Increased carbon monoxide in exhaled air of patients with cystic fibrosis

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Abstract

Background—Inflammation, oxidative stress, and recurrent pulmonary infections are major aggravating factors in cystic fibrosis. Nitric oxide (NO), a marker of inflammation, is not increased, however, probably because it is metabolised to peroxynitrite. Exhaled carbon monoxide (CO), a product of heme degradation by heme oxygenase 1 (HO-1) which is induced by inflammatory cytokines and oxidants, was therefore tested as a non-invasive marker of airway inflammation and oxidative stress.

Methods—Exhaled CO and NO concentrations were measured in 29 patients (15 men) with cystic fibrosis of mean (SD) age 25 (1) years, forced expiratory volume in one second (FEV1) 43 (6)% predicted, 14 of whom were receiving steroid treatment.

Results—The concentration of exhaled CO was higher in patients with cystic fibrosis (6.7 (0.6) ppm) than in 15 healthy subjects (eight men) aged 31 (3) years (2.4 (0.4) ppm, mean difference 4.3 (95% CI 2.3 to 6.1), p<0.001). Patients not receiving steroid treatment had higher CO levels (8.4 (1.0) ppm) than treated patients (5.1 (0.5) ppm, mean difference 3.3 (95% CI –5.7 to –0.9), p<0.01). Normal subjects had higher NO levels (6.8 (0.4) ppb) than patients with cystic fibrosis (3.2 (0.2) ppb, mean difference 3.8 (95% CI 2.6 to 4.9), p<0.05) and were not influenced by steroid treatment (3.8 (0.4) ppb and 2.7 (0.3) ppb for treated and untreated patients, respectively, mean difference 0.8 (95% CI –0.6 to 2.3), p>0.05). Patients homozygous for the AF508 CFTR mutation had higher CO and NO concentrations than heterozygous patients (CO: 7.7 (1.8) ppm and 4.0 (0.6) ppm, respectively, mean difference 3.7 (95% CI –7.1 to –0.3), p<0.05; NO: 4.1 (0.5) ppb and 1.9 (0.7) ppb, respectively, mean difference 2.2 (95% CI –3.7 to –0.6), p<0.05).

Conclusions—High exhaled CO concentrations in patients with cystic fibrosis may reflect induction of HO-1. Measurement of exhaled CO concentrations may be clinically useful in the management and monitoring of oxidation and inflammatory mediated lung injury.

(Thorax 1999;54:917–920)

Keywords: carbon monoxide; nitric oxide; cystic fibrosis; inflammation; oxidative stress

Cystic fibrosis is characterised by recurrent respiratory tract infections leading to damage to the airways, development of bronchiectasis, and progressive airflow obstruction. Infection plays a central role in the progression of the disease and is associated with increased concentrations of several cytokines in the bronchoalveolar lavage fluid, such as interleukin 8 (IL-8), IL-1β, and tumour necrosis factor α (TNF-α), recruitment of neutrophils into the airways, and oxidative stress. Deficiencies in circulating antioxidants, such as glutathione, vitamin E, serum albumin, and iron, have been reported in cystic fibrosis. These deficiencies cause an inability to counterbalance the high production of free radicals, particularly during pulmonary infections, and increased oxidative damage is generated.

Nitric oxide (NO) may play an important role in regulating airway function and in the pathophysiology of inflammatory airway disease. Exhaled NO has been shown to be a marker of inflammation in asthma and bronchiectasis. However, despite chronic airway inflammation in cystic fibrosis, exhaled NO levels appear to be lower than normal, possibly because of a reduced expression of the inducible form of NO synthase (iNOS), trapping of NO in the mucous layer, or because NO is metabolised to peroxynitrite making this measurement of little use for monitoring lung inflammation in cystic fibrosis.

CO is a product of heme degradation by heme oxygenase (HO). Two isoforms of HO have been described—the constitutive HO-2 and the inducible HO-1 which is ubiquitously distributed. The latter is activated by a variety of pro-inflammatory cytokines, endotoxin, and reactive oxidant species (ROS), and is part of the protective response to oxidative stress.

We investigated whether patients with cystic fibrosis have higher exhaled levels of CO than healthy control subjects and whether the levels of exhaled CO and NO are reduced in patients receiving regular inhaled corticosteroids which suppress inflammation in the airways of asthmatic patients.

Methods

Patients

Patients were recruited from the adult Cystic Fibrosis Clinic at the Royal Brompton Hospital. Those colonised with Burkholderia cepacia, methicillin resistant Staphylococcus aureus (MRSA), or with acute chest infection or disease exacerbation were excluded from the study. Twenty nine patients with cystic fibrosis (15 men) of mean (SD) age 25 (1) years, forced...
expiratory volume in one second (FEV1) 43 (6)% predicted, 15 of whom were receiving treatment with inhaled steroids, and 15 control subjects (eight men) aged 31 (3) years were recruited (table 1). The genotype of 15 patients was investigated by genomic DNA analysis. Fourteen patients were colonised with Pseudomonas aeruginosa. None of the patients had clinical signs of pancreatic insufficiency. All patients were life long non-smokers, the smoking status of all the subjects was confirmed by nicCheck (DynaGen Inc, Cambridge, Massachusetts, USA) which detects nicotine and its metabolites in urine. Active and passive smokers (smoke exposure for more than 30 min/day) were excluded from the study.

EXHALED CO

Efalhced CO was measured using a modified analyser (EC50-MICRO Smokerlyzer CO Monitor, Bedfont Scientific Ltd, Upchurch, Kent, UK) sensitive to 1–500 parts per million (ppm, by volume) CO adapted for on-line recording. The subjects exhaled slowly from total lung capacity with a constant flow (5–6 l/min) against a resistance of 3 (0.4) mm Hg over 20–30 s.30 Two successive recordings were made and maximal values were used in all calculations. Ambient CO levels were recorded before each measurement.

EXHALED NO

Exhaled NO was measured using a chemiluminescence analyser (model LR2000; Logan Research, Rochester, UK) sensitive to NO from 1 to 5000 ppb (by volume) and with a resolution of 0.3 ppb which was designed for on-line recording of exhaled NO concentration as previously described.31 The analyser was calibrated using certified NO mixtures (90 ppb and 436 ppb) in nitrogen (BOC Special Gases; Guildford, UK). Measurements of exhaled NO were made by slow exhalation (5–6 l/min) from total lung capacity for 20–30 s against a resistance of 3 (0.4) mm Hg.

STATISTICAL ANALYSIS

Comparisons between groups were made by one way analysis of variance (ANOVA) and data were expressed as mean (SE) values with confidence intervals of differences. A p value of <0.05 was considered significant.

Results

EXHALED CO

Exhaled CO levels were increased in patients with cystic fibrosis (6.7 (0.6) ppm) compared with the control group (2.4 (0.4) ppm, mean difference 4.3 (95% CI 2.3 to 6.1), p<0.01; fig 1). Untreated patients had higher CO levels than steroid treated patients (8.4 (1.0) ppm and 5.1 (0.5) ppm, respectively, mean difference 3.3 (95% CI –5.7 to –0.9), p<0.01). There was a tendency for patients colonised with P aeruginosa to have lower levels of exhaled CO than non-colonised patients (6.3 (0.9) ppm and 7.0 (0.9) ppm, respectively, mean difference 0.8 (95% CI –2.3 to 0.6), p>0.05), but this difference was not significant. The homozygous genotype for the ΔF508 mutation. All of the subjects were homozygous for the ΔF508 mutation. The homozygous genotype (n = 6) (7.7 (1.8) ppm and 7.8 (1.9) ppm, respectively, mean difference 0.1 (95% CI –1.9 to 0.7) ppm, p<0.01) and heterozygous genotype (n = 6) (7.3 (1.8) ppm and 7.4 (1.8) ppm, respectively, mean difference 0.1 (95% CI –1.9 to 0.7) ppm, p=0.05). There was no difference in disease severity in these two groups of patients.

EXHALED NO

Exhaled NO levels were lower in both steroid treated patients (3.6 (0.4) ppb) and untreated patients (2.7 (0.3) ppb) than in the control
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In cytokines such as IL-1β and TNF-α which are known to upregulate the inducible form of NO synthase (iNOS), exhaled NO levels are not increased in patients with cystic fibrosis. The reason for this may be the absence of detectable immunoreactivity for iNOS in lung epithelium. Alternatively, high levels of CO may inhibit iNOS activity9,10 and therefore reduce the levels of exhaled NO. Decreased exhaled NO concentrations may also be due to retention and metabolism of NO within the airways as shown by an increased production of NO metabolites (nitrates and nitrates) in tracheal secretions. Excess NO may be metabolised locally and may activate HO-1 and CO production before it has an opportunity to diffuse into the airway lumen.

Heme oxygenase is present in the pulmonary vascular endothelium and alveolar macrophages and can be upregulated by oxidative stress and pro-inflammatory cytokines, increasing the production of CO. The high levels of exhaled CO found in patients with cystic fibrosis may be due to oxidation induced HO-1 hyperactivity and therefore the measurement of exhaled CO may reflect inflammation and oxidative stress. This is further confirmed by the finding of lower CO levels in patients on steroid treatment. In fact, by reducing inflammation, attenuating the release of oxidants by inflammatory cells, and suppressing pro-inflammatory cytokine production, steroids may attenuate HO-1 expression and therefore the synthesis of CO.

Cystic fibrosis is characterised by relapsing airway infection and neutrophil recruitment. Considering that neutrophil infiltration of the airways is associated with release of both free radicals and inflammatory cytokines, the use of different markers to separate inflammation from oxidative stress may prove difficult. iNOS is activated by inflammatory cytokines, whereas HO-1 is induced both by cytokines and oxidants, so exhaled NO may reflect inflammation, while CO may be a marker of inflammation and oxidative stress. However, NO induces the generation of CO, activating the transcription of the HO-1, while high levels of CO reduce NO synthesis, inhibiting iNOS. The reciprocal interaction of NO and CO may reduce their sensitivity to detect inflammation and oxidative stress specifically.

Although H2O2 levels are similar in patients with cystic fibrosis and normal subjects, other ROS compounds such as superoxide anion may activate HO-1. Furthermore, H2O2 may still modify HO-1 activity before being metabolised by ROS scavenger such as myeloperoxidase and catalase which are known to be higher in this group of patients. The level of exhaled CO is a non-specific marker of oxidative stress in the airways, therefore different levels are not likely to be found in patients colonised with different bacteria. This hypothesis is confirmed by the finding of similar levels of exhaled CO and NO whether or not patients were colonised with P aeruginosa.

Approximately 70% of cystic fibrosis chromosomes carry a phenyalanine deletion at amino acid position 508 (ΔF508). We have found lower CO and NO levels in patients homozygous for the ΔF508 mutation. Considering the growing interest in gene therapy in...
cystic fibrosis, further studies are needed to investigate the role of CO levels in the assessment of effective therapeutic gene delivery or to confirm the diagnosis in patients with borderline sweat tests where more extensive genetic analysis is not available.

Measurement of exhaled CO levels may be a means of detecting and monitoring cytokine mediated inflammation and oxidant stress in the lower respiratory tract and of assessing the efficacy of treatment. Because CO measurement is simple and non-invasive it can be repeated and can be used in children and in patients with severe disease. The increase in exhaled CO levels observed in this study may be of pathophysiological significance. It will be necessary to intervene with specific inhibitors of HO in the future to explore this possibility further.

Supported by grants from the University of Milan School of Respiratory Disease and the British Lung Foundation (UK).