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Oxidative Stress in Cystic Fibrosis

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Abstract

Airway infection leads to progressive damage of the lungs in patients with cystic fibrosis (CF). Oxidative stress has been implicated as a causative factor in the aetiology of that process. Supplementation with antioxidant micronutrients (vitamin E, vitamin C, β -carotene and selenium), docosahexaenoic acid or glutathione might therefore be helpful in maintaining an adequate redox balance. Current literature suggests a relationship between oxidative status and lung function. In this chapter we will summarize the main pathways of oxidative stress, focusing on results of new antioxidant treatments.

Keywords: Oxidative stress, fat-soluble vitamins, β -carotene, glutathione, essential fatty acid, antioxidants

1. Introduction

1.1. Oxidative stress pathways in cystic fibrosis

Colonization of the respiratory tract by bacterial pathogens in the mucus of CF patients leads to a sustained inflammatory response characterized by massive influx of polymorphonuclear neutrophils and the activation of macrophages, eosinophils, monocytes and lymphocytes. An integral part of this inflammation is the production and release of free radicals such as superoxide (O_2^-) and hydroxide (OH^-), which can induce oxidative stress. In fact, elevated levels of proinflammatory cytokines, especially interleukins (IL-1 β , IL-6, IL-8), tumour necrosis factor- α and potent neutrophil chemoattractants found in bronchoalveolar lavage, are

involved in the production of pro-oxidants leading to apoptosis and tissular damage. Conversely, the production of immunosuppressive cytokine IL-10 with anti-inflammatory properties is reduced or even suppressed [1].

Thus, the severe and recurrent respiratory inflammation ultimately leads to excessive activated neutrophils and macrophages, which contribute to the generation of free radicals. Furthermore, defects in the Cystic Fibrosis Transmembrane Regulator (CFTR) can directly affect transport and glutathione homeostasis, while maldigestion and malabsorption related to exocrine pancreatic insufficiency impair the absorption of fat-soluble vitamins and antioxidants. It has been suggested that the chloride channel CFTR also regulates glutathione, disturbing the balance between pro- and anti-oxidants and promoting oxidative stress, which may play an important role in Cystic Fibrosis Related Diabetes, a serious complication associated with a dramatic increase in morbidity and mortality [2].

Although the cause of CF is well established, the pathogenesis of this progressive multisystemic disease is not yet fully understood. In fact, the broad spectrum of phenotypes and severity in CF patients that carry the same combination of mutations suggests additional environmental or genetic factors.

The CFTR dysfunction in the pancreas causes exocrine pancreatic insufficiency in almost 90% of patients with CF. This leads to fat malabsorption, which explains the difficulty to gain or at least maintain weight, and the high incidence of fat-soluble-vitamins and antioxidant (vitamins A, E, and D and carotenoids) deficiency, and also essential-fatty-acids deficiency. Obviously, the reduced availability of dietary antioxidants may further increase oxidative stress in CF patients, which apparently plays an important role in multiorgan pathophysiology of CF.

Consequently, the products of lipid peroxidation, which are markers of oxidative stress, have been detected in exhaled breath condensate, as well as in blood and urine of CF patients. Thus products of lipid peroxidation are unstable molecules that can reach distant sites to exert various effects, including activation of the fibroblast cells in the presence of inflammation, which further increases oxidative stress [3].

2. Management of oxidative stress in cystic fibrosis

Nutrition plays an essential role in the survival and quality of life of CF patients. CF patients have high caloric requirements due to an increased resting energy expenditure (REE), bacterial infection, and malabsorption. REE is higher in CF patients with a more severe phenotype. Lung function and nutritional status are closely correlated, and the severe weight loss can lead to a decrease in lean body mass, with consequences for respiratory muscles. There is a significant correlation between growth retardation and the severity of pulmonary involvement [4]. Recently, Yen et al. found that greater weight at age four years is associated with greater height, better pulmonary function, fewer complications of CF, and better survival through the age of 18 years. Furthermore, greater weight for age in the peripubertal period is associated, on

average, with improved tempo and timing of pubertal height growth [5]. Long-term nutritional management is as integral a part of modern care as pulmonary therapy, and is intimately linked to pulmonary outcomes.

Dietetic management is based on the replacement of pancreatic enzymes and fat-soluble vitamins (A, D, E, K), together with a high-protein diet and high calorie intake (120–125% of the normal recommended daily allowance). The recommended Body Mass Index (BMI) for adult CF patients is ≥ 22 kg/m² for females and ≥ 23 kg/m² for males. Overnight enteral nutrition via nasogastric tube or gastrostomy provides supplementary nutritional support when BMI is suboptimal [6].

a. Docosahexaenoic acid (DHA)

Essential fatty acid (EFA) imbalance has been identified in CF patients and is characterized by a decrease in docosahexaenoic acid (DHA) and linoleic acid and an increase in arachidonic acid (AA) [7]. These characteristics were mainly attributed to intestinal malabsorption due to exocrine pancreatic insufficiency. In the last few years, new mechanisms have been proposed, such as intensification of the β -oxidation of polyunsaturated fatty acids (PUFAs), inadequate dietary EFA consumption, the possibility of an intrinsically defective EFA metabolism in CF epithelial cells, an increase in the production of proinflammatory eicosanoids, a rise in the peroxidation of PUFAs, and finally an impairment of desaturases or hepatic lipase activity [8].

It has been observed that long-term intake of the daily mixtures of fatty acids (eicosapentaenoic, docosahexaenoic, linoleic and γ -linolenic acid) at a low dose has a positive effect on lung function and inflammation in adult CF patients. The total number of exacerbations after a year of supplementation was reduced, while the lean-body-mass and lung-function parameters measured by spirometry were increased. In addition, supplementation led to improved parameters of oxidation, inflammation (IgG and IgM) and other clinical parameters [9]. 8-isoprostane, a free-radical product of lipid peroxidation which is a consequence of oxidative stress, appears to be a prognostic factor in deterioration of lung function in a short time in patients infected with *Burkholderia cenocepacia*. The measured concentration of 8-isoprostane in exhaled breath condensate of 24 patients did not show any significant relationship with the clinical parameters during a one- and three-year study [10].

Forty-three CF patients were enrolled in a randomized double-blind placebo-controlled study with three fatty-acid blends containing mainly n-3 or n-6 FA, or saturated fatty acid acting as placebo [11]. After three months, in the omega-3 fatty-acid-supplemented group, a significant decrease in the inflammatory markers, erythrocyte sedimentation rate and IL-8 was reported. Another, longer-term study (17 participants) demonstrated a significant increase in essential-fatty-acid content in neutrophil membranes and a significant decrease in the leukotriene B₄ to leukotriene B₅ ratio in participants taking omega-3 supplements compared to the placebo [12].

b. Fat-soluble vitamins

Supplementation of CF patients with vitamin E and β -carotene has been effective in preventing oxidative lung damage [13], as seen by a decrease in lipid peroxidation products. It is believed that these antioxidants have an important role in maintaining or restoring essential-fatty-acid

status by protecting polyunsaturated fatty acid from oxidative degradation, as their supplementation augments levels of plasma polyunsaturated fatty acid.

Carotenoids

Levels of plasma carotenoids such as β -carotene, β -cryptoxanthin, and total lycopene are significantly lowered in CF patients and this has been associated with higher susceptibility to lipid peroxidation. Rust et al. [14] examined the effect of long-term oral β -carotene supplementation in patients with CF. Patients of the CF supplementation group received 1 mg β -carotene/kg body weight/day (maximally 50 mg β -carotene/day). During high-dose treatment, a significant decrease in the MDA level and a correction of total antioxidative capacity was observed.

Renner et al. reported distinct clinical benefits from high-dose (1 mg/kg body weight/ day, maximum 50 mg/day) supplements. Their patients required significantly fewer antibiotics during the phase of high-dose β -carotene supplementation and showed a decrease in pulmonary exacerbations [15]. Lepage et al. reported that the two-month supplementation of CF patients with 4.42 mg β -carotene, three times per day, led to the normalization of increased MDA level and increased plasma β -carotene from 0.08 ± 0.03 to 3.99 ± 0.92 μM [16].

At the same time, toxicity issues have been raised for supplementation with water-miscible vitamin A formulations in CF patients, which may increase serum retinol and possible risk of CF-associated liver and bone complications [17]. However, β -carotene supplementation seems to be safe since it does not affect serum concentrations of other carotenoids and retinol [14]. Recent studies have analysed the use and safety of a new CF polyvitamin (AquADEKs®), which comprises almost 90% vitamin A in retinol form. Patients on AquADEKs® maintained a high level of serum β -carotene, but serum retinol was not above the normal levels. β -carotene levels were associated with lung function and better nutritional status [18,19], while lipid peroxidation markers were not affected [20].

Vitamin E (α -tocopherol)

α -tocopherol acts as a membrane antioxidant closely associated with polyunsaturated fatty acids. Vitamin E's antioxidative properties might be helpful in reducing the negative effects of free radicals. Current recommended supplementation of vitamin E in CF patients only includes α -tocopherol. Supplementation with high levels of α -tocopherol alone may result in further imbalances in CF patients: such supplementation has been shown to deplete γ -tocopherol in the blood and tissues [21]. Papas et al. [16] evaluated vitamin E supplementation with mixed tocopherols. The increase in the blood of levels of γ -tocopherol may be particularly important for CF patients due to its function as a scavenger of reactive nitrogen species and its synergistic effects with α -tocopherol [22]. Cystic fibrosis is characterized by neutrophil-dominated airway inflammation. Activated neutrophils release oxidants, proteases, and cytokines, further sustaining and increasing the inflammatory response and causing direct injury to the lungs. Improved antioxidant capacity with γ -tocopherol, especially if present in the lungs, could potentially decrease oxidant-mediated damage and limit the cytokine-mediated neutrophil recruitment. It has also been reported that reduced serum levels of vitamin E are associated with an increased rate of pulmonary exacerbations in CF [23].

c. Other vitamins and micronutrients

Vitamin C

Vitamin C is present in the respiratory lining fluid of human lungs, and local deficit occurs during oxidative stress. Experimental findings confirm that vitamin C induced the openings of CFTR Cl⁻ channels without a detectable increase in intracellular cyclic AMP levels. Vitamin C instilled into the nasal epithelium of human subjects effectively activates Cl⁻ transport, too.

The pool of vitamin C in the respiratory tract represents a potential nutraceutical and pharmaceutical target for the complementary treatment of oxidative stress in patients with CF [24]. In a study by Winklhofer-Roob et al. [25] on 122 children and young adults with CF, ascorbic-acid concentrations decreased with age, and low vitamin C levels were associated with the highest indexes of inflammation, so the authors concluded that ascorbic acid could interact with an inflammation-amplifying circle of activation of alveolar neutrophils and macrophages.

Water-soluble vitamins seem to be well absorbed by patients with CF, but there is documented evidence of poor dietary intake. A supplement of at least 50–100 mg vitamin C/day should be prescribed for patients with an unbalanced diet, or if there is evidence of deficiency.

Selenium

Dietary intake of selenium is inversely related to inflammatory markers such as sialic acid and triacylglycerol [26]. Moreover, a possible role of selenium in the modulation of serum complement 3, which may be an early marker of metabolic syndrome manifestations, has also been documented.

Wood et al. carried out an eight-week, double-blind, randomized intervention trial, providing two groups of patients with low- and high-dose vitamin supplements (500 µg vitamin A and 10 mg vitamin E vs. 500 µg vitamin A, 25 mg β-carotene, 200 mg vitamin E, 300 mg vitamin C and 90 µg selenium). They demonstrated significant improvement of clinical indicators after treatment. Increased serum β-carotene, selenium, and fatty-acid concentrations were linked to improved lung function [27].

Consensus regarding supplementation of antioxidants in CF to include selenium is yet to be established. Two studies have attempted to demonstrate successfully how the administration of 2.8 µg/kg/day and 90 µg/day of selenium can decrease oxidative stress in CF [28].

d. Glutathione and n-acetylcysteine

The discovery that CF is associated with significantly diminished efflux of reduced glutathione (GSH) from most cells in the body [29] offers a new perspective on the pathophysiology of this disease. GSH plays several important roles; among the most important are the following: 1) primary water-soluble antioxidant; 2) mucolytic capable of cleaving disulphide bonds; and 3) regulator of immune-system function [30].

The relationship between redox ratio (GSH:GSSG) and total glutathione (GSH+GSSG) and the initiation of inflammation is well established [29,30]. GSH is also an important component of the epithelial lining fluid of the intestines, helping to keep intestinal mucus thin, serving to

defend the intestinal system against reactive oxygen species, and keeping inflammation in check under normal circumstances [31].

In a recent placebo-controlled, randomized, double-blinded, clinical trial in 44 paediatric CF patients aged between 18 months and 10 years [32], treatment with oral glutathione (65 mg/kg/day) increased weight and BMI z score and improved measures of gut inflammation (faecal calprotectin) over the course of six months, without adverse side effects. The authors therefore concluded that Oral GSH might primarily be beneficial in those children with more severe inflammation of the gut, and suggested that early intervention with oral glutathione in young CF children with growth failure could forestall decline in pulmonary function in later years.

Several studies have investigated the potential therapeutic role of inhaled GSH in patients with CF. Three short-term clinical trials, including a placebo-controlled one, have shown the tolerability and efficacy of inhaled GSH on pulmonary function in these subjects [33-34]. A recent 12-month randomized single-blind placebo-controlled trial demonstrated the efficacy of inhaled GSH (600 mg twice daily) on lung function in CF adults [35]. Three months of therapy with inhaled GSH resulted in a statistically significant improvement in percentage-predicted FEV₁, measured as a pre-post difference from baseline values, when compared to the placebo, which persisted at six and nine but not at 12 months. A reduced compliance with therapy in adult patients could explain the decrease in FEV₁ values registered in the last visit. The best improvements in functional parameters were registered in the subgroup of patients with moderate lung disease (FEV₁ below 81%). These results are in concordance with those reported by Griese et al. [36], who showed a significant increase of FEV₁ absolute values (but not when expressed as percentage-predicted) from the baseline after three months of GSH therapy.

N-acetylcysteine (NAC), a well-known cysteine donor for the synthesis of glutathione, has been used in different diseases to treat GSH deficiency [37]. High-dose oral NAC has been shown to increase neutrophil GSH levels, decrease airway neutrophil recruitment and reduce neutrophilic release of airway elastase in CF patients [38]. Skov et al. demonstrated that high-dose oral NAC (1200 mg x 2/day for 30 days) in CF patients with chronic *P. Aeruginosa* infection decreased the level of oxidized vitamin C [39].

Indications of a positive effect of NAC treatment on the lung function of a subgroup of CF patients have previously been published [40]. Recently, a placebo-controlled randomized clinical trial (70 CF patients) was conducted in the USA to study the effect of oral NAC on lung inflammation (ClinicalTrials.gov Identifier: NCT00809094). Oral NAC was administered in a dose of 1800 mg/day divided into two dosages over a period of 24 weeks and the effects on the sputum levels of human neutrophil elastase (HNE) were assessed as a primary end-point. While no statistical significant difference was found between the two groups with regard to the primary end-point, an improvement in the predicted FEV₁% was observed in the NAC-treated group.

A recent Cochrane review on the use of thiol derivatives, such as NAC, did not find sufficient evidence to recommend the use of these compounds in the management of CF lung disease, but concluded that further studies were warranted [41].

γ -Glutamylcysteine ethyl ester (GCEE) is another potentially interesting GSH pro-drug, which has proved to have some efficacy in the amelioration of oxidative stress, e.g., in experimental myocardial infarction [42] and central-nervous-system conditions (see, e.g., [43]). However, GCEE has not been investigated in CF yet.

New CFTR modulation therapies are being designed to correct the function of the defective protein (CFTR) made by the CF gene, allowing chloride and sodium to move properly in and out of cells lining the lungs and other organs. N6022 is a new injectable compound that modulates the function of the defective CFTR protein and decreases inflammation in the lung. N6022 is the first of a new class of compounds that increase levels of an important signalling molecule in the body called S-nitrosoglutathione, or GSNO. These novel compounds have been shown in preliminary results (Phase 1b trial) to increase the amount of CFTR that reaches the cell membrane and to stabilize CFTR so that its function can be improved.

3. Conclusion

In conclusion, there appears to be conflicting evidence regarding the clinical effectiveness of antioxidant supplementation in CF patients. Based on the available evidence, glutathione (administered either orally or by inhalation) and high doses of β -carotene appear to improve lung function in some cases and decrease oxidative stress. Further studies, especially in very young patients, examining clinically relevant outcomes, dose levels and other promising therapies like CFTR modulation, are necessary before a firm conclusion can be made regarding the effects on oxidative stress in these patients.

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