is an aerosolized formulation of the monobactam antibiotic aztreonam and lysine. In contrast, the intravenous formulation of aztreonam contains arginine which can cause airway inflammation. The initial randomized controlled trial of inhaled aztreonam was performed in 211 individuals with CF 6 years of age or older with *P. aeruginosa* infection (2). After a 28 day course of tobramycin inhalation solution (TIS), patients were randomized to receive either 75 mg of inhaled aztreonam three times daily or placebo for 28 days. Inhaled aztreonam treatment increased the median time to requiring additional anti-pseudomonal antibiotics for symptoms of pulmonary exacerbation by 21 days, compared with placebo (p=0.007). In addition, inhaled aztreonam improved mean symptom scores (p=0.02), forced expiratory volume in 1 second (FEV_1) (6.3%, p=0.001) and sputum *P. aeruginosa* bacterial density (-0.66 $\log_{10}$ CFU/g, p=0.006) compared with placebo. The drug was well tolerated and the in vitro susceptibilities of *P. aeruginosa* isolates to aztreonam from baseline to end of therapy were comparable. Similar improvements in respiratory symptoms, lung function and microbiological outcomes were also observed in another Phase II trial of inhaled aztreonam in CF patients with moderate to severe lung disease and no recent use of anti-pseudomonal antibiotics or azithromycin (3). In CF individuals greater than 6 years of age and mild lung disease (FEV_1>75% predicted), however, the effects of inhaled aztreonam treatment were more modest with no statistically significant improvement in respiratory symptoms and only a 2.7% improvement in relative FEV_1% predicted (p=0.021) compared to placebo treatment (4). When compared to TIS in an open-label, parallel-group international trial in 273 patients with CF, after 3 cycles of inhaled aztreonam (28 day on; 28

days off), the mean change in FEV_1 was 2.05% for aztreonam compared to -0.66% for TIS (p=0.002) (5). Patients in the aztreonam arm also had fewer respiratory hospitalizations and additional anti-pseudomonal antibiotics compared to the TIS arm. However, it is important to note that over 85% of subjects had already received at least 3 cycles of TIS before randomization and the beneficial effects of inhaled tobramycin are known to diminish over time. This so-called honeymoon effect is not restricted to aminoglycosides and is not explained by the development of in vitro resistance (6). In addition to studies in CF patients with *P. aeruginosa* infection, the efficacy of inhaled aztreonam has been examined in CF individuals with chronic *Burkholderia cepacia* complex infection (7). A double-blind, placebo-controlled, 24 week trial of continuous inhaled aztreonam versus placebo treatment was performed in 100 CF patients with chronic *Burkholderia* spp. infection. By 24 weeks of treatment, there was no significant difference between arms in terms of change in FEV_1, number of respiratory exacerbations or hospitalizations. Importantly, there was also no significant decrease in sputum bacteria density change in treated patients compared to placebo. In vitro studies have demonstrated that aztreonam, as a ß-lactam that functions best against rapidly dividing organisms, may not have very good activity against CF organisms when grown as a biofilm, in contrast to aminoglycosides, for example (8).

Levofloxacin inhalation solution

Aerosolized levofloxacin is another new pharmaceutical agent developed for the teatment of *P. aeruginosa* pulmonary infections in patients with CF. Levofloxacin is a second generation fluoroquinolone that causes cell death by inhibiting topoisomerase, an enzyme required for DNA synthesis (9). It has properties that render it particularly effective against bacterial infections in

the CF lung. Thickened mucus layers in the lung of CF patients have been shown to contain areas of low oxygen tension, for example (10). In vitro studies of the effect of oxygen limitation on antimicrobial activity against *P. aeruginosa* demonstrate that, unlike tobramycin, amikacin and aztreonam, *P. aeruginosa* minimum inhibitory concentration (MICs) and time-kill curves for levofloxacin are similar under aerobic and anaerobic conditions (11). The same investigators have confirmed that, in comparison to aztreonam and aminoglycosides, levofloxacin has the most rapid rate of killing among *P. aeruginosa* isolates, is more potent against *P. aeruginosa* biofilms and its bactericidal activity is not affected by sputum (12). Additional in vitro studies have suggested that inhaled levofloxacin may also have anti-inflammatory effect (13). Levofloxacin produced a dose-dependent reduction of both TNF-α and *P. aeruginosa* lipopolysaccharide-induced IL-6 and IL-8 levels in cultured human airway epithelial cells. Phase I studies of safety, tolerability and pharmacokinetic parameters of inhaled levofloxacin in stable CF patients showed the drug to be safe, resulting in low serum levels (approximately 16% of serum levels after an oral dose). This should result in a very low risk of systemic toxicities such as tendinopathy or arthropathy. However, very high sputum concentrations were achieved, in the order of 4,000 µg/ml (14). A subsequent phase II randomized, placebo controlled study of 151 CF patients with *P. aeruginosa* infection demonstrated that inhaled levofloxacin 240 mg twice daily for 28 days (delivered via eFlow nebulizer) resulted in an 8.7% increase in FEV_1 percent predicted compared to placebo (p=0.003) (15). A phase III, open-label, randomized non-inferiority study comparing inhaled levofloxacin 240 mg twice daily versus tobramycin inhalation solution 300 mg twice daily over three 28 day on/off cycles in 282 CF patients with chronic *P. aeruginosa* infection demonstrated no significant difference in the change in FEV_1% predicted between the groups (16). In addition, although there was no significant difference

between the groups in time to symptom-defined exacerbation, subjects treated with inhaled levofloxacin solution had a longer time to adminstration of antibiotics (p=0.04) and a lower proportion of subjects hospitalized for a respiratory exacerbation (p=0.04) than those treated with inhaled tobramycin. The number of adverse events were similar between the two groups; dysgeusia (taste distortion) was the most common adverse event in the inhaled levofloxacin group.

Tobramycin inhalation powder

In addition to new types of antibiotics of differing classes, there have also been developments in the way these drugs are formulated and delivered. A currently available example is tobramycin inhalation powder (TIP). New developments in particle manufacturing have allowed tobramycin to now be formulated as a dry powder. Tobramycin inhalation powder (TIP) has the advantages of being able to be delivered very quickly (in approximately 5 minutes) using a "podhaler^{TM}" inhalation device and having good lung delivery (17). Initial pharmacokinetic studies demonstrated that TIP could achieve higher maximal sputum concentrations (measured 30 minutes after TIP administration) (1,092$\pm$1,052 μg/g) compared to TIS (737$\pm$1,028 μg/g), although with a wide standard deviation (18). In the phase III EVOLVE trial, TIP (112 mg twice daily) was compared to placebo in CF patients chronically infected with *P. aeruginosa* (19). In comparison to placebo, TIP significantly improved FEV_1, reduced sputum *P. aeruginosa* density and decreased respiratory related hospitalization. The most common adverse event was cough but this was higher in patients receiving placebo (26%) versus TIP (13%). TIP was not associated with ototoxicity or nephrotoxicity. Subsequently, the EAGER trial was conducted: a non-inferiority study to assess the safety and efficacy of TIP (112 mg

twice daily) compared to TIS (300 mg/5 ml twice daily) in chronic *P. aeruginosa* infected CF patients (20). TIP was comparable to TIS with similar increases in FEV_1 and similar rates of hospitalizations for respiratory-related events. Sputum *P. aeruginosa* density was reduced more in TIP-treated patients relative to TIS-treated patients (-1.6 $\log_{10}$ vs -0.92 $\log_{10}$ CFU/g for mucoid isolates and -1.77 $\log_{10}$ vs -0.73 $\log_{10}$ CFU/g for nonmucoid isolates). In addition, TIP achieved higher mean sputum concentrations (measured 30 minutes post dose) than TIS (1979±2770 µg/g vs 1074±1182 µg/g). The most common adverse event reported was cough which was higher in TIP (25%) than TIS (4%) treated patients. However, the rate of bronchospasm, nephrotoxicity, ototoxicity and serious adverse events were similar in both groups.

Colistin inhalation powder

Colistimethate sodium (colistin), a cationic polypeptide antibiotic (a polymyxin) which functions by disrupting the bacterial cell membrane, can also be delivered as a dry powder. It is commercially known as Colobreathe dry powder for inhalation. Colobreathe is an encapsulated dry-powder formulation administered using a hand-held inhaler (Turbospin). Colobreathe comes as capsules containing the equivalent of 125 mg colistimethate sodium (1 662 500 IU) in fine particle form. One inhaled capsule delivers the equivalent of a nebulised dose (≥ 2 MU). A Phase II, randomized open-label study was conducted in CF patients ages 6 years and older with chronic *P. aeruginosa* infection (21). After at least two 28 days of on-off TIS cycles, 380 subjects were randomized to either one capsule of Colobreathe dry powder for inhalation twice daily (without interruption) or three 28 day cycles of TIS (300 mg/5 ml) delivered via PARI nebulizer twice daily for a total study duration of 24 weeks. After 24 weeks, the adjusted mean

difference between Colobreathe versus TIS in change in FEV_1% predicted was -0.97% (95% CI -2.74% to 0.86%), favouring TIS. This did not exceed the preset non-inferiority limit of the 95% CI (-3%). Few colistin-resistant *P. aeruginosa* isolates emerged and adverse events were similar in both groups. Although more patients in the Colobreathe group rated their device as easy to use compared to the TIS group, the improvement in lung function over the 6 month study period was negligible in both treatment arms.

Inhaled liposomal amikacin

Another novel method of delivery is the packaging of antibiotics within liposomes for aerosolization. An example of this is inhaled liposomal amikacin, otherwise known as Arikace. Arikace contains high concentrations of aqueous, water soluble amikacin, an aminoglycoside, encased within a liposome (22). The liposome is composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol which makes it very similar to surfactant, allowing it to be cleared from the lungs through a common pathway (22). Because the liposome is very small and has a neutral charge (shielding the positively charged amikacin from the negatively charged CF sputum), the drug is able to effectively penetrate into CF sputum and bacterial biofilms (23). Once at the site of infection, liposomes release the active drug, amikacin, upon exposure to rhamnolipids, a by-product of the *P. aeruginosa* bacteria itself. Phase I studies were done in 24 CF patients with chronic *P. aeruginosa* infection who received 500 mg of liposomal amikacin by inhalation via the PARI LC STAR nebulizer once daily for 14 days (24). The relative change from baseline in forced expiratory volume in 1 second (FEV_1) % predicted was 10.8% ($p<0.001$) and 5.62% ($p=0.073$) on days 7 and 14, respectively. However, there was no statistically significant change in the sputum *P. aeruginosa* bacterial density. Follow up Phase II studies

were then performed comparing multi-dose Arikace delivered via eFlow nebulizer to placebo for 28 days in CF patients with chronic *P. aeruginosa* infection (25). Overall, Arikace was well tolerated. Subjects receiving 280 mg of Arikace once daily had an increase in FEV_1 from baseline of 0.10±0.128 L compared to an increase of 0.011±0.101 L (p=0.009) in the placebo group. Patients receiving 560 mg Arikace had an FEV_1 increase from baseline of 0.081±0.161 L compared to 0.011±0.101 L (p=0.033) in the placebo group and this increase was maintained at day 56, even after 28 days off study drug (0.093±0.203 L vs -0.032±0.119 L, p=0.003). Mean *P. aeruginosa* sputum density was also significantly reduced in this treatment group at day 14, day 28 and even at day 35 (7 days off treatment). A Phase III randomized, open label trial comparing Arikace to TIS in CF patients with *P. aeruginosa* infection has recently been completed but the results have not yet been published (clinicaltrials.gov identifier NCT01315678). In addition, the ability to deliver drug into biofilm structures within CF sputum may be beneficial in treating other multidrug resistant pathogens in CF such as *Mycobacterium abscessus*. A Phase II trial (clinicaltrials.gov identifier NCT01315236) is currently underway, in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), to study the use of Arikace for the treatment of recalcitrant nontuberculous mycobacteria lung disease in both CF and non-CF patients.

Implications of antimicrobial resistance

Definition of resistance and effects of sub-inhibitory drug concentrations

Current definitions of bacterial resistance rely on antibiotic concentrations achieved by systemic therapy (oral or intravenous drug administration). "Breakpoints' by which resistance is

defined by the Clinical Laboratory Standards Institute (CLSI) are therefore generally quite low, requiring, for example, a minimum inhibitory concentration (MIC) of tobramycin of ≤ 4 μg/ml for a *P. aeruginosa* isolate to be deemed susceptible (26). However, aerosolized antibiotics can achieve significantly higher sputum concentrations than intravenous antibiotics, in the order of 100 fold greater (27). Thus, the clinically relevant definitions of resistance based on nebulized delivery have yet to be determined (28).

Although aerosolized antibiotics have the advantage of being able to deliver high intrapulmonary concentrations of drug, antimicrobial resistance can still develop. There can be areas of atelectasis and mucous plugging causing certain areas of the CF lung to be under-ventilated resulting in sub-inhibitory concentrations of antibiotics. Sub-inhibitory concentrations of antibiotics have been shown to increase the mutation frequency for *P. aeruginosa* as well as promote the formation of biofilm, leading to the selection of antimicrobial resistance (29). This is particularly true in the case of systemic administration of antibiotics, where the pulmonary drug concentrations are even lower, often far below the minimum inhibitory concentrations for organisms such as *P. aeruginosa*. However, certain antimicrobials such as azithromycin have been shown to have effects against *P. aeruginosa* even at sub-inhibitory concentrations. Sub-inhibitory azithromycin concentrations can suppress motility and the production of several virulence factors, including proteases, pyocyanin, exotoxin A, phospholipase C and exopolysaccharides as well as the production of quorum sensing signal molecules (30).

Consequences of antimicrobial resistance

Antimicrobial resistance may still be relevant in CF and has potential clinical implications. Although in vitro antimicrobial susceptibility testing, whether done on planktonic

(31) or biofilm (32) growth of bacteria, has been shown not to correlate with clinical outcomes, greater drops in sputum bacterial density of *P. aeruginosa* do correlate, albeit to varying degrees, with increases in FEV_1 (33). In addition, the development of antimicrobial resistance contributes to the persistence of bacteria within the CF lung and chronic infection with pathogens such as *P. aeruginosa*, *Stenotrophomonas maltophilia* and methicillin-resistant *Staphylococcus aureus* (MRSA), is associated with worse clinical outcomes (34-36). Finally, there is evidence to suggest that CF patients infected with multi-drug resistant organisms have worse survival following lung transplantation (37).

The development of antimicrobial resistance is a particular problem in the long-term treatment of chronic CF pulmonary infections due to pathogens such as nontuberculous mycobacteria. Infection with bacteria of the *M. abscessus* complex, consisting of *M. abscessus*, *M. bolletti* and *M. massiliense*, are the most concerning, associated with more rapid lung function decline and death in CF (38-39). Empiric therapy often consists of a macrolide such as azithromycin or clarithromycin, an aminoglycoside such as amikacin and cefoxitin intravenously. However, *M. abscessus* subspecies *abscessus* often have an erythromycin ribosomal methylation (*erm*) gene which encodes for enzymes that methylate the 23S ribosomal RNA within the 50S ribosomal subunit, thus reducing the affinity of macrolides for their specific target in impairing protein synthesis (40). Although expression of the *erm* gene can be variable, its detection though molecular methods would preclude the use of a macrolide based regimen in the treatment of these infections. Even in the absence of the *erm* gene, *M. abscessus* species may resistant to macrolides through other mechanisms, such as efflux pumps (41). In vitro antimicrobial susceptibility testing can involve delays, however, requiring incubation of *M. abscessus* isolates with macrolides for 14 days to detect inducible mechanisms of resistance. A

more rapid, molecular technique that could detect all the genes encoding mechanisms of antimicrobial resistance within a bacterial genome has potential future clinical applications.

Effects on the microbiome

Antibiotics not only have effects on the predominant bacterial pathogen in an individual's lung, but also on the multitude of other species that are known to exist as part of the CF lung microbiome. Changes in the microbiome in one location of the body, such as the gut, may even influence the bacterial composition in the lung (42). It is now well recognized that the CF pulmonary microbiota is composed of a diverse polymicrobial community that interact with one another and are influenced by repeated, spectrum courses of antimicrobial therapy (43). Organisms that are not routinely identified on CF cultures, such as anaerobes, have been shown, not surprisingly, to have reduced susceptibility to antibiotics typically used to treat CF patients (44). In a study of adult CF patients, cultured *Prevotella* isolates were significantly more resistant in vitro to amoxicillin-clavulanate and doxycycline than isolates from healthy adults (45). Use of antibiotics is the primary driver of decreased microbiome diversity in the CF lung (46). In an analysis of the effect of antibiotic use on microbial diversity in CF, intravenous antibiotic use, rather than oral or inhaled, had the highest probability of effecting greater change in community diversities, presumably due to the type of antibiotic used (eg. meropenem), with wide spectrums of action (47). Several investigators have studied the changes in the composition of the pulmonary microbiome at the onset of a CF exacerbation and have failed to identify any clear pattern to explain their occurrence (48-49). One group did observe an increase in the relative abundance of *Gemella* species at exacerbation, suggesting that this anaerobe may be involved in triggering a pulmonary exacerbation (50). Antimicrobial treatment of pulmonary

exacerbations has been associated with very early effects on the CF microbiome (as early as day 3 of treatment) including significant reductions in the relative abundance of *Pseudomonas* which correlates with improvements in FEV_1% predicted (51-52). In contrast, the decrease in anaerobic bacteria with antibiotic treatment in more variable and less strongly associated with improvements in lung function (52-53). Regardless, the pulmonary microbiome quickly returns to pre-treatment states following the end of antimicrobial treatment, indicating that it is quite resilient to change (49). Whether these combined results suggest inadequate treatment of dominant pathogens such as *P. aeruginosa* or potentially more appropriate, alternative targets such as anaerobes, is currently unknown. To answer these questions, further research, using large numbers of longitudinally collected samples at multiple time points with detailed corresponding antibiotic history, is required.

Non-antibiotic treatments

Antimicrobial resistance might be further prevented by using non-antibiotic treatments which have activity against bacterial species; these include the use of antibiotic adjuvants. These are a diverse group of novel agents which act by increasing the organism's antibiotic susceptibility; by reducing its virulence; or by rendering the organism more susceptible to the host immune system (54). These include agents such as gallium, antimicrobial peptides and anti-biofilm compounds. Additionally, vaccination strategies against *P. aeruginosa* have been attempted to prevent initial infection with this organism.

Gallium metal

Gallium metal, structurally similar to iron (Fe), can substitute itself in many of the Fe dependent pathways essential in the growth and functioning of many bacteria such as *P. aeruginosa* (55). In such a way, gallium can inhibit the growth of bacteria such as *P. aeruginosa*. In vitro studies have shown that a liposomal gentamicin formulation with gallium metal is more effective than gentamicin alone in eradicating antibiotic-resistant *P. aeruginosa* isolates from CF patients, grown planktonically or as a biofilm (56). In fact, the liposomal gallium-gentamicin combination was the only formulation that completely eradicated biofilms and blocked quorum-sensing molecules at very low concentrations of gentamicin (0.94 mg/L). Gallium is thus currently being investigated for its antibacterial properties and its potential applications in the treatment of pulmonary infections in CF.

Antimicrobial peptides

Another example of non-antibiotic compounds with antibacterial effects are antimicrobial peptides. Antimicrobial peptides, also known as defensins, are endogenous peptides that are produced by myeloid and epithelial cells and are part of the innate immune system and the first line of host defense (57-58). Antimicrobial peptides disrupt the bacterial cell membrane causing lysis and death and thus have activity against a wide variety of microbial species (Gram-positive, Gram-negative organisms, fungi), attractive features in the treatment of CF pulmonary infections (59). Specific peptides such as cathelicidin peptides and lactoferrin have been shown to have activity against CF clinical isolates of *P. aeruginosa* and *Burkholderia cepacia* complex (60-61). However, despite their rapid and wide spectrum of activity, even mechanisms of resistance against antimicrobial peptides have been identified (62-63).

Anti-biofilm compounds

Much of the difficulty encountered in treating pulmonary infections in CF patients is due to the fact that bacteria such as *P. aeruginosa* grow as a biofilm in the airways of CF patients with chronic pulmonary infections (64-65). When infection becomes chronic, *Pseudomonas* grow as biofilms; namely, bacterial populations embedded in a complex matrix comprising exopolysaccharide (bacterial alginate), airway mucins and neutrophil-derived DNA. The biosynthesis and regulation of bacterial alginate (which influences the architecture of the biofilm) is complex and involves mutations in bacterial regulatory genes (muc mutations) and an important population-based regulatory system known as quorum sensing (QS) (66-68). The quorum-sensing genes (*Las*R and *Rhl*R) encode for virulence factors such as proteases, rhamnolipids, hemolysins, iron-scavenging pigments, catalase and superoxide dismutase (69). Biofilms are highly resistant to killing by antibiotics for many reasons. This antimicrobial resistance is due in part to the polysaccharide matrix which retards the diffusion of antibiotics, but more importantly results in a slow rate of bacterial growth within the matrix, and in the case of aminoglycosides, to electrolyte interference with antibiotic activity (70). Much work has therefore been done to develop compounds that can inhibit or disrupt bacterial biofilms to allow antibiotics to work. An example of this is alginate oligosaccharides (OligoG), a biopolymer which is found in brown algae. In vitro studies have demonstrated that OligoG can not only inhibit biofilm formation and disrupt established 24-hour biofilms but also potentiate antibiotic effects, decreasing the minimum inhibitory concentration (MIC), up to 512-fold, for macrolides, β-lactams and tetracyclines, against important multidrug resistant pathogens such as *P. aeruginosa* and *B. cepacia* complex (71). Although its mechanism of action is not yet fully understood, it is postulated that it disrupts the bacterial membrane by chelating cations, allowing

antibiotics to effectively penetrate the cell. Phase II studies of inhaled dry powder OligoG in adult patients with CF are currently underway (clinicaltrials.gov identifier NCT02157922). Another potentially useful anti-biofilm agent is garlic and garlic extracts, in particular, the compounds allicin and ajoene. Using high-throughput screening, garlic was discovered to inhibit quorum sensing through a presently unknown mechanism (72). When *P. aeruginosa* was grown in vitro in continuous-culture flow chambers, the garlic-treated biofilms, in comparison to untreated controls, were susceptible to both tobramycin and polymorphonuclear leukocyte killing (73). In addition, in a mouse model of *P. aeruginosa* pulmonary infection, the treated group had a greater decrease in lung bacterial burden compared to the placebo group. When tested in a trial of 26 CF patients, there was a greater decline in FEV_1 from baseline for patients in the placebo arm compared to the garlic treated arm and a greater increase in weight in the garlic treated arm compared to the placebo arm, although neither of these differences between treatment groups was significant (74). With this suggestion of improvement with garlic treatment, larger trials investigating its potential benefit in CF are warranted. The potential value of trials of natural products, either alone or, more likely, as adjuvants to present antimicrobial therapy, has recently been emphasized by the killing potential of pure allicin and allicin-containing aqueous garlic extract against the *Burkholderia cepacia* complex, a multiresistant and life-threatening pathogen for which there are few treatments (75). Another potential advance in the treatment of *Burkholderia*, comes from deliberately targeting the organism's unusual lipopolysaccharide and demonstration of the potent antimicrobial properties of the novel LPS inhibiting agent, CHIR090 (76).

Immunization against P. aeruginosa

Given the intrinsic difficulties in treating established *P. aeruginosa* chronic infections in the CF lung, preventing initial infection through immunization is an attractive alternative. Multiple attempts have been made to develop vaccines using different antigenic components of the *P. aeruginosa* bacterium, including outer membrane proteins, surface polysaccharides and flagellar proteins. Antibody studies done in CF patients have demonstrated that IgG, IgM and IgA against *P. aeruginosa* whole cell and specific outer membrane proteins can be detected in serum and bronchoalveolar lavage fluid following both upper and lower respiratory tract infection, suggesting potential vaccine antigens (77). Surface polysaccharides are another possible antigenic option but polysaccharides such as alginate, which is a conserved antigen across many *P. aeruginosa* strains, is poorly immunogenic in humans and does not induce the production of protective antibodies. In an effort to boost its immunogenicity, *P. aeruginosa* polysaccharides have been conjugated to toxins as well as flagellar proteins as vaccines (78-79). Yearly immunization with O-polysaccharide-toxin A conjugate vaccine against *P. aeruginosa* in 25 CF patients induced sustained but low levels of vaccine-specific serum IgG antibodies (79). In contrast, *P. aeruginosa* flagella vaccines produce high and long-lasting serum antibody titers against flagella antigens (80). In the largest randomized, placebo controlled trial of a bivalent flagella *P. aeruginosa* vaccine in 483 CF patients, the vaccine produced high and long-lasting serum antiflagella IgG titers (81). After 4 intramuscular vaccinations, the relative risk of *P. aeruginosa* infection in the per-protocol group was 0.66 (95% CI: 0.46-0.93, p=0.02) compared to placebo, indicating that vaccination provided a 34% degree of protection (82). Of note, some vaccinated patients were infected with *P. aeruginosa* strains that expressed flagella antigens not included in the vaccine, suggesting that a multivalent flagella vaccine preparation may be required. Although the results of this vaccine trial appeared promising, another randomized,

double-blind controlled trial conducted by industry (Aerugen Berna Vaccine) of a polyvalent (exotoxin-A-polysaccharide) *Pseudomonas* vaccine in 476 participants was prematurely terminated with no reported results, raising the concern of publication bias as noted in a Cochrane review (83). Detecting the efficacy of *P. aeruginosa* vaccines in clinical trials may have also been affected by the increasing and, in some centers, continuous use of one or more nebulized antibiotics, and a reduced frequency of exacerbations. Thus, although immunization against *P. aeruginosa* in CF patients cannot yet be recommended (83), it bears promise that deserves ongoing investigation. Mucosal immunization, reflecting the actual route of infection, may be more effective than intramuscular or subcutaneous vaccination. *P. aeruginosa* vaccination in 48 healthy volunteers demonstrated a significant rise in IgA and IgG in the lower airways only after nasal and oral, not systemic, vaccination (84). In addition, prophylaxis against *P. aeruginosa* infection using anti-*P. aeruginosa* antibodies has also been tried in CF. Gargling with anti-*Pseudomonas* IgY, isolated from the egg yolks of hens immunized with *P. aeruginosa*, has been shown to decrease the number of *P. aeruginosa* infections in a small number of individuals with CF (85), presumably by preventing initial colonization of the organism in the oropharynx. A phase III study to evaluate the clinical efficacy and safety of avian polyclonal anti-*Pseudomonas* antibodies (IgY) in the prevention of recurrence of *P. aeruginosa* infection in CF patients is currently underway (clinicaltrials.gov identifier NCT01455675). At present, there is no commercially-available anti-pseudomonal vaccine.

Lifetime antimicrobial burden and toxicity

Burden of care and adherence

Although the repeated and long-term use of antibiotics has been crucial in slowing lung function decline and improving survival in people with CF, it has added a significant burden of care in these individuals. In addition to pancreatic enzymes, vitamins, physiotherapy, inhaled mucolytics and possibly inhaled glucocorticoids and/or bronchodilators, patients may also be taking inhaled tobramycin, oral azithromycin, in addition to the prescribed oral and intravenous antibiotics for pulmonary exacerbations. CF patients with chronic *P. aeruginosa* infection are frequently on inhaled tobramycin for life, given that there are no clear guidelines or data as to when to stop this therapy. Furthermore, with the advent of other anti-pseudomonal inhaled antibiotics such as aztreonam, some patients are now being treated with a second aerosolized antibiotic in the "off month"of the tobramycin cycle as an attempt to maintain FEV_1, adding to the complexity of their treatment plan. More rapid, efficient nebulizers and dry powder delivery systems such as the podhaler are helpful in terms of reducing the time required for patients to do their therapies and hopefully increasing their compliance and adherence. Adherence to therapies is an important component to the improvement of outcomes; high adherence with tobramycin inhalation solution, for example, has been shown to be associated with a decreased risk of hospitalization (86). Unfortunately, studies have demonstrated that only 32% of CF patients are fully adherent to prescribed nebulized antibiotic treatment (87). Chip-based recording nebulizers can store data such as treatment date, time, duration and completeness of dose, that can then be downloaded to generate an accurate report of adherence (88). Using this type of information, it is possible to provide patients with feedback and adjust treatment schedules to hopefully improve compliance (89).

Antimicrobial toxicities

In addition to the burden of lifelong antimicrobial therapies, CF patients have to contend with the associated toxicities as well. These toxicities are most apparent for aminoglycosides, for which the primary side effects are nephrotoxicity and ototoxicity. Intravenous aminoglycosides are a mainstay of therapy for the treatment of pulmonary exacerbations in CF. Serum drug levels should be monitored to avoid nephrotoxicity. Given that aminoglycosides function in a concentration dependent manner, once daily dosing is likely to be more efficacious as well as associated with fewer toxicities (90). Minimizing long-term nephrotoxicity is important with respect to lung transplantation as well because renal impairment is a strong predictor of decreased survival following lung transplantation in CF (91). The ototoxicity seen with aminoglycosides can either be a sensorineural hearing loss or a vestibular toxicity and is associated with drug accumulation within the inner ear cells with prolonged use. For the treatment of nontuberculous mycobacterial pulmonary infections in individuals with CF, in which aminoglycosides such as amikacin are frequently used for a significant portion of the treatment course, intravenous administration should be limited to 4 weeks with the remainder of the therapy given through inhalation. Even with aerosolized administration of aminoglycosides, hearing loss has been reported in CF patients with prolonged use (92). In addition to the toxicities specifically seen with aminoglycoside use, other adverse events in CF patients from prolonged intravenous antimicrobial therapy include thromboses secondary to central catheter placement, for example.

Conclusions

The advantages of aerosolized therapy suggest that this is an important area for future drug development. Antimicrobials should also be designed to be effective in the conditions encountered in the CF lung such as against bacterial biofilm growth, under anaerobic conditions and be able to penetrate sputum. With the discovery of small molecules that can restore the function of the cystic fibrosis transmembrane conductance regulator (CFTR), it is hoped that one day individuals with CF will no longer be afflicted with these difficult to treat pulmonary infections caused by opportunistic, multidrug resistant pathogens. However, until the underlying defect can be corrected in all CF patients, antimicrobial treatments that decrease the bacterial contribution to the underlying pulmonary hyper-inflammatory state are vital to the quality of life and survival of individuals with CF.

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