Abnormal n-6 fatty acid metabolism in cystic fibrosis contributes to pulmonary symptoms

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ABSTRACT

Cystic fibrosis (CF) is a recessively inherited fatal disease that is the subject of extensive research and ongoing development of therapeutics targeting the defective protein, cystic fibrosis transmembrane conductance regulator (CFTR). Despite progress, the link between CFTR and clinical symptoms is incomplete. The severe CF phenotypes are associated with a deficiency of linoleic acid, which is the precursor of arachidonic acid. The release of arachidonic acid from membranes via phospholipase A2 is the rate-limiting step for eicosanoid synthesis and is increased in CF, which contributes to the observed inflammation. A potential deficiency of docosahexaenoic acid may lead to decreased levels of specialized pro-resolving mediators. This pathophysiology may contribute to an early and sterile inflammation, mucus production, and to bacterial colonization, which further increases inflammation and potentiates the clinical symptoms. Advances in lipid technology will assist in elucidating the role of lipid metabolism in CF, and stimulate therapeutic modulations of inflammation.

1. Introduction

Cystic fibrosis (CF) is a metabolic recessive hereditary disease with the most common mutation, dF508, identified at chromosome 7 in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR) [1]. To date, roughly 2000 mutations have been identified; however, less than a tenth of those are associated with a clinical CF phenotype. The distribution of mutations varies in different ethnic populations and combinations with frequent complex alleles further influencing the phenotype [2–4]. The CFTR is a chloride channel, which also transports bicarbonate and glutathione, and its defect function in CF together with abnormal mucus production impairs the composition and transport of the airway surface liquid layers [5]. Furthermore, the abnormal CFTR influences the epithelial sodium channel (ENaC) resulting in an increase in the absorption of sodium, which aggravates the dehydration of secretion and the impaired airway fluid transport [6]. Pulmonary infections, especially with Pseudomonas aeruginosa, and metabolic changes involving calcium transport and arachidonic acid (ARA) further influence the airway mucus production [7–10]. Impaired phagocytosis and strong neutrophil inflammation are characteristics of CF and are at least partly related to Ps. aeruginosa-mediated eicosanoid stimulation of alveolar macrophages [11, 12]. Using high-throughput techniques, correctors and potentiators of the CFTR are under development to improve the channel function, and the new combination drugs have shown promising improvement in patients with specified mutations [13].

The most prominent clinical symptoms in CF are related to the airway system and the gastrointestinal tract, including liver and pancreas, and are characterized by sticky and tenacious mucus, explaining the alternative expression of the disease as mucoviscidosis. The development of transgenic animals has increased the ability to study the basic influence of an absent or dysfunctional CFTR upon metabolism and symptomatology. The access to transgenic pigs and ferrets has especially improved modeling of the human disease compared to transgenic rodents [14].

The influence of gene modifications upon the phenotype has been discussed [2, 15] and altered DNA methylation levels have been shown in nasal epithelium and blood cell samples from patients with CF, suggesting the influence of epigenetics [16, 17]. These changes might explain differences of disease severity in siblings with the same mutations.

2. The link between the CFTR channel and clinical symptoms

The general view to explain the clinical characteristics of the disease is that the abnormal CFTR channel function makes the secretion thick and difficult for normal transportation by cilia and thereby causes stasis in the airways favoring bacterial colonization. Many more channels and...
signaling pathways are likely involved; however, the link to the different clinical symptoms is incomplete [18, 19]. A number of the disease features are difficult to explain from a pure channel dysfunction, for example the abnormal sialylation [20], abnormal glucose/insulin homeostasis [21], abnormal bone mineralization [22] and abnormal lipid metabolism [23]. Oxidative stress as an effect of infection is common, but it is also discussed to be more primarily connected to the type of mutation [24]. Recent data suggest a direct effect of CFTR modifying lipid mediators [25–27], and metabolomics approaches offer the potential to identify new relevant pathways in disease [28–33].

3. The characteristic airway disease

Airway symptoms comprise both the sinus and the lungs. It has long been controversial as to whether the inflammation or the infection is the primary cause of the airway pathology [19, 34–36]. Recent research, including that in transgenic animal models and newborn screening programs, has supported the view that the inflammation is primary to infection [37–39]. This was also illustrated in a study of CF and non-CF human fetal grafts, where IL-8 production was increased 8-fold in the sterile CF graft. A significant increase of focal subepithelial clusters of leukocytes was also found relative to the non-CF grafts [40].

Several studies indicate a characteristic high neutrophil-mediated inflammation in CF [41–43]. Impaired phagocytosis in dysfunctional airway neutrophils and macrophages contributes to the disease [43–48]. This might be interpreted as an imbalance between the anti-infectious and the pro-inflammatory functions of the neutrophils [47], which was not modulated by in vitro treatment with docosahexaenoic acid (DHA) [48]. This imbalance together with impaired specialized pro-resolving lipid mediator (SPM) production can prolong the inflammation [25–27, 45–47].

Abnormal pulmonary surfactant composition and activity have also been reported [49–52], particularly shifts in the phospholipid profiles. The most consistently observed alterations include a decrease in the ratio between phosphatidylcholine and sphingomyelin [49–51] and an increase in phosphatidyl-inositol [49, 50, 52]. It is unclear the extent to which these changes are related to basic disturbances or to infection. The changed ion transport and low pH in small airways impair the killing of bacteria [53]. The host defenses are dependent on a balance between the surfactant proteins and surfactant lipids to modulate the immune response to bacteria [54]. Decreased surfactant phospholipid levels have been associated with chronic Ps. aeruginosa infection, activating a reduced promoter activity of phosphocholine cytidylyltransferase, the rate regulatory enzyme required for the major surfactant phospholipid, dipalmitoylphosphatidylcholine [55]. Furthermore, an impaired clearance of apoptotic cells are related to changes in the surfactant proteins SP-A and SP-D [56].

The expression of CFTR and ENaC are more prominent distally in healthy individuals; CFTR is more expressed in type II alveolar cells, while ENaC expression is greater in type I alveolar cells. These patterns suggest that CF might have an early and important disturbance in the alveolar secretion besides the more extended abnormal mucus in goblet cells and submucosal glands [53, 57, 58]. The field is developing a better understanding of the surfactant pathology in order to improve pulmonary symptoms, especially by studies in transgenic pigs.

In contrast to what would be expected in the inflamed and infectious airways, exhaled nitric oxide NO is decreased in CF [59]. No explanation has been found; however, arginine activity was reported to increase in CF cell lines, reducing the available substrate for NO synthase [60]. Another study found that low nasal and exhaled NO levels were associated with the more severe genotypes [61]. Non-pseudomonas infected patients showed an inverse correlation between nasal NO and the ratios of serum phospholipid concentrations of AA to DHA and oleic acid (OA) to linoleic acid (LA) [49, 61], with both ratios characteristically increasing in CF.

It is unclear why the infection in CF usually starts with Staphylococcus aureus [62], and is then followed by the facultative microorganism Ps. aeruginosa [63]. Although these are the major pathogens, the patients can also be chronically infected by other bacteria, and they have a higher prevalence of atypical mycobacteria and fungi [64, 65]. Accordingly, the patients have a high load of antibiotic and mucolytic treatments, especially because the survival is limited by the progressive pulmonary disease and seldom by failure of other organs (e.g., liver cirrhosis). In severe cases, lung and/or liver transplantation can now prolong the life of many patients [66].

One problem in studying CF is the recurrent exacerbations of the airway infection due to the chronic bacterial colonization, often triggered by viral infections and other clinical events [67]. The inflammatory response appears to be increased in CF patients with a higher IL-8 and neutrophil response, regardless of pathogens compared to respiratory infections in other individuals [41]. These results might be explained by dysfunctional feed-back mechanisms in CF cells, as discussed above, including the regulation of lipid mediators. Studies have reported that CFTR knockout mice had higher levels of cyclooxygenase-2 (COX-2) and NKB activity and that a negative regulation of COX-2/PGE2 was lacking in these animals [68]. A changed macrophage profile and function have also been suggested to impair the inflammatory response [69, 70]. Studies in transgenic animals are valuable, because investigations can be initiated in fetal life to follow disease development [40, 71]; however, species-dependent differences in development, morphology and immunity between rodent, pigs and ferrets compared to humans also restrain the comparisons [14].

4. Inflammation and fatty acid metabolism

If the inflammation is the primary cause of the airway disease, the responsible factors have to be defined. Inflammation is a defense system under strict control during healthy conditions. The balance between long-chain PUFA-derived pro-inflammatory and pro-resolving mediators is an important component of the inflammatory homeostasis. The most studied PUFAs are ARA, a n-6 fatty acid, and the n-3 fatty acids eicosapentaenoic acid (EPA) and DHA [72]. As schematically shown in Fig. 1, these fatty acids are synthesized from the essential fatty acids, LA and α-linolenic acid (ALA), respectively. Their relative rate of conversion is influenced by the balance between the individual fatty acids, because the substrates compete for the same enzymes. High levels of ARA have been associated with inflammation due to its conversion to pro-inflammatory eicosanoids, but the view that high LA will lead to enhanced development [40, 71]; however, species-dependent differences in development, morphology and immunity between rodent, pigs and ferrets compared to humans also restrain the comparisons [14].

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Although less studied than ARA, LA is also converted to a suite of downstream putative lipid mediator products that have been suggested to be involved in the inflammatory process, but whose functions are largely unknown [86]. These compounds are broadly termed octadecanoids and include the hydroxyoctadecadienoic acids (HODEs), epoxy- and dihydroxyoctadecenooic acids (EpOMEs and DiHOMEs), and trihydroxyoctadecenooic acids (TriHOMEs), as well as recently described microbial products [87]. The HODEs have reported PPARγ antagonist activity [88] and are considered to be general markers of autooxidation [29]. The vicinal diol 12,13-DiHOME is the soluble epoxide hydrolase (sEH) product of the corresponding LA-derived epoxide (12(13)-EpOME) and has been reported to impede immune tolerance and be anti-inflammatory properties of lipid mediators derived by cytochrome P450 [92, 93]. In addition, anti-inflammatory properties of lipid mediators derived by cytochrome P450 activity may be involved; however, these compounds have been less studied in CF [86]. A new group of esterified eicosanoids attached to phosphatidylethanolamines (Elovl2 and 5). Peroxisomal β-oxidation (β-oxid); Dihomo-γ-linolenic acid (DHGLA); Arachidonic acid (AA), Eicosapentaenoic acid (EPA); Docosapentaenoic acid (DPA); Docosahexaenoic acid (DHA). The major metabolites are shown in text boxes.

5. Linoleic acid deficiency

It has been known for >50 years that patients with CF have deficient circulating concentrations of LA [99] and this has been confirmed in numerous studies and in many tissues [79, 100, 101]. Later studies have shown that the degree of this deficiency is related to the type of mutation; the mutations associated with more severe phenotypes are more prone to have LA deficiency [99, 100] with a compensatory increase in OA [102, 103]. The deficiency is present already at birth and also illustrated by an increase of eicosatrienoic acid (Mead acid; 20:3n-9) and the ratio between Mead acid and ARA [104, 105]. Mead acid can be similarly converted to eicosanoid analogs, including LTB4 and LTC4 [106]. Longitudinal studies in infants identified by neonatal screening have shown that the LA deficiency appears to be less in toddlers and increase again in prepuberty [105], and that children with the lowest concentrations of LA initially had difficulties to grow in spite of high energy intake compared to those with consistent high LA concentrations [107]. These authors also showed that patients with higher concentrations of LA did not need very high energy intake to grow [108]. This is of interest because pulmonary symptoms are very strongly correlated to nutrition.

There is no consistent increase of ARA and decrease of DHA in plasma, but the balance, as reflected in the ratio between these fatty acids, is usually increased [101–103]. In addition, patients without pancreatic insufficiency and heterozygotes seem to have a mild lipid abnormality [109, 110]. The strong association between severe phenotype with pancreatic insufficiency and low LA concentrations explains why the deficiency was for a long-time referred to as fat malabsorption, which was more pronounced in the earlier years when there was less efficient pancreatic enzyme replacement therapy. Later research has shown that there might be other explanations for the low LA concentrations, as will be discussed below.

6. The cause of linoleic acid deficiency in CF

One explanation for the low LA concentration might be a high turnover of LA by increase of the enzymes to transform LA to long-chain PUFAs via desaturation and elongation [111]. For example, specific genotypes of fatty acid desaturases (FADS) are associated with variability in the conversion of LA to ARA and the subsequent formation of eicosanoids [112]. A high turnover of phosphatidylcholine, an
important constituent of membranes, has been reported in CF [113–115]. An increase of the transforming enzymes has been suggested to be the primary explanation of the lipid abnormality [111]; however, this cannot explain the defective inhibition of PLA2 by dexamethasone, resulting in increased liberation of ARA compared to controls [116]. This inhibition was posttranscriptional because the synthesis of thymidine was unaffected and suggested to depend on a defective regulation of PLA2 by lipomodulin (lipocortin, annexin 1) contributing to the clinical symptoms in CF [117]. Borot et al. [118] confirmed the link between CFTR, annexin 1, p11 and the increase of PLA2 in a cell line with a CFTR inhibitor. In an elegant study, Dalli et al. [119] presented a hypothesis that the abnormal inflammation related to defective CFTR function could be linked to impaired annexin 1 handling in the cell. The association between abnormal annexin and inflammation had further been supported in CF KO mice, in which annexin 1 was undetectable in lungs and pancreas associated with an upregulation of PLA2 [120]. The same authors reported that in nasal biopsies from CF patients, the annexin 1 expression was less attenuated in those with a mild phenotype. Annexin 1 expression was also found to be downregulated in a CF mouse model with tendon inflammation [121]. The control of ARA liberation by annexin 1 is dependent on the interaction with p11 of the S100 family of calcium-binding proteins [122], which can be induced by dexamethasone [123]. This potential link to the impaired PLA2 inhibition in CF requires further study.

The defective inhibition of ARA liberation has also been confirmed in other cell systems [115, 124–126]. Increased release of ARA can explain the elevation in eicosanoid levels, because ARA release is the rate-limiting step for eicosanoid synthesis [127]. Metabolites of both prostaglandins and thromboxane B2 have been found increased in plasma and urine, which indicates a systemic increase in eicosanoid synthesis [79–82]. Increases of PGE2 and PGF2α were hampered by LA supplementation [85, 81]. Dexamethasone did not inhibit calcium ionophore A23187-induced production of LTB4 and LTC4 in leukocytes of CF patients [128]. This further supported previous observations of an impaired ARA release and the associated impact on the prostaglandin system. Resistance to dexamethasone response in epithelial CF cells has also been reported by others [129].

Infection can stimulate the inflammatory process, but higher concentrations of ARA were found in bronchial secretions of stable CF patients compared to other pseudomonas infected non-CF patients [130]. Elevated ARA was also found in sputum from adult CF patients [30]. In lung phospholipids of CF mice, phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol containing ARA were increased although the authors found that generally the n-6 fatty acids in phosphatidylcholine were decreased [131]. Further support for an increased ARA is provided by Dif et al. [132], who reported increased cPLA2 activation in bronchial epithelial cells from CF patients and further that silencing of CFTR in human bronchial epithelial cells induced PLA2 activation. The same group has also shown that bronchial cells from CF patients with the dF508 mutation released more PGE2 than control cells on LPS stimulation, related to higher COX-2 expression [133]. An absence of negative regulation of CFTR on COX-2/PGE2 might further increase inflammation [68]. PGE2 can induce the expression of IL-6 and IL-8 in T lymphocytes and airway epithelial cells, respectively [134, 135]. These cytokines, usually increased in the serum of CF patients, have also been shown to be associated with an increased ARA/DHA ratio in the alveolar cells [136]. This suggests that the lipid abnormality and the effect of infection cooperate in the abnormal airway milieu, as illustrated by an improvement in the LA status after lung transplantation [137]. Interestingly, ivacarot treatment, which has a good effect on pulmonary function in patients with GS11D mutations, showed a trend to lower ARA and prostaglandin levels [138]. Accordingly, data indicate a high degree of inflammation associated with increased availability of ARA, which might be reflected in a high turnover of LA, resulting in low serum concentration in the plasma phospholipids [117].

7. Arachidonic acid and mucus
The sticky and tenacious mucus has been associated to the impaired channel activity by decreased bicarbonate, lowering the pH and inhibiting ENAC, but that has not fully explained the airway disease. The concentrations of the mucins MUC5B and MUC5AC appear to be differently regulated [139]. MUC5B is flushed by chloride and bicarbonate rich fluid, which raises the pH and thereby pulls MUC5B into a linear polymer. These bundles of MUC5B are then transported by cilia. MUC5AC mucins produced from the goblet cells coat the bundles, which has been suggested to slow their transport and separate them from the fast transporting airway surface liquid flow. It has been shown that CFTR silences the MUC5AC expression in bronchial human epithelial cells and that the expression was enhanced by ARA and reduced by cPLA2 inhibition [132]. Interestingly, inhibition of the CFTR chloride transport function had no effect on MUC5AC expression. The same authors also showed that in a CF mouse model, both basal and LPS-induced MUC5AC expression were associated with increased cPLA2 activity and that increased PLA2 activity was also found in bronchial explants from CF patients [133]. The sticky mucus might thus be influenced by non-linear MUC5B bundles from the defective CFTR channel function and an increase in MUC5AC mucus due to an elevated concentration of ARA, which further hampers mucus transport.

8. n-6 fatty acids in relation to bacterial colonization
Factors behind the characteristic bacterial colonization are incomplete and new studies in transgenic animals suggest more complicated impairment in the host defense to infection [140], as also described above (Section 3). The chronic colonization with pseudomonas has been suggested to be associated with an impaired CFTR function limiting the possible internalization and lysosome digestion of bacteria [141]. Pseudomonas is a facultative bacteria and not unusual in other lung diseases. Its growth is favored by hypoxia, which is always present in advanced disease, but might also be relevant at the alveolar level [95]. However, the staphylococcus infection usually precedes the pseudomonas colonization and often also persists in parallel. In a rabbit alveolar macrophage experimental model containing labeled ARA, the individual eicosanoid profiles produced as well as the overall amounts differed based upon the bacteria species [11]. Furthermore, bacterial phagocytosis was inhibited by PGE2 binding to G protein coupled E-prostanoid receptors increasing intracellular CAMP in a human macrophage-like cell line [12]. A virulence factor in Ps. aeruginosa (e.g., flagellin) was crucial for the bacterial influence on mucus production and IL-8 secretion in human epithelial cells [8]. Flagellin has been identified as the binding factor of pseudomonas to MUC 1 on the epithelial cells. Conversely, pseudomonas infection may increase MUC 1, which has an anti-inflammatory action by preventing overexpression of inflammatory mediators [7]. The interaction is complex and early studies in transgenic animals are important to distinguish basic disturbances from a surplus of bacterial infections. It may be debated that the staphylococcus infection has been neglected due to the parallelism between the deteriorated lung function and the chronic pseudomonas infection. St. aureus is much more aggressive in cell systems and caused a hundred-fold higher respiratory burst than pseudomonas in an in vitro system [142]. Bacterial growth can be modulated by lipid substrate [143], and there are some interesting data suggesting that the imbalance between LA and the compensatory increase of OA seen in CF might stimulate growth of St. aureus [144, 145]. The n-6-and n-3 fatty acids might also inhibit growth of Ps. aeruginosa [146] and increase macrophage phagocytosis [147]. Intravenous administration of either γ-linolenic acid (18:3n-6) or ARA enhanced antimicrobial bacterial killing in experimental sepsis caused by multidrug-resistant Ps. aeruginosa [148]. Accordingly, the fatty acid status in patients with CF can have important implications for the treatment of their bacterial infections. It can be speculated that the regular supplementation of LA in a
majority of Swedish patients with CF during the last decades of the 20th century contributed to the relatively low need of antibiotic treatments and well-preserved lung function [149].

Recent studies have discussed the importance of interactions between the intestinal and pulmonary microbiota - the gut-lung axis [150]. Different types of interaction have been suggested, including transfer of bacterial metabolic products or immunological factors [87]. A further theoretical possibility is that the habitat for growth of special bacteria may be similar in intestinal and pulmonary milieu by changes in membrane lipid composition. In CF, the effects of probiotics have been studied; however, the impact on pulmonary symptoms has to date not motivated a general recommendation [151].

9. Clinical effect of linoleic acid supplementation on pulmonary function

Several clinical studies have shown that a hypercaloric diet, 120-200% of recommendations, is seldom sufficient to achieve good clinical nutrition in patients, despite the fact that such diets also increase the associated LA intake [152]. This has been repeatedly demonstrated and recently illustrated in a study with aggressive nutritional treatment of newborns with CF diagnosed by newborn screening [153]. Supplementation of LA together with high energy intake can improve the clinical status and even lower the energy demand [106, 154–157]. One study showed that supplementation increased the LA fraction in the urine [78] compared to initial values. A concern for stimulation of inflammation by supplying LA, due to it being a precursor to ARA, has resulted in hesitation to supply LA and therefore large clinical studies are scarce [160]. This fear may be unfounded because studies in different systems have shown that LA and ARA concentrations have an inverse association [74, 161–163]. This seems also to be particularly relevant for patients with CF [78, 164] (Fig. 2). Furthermore, in Sweden, a large portion of the CF population has since the 1970’s been supplemented with LA [148], which has probably contributed to excellent clinical results, including well preserved lung function into adulthood [66, 165]. However, controlled randomized long-term studies are necessary to obtain support for a beneficial effect of LA supplementation, which might be an inexpensive adjuvant relative to more costly therapy.

10. Conclusion and perspectives

There are increasing indications that the lipid abnormality might have a profound influence on the clinical symptoms in CF, not only the pulmonary disease focused on in this perspective. The early inflammation and many symptoms can be associated with abnormal n-6 metabolism, in terms of both the increased AA metabolism and the LA deficiency. Recent animal studies further support inflammation as primary to infection, but the latter undoubtedly adds to this imbalance by further releasing ARA and inflammatory eicosanoids. During these circumstances, interaction with the SPMs would be of importance and more knowledge about the different lipid mediators might explain much of this interference. New insights into lipid metabolism in CF can be obtained with modern technology and prospective randomized controlled intervention studies are warranted and planned. LA supplementation may have potential as an inexpensive adjuvant treatment to the developing modulators of the channel function.

Declaration of competing interest

The authors state that they have no conflicts of interest to declare.

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