Dose-dependent onset and cessation of action of inhaled budesonide on exhaled nitric oxide and symptoms in mild asthma

S A Kharitonov, L E Donnelly, P Montuschi, M Corradi, J V Collins, P J Barnes

Background: Dose dependent anti-inflammatory effects of inhaled corticosteroids in asthma are difficult to demonstrate in clinical practice. The anti-inflammatory effect of low dose inhaled budesonide on non-invasive exhaled markers of inflammation and oxidative stress were assessed in patients with mild asthma.

Methods: 28 patients entered a double blind, placebo controlled, parallel group study and were randomly given either 100 or 400 µg budesonide or placebo once daily, inhaled from a dry powder inhaler (Turbohaler), for 3 weeks followed by 1 week without treatment. Exhaled nitric oxide (NO), exhaled carbon monoxide (CO), nitrite/nitrate, S-nitrosothiols, and 8-isoprostanes in exhaled breath condensate were measured four times during weeks 1 and 4, and once a week during weeks 2 and 3.

Results: A dose-dependent speed of onset and cessation of action of budesonide was seen on exhaled NO and asthma symptoms. Treatment with 400 µg/day reduced exhaled NO faster (–2.06 (0.37) ppb/day) than 100 µg/day (–0.51 (0.35) ppb/day; p<0.01). The mean difference between the effect of 100 and 400 µg budesonide was –1.55 ppb/day (95% CI –2.50 to –0.60). Pretreatment NO levels were positively related to the subsequent speed of reduction during the first 3–5 days of treatment. Faster recovery of exhaled NO was seen after stopping treatment with budesonide 400 µg/day (1.89 (1.43) ppb/day) than 100 µg/day (0.49 (0.34) ppb/day, p<0.01). The mean difference between the effect of 100 and 400 µg budesonide was 1.40 ppb/day (95% CI –0.49 to 2.31).

Conclusion: Measurement of exhaled NO levels can indicate a dose-dependent onset and cessation of anti-inflammatory action of inhaled corticosteroids in patients with mild asthma.
Table 1  Onset of action of inhaled budesonide on exhaled markers, lung function, and symptoms in patients with mild asthma

<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>NO (ppb)</th>
<th>CO (ppm)</th>
<th>NO2/NO (µM)</th>
<th>S-NO (µM)</th>
<th>8-iso (pg/ml)</th>
<th>FEV1 (% pred)</th>
<th>PEF (l/min)</th>
<th>Symptom score (units)</th>
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<tbody>
<tr>
<td><strong>Budesonide 400 µg/day</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0</td>
<td>20.8</td>
<td>7.5</td>
<td>2.6</td>
<td>57.4</td>
<td>1.0</td>
<td>10.4</td>
<td>90</td>
<td>460 (392 to 528)</td>
</tr>
<tr>
<td>3</td>
<td>15.5</td>
<td>5.0</td>
<td>3.0</td>
<td>48.3</td>
<td>0.5</td>
<td>8.4</td>
<td>95</td>
<td>467 (398 to 537)</td>
</tr>
<tr>
<td>5</td>
<td>12.9</td>
<td>2.5</td>
<td>2.7</td>
<td>39.2</td>
<td>0.4</td>
<td>8.0</td>
<td>95</td>
<td>471 (403 to 539)</td>
</tr>
<tr>
<td>7</td>
<td>13.5**</td>
<td>10.0</td>
<td>9.0</td>
<td>36.2</td>
<td>0.6**</td>
<td>11.9</td>
<td>92</td>
<td>468 (390 to 546)</td>
</tr>
<tr>
<td>14</td>
<td>14.3**</td>
<td>10.0</td>
<td>10.2</td>
<td>41.2**</td>
<td>0.1**</td>
<td>10.7</td>
<td>94</td>
<td>481 (421 to 540)</td>
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<tr>
<td>21</td>
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<td>8.5</td>
<td>8.0</td>
<td>45.2</td>
<td>0.5**</td>
<td>7.2</td>
<td>94</td>
<td>449 (387 to 510)</td>
</tr>
<tr>
<td><strong>Budesonide 100 µg/day</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>5.0</td>
<td>3.4</td>
<td>51.4</td>
<td>0.9</td>
<td>11.6</td>
<td>94</td>
<td>522 (480 to 564)</td>
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<td>3.3</td>
<td>41.9</td>
<td>0.9</td>
<td>12.6</td>
<td>93</td>
<td>500 (448 to 559)</td>
</tr>
<tr>
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<td>3.3</td>
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<td>9.1</td>
<td>92</td>
<td>507 (459 to 556)</td>
</tr>
<tr>
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<td>10.0</td>
<td>2.8</td>
<td>38.2</td>
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<td>10.7</td>
<td>95</td>
<td>517 (473 to 560)</td>
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<tr>
<td>14</td>
<td>12.4**</td>
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<td>34.2</td>
<td>1.0</td>
<td>9.4</td>
<td>93</td>
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<td><strong>Placebo</strong></td>
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<td>2.8</td>
<td>43.5</td>
<td>1.0</td>
<td>10.7</td>
<td>93</td>
<td>502 (455 to 549)</td>
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</table>

NO = nitric oxide; CO = carbon monoxide; NO2 = nitrate; NO3 = nitrite; SNO = nitrosi-thiols; 8-iso = 8-isoprostane; FEV1 = forced expiratory volume in 1 second; PEF = peak expiratory flow. Data are mean (95% CI). * p < 0.05, ** p ≤ 0.01 v pretreatment baseline (day 0) value.

**Outcomes and measures**

Exhaled NO was measured. The primary outcome was magnitude and direction of maximal changes of exhaled NO, as a marker of bronchial inflammation. Secondary outcomes included daily symptom scores, lung function, and exhaled NO and CO levels. Exhaled NO was measured 1 hour after the first dose of either 100 µg once a day (days 1, 3, 5, and 7) and at the end of the last week of no treatment. In addition, weekly symptom scores were assessed 1 hour after the first dose of both 100 µg once a day (days 1, 3, 5, and 7) and at the end of the last week of no treatment. At all visits measurements of 8-isoprostane and 8-iso levels were collected on days 1, 3, 5, and 7.

**Study design**

This was a single-blind, parallel group, placebo-controlled, randomized trial. The study ran for 2 weeks. Placebo treatment was continued for 2 weeks. At the end of weeks 2 and 3, all patients received 100 µg budesonide once a day and group 2 received 100 µg budesonide once a day and group 3 received placebo once a day in the morning via a dry powder inhaler (Turbohaler) 2 weeks apart. Placebo was added to regular treatment with inhaled or oral corticosteroids and those patients received an additional 2 hours of bronchodilator therapy 4 hours before any assessment. Patients not taking inhaled corticosteroids did not consume any bronchodilators for 6 hours before any assessment.
clips. Condensate was stored at –70°C in a 2 ml sterile plastic tube. The levels of both nitrite and nitrate in the condensate were measured according to the method of Mishko et al.15 using a fluorimeter (Labtech, Uckfield, UK), as previously described.16 Nitrosothiols were assessed using the Oxonon nitrosothiol detection kit (Alexis Biochemicals, Nottingham, UK), and 8-isoprostanate by a specific enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, USA), as previously described.14 16

Lung function

FEV1 was measured with a dry spirometer (Vitalograph-S, Vitalograph Ltd, Buckingham, UK). The best value of three morning and evening peak flow (PEF) measurements was determined and expressed as a percentage of the predicted value. The highest of the three morning and evening peak flows was expressed as a percentage of the predicted value. The primary objective of the study was to assess the onset and duration of anti-inflammatory action of budesonide (100 µg or 400 µg once daily) vs placebo. The primary variable for this comparison was therefore the speed of changes in exhaled NO, a marker of airway inflammation. There were several secondary variables including lung function parameters, markers in exhaled breath condensate, and asthma symptoms.

To compare the speed of onset and duration of action of three different treatments, we have assessed the magnitude and the direction of maximal positive or negative changes between day 1 and days 3 or 5 in exhaled NO and CO, NO2/NOx in exhaled breath condensate, and FEV1 and PEF during the first week of treatment and during the last week of the study when treatment was stopped (changes between day 21 and days 24 or 26). For example, with maximal exhaled NO reduction of 8 ppb (from 18 ppb at day 1 to 10 ppb at day 5), the calculated speed of NO changes was –1.6 ppb/day (–8 ppb divided by 5 days). If exhaled NO increased from 10 ppb (day 21) to 18 ppb (day 26), the calculated speed was +1.6 ppb/day (+8 ppb divided by 5 days).

It allowed us to look not only at the direction of fast changes in inflammatory markers, symptoms and lung function, but also to express the data individually in terms of speed as a summary measure, and therefore to compare speed of onset or cessation of action of three different treatments by direct statistical comparison (two tailed unpaired t test). Slower more “chronic” changes of inflammatory markers in exhaled air, condensate, lung function, and symptoms were assessed in a similar fashion by comparing their maximal positive or negative changes after 7, 14, and 21 days of treatment with the levels before treatment.

The comparison between treatment groups to determine whether the maximum effect is similar and happens at the same time, or whether the effect is different, dose-dependent, and happens at different time points, was made by two tailed unpaired t test for the two groups. Matthews17 has described a similar approach to the analysis of serial measurements in medical research.

Because of the variability of exhaled NO measurements in the study patients (n=28, mean (SD) 18.2 (6.1) ppb (95% CI 15.8 to 20.5)), we calculated that the sample size of 11 would have 70% power to detect a difference in mean values of 35% (6.36 ppb). Similar calculations were made for exhaled NO2/NOx (mean (SD) 52.86 (14.18) µM (95% CI 47.36 to 58.36) measurements (80% power to detect a difference in means of 35% (18.5 µM)); exhaled 8-isosprostanate (mean (SD) 13.01 (4.47) pg/ml (95% CI 11.28 to 14.75) measurements (60% power to detect a difference in means of 35% (4.55 pg/ml)); exhaled CO (mean (SD) 2.89 (0.79) ppm (95% CI 2.41 to 3.37).
**RESULTS**

**Exhaled gases**

Nitric oxide and carbon monoxide

Baseline exhaled NO values were similar in all groups (table 1). The onset of action of inhaled budesonide on exhaled NO was dose-dependent, both within the initial phase (first 3–5 days of treatment) and during treatment weeks 1, 2, and 3 (fig 1A). The reduction in exhaled NO during the first 3–5 days was thus significantly faster in the group receiving 400 µg/day budesonide (–2.06 (0.37) ppb/day) than in the group receiving 100 µg/day (–0.51 (0.35) ppb/day; p<0.01) or in the placebo group (–0.89 (0.87) ppb/day). The mean difference between the effect of 100 and 400 µg budesonide was –1.55 ppb/day (95% CI –2.50 to –0.60). The speed of the reduction in exhaled NO during the following 3 weeks of treatment was also dose-dependent, but slower. There was a significant difference between the effect of 400 µg/day (–0.90 (0.13) ppb/day) and 100 µg/day (–0.54 (0.08) ppb/day; p<0.05). The mean difference between the effect of 100 and 400 µg budesonide was –0.36 ppb/day (95% CI –0.53 to –0.18). The effect of both doses was also different from placebo (0.26 (0.32) ppb/day; p<0.01 and p<0.01, respectively).

Recovery of exhaled NO levels back to pretreatment levels after treatment was stopped was faster in patients treated with 400 µg/day budesonide (1.89 (1.43) ppb/day) than 100 µg/day (0.49 (0.34) ppb/day, p<0.01; fig 1B). The mean difference between the effect of 100 and 400 µg budesonide was 1.40 ppb/day (95% CI –0.49 to 2.31). Full recovery of the exhaled NO levels in both groups of patients treated with budesonide was completed by the end of the first week of no treatment (table 2).

There was a weak but significant positive correlation between the pretreatment levels of exhaled NO and the speed of subsequent NO changes during the initial phase (first 3–5 days) of treatment with both doses of budesonide (fig 1A). This correlation became stronger in patients treated with the higher dose of budesonide during weeks 1, 2, and 3 of treatment, but was lost in the group treated with 100 µg budesonide. No correlation was seen between the “achieved” exhaled NO levels (at the end of week 3 of treatment) and the speed of the subsequent NO recovery during the last week of the study with no treatment (fig 1D).

There was no effect of either treatment or placebo on exhaled levels of CO, either during the onset or cessation of their action (tables 1 and 2). No changes compared with baseline were seen in either exhaled NO or CO levels 3 hours (23.0 (3.8) ppb and 3.0 (0.26) ppm, respectively, p=NS) or 6 hours (22.9 (3.33) ppb and 2.7 (0.35) ppm, respectively, p=NS) after the first dose of 400 µg budesonide; 100 µg budesonide also had no effect: NO (18.3 (1.48) ppb at 3 hours and 18.2 (1.69) ppb at 6 hours, p=NS); CO (3.0 (0.43) ppm at 3 hours and 3.4 (0.22) ppm at 6 hours, p=NS). Placebo also had no effect (data not shown).
Dose-dependent effects of inhaled budesonide in mild asthma

NO₂ were not dose-dependent. There was a further reduction in µM/day dose of budesonide caused a faster reduction in NO₂/NO₃ levels was achieved by the end of the first week of treatment. The lower levels in exhaled breath condensate during weeks 1, 2, and 3 of treatment. The mean difference between the effect of 100 and 400 µg/day budesonide was –0.83 µM/day (95% CI –1.66 to 0.01). However, the effect of budesonide was not different from placebo (–0.23 (0.44) µM/day). A significant reduction in exhaled S-nitrosothiols compared with pretreatment levels was only seen at the end of treatment weeks 1 and 3 with the higher dose of budesonide (table 1).

**Lung function and symptom score**

Lung function (FEV₁, and PEF) did not change significantly in any of the patient groups (tables 1 and 2, fig 3A). Although most of the patients had mild and occasional asthma

**Exhaled breath condensate**

NO₂/NO₃

There was rapid reduction in NO₂/NO₃ in exhaled breath condensate (within 3–5 days) following treatment with budesonide at 400 µg/day (–4.82 (0.99) µM/day; p<0.05) and 100 µg/day (–3.55 (1.14) µM/day; p<0.05; fig 2A). These changes, however, were not dose-dependent. There was a further reduction in NO₂/NO₃ during weeks 1, 2, and 3 of treatment. The lower dose of budesonide caused a faster reduction in NO₂/NO₃ levels (–1.73 (0.44) µM/day) than the higher 400 µg/day dose (–0.82 (0.12) µM/day; p<0.05; fig 2B). The mean difference between the effect of 100 and 400 µg budesonide was 0.91 µM/day (95% CI 0.11 to 1.71).

Recovery of NO₂/NO₃ levels in exhaled breath condensate was significantly faster in both the group given budesonide 400 µg/day (4.03 (2.07) µM/day, p<0.05) and those treated with 100 µg/day (1.70 (1.79) µM/day, p<0.05) compared with placebo (–4.93 (1.39) µM/day). A complete recovery of NO₂/NO₃ levels was achieved by the end of the first week of no treatment (table 2).

**Exhaled 8-isoprostane**

No changes in exhaled 8-isoprostanes were seen at any period of the study in either of the groups (tables 1 and 2).

**S-nitrosothiols**

Significantly faster reduction in S-nitrosothiols in exhaled breath condensate was seen in patients treated with 400 µg/day budesonide (–0.67 (0.20) µM/day) than in those treated with 100 µg/day (0.08 (0.24) µM/day, p<0.05). The mean difference between the effect of 100 and 400 µg budesonide was –0.83 µM/day (95% CI –1.66 to 0.01). However, the effect of budesonide was not different from placebo (–0.23 (0.44) µM/day). A significant reduction in exhaled S-nitrosothiols compared with pretreatment levels was only seen at the end of treatment weeks 1 and 3 with the higher dose of budesonide (table 1).

![Figure 2](image-url) Speed of change in nitrite/nitrate in exhaled breath condensate during (A, B) the onset and (C) the cessation of action of budesonide 100 µg/day or 400 µg/day or placebo.

![Figure 3](image-url) Speed of change in peak expiratory flow (PEF) during the onset (A) and cessation (C) of action of 100 µg/day budesonide, 400 µg/day budesonide, and placebo. Speed of change in symptom score during the onset (B) and cessation (D) of action of 100 µg/day or 400 µg/day budesonide or placebo.
symptoms (table 1), a significant dose-dependent difference was seen in the speed of reduction of symptom scores between the groups: 400 µg/day budesonide (−0.1 (0.05) units/day); 100 µg/day budesonide (0.11 (0.05) units/day; p<0.05). The mean difference between the effect of 100 and 400 µg budesonide was −0.16 units/day (95% CI −0.36 to 0.03). The effect of the higher dose of budesonide was also significantly different from placebo (0.28 (0.14) units/day; p<0.05). All of the symptomatic patients became symptom free between weeks 1 and 3 of treatment with 400 µg/day, although two patients treated with the lower dose of budesonide and two in the placebo group became slightly more symptomatic towards the end of the treatment period (fig 3B).

Patients treated with the lower dose of budesonide had a further reduction in their asthma symptoms after treatment was stopped (−0.15 (0.07) units/day). In contrast, patients treated with 400 µg/day became more symptomatic shortly (within 3–5 days) after treatment was stopped (0.10 (0.06) units/day, p<0.05), although by the end of the last week of the study most of the patients in all groups had fewer symptoms than before the study (table 2). The mean difference between the effect of 100 and 400 µg budesonide was −0.15 units/day (95% CI −0.30 to 0.01).

**DISCUSSION**

This is the first study to show the dose-dependent onset and cessation of anti-inflammatory action of inhaled corticosteroids on exhaled NO and asthma symptoms in patients with mild asthma. We have also shown that higher pretreatment levels of exhaled NO were related to the faster onset of action of budesonide. The speed of recovery of exhaled NO levels and return of asthma symptoms after treatment was stopped was similar to the speed of onset of the anti-inflammatory action of steroids. There were no changes in exhaled levels of NO and CO 2 and 6 hours after a single dose of either 100 or 400 µg budesonide. Rapid reduction and recovery of nitrate/nitrite in exhaled breath condensate during the onset and cessation of action of budesonide was not dose-dependent, and a significant reduction in S-nitrosothiols in the exhaled breath condensate was seen only in patients treated with the higher dose of budesonide. There were no changes in exhaled CO levels, lung function, or 8-isoprostane levels in exhaled breath condensate during the study.

**Exhaled NO**

It is difficult to show a dose-dependent effect of inhaled corticosteroids in clinical studies. A large number of patients (n=473) may be required, as well as a long treatment period (12 weeks) with a wide range of doses (200 v 400 v 800 v 1600 µg/day budesonide) to demonstrate a statistically significant dose-response effect on lung function (FEV 1 and PEF). If more direct measurements of airway inflammation are used such as changes in bronchial reactivity after allergen challenge, the number of patients required may be smaller (n=26) and the treatment period with similar doses of steroids may be shorter (3 weeks), but the dose-related effect will only be seen between the steroids and placebo and not between the doses. If bronchial reactivity and sputum cosinophilia following an allergen challenge are used as outcome measurements, the dose-dependent effect of low and high doses (100 v 200 v 800 µg/day) of mometasone furoate can be seen in a smaller group of patients (n=12) treated for a shorter time (6 days).

The problem is that neither of the above approaches resembles the clinical situation. Furthermore, these approaches could not easily be used either in hospital or primary care to adjust and to monitor treatment with corticosteroids.

We have shown that the acute (within 3–5 days of treatment) and chronic (7–21 days) reduction in exhaled NO is dose-dependent in patients with mild asthma treated with low doses of budesonide. Serial exhaled NO measurements, as we recently suggested,1 may therefore be of use to study the onset and duration of action of inhaled corticosteroids and patient compliance. It is still unclear whether exhaled NO levels can be used to study the effect of higher doses of inhaled corticosteroids. We did not find any further reduction in NO levels in patients with mild asthma treated with 1600 µg/day budesonide for 3 weeks, although in a separate study a dose-dependent reduction in NO levels was found in patients treated with 100 and 400 µg/day budesonide for 3 weeks.2

The onset of action of steroids on exhaled NO may depend not only on the dose, but also on asthma severity and the route of dose administration. For example, oral prednisolone (30 mg/day) reduced exhaled NO levels by 22% within 72 hours in mild asthmatics,3 but higher doses (180–500 mg) caused a 36% reduction within 50 hours in patients with acute severe asthma.4 All of our patients were mild asthmatics, randomly selected for treatment, and none of the baseline parameters differed significantly between the groups. Differences in the reduction in exhaled NO and faster symptomatic improvement between the groups can therefore be attributed to the dose-dependent effect of the treatment rather than to differences in asthma severity.

Exhaled NO levels may be reduced very rapidly—for example, within 6 hours after a single high dose (8 mg) of nebulised budesonide in patients with moderate symptomatic asthma.5 However, we did not find any changes in either exhaled NO or CO levels 3 and 6 hours after a single dose of 100 or 400 µg budesonide. It is well established that exhaled NO will be gradually reduced during the first week of regular treatment with inhaled corticosteroids,6 with maximal reduction at 3 or 4 weeks.7

There are several possible explanations for the “fast” and “slow” reduction of exhaled NO levels by corticosteroids. For example, steroids may directly inhibit inducible NO synthase (iNOS).8 However, this does not appear to work in primary human airway epithelial cells.9 Indirect inhibition may be achieved by suppression of nuclear factor-κB (NF-κB)10 by inhibiting the release of proinflammatory cytokines such as tumour necrosis factor α (TNF-α) and interleukin (IL)-1β which activate iNOS.11 These mechanisms are fast. For example, NF-κB suppression may be seen within 30 minutes,12 leading to downregulation of iNOS activity and reduction of NO in exhaled air. Local interactions between NO and superoxide anions are also important factors as oxidative stress may be responsible for steroid resistance in severe asthma. We have shown that inhaled steroids reduce NO levels in exhaled breath and this is correlated with a reduction in nitrotyrosine, a stable product formed from the interaction between NO and superoxide anions, in bronchial biopsy specimens from patients with mild asthma.13

We have speculated that, by knowing the pretreatment exhaled NO levels and the dose of steroids, the speed of their effect may be estimated. Indeed, we have found a weak but significant correlation between the levels of exhaled NO before treatment and the subsequent speed of action of corticosteroids (fig 1B).

An important question is how fast exhaled NO levels take to recover when steroid treatment is stopped. Budesonide has a relatively low glucocorticoid receptor affinity and the half-life of active steroid receptor complexes is 5 hours.14 Exhaled NO levels in our study recovered rapidly during the first 3–5 days in all patients treated with budesonide, and the full recovery was completed by the end of the week off treatment. Interestingly, the faster recovery of exhaled NO levels in patients treated with 400 µg/day budesonide (fig 1B) was independent of the degree of the reduction in exhaled NO by the end of week 3 of treatment (fig 1D). The assumption that the effect of 400 µg/day budesonide would last longer if treatment was stopped does not therefore appear to be true.

We have previously suggested that exhaled NO levels can be used as a marker for loss of control of asthma15 because an
increase in NO levels and asthma symptoms may be seen before a deterioration in airway hyperresponsiveness and lung function or an increase in sputum eosinophils during an exacerbation of asthma. It has recently been shown that exhaled NO levels above 13 ppb have a sensitivity of 0.67 and a specificity of 0.65 to predict a step up in treatment. Interestingly, our patients treated with 100 µg/day budesonide were slightly more symptomatic by the end of week 3 of treatment and had higher levels of exhaled NO (13.4 ppb) than during the previous week of treatment (table 1). This may suggest that a step up in treatment would have been beneficial, but long term studies are needed using exhaled NO levels to direct treatment.

There is evidence that symptom driven dosing with combination inhalers (inhaled steroid and long acting β₂ agonists) may be used in the future when the dose of the steroid could be determined by the degree of symptoms at a particular time. We suggest that the high sensitivity of exhaled NO may be used to adjust doses based on control of inflammation in asthma treatment. This is important as the long acting β₂ agonist may control symptoms and therefore mask the underlying inflammation which may not be adequately suppressed by corticosteroids. Portable, simple, and inexpensive exhaled NO analysers (based on measurements other than the chemiluminescence principle of NO detection) could be available in the next few years, making this approach feasible in the future.

**Exhaled CO**

There were no significant changes in exhaled CO levels in any patients during the study except a reduction after 3 weeks of 400 µg/day budesonide which may be a reflection of a modest improvement in oxidative stress in asthma. It is likely that CO might be used as a practical marker to detect and to monitor exacerbations of asthma, but its use as a marker of airway inflammation in patients with mild asthma appears to be limited.

**Exhaled nitrite/nitrate and S-nitrosothiols**

The reduction in nitrite/nitrate levels in exhaled condensate during the onset and cessation of action of corticosteroids was also fast but was not dose-dependent. This may be a reflection of a rather complex process of nitrite/nitrate formation and metabolism, and the greater variability in the measurements compared with exhaled NO.

NO synthase activity is the major source of NO which reacts with superoxide anions (O₂⁻) to form peroxynitrite (ONOO⁻) and consequently 3-nitrotroline, which is present in asthmatic airways and is increased in exhaled breath condensate in patients with mild asthma. We have shown that there is a relationship between exhaled NO levels, INOS activity, and 3-nitrotyrosine in epithelial cells of asthmatic patients and their reduction after treatment with inhaled corticosteroids. The 3-nitrotyrosine levels in exhaled breath condensate were further increased after steroid withdrawal and subsequent exacerbation in patients with moderate/severe asthma.

Despite the attractive notion that NO synthase activity and ONOO⁻ formation may be the major sources of nitrite/nitrate formation and other mechanisms may also contribute. Thus, NO may be formed independently of enzymes from nitrite under acidic conditions or it may be generated by peroxidases such as eosinophil peroxidase (EPO) and myeloperoxidase (MPO) in the presence of nitrite and hydrogen peroxide. This association, which is found in asthma and other inflammatory disorders, is of importance because peroxidases such as MPO and EPO can efficiently generate nitrate proteins through mechanisms independent of ONOO⁻. EPO can use nitrite as a substrate to nitrate protein tyrosyl residues or to be an even more potent catalyst of 3-nitrotyrosine formation than MPO, raising the possibility that EPO may be responsible for 3-nitrotyrosine formation in asthma.

High levels of nitrate have been found in exhaled breath condensate and sputum of asthmatic patients, especially during acute exacerbations. Patients treated with low doses of budesonide had slightly higher exhaled NO levels by the end of the treatment period, which may be explained by a greater variability in the levels of nitrate/nitrite in exhaled breath condensate.

NO can react with sulphhydryl groups to yield S-nitrosothiols which play an intermediate role in cell metabolism or serve as a carrier for NO. A significant reduction in exhaled S-nitrosothiols compared with pretreatment levels was only seen at the end of weeks 1 and 3 of treatment with high dose budesonide. The speed of recovery of the S-nitrosothiols was similar to the changes in exhaled NO and nitrite/nitrate, suggesting that attenuation of NO production by steroids is the predominant mechanism for the changes in S-nitrosothiols.

**Exhaled 8-isoprostane**

F₂-isoprostanes are increased in plasma and bronchoalveolar lavage fluid of asthmatic patients, and levels of 8-isoprostane are approximately doubled in patients with mild asthma compared with normal subjects, and increased about threefold in those with severe asthma, irrespective of treatment with corticosteroids. This makes 8-isoprostane a useful marker of asthma severity, in contrast to exhaled NO, in stable well controlled asthmatics treated with steroids. The lack of effect of corticosteroids on exhaled 8-isoprostane in this study provides evidence that inhaled corticosteroids may not be very effective in reducing oxidative stress. Alternatively, the lack of effect of corticosteroids on inhibiting oxidative stress could be a function of their dose.

**Symptoms and lung function**

The advantage of exhaled NO measurements is that the changes in NO levels during steroid treatment tend to precede the improvement in symptoms, FEV₁, and sputum eosinophilia. We have shown that the speed of improvement in asthma symptoms was also dose-dependent. The higher dose of budesonide reduced NO levels and stopped night time asthma symptoms within 3 days in almost all patients. A simultaneous and modest improvement in FEV₁ did not show significant dose dependency.

The fact that patients treated with 100 µg/day budesonide were slightly more symptomatic by the end of week 3 of treatment and had exhaled NO levels higher than during the previous week of treatment (table 1) suggests that a step up in treatment may be beneficial for these patients, but more studies are needed using exhaled NO to direct treatment.

The recovery in asthma symptoms and in exhaled NO levels was faster in the group treated with 400 µg/day budesonide than in those treated with 100 µg/day. For both groups recovery of NO and asthma symptoms preceded the return of FEV₁ to the pretreatment level, giving further support to the possibility that exhaled NO may serve as a fast responding indicator of patient compliance with treatment.

**Conclusions**

We have shown a rapid and dose-dependent onset of the anti-inflammatory effect of inhaled budesonide on exhaled NO levels and symptoms in well controlled mild asthmatic patients. These positive effects of budesonide were associated with a reduction in nitrite/nitrate and S-nitrosothiols in exhaled breath condensate. We have also shown that the effects of inhaled budesonide in sustaining its anti-inflammatory effect after its discontinuation is of short duration. Measurement of exhaled NO levels, nitrite/nitrate, and S-nitrosothiols in exhaled breath condensate may provide a rational basis for dose finding studies of anti-inflammatory drugs and antioxidants, and are important end points in clinical trials of these drugs in asthma.

The changes in exhaled NO
levels and other markers may further have clinical relevance in assessing compliance and therapeutic response.

Exhaled NO may also be useful in patients using fixed combination inhalers (corticosteroids and long-acting β₂ agonist) to ensure that inflammation is controlled, as this may be difficult to assess from symptoms when a long-acting bronchodilator is used at the same time as an inhaled corticosteroid.

References

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