Normal Bronchial Blood Flow in COPD Is Unaffected by Inhaled Corticosteroids and Correlates With Exhaled Nitric Oxide*

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Background: In COPD patients, there is reduced vascularity and inflammation of the bronchi, which may have opposite effects on bronchial blood flow (QAW). We studied the relationship of QAW with the fraction of exhaled nitric oxide (FENO), which is a potent vasodilator. We also investigated the vascular response to budesonide and a β2-agonist.

Methods: We measured QAW in 17 patients with COPD (mean [± SEM] age, 67 ± 3 years; 10 male patients; mean FEV1, 57 ± 3% predicted; mean FEV1/FVC ratio, 54 ± 4%), all of whom were ex-smokers, and in 16 age-matched nonsmoking volunteers (mean age, 64 ± 4 years) and compared this to FENO. QAW was measured using the acetylene dilution method.

Results: Mean QAW was similar in patients with COPD (34.29 ± 1.09 µL/mL/min) compared to healthy subjects (35.50 ± 1.74 µL/mL/min; p > 0.05) and was not affected by long-term treatment (35.89 ± 1.63 µL/mL/min) or short-term treatment (32.50 ± 1.24 µL/mL/min; p < 0.05) with inhaled budesonide. QAW positively correlated with the diffusion of carbon monoxide (ie, carbon monoxide transfer coefficient: r = 0.74; p < 0.05). FENO levels were mildly elevated in steroid-treated patients (10.89 ± 0.87 parts per billion [ppb]) and untreated patients (9.40 ± 0.86 ppb) compared to the control group (8.22 ± 0.57 ppb; p < 0.05) and were correlated with QAW (r = 0.6; p < 0.05). Ten minutes after the inhalation of 200 µg of albuterol, QAW was more elevated in healthy control subjects (59.33 ± 2.40 µL/mL/min) compared to COPD patients (38.00 ± 0.58 µL/mL/min; p < 0.05), indicating that COPD patients may have a reduced bronchial vascular reactivity.

Conclusions: QAW is normal in COPD patients and is not affected by therapy with inhaled corticosteroids or β2-agonists. In addition, QAW correlates with levels of FENO, which may have a regulatory role.

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Key words: bronchial blood flow; COPD; inflammation; nitric oxide

Abbreviations: DS = dead space; Δε°Τ = exhaled breath temperature gradient; FENO = exhaled nitric oxide; NO = nitric oxide; ppb = parts per billion; QAW = bronchial blood flow; Tlco = carbon monoxide transfer coefficient

COPD is characterized by progressive, largely irreversible airflow limitation, leading to disability and mortality. During this process, the airways of COPD patients may show a number of changes including a modest increase in airway smooth muscle mass, hypertrophy of mucus-secreting glands, mucous metaplasia, squamous metaplasia, and parenchymal destruction. Despite a large number of studies investigating the pathology of COPD, airway vasculature has received little attention, partly because of the difficulty in measuring bronchial blood flow (QAW) in patients. However, some initial histologic studies2,3 have shown decreased bronchial vascularity, and, more recently, it was confirmed that the number of vessels in the airways of COPD patients is not significantly elevated,4 despite the presence of airway inflammation, which normally causes vascular proliferation. This is in sharp contrast to the situation in asthma patients, in whom increased vascularity has been documented using several techniques.5–7 Furthermore, the vascular changes in COPD patients seem to be associated with a reduc-
tion of vasomotor reactivity not only systemically,\(^8\) but also in the airways.\(^9\) These changes in vascular function may play a role in the pathophysiology of the disease.

The bronchial circulation arises from the aorta and forms a peribronchial plexus of vessels which is part of the systemic circulation and does not take part in gas exchange. The branches then penetrate the muscular layer to form a submucosal network. Capillary engorgement and/or leakage in this circulatory bed could directly alter airway wall thickness and bronchial diameter. Inflammatory mediators released in COPD patients may contribute to bronchial vasodilation,\(^10\) and potential candidates include bradykinin,\(^11\) prostaglandins,\(^12\) and neuropeptides released by sensory nerves.\(^13\) Platelet activating factor may also increase bronchial vascular blood flow.\(^14\) On the other hand, QAW may be reduced because of low bronchial vascularity,\(^2\) the vasoconstrictive effect of endothelins,\(^15\) and hyperinflation, which decreases QAW. Interestingly, samples of induced sputum from patients with emphysema have reduced levels of vascular endothelial growth factor,\(^16\) which is a trophic compound that is required for endothelial and vascular survival.

In a previous study,\(^17\) we showed that patients with COPD have reduced exhaled breath temperature gradients (ΔT\(_{\text{e}}\)) compared to healthy subjects. Because QAW is the main determinant of exhaled breath temperature, in the current study we hypothesized that COPD patients had reduced QAW despite the presence of airway inflammation, which normally drives airway vascular proliferation. Furthermore, considering that these vascular anatomic and functional changes may play a role in the pathogenesis of the disease, we studied the correlation of QAW with exhaled nitric oxide (FENO), which had never been investigated before in any other study. Even though FENO may not be a reliable marker of inflammation in COPD patients, elevated nitric oxide (NO) levels correlate with an increased bronchodilator response and steroid responsiveness;\(^18\) therefore, its measurement may be of interest when investigating the short-term effect of corticosteroids. Furthermore, because NO has vasodilating properties, it may have a role in the regulation of QAW; therefore, its measurement goes beyond its value as a marker of inflammation, which is controversial in COPD patients, but remains interesting for its vasoactive properties.

In this study, we also investigated the short-term effect of therapy with budesonide, an inhaled corticosteroid with antiinflammatory properties. In addition, because it has recently been suggested\(^9\) that COPD patients may have an impaired vascular reactivity, we studied the short-term effects of albuterol therapy on QAW.

**Materials and Methods**

**Patients**

All the patients who were enrolled in the study met the Global Initiative for Chronic Obstructive Lung Disease criteria for the diagnosis of COPD\(^10\) and had moderate (n = 12) or severe (n = 5) disease severity with a moderate degree of emphysema, as assessed by the carbon monoxide transfer coefficient (T\(_{\text{LCO}}\)) in 10 patients and CT scan in 4 patients. All of the patients were ex-smokers with a history of smoking equivalent to at least 20 pack-years. Active and passive smokers (i.e., smoke exposure for >0.5 h/d) were excluded from the study. None of the patients had a history of atopy, as assessed by clinical history and a skin-prick test, or a significant reversibility of airflow obstruction (i.e., >15% or >200 mL) after the inhalation of 400 µg of albuterol via a metered-dose inhaler. The patients were in a stable state, and symptoms of COPD, especially the grade of dyspnea and sputum volume, were unchanged in the previous 3 months. All of the patients enrolled in this study were afebrile.

Seventeen COPD patients (mean ± SEM age, 67 ± 3 years; 10 male patients), all of whom were confirmed ex-smokers, were studied (9 had received steroid treatment for at least 3 months) [Table 1]. Steroid-treated patients received treatment with inhaled fluticasone propionate, 500 µg/d (n = 5), and beclometasone, 500 µg/d (n = 4). None of 16 nonsmoking control subjects (12 men; mean age, 64 ± 4 years), some of whom had been included in previous studies, had a history of respiratory or cardiovascular disease. There was no history of upper respiratory tract infection for at least 4 weeks before the study. In order to reduce the effect of ambient contamination, all subjects had at least 30 min rest before QAW and FENO were measured.

**FENO Measurements**

FENO was measured using a modified chemiluminescence analyzer (model LR2000: Logan Research; Rochester, Kent, UK), as previously described.\(^20\) The analyzer was calibrated using certified NO mixtures (50 parts per billion [ppb]) in nitrogen (BOC Special Gases; Guildford, UK).

**QAW**

As previously published,\(^5\) we modified a validated\(^21\) soluble inert-gas uptake method to measure QAW, using acetylene.
Table 1—Patient Characteristics*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Not Steroid-Treated Patients (n = 8)</th>
<th>Steroid-Treated Patients (n = 9)</th>
<th>Healthy Subjects (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>59 ± 6</td>
<td>71 ± 3</td>
<td>64 ± 4</td>
</tr>
<tr>
<td>Sex</td>
<td>Male 5</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Female 3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>59 ± 6</td>
<td>71 ± 3</td>
<td>95 ± 9</td>
</tr>
<tr>
<td></td>
<td>TlCO, % predicted (n = 10) 70 ± 6 (n = 6)</td>
<td>68 ± 6 (n = 4)</td>
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</tr>
<tr>
<td>Smokers</td>
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</tr>
<tr>
<td>Ex-smokers</td>
<td>8</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Therapy</td>
<td>Inhaled β-adrenergics 8</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Theophylline 0</td>
<td>0</td>
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<td></td>
<td>Inhaled steroids 0</td>
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<td>0</td>
</tr>
<tr>
<td></td>
<td>Oral steroids 5</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

*Values are given as the mean ± SEM or No. NA = not applicable.

rather than the potentially explosive dimethylether.11,22 The subjects were sitting in front of a valve system initially inhaling (through a mouthpiece with nose clips on) room air and then a gas mixture from a plastic (Teflon; DuPont; Wilmington, DE) bag containing 35% O₂, 0.3% acetylene, 3% sulfur hexafluoride, and a balance of nitrogen. The subjects inhaled into a mass spectrometer at a constant exhalation flow rate (5 to 6 L/min). During exhalation, the concentration of acetylene and sulfur hexafluoride were measured directly online by a mass spectrometer; the exhaled gas volume was also measured. The anatomic dead space (DS) was calculated using the Fowler method. QAW was calculated from the Fick principle (ie, dilution of acetylene concentration) in the anatomic DS, with the area under the curve being inversely proportional to the QAW. QAW was expressed in microliters per milliliter per minute, representing the volume of blood per volume of DS per time. The effect size was chosen based on our previous experience in the measurement of the same variables.3

Statistical Analysis

Comparisons between groups were made by two-way analysis of variance, and data were expressed as the mean ± SEM and confidence intervals of differences. Significance was defined as a p value of < 0.05.

For a magnitude of the hypothesized effect of 60 and a power of 80%, the number of subjects to be included in this study was 16. The effect size was chosen based on our previous experience in the measurement of the same variables.3

Study Design

The study was approved by the Brompton and Harefield NHS Trust Ethics Committee. After a clinical examination was carried out, QAW and FENO were measured after at least 30 min of rest in the laboratory. This was followed by spirometry. The experiments were carried out before the administration of the morning medications. Because we have previously shown3 that the vasodilating activity of β₂-agonists lasts only 20 to 30 min, it seems reasonable to assume that the vascular activity of the inhalers taken the night before was unremarkable at the time of the tests.

Five steroid-naive COPD subjects, and eight healthy volunteers agreed to have QAW measured after the inhalation of budesonide, 400 μg, or placebo, which were administered in three different visits. The measurements were repeated at 30 min, 1 h, and 2 h after budesonide and placebo inhalation; at baseline and every 10 min for half an hour after the albuterol inhalation; and then at 1 h. The time points for the measurements were based on our previous experience with the vascular activity of inhaled corticosteroids.3

Results

QAW

QAW was not significantly different in patients with COPD (34.29 ± 1.09 μL/mL/min) compared to that in healthy subjects (35.50 ± 1.74 μL/mL/min;
p > 0.05). In addition, QAW was not affected by long-term treatment with corticosteroids (treated patients, 35.89 ± 1.63; untreated patients, 32.50 ± 1.24 μL/mL/min; p < 0.05) [Fig 1]. QAW was not correlated with airway obstruction as assessed by FEV₁; however, there was a positive correlation with TLCO (r = 0.74; p < 0.05) [see Fig 3, left, A].

**Effect of Albuterol**

Ten minutes after the inhalation of 200 μg of albuterol, QAW was significantly elevated from baseline both in healthy control subjects (59.33 ± 2.40 μL/mL/min) and COPD patients (38.00 ± 0.58 μL/mL/min; p < 0.05). This increase was significantly higher and lasted for a longer time in healthy subjects (50 min) compared to COPD patients (10 min) [Fig 2, left, A]. The response to albuterol was not significantly different in patients receiving long-term treatment with inhaled corticosteroids compared to steroid-naïve patients.

**Effect of Budesonide**

QAW was unchanged after the inhalation of 400 μg of budesonide at every time point. There were no significant differences between COPD patients and healthy subjects (Fig 2, right, B).

**FENO**

FENO levels were similarly elevated in steroid-treated patients (10.89 ± 0.87 ppb) and untreated patients (9.40 ± 0.86 ppb) compared to the control subjects (8.22 ± 0.57 ppb; p < 0.05) and were correlated with the levels of QAW (r = 0.6; p < 0.05) [Fig 3, right, B]. There was a tendency for higher exhaled FENO concentrations in patients with a more relevant smoking history expressed in pack-years (r = 0.4; p > 0.05), but this was not significant.

**DISCUSSION**

We have demonstrated that patients with COPD have a similar QAW compared to healthy subjects and reduced vascular reactivity. Furthermore, for the first time we found a positive correlation between QAW and FENO. We confirm that patients with COPD have an abnormal bronchial vascular reactivity that is steroid-resistant, and we suggest that treatment with NO may have a role in the regulation of QAW.

QAW was measured using the acetylene dilution method, as previously shown.⁵ This method is non-invasive, whereas the use of microspheres, which is considered the “gold standard” for the measurement of blood flow, is invasive and presents limitations such as the recirculation of the radioactive spheres.

Contrary to what has been shown in asthma patients in whom QAW levels are increased,⁶,⁷ patients with COPD have normal QAW levels. This may reflect the normal vascularity of the bronchial vessels⁴ and the low blood supply caused by intimal proliferation and hyperplasia.⁵ Initial studies by Cudkowicz,⁶ who performed the first postmortem bronchial arteriograms in 18 cases of emphysema, observed narrowing or obliteration of the intrapulmonary bronchial arteries with diminished pleural branches. In patients with chronic bronchitis, microscopic examination revealed medial hyperplasia and intimal proliferation of varying severity. These results are in keeping with those from our previous investigations²⁴ showing reduced Δe°Ts of the exhaled breath of COPD patients. This may be due to the normal or reduced vascularity of the airways and the blunted vascular reactivity.⁹ More specifically, we suggest that despite the presence of airway inflammation in COPD patients, the bronchial vessels, which are reduced or normal in number, do not respond to the inflammatory vasodilating stimuli.

QAW may affect heat exchange in the airways and ΔeTs, as indicated by the positive correlation between these two parameters and the response to vasoactive medications.⁵ However, heat exchange in the bronchial tree may be also affected by other variables, including the presence of mucus and the thickness of the bronchial wall. This may explain the finding of low ΔeT levels and normal QAW levels in COPD patients.

It has been well-established by *in vitro* and *in vivo* studies that β₂-adrenergic agonists cause vasodilation predominantly by endothelial NO synthase ac-
tivation and NO release; β2-adrenergic agonists are commonly used to investigate endothelium-dependent vasodilation.9 As previously shown for Δe°T,5 QAW levels were also increased in steroid-naive COPD patients after the inhalation of salbutamol, a known bronchial vasodilator.25 This differs from what we found in asthma patients, in whom the inhalation of salbutamol did not affect Δe°T or QAW,5 probably because in patients with asthma the vessels are already maximally dilated by inflammatory mediators and the QAW cannot be further increased.25 The finding of a lower post-β2-adrenergic agonist therapy QAW increase in COPD patients compared to healthy subjects supports the hypothesis that steroid-naive COPD patients have normal or reduced vascularity of the airways and blunted vaso-reactivity. This may be due to endothelial dysfunction resulting in abnormal relaxation responses, which have been demonstrated in the brachial, carotid, and coronary arteries, suggesting the presence of systemic vascular dysfunction.8 Further studies are required to investigate the short-term effect of albuterol inhalation in COPD patients who have received long-term treatment with corticosteroids, which may restore the vascular sensitivity to β2-adrenergic agonists.9

In this cross-sectional study, QAW was not different in corticosteroid-treated compared to untreated COPD patients, despite previous reports26 of steroids reducing QAW levels22 when used at the same doses as in our study. This is consistent with a previous study5 published by our group showing that steroid-treated asthmatic patients have similar Δe°Ts compared to untreated patients. As with long-term treatment with corticosteroids, the short-term inhalation of budesonide was equally ineffective in lowering QAW, which remained unchanged in COPD patients and healthy subjects. Even though a dose-response study would be required to confirm this finding, it is interesting that the administration of budesonide at the same dose effectively reduced QAW in asthmatic patients26 but not in our group of COPD patients. One hypothesis is that in our COPD patients the vasoconstrictive action of inhaled corticosteroids may have been balanced by β2-agonist-induced vasodilatation, resulting in minimal changes in bronchial artery diameter and blood flow, and therefore in no changes of QAW and Δe°T. Taken together, the insensitivity of the bronchial circulation of COPD patients to the inhalation of salbutamol and budesonide is confirmation that this group of patients has reduced vascular reactivity.

This is the first study in which FENO and QAW were measured in the same group of COPD patients. Elevated levels of FENO in patients with asthma27 and in those with interstitial lung disease28 are likely to be due to the activation of the inducible form of NO synthase and therefore may reflect airway inflammation. Even though in COPD patients the measurement of exhaled FENO as a noninvasive marker of inflammation has previously been investigated5 and criticized, elevated NO levels correlate with an increased bronchodilator response and steroid responsiveness, as shown by Papi et al18 and therefore may, to some extent, reflect airway inflammation.18,29–32 In the present study, FENO levels were marginally elevated in COPD patients and were not influenced by steroid treatment, which is in accordance with our previous data.33 This is consistent with the finding that inflammation in COPD

Figure 2. Short-term effect of budesonide inhalation (400 μg) [left, A] and albuterol (200 μg) [right, B] on QAW levels in healthy subjects (□) and in patients with COPD (●).
patients is not suppressed by inhaled or oral corticosteroids, even at high doses. For the first time, we were able to show a positive correlation between FENO and QAW. Even though NO may not be a reliable marker of airway inflammation in COPD patients, this gas is a potent vasodilator and may play a role in the regulation of bronchial vasomotor tone. This is confirmed by the correlation between FENO and QAW, which shows that patients with higher levels of NO may have bronchial vasodilatation and an increased QAW. Unfortunately, in the current study, we were unable to establish the contribution of NO produced by the bronchial vasculature compared to the pulmonary circulation. In this study, FENO was not measured after the inhalation of albuterol or budesonide. However, there is evidence that catecholamines may have an anti-inflammatory effect through the NO pathway and we have previously shown that therapy with inhaled corticosteroids reduces the levels of FENO and causes vasoconstriction in asthmatic patients but not in COPD patients, which is in keeping with the current findings.

In COPD patients, the final QAW may depend on the balance between proinflammatory mediators causing vasodilation and the reduced vascularity associated with the progression of the disease. It is noteworthy that even though the patients enrolled in this study were predominantly sputum producers, as shown by the low degree of emphysema (as assessed by CT scan and TLco), their QAW levels were normal. We assume that this may be due to bronchial tissue remodeling in COPD patients, which, in contrast with the situation in asthma patients, does not include a relevant proliferation of the vessels. Interestingly, patients with lower TLco values had lower levels of QAW, possibly as a result of parenchymal and therefore vascular vessel loss. This is the first time that a correlation between QAW and TLco has been reported; we think that this is of interest because it provides an insight into the relationship between the extent of lung parenchymal loss and QAW, which had never been studied before.

We were able to confirm that patients with COPD do not have increased vascularity of the airways despite the presence of chronic airway inflammation; in addition, these patients have an abnormal bronchial vascular reactivity that is resistant to $\beta_2$-adrenergic agonist and corticosteroid treatment. We also showed for the first time that NO may play a role in the regulation of QAW and that this may be due to the vasodilating capabilities of this gas.

Prospective studies are necessary to investigate the association of QAW, disease progression, and exacerbation. Further studies are necessary to investigate the correlation of these new measurements with other markers of inflammation in exhaled breath condensate and induced sputum samples, and their clinical utility in the follow-up of patients with COPD.

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