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Original Article

The effect of short-term, high-dose oral N-acetylcysteine treatment on oxidative stress markers in cystic fibrosis patients with chronic *P. aeruginosa* infection — A pilot study

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Abstract

Background: Patients with cystic fibrosis (CF) and chronic *Pseudomonas aeruginosa* lung infection have increased oxidative stress as a result of an imbalance between the production of reactive oxygen species caused by inflammation and their inactivation by the impaired antioxidant systems. Supplementation with anti-oxidants is potentially beneficial for CF patients.
Methods: The effect of 4 weeks of oral N-acetylcysteine (NAC) treatment (2400 mg/day divided into two doses) on biochemical parameters of oxidative stress was investigated in an open-label, controlled, randomized trial on 21 patients; 11 patients in the NAC group and 10 in the control group. Biochemical parameters of oxidative burden and plasma levels of antioxidants were assessed at the end of the study and compared to the baseline values in the two groups.
Results: A significant increase in the plasma levels of the antioxidant ascorbic acid ($p = 0.037$) and a significant decrease in the levels of the oxidized form of ascorbic acid (dehydroascorbate) ($p = 0.004$) compared to baseline were achieved after NAC treatment. No significant differences were observed in the control group. The parameters of oxidative burden did not change significantly compared to baseline in either of the groups. A better lung function was observed in the NAC treated group with a mean (SD) change compared to baseline of FEV1% predicted of 2.11 (4.6), while a decrease was observed in the control group (change −1.4 (4.6)), though not statistically significant.
Conclusion: Treatment with N-acetylcysteine 1200 mg × 2/day for 30 days significantly decreased the level of oxidized vitamin C and increased the level of vitamin C (primary end-points) and a not statistically significant improvement of lung function was observed in this group of patients.

Keywords: N-acetylcysteine; Antioxidants; Oxidative stress; Ascorbic acid

Abbreviations: AA, ascorbic acid; DHA, dehydroascorbic acid (oxidized form of AA); MDA, malondialdehyde; NAC, N-acetylcysteine; ROS, reactive oxygen species; 8isoP, isoprostane; GSH, glutathione; CI, confidence interval.

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1. Introduction

From early childhood, patients with cystic fibrosis (CF) have recurrent and chronic respiratory tract infections characterized by polymorphonuclear neutrophil (PMN) inflammation. Counts of PMNs in CF airway fluid have been found to be thousands of times higher than normal [1,2]. Sputum neutrophil counts and elastase activity correlate well with clinical measures of CF lung dysfunction, such as declining forced expiratory volume in 1 s (FEV1) or forced vital capacity (FVC) [3], which is consistent with neutrophils playing a central role in CF airway destruction. A consequence of the PMN-dominated inflammation is the release of proteases and reactive oxygen species (ROS). The neutrophils continuous interaction with bacterial products and their inability to engulf bacteria embedded in biofilms contribute to this exaggerated ROS production. Subsequently, ROS lose their physiological role in killing pathogens and turn into toxic effectors responsible for damaging the pulmonary epithelium as well as of other components of the lung parenchyma. Importantly, ROS can also modify the antioxidant homeostasis of extracellular fluids and epithelia causing the immune-inflammatory imbalance observed in the CF lung [4].

Besides the increased consumption of antioxidants caused by the exaggerated production of ROS [5], patients with CF have an impaired absorption of dietary antioxidants in the gut [6–10] and the inability to efflux glutathione (GSH) into the extracellular milieu of the lung [11]; the most abundant intracellular antioxidant.

Thus, the high ROS production and impaired antioxidant systems explain the systemic redox imbalance observed in CF for which evidence is available in the literature [4,12]. It has been shown [13] that this redox imbalance affects circulating neutrophils before they migrate to CF airways, as evidenced by marked basal intracellular GSH deficiency.

N-acetylcysteine (NAC) is a cysteine prodrug and can be considered a GSH precursor [14] and oral administration of NAC replenishes the cellular levels of GSH [15]. 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 [13].

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 [16]. Indications of a positive effect of NAC treatment on the lung function of a subgroup of CF patients have previously been published in our center [17].

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 2700 mg/day divided into three 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, a slight improvement in the lung function FEV1% predicted (95% CI) was observed in the NAC treated group with a change of 1.05 (−26.16 to 25.73) while a significant decrease of −5.62 (−24.54 to 19.69) of the lung function was observed in the placebo-treated group. A larger randomized, placebo-controlled clinical study (153 CF patients) investigating the effect of inhaled GSH for 6 months similarly showed a significant improvement in lung function at three months, although differences between the two groups, failed to reach statistical significance after 6 months [18].

Like GSH, ascorbic acid (AA) is an important antioxidant of the lung and GSH also plays a central role in AA recycling [19]. In a guinea-pig model of oxidative stress caused by low plasma levels of AA, we have previously demonstrated, that biofilm lung infection with *Pseudomonas aeruginosa* is characterized by a worsening of the PMN-dominated inflammatory response in the lung [20]. In this animal model, the low AA levels lead to an increased oxidative burst from PMNs [20], indicating that an impaired antioxidant system can in turn exacerbate the inflammatory response. This raised the hypothesis that improved AA status can decrease the inflammation in the lung. One way of improving the AA status is by GSH supplementation as GSH facilitates AA recycling and homeostasis. GSH provides $2H^+$ and $2e^-$ which react with the oxidized form of AA (dehydroascorbic acid DHA) and maintain AA on its reduced form [19]. As NAC is a source of GSH, we hypothesized that high-dose oral NAC, as a source of GSH, would increase the antioxidant capacity of the plasma and subsequently decrease the levels of oxidative burden markers. Although used by many CF patients, especially as a mucolytic agent, no data on the effect of NAC treatment on oxidative stress markers are available.

The aim of this study was to investigate the effect of high dose, orally administered NAC on oxidative stress markers in urine (8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydro-guanosine (8-oxoGuo)) and plasma malondialdehyde (MDA) and 8-isoprostane (8-isoP) as well as on the plasma antioxidant levels (ascorbic acid (AA), dehydroascorbic acid (DHA) and alpha- and gamma-tocopherols) as primary end-points. Lung function changes were secondary endpoints of the study. This study was intended as a pilot study enabling proper power calculations necessary for number of CF patients to be included in a larger phase II clinical study in CF patients.

2. Material and methods

2.1. Patients

An open-label, controlled, randomized study was conducted at the Copenhagen CF Center (Eudract CT nr.: 2007-001401-15). The protocol was reviewed and approved by the Committee on Health Research Ethics in the Capital Region of Denmark. All subjects provided written informed consent.

Inclusion criteria were: adult CF patients (CF defined by positive [>60 mM Cl_2] sweat chloride test and/or two disease-causing mutations) with chronic *P. aeruginosa* lung infection, at the end of a two-week intravenous antibiotic treatment. Exclusion criteria were: hypersensitivity to N-acetyl cysteine, prior lung transplantation or if on lung transplant waiting list, patients who