Increased carbon monoxide in exhaled air of patients with cystic fibrosis

P Paredi, P L Shah, P Montuschi, P Sullivan, M E Hodson, S A Kharitonov, P J Barnes

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)% of 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.

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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 countervail 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.

NO 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, antioxidants, NO, 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 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
Table 1 Mean (SE) characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal controls (n=15)</th>
<th>Steroid treated (n=15)</th>
<th>Steroid untreated (n=14)</th>
<th>ΔF508 hom (n=9)</th>
<th>ΔF508 het (n=6)</th>
<th>Pseud positive (n=14)</th>
<th>Pseud neg (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31 (3)</td>
<td>22 (11)</td>
<td>28 (6)</td>
<td>24 (9)</td>
<td>23 (9)</td>
<td>27 (9)</td>
<td>24 (6)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>8/7</td>
<td>5/10</td>
<td>10/4</td>
<td>3/6</td>
<td>4/2</td>
<td>4/10</td>
<td>11/4</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>96 (7)</td>
<td>39 (7)</td>
<td>42 (6)</td>
<td>48 (9)</td>
<td>59 (9)</td>
<td>58 (9)</td>
<td>51 (9)</td>
</tr>
<tr>
<td>Steroid treatment</td>
<td>0</td>
<td>15</td>
<td>0</td>
<td>9</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

hom = homozygous; het = heterozygous; Pseud positive = patients colonised with Pseudomonas aeruginosa; Pseud neg = patients not colonised with Pseudomonas aeruginosa; FEV₁ = forced expiratory volume in one second.

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 CFTR mutation (n = 9) was associated with higher exhaled CO levels than the heterozygous genotype (n = 6) (7.7 (1.8) ppm and 4.0 (0.6) ppm, respectively, mean difference 3.7 (95% CI –7.1 to –0.3), p<0.01, fig 2).

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 group (4.3 (0.6) ppb) (p<0.05).
Carbon monoxide in cystic fibrosis

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.37 Alternatively, high levels of CO may inhibit iNOS activity37 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.19 20 Excess NO may be metabolised locally and may activate HO-1 and CO production21 before it has an opportunity to diffuse into the airway lumen.

Heme oxygenase is present in the pulmonary vascular endothelium26 and alveolar macrophages27 and can be upregulated by oxidative stress27 and pro-inflammatory cytokines,21 22 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,39 attenuating the release of oxidants by inflammatory cells,40 and suppressing pro-inflammatory cytokine production,41 steroids may attenuate HO-1 expression42 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 radicals5 and inflammatory cytokines,1 the use of different markers to separate inflammation from oxidative stress may prove difficult. iNOS is activated by inflammatory cytokines42 whereas HO-1 is induced both by cytokines42 43 and oxidants,44 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-1,45 while high levels of CO reduce NO synthesis, inhibiting iNOS.47 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-1.23 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.45

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).4 We have found average CO levels in patients homozygous for the ΔF508 mutation. Considering the growing interest in gene therapy in

Discussion

We have found that, in patients with cystic fibrosis, exhaled CO concentrations are increased while NO concentrations are decreased compared with normal subjects and that steroid treatment is associated with lower NO levels (4.1 (0.5) ppb) than the heterozygous genotype (1.9 (0.4) ppb, mean difference 2.2 (95% CI –3.7 to 0.6), p<0.05). Neither exhaled NO nor CO concentrations were correlated with FEV1 (r = 0.03, p = 0.89 and r = –0.07, p=0.79, respectively).

Increased levels of reactive oxygen species and oxidative damage have been demonstrated in cystic fibrosis.4 This is possibly due to increased concentrations of oxidation products12 13 and a defect in intracellular oxygen metabolism.19 Glutathione, the major antioxidant in the lung, is reduced both in the epithelial lining fluid of the lung and in the plasma, possibly as a consequence of the basic genetic defect.4 The damaging effects of glutathione deficiency are exacerbated by the recurrent infections leading to inflow of activated neutrophils releasing free radicals.19 Therefore, circulating and tissue antioxidants are not able to counterbalance the increased generation of free radicals, particularly during bouts of acute pulmonary infection. The increased lung oxidation may stimulate heme catabolism by inducing HO-1 activity as reflected by increased levels of exhaled CO.

Inflammation also has an important role in cystic fibrosis.36 However, despite an increase in cytokines such as IL-1β and TNF-α which are known to upregulate the inducible form of NO synthase (iNOS), exhaled NO levels in group (6.8 (0.4) ppb, p<0.05) with mean differences of 3.1 (95% CI 1.6 to 4.6) and 4 (95% CI 2.5 to 5.5), respectively (p<0.05, fig 3). Colonisation with P aeruginosa did not influence exhaled NO levels (2.7 (0.3) ppb and 3.6 (0.4) ppb for colonised and non-colonised patients, respectively, mean difference 0.8 (95% CI –2.3 to 0.6), p>0.05). The homozygous genotype for the ΔF508 CFTR mutation was associated with higher NO levels (4.1 (0.5) ppb) than the heterozygous genotype (1.9 (0.4) ppb, mean difference 2.2 (95% CI –3.7 to 0.6), p<0.05). Neither exhaled NO nor CO concentrations were correlated with FEV1, (r = 0.03, p = 0.89 and r = –0.07, p=0.79, respectively).

![Figure 3](http://thorax.bmj.com/ on March 18, 2022 by guest. Protected by copyright.)

**Figure 3** Concentrations of exhaled nitric oxide (NO) in normal subjects, patients with cystic fibrosis (CF) on steroid treatment, and untreated patients.

- Normal
- CF steroid
- Untreated
- Treated

<table>
<thead>
<tr>
<th>Condition</th>
<th>NO (ppb)</th>
<th>p-value</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF steroid</td>
<td>0.8</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated</td>
<td>0.9</td>
<td>&lt;0.05</td>
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</tbody>
</table>

CO production25 and oxidants,27 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-1,23 while high levels of CO reduce NO synthesis, inhibiting iNOS.47 The reciprocal interaction of NO and CO may reduce their sensitivity to detect inflammation and oxidative stress specifically.

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Approximately 70% of cystic fibrosis chromosomes carry a phenyalanine deletion at amino acid position 508 (ΔF508).4 We have found average CO 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.

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