OXIDATIVE STRESS AND ANTIOXIDANT THERAPY IN CYSTIC FIBROSIS IN CHILDREN

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Abstract

Cystic fibrosis (CF) is caused by a defect of CFTR gene. The presence of defective CFTR appears to produce a redox imbalance in epithelial cells and extracellular fluids and causes an abnormal generation of reactive oxygen species (ROS). Airway surface liquid (ASL) in CF bronchi is characterized by increased concentration of ROS, lowered levels of glutathione and reduced nitric oxide. The increase of pro-oxidative species in ASL contributes to the progressive lung tissue damage and to the amplification of the inflammatory response in CF airways. Oxidative stress and inflammation can affect surfactant biophysical activity leading to early alterations of lung function and involving both lipid and protein components. Surfactant protein D becomes unable to agglutinate bacteria when it is modified by oxidation, which facilitates pathogen colonization in the lung. Antioxidant supplements might reduce the oxidative damage in the lungs. Different antioxidants as vitamin E, β-carotene and ω-3 fatty acids have observed to alleviate selected biochemical signs of oxidative stress. Glutathione - the major antioxidant shield in the epithelial lining fluid, in people with CF is not released into the lungs properly. Administration of glutathione improve lung function in some cases and lower oxidative stress. Some enzymes which help antioxidants work are dependent on selenium; so selenium supplements aim to stimulate antioxidant action. Vitamin C decreases with age in people with cystic fibrosis, so vitamin C supplements aim to rebuild these levels. Conclusion: antioxidant supplementation may prove beneficial in slowing the rate of deterioration in pulmonary function when combined with current therapies.

Key words: cystic fibrosis, children, oxidative stress, antioxidants.

Cystic fibrosis (CF) is the most common hereditary disease of the Caucasian population that involve multiple organs, especially the respiratory and the digestive systems. The defect in CF is the loss of function of the transmembrane conductance regulator (CFTR) protein. The disease severity is the consequence of environmental and genetic factors. Among them, the oxidative stress (OS) play an important role in the evolution of CF, with susceptibility to oxidative damage, decline of pulmonary function and impaired lung antioxidant defense.1

Oxidative stress represent an imbalance between oxidant production and antioxidant defense, resulting in an increase in the steady-state levels of oxidized cellular macromolecules.2 Oxidants are derived from an NADPH-oxidase that reduces molecular oxygen to superoxide. Thr superoxide dismutates to hydrogen peroxide which is used by the heme enzyme myeloperoxidase to oxidize chloride and thyocyanate to hypochlorus acid and hypochoynicite.3

Patients with CF are exposed to chronic OS due to an overproduction of reactive oxygen species (ROS) as a result of chronic activation of neutrophils and macrophages and of impaired antioxidant status which is not confined to the fat soluble antioxidants vitamin E and carotenoids.4

The basic CF genetic defect itself is a source of OS. Class II CFTR mutation, such as ΔF508, cause accumulation of misfolded CFTR protein in the endoplasmatic reticulum (ER) resulting in OS. CFTR transports the glutathione – the major antioxidant in the lung, and mutations in CFTR lead to low levels of this antioxidant in airway surface liquid. It was demonstrated that CFTR expression and function are modulated by OS. CFTR can impair cell volume and pH regulation, transepithelial transport, membrane conductance and the glutation (GSH)‑related antioxidant and detoxication activity in the extracellular milieu.5 Furthermore, CFTR dysfunction drives mitochondrial defects, alterations in oxidative phosphorilation, calcium homeostasis, OS, apoptosis and innate immune response.1 The respiratory tract is protected from infection by ROS generated by phagocytic and epithelial cells. As a result of chronic pulmonary infections and digestive malabsorption, appear an imbalance between the production of ROS (superoxide and hydrogen peroxide) and their inactivation by protective systems.6 An oxidative environment influences intracellular signaling events leading to apoptosis, increased synthesis and secretion of mucin and alterations in ion transport. The constitutive defec of GSH metabolism with a lowered intake and absorption of fat‑soluble antioxidant vitamins contribute to a defective antioxidant protection which exacerbate OS indices.5

Oxidative unbalance in CF is characterized by increased concentrations of ROS, lowered levels of glutathione (GSH) and reduced nitric oxid (NO).The increase of pro-oxidative species in airway surface liquid contributes to the progressive lung tissue damage and to the amplification of the inflammatory response in airways characterized by the release of chemokines and cytokines (IL-6, IL-8), NADPH oxidase, mieloperoxidase, lactoperoxidase (fig. 1.)7

Neutrophils are a major pro-inflammatory cell in CF. Activated neutrophils are a major source of free radicals and are considered a potent mechanism for killing organisms and damage the pulmonary epithelium. Activated neutrophils migrate into the airways to attack invading bacteria and release in their microenvironment ROS (superoxide anion, hydrogen peroxide, hydroxyl free radical), mainly by the activation of the NADPH oxidase.5,7,8 Some bacteria such as P. aeruginosa
generate oxygen radicals though the release of pyocyanin and other phenazine pigments. Thus, the CF airway is exposed to oxidants derived from inflammatory and infectious processes.8

![Diagram](image)

Fig.1. Oxidative unbalance in conductive airways of patients affected by cystic fibrosis.5

The cumulative effect of repeated infections is responsible for the decline of lung function in CF. In particular, the production in excess of oxygen free radicals overloads the antioxidant defences and oxidises membrane components of lung cells, contributing to the decrease in lung function.7 Other studies suggest that the increased OS is in response to inflammation associated with infection, rather than part of the primary defect.9

In the presence of chronic inflammatory syndrome, ROS may lose the physiological role in the killing of pathogen. ROS can modify the thiol homeostasis of extracellular fluids and epithelia and promote the activation of MAPK signaling pathways.5 The markers of free radical-mediated damage in plasma of CF patients (lipid hydroperoxides, malondialdehyde, protein carbonyls) are present in patients with normal concentrations of circulating antioxidants, indicating that oxidative damage is the result of elevated rates of free radical production. OS and inflammation in CF can affect surfactant biophysical activity, thus leading to early alteration of lung function. Oxidative damage of surfactant involve both protein and lipid components.5

An other explanation for increases of OS markers is mitochondrial generation of oxidants. Mitochondria are the primary source of intracellular OS and can produce intramitochondrial ROS. Increases of intracellular level of Ca2+ can stimulate mitochondrial generation of ROS / reactive nitrogen species (RNS) and via protein kinase C activation may increase NADPH oxidase-dependant generation of free radicals and thus induce OS and ultimately apoptosis.10

Measurement of the mitochondrial aconitase and fumarase activities is useful to identifying mitochondrial oxidative stress. A decrease in aconitase activity without a concomitant decrease of fumarase suggests the presence of OS. Measurement of oxidized deoxyguanosine in the mitochondrial genome is a second specific marker for mitochondrial OS. CFTR play a role in modulating mitochondrial GSH levels in lung epithelium and is a contributing factor of lung OS.2

Normal airways are able to cope with OS through a variety of mechanisms, including the absorption and/or biosynthesis of antioxidants. Brown et al. demonstrated that the products of lipid and protein oxidation are present in CF patients with normal concentrations of circulating antioxidants, indicating that oxidative damage is the result of elevated rates of free radical production.7

CF-related diabetes is the most common complication. Recent evidence has confirmed that CFTR is an important regulator of insulin secretion by islet β-cell. In diabetes, hyperglycemia and hyperlipidemia are emerging factors which through complex mechanisms lead to oxidative stress. The combination of increased OS and accumulation of misfolded CFTR proteins in the ER of the β-cell may lead to endoplasmic reticulum stress and eventual apoptosis of this cell lineage. This effect can be potentiated by the malabsorption of dietary antioxidant in CF patients. The expression of the endogenous antioxidants is low in β-cells and this situation sets them up as easy targets for free radical production and OS. GSH deficiency is known to cause oxidative damage in the pancreas and is associated with the onset of diabetes. Since increased OS and altered Ca2+ homeostasis are found in CF, it is believed that both elements could be involved in insulin deficiency observed in CFRD. As the ER plays an important role in the regulation of Ca2+ homeostasis, it is possible that the combination of OS with ER stress during the course of CF further decreases insulin secretion. The multiple-organ dysfunction of CFTR protein is directly associated with an increase in OS which can alter glucose tolerance by reducing insulin secretion or inhibiting its signalling pathways then leading to CFRD.10 (fig. 2.)

Quantification of OS can be assessed by the detection of lipid peroxidation end-products deriving from the degradation of polyunsaturated fatty acids. Several markers for lipid peroxidation are isoprostanes and aldehydes (malonaldehyde, lipid-adducts 4-hydroxynonenal). Oxidative stress biomarkers have been detected in exhaled breath condensates, bronchoalveolar lavage fluid and blood from CF patients. These lipid peroxidation products were also found to be increased in peripheral blood, plasma and urine, suggesting that oxidative stress originating from lungs can shift through other organs.10
Fig. 2. The links between CFTR dysfunction, OS and occurrence of CFRD in CF.\textsuperscript{10}

Antioxidants in CF

Major antioxidants are:
- enzymatic: superoxide dismutase, catalase and glutathione peroxidase.
- nonenzymatic: ascorbate, urate, α-tocopherol, reduced glutathione (GSH), albumins and mucins.

In the normal lung, ascorbate and GSH are present in high concentrations in bronchoalveolar lavage fluid, but in the upper airways concentrations are lower and mucin may represent the major antioxidant. α-tocopherol is present in low concentrations in the airway surface fluid.\textsuperscript{7}

Glutathione – a tripeptide composed of L-cysteine, L-glutamic acid and glycine, is the major antioxidant in the epithelial lining fluid of the lung and protects this area from OS.\textsuperscript{8,9} It is part of intracellular defense system which protects the epithelium against the injuries and inflammation present in CF.\textsuperscript{11} Cells can be protected against OS by extracellular glutathione through the degradation catalysed by the exo-enzyme γ-glutamyl transpeptidase and its de novo synthesis within the cytosol via the γ-glutamyl cycle.\textsuperscript{9}

Oxidant-antioxidant imbalance in CF was confirmed in different studies.\textsuperscript{6,12} A sub-optimal antioxidant protection represent an important contributor to OS and to the poor control of immuno-inflammatory pathways in patients with CF.

In CF there are low levels of total radical-trapping antioxidants parameters that are accompanied by low concentrations of vitamins A, E, β-carotene and oligoelements (selenium, zinc, copper) in plasma.\textsuperscript{5,6} Pancreatic insufficiency also increase susceptibility to deficiencies in lipophilic antioxidants. The presence of malabsorption explains the high incidence of fat-soluble antioxidant deficiencies (vitamin, carotenoids) and essential fatty acid deficiency.\textsuperscript{10}

Roum J.H. cited by Hector et al.\textsuperscript{13} demonstrated that glutathione levels are decreased in patients with advanced CF lung disease, whereas in younger CF children was found only a tendency to lower levels. The analyses of bronchoalveolar lavages have revealed the presence of decreased levels of GSH in the alveolar epithelial lining fluid of CF patients.\textsuperscript{5} CFTR has an important role in the extracellular transport of GSH. CFTR transports glutathione, this being the explanation for the low glutathione level in CF airways. Alternatively, activated neutrophils are capable of oxidising and disabling glutathione.\textsuperscript{13}

Different authors found a decrease of glutathione concentration in the epithelial lining fluid of CF patients with pulmonary inflammation. The glutathione deficiency is associated with changes in other antioxidants, increased OS with pancreatic and hepatic damage, increased lipid peroxidation and enhanced protein oxidation, denaturation and aggregation.\textsuperscript{10} Administration of glutathione, orally or by inhalation, improves lung function and decreases OS.\textsuperscript{14}

Vitamin E is an antioxidant that protects the lipid fraction of cell membrane from a free radical oxidative injury. Numerous patients with CF presents biochemical vitamin E deficiency defined as plasma concentrations below the mean -2 SD.

Lowered vitamin E level was found in CF in association with lowered levels of other liposoluble vitamins such as A and D. Increased number of exacerbations is correlated to lower plasma vitamins E and A.\textsuperscript{15} The clinical setting of parenteral iron therapy demonstrates that only vitamin E status higher than normal protects against acute transition metal ion-induced oxidative-stress.\textsuperscript{4}

Carotenoids (β-carotene, total lycopene) are lowered in CF and are associated with higher susceptibility to lipid peroxidation. Impaired vitamin E and β-carotene statuses are associated with enhanced susceptibility to oxidation of LDL and increased of lipid peroxidation.\textsuperscript{4} Rust et al. cited by Galli\textsuperscript{5} demonstrated that the long-term oral supplementation with 1 mg/kgBW/day restore the level of this carotenoid, confirming the need of high doses of this fat-soluble factor.

Co-enzyme Q10 (CoQ) or ubiquinone is a lipid-soluble component of the mitochondrial electron transport chain. Maleabsorption or oxidation of CoQ may result in decreased circulating levels in patients with CF, possible resulting in decreased ATP production or protection against OS or both.\textsuperscript{5}

Several antioxidants have been shown to have mucolytic and anti-inflammatory properties. Zinc and vitamin C help increase epithelial chloride secretion through CFTR-dependent and independent pathways. Zinc is important in antioxidant
defenses due to its association with superoxide dismutase (SOD) and a cytosolic copper-zinc SOD. Selenium has antioxidant function, but also other functions including neutrophil response to pathogens, cell cycling and metabolism of carcinogens.8

Patients with CF whose lungs are colonized by P. aeruginosa are at increased risk of iron-mediated ROS formation because elastase from the bacteria cleaves transferrin and lactoferrin. Transferrin and lactoferrin cleavage products appear in the epithelial lining fluid of patients with CF and P. aeruginosa. The iron released from these cleaved protein can catalyze hydroxyl radical formation.8

Conclusions

Oxidative stress characterizes CF patients from the pediatric age and it progressively increases over the years. Patients with CF and pancreatic insufficiency need a careful monitoring of redox balance due to the risk of OS.

The free radical-mediated damage may play a role in the increase in pulmonary dysfunction in patients with CF.

Normal levels of antioxidant defences in CF are insufficient to protect against the oxidative stress that the patients experience from repeated infections. So, additional antioxidant supplementation may prove beneficial in slowing the rate of deterioration of pulmonary function when is combined with current therapies and may help maintain an oxidant-antioxidant balance. The daily intake of antioxidants and the use of pharmacological inhibitors aims at suppressing chronic activation of stress-sensitive signalling pathways may be important in preventing the onset of CFRD.

References


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