Novel Prospects for Managing Cystic Fibrosis

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Abstract

Although loss of function mutations in an epithelial chloride channel (cystic fibrosis transmembrane conductance regulator – CFTR) is known to cause cystic fibrosis (CF), it remains unclear how this dysfunction leads to the clinical picture of CF. Downstream effects of CFTR dysfunction that are believed to contribute to CF pathogenesis include: increased activity of an epithelial sodium channel (ENaC), which depletes airway surface liquid and renders mucus more viscous; increased epithelial oxidative stress largely attributable to subnormal nrf2 activity; increased epithelial production of pro-inflammatory cytokines, driven in part by oxidative stress and overactivation of NF-kappaB; and decreased nitric oxide bioactivity. It may prove feasible to address each of these pathogenic mechanisms with drugs and nutraceuticals which are currently available. AMPK activators such as metformin and berberine, as well as the IKKbeta inhibitor salicylate, have the potential to decrease ENaC activity, while also blunting pro-inflammatory cytokine production via inhibition of NF-kappaB. Phase 2 inducers such as lipoic acid may amplify epithelial expression of antioxidant enzymes and glutathione. Supplementation with N-acetylcysteine likewise can promote glutathione synthesis in lung epithelium, and antioxidants such as spirulina-derived phycocyanobilin, astaxanthin, and melatonin may confer additional protection to oxidant-stressed epithelium. Supplemental citrulline may aid pulmonary function in CF by boosting NO bioactivity. While it seems unlikely that any one of these measures in isolation would confer major clinical benefit in CF, a functional combination of these may have greater potential in this regard.

Pathogenic Mechanisms in Cystic Fibrosis

Cystic fibrosis (CF), the most common lethal genetic disorder in Caucasian populations, is caused by homozygous loss of function of a chloride channel, the CF transmembrane conductance regulator (CFTR).[1, 2] The responsible mutations can cause failure of chloride transport, or prevent trafficking of the channel to the apical plasma membrane. The downstream consequences of this which are suspected to play a pathogenic role in CF include:

Increased function of the ENaC sodium channel.[3] This leads to increased uptake of sodium ions and fluid by bronchial epithelial cells, such that airway surface liquid is depleted.[4] Consequently, airway mucus is more viscous and less readily swept out by ciliary action, setting the stage for airway occlusion and persistent bacterial infections. This increase in ENaC activity may reflect the fact that CFTR physically associates with ENaC in a way that precludes the proteolytic activation of the latter.[5] Transgenic mice with ENaC overexpression in bronchial epithelium develop a clinical syndrome quite analogous to human CF, suggesting that ENaC overactivity may be a central mediator of CF pathogenesis.[3, 4, 6] However, this view is still somewhat controversial, and it seems likely that ENaC overactivity is not the sole reason why CFTR dysfunction is pathogenic.[7] Intriguingly, some patients with CF-like clinical syndromes who nonetheless express a functional CFTR channel have been found to make variant forms of ENaC with increased open probability.[8-10]

Increased oxidative stress in bronchial epithelial cells.[11] This reflects, in large part, down-regulated expression of a number of antioxidant enzymes whose synthesis is promoted by the Nrf2 transcription factor.[12] For reasons that remain unclear, Nrf2 expression and activity are decreased in CF epithelial cells.[13] However, Nrf2 does respond normally to an activating phase 2 stimulus in these cells, such as tert-butylhydroquinone. Whether superoxide production in CF epithelium is elevated in less clear. Mitochondria are under increased oxidative stress owing to a stark deficiency of intramitochondrial glutathione (possibly reflecting a role for CFTR in mitochondrial glutathione import), and damaged mitochondria are more prone to overproduce superoxide.[14] The glutathione content of airway surface liquid, thought to play an antioxidant and anti-infectious role, is also low, since CFTR mediates its export.[12, 15] Superoxide can also be generated in bronchial epithelium by the complexes Duox1 and Duox2, variant forms of NADPH oxidase.[16] Infiltrating activated neutrophils also contribute to the bronchial oxidative stress load in CF.

Increased production of pro-inflammatory cytokines by the bronchial epithelium. CF epithelial cells are more sensitive to pro-inflammatory activating stimuli such as IL-1 or TNF-alpha, and feedback mechanisms which dampen response over time in normal epithelium are less effective in CF epithelium.[17-21] This pro-inflammatory response is associated with increased activation of the NF-kappaB transcription factors, and there is experimental evidence that hydrogen peroxide-mediated up-regulation of NF-kappaB activation is largely responsible for pro-inflammatory bias of CF epithelium.[13, 18, 22] This situation is of course compounded when persistent infections in CF provoke epithelial inflammation.

Diminished levels of nitric oxide (NO) in CF airways.[23] NO functions physiologically in the lung to regulate bronchial and vascular smooth muscle tone. The reduced levels of airway NO in CF may reflect increased oxidant scavenging of NO, but reduced effective activity of bronchial NO synthases may also play a role. In particular, elevated lung levels of the NO synthase antagonist, asymmetric dimethylarginine, and a marked increase in plasma arginase, may compromise lung NO production in CF.[24, 25] These abnormalities of arginine metabolism and NO production resolve to a degree with effective antibacterial therapy. The fact that inhalation of nebulized arginine acutely improves lung function in CF suggests that deficient NO production is of pathogenic importance in this disorder.[26] However, a trial of oral arginine by the same group failed to observe comparable benefit, likely owing to inadequate delivery of arginine to bronchial epithelium.[27]

Diminished export of hypothiocyanite.[28] This labile agent is produced within bronchial epithelial cells, and is exported to the airway surface liquid, where it exerts potent antibacterial activity. This export is mediated via CFTR, and hence this protective mechanism is compromised in CF.

Fortunately, certain nutraceuticals and drugs have the potential to address some (though regrettably not all) of these pathogenic mechanisms in CF.

AMPK Activators and Salicylate May Suppress ENaC and NF-kappaB Overactivity

AMP-activated kinase (AMPK) has been shown to oppose ENaC activity. In part, this reflects the fact that AMPK catalyzes an activating phosphorylation on the ubiquitin ligase Nedd4-2, which promotes the proteasomal destruction of ENaC and hence diminishes its expression in the plasma membrane.[29, 30] Other research suggests that AMPK may act more directly to suppress the channel function of existing ENaC.[31] AMPK also has the potential to suppress NF-kappaB activity by several mechanisms; in

particular, AMPK promotes activation of Sirt1, which removes an activating acetyl group from c-Rel.[32-34] Hallows and colleagues, who have proposed that the AMPK activator metformin should be tested clinically in CF, have demonstrated that both metformin and the AMPK activator AICAR can diminish ENaC activity in CF epithelial cells, increase the height of airway surface liquid on this cells, and suppress the production of pro-inflammatory cytokines by CF epithelial cells challenged with lipopolysaccharide.[20, 35] The herbal compound berberine may have potential analogous to metformin in this regard, as it likewise functions as an AMPK activator, and has clinical anti-diabetic activity comparable to that of metformin.[36-38]

IkappaB kinase-beta (IKKbeta) activity is upregulated in CF epithelium, serving as a key activator of NF-kappaB.[39] The IKKbeta inhibitor parthenolide suppresses IL-8 production when CF epithelial cells are challenged with IL-1 or TNF-alpha. In vivo, parthenolide pre-treatment inhibits bronchial neutrophil influx and cytokine production in mice administered intratracheal LPS.[21] Hence, IKKbeta inhibitors may have potential for dampening the exuberant bronchial inflammatory response in CF. Moreover, IKKbeta confers an inhibitory phosphorylation on Nedd4-2, and thereby tends to increase the expression and activity of ENaC.[40] Hence, IKKbeta inhibitors may share the ability of AMPK activators to suppress both bronchial inflammation and ENaC overactivity, key pathogenic factors in CF. Although parthenolide per se is not available as a nutraceutical (its herbal source feverfew is probably too dilute a source to be useful in this regard), and various novel drugs capable of inhibiting IKKbeta have not yet achieved clinical approval, the venerable natural anti-inflammatory agent salicylate can function clinically as an IKKbeta inhibitor; indeed, this appears to be the basis of its utility in rheumatoid arthritis and type 2 diabetes.[41-44] Moreover, there is recent evidence that clinical concentrations of salicylate can achieve a mild allosteric activation of AMPK that is complementary to the activation achieved by agents such as metformin or berberine that raise ADP/AMP levels.[45] Hence, salicylate – preferably in the form of the well-tolerated pro-drug salsalate[46] – may merit clinical evaluation in CF. Unlike aspirin and the NSAIDs, salicylate achieves only a weak and transitory inhibition of cycloxygenases, and hence does not confer the increase risks for GI ulceration or renal damage seen with chronic NSAID therapy.[47, 48] The dose-limiting side effect of salicylate is fully reversible ototoxicity (tinnitus, mild hearing loss) that can usually be avoided or corrected by proper dose titration.[44, 49]

Phase 2 Inducers – Lipoic Acid

As noted, subnormal activity of Nrf2 appears to be largely responsible for the endogenously generated oxidative stress in CF epithelial cells. Nonetheless, activation of Nrf2 by the phase 2 inducer tert-butylhydroquinone appears to be normal.[13] This suggests that clinical phase 2 inducers might be capable of alleviating some of the oxidative stress that promotes bronchial inflammation in CF. Many phytochemicals are known to have phase 2 inducing activity, and broccoli sprouts rich in sulforaphane precursors have been developed for this purpose.[50, 51] But the most clinically established phase 2 inducer appears to be lipoic acid, whose clinical activity in diabetic neuropathy seems likely to reflect phase 2 induction.[52-56] The well known ability of lipoic acid to boost glutathione levels in rodent tissues may also be attributable to a phase 2 effect, as the rate-limiting enzyme for glutathione synthesis, gamma-glutamylcysteine synthase is phase 2 inducible.[52, 57] Lipoic acid, in dose schedules beneficial in diabetes, may therefore merit clinical study in CF.

Ancillary Antioxidant Measures

Increased synthesis of glutathione in CF epithelium has the potential to oppose the pro-inflammatory impact of oxidative stress, and might also alleviate to some degree the deficit of glutathione in mitochondria and airway surface liquid. While effective phase 2 induction can promote such synthesis, intracellular cysteine availability is rate-limiting in this regard. Hence, supplementation with N-acetylcysteine (NAC) or L-cystine, which can boost intracellular cysteine, may also be useful for promoting glutathione synthesis in CF epithelium.[58, 59] Several clinical trials have evaluated supplemental NAC, in doses as high as 3000 mg daily, in CF.[60] This therapy is well tolerated and does succeed in increasing sputum glutathione level.[61] One 4 week study found that NAC treatment decreased pulmonary neutrophil burden and sputum elastase activity, an important prognostic factor in CF.[62] However, a longer study failed to confirm this anti-inflammatory impact, and no such study has shown an impact on pulmonary function, possibly reflecting the fact that very long-term administration would be required fro such benefit.[61] While NAC supplementation clearly is no magic bullet for CF, it may well find a role as a component of a more comprehensive therapeutic strategy.

The extent to which Duox1/2 contribute to oxidative stress in CF is not yet clear, but the NAPDH oxidase activity of infiltrating neutrophils is clearly important in this regard. Free bilirubin functions physiologically as an inhibitor of NADPH oxidase, and the apparent ability of heme oxygenase-1 induction to control Duox1 activity suggests that bilirubin likewise may inhibit this complex.[63-66] Bilirubin's precursor biliverdin functions as an inducer of IL-10, an anti-inflammatory cytokine whose production is diminished in CF.[19, 67-69] Epidmiologically, serum bilirubin correlates inversely with risk for COPD; in rats, biliverdin administration provides protection from LPS-induced lung injury.[70, 71] Although bilirubin and its much more soluble precursor biliverdin are not available for clinical use, there is recent evidence that the biliverdin homolog phycocyanobilin (PhyCB), richly supplied by cyanobacteria such as spirulina, can mimic the antioxidant and anti-inflammatory effects of biliverdin/bilirubin; this may explain the versatile and potent anti-inflammatory effects of oral spirulina or phycocyanin (the spirulina proteins whose chromophore is PhyCB) in numerous rodent studies.[72, 73] Spirulina or PhyCB-enriched spirulina extracts may be worthy of consideration as a component of antioxidant supplementation in CF.

While there have been many efforts to improve vitamin E status in CF, the carotenoid astaxanthin appears to have far greater efficacy than vitamin E as a protector of biological membranes, and might have particular potential for protecting mitochondrial membranes from the elevated oxidant load associated with CF.[74-78] Indeed, mitochondrial protection may underlie the favorable impact of astaxanthin pre-administration on ischemia-reperfusion damage in pre-clinical studies.[79] Astaxanthin has not yet been studied in CF; it presumably would have greater efficacy if administered in micellized form to insure better absorption in CF patients.

The outstanding antioxidant potential of high-dose folate (10-80 mg daily) has so far been grievously neglected by clinical researchers.[80-83] The tetrahydro forms of folate generated after intracellular uptake of this vitamin have versatile oxidant-quenching activity; in vascular endothelium, their ability to quench peroxynitrite-derived radicals helps to prevent the oxidative uncoupling of NO synthase, a phenomenon which possibly contributes to NO deficiency in CF.[84-87] The impact of folate on oxidative stress and NO synthesis in CF epithelial cells merits evaluation.

In many cells, melatonin exerts an inductive effect on antioxidant expression via nuclear melatonin receptors.[88] In light of the fact that Nrf2-mediated induction of antioxidant enzymes is compromised in CF epithelium, it would be of interest to determine whether melatonin administration might have the potential to "pinch hit" in this regard.

Citrulline – A Substrate for NO Synthase

Because citrulline is immune to the the degradative activity of arginase in the GI tract and plasma, supplemental citrulline has shown a greater capacity to boost tissue arginine levels – and support NO synthesis when ADMA levels are elevated – than has supplemental arginine.[89, 90] Hence, it would be worthwhile to determine whether citrulline supplementation could boost bronchial NO production in CF patients and thereby improve pulmonary function, as has been reported with inhaled arginine.

Overview

These considerations suggest that metformin or berberine, salsalate, lipoic acid, NAC, citrulline, and a range of non-vitamin antioxidants (PhyCB, astaxanthin, high-dose folate, melatonin) merit evaluation in CF epithelial cells and, if promising results warrant it, clinical CF. These agents have the potential to address the ENaC overactivity, the oxidative stress, the exuberant production of pro-inflammatory cytokines, and the NO underactivity that appear to play a significant role in CF pathogenesis. Not unlikely, it may prove necessary to employ a complementary array of such measures to achieve clinically important benefit in CF.

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Addendum: Dosage Suggestions

Based on previous clinical use, or extrapolation from animal studies, the following dose ranges are suggested for those who wish to use the indicated drugs/nutraceuticals in clinical studies or open treatment with cystic fibrosis patients. These measures should be viewed as unproven adjuvants, and not as substitutes for clinically proven therapies.

Metformin – 500 mg 2-3 times daily, or 850 twice daily

Berberine – 500 mg 2-3 times daily

Salsalate – 1.5-2.25 g, twice daily (try lower dose first,

Lipoic Acid – 600-900 mg twice daily

N-Acetylcysteine – 600-1200 mg twice daily

Spirulina – 15g once or twice daily

Astaxanthin – 8 mg twice daily

Folate – 20-80 mg daily

Melatonin – 10-20 mg at bedtime

Citrulline Powder – 3 g twice daily