L-Arginine Deficiency in Cystic Fibrosis Lung Disease

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Abstract: Cystic fibrosis affects multiple organs but lung disease remains the major determinant of patient morbidity and mortality. Cystic fibrosis lung disease is characterized by chronic infection and inflammation. The amino acid L-arginine is substrate for both nitric oxide synthases and arginases. The activity of arginase in sputum is increased while the production of nitric oxide is reduced in the cystic fibrosis airways. Nitric oxide deficiency in cystic fibrosis contributes to the susceptibility to certain bacterial infections of the airways. Arginase activity in the cystic fibrosis airways correlates with nitric oxide production and pulmonary function. Treatment leading to a decrease in arginase activity also results in increased airway nitric oxide production and improved pulmonary function in cystic fibrosis. Similarly, L-arginine supplementation is followed by increased airway nitric oxide production, and nebulized inhaled L-arginine not only improved exhaled nitric oxide concentrations but also increased pulmonary function in patients with cystic fibrosis. These finding suggest that treatment targeting the imbalance of nitric oxide synthase and arginase in the cystic fibrosis airways may improve host defense as well as airway obstruction in these patients.

Keywords: Cystic fibrosis, nitric oxide, L-arginine, arginase.

CYSTIC FIBROSIS

Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene and is among the most common of the fatal genetic diseases. Cystic fibrosis affects multiple organs but lung disease is the major determinant of morbidity and mortality in CF patients. Abnormal ion transport across respiratory epithelium is associated with increased viscosity of airway secretions and mucus retention in the CF lungs. Chronic airway inflammation and infection are characteristics of CF lung disease [1].

NITRIC OXIDE IN CYSTIC FIBROSIS

Nitric oxide (NO) is involved in regulation of bronchomotor tone, perfusion, ciliary beating, antimicrobial defense and other aspects of lung biology [2-4]. The concentrations of NO are decreased in the CF airways and reduced exhaled NO is associated with poor pulmonary function [5, 6]. Reduction of NO formation in CF is associated with airways obstruction and increased susceptibility to infections of the lung [7,8]. NO has both antiviral and antibacterial properties, and is important in the killing of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are common pathogens in CF lung disease. NO is formed by three NO synthase isoforms (NOS1-3) that are each expressed in different cell types, but all three are expressed constitutively in normal airway epithelium. NOS2 which is inducible by inflammatory cytokines and bacterial LPS [9] is believed to contribute effectively to the antimicrobial properties of the airways. While airway epithelial expression of NOS2 is similar between otherwise healthy young CF patients and normal children [10], the expression of NOS2 is reduced in chronic CF lung disease. Attenuated NOS2 expression and function is believed to contribute to infections of CF airways with *P. aeruginosa* [11,12]. The observation that low-producer gene variants in *NOS1* and *NOS3* were also associated with increased risk for *P. aeruginosa* infections in CF patients however suggests that the emergence of *P. aeruginosa* may be related to low NO formation in general and not specifically to NOS2 deficiency in CF [13-15]. Low-producer gene variants in the *NOS1* gene were also found to be associated with more progressive CF lung disease, as reflected by an increased annual decline in pulmonary function [16].

REASONS FOR REDUCED NITRIC OXIDE IN CF

The mechanisms resulting in the low fraction of NO in exhaled air ($F_{E_{NO}}$) of patients with CF are incompletely understood but may include mechanical retention of NO in CF airway secretions, consumption of NO by denitrifying bacteria, reduced formation of NO due to a lack of NOS2 expression in airway epithelium, and conversion of NO to metabolites such as peroxynitrite by, for instance, neutrophil-derived superoxide. Substrate limitation for NOS may result in uncoupling of the enzyme with subsequently production of superoxide anion and peroxynitrite. Evidence for reduced systemic availability of L-arginine, the substrate for NOS, was found in CF patients. Patients with a pulmonary exacerbation had reduced L-arginine concentrations in plasma which normalized after antibiotic treatment. Decreased plasma L-arginine before treatment was associated with increased circulating arginase I concentrations [17]. Of interest, plasma ornithine, the product of arginase activity, was increased after treatment for an exacerbation and the relative bioavailability of L-arginine remained significantly reduced in the patients despite antibiotic treatment [17].

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INCREASED ARGINASE ACTIVITY IN CF LUNG

The activity of arginase was found to be higher in CF sputum samples compared to healthy controls, was lower in CF patients treated with systemic corticosteroids when compared to patients not treated with steroids, and was higher in patients presenting with a pulmonary exacerbation when compared to clinically stable CF patients. Intravenous antibiotic treatment resulted in a significant decrease in sputum arginase activity in both CF patients treated for an acute exacerbation or electively however, remained significantly increased when compared to sputum from healthy control subjects [18]. Further more, sputum arginase activity and pulmonary function (FEV_1) was found to have a negative correlation which suggested that increased arginase activity resulted in decreased formation of bronchodilator NO. This was supported by a significant positive correlation between FE_{NO} and pulmonary function (FVC and FEV_1) in clinically stable CF patients, as well as a significant negative correlation of arginase activity and FE_{NO} in patients presenting with a pulmonary exacerbation. Interestingly, changes in sputum arginase activity during antibiotic treatment were negatively correlated to changes in FEV_1 confirming an important role of arginase activity in CF-related airway NO deficiency and pulmonary function [18].

PSEUDOMONAS AERUGINOSA AND NITRIC OXIDE

P. aeruginosa is capable of anaerobic growth by respiration using the NO metabolites nitrate (NO_3^-) or nitrite (NO_2^-) as terminal electron acceptors [19]. NO_3^- and NO_2^- are present in CF airway surface liquid (ASL) [20-23] and sputum [22, 24], where NO_3^- levels have been estimated to be as high as 1 mM [22]. These concentrations are permissive for anaerobic growth of *P. aeruginosa* both *in vitro* and *in vivo* [25], where the bacterium controls the environmental level of toxic NO by synthesis of protective NO reductase (NOR) and nitrite reductase (NIR) [19,26]. As CF lung disease progresses mucoid *P. aeruginosa* strains emerge and become the predominant opportunistic pathogens [27], which are inherently resistant to antibiotics [28] and phagocytic neutrophils [29]. The mechanisms behind the phenotypic switch to the mucoid form are incompletely understood, however one mechanism of mucoid conversion in CF isolates is via mutations in the *mucA* gene [30]. More than 80% of mucoid *Pseudomonas* isolates from CF patients in North America have been shown to possess *mucA* mutations [31]. Interestingly, NIR and NOR activity are remarkable low in *mucA* mutant *P. aeruginosa*, making this pathogen particularly susceptible to NO-mediated killing [26]. *MucA* mutant bacteria also have a markedly reduced capacity to remove NO generated aerobically from S-nitrosoglutathione [32]. In addition, it was shown that 15 mM NO_2^- kills *mucA* mutant *P. aeruginosa* in CF airways at pH 6.5 under anaerobic conditions [26]. *In vitro* experiments on the dose-effect relationship between NO_2^- concentration and killing of mucoid *P. aeruginosa* showed that the LD_{50} was approximately 3 mM NO_2^- after 24 hours. These findings support previous studies demonstrating that overproduction of NO by anaerobic *P. aeruginosa* biofilms results in metabolic suicide of these bacteria, an event that was preventable by an NO scavenger

[33]. *In vitro* enhancement of antibiotic susceptibility of *P. aeruginosa* in biofilms had also been reported with L-arginine, NO_3^- or NO_2^- supplementation; presumably through an NO mediated mechanism [34]. Enhancement of endogenous NO production, for instance through supplementation of L-arginine, the substrate of NO synthases, may therefore be a viable treatment option to enhance host defense against certain CF pathogens such as *P. aeruginosa*.

TREATMENT STUDIES WITH L-ARGININE

It is currently unknown whether bacterial arginase contributes significantly to total L-arginine conversion to L-ornithine, but it is conceivable that increased pulmonary arginase activity results in reduced availability of L-arginine for NO synthesis in the CF airways. Consistent with the hypothesis that low L-arginine levels contribute to the reduced formation of NO in CF airways, supplementation of L-arginine either orally, intravenously or by inhalation resulted in a significant increase of airway NO formation in CF patients [7, 35, 36]. The inhalation of a single dose of nebulized L-arginine in patients with CF not only increased exhaled NO but also resulted in a sustained improvement in pulmonary function [36]. As these preliminary clinical studies on L-arginine supplementation were safety studies and not powered for efficacy, the results have to be interpreted with caution. Possible side effects of arginase-related ornithine metabolic products such as proline, the precursor of collagen production, as well as the polyamines which may contribute to airway remodeling after increasing the L-arginine availability in the CF airways, have not been studied yet. Of interest, the inhalation of a single dose of L-arginine did not result in a measurable increase of either L-citrulline or L-ornithine in sputum, although sputum L-arginine increased significantly. In contrast, plasma L-arginine and L-citrulline remained unchanged, while the concentrations of L-ornithine in plasma increased significantly after inhalation of L-arginine [36].

The effect of L-arginine supplementation on host defense and bacterial colonization of CF airways has also not yet been studied in clinical trials. In a rat model of chronic infection with *P. aeruginosa*, L-arginine supplementation significantly reduced tissue damage, inhibited neutrophil recruitment and reduced the pro-inflammatory cytokine interleukin (IL)-1β, while inhibition of NO production in this model worsened lung damage [37]. Further studies are needed to evaluate effects and potential side effects of L-arginine supplementation and increased airway NO production in CF patients. As specific arginase inhibitors become available, blocking arginase could potentially be useful to enhance the effect of L-arginine therapy in patients with CF.

ABBREVIATIONS

CF	=	Cystic fibrosis
CFTR	=	Cystic fibrosis trans-membrane conductance regulator
FE_{NO}	=	Fraction of nitric oxide in exhaled air
FEV1	=	Forced expiratory volume in one second
FVC	=	Forced vital capacity

IL   =   Interleukin

NIR   =   Nitrite reductase

NO   =   Nitric oxide

NO$_3$ =   Nitrate

NO$_2$ =   Nitrite

NOR   =   NO reductase

NOS   =   Nitric oxide synthase

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