

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 $\times$ 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. © 2014 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.

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; IisoP, 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

received NAC in the last 30 days, patients with recent hemoptysis or an abnormal liver function test (ALAT) more than twice the normal range (10–70 U/L). After written consent, patients were consecutively randomized to one of the two groups (receiving NAC or control group) 1:1. Patients were included in the study over 2 years (March 2011 to August 2013).

3. Study design

Twenty-one CF patients with chronic *P. aeruginosa* lung infection were included in the study (12 males and 9 females), median age 39 years (range 25–61 years). Chronic *P. aeruginosa* infection was defined as the persistent presence of *P. aeruginosa* for at least 6 consecutive months, or less when combined with the presence of two or more *P. aeruginosa* precipitating antibodies. All patients were controlled on a regular monthly basis and each patient had an average of 10 sputum cultures per year. Eleven patients were $\Delta F508$ homozygotes (6 in NAC and 5 in control group) and ten heterozygotes (4 in NAC and 6 in control group). Pulmonary function tests were performed according to international recommendations [21] measuring FEV1, expressed as a percentage of predicted values for sex and height using reference equations from Wang or Hankinson [22,23].

The lung function at baseline of the patients in the NAC group was FEV1 (% predicted) mean (95% CI) 58.36% (46.26; 70.46) and of the patients in the control group 53.7% (37.6; 69.8). The control group did not receive placebo medication and was therefore aware of the group to which they were assigned. Patients in the NAC group received oral treatment with N-acetylcysteine, tablets of 600 mg effervescent (Mucolysin® 600 produced by Sandoz A/S), 2 tablets twice a day (a total daily dose of 2400 mg) for 4 weeks. All other medication was continued, including inhalation with pulmozyme, bronchodilators with β_2 agonists and colistin and per oral treatment with ciprofloxacin and azithromycin. There were no differences between the two groups in terms of additional medication, with the exception of three patients in the intervention group receiving low-dose (5 mg $\times$ 1) prednisone. With two exceptions in the control group, all patients (19/21 90%) were pancreatic insufficient and all were advised to continue daily supplementation with multivitamin (2 capsules of AquADEKs®).

Two of the patients belonging to the NAC group did not complete the study: One due to adverse events (intestinal pain) and one due to lack of compliance. These two patients were excluded from the final analysis, thus the effect of the treatment with NAC was evaluated in 9 CF patients compared to 10 CF controls.

3.1. Measurements of oxidative stress

The primary efficacy end-points were changes compared to baseline in the level of oxidative stress markers, including lipid peroxidation: plasma malondialdehyde (MDA) and 8-isoprostane (8-isoP) and urinary excretion of 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo), as well as plasma antioxidant levels:

vitamin C or ascorbic acid (AA) and oxidized vitamin C (dehydroascorbic acid (DHA), alpha- and gamma-tocopherols).

The secondary efficacy end-points were: lung function (FEV1 and FVC) and as inflammatory parameter: oxidative burst in the PMNs.

Blood samples and 24-hour urine samples were collected when patients entered the study (baseline values) and 4 weeks later.

3.2. Plasma AA and DHA measurements

Blood samples were immediately centrifuged (16,000 $\times g$, 1 min). One 100 μ L plasma aliquot was acidified with an equal volume of 10% m-phosphoric acid containing 2 mM EDTA, briefly vortex mixed, centrifuged and the supernatant frozen at -80°C [24]. AA and total vitamin C were measured by HPLC with colorimetric detection and the fraction of DHA was calculated by subtraction using uric acid as endogenous standard as described in details elsewhere [25].

3.3. MDA measurements

Plasma (100 μ L) MDA analysis was accomplished by reverse-phase HPLC with fluorescence detection of the genuine MDA (thiobarbituric acid)₂ adduct as described previously [26]. 8-isoP was assessed as per the manufacturer's recommendation (Cat no.: 516351, Cayman Chemicals, USA). The method is based on a competitive enzyme immunoassay using 8-isoP and an isoprostane conjugate. The concentration of bound conjugate, which is developed with Ellman's reagent and quantified spectrophotometrically, is inversely proportional to the concentration of 8-isoP in the sample. Alpha- and gamma-tocopherols were analyzed by HPLC with colorimetric detection as modified from Sattler and coworkers [27].

3.4. 8-oxodG and 8-oxoGuo measurements

The urinary content of the oxidized nucleosides 8-oxodG and 8-oxoGuo was quantified using a modified version of an ultra performance liquid chromatography and tandem mass spectrometry (UPLC-MS/MS) assay, described in detail elsewhere [28]. Briefly, the frozen urine samples were thawed, mixed and heated to 37°C for 5 min to re-dissolve possible precipitate and centrifuged at 10,000 $\times g$ for 5 min. All further sample preparation was performed using a Biomek 3000 robot (Beckman Coulter, CA, USA). 110 μ L of each urine sample or calibration standard were mixed with 90 μ L 100 mM lithium acetate buffer and 90 μ L of 50 nM internal standard. The chromatographic separation was performed on an Acquity I-class UPLC system (Waters Corp., Milford, USA) using an Acquity UPLC BEH Shield RP18 column (1.7 μ m, 2.1×100 mm; Waters Corp.) with a column temperature of 4°C . The mass spectrometry detection was performed on a Xevo-TSQ triple quadrupole mass spectrometer (Waters Corp., Milford, USA), using electrospray ionization in the positive mode for 8-oxodG and negative ionization mode for 8-oxoGuo. Calibration standards ranged from 1 to 60 nM. As internal standards, stable

isotope-labeled 8-oxodG and 8-oxoGuo, [$^{15}\text{N}_5$]8-oxodG and [$^{15}\text{N}_5$] 8-oxoGuo, were used. To confirm the presence of the analyte and the absence of false contributions from co-elution of similar compounds in the urine samples, two specific fragments of each analyte were included in the analysis. The average within-day variation (RSD, %) estimated from the method validation was 2.3% for 8-oxoGuo, and 3.8% for 8-oxodG. The average recovery was 103.7% and 104.8%, respectively. The 8-oxodG and 8-oxoGuo urinary excretion was normalized to the urinary creatinine concentration, quantified by Jaffe's reaction. The average within-day and between-day variation (RSD, %) estimated from the method validation was 2.3% and 9.0% for 8-oxoGuo, respectively, and 3.8% and 7.4% for 8-oxodG.

8-oxodG and 8-oxoGuo were normalized against urinary creatinine concentrations.

3.5. Oxidative burst in PMN measurements

The respiratory burst of PMNs was estimated by a modified flow cytometric assay [20] for the intracellular content of H_2O_2 according to the fluorescence intensity from oxidized 123-dihydrorhodamine (DHR) [29].

3.6. Statistical analysis

Statistical analysis was performed by GraphPad Prism 6.04. The Gaussian distribution of the values was tested by D'Agostino & Pearson omnibus normality test. On normally distributed values, one-tailed *t*-test on paired samples was used to compare the changes of the measured parameters in the end of the study compared to baseline in NAC treated and untreated (control) patients. The level of significance was 5%. The differences between the measured parameters in the two groups at baseline were tested by two-tailed unpaired *t*-test.

4. Results

At baseline, CF patients in the NAC and control groups had similar levels of oxidative burden markers and plasma antioxidant levels. Thus, no significant differences in the levels of oxidative stress markers in plasma (MDA, 8isoP) and urine (8oxodG, 8 oxodGuo) and of the plasma levels of antioxidants (ascorbate, dehydroascorbate, alpha- and gamma-tocopherols), were observed between the two groups (Table 1). Additionally, lung function of the patients was similar in the two groups at baseline (Table 1).

The patients in the intervention group received NAC in a mean dose of 36 mg/kg/day (max 59 mg/kg/day and min 25.8 mg/kg/day).

After 4 weeks of NAC treatment, a significant increase in the plasma level of ascorbic acid (AA) ($p = 0.037$) and decrease in the level of oxidized ascorbic acid (DHA) ($p = 0.004$) compared to baseline were observed, while no significant changes were observed in the control group (Fig. 1 and Table 2).

A decrease in the serum levels of alpha- and gamma-tocopherols was observed in both groups, with a significant

decrease ($p = 0.03$) in the levels of gamma-tocopherol in the NAC treated group.

A non-significant decrease in the oxidative burst of the PMNs was found in the end of the study compared to baseline in both groups (data not shown).

An improvement compared to baseline in the FEV1 (% predicted) mean (95% CI) +2.11 (−1.44; 5.66) was observed in the NAC treated group while a decrease was observed in the control group mean (95% CI) (−1.4 (−4.7; 1.9)), both changes did not reach the level of significance ($p > 0.05$) (Table 2).

4.1. Safety and adverse events profile

One patient stopped treatment with NAC due to stomach pain. No other adverse events were observed.

5. Discussions

Oral treatment with NAC 1200 mg $\times$ 2 daily for 30 days significantly decreased the level of oxidized vitamin C and increased the level of vitamin C in patients with CF in the present study. The significant increase in the plasma levels of AA and decrease in the DHA levels under treatment with NAC are in accordance with the role played by GSH in the ascorbate homeostasis [19]. In short, it is known from in vitro assays that cells take up exogenous DHA, and in the presence of GSH convert it to AA in the cytoplasm. Our data demonstrate that the redox coupling between GSH and DHA in the regeneration of AA exposed to an oxidative challenge is well functioning in CF patients, explaining the lower plasma levels of DHA and the higher AA levels compared to baseline, following NAC administration.

NAC has been used in our study as an antioxidant agent in a mean dose that was calculated to be 36 mg/kg/day. The optimal dosage as anti-oxidant agent in CF is not known but this dosage is higher than what is usually recommended as mucolytic agent (400–1200 mg/day). Taking into account the benign side-effect profile of NAC, the dosage can probably be safely increased to 50 mg/Kg/day [30].

According to in vitro data, vitamin C (AA) spares for vitamin E (tocopherol) [19] and the increased plasma AA levels after NAC treatment could thus be expected to cause an increase in the alpha-tocopherol levels. However, conflicting results have been reported from in vivo studies and Burton et al. [31] failed to find the sparing effect in a well-controlled experiment conducted in an animal model.

The mean AA serum levels at baseline were 79.77 $\mu\text{mol/L}$ in the NAC group and 76.62 $\mu\text{mol/L}$ in the control group (Table 1), both values being above 50 $\mu\text{mol/L}$, which is considered the protective level for free radical disease. The mean serum alpha-tocopherol levels at baseline were 29.73 $\mu\text{mol/L}$ in the NAC group and 29.70 $\mu\text{mol/L}$ in the control group (Table 1), which is within the normal ranges reported from healthy non-CF populations (24.06 $\mu\text{mol/L}$) [32]. The low levels of alpha-tocopherol frequently reported in CF patients [33,34], were thus not found in our adult CF population. The mean gamma-tocopherol serum levels at baseline were 3.52 $\mu\text{mol/L}$ in the

Table 1

Baseline levels of measured parameters mean (95% CI) in NAC treated and control groups. Unpaired *t*-test was used to analyze the differences between the two groups. The patients in the two groups have similar levels of the measured parameters at baseline.

Group (number of patients)	Plasma antioxidants				Plasma markers of oxidative burden		Urinary markers of oxidative burden	
	Ascorbic acid (μmol/L)	Oxidized ascorbic acid (% of total)	Alpha-tocopherol (μmol/L)	Gamma-tocopherol (μmol/L)	MDA (μmol/L)	8-isoP (ng/L)	DNA 8oxodG (nM/mM creatinine)	RNA 8oxodGuo (nM/mM creatinine)
NAC (11)	79.77	4.97	29.73	3.52	0.30	53.31	1.54	3.73
Mean (95% CI)	(58.7; 100.8)	(3.73; 6.21)	(22.48; 36.98)	(2.26; 4.77)	(0.21; 0.38)	(45.64; 60.99)	(0.99; 2.09)	(2.49; 4.96)
Control (10)	76.62	5.25	29.70	2.36	0.25	57.20	1.19	3.31
Mean (95% CI)	(60.82; 92.42)	(3.9; 6.6)	(21.8; 37.6)	(0.98; 3.72)	(0.22; 0.28)	(49.6; 64.7)	(0.87; 1.5)	(2.72; 3.89)
p	0.79	0.73	0.99	0.17	0.26	0.42	0.18	0.52

Group (number of patients)	FEV1 (% predicted)	FVC (% predicted)
NAC (11)	58.36	94.18
Mean (95% CI)	(46.26; 70.46)	(80.81; 107.6)
Control (10)	53.7	90.20
Mean (95% CI)	(37.6; 69.8)	(69.83; 110.6)
p	0.60	0.71

NAC group and 2.36 μmol/L in the control group; notably lower than what has been reported in healthy non-CF population (4.9 μmol/L in the corresponding age group) [32], but similar to reports from CF patients receiving AquADEKS® supplements [35].

It has been shown that vitamin E levels increase after antibiotic treatment as a result of bronchial inflammation

control [36]. Based on these observations, it may be speculated, that the vitamin E levels were likely to be at their highest level at baseline in our study, as all CF patients were included in the study at the end of a 14 day antibiotic treatment. The decrease in the alpha- and gamma-tocopherol levels observed in both groups after 4 weeks may thus be explained by the dynamics of vitamin E during the course of the disease.

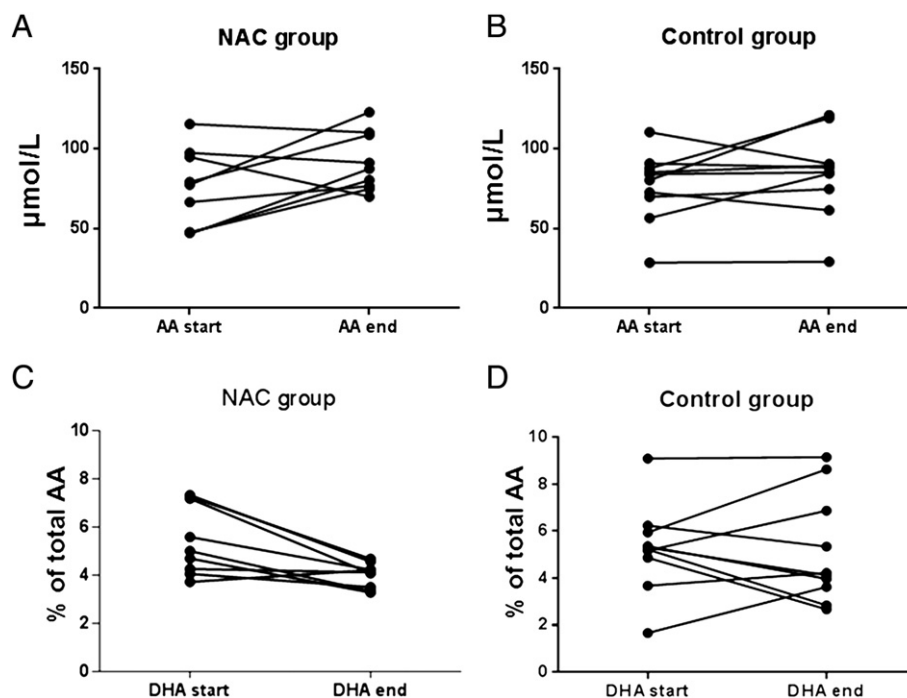


Fig. 1. Changes in the plasma levels of ascorbic acid (AA) and dehydroascorbate (DHA) at the end of the study compared to baseline in the NAC treated group (A and C) and in the control group (B and D, respectively). Significant higher plasma levels of ascorbate ($p = 0.037$) and lower of oxidized ascorbate ($p = 0.004$) compared to baseline were found in the NAC treated patients but not in the control group. Paired *t*-test was used to analyze the differences between the levels at the end of the study compared to baseline.

Table 2

Differences in plasma and urine markers and lung function after 4 weeks compared to baseline in the NAC treated and control group. The p value represents the significance of the difference between levels at baseline and end of the study (t-paired, one tailed).

Group (number of patients)	Plasma antioxidants				Plasma markers of oxidative burden		Urinary markers of oxidative burden	
	Ascorbic acid ($\mu\text{mol/L}$)	Oxidized ascorbic acid (% of total)	Alpha-tocopherol ($\mu\text{mol/L}$)	Gamma-tocopherol ($\mu\text{mol/L}$)	MDA ($\mu\text{mol/L}$)	8-isoP (ng/L)	DNA 8oxodG (nM/mM creatinine)	RNA 8oxodGuo (nM/mM creatinine)
NAC (9)	16.66	−1.45	−5.88	−1.46	−0.04	1.84	0.1	−0.11
Mean (95% CI)	(−2.07;35.39)	(−2.39; −0.51)	(−15.66;3.88)	(−3.07;0.16)	(−0.14; 0.06)	(−8.28;11.96)	(−0.55;0.75)	(−1.06;0.83)
	p = 0.037	p = 0.004	p = 0.099	p = 0.035	p = 0.200	p = 0.340	p = 0.329	p = 0.394
Control (10)	7.72	−0.10	−3.96	−0.48	0.02	−5.73	−0.26	−0.58
Mean (95% CI)	(−6.16;21.62)	(−1.38;1.17)	(−10.55;2.63)	(−1.88; 0.92)	(−0.03;0.07)	(−13.54;2.07)	(−0.61;0.09)	(−1.43;0.27)
	p = 0.120	p = 0.427	p = 0.100	p = 0.230	p = 0.224	p = 0.065	p = 0.065	p = 0.079

Group	FEV1 (% predicted)	FVC (% predicted)
NAC (9)	2.11 (−1.44;5.66)	1.44 (−3.38;6.27)
Mean (95% CI)	p = 0.104	p = 0.255
Control (10)	−1.4 (−4.7; 1.9)	0.1 (−6.03; 6.23)
Mean (95% CI)	p = 0.182	p = 0.486

In addition, the effect of GSH on tocopherol levels is thought to be indirect through the sparing of vitamin E by vitamin C and it is therefore not surprising, that such effects were not observed following a relatively short study period. An explanation for the significant decrease in the plasma levels of gamma-tocopherol in the NAC treated group is not available at the present time.

The mechanisms by which ROS cause tissue injuries are many, and among them it is important that the role played by ROS attacks on polyunsaturated fatty acids of lipid structures (membranes) and DNA [6,8,10,37–39].

Malondialdehyde (MDA) is an end product of the oxidation and decomposition of unsaturated fatty acids and 8-iso prostaglandin F $_{2\alpha}$ (8 isoprostane) is produced by free-radical catalyzed peroxidation of arachidonic acid.

MDA levels have been found to be increased in plasma of CF patients compared to controls in some studies [34,40,41]. However, this was not found in a large cohort of CF patients [36] using sensitive methods for MDA measurement. It has been suggested, that due to the fast elimination from plasma, measuring lipid peroxidation in the epithelial lining fluid is more appropriate when approximating the severity of a lesion at the site of inflammation [1], however this involves invasive procedures and is cumbersome. 8 isoprostane levels were found to be increased in the plasma of CF patients [42,43]. Due to the large diversity of the measurement methods for MDA and 8 isoP among various laboratories [44], no comparison of the data in our study with previously published studies was performed. In our study, no decreases in the levels of lipid peroxidation markers (MDA and 8isoP) were observed after NAC administration. As the main antioxidant which prevents lipid peroxidation is tocopherol, present in cell membranes and as NAC supplementation did not cause an improvement in the tocopherol levels, it is not surprising, that a decrease in the lipid peroxidation markers was not observed.

Urinary 8oxodG levels, as a marker of DNA oxidation, have been reported to be higher in CF patients compared to controls [38]. No data on the 8oxodGuo levels, as a marker of RNA oxidation, are available in the literature, our study being the first reporting these measurements in CF patients.

The urinary levels of DNA and RNA oxidation products (8oxodG and 8oxodGuo) did not show significant changes compared to baseline in either of the groups, though a trend towards lower urinary levels compared to baseline was observed in the control group. This is probably due to the chronic inflammation, present for several decades in these CF patients, contributing to the overall oxidative stress and plausibly overwhelming the antioxidant system, despite higher AA levels.

Both, the small number of CF patients included in the study and the short period of treatment (4 weeks) are draw-backs of our trial, which was meant as a pilot study.

Due to the intensive treatment of CF patients with chronic *P. aeruginosa* infection, intervention studies with antioxidants are difficult to perform and require large number of patients in each group in order to find significant changes in the measured parameters and require multicenter studies [18].

Due to difficulties in recruiting patients for a satisfactory matched paired design study in our CF center, we were not able to perform suitable power calculations for future studies. To circumvent the problem of variability between individuals in the two groups, a placebo-controlled, cross-over design study could be conducted in our CF center in the future. This kind of design has been used in a previous study on the effect of NAC in CF patients [17]. Using changes in FEV1 during treatment from our pilot study with NAC we calculated that a sample size of 40 patients will need to enter this two-arms (NAC and placebo) crossover study. Based on the data collected in this pilot study, we suggest a treatment period of 6 months with NAC in a dose of 50 mg/kg/day.

Since it has been shown that inflammation in CF patients starts as early as infancy [45–47], clinical trials aiming at correcting the oxidant/antioxidant imbalance in young children, especially in countries with newborn screening, could be considered.

In conclusion, in accordance with our hypothesis, this pilot study demonstrated that supplementation with the GSH precursor, NAC, improved the antioxidant capacity of plasma by increasing AA levels and decreasing the DHA levels. No further effect on the vitamin E levels and markers of lipid peroxidation were observed. The tendency towards a better lung function in this pilot study which is very much underpowered and after NAC supplementation for a short period of time (4 weeks) is encouraging and calls for new multi-centered placebo-controlled trials on a large population of CF patients that are matched in accordance with sex, age and lung function.

Conflict of interests

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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