Increased levels of exhaled carbon monoxide in bronchiectasis: a new marker of oxidative stress

I Horvath, S Loukides, T Wodehouse, S A Kharitonov, P J Cole, P J Barnes

Abstract

Background—Bronchiectasis is a chronic inflammatory lung disease associated with increased production of oxidants due mostly to neutrophilic inflammation. Induction of heme oxygenase (HO-1) by reactive oxygen species is a general cytoprotective mechanism against oxidative stress. HO-1 catabolises heme to bilirubin, free iron and carbon monoxide (CO). Exhaled CO measurements may therefore reflect an oxidative stress and be clinically useful in the detection and management of inflammatory lung disorders. Methods—The levels of exhaled CO of 42 non-smoking patients with bronchiectasis treated or not treated with inhaled corticosteroids were compared with CO levels in 37 normal non-smoking subjects. Results—Levels of exhaled CO were raised in patients with bronchiectasis, both those treated with inhaled corticosteroids (n = 27, median 5.5 ppm, 95% CI 5.16 to 7.76) and those not treated with inhaled corticosteroids (n = 15, median 6.0 ppm, 95% CI 4.74 to 11.8), compared with normal subjects (n = 37, median 3.0 ppm, 95% CI 2.79 to 3.81, p = 0.0024). There was no correlation between exhaled CO and HbCO levels (r = 0.42, p = 0.12) in normal subjects (n = 7), nor between the urine cotinine concentration and exhaled CO levels (r = 0.2, p = 0.12). Conclusions—Increased levels of exhaled CO may reflect induction of HO-1 and oxidative stress in bronchiectasis. Measurement of exhaled CO may be useful in the management of bronchiectasis and possibly other chronic inflammatory lung disorders.

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Keywords: exhaled carbon monoxide; bronchiectasis; heme oxygenase

Oxidative stress and reactive oxygen species have been implicated in the pathogenesis of many pulmonary diseases including chronic inflammatory lung disorders such as asthma, chronic obstructive pulmonary disease (COPD), and bronchiectasis. Reactive oxygen species, including the superoxide anion (O₂⁻), hydroxyl radicals, and hydrogen peroxide (H₂O₂), are produced by activated immune and inflammatory cells and cause oxidation of nucleic acids, proteins, and membrane lipids. One of the mechanisms protecting against an oxidative stress is the induction of a stress response protein, heme oxygenase (HO). HO-1, an inducible form of HO, catalyses the degradation of heme to bilirubin, and consequently to the anti-oxidant bilirubin, producing free iron and carbon monoxide (CO) which has several biological activities including stimulation of guanylate cyclase. Some other inflammatory mediators such as cytokines and nitric oxide (NO) are also released during chronic inflammation and are able to induce HO-1 expression.

Exhaled NO levels are raised in patients with bronchiectasis, reflecting the chronic inflammatory process. A vicious circle of microbial colonisation and host derived inflammation leads to damage of adjacent normal lung tissue with neutrophilic infiltration and high levels of proinflammatory cytokines present in airway secretions, resulting in overproduction of O₂⁻ and other oxidants.

We therefore postulated that measurement of CO levels in the breath of patients with bronchiectasis may reflect HO-1 activation and may provide useful information on the control of inflammation and the assessment of new anti-inflammatory treatments.

Methods

Patients

Forty two non-smoking patients (16 men) of mean age 45 (15) years, forced expiratory volume in one second (FEV₁) 59 (19.5)% predicted, carbon monoxide transfer factor (TLCO) 88 (4.4)% predicted with clinically and radiologically diagnosed bronchiectasis confirmed by high resolution computed tomographic (CT) scanning of the thorax were studied. All were clinically stable and had no evidence of acute infective exacerbations for at least four weeks prior to the study. Bronchiectasis was believed to be secondary to tuberculosis in two cases, to primary ciliary dyskinesia in 10, to IgA deficiency in two cases, to a deficiency of a subclass of IgG in three, and with no definite antecedent cause identified in the remaining patients (idiopathic). Patients with cystic fibrosis, allergic bronchopulmonary aspergillosis, asthma, and atopic diseases were excluded. Fifteen patients were taking regular inhaled β₂ agonists and 27 were on inhaled corticosteroids (fluticasone propionate, 500–2000 µg daily). Thirty seven normal non-smoking subjects (20 men) of mean age 33 (17.1) years were free of respiratory infections for at least six weeks before starting the study, had no history of chronic cardiovascular or respiratory disease, and were not receiving any regular medication. These subjects had a negative history of allergy (negative skin prick tests to common allergens), normal spirometric
values (FEV\textsubscript{1} 94 (4.9)% predicted), and normal bronchial reactivity with a provocative concentration of methacholine causing a 20% fall in FEV\textsubscript{1} (PC\textsubscript{20}) of >32 mg in all subjects.

The subjects were tested by NicCheck I (DynaGe Inc, Cambridge, Massachusetts, USA) which determines the levels of cotinine in urine to ensure non-smoking status. Active and passive smokers (smoke exposure for more than 0.5 hour/day) were excluded from the study. In addition, HbCO was measured in blood in seven normal volunteers and compared with their exhaled CO levels.

The study was approved by the ethics committee of the Royal Brompton Hospital.

**MEASUREMENTS**

FEV\textsubscript{1} was measured using a dry spirometer (Vitalograph, Buckingham, UK) and the best value of three manoeuvres was expressed as a percentage of the predicted value. Airway responsiveness was measured by methacholine provocation challenge (Dosimeter MB3; MEFAR, Boveza, Italy). The PC\textsubscript{20} was calculated by interpolation of the logarithmic dose-response curve.

Exhaled CO was measured using a modified Micro Smokerlyser (Bedfont Scientific UK) sensitive to CO from 0 to 500 parts per million (ppm, by volume), adapted for on-line recording of CO concentration and integrated with a chemiluminescence analyser (Model LR2000, Logan Research, UK) to control exhalation parameters. Exhaled CO was not measured on the day of TLCO assessment to exclude the influence of inhaled gas mixture containing 0.3% CO on exhaled CO levels. The subjects exhaled slowly from functional vital capacity with a constant flow (5–6 l/min) against a low resistance (3 (0.4) mm Hg) over 20–30 s into the analyser. Two successive recordings were made and the highest value was used in all calculations. Ambient CO levels were recorded before the measurements.

**STATISTICAL ANALYSIS**

The data are presented as medians and ranges. The results of the studied groups were compared by the Mann-Whitney U test and estimates of the differences between groups were expressed as medians and 95% confidence intervals (CI). Spearman’s rank correlation coefficient was used to test the relationships between variables. A p value of <0.05 was considered significant.

**Results**

Exhaled CO was detectable in all subjects. The levels of exhaled CO were significantly increased in patients with bronchiectasis (n = 42) compared with healthy subjects (median 6.0 ppm, 95% CI 5.58 to 8.57 ppm versus median 3.0 ppm, 95% CI 2.79 to 3.81 ppm, p = 0.0024; fig 1). There was no difference in exhaled CO levels between 27 steroid treated patients and 15 non-treated patients (median 5.5 ppm, 95% CI –5.16 to 7.76 ppm versus median 6.0 ppm, 95% CI 4.47 to 11.8 ppm, p = 0.08). There was a tendency for a negative correlation between lung function as assessed by FEV\textsubscript{1} and exhaled CO levels (r = –0.33, p = 0.46; fig 2). There was no correlation between exhaled CO and HbCO levels (r = 0.42, p = 0.12) in normal subjects (n = 7). The urinary levels of cotinine were compared with exhaled CO concentrations in all normal subjects and patients with bronchiectasis and no correlation was found (r = 0.2, p = 0.12).

**Discussion**

The results of this study show that exhaled CO is increased in patients with bronchiectasis, irrespective of their anti-inflammatory treatment, which might be a reflection of oxidative stress and HO-1 activation.

The source of CO in exhaled air of non-smoking subjects is uncertain. It is likely that exhaled CO derives from an endogenous source since the inhaled HO inhibitor, tin- mesoporphyrin, significantly inhibits the concentration of CO (unpublished observations). The technique used in the present study with a single expiratory manoeuvre against resistance is known to minimise the upper respiratory and ambient air contribution to the exhaled CO.

Bronchiectasis is a chronic inflammatory airway disease which is characterised by neutrophil dominant airway inflammation and damage to bronchial epithelial cells. Increased HO-1 protein expression may be due to the induction of the enzyme by inflammatory cytokines and oxidants such as interleukins, tumour necrosis factor-\(\alpha\), interferon-\(\gamma\), and \(\text{H}_2\text{O}_2\) which are capable of inducing HO-1 expression in cell lines and tissues. The increase in exhaled CO levels in patients with...
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