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). Symptom improvement was dose-dependent, although symptoms returned faster in patients treated with 400 µg/day. A significant reduction in exhaled nitrite/nitrate and S-nitrosothiols after budesonide treatment was not dose-dependent. There were no significant changes in exhaled CO or 8-isoprostanes in breath condensate.

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>NO&lt;sub&gt;2&lt;/sub&gt;/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>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>208 (15.3 to 26.3)</td>
<td>3.2 (2.6 to 3.9)</td>
<td>57 (47 to 62)</td>
<td>1.0 (0.52 to 1.54)</td>
<td>1.0 (0.4 to 1.43)</td>
<td>90 (83 to 96)</td>
<td>460 (392 to 528)</td>
<td>0.7 (0.12 to 1.34)</td>
</tr>
<tr>
<td>3</td>
<td>155.0 (10.4 to 20.6)</td>
<td>3.0 (2.1 to 4.0)</td>
<td>48 (35 to 62)</td>
<td>–</td>
<td>8.4 (0.95 to 15.9)</td>
<td>95 (88 to 102)</td>
<td>467 (398 to 528)</td>
<td>0.5 (0.2 to 0.9)</td>
</tr>
<tr>
<td>5</td>
<td>129.0 (9.1 to 16.7)</td>
<td>2.7 (2.2 to 3.2)</td>
<td>39 (25 to 53)</td>
<td>–</td>
<td>8.0 (6.0 to 10.1)</td>
<td>95 (89 to 100)</td>
<td>471 (402 to 539)</td>
<td>0.5 (0.1 to 1.17)</td>
</tr>
<tr>
<td>7</td>
<td>135.3 (9.0 to 16.7)</td>
<td>2.8 (2.1 to 3.0)</td>
<td>36* (20 to 53)</td>
<td>0.6 (0.35 to 0.84)</td>
<td>11.9 (5.7 to 18.2)</td>
<td>92 (83 to 101)</td>
<td>468 (390 to 546)</td>
<td>0.4 (0.02 to 0.70)</td>
</tr>
<tr>
<td>14</td>
<td>143.5 (10.2 to 18.4)</td>
<td>2.8 (1.9 to 3.7)</td>
<td>41* (26 to 55)</td>
<td>–</td>
<td>10.7 (6.8 to 14.9)</td>
<td>94 (86 to 101)</td>
<td>481 (421 to 540)</td>
<td>0.4 (0.09 to 0.82)</td>
</tr>
<tr>
<td>21</td>
<td>114.0 (8.5 to 14.3)</td>
<td>2.4* (1.6 to 3.3)</td>
<td>45 (26 to 54)</td>
<td>0.5* (0.27 to 0.75)</td>
<td>7.2 (3.9 to 10.5)</td>
<td>94 (84 to 105)</td>
<td>449 (387 to 510)</td>
<td>0.3 (0.16 to 0.71)</td>
</tr>
<tr>
<td>Budesonide 100 µg/day</td>
<td>0</td>
<td>17.3 (10.3 to 24.4)</td>
<td>3.1 (2.0 to 4.2)</td>
<td>43 (15 to 70)</td>
<td>1.0 (0.53 to 1.41)</td>
<td>94 (86 to 101)</td>
<td>511 (472 to 550)</td>
<td>0.1* (0.11 to 1.83)</td>
</tr>
<tr>
<td>Placebo</td>
<td>0</td>
<td>14.3 (11.4 to 17.2)</td>
<td>3.1 (2.0 to 4.1)</td>
<td>48 (33 to 63)</td>
<td>0.8 (0.10 to 1.73)</td>
<td>12.8 (11.6 to 23.9)</td>
<td>93 (81 to 105)</td>
<td>502 (455 to 549)</td>
</tr>
</tbody>
</table>

NO=nitric oxide; CO=carbon monoxide; NO<sub>2</sub>=nitrite; NO= nitrate; S-NO=S-nitrosothiols; 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.
Dose-dependent effects of inhaled budesonide in mild asthma

To compare the speed of onset and duration of action of three different treatments, we have assessed the magnitude and the direction of maximal negative or positive changes between day 1 and days 3 or 5 in exhaled NO and CO, NO\textsubscript{2}/NO\textsubscript{3} in exhaled breath condensate, and FEV\textsubscript{1} and PEF during the first week of treatment and during the last week of the study. The primary variable for this comparison was 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.

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. 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 negative or positive 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. Matthews\textsuperscript{5} 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% (18.5 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 negative or positive changes after 7, 14, and 21 days of treatment with the levels before treatment.

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Cessation of action of inhaled budesonide on exhaled markers, lung function, and symptoms in patients with mild asthma

Table 2  Cessation of action of inhaled budesonide on exhaled markers, lung function, and symptoms in patients with mild asthma

<table>
<thead>
<tr>
<th>Days</th>
<th>NO (ppb)</th>
<th>CO (ppm)</th>
<th>NO2/NO3− (µM)</th>
<th>8-iso (pg/ml)</th>
<th>FEV1 (% pred)</th>
<th>PEF (l/min)</th>
<th>Symptom score (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Budesonide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400 µg/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last day of treatment (day 21)</td>
<td>11.4 (8.5 to 14.3)</td>
<td>2.4 (1.6 to 3.3)</td>
<td>45 (26 to 54)</td>
<td>7.2 (3.9 to 10.5)</td>
<td>94 (84 to 105)</td>
<td>449 (387 to 510)</td>
<td>0.3 (-0.16 to 0.71)</td>
</tr>
<tr>
<td>No treatment (day 24)</td>
<td>15.9* (12.6 to 19.1)</td>
<td>2.82 ± 0.35</td>
<td>51 (41 to 62)</td>
<td>11.7 (2.1 to 21.3)</td>
<td>95 (84 to 106)</td>
<td>458 (401 to 515)</td>
<td>0.5 (0.19 to 0.90)</td>
</tr>
<tr>
<td>(day 26)</td>
<td>19.7** (16.6 to 22.7)</td>
<td>3.5* (2.6 to 4.3)</td>
<td>50 (35 to 65)</td>
<td>10.4 (5.4 to 15.4)</td>
<td>88 (77 to 100)</td>
<td>437 (366 to 508)</td>
<td>0.5 (-0.10 to 1.01)</td>
</tr>
<tr>
<td>(day 29)</td>
<td>18.4* (14.6 to 22.2)</td>
<td>3.1 (2.1 to 4.1)</td>
<td>56* (51 to 61)</td>
<td>9.9 (5.6 to 14.3)</td>
<td>90 (81 to 100)</td>
<td>451 (392 to 511)</td>
<td>0.3 (-0.04 to 0.59)</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last day of treatment (day 21)</td>
<td>9.5 (9.5 to 22.4)</td>
<td>2.9 (1.6 to 4.2)</td>
<td>53 (42 to 65)</td>
<td>14.7 (8.5 to 20.8)</td>
<td>88 (76 to 105)</td>
<td>487 (361 to 613)</td>
<td>0.02 (-0.03 to 0.06)</td>
</tr>
<tr>
<td>No treatment (day 24)</td>
<td>14.9 (9.5 to 20.3)</td>
<td>2.9 (1.6 to 4.2)</td>
<td>53 (42 to 65)</td>
<td>14.7 (8.5 to 20.8)</td>
<td>88 (76 to 105)</td>
<td>487 (361 to 613)</td>
<td>0.02 (-0.03 to 0.06)</td>
</tr>
<tr>
<td>(day 26)</td>
<td>15.2 (9.0 to 19.6)</td>
<td>3.0 (1.7 to 4.4)</td>
<td>38 (27 to 53)</td>
<td>11.3 (7.3 to 15.4)</td>
<td>92 (84 to 101)</td>
<td>510 (305 to 715)</td>
<td>0.02 (-0.03 to 0.06)</td>
</tr>
<tr>
<td>(day 29)</td>
<td>16.6 (9.9 to 22.3)</td>
<td>3.6 (2.6 to 4.5)</td>
<td>45 (32 to 77)</td>
<td>9.9 (2.9 to 16.8)</td>
<td>93 (88 to 98)</td>
<td>507 (457 to 556)</td>
<td>0.7 (0.05 to 1.41)</td>
</tr>
</tbody>
</table>

NO=nitric oxide; CO=carbon monoxide; NO2=nitrite; NO3−=nitrate; S-NO=S-nitrosothiols; 8-iso=8-isoprostane; FEV1=forced expiratory volume in 1 second; PEF=peak expiratory flow.

* p<0.05, ** p<0.01 vs treatment (day 21) value.

RESULTS

Exhaled gases

Nitric oxide

Baseline exhaled NO values were similar in all groups (table 1). The onset of action of inhaled budesonide was dose-dependent, both within the budesonide and placebo groups (fig 1A). The reduction in exhaled NO during the first 3–5 days of treatment was thus significantly faster in the group receiving 400 µg of budesonide than for the group receiving 100 µg (fig 1B). The mean difference between the reduction in exhaled NO in the group receiving 400 µg of 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 treatment (table 2). No change in the pretreatment levels of exhaled NO and the speed of the subsequent NO recovery during the last week of treatment (table 2).

There was no significant difference in the pretreatment levels of exhaled NO between the groups, with the baseline levels of exhaled NO and the speed of subsequent NO changes during the initial phase (first 3–5 days) being similar between the groups. No changes in the pretreatment levels of exhaled NO were seen in either exhaled NO or CO levels 3 hours after the first dose of budesonide (3.8 ppb and 3.0 µM, respectively, p<NS). There were no changes in the pretreatment levels of NO or CO between the baseline and the day of treatment, but was lost in the group treated with 100 µg of budesonide after 1.40 ppb/day, 100 µg (1.01 ppb/day; p<0.05). The mean difference between the effect of 100 and 400 µg of budesonide was also significantly different from placebo (0.26 (0.32) ppb/day; p<0.01, respectively).

Placebo also had no effect (data not shown).

The onset of action of inhaled budesonide on exhaled NO levels in both groups was dose-dependent, both within the budesonide and placebo groups (fig 1A). The reduction in exhaled NO during the last week of treatment (day 21) value.

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Placebo also had no effect (data not shown).
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<0.01) and 100 µg/day (−3.55 (1.14) µM/day; p<0.05) compared with placebo. A further 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) was seen at the end of treatment weeks 1 and 3 with the higher dose of budesonide (table 1). There was a significantly faster reduction in NO₂⁻/NO³⁻ levels in exhaled breath condensate during the onset (A, B) and cessation (C) of action of 100 µg/day or 400 µg/day budesonide or placebo.

**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).

**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

![Figure 2](http://thorax.bmj.com/)  
**Figure 2.** 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](http://thorax.bmj.com/)  
**Figure 3.** 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 3 and 6 hours after a single dose of either 100 or 400 µg budesonide. Rapid reduction and recovery of nitrite/nitrate 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 µg 400 µg 800 µg 1600 µg/day budesonide) to demonstrate a statistically significant dose-response effect on lung function (FEV1, 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 spumus eosinophilia following an allergen challenge are used as outcome measurements, the dose-dependent effect of low and high doses (100 µg 200 µg 800 µg/day) of montelukast 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 first 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, 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.

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 dosing. For example, oral prednisolone (30 mg/day) reduced exhaled NO levels by 22% within 72 hours in mild asthmatics, but higher doses (180–500 mg) caused a 36% reduction within 50 hours in patients with acute severe asthma. 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. 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, with maximal reduction at 3 or 4 weeks.

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). However, this does not appear to work in primary human airway epithelial cells. Indirect inhibition may be achieved by suppression of nuclear factor-κB (NF-κB) by inhibiting the release of proinflammatory cytokines such as tumour necrosis factor α (TNF-α) and interleukin (IL)-1β which activate iNOS. These mechanisms are fast. For example, NF-κB suppression may be seen within 30 minutes, 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.

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. 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 therefore appear to be true.

We have previously suggested that exhaled NO levels can be used as a marker for loss of control of asthma because...
increase in NO levels and asthma symptoms may be seen before a deterioration in airway hyperresponsiveness and lung function\(^a\) or an increase in sputum eosinophils\(^b\) 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.\(^c\) 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 β\(_2\) 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 β\(_2\) 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,\(^d\, e\) but its use as a marker of airway inflammation in patients with mild asthma appears to be limited.\(^f\)

**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 (O\(_2^-\)) to form peroxynitrite (ONOO\(^-\))\(^g\) and consequently 3-nitrotyrosine, which is present in asthmatic airways\(^h\) and is increased in exhaled breath condensate in patients with mild asthma.\(^i\) 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.\(^j\) 3-nitrotyrosine levels in exhaled breath condensate were further increased after steroid withdrawal and subsequent exacerbation in patients with moderate/severe asthma.\(^k\)

Despite the attractive notion that NO synthase activity and ONOO\(^-\) formation may be the major sources of nitrite/nitrate formation in exhaled breath condensate in patients with mild asthma,\(^l\) 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.\(^j\) 3-nitrotyrosine levels in exhaled breath condensate were further increased after steroid withdrawal and subsequent exacerbation in patients with moderate/severe asthma.\(^k\)

High levels of nitrite have been found in exhaled breath condensate\(^m\) and sputum\(^n\) of asthmatic patients, especially during acute exacerbations.\(^o\) 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 nitrite/nitrate in exhaled breath condensate.

NO can react with sulphydryl groups to yield S-nitrosothiols\(^p\) 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-isoprostanone**

8-isoprostanes are increased in plasma\(^q\) and bronchoalveolar lavage (BAL) fluid of asthmatic patients,\(^s\) and levels of 8-isoprostanone 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.\(^t\) This makes 8-isoprostanone 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-isoprostanone 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, FEV\(_1\), and sputum eosinophilia.\(^t\) 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 FEV\(_1\) 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 FEV\(_1\) 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.

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REFERENCES


