Airway nitric oxide output is reduced in bronchiectasis

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KEYWORDS
Bronchiectasis; Inducible nitric oxide synthase; Glucocorticoid

Summary

\textit{Background:} Increased concentrations of exhaled nitric oxide (NO) have been detected in inflammatory lung diseases including asthma and have been attributed to increased expression and activity of inducible nitric oxide synthase (iNOS) within the airways. However, previous studies of exhaled NO in patients with bronchiectasis have yielded conflicting results, with reports of both increased and normal NO values. Recent evidence from animal models suggests that chronic airway infection reduces NO production within the lung, despite causing increased iNOS expression. We tested the hypothesis that, in human subjects with bronchiectasis, chronic airway infection reduces NO output from the conducting airways.

\textit{Methods:} Using a recently described two-compartment model, we measured separately the contributions of the conducting airways and the alveoli to exhaled NO in nine patients with stable bronchiectasis and eight control subjects before and after inhaled glucocorticoid therapy.

\textit{Results:} We found that airway NO output was significantly lower in bronchiectasis than in normal airways whereas NO output from the alveoli was similar to that of control subjects. High-dose inhaled glucocorticoid therapy did not alter airway or alveolar NO production.

\textit{Conclusions:} These findings demonstrate that, in patients with bronchiectasis, airway NO output is reduced and that iNOS does not contribute significantly to airway NO production.

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Abbreviations: NO, nitric oxide; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; CT, computerised tomography; CF, cystic fibrosis

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Introduction

Nitric oxide (NO) produced in the lungs can be detected in exhaled gas from normal lungs and altered NO excretion from the lungs has been reported in respiratory diseases.\(^1\)\(^2\) In particular, expression of the high output isoform of nitric oxide synthase (iNOS or type II NOS) is increased in asthma\(^3\) and is associated with abnormally elevated NO concentrations in exhaled gases.\(^4\)\(^5\) Exhaled NO may be reduced towards normal by inhalation of selective inhibitors of iNOS or glucocorticoids that can inhibit the expression of this inducible isoform.\(^6\)\(^7\)\(^8\) Increased concentrations of exhaled NO in association with increased expression of iNOS have also been reported in several other lung diseases.\(^9\)\(^-\)\(^11\) Taken together, these findings suggest that increased exhaled NO in these diseased airways results from increased iNOS activity in the airways. Other mechanisms that have been postulated include increased airway diffusing capacity for NO, altered neuronal NOS (nNOS) activity and altered pH of airway lining fluid.\(^12\)\(^-\)\(^14\) Thus, it has been suggested that measurement of NO in exhaled air may be a useful non-invasive index of inflammation in the respiratory tract in a variety of inflammatory lung diseases.\(^15\)

The airways of patients with bronchiectasis are chronically infected and inflamed and demonstrate increased expression of iNOS,\(^16\) suggesting that exhaled NO should be increased. However, studies in patients with bronchiectasis, which have measured exhaled NO concentrations at a single exhaled flow rate, have yielded conflicting results with reports of both elevated and normal values.\(^16\)\(^-\)\(^18\) Recent reports of chronic airway infection in rodent models found reduced NO production in the lung despite increased iNOS expression.\(^21\)\(^-\)\(^23\)

The previous conflicting reports of exhaled NO in bronchiectasis, and the apparent discrepancy with rodent studies, may have arisen because of the methods used for measuring exhaled NO, in particular the flow dependency of these methods. More recently, a two-compartment model of pulmonary NO output has been described which allows the separate measurement of two flow independent parameters, arterial NO output and alveolar NO concentration.\(^13\)\(^24\)\(^-\)\(^26\) We undertook the present study to test the hypothesis that chronic infection of the airways leads to a reduction in NO output from the conducting airways, as predicted by our previous studies in a rodent model.\(^22\)\(^23\)

Using the two-compartment model described above, we determined the separate contributions of NO output from the conducting airways and from the gas exchange regions of the lungs of bronchiectatic patients and compared them with control subjects. In addition, we examined the effect of inhaled corticosteroids on these two sources of NO. Some of the results of these studies have been previously reported in the form of an abstract.\(^27\)

Methods

Recruitment of subjects

The study protocol was reviewed and approved by the Ethics Committee of St. Vincent’s University Hospital and informed written consent was obtained from all participating sub-

jects. Patients with chronic, stable purulent bronchiectasis, proven by CT scan were recruited from the outpatient department of the hospital. Subjects were excluded if they had a diagnosis of cystic fibrosis, primary ciliary dyskinesia, situs inversus, chronic bronchitis, atopy, asthma, paranasal sinusitis, seasonal allergic disease, allergic bronchopulmonary aspergillosis, a family history of bronchiectasis or other lung disease. Other exclusion criteria were current treatment with oral corticosteroids, evidence of *Pseudomonas aeruginosa* in sputum (excluded by repeated sputum culture), a respiratory tract infection in the 2 weeks preceding recruitment, peripheral blood eosinophilia or immunoglobulin deficiency.

The control subjects were healthy, non-smoking volunteers recruited from the hospital staff without history of any chronic pulmonary disease, atopy, paranasal sinusitis, or recent respiratory tract infection and who were not taking any medications. They all had normal spirometry.

Study protocol

Baseline spirometry and exhaled NO were recorded in all subjects on entry to the study. Those subjects with bronchiectasis who were already taking prescribed inhaled glucocorticoid medication prior to enrolment underwent a two-week washout period before baseline measurements were taken; other prescribed medications remained unchanged. Following baseline measurements, all subjects took a two-week course of inhaled fluticasone (metered dose inhaler, total dose 1000 μg twice daily) via a spacer device. Pulmonary function testing and exhaled NO measurements were then repeated.

Measurement of exhaled NO

Online exhaled NO measurements were made using a Sievers 280 chemiluminescence analyser (Sievers Instruments, Boulder, CO, USA). The analyser was calibrated daily with a known NO concentration (BOC gases, Dublin, Ireland) and before each subject with NO-free air (Sievers zero-air-filter). The sensitivity of the analyser to NO ranged from <1 to 500,000 parts per billion (ppb) with a resolution of 1 ppb and a response time of 200 ms. Ambient NO was <3 ppb on each day that measurements were taken.

Seated subjects inhaled maximally via their mouths to total lung capacity, breath-held for 6–10 s and then exhaled against a fixed resistance, whilst maintaining a constant mouth pressure; nose clips were not used. Mouth pressure was maintained constant by means of a visual feedback device. The manoeuvre was included for analysis if the pressure was within 5% of the target value for a minimum of 6 s during exhalation while a stable concentration of NO was observed during a plateau phase. The mean NO concentration of three adequately performed exhalations which were within 5% of each other was taken to be the average NO concentration at that flow rate. Four exhaled flow rates (100, 150, 200 and 250 ml/s) were examined in each subject by using four fixed resistances (Sievers, Boulder, CO, USA). NO output was calculated as the product of steady-state NO concentration and flow rate, corrected to BTPS. A straight line was fitted to the plot of NO output against flow by least
squares regression. The intercept of this line equalled
airway NO output and the slope equalled alveolar NO
concentration.24

Reproducibility of exhaled NO measurements in healthy
controls
Reproducibility of exhaled NO measurements over the same
four flow rates was previously confirmed in group of 20
healthy female adults (age range 24–31 years) using the
same methodology. Subjects without history of atopy or
respiratory disease were recruited from among the staff and
medical students of the hospital. They had no history or
symptoms suggestive of asthma and pulmonary function
tests were normal. There was no overlap with the control
subjects from the bronchiectasis study. Exhaled NO mea-
surements were recorded in the morning on three occasions
at intervals of one week and reproducibility was confirmed
by Bland–Altman analysis.28 The mean difference in airway
NO production measured on the first and last occasions in a
single individual was 1.1 (15.6) nl/min. The limits of
agreement were 4.5 and –41.1. The mean difference in
alveolar NO concentration was –0.2 (2.4) ppb. The limits of
agreement were 4.5 and –4.9 in the Bland–Altman analysis.
The median alveolar NO concentrations on the first and
last day were 1.6 (0.1–4.5) ppb and 1.2 (0.1–4.1) ppb,
respectively. The median airway NO output on the first and
last day was 48.5 (27.4–87.2) nl/min and 54.2 (20.8–87.8)
nl/min, respectively.

Statistical analysis
Kolmogorov–Smirnov tests of normality were performed in
order to test the distributions of data prior to analysis. Both
alveolar NO and airway NO were normally distributed. The
values shown are means ± standard deviations or medians
and ranges. Statistical comparisons are made using paired
and unpaired t-tests as appropriate. A p-value of less than
0.05 was accepted as statistically significant.

Results

Subject characteristics

Twelve patients with bronchiectasis were recruited over an
eight month period. Three were subsequently excluded; one
due to non-compliance, one due to intercurrent lower
respiratory tract infection and one due to an inability to
correctly perform the exhaled NO measurements. Nine
patients completed the study. All subjects were lifelong
non-smokers or ex-smokers for at least 1 year. The median
age (range) of the patient group was 54 (37–63) years and
they were all females. Four had developed bronchiectasis
secondary to severe childhood pneumonia, one from
documented childhood measles pneumonia and one due to
tuberculosis in young adulthood. Three cases were idio-
patic. All were sputum producers of between one and two
eggcupfuls per day. The results of pulmonary function
testing and dyspnoea scores are shown in Table 1.

Six patients were taking prescribed inhaled steroids prior
to enrolment; these were withdrawn for a two-week period
prior to entry in accordance with the study protocol. Other
medications used to treat their airways disease included β-2
agonists and inhaled anticholinergics.

Eight control subjects were recruited. They had a median
age of 46 (33–55) years and a 7:1 female predominance.
Only subjects who complied with the inhaled steroid
medication for two weeks as assessed by interview and
change in canister weight were included. Figure 1 shows the
method of computing NO output in a sample control and
bronchiectatic subject.

Exhaled NO measurements

Exhaled NO was measured in nine subjects with bronchiec-
tasis and eight age-matched controls. There was a linear
relationship between exhaled NO output and flow rates
(Fig. 1) for all bronchiectasis patients ($r^2 = 0.81 ± 0.17$;
$p < 0.001$) and control subjects ($r^2 = 0.86 ± 0.16$; $p < 0.001$)
examined.

At baseline, mean airway NO production (Fig. 2a) was
31.8 (± 15) nl/min in subjects with bronchiectasis, signifi-
cantly lower ($p < 0.02$) than in controls (52.5 ± 17.3 nl/min).
However, the mean alveolar NO concentration (Fig. 2b) in
the diseased group (2.5 ± 2.0 ppb) did not differ significantly
from the control group (2.4 ± 1.6 ppb).

Following two weeks of inhaled steroids, mean airway NO
production was 36.1 (± 22.8) nl/min in the bronchiectasis
group and 58.6 (± 34.6) nl/min in the control group, neither
of which was significantly different from the respective pre-
steroid values. Similarly, the mean alveolar NO concentra-
tion in the diseased group was 2.9 (± 1.6) ppb and in the
control group was 1.3 (± 1.4) ppb, neither of which had
changed significantly from baseline values.

| Table 1 Pulmonary function tests and dyspnoea score. |
|-----------------|-----------------|-----------------|-----------------|
|                 | Bronchiectatics (n = 9) | Controls (n = 8) |
|                 | Pre-steroid | Post-steroid | Pre-steroid | Post-steroid |
| FEV1 (L)        | 1.7±0.5     | 1.7±0.5     | 3.1±0.5     | 3.2±0.5     |
| FVC (L)         | 2.2±0.5     | 2.3±0.5     | 3.9±0.7     | 3.9±0.6     |
| FEV1 (% pred)   | 70.0±17.6   | 70.8±18.0   | 96.9±5.8    | 98.1±7.6    |
| FVC (% pred)    | 81.6±10.9   | 82.4±13.6   | 95.7±6.0    | 95.2±5.5    |
| FEV1/FVC (%)    | 73.4±13.1   | 73.7±12.9   | 80.3±2.9    | 81.1±2.6    |

Values shown are means ± standard deviation. Pre-steroid indicates values prior to a two-week course of inhaled steroid. Post-steroid
indicates values recorded after a two-week course of inhaled steroid. % pred, percentage of predicted value.
Mean NO concentrations at all 4 flow rates are shown in Table 2. To allow comparison with previous studies, mean exhaled NO concentrations at a fixed flow rate of 250 ml/s are presented in this table. There was no significant difference between bronchiectasis and controls at baseline, in marked contrast to the significantly lower values of airway NO output observed in this study (Fig. 2a). Inhaled steroid therapy did not significantly alter exhaled NO concentrations at 250 ml/s in either group.

To allow for comparison with future studies, we report mean NO output at a flow rate of 50 ml/s (Table 3). This flow rate corresponds to 3.0 l/min on the plots of flow rate versus NO output in Fig. 1. There was a significant difference between the two groups; prior to inhaled steroids, exhaled NO output was statistically lower in the bronchiectatic group compared to the control group ($p<0.05$). Since exhaled NO concentration at low flow rates (50 ml/s) is largely determined by NO output from the airways, this fits very well with our finding of reduced bronchial NO output in bronchiectasis using the two-compartment model.

**Discussion**

This is the first study that reports the separate measurements of airway NO output and alveolar NO concentration in humans with chronic suppurative bronchiectasis not caused by cystic fibrosis. We were interested to separately measure conducting airway NO output because this is a major site of infection and inflammation in bronchiectasis. The data show that airway NO output was decreased in bronchiectasis compared to controls, while alveolar NO concentrations were similar in both groups. We have also demonstrated that inhaled steroids did not alter airway NO output in these patients.

The standard approach in previous studies has been to report NO concentration at a fixed expiratory flow rate while nasal NO was excluded. However, this approach does not provide information about the separate sources of the NO in the lower airways. Moreover, the values observed are dependant on the chosen flow rate. More recent approaches have provided additional important insights into the sites of excretion of exhaled NO within the lungs. George and co-workers first described a two-compartment model in normal subjects, an approach which others have subsequently found to provide a good match with empirically derived data in both normal subjects and those with respiratory disease. Using this two-compartment model, we were able to separately measure airway NO output and alveolar NO concentration in control subjects and in those with chronic supplicative bronchiectasis. The values that we observed in our normal subjects were similar to those reported previously. In both our subject groups, there was a linear relationship between exhaled NO output and flow rates (see Results section) suggesting that the two-compartment model is applicable in this disease condition (Fig. 1). This is in agreement with the reports of others in both asthma and CF patients.

Exhaled NO concentrations in bronchiectasis have previously been reported to be similar in control and bronchiectatic subjects following measurement at a single fixed expiratory flow rate of 250 ml/s. For direct comparison, we have reported our results in both groups at this single flow rate at baseline (Table 2) and we found similar exhaled NO concentrations in control and bronchiectatic subjects. At this relatively high flow rate, exhaled NO is determined largely by the alveolar NO concentration. Thus, the similarity of exhaled NO concentrations in this flow rate in bronchiectasis and control subjects is in good agreement with our finding that alveolar NO concentrations were not significantly different in these two groups using the two-compartment model.

Kharitonov and colleagues have previously reported increased exhaled NO concentrations in bronchiectasis in contrast to our findings and previous reports. However, peak rather than plateau expiratory NO concentrations were recorded in that study while subjects wore a nose-clip. Differences in patient characteristics may also...
have contributed to the differences in results. For this reason, we carefully excluded all patients who might have had an additional disease known to alter iNOS expression and activity.

Tsang et al. 20 found no difference in exhaled NO concentrations between bronchiectatic and control subjects when measured at a single flow rate of 50 ml/s. Bronchiectasis patients with Pseudomonas infection had lower levels of exhaled NO than their counterparts. We excluded patients with Pseudomonas infection from our study and by measuring exhaled NO over a range of flow rates we still found a statistically significant reduction in airway NO output (but not in alveolar NO concentration) in bronchiectasis compared to controls. The discrepancy in results may be contributed to by differences in methodology or by the numerous exclusion criteria of our study.

Six patients with bronchiectasis underwent a two-week washout period prior to enrolment in our study. It has been shown that exhaled NO levels recover rapidly after cessation of inhaled budesonide in mild asthmatics with complete recovery of NO levels by the end of the first week.37 Based on this, a two-week washout period should have been adequate to allow accurate baseline NO measurements to be recorded at time of enrolment. It could be argued, however,

### Table 2. Exhaled NO concentrations at four different expiratory flow rates.

<table>
<thead>
<tr>
<th>Expiratory flow rate (ml/s)</th>
<th>P-value</th>
<th>Bronchiectasis (n = 9)</th>
<th>Controls (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-steroid</td>
<td>Post-steroid</td>
</tr>
<tr>
<td>100</td>
<td>0.1</td>
<td>8.0±3.1 (3.8–13.7)</td>
<td>8.7±5.2 (3.5–20.2)</td>
</tr>
<tr>
<td>150</td>
<td>0.7</td>
<td>6.2±2.2 (3.2–9.5)</td>
<td>6.8±3.5 (2.8–9.6)</td>
</tr>
<tr>
<td>200</td>
<td>0.3</td>
<td>5.5±2.3 (2.4–9.5)</td>
<td>5.7±3.5 (3.7–8.8)</td>
</tr>
<tr>
<td>250</td>
<td>0.5</td>
<td>4.8±2.0 (2.2–8.7)</td>
<td>5.1±2.1 (3.0–7.9)</td>
</tr>
</tbody>
</table>

Values shown are means±standard deviations and ranges. Pre-steroid indicates values prior to a course of inhaled steroids. Post-steroid indicates values recorded after a two-week course of inhaled steroid. NO concentrations are reported as parts per billion. P values shown are for pre-steroid comparisons between both groups.

### Table 3. NO output at 50ml/s calculated from 2-compartment model plots.

<table>
<thead>
<tr>
<th></th>
<th>Bronchiectasis (n = 9)</th>
<th>Controls (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-steroid (nl/min)</td>
<td>38.4±17.1**</td>
<td>59.6±19.1</td>
</tr>
<tr>
<td>Post-steroid (nl/min)</td>
<td>54.8±18.6</td>
<td>68.8±16.2</td>
</tr>
</tbody>
</table>

Values shown are means±standard deviations and ranges. Pre-steroid indicates values prior to a course of inhaled steroids. Post-steroid indicates values recorded after a two-week course of inhaled steroid. NO output at 50 ml/s is reported in nl/min. **P<0.05 versus pre-steroid control value.
that a longer wash-out period may have resulted in increased baseline NO levels and consequently in a reduction in exhaled NO after inhalation of glucocorticoids. Our findings that inhaled steroids have no significant effect on exhaled NO in subjects with bronchiectasis are consistent with a recent report. iNOS expression is reported to be increased in the airways of patients with bronchiectasis compared to controls. However, only three subjects with bronchiectasis were analysed in that particular study. We and others have previously reported in animal studies that chronic airway infection leads to increased iNOS expression but not excess NO production in intact lungs. A similar suppression of iNOS activity may occur in chronic airway infection in humans thus accounting for our present observation. An alternative explanation is that NO production is increased within the airway wall but not excreted into the airway lumen due to increased local metabolism by superoxide radicals in the presence of infection. This rapid reaction, reducing NO to peroxynitrite, would prevent NO reaching the airway lumen and reduce exhaled NO. However, we have recently shown in animal studies that there was no evidence of augmented iNOS activity or peroxynitrite production despite increased iNOS expression.

Glucocorticoids inhibit the expression of iNOS but not that of the constitutive NOS isoforms. Moreover, inhaled glucocorticoids inhibit iNOS expression and reduce exhaled NO in asthma. If iNOS were a significant contributor to exhaled NO in bronchiectatic airways, then NO production should be reduced after a course of inhaled steroid therapy. We found no significant change in airway NO production or alveolar NO concentration in the bronchiectatic patients after a two-week course of inhaled steroids, suggesting that iNOS was not contributing to airway NO output in bronchiectasis. Our observation that inhaled steroids did not decrease airway or alveolar NO production in normal subjects is compatible with previous results.

Reduced airway NO could play a significant role in the pathogenesis of bronchiectasis. High concentrations of NO may have an important anti-microbial role in airway defence and loss of this action could facilitate chronic airway infection, as has been suggested in cystic fibrosis. Normal airway NO production is required for sodium secretion by the airway epithelium. Reduced NO leads to excessive sodium uptake by these cells, an action that could lead to excessively viscous sputum, impaired sputum clearance and thus susceptibility to chronic bacterial infection. Low NO concentrations could also lead to lung damage through loss of its anti-inflammatory function, which could contribute to an excessive response to pulmonary infection resulting in tissue damage. The extent to which these mechanisms contribute to the pathogenesis of non-CF bronchiectasis remains to be determined.

In conclusion, we have shown that, despite the previous demonstrations of increased iNOS expression in bronchiectasis, airway NO output was reduced in these patients. We have also demonstrated that inhaled steroid did not further reduce the airway NO which suggests that iNOS did not contribute to exhaled NO in this disease. The reduced airway NO output in bronchiectasis may contribute to the pathogenesis of this disease.

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References


