Effect of oral L-arginine on airway hyperresponsiveness to histamine in asthma

H W F M de Gouw, M B Verbruggen, I M Twiss, P J Sterk

Abstract

Background—Nitric oxide (NO) may exert protective properties within the airways of asthmatic patients. It was postulated that airways obstruction in asthma may be associated with endogenous NO deficiency caused by limited availability of NO synthase substrate.

Methods—In a double blind, crossover study 14 asthmatic patients received pretreatment with oral L-arginine (50 mg/kg body weight) or placebo prior to histamine challenge. Histamine challenge was performed until a 50% fall in forced expiratory volume in one second (FEV₁) occurred and the response was expressed as the provocative concentration causing a 20% fall in FEV₁ (PC₂₀) and as the dose–response slope (maximal % fall in FEV₁/cumulative dose (µmol)).

Results—Pretreatment with L-arginine did not affect PC₂₀ histamine (mean change in doubling dose 0.18 (95% confidence interval (CI) –0.36 to 0.71), p = 0.5) but the dose–response slope to histamine was slightly reduced (mean change: 0.7 (95% CI 0.6 to 0.9), p = 0.016).

Conclusions—Oral L-arginine does not influence airway hyperresponsiveness to histamine as reflected by PC₂₀, although the dose–response slope is slightly reduced in patients with asthma. This indicates only marginal, clinically unimportant limitation of NO synthase substrate in asthma.

Keywords: nitric oxide; asthma; airway hyperresponsiveness

Evidence is accumulating that endogenous nitric oxide (NO) is involved in the pathophysiology of asthma. NO is formed by NO synthase (NOS) using L-arginine as a substrate. It has been shown that the level of NO in exhaled air is increased in subjects with asthma, and that it varies with disease severity. However, the functional role of NO in the pathophysiology of asthma is still unclear.

Recent studies on the modulation of endogenous NO production have revealed protective features of NO during episodes of airways obstruction. Increased airway hyperresponsive-ness to non-sensitising stimuli has been observed following inhalation of NOS inhibitors in mildly asthmatic subjects, whereas this could not be found in patients with more severe asthma.

Based on these findings, it can be postulated that airways obstruction in asthma is associated with an endogenous NO deficiency due to limited availability of NOS substrate. We therefore examined the protective effect of oral supplementation of the natural substrate of NOS (L-arginine) on relatively mild and more severe histamine induced airways obstruction in patients with asthma.

Methods

The study subjects comprised 14 non-smoking, atopic, asthmatic patients using inhaled short acting β₂ agonists on demand only. They had episodic chest symptoms, their baseline forced expiratory volume in one second (FEV₁) ranged from 82% to 115% predicted, and all were hyperresponsive to inhaled histamine (provocative concentration causing a fall in FEV₁ of 20% (PC₂₀) 0.04–6.5 mg/ml). They were asked to refrain from using bronchodilators for at least eight hours before testing.

In a double blind, randomised, crossover study the patients received pretreatment with oral L-arginine or placebo on two separate days separated by an interval of 1–2 weeks. Exhaled NO, FEV₁, blood pressure, and heart rate were measured before (baseline) and at 30, 60, and 90 minutes following pretreatment, after which a histamine challenge was performed. Gelatin capsules containing L-arginine (50 mg/kg body weight; Bufa, Uitgeest, The Netherlands) or placebo (cellulose) were given 90 minutes before standardised histamine challenges using the two minute tidal breathing method. To examine the effects of L-arginine on mild as well as severe degrees of airways obstruction, the challenge test was continued until a 50% fall in FEV₁ from baseline was obtained or when the highest dose was reached. The response was expressed as PC₂₀ as a measure of airways sensitivity, whilst the dose-response slope was calculated (maximal % fall in FEV₁ maximal dose of histamine (µmol)) as a measure of airways reactivity.

Exhaled NO was measured using a chemiluminescence analyser (Sievers, Boulder, Colorado).
Table 1  Effect of pretreatment with placebo or L-arginine on FEV\(_1\), exhaled NO levels, PC_{20}, and dose-response slope to histamine

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>L-arginine</th>
<th>Difference between l-arginine and placebo‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FEV(_1) (% predicted)</strong></td>
<td>92.8 (85.8 to 99.9)</td>
<td>96.6 (87.9 to 105.4)</td>
<td>3.8% predicted (95% CI 0.9 to 6.6)†, p&lt;0.05</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td>95.1 (88.3 to 102.0)</td>
<td>95.3 (88.1 to 102.6)</td>
<td>−0.3 (−3.0 to 2.3)†, p&gt;0.2</td>
</tr>
<tr>
<td><strong>30 min</strong></td>
<td>95.4 (85.8 to 100.9)</td>
<td>93.3 (85.5 to 101.3)</td>
<td>−2.3% predicted (95% CI −3.6 to 0.7)†, p&gt;0.2</td>
</tr>
<tr>
<td><strong>60 min</strong></td>
<td>95.2 (87.9 to 102.5)</td>
<td>94.0 (87.2 to 105.0)</td>
<td>−1.3% predicted (95% CI −2.3 to 0.7)†, p&gt;0.2</td>
</tr>
<tr>
<td><strong>90 min</strong></td>
<td>17.4 (12.5 to 22.3)</td>
<td>19.9 (13.0 to 26.8)</td>
<td>2.4% predicted (95% CI 1.2 to 3.7)†, p&lt;0.05</td>
</tr>
<tr>
<td><strong>Exhaled NO (ppb)</strong></td>
<td>19.0 (13.9 to 24.2)</td>
<td>21.8 (14.8 to 28.7)</td>
<td>2.6% predicted (95% CI 1.6 to 3.7)†, p&lt;0.05</td>
</tr>
<tr>
<td><strong>30 min</strong></td>
<td>18.0 (14.0 to 22.0)</td>
<td>21.6 (14.6 to 28.5)</td>
<td>3.5% predicted (95% CI 2.5 to 4.5)†, p&lt;0.05</td>
</tr>
<tr>
<td><strong>60 min</strong></td>
<td>19.5 (15.5 to 23.5)</td>
<td>22.0 (15.5 to 28.3)</td>
<td>2.5% predicted (95% CI 1.5 to 3.5)†, p&lt;0.05</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td>7.9 (3.4 to 18.2)</td>
<td>10.8 (4.8 to 24.5)</td>
<td>2.5% predicted (95% CI 1.5 to 3.5)†, p&lt;0.05</td>
</tr>
</tbody>
</table>

FEV\(_1\) = forced expiratory volume in one second; ppb = parts per billion; PC\(_{20}\) = provocative concentration causing a 20% fall from baseline FEV\(_1\), DRS = dose-response slope.

This is the first study of the effects of L-arginine supplementation on upper airway hyperresponsiveness in patients with asthma. Previous animal studies have demonstrated the ability of L-arginine to reverse the enhanced bronchoconstriction to non-sensitising stimuli following inhibition of NO synthesis at the level of the NOS and to reduce contractility to histamine in vitro. In addition, they provided indirect evidence for an association between endogenous NO deficiency and the increase in airway sensitivity and reactivity following either respiratory virus infection or allergen exposure in guinea pigs. Our results do not confirm these findings, although a slight improvement in airway reactivity occurred following supplementation with oral L-arginine.

It is unlikely that our results can be explained by measurement errors since they were obtained using validated methodology for challenge testing and measurement of exhaled NO. The dose and timing of L-arginine pretreatment were based on a previous report which showed increased levels in exhaled NO two hours after ingestion of 50 mg/kg L-arginine in asthmatic subjects. In this study, however, we were not able to detect an increase in exhaled NO. Since our patients did not have an L-arginine deficient or weighted diet, we cannot exclude the possibility that these subjects had little or no NOS substrate limitation. Finally, since high concentrations of L-arginine have been shown to act as a non-competitive antagonist of the contractile effect of histamine in vitro, we cannot exclude the contribution of the anti-histamine effects of L-arginine to the present findings.

Besides having immunomodulatory and cytotoxic properties, NO is known to exert protective effects within the airways. This “relaxant” effect is likely to be derived from constitutive NOS activity (cNOS), of which neuronal NOS (nNOS) in inhibitory non-adrenergic non-cholinergic (iNANC) nerves seems to predominate. It has been suggested that a deficiency of endogenous “relaxant” NO is one of the underlying mechanisms for increased bronchoconstrictor stimuli in asthma. This may be explained either by a reduction in NOS activity and/or reduced local availability of the NOS substrate. Asthma has been associated with increased exhaled NO levels, of which the major part seems to derive from local inducible NOS (iNOS). Since our patients did not have an L-arginine deficient or weighted diet, we cannot exclude the possibility that these subjects had little or no NOS substrate limitation. Finally, since high concentrations of L-arginine have been shown to act as a non-competitive antagonist of the contractile effect of histamine in vitro, we cannot exclude the contribution of the anti-histamine effects of L-arginine to the present findings.

Discussion

These results show that oral L-arginine does not affect airway hyperresponsiveness to inhaled histamine in patients with asthma, although a slight reduction in airway reactivity (as indicated by the dose-response slope) was seen. These findings suggest only marginal limitation of NOS substrate in asthmatic subjects in vivo and therefore argue against a clinically relevant deficiency of endogenous NO in asthma.
Nase activity is favoured over NO synthesis. However, at present there is no evidence of increased arginase levels and/or activity in asthma. Finally, Chakder and colleagues reported that continuous iNANC activation in smooth muscle strips caused significant decreases in levels of L-arginine. It can be speculated that in asthma the iNANC system may be perpetually activated, thereby exhausting its own bronchodilator features. It remains to be elucidated whether these in vitro findings can be extrapolated to the human situation.

This work was funded by the Netherlands Asthma Foundation (grant 96.10).