



Antibiotic treatment of CF lung disease: From bench to bedside

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Abstract

Chronic infection of the respiratory tract is a hallmark of cystic fibrosis (CF). Antibiotic treatment has been used as one of the mainstays of therapy and together with other treatment modalities has resulted in increased survival of CF patients. Increasing resistance of CF-specific pathogens to various classes of antibiotics explains the need for novel antibiotic strategies. This review focuses on the future development of new antibiotic therapies, including: (1) New targets, (2) novel antibiotic regimens in CF, (3) new antibiotics, and (4) other investigational therapies. In addition, we briefly summarize developments in the area of microbial diagnostics and discuss interactions between the complex pulmonary microflora.

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1. Introduction

The projected life expectancy of individuals with cystic fibrosis (CF) has increased significantly during the last 20 years and is expected to be >50 years for individuals born in 2000 [1]. The development of novel antibiotic drugs and therapeutic regimens has contributed greatly to advances in the treatment of CF patients [2]. However, these improvements are limited by several factors: (1) Chronic infection with typical CF-specific microorganism cannot be cured and pulmonary infectious disease still causes the majority of mortality and morbidity in CF. (2) The interaction between the host and pathogens is complex in CF lung disease. Young patients are often infected with *Haemophilus* spp. and *Staphylococcus aureus*. Subsequently, *Pseudomonas aeruginosa* becomes the predominant pathogen. *Burkholderia cepacia* complex and *Stenotrophomonas maltophilia* also infect CF airways [3].

These and other bacteria adapt to the CF-specific environment. In addition to the broad antibiotic resistance of some of these microorganisms, biofilm formation and the development of cellular resistance mechanisms, such as the induction of efflux pumps, attenuates the effectiveness of antibiotic therapy. Multidrug resistance (MDR) is a clinically relevant problem. While this review focuses on bacterial infection, fungal and viral infections are recognized as clinically relevant to the development of CF lung disease [4].

2. New diagnostic approaches

2.1. Analysis of emerging pathogens and of the CF microbiome

The bacteria most frequently isolated from the sputum of patients with CF such as *S. aureus*, non-capsulated *H. influenzae* and *P. aeruginosa* are identified by standard aerobic microbiological methods. Moreover, individuals with CF are susceptible to chronic respiratory tract infection with fast-growing mycobacteria and gram-negative bacterial species that are intrinsically resistant to a broad range of antimicrobial agents and usually are poor air-

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way colonizers and not pathogenic for healthy persons. These emerging and/or rare CF pathogens include *Mycobacterium chelonae*, *Mycobacterium abscessus*, *Streptococcus* spp., *Stenotrophomonas maltophilia*, *Achromobacter* (*Alcaligenes*) *xylosoxidans*, *Inquilinus limosus* and several species within the genera *Burkholderia*, *Ralstonia* and *Pandoraea*. The majority of these species are not included in automated identification systems. Hence, definitive diagnosis requires 16S rDNA sequencing and thorough expertise in CF microbiology. Recent culture-independent DNA-based profiling of CF respiratory secretions uncovered a further layer of complexity of CF microbiology [5,6]. The bacterial community within the CF lung was found to be polymicrobial in nature including a range of anaerobic species primarily within the genera *Prevotella*, *Veillonella*, *Propionibacterium* and *Actinomyces* [7,8]. Although the clinical relevance of anaerobic bacteria for CF lung infection is an open question, we can envision that CF microbiology will soon become much more complex than the routine clinical microbiology than it is now.

2.2. Sampling of upper and lower CF airways

Besides the ever-growing list of CF-associated bacterial pathogens, the modes of sampling have recently re-emerged as a timely issue of CF diagnostic microbiology. How many samples should be collected from which site by applying which technique? Typically, just one throat swab, spontaneously expectorated sputum sample or induced sputum will be processed at the patient's visit to the CF clinic. However, according to culture-independent 16S rDNA profiling a single expectorated or induced sputum sample represents only approximately 60% of the bacterial species found through the comprehensive analysis of five samples collected in series on the same day [9]. These data suggest that sampling in greater depth may be a more effective strategy. It remains to be seen whether such a procedure is practically feasible for routine patient care.

Sputum bacteriology is taken as a surrogate for the microbial colonisation of the lower airways. The microbiome of the upper airways is usually not assessed. A recent study challenges this attitude [10]. The authors sampled the upper airways of CF patients by nasal lavage. They found identical *S. aureus* and *P. aeruginosa* genotypes colonising the upper and lower airways of CF patients [10]. This result suggests that the upper airways are a gateway for the acquisition of common CF pathogens and act as a reservoir for them. Because nasal lavage requires training and active cooperation, it is not applicable to all age groups. However, it is sufficiently rapid and easy to become widely introduced into the clinic.

2.3. Assessment of clonal variants

A further characteristic feature of CF isolates that impedes the straightforward identification of taxa is their broad spectrum of numerous and often atypical phenotypes. Morphotypes of a single clone may differ substantially in their antimicrobial susceptibility. Of particular significance for

antimicrobial treatment are small colony variants [11] and hypermutable strains [12] that often confer multidrug-resistant phenotypes to the polymicrobial community of CF lungs. Hence, the presence of these phenotypic variants in bacteriological specimens and their antimicrobial susceptibility should be communicated to the CF physician.

A further challenge is the different lifestyle of the microbes in the CF lungs that either may thrive in the planktonic state or may be embedded in intraluminal biofilms [13]. Standard susceptibility testing assesses the effect of antimicrobial drugs on planktonic bacteria in the late exponential or early stationary growth phase. Hence, *in vitro* tests mimic only part of the microbial community in CF airways. However, this deficit has been recognized by researchers who set up protocols for susceptibility testing of biofilm-grown pathogens such as the Calgary Biofilm Device [14] or a bacterial viability stain in combination with automated confocal laser scanning microscopy [15].

3. Novel antibiotic regimens in CF

As chronic *P. aeruginosa* infection is one of the major factors contributing to progressive deterioration of lung function in CF patients, pulmonary exacerbations due to *P. aeruginosa* are treated with IV antibiotics, mostly combinations of a beta-lactam, such as ceftazidime, and an aminoglycoside, such as tobramycin. In conventional treatment regimens, both antibiotics are administered in the form of thrice-daily short infusions, but new treatment regimens are sought to decrease toxicity and improve efficiency, especially as multiple drug resistance develops. Aminoglycosides have concentration-dependent antibacterial activity and a post-antibiotic effect, whereas beta-lactam antibiotics display time-dependent antibacterial activity. Once daily tobramycin provides a greater peak concentration that improves efficacy while reducing overall exposure to the drug and hence, decreasing the risk of toxicity. Efficacy was shown to be comparable for once and thrice-daily dosing of tobramycin in CF [16]. Although decreased nephrotoxicity was demonstrated only in non-CF patients, the US CF Foundation recommends once-daily dosing of aminoglycosides to avoid the risk of toxicity [17]. There are limited clinical data about the toxicity of continuous ceftazidime infusions in CF patients, but recent studies demonstrate that continuous infusion does not increase drug toxicity and appears to be as effective as short infusions. Importantly, continuous ceftazidime infusions give better results in CF patients harboring resistant isolates of *P. aeruginosa* [18]. Such a treatment regimen for IV antibiotics might also facilitate treatments when they take place at home, making it possible for the nurse to visit the patient only once per day. It also allows patients to continue to attend school or work if their general status permits during IV antibiotic treatment.

Outcomes in CF patients with chronic pulmonary Pseudomonal infection can be improved by the daily use of nebulized anti-Pseudomonal antibiotics, especially tobramycin. However, long nebulization times may adversely impact on

adherence to the treatment regimen. New nebulizers are expected to reduce drug nebulization times. For example, delivery of tobramycin solution for inhalation (TSI) via the PARI eFlow[®] rapid nebulizer (PARI GmbH, Starnberg, Germany) is quicker (e.g. ~4 min) than the same dose of TSI administered via the Pari LC[®] PLUS nebulizer (PARI GmbH) powered by the DeVilbiss Pulmo-Aide[®] compressor (DeVilbiss, Philadelphia, USA) (e.g. ~15 min). The systemic exposure and sputum concentration of tobramycin from eFlow rapid and LC PLUS appear to be broadly similar [19].

Prevention of chronic *P. aeruginosa* infection by antibiotic therapy initiated shortly after developing a new *P. aeruginosa* infection is part of the standard care of CF patients. It is usually effective, but the optimal therapeutic regimen and duration for early treatment remains unclear. The EarLy Inhaled Tobramycin for Eradication (ELITE) study has been conducted in 98 CF patients (aged ≥ 6 months) with early *P. aeruginosa* infection to assess the efficacy and safety of two regimens (28 and 56 days) of TSI (TOBI[®]) [20]. It concluded that TOBI[®] twice daily for 28 days is as effective as 56 days and well tolerated therapy for early *P. aeruginosa* infection in CF patients.

The results of the EPIC (Early Pseudomonas Infection Control) clinical trial were presented at the 2009 North American Cystic Fibrosis Conference [21]. The study enrolled 304 children with CF aged 1 to 12 years to evaluate early intervention with inhaled and oral anti-Pseudomonal therapy at first isolation of *P. aeruginosa* from respiratory cultures. Two different antimicrobial treatment regimens were investigated: (1) culture-based therapy (i.e. treatment based on microbiological findings of *P. aeruginosa* positive respiratory cultures) and (2) cycled antibiotic therapy (i.e. treatment provided systematically in quarterly cycles until the end of the 18-month study period). Inhaled tobramycin was associated with oral ciprofloxacin or with placebo. No difference was observed between the treatment groups in the proportion of recurrent *P. aeruginosa* positive cultures, in time to pulmonary exacerbation requiring antibiotics or in other measures of clinical efficacy (change in mean weight or in mean FEV₁ from baseline) [21].

Besides antibiotic treatment for *P. aeruginosa*, particular attention should be paid to patients colonized with methicillin resistant *S. aureus* (MRSA). Although there is less clinical evidence than for eradication of *P. aeruginosa*, eradication of MRSA is achievable in the majority of patients even when significant time has lapsed from initial isolation. In some instances, up to 3 courses of antibiotics were required to achieve eradication [22]. The most frequent regimens included linezolid or rifampicin in association with fucidin, trimethoprim or doxycycline.

4. Antibiotics in the pipeline

The anti-infective therapeutics pipeline contains mainly new inhaled formulations of existing antibiotics to improve delivery times, which will facilitate adherence to treatment. The pipeline also contains antibiotics with different mecha-

nisms of action against *P. aeruginosa* and other CF pathogens to improve their efficacy.

Aztreonam lysine for inhalation (AZLI) is now available to CF patients. It was evaluated in 246 CF patients (>6 years) on maintenance treatment for *P. aeruginosa* airway infection in a randomized, double-blind, placebo controlled study [23]. Patients were treated with 75 mg AZLI or placebo, twice or three times daily for 28 days and then monitored for 56 days. AZLI treatment delayed the time until inhaled or IV anti-Pseudomonal antibiotics were required, improved respiratory symptoms and pulmonary function and was well tolerated.

New drug formulations have appeared, such as Tobramycin Inhalation Powder (TIP), a novel dry-powder formulation designed to deliver a high dose of tobramycin topically to the lungs for management of chronic *P. aeruginosa* infections. Serum tobramycin pharmacokinetics (PK) profiles were similar for TIP and TSI. Four capsules of 28 mg TIP (total tobramycin dose 112 mg) produced comparable systemic exposure to 300 mg TSI, in less than one-third of the administration time. The most common adverse events associated with TIP were cough and dysgeusia [24]. The results of a phase 3 study are expected shortly.

Inhalable dry powder is also being developed for ciprofloxacin, alone or in association with recombinant deoxyribonuclease [25]. Co-delivery of antibiotics and DNase using an inhalable particle system may be a promising strategy for local anti-Pseudomonal therapy in CF airways.

Arikace[™] is a sustained-release lipid formulation of amikacin for inhalation, delivered in 10–13 minutes by Pari eFlow[®] [26]. In a phase 2 study, Arikace[™] administered once daily for 28 days was well tolerated. Patients receiving Arikace[™] had superior clinical benefit and prolonged time to exacerbation as compared to placebo. Patients receiving 560 mg of Arikace[™] demonstrated improvement in lung function over baseline while patients on placebo declined over time. A treatment effect of 14% of FEV₁ % predicted was observed at two months after discontinuing study drug.

Liposome entrapment of other antibiotics, such as tobramycin and polymyxin B, may be a promising therapeutic strategy for CF lung infections. This formulation reduces antibiotic inhibition up to 100-fold and the colony-forming units (CFU) of endogenous *P. aeruginosa* in sputum by 4-fold compared to the conventional antibiotic [27]. Liposomes are often used to improve the pharmaceutical preparation of lipophilic substances and are also known to increase penetration into biofilms [28]

Aerosolized levofloxacin is more potent than aminoglycosides and aztreonam against *P. aeruginosa* biofilms. High concentrations of levofloxacin readily achievable in the lung following aerosol delivery might be useful for the management of pulmonary infections in CF patients [29].

Finally, a new anti-Pseudomonal cephalosporin, CXA-101, seems promising for patients with CF, bettering the activity of ceftazidime against resistant *P. aeruginosa* strains and retaining activity against those with carbapenem resistance [30].

5. Novel microbial targets

Novel microbial targets are urgently needed to overcome rising antibiotic resistance of important human pathogens. However, evidence from previous antimicrobial screenings, *in silico* analysis, and experimental target evaluation suggests that the number of novel bacterial targets might be severely limited [31]. An exhaustive screening in enterobacteria, for example, revealed that the targets, which were identified to be essential for the bacterial cell almost all belonged to metabolic pathways that are inhibited by current antibiotics or that have previously been considered for antimicrobial development [32].

Current research projects in the field of antimicrobial treatment of bacterial infections in CF screen small molecule libraries, design antisense nucleic acids or investigate antimicrobial peptides for their activity against typical CF pathogens. Adhesins [33] and the c-di-GMP signalling network [34] are discussed as novel targets for antimicrobial treatment. An active field is the search for quorum-sensing inhibitors [35,36]. Based on experience in Japan on the efficacy of long-term treatment of chronic *P. aeruginosa* pulmonary infections with azithromycin [37], this well-known macrolide was recently introduced into the CF clinic. One major mode of its multiple actions is the inhibition of quorum sensing [38]. Thus, drugs that have been in clinical use for many years are being re-evaluated as beneficial agents for antimicrobial therapy in CF. Other examples are the increasing use of systemic colistin that disrupts the gram-negative inner membrane for the treatment of multi-resistant *P. aeruginosa* [39] or systemic temocillin, a 6-(α)-methoxy-carboxypenicillin with exceptional stability in the presence of most β -lactamases, for the treatment of pulmonary infections with the *B. cepacia* complex [40].

6. Phage-based antibacterial therapy

Phage-based therapies are based on the natural interaction between (bacterio-)phages and their host, the bacterial cell. Lytic phages kill their bacterial host following amplification and release progeny phages [41]. The possibility of applying the bacteriolytic activity of phages to treat infections was recognized as early as the 1920s [42]. Several non-randomized studies claimed efficacy, however, the quality of many of these studies was low. Potential advantages are: (1) phage-based therapies would be complementary to classical antibiotic approaches, (2) current data indicate low toxicity, (3) bacterial strains resistant to known antibiotics might be susceptible to phage based therapies. Obstacles to phage-based therapies include the narrow host range of an individual phage. To overcome this problem, many studies have been performed with phage cocktails with multiple components. This will cause problems with drug approval by drug regulation authorities. The principle of phage therapy is also not patentable, resulting in difficulties protecting intellectual property and dissuading investment in this area. While some groups and small companies work in this direction, the translation into clinical medicine appears difficult.

7. Augmentation of the immune system

The modulation of immunity is an intriguing concept for treatment of CF lung disease. However, it is difficult to achieve due to the complexity of the interactions between host and microbe. Inflammation in CF lung disease is largely caused by the recognition of bacterial structures by pattern recognition receptors such as Toll-like receptors (TLRs). Therapeutic targeting of innate immunity with TLR agonists and antagonists might allow increasing antibacterial activity and decrease inflammatory responses [43].

The protease/antiprotease system interaction with several inflammatory cascades is severely dysregulated in CF lung disease. Supplementation of alpha-1-antitrypsin resulted in increased bacterial killing in a preclinical model [44] as well as in CF clinical trial [45].

Vitamin D (VitD) has multiple activities in addition to its regulatory role in calcium homeostasis. Importantly, it is now recognized that VitD regulates the immune system, impacting on many cell types of the adaptive and innate immune systems [46]. In macrophages [47] and airway epithelial cells [48], VitD derivatives increase antibacterial activity and modulate inflammation.

In experimental systems, the immune system can also be modulated to either increase antimicrobial activity or decrease inflammation. For example, pretreatment with inactivated *Hemophilus* protected a murine model of infection against death [49,50]. Also the modulation of TLRs can be applied in experimental systems to modulate inflammation [43].

8. Summary

The exact mechanisms that link mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) with infectious lung disease in CF are unknown. However, development of antibiotic therapy is the most important reason for improvements in the prognosis of individuals with CF. Novel developments in antibiotic therapy include the detection of (a limited number of) new targets, the development of new antibiotics, and the evaluation of the clinical use of novel regimens. One current focus is topically, inhaled antibiotics. While this appears promising, the likelihood is that there will be a limited number of new antibiotics available in the next few years, especially for gram-negative bacteria [51]. Many small companies have stopped their work in this area and the pharmaceutical industry has also significantly reduced its antibiotic research and development programme. The development of novel antibiotics and the evaluation of their clinical application remains a major issue for the treatment of infectious lung disease, such as that in CF.

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Conflict of interest

The authors have no conflict of interest.

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