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New and Emerging Therapies for Pulmonary Complications of Cystic Fibrosis

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Abstract

In the decade since the gene for cystic fibrosis (CF) was discovered, research into potential therapeutic interventions has progressed on a number of different fronts. The vast majority of morbidity and mortality in CF results from inflammation and infection of the airways. Direct delivery of antibacterials to the airway secretions via a nebuliser is an attractive therapeutic option, and a novel formulation of tobramycin designed for such a purpose has been demonstrated to improve spirometry and decrease the need for intravenous antibacterials. In addition, early clinical trials are studying the effects of small peptides with antibiotic properties (defensins) delivered directly to the airways.

Inflammation, whether secondary to infection or an independent feature of CF, leads to progressive bronchiectasis. Anti-inflammatories such as prednisone and possibly ibuprofen have been shown to decrease the rate of respiratory decline in patients with CF but have tolerability profiles that limit clinical usefulness. Macrolides also have anti-inflammatory properties and clinical trials are now ongoing to assess the efficacy of these agents in CF.

Multiple agents, including uridine triphosphate (UTP), genistein, phenylbutyrate and CPX (cyclopentyl dipropylxanthine), have been demonstrated in cell culture to at least partially correct the primary defect of ion transport related to mutations in the cystic fibrosis transmembrane conductance regulator (CFTR). No agent of this class has yet demonstrated clinical effectiveness, but several are in preclinical and early clinical trials.

Finally, gene therapy that allows for the incorporation and expression of wild-type CFTR in respiratory epithelial cells would be definitive therapy for CF. However, multiple barriers to delivery and expression need to be overcome. With research proceeding on these multiple fronts, new therapies for pulmonary complications promise to continue to increase the life expectancy of individuals with CF.

Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations of a gene on the long arm of chromosome 7. This mutation results in the lack of expression and/or function of the cystic fibrosis transmembrane conductance regulator (CFTR), which primarily functions as a cyclic adenosine monophosphate (cAMP)-regulated chloride channel. Although CFTR is normally expressed on all epithelial cells, the major clinical manifestations of CF result from abnormalities in the airway epithelium and from exocrine pancreatic insufficiency. With the advent of pancreatic enzyme replacement

and fat soluble vitamin supplementation, nutrition can now be maintained in most patients with CF. The median life expectancy in CF has increased from early childhood to over 30 years in the last several decades.[1] Progressive lung disease, characterised by chronic inflammation, infection and bronchiectasis, remains the most common cause of morbidity and mortality in CF, resulting in over 90% of deaths in individuals with the disease.

Therapies for CF-related lung disease have largely focused on alleviating the effects of chronic inflammation and infection in the airways. In the US and many other nations, the majority of CF care takes place under the auspices of comprehensive care centres. Much of the improvement in survival in CF relates to the availability and use of antibacterials effective against *Pseudomonas* spp., which infect the airways of more than 80% of adults with CF. Bacterial load and inflammatory mediators can also be reduced by enhancing airway clearance. Recognising that chronic inflammation leads to permanent destruction of airway integrity, anti-inflammatory therapies have been tried in CF with limited success. Therapies designed to correct the basic defect of ion transport have been studied in cell culture and are beginning to enter into early clinical trials. Finally, gene therapy that results in the expression and function of CFTR in airway epithelial cells would represent the ultimate treatment for pulmonary CF, although it would best be provided very early in the course of the disease.

Research in the field of CF is extremely broad based, ranging from basic research aiming to explain the nature of the disease from a genetic and molecular perspective to research in organ transplantation. Potential therapeutic interventions exist throughout multiple stages in the pathophysiology of the disease (fig. 1). A comprehensive discussion of all of the many promising therapies still in preclinical and early clinical trials is not possible in this limited format. A summary of ongoing clinical research can be found at the United States CF Foundation website at http://www.cff.org/research00.htm. This is not a systematic review of the evidence supporting current standard of practice in CF. Information of this sort can be found in the Clinical Practice Guidelines for CF and the Consensus Conference Statements of Concepts in Care, both published in the US by the CF Foundation. In this review of new and emerging therapies in CF, we have focused our attention on those interventions that have either recently been demonstrated to be effective in large, randomised controlled studies or that have entered into clinical trials over the last several years.

1. Antimicrobials

The microbiology of CF airways is relatively predictable, with *Hemophilus influenzae* giving way to *Staphylococcus aureus* early in the course of the disease. By the onset of adolescence, *Pseudomonas aeruginosa* becomes the predominant organism. Antimicrobial therapy can be used on either a scheduled (maintenance) basis or reserved only for acute pulmonary exacerbations of the disease.

Antimicrobial therapy in CF is universally employed for pulmonary exacerbations of the disease, which are manifested clinically by acute worsening in pulmonary symptoms. Treatment for pulmonary exacerbations should be targeted to the predominant pathogenic organisms isolated from patient sputum and guided by susceptibility testing. In the case of *P. aeruginosa* and other Gram-negative rods, use of two antibacterials from different classes (e.g. a β-lactam and an aminoglycoside) is recommended.[2] The usual course of treatment is 14 to 21 days.

Increasing antibacterial options for patients with CF remains important because resistance to multiple agents becomes common as the disease progresses. The last major advance in this regard came with the advent of quinolone antibacterials, which not only added a new class of drugs to the anti-pseudomonal armamentarium but also provided an oral delivery route. Unfortunately, new additions to the quinolones, as well as to the β-lactam and aminoglycoside classes have been of more limited value in CF, as resistance often develops to multiple drugs in a particular class.

Maintenance antibacterial therapy for CF has been theoretically attractive, offering the potential

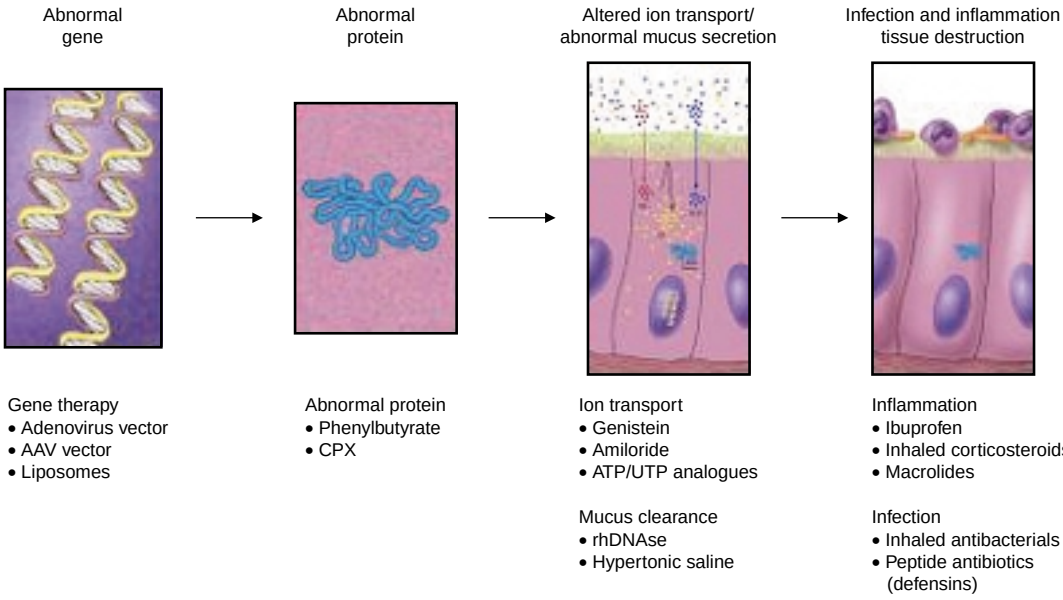

Fig. 1. New and emerging therapeutic approaches to cystic fibrosis as they relate to the pathophysiological stages of the disease. **AAV** = adeno-associated virus; **ATP** = adenosine triphosphate; **CPX** = cyclopentyl dipropylxanthine; **UTP** = uridine triphosphate. Modified from the CF Foundation.

to decrease symptoms and slow the progression of lung disease. Unfortunately, the long term use of cephalosporins for the treatment of *S. aureus* appears to increase the likelihood of developing *P. aeruginosa* infection[3] and long term quinolone use appears to be associated with the development of quinolone-resistant strains of *P. aeruginosa*.

The most important recent advance in maintenance antibacterial therapy for CF has come not with the development of a new class of drug but through the formulation of a nebulised aminoglycoside. In a large, randomised, controlled clinical trial, a pH balanced and preservative free formulation of tobramycin (TOBI®)**1** improved forced expiratory volume in 1 second (FEV_1), decreased bacterial density and decreased the risk of hospitalisation in patients with CF with moderate to severe airflow obstruction and infected with *P. aeruginosa*.[4] Nebulised tobramycin 300mg was administered twice-daily for 28 days followed by 28 days off the drug, with this cycle being repeated 3 times in the initial study. The intermittent use of the drug was chosen in an attempt to avoid the development of resistance that has limited the use of other maintenance therapies in CF.

Longitudinal follow up has demonstrated a trend toward increased levels of tobramycin resistance in *P. aeruginosa* isolates in patients receiving nebulised tobramycin, but not an increase in high level resistance that would render the therapy ineffective.[5] Toxicity appears to be minimal, especially compared with intravenous tobramycin, although we have noted a small percentage of patients who experience significant bronchospasm with administration of the drug. This is often amenable to pretreatment with inhaled β_2-agonists. Clinicians also need to be aware that systemic absorption of nebulised tobramycin occurs in varying degrees.

Although virtually any antibacterial could conceivably be given via nebulisation, airway irritation and inconsistent drug delivery are problematic.[6] Other than tobramycin, colistin is probably

1. Use of trade name is for product identification only and does not imply an endorsement.

the most commonly used aerosolised antibiotic in CF. Colistin clearly causes bronchospasm in a significant percentage of patients, a toxicity that does not appear to be related to the tonicity of the preparation.[7] In patients who tolerate the drug, symptoms and spirometry appear to be improved[8] and colistin may be of particular value in those patients with otherwise pan-resistant organisms.[9]

An intriguing hypothesis regarding the pathogenesis of CF may lead to a unique form of therapy for the disease. It has been suggested that antimicrobial peptides (defensins) normally present in the airway surface fluid and important for the prevention of bacterial colonisation may be inactivated by low salt concentrations seen in CF.[10] The functional loss of defensin activity could explain the link between chloride channel function and infection in CF. The question of whether the salt concentration of CF sputum differs significantly from that of normal airway secretions remains an open one and the role of defensins in the pathogenesis of CF is far from clear. However, the theory carries with it obvious therapeutic implications, and has spurred interest in the isolation and delivery of salt-stable antimicrobial peptides. Early phase clinical studies have begun.

2. Anti-Inflammatory Drugs

Airway inflammation in CF leads eventually to the bronchiectasis and irreversible airflow obstruction characteristic of the disease. Although inflammation accompanies airway infection, there is evidence that the process may precede infection in CF.[11] Recognition of the importance of airway inflammation has led to clinical trials of common anti-inflammatory medications, most notably corticosteroids and the nonsteroidal anti-inflammatory agent, ibuprofen. In addition, observations regarding the apparent anti-inflammatory effect of macrolide antibiotics in Japanese panbronchiolitis[12] has led to preliminary clinical trials of these agents in patients with CF.

Oral corticosteroids offer the potential to ameliorate progressive lung disease in CF but long term use is associated with a wide array of adverse effects.[13] In a large, randomised trial of alternate day prednisone (1 or 2 mg/kg once daily) over a 4-year period in children with CF, lung function (as measured by FEV_1) was better preserved in the treatment groups compared with placebo.[14] Unfortunately, bone growth was significantly reduced and the incidence of abnormal glucose metabolism markedly increased in the treatment groups as well. Persistent concern about the adverse effects of prednisone have limited its clinical usefulness.[15]

Inhaled corticosteroids (ICS) offer the potential advantage of limited systemic toxicity while decreasing airway inflammation. However, the efficacy of ICS in CF has yet to be demonstrated according to a recent systematic review of several small trials.[16] Larger trials are now ongoing.

Nonsteroidal anti-inflammatory agents also offer the potential of the positive effects on lung function seen with systemic corticosteroids with, perhaps, a better safety profile. In one clinical trial, high-dose ibuprofen was associated with a slowing in the loss of lung function (as measured by FEV_1) in patients with CF compared with placebo.[17] Although the study investigators did not note significant adverse events, others have reported renal toxicity.[18] The lack of corroborating studies[19] and concern over the safety of long term ibuprofen use has limited the use of the drug to less than 10% of patients with CF.[15]

Recently, there has been significant interest in long term macrolide therapy in CF. Macrolides are thought to have a negligible antibacterial effect on *P. aeruginosa* and other Gram-negative pathogens common in CF, and it has been postulated that any improvement in lung function in CF attributable to these drugs would have to be as a result of another mechanism of action. Some basic science supports the notion that macrolides have anti-inflammatory properties.[20,21] No published clinical trials to support the use of macrolides in CF exist currently, although several randomised trials are on-going.[22] A large, multi-centred phase III trial was announced at the 2000 North American CF Foundation Conference.

3. Airway Clearance

Traditional mechanical methods to improve airway clearance in patients with CF have included postural drainage, autogenic drainage and chest percussion. More recent options include the Flutter®[1] device for airflow oscillation[23,24] and the ThAIRpy®[1] vest,[25] which uses pneumatic compression to produce high-frequency chest wall oscillation. No particular approach to airway clearance has proven to be uniformly better than the others, and objective and subjective improvement is often quite idiosyncratic. A large trial designed to find equivalency between Flutter®, the vest and conventional chest physiotherapy is underway.

The high viscosity of sputum in CF has led to the use of mucolytics to assist in airway clearance. Multiple drugs, including acetylcysteine, guaifenesin and iodinated products have been used but not rigorously tested in patients with CF. The observation that sputum from patients with CF has an extremely high DNA content that significantly increases viscosity led to the development of a mucolytic specifically for CF, a recombinant form of DNase. In a large, multi-centred, randomised controlled trial, once daily DNase 2.5mg via nebuliser was associated with improved spirometry and decreased days of antibacterial therapy.[26] No increased benefit was seen in the twice-daily administration arm.

Recent reports have suggested that simple inhalation of nebulised hypertonic saline increases mucociliary clearance in patients with CF.[27,28] A short trial of hypertonic saline therapy versus isotonic saline in patients CF demonstrated a significant improvement in spirometry over a 3-week period.[29] No adverse events were reported. Long term trials of hypertonic saline are lacking. The low cost and toxicity of this therapy would make it quite appealing if long term benefit can be demonstrated.

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4. Correcting the Basic Defect

How the basic abnormality of defective ion transport (diminished or absent chloride secretion and the hyperabsorption of sodium) leads to clinical disease in CF remains unclear, but it is postulated that correction of the basic defect may prove to be effective in ameliorating the disease. Interventions designed to correct the basic defect can be categorized into one of two basic approaches, either attempting to improve the function of mutant CFTR or, alternatively, trying to modulate chloride and sodium transport through non-CFTR pathways. Although there are multiple agents with demonstrable *in vitro* activity in each of these areas, no clinically effective agent has yet been identified.[30]

Attempts to improve the function of mutant CFTR are generally directed at the most common mutation, deltaF508, which accounts for approximately 85% of abnormal alleles in North America. In patients who are homozygous for deltaF508, mutant CFTR may be degraded during processing in the endoplasmic reticulum, does not appear to reach the cell surface in significant amounts and, even if present on the cell surface, is not as easily activated as wild-type CFTR. 4-Phenylbutarate and several xanthines, particularly 8-cyclopentyl-1,3-dipropylxanthine (CPX) have the ability to increase chloride efflux in airway epithelial cells in culture.[31] Neither has yet demonstrated clinical efficacy in early trials. The isoflavone genistein can activate both mutant and wild-type CFTR, and may potentiate the effects of drugs that increase mutant CFTR presence on epithelial cell surfaces.[31,32]

Several agents have the ability to alter cellular ion transport independent of CFTR in a manner that may compensate for the absence of CFTR function. Adenosine triphosphate (ATP) and uridine triphosphate (UTP), purinergic agonists, can stimulate chloride secretion via a separate chloride channel. Amiloride can block the sodium absorption that accompanies decreased chloride excretion in CF. The instability of ATP and UTP currently profoundly limit clinical application, although

more stable analogues are currently under development. Amiloride, which also has a short half-life, has not been demonstrated to have clinical efficacy despite a small pilot study a decade ago suggesting benefit.[33]

In summary, the attempt to correct the basic defect remains a logical and intriguing arena of research, but has yet to provide a clinically useful therapy for CF.

5. Gene Therapy

No potential therapy holds as much promise in CF as placing functional CFTR into cell surface membranes. If airway epithelial cells can be provided with functioning CFTR early in the course of the disease, it is presumed that most or all of the destructive effects of inflammation and infection could be avoided. Despite early optimism that such a goal would be achievable in a relatively short timeframe, a decade of research and early clinical trials has brought us only incrementally closer to clinically successful gene therapy for CF.[34]

In principle, gene therapy requires that a normal copy of the missing or mutated gene be placed within the appropriate cell with a promoter that would assure that the gene is transcribed to allow for normal gene product to be manufactured and processed by the cell. Several steps are necessary for success. Firstly, the identification and cloning of the gene, which was accomplished in CF over 10 years ago.[35] Next, the appropriate target cells must be identified. For CF, it remains unclear whether the columnar epithelial cells or serous cells of the submucosal glands are more important in the pathogenesis of the disease. Next, the gene must be delivered in such a fashion that the DNA is incorporated into the cell. Then, gene transcription must occur, and the gene product must be manufactured and expressed in sufficient quantities to ameliorate the disease. Finally, either the gene must persist in target cells or the process of gene transfer must be repeatable. In CF, the last three steps have proven problematic.

Delivery of DNA coding for CFTR can potentially be accomplished through a variety of vectors. Adenovirus vectors are hampered by low gene transfer efficiency[36,37] and are highly immunogenic, with subsequent administrations losing efficiency.[38] Although adeno-associated virus (AAV) vectors may be less immunogenic, the small size limits the amount of DNA that can be transferred. Liposomal vectors, which complex DNA with cationic lipids, offer an alternative to viral vectors and have produced transient expression of CFTR in patients with CF.[39] Transfer efficiency is again very low, but it does appear possible to use repeated administrations.[40]

Any potential promise of gene therapy remains years off and it seems reasonable to assume that optimal benefit from such therapy will be provided to patients early in the course of the disease, before irreversible bronchiectasis has developed.

6. Conclusion

The development of new therapies for the treatment of lung disease in CF is progressing on several fronts. Novel antibacterial agents and a focus on direct delivery to the airway, along with an improvement in airway clearance, will aid in minimising the damage caused by chronic airway infection. An improvement in the safety profile of newer anti-inflammatories, including macrolide antibiotics, may make the use of these agents more common in CF. Attempts to correct the basic defect of ion transport have been successful in cell culture and are moving toward clinical trials. Finally, the promise of gene therapy looms as an eventual definitive approach to alleviating the morbidity and mortality associated with this still life-shortening disease.

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