Therapeutic Approaches to Acquired Cystic Fibrosis Transmembrane Conductance Regulator Dysfunction in Chronic Bronchitis

George M. Solomon[1,2*], S. Vamsee Raju[1,2*], Mark T. Dransfield[1,3], and Steven M. Rowe[1,2,3,4,5]

[1]Department of Medicine, [2]Gregory Fleming James Cystic Fibrosis Research Center, [3]University of Alabama at Birmingham Lung Health Center, [4]Department of Pediatrics, and [5]Department of Cell Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, Alabama

Abstract

Chronic obstructive pulmonary disease is a common cause of morbidity and a rising cause of mortality worldwide. Its rising impact indicates the ongoing unmet need for novel and effective therapies. Previous work has established a pathophysiological link between the chronic bronchitis phenotype of chronic obstructive pulmonary disease and cystic fibrosis as well as phenotypic similarities between these two airways diseases. An extensive body of evidence has established that cigarette smoke and its constituents contribute to acquired dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) protein in the airways, pointing to a mechanistic link with smoking-related and chronic bronchitis. Recent interest surrounding new drugs that target both mutant and wild-type CFTR channels has paved the way for a new treatment opportunity addressing the mucus defect in chronic bronchitis. We review the clinical and pathologic evidence for modulating CFTR to address acquired CFTR dysfunction and pragmatic issues surrounding clinical trials as well as a discussion of other ion channels that may represent alternative therapeutic targets.

Keywords: chronic obstructive pulmonary disease; cystic fibrosis transmembrane conductance regulator; chronic bronchitis

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*These authors contributed equally.

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Correspondence and requests for reprints should be addressed to Steven M. Rowe, M.D., M.S.P.H., MCLM 706, 1918 University Boulevard, Birmingham, AL 35294-0006. E-mail: smrowe@uab.edu

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Chronic obstructive pulmonary disease (COPD) is a major international health concern and the third leading cause of death in the United States (1). Most cases are due to cigarette smoking, although other environmental exposures can also cause disease, including heavy metals and toxic fumes such as pollution or diesel particulates. Alpha-1 antitrypsin deficiency is also responsible for approximately 2 to 3% of cases and can precipitate an early presentation, especially in the context of smoking (2). COPD is associated with two predominant clinical phenotypes, emphysema and chronic bronchitis; however, they often overlap, and the distinction in most patients is imprecise (3–5). Chronic bronchitis is particularly troubling as it can often dominate symptoms, is associated with pulmonary exacerbations, and presently has few treatment options (4, 5). As chronic bronchitis shares many clinical and pathologic features with cystic fibrosis (CF), an archetypal obstructive lung disease caused by genetic mutations in the cystic fibrosis transmembrane conductance regulator (CFTR), it is possible that there are common mechanisms and shared therapeutic strategies. This has been bolstered by the recognition that cigarette smoking and COPD itself induce an acquired state of CFTR protein dysfunction in patients with normal CFTR genes. Furthermore, the discovery of CFTR potentiators, including the recently Food and Drug Administration–approved agent ivacaftor, which can also activate wild-type CFTR, has generated excitement that ion transport activation may represent an important therapeutic target for the treatment of COPD (6). Here we review the potential contribution of acquired

CFTR dysfunction to the pathophysiology of COPD, and in particular chronic bronchitis, with a focus on potential therapies.

Acquired CFTR Dysfunction in Smoking

There is a longstanding interest in the effects of cigarette smoking on epithelial ion transport since Welsh reported that cigarette smoke extract decreased chloride (Cl^-) secretion across canine airway epithelium, even before CFTR had been identified (7). Years later, Kreindler and colleagues established that cigarette smoke extract decreases CFTR-dependent Cl^- secretion *in vitro* (8). Cantin and colleagues confirmed these findings in Calu-3 monolayers, an airway glandular cell line, and showed that the effects of cigarette smoke were not specific to pulmonary epithelium by demonstrating similar effects on CFTR expression and function in the intestinal epithelial cell line T84 (9). Moreover, in a retrospective analysis using nasal potential difference (NPD) involving men with and without a history of smoking and the absence of CFTR genetic mutations, healthy smokers exhibited CFTR deficiency, as observed by a blunted NPD response to Cl^--free isoproterenol perfusion, indicating reduced CFTR activity *in vivo*. Administration of cigarette smoke to the nares of healthy smokers caused an acute blockade of CFTR activity, as measured by NPD, establishing the causality of cigarette smoke and indicating the kinetics of this reaction are relatively rapid (10).

Emerging data from multiple prospective studies have established that acquired CFTR dysfunction caused by smoking is not limited to healthy smokers; it also affects patients with COPD and correlates with disease severity and clinical symptoms. Cigarette smokers and patients with COPD exhibited reduced CFTR function by NPD, and this was accompanied by reduced mRNA transcript levels from the nares (6, 11). Furthermore, lower airway potential difference measured via bronchoscopy was also reduced in a distinct cohort; functional decrements were confirmed by Western blot of endobronchial biopsies indicating reduced protein expression (11). In each of these studies, patients with COPD who stopped smoking for at least 1 year did not exhibit CFTR dysfunction, although there was some variability between subjects, particularly in the lower airway where other factors such as hypoxia, neutrophilic inflammation, and goblet cell hyperplasia may also contribute to CFTR deficits (12, 13). Furthermore, CFTR dysfunction was also associated with symptoms of chronic bronchitis, supporting the notion that abnormal epithelial anion transport may contribute to this phenotype, which like CF is plagued by chronic mucus hypersecretion (6, 11). Supporting the overall hypothesis, the expression of CFTR was positively correlated with FEV_1 in the lungs of patients with COPD, whereas expression of negative regulators of airway hydration, including ENaC, was negatively correlated to lung function (14).

Although reduced CFTR activity in the airway was supported by previous studies in the epithelial cells, surprisingly, deficient CFTR activity was not limited to the respiratory epithelium *in vivo*. Rather, systemic (nonrespiratory) CFTR dysfunction was established by elevated sweat chloride concentration in smokers and patients with COPD and was also observed using intestinal current measurements in a confirmatory analysis in this patient population (15, 16). β-adrenergic–stimulated sweat rate, a parameter dependent on CFTR-mediated anion secretion in the coil of the sweat gland, confirmed these findings and also replicated the abnormality in sweat chloride (16). Interestingly, both sweat chloride and sweat rate were altered in former smokers with COPD, suggesting residual CFTR dysfunction is present in this population, a finding distinct from parameters measured on the airway surface. The reason for the difference in these tissues is unknown but may be due to the relative sensitivity of the sweat assays for detecting small degrees of CFTR dysfunction or differences in the biology of the sweat gland from the respiratory epithelium. Each of these studies excluded patients who harbor one or more genetic mutations in CFTR, indicating inheritance was not a factor in these diseases. Sweat rate was also shown to partially recover after smoking cessation, an indicator of causality, although this was not evident until 2 to 3 weeks after quitting, suggesting some time is required to demonstrate improved CFTR activity (17).

There are thousands of noxious substances in cigarette smoke, but a few constituents dominate its toxic effects. Current evidence implicates acrolein and cadmium as cigarette smoke constituents that block CFTR function *in vitro*, and they can also reach detectable levels in humans (15, 18). These agents may be potentially amenable to therapeutic intervention.

The mechanistic basis of reduced CFTR dysfunction on a molecular level continues to evolve, as more than one pathway likely contributes, including those that effect CFTR mRNA expression, protein stability, and channel gating. *In vitro* and *in vivo* studies have shown cigarette smoke affects mRNA levels (6, 10). CFTR expression was found to be altered by up-regulation of miR-101, a repressive miRNA that modulates CFTR 3′-UTR to affect its transcription (19). Epigenetic effects have also been postulated (20, 21) as well as changes in protein expression (6, 10). In addition, CFTR surface expression is altered by sequestration of channels into an aggresome-like perinuclear (but not lysosomal) compartment that was driven by smoke-induced spikes in intracellular Ca^{2+} mobilization (10, 22). Finally, cigarette smoke has been shown to directly reduce channel gating as measured by altered open probability (23); this was recapitulated by acrolein exposure (15). In addition, cigarette smoke induces transforming growth factor-β_1, which modulates CFTR activation (via β-adrenergic stimulation) via endogenous stimulation (24). Because smoking also increases mucus expression and reduces cilia beat frequency and ciliogenesis, even small decrements in CFTR function are likely to impose marked effects on airway physiology and mucus clearance (25).

Therapeutic Challenges in Chronic Obstructive Pulmonary Disease

Although COPD is among the most common causes of death worldwide, no current pharmacologic treatments alter its natural history (26–28). Although bronchodilators (frequently in combination with inhaled corticosteroids) are the mainstay of treatment and do reduce exacerbations, improve dyspnea, and increase quality of life, they do not definitively alter mortality (29–31). In addition, inhaled steroids in COPD are associated with an increased risk of pneumonia and do not alter mucus obstruction or its clearance (29, 32, 33).

Mucolytic therapy for COPD has yielded marginal benefits thus far (34–36). This has been attributed to the lack of efficacy of currently available agents, poor bioavailability, and failure to deliver the drug to the distal airways where mucus obstruction is observed (37–40). An improved approach that targets the small airways (with a potent and orally available agent) could be expected to overcome these limitations and thus improve mucus stasis, an important cause of residual airway obstruction in COPD that cannot be addressed by bronchodilators alone, and a feature closely linked to mortality and lung function decline (41–43).

Like lung cancer and coronary artery disease, the need for new COPD therapies is well accepted, despite evidence that cigarette smoking is the major contributing and modifiable risk factor (44, 45). Even after cessation, COPD continues to progress, and airflow obstruction is present in a substantial subset of ex-smokers (i.e., ~4–5 million people), of whom up to half exhibit chronic bronchitis (46).

Crossover between Therapies for Cystic Fibrosis and Chronic Obstructive Pulmonary Disease

The shared pathophysiologic features between CF and COPD have led to a number of prior investigations examining the potential role of therapeutics developed for patients with one of these airway diseases for use in the other. Although not all efforts have been successful (47–50), randomized trials have demonstrated the shared benefits of intravenous corticosteroids during exacerbations (51, 52), chest physiotherapy (53–55), and, most recently, the chronic administration of azithromycin to reduce acute exacerbations (56, 57). This experience suggests that some agents effective in CF may also be efficacious in COPD.

Rescue of CFTR Function: A New Paradigm in Cystic Fibrosis Care

"CFTR potentiators" are a class of agents developed to correct the underlying cause of CF and principally target surface mutations. CFTR mutations can be grouped into several categories based on the molecular defect. Class I and II mutations are generally severe and result in relatively little protein product at the cell membrane. In comparison, class III (severe gating mutants, e.g., G551D), class IV (altered conductance), and class V (reduced expression) CFTR defects (58–60) have relatively preserved cell surface expression and more closely resemble the situation in COPD. The archetype agent ivacaftor (formerly VX-770) robustly activates multiple CFTR forms, including wild type, G551D (a class III mutation), and other mutations with residual expression or function (class III, IV, and V) in primary airway epithelial monolayers, but only if they are located at the cell surface in sufficient number (61–63). Ivacaftor is now Food and Drug Administration approved for treatment of CF with several mutations. Ivacaftor confers robust activation of G551D to markedly improve CFTR function, mucociliary clearance, pulmonary function, and exacerbation frequency in patients with CF (64); positive effects are also observed in the class IV allele R117H (65).

Although CFTR potentiators were originally developed to restore activity to mutant CFTR, some CFTR potentiators also augment wild-type CFTR function (6, 61). For example, both ivacaftor and other CFTR potentiators activate wild-type CFTR by augmenting open channel probability (62, 66, 67), which is reduced by smoke exposure (23). Because CFTR regulates airway surface liquid (ASL) depth (68), which in turn determines mucociliary clearance (69–71), it follows that potentiation of CFTR anion secretion should result in augmented mucus transport compared with resting conditions (72, 73).

CFTR Potentiators Reverse Acquired CFTR Abnormalities and Augment Mucociliary Transport *In Vitro*

In smoke-exposed epithelial cells, ivacaftor potentiated CFTR-dependent short-circuit current, regardless of prior smoke exposure and to levels that exceeded wild-type CFTR activity (6) Ivacaftor also potentiated CFTR-dependent currents in normal explanted human trachea. The effects of ivacaftor were similar whether the individual was an active smoker or nonsmoker, providing evidence of strong activity of ivacaftor in tissue (6). Confocal microscopy revealed ivacaftor increased ASL depth in smoke-exposed epithelia and caused a large increase in mucociliary transport rate (MCT) (6, 15). Hypertonic saline, which increases ASL depth *in vitro* by osmotic force, also improved MCT *in vitro*, indicating CFTR can be circumvented (10). Of note, all studies examining the effect of potentiators on ASL depth and MCT were performed with "chronic" (24 h) exposure, indicating concerns regarding the effect of ivacaftor on CFTR stability did not substantially diminish treatment benefit (74). In total, these data strongly indicate that CFTR potentiators and other agonists of ion transport can reverse CFTR dysfunction induced by smoking, augmenting ASL depth and increasing MCT in airway epithelia (6). Whether this is principally due to CFTR-mediated chloride secretion and its resultant effects on ASL hydration, or partial inhibition of CFTR-dependent bicarbonate transport, which can influence mucus viscosity (75) and adhesion to the airway surface (76) observed in the latest models of the CF airway, deserves exploration.

Roflumilast and Its Role in Chronic Bronchitis

Roflumilast is a phosphodiesterase-4 (PDE-4) inhibitor approved for the treatment of frequent exacerbations in patients with COPD with chronic bronchitis (77). It was advanced for testing as a putative antiinflammatory, because PDEs are highly expressed in a variety of inflammatory cells. Interestingly, roflumilast was only shown to be effective in patients with chronic bronchitis, raising questions as to whether antiinflammatory properties were the sole reason for its therapeutic benefit (78). As a PDE inhibitor, roflumilast also increased cellular cAMP levels *in vitro* and activated CFTR by augmenting its phosphorylation in a manner that was competitive with cAMP-dependent stimuli, a distinction from CFTR potentiators (79). Roflumilast is relatively specific for PDE4 and has minimal effects on cGMP. Roflumilast was also effective in restoring CFTR function in human bronchial epithelial cells exposed to cigarette smoke (80). Downstream of CFTR augmentation, roflumilast augmented ASL depth, including cells affected by cigarette smoking (79, 80). Although Tyrrell and colleagues did not observe an increase in cAMP or ASL depth when human

bronchial epithelial (HBE) cells were treated with roflumilast alone, roflumilast did augmented cAMP levels and ASL hydration in presence of adenosine, a physiologic activator of cAMP and CFTR in the airways (80). Moreover, roflumilast was found to be superior to β-agonists such as salmeterol and isoproterenol in restoring ASL height. Currently, there are no published reports regarding the effect of roflumilast on CFTR expression.

Roflumilast-N-oxide, the more stable and potent metabolite of roflumilast, protected against cigarette smoke–induced loss of ciliated epithelium and the rate of ciliary beating (81). Taken together, the bioactivity of roflumilast on CFTR function suggests it may partially explain its therapeutic benefit in patients with COPD with chronic bronchitis and frequent exacerbations, because this phenotype is most responsive to roflumilast therapy and also is associated with an increased prevalence of CFTR dysfunction (11, 15); this also adds additional credence to the hypothesis that CFTR potentiation may be beneficial in COPD. Interestingly, CFTR activation and fluid secretion in the intestine was also shown to occur with roflumilast and could explain the adverse effect of diarrhea commonly observed in the clinic (79). Inhaled PDE inhibition could be an approach to circumvent this side effect.

Patient Selection for Trials with CFTR Potentiators and Activators

The heterogeneity of COPD pathophysiology and phenotypic expression has been a challenge to drug development and raises the importance of patient selection for the evaluation of experimental therapeutics (82). Selection criteria to target the COPD subphenotypes likely to be responsive to CFTR or other ion transport pathways will be imperative, especially in the early phases of drug development. The presence of smoking would be logical criterion for patient selection, because smoking clearly contributes to acquired CFTR dysfunction. However, we posit the presence of chronic bronchitis would be more clinically relevant and possibly more feasible, as this clinical criterion is associated with CFTR dysfunction and also with mucus hyperplasia, which together contribute to impaired mucociliary clearance in the small airways (4, 5, 83). The presence of chronic bronchitis also carries a much higher risk of exacerbation and morbidity (3, 41), representing significant unmet medical need not addressed by current therapies. The benefits of CFTR activation may be generalizable beyond patients with COPD with chronic bronchitis, however, as even patients without chronic mucus hypersecretion exhibit airways obstruction due to mucus impaction of the small airways, as detected by endobronchial biopsy (43). Of the two clinical criteria noted, the presence of chronic bronchitis may be most appropriate, because new treatments that encourage continued smoking by abating symptoms could be problematic.

Laboratory studies could also be used to select patients likely to respond to CFTR potentiators or other anion transport activators. An obvious strategy would be to populate the study with individuals with clear evidence of CFTR dysfunction. Sweat chloride would be highly feasible for this purpose, because the test is reproducible, well standardized, and is sensitive to even modest degrees of CFTR dysfunction (15, 84). Our previous studies indicate that 40 to 50% of patients with Global Initiative for Chronic Obstructive Lung Disease stage II or greater COPD demonstrate significant elevation of sweat chloride (although still below the diagnostic criteria of CF) compared with healthy nonsmokers (15). NPD could also be performed and has the potential advantage of reflecting the status of CFTR function in the airway (6). It is not clear whether CF-carrier status would impact likelihood of treatment response. So far, relatively small studies have not consistently detected an association between chronic bronchitis and CFTR carrier status (85), although it is possible an effect of CFTR mutations could be confirmed with larger study cohorts. On the other hand, the presence of an allele that does not respond to a CFTR potentiator (at least as compared with a wild-type channel) may also diminish the treatment effect.

Biomarkers of CFTR and Other Indicators of Clinical Efficacy

Experience in CF indicates that improved CFTR function measured by sweat chloride or NPD predicts improved lung function on a group-wise basis with CFTR potentiator therapy (84). However, this has not yet been proven in COPD; thus, a comprehensive approach to clinical endpoints and biomarkers downstream of CFTR seem warranted. Other measures of CFTR function besides these two endpoints that could be considered include function of the secretory coil of the sweat gland using β-adrenergic–stimulated sweat secretion. This assay is sensitive to small decrements in normal CFTR function (86) and to the COPD phenotype among smokers (16); furthermore, β-adrenergic sweat rate improves in healthy smokers on initiating a smoking cessation program, indicating the assay is dynamic within an individual (17). Other markers intermediate to clinical efficacy could also be useful. Augmentation of CFTR activity in COPD, if acquired CFTR dysfunction contributes to COPD pathogenesis, should confer improved mucociliary clearance, as observed with ivacaftor treatment in patients with the G551D CFTR mutation (64); this can be measured by clearance of Tc99-radiolabeled particles (72). Other indicators could include resolution of hyperinflation, small airway obstruction, or bronchial wall thickening as detected by changes in high-resolution computed tomography scan. These studies have also been illustrative in patients with CF treated with ivacaftor, even in relatively short-term studies (87, 88). Patient symptoms could also be informative, as there are a number of validated outcome measures in COPD that quantify mucus production (89–91). Ultimately, clinical efficacy will have to be demonstrated by changes in spirometry and exacerbation frequency, but these endpoints require relatively large studies. Even in CF, CFTR biomarkers have not predicted whether a patient experiences improved lung function on an individual basis (92). Thus, there is much to be learned about whether an improvement in a short-term biomarker can be used to select patients likely to benefit from prolonged administration of a new COPD therapy.

Potential Contribution of Other Ion Channels and Their Therapeutic Potential

Although mucosal defects in CF highlight the pathophysiologic role played by CFTR, there are several other epithelial channels known to conduct chloride across the

airway surface. These channels function in concert to generate a luminal chloride gradient that drives paracellular sodium and water to sustain isosmotic mucosal secretions. As such, epithelial chloride channels do not function in isolation, and they remain electrochemically in tandem with other apical Na^+ channels, anion exchangers, basolateral Na^+/K^+ pumps, and K^+ channels to maintain luminal osmolality.

Calcium-activated chloride channels (CaCC) are a large family of apical chloride channels that are activated in response to increased intracellular Ca^{2+} (93). Although initially associated with Ca^{2+} mobilization by muscarinic stimulation, they are now known to respond to a wide variety of Ca^{2+} agonists (94). Attempts to define molecular identity of CaCCs have identified several interesting candidates (e.g., CLCA1, CLC3, hTTYH3) over three decades (95). Three independent laboratories identified TMEM16A (also known as anactomin-1) with convincing properties of a CaCC (96–98). Gene silencing and murine knockout models have since validated its pharmacologic selectivity and tissue distribution (99, 100). Nearly 50% of uridine 5′-triphosphate (UTP)-stimulated airway CaCC activity was attributed to TMEM16A, and its pharmacologic inhibitors were found to abolish only the transient CaCC currents (101). Identity of other CaCC channels remains elusive, and successful characterization of TMEM16A highlights the likelihood of discovering additional members using advanced experimental and theoretical approaches. Because alternated chloride channels continue to remain a prime focus for developing CF therapy, TMEM16A, with its established biology, has been at the forefront of such efforts (102, 103).

SLC26A is another large family of anion transporters known to mediate epithelial Cl^- and HCO_3^- secretion. Members of this family are of significant interest to CF research, because they directly interact with CFTR domains and mutually augment anion secretion by sharing regulatory proteins such as protein kinase A (104, 105). SLC26A9 is one such apical channel that is responsive to cAMP/protein kinase A–mediated stimulation and accounts for basal ion secretion after inhibition of epithelial sodium absorption (106). Interestingly, SLC26A9 is known to function synergistically with wild-type CFTR (but not with mutated CFTR) to conduct anion transport (105). Thus, interactions between CFTR and SLC26A channels might be exploited in acquired decrements of the CFTR channel. As such, alternate chloride channels are an attractive drug target that can augment anion secretion independent of CFTR (107), potentially circumventing acquired CFTR dysfunction. The potential of the epithelial sodium channel (ENaC), a key ion channel that regulates ASL depth and mucus hydration on the epithelial cell surface, as a therapy in COPD is reviewed elsewhere in this issue.

Conclusions

Previous work indicates that cigarette smoking, the most common cause of chronic bronchitis, results in a functional CFTR defect in the airways and also systemically. The success of CFTR modulators for the treatment of CF has resulted in significant interest in determining whether these agents may also be useful in chronic bronchitis. Preclinical studies demonstrate the capacity of CFTR potentiators to restore airway surface liquid defect and augment mucociliary transport in human cells and tissues by augmenting CFTR function, suggesting their potential as a therapeutic agent in the disease. Although disease heterogeneity is a challenge, several tools for patient selection are apparent, including targeting patients with COPD with chronic bronchitis or enriching the population for individuals with abnormal CFTR function. Tools to detect efficacy include measures of CFTR activity, physiologic assays of mucus clearance, and measures of clinical efficacy. The confluence of an improved understanding of airway biology, comprehensive patient phenotyping, and availability of new therapies provides new opportunities to explore CFTR modulators and other ion transport agonists as new therapeutic agents that target COPD at the level of the airway epithelium. ■

Author disclosures are available with the text of this article at www.atsjournals.org .

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