Effect of topical steroids on nasal nitric oxide production in children with perennial allergic rhinitis: a pilot study

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It has been hypothesized that concentrations of exhaled nitric oxide (NO) may be related to the extent of cytokine-mediated airway inflammation. Recent findings indicate the nasal airways as an important site of NO production.

Our objective was to evaluate whether children with allergic rhinitis show different nasal NO levels when compared with normal healthy subjects and the effect of topical steroids and anti-histamine therapy.

We have measured the concentration of NO drawn from the nose of 21 children (5-17 years old) affected by perennial allergic rhinitis (house dust mite) out of therapy for at least 3 weeks. Thirteen children were then treated with nasal beclomethasone propionate (BDP) (400 μg daily) and eight subjects with nasal anti-histamine levocabastine (200 μg daily). Measurements were performed before and after 10 days of treatment. As a control group we evaluated 21 healthy children aged 5-15 years. To measure NO we used a chemiluminescence analyser.

Before treatment the whole group of children with allergic rhinitis showed a mean (± SEM) nasal NO concentration of 267 ± 18 ppb, significantly higher (P<0.01) than the control group (186 ± 15 ppb). The group of children treated with BDP showed, after 10 days of therapy, a significant (P<0.05) decrease of nasal NO concentration (271 ± 21 ppb vs. 212 ± 20 ppb). Indeed, in the group treated with levocabastine, nasal NO concentrations did not present a significant difference (P not significant) compared with baseline (261 ± 33 ppb and 252 ± 31 ppb, respectively).

These data suggest that (1) children with allergic rhinitis have higher levels of nasal NO than non-atopic controls and (2) intranasal steroid therapy significantly reduces nasal NO production in children with allergic rhinitis. We speculate that the allergic inflammatory response may influence the nasal NO levels and that NO measurements may be a useful marker of nasal inflammation.

Introduction

Nitric Oxide (NO) is a gas produced in mammalian airways and there is convincing evidence that it plays a key role in the regulation of a wide variety of airway functions. NO is derived from the guanidine nitrogen of L-arginine by the action of nitric oxide synthase (NOS), which exists in constitutive (cNOS) and inducible (iNOS) isoforms (1). The cNOS isoforms produce small amounts of NO which act locally. cNOS isoforms are expressed in endothelial cells (type III NOS), in the peripheral nervous system (type I NOS) and in other cells such as neutrophils, platelets and mast cells (2). The NO produced by these cNOS isoforms has a predominant role of a homeostatic molecule and it is mainly involved as mediator in the endothelium-dependent vasodilation and in the regulation of arterial tone. The iNOS isoforms (type II NOS) are calcium-independent enzymes and may be expressed after exposure to pro-inflammatory cytokines as well as to endotoxin or to oxidant substances. Induction of iNOS produces larger amounts of NO than the activation of cNOS. iNOS has been described in macrophages, epithelial cells, smooth muscle cells, endothelial cells and neutrophils. Steroids inhibit the induction of iNOS but have no effect on cNOS (1).

A number of observations determined that NO content in the expired air of asthmatics who were not receiving treatment with glucocorticoids was greater than exhaled NO of normal subjects and of asthmatics treated with steroids (3). This finding, coupled with the demonstration of enhanced type II NOS immunoreactivity in the airway epithelium of patients with asthma (4), raised the interesting possibility that measurement of exhaled NO could be a useful index of airway inflammation.
High concentrations of NO have been detected in the nose and it has been suggested that it is produced by the nasal pharyngeal mucosa (5). Recently, immunocytochemical studies of nasal biopsies have identified the presence of iNOS also in the human nasal mucosa (6) and a possible role for type II NO synthase in mediating the inflammatory response of nasal mucosa has been suggested.

Few studies have evaluated the role of NO in patients with allergic rhinitis (7,8) and the effect of therapy on NO production is not defined yet. The aim of this study is to evaluate (1) whether children with perennial allergic rhinitis show different nasal NO levels when compared with normal healthy subjects and (2) whether a topical course of steroid and anti-histamine treatments may alter nasal NO levels.

**Patients and Methods**

The study was performed in 21 children (mean age 9.6 ± 0.6 years, range 5-17 years) with allergic rhinitis for at least 2 yr. Children were eligible for our study if affected by perennial allergic rhinitis with characteristic symptoms (nasal pruritus, sneezing, rhinorrhea, obstruction) and a positive skin test (>3 mm wheal) for Dermatophagoides pteronyssinus and Dermatophagoides farinae. Exclusion criteria from the study included a concomitant allergy to seasonal pollens, treatment with any drugs and/or infection of the upper respiratory tract in the 3 weeks previous to the study. All children attended the Paediatric Pulmonology-Allergology outpatient clinic of the Department of Paediatrics of Padova.

Children were randomly allocated to receive a 10 day course of (1) a topical glucocorticoid, beclomethasone dipropionate nasal spray (BDP group) or (2) a topical anti-histamine drug, levocabastine nasal spray (anti-H group). Children of the BDP group were treated with a dosage of 200 μg twice daily of beclomethasone nasal spray (Becotide®, Glaxo) and children of the anti-H group with a dosage 100 μg twice daily of levocabastine nasal spray (Levostab®, Janssen).

Evaluation of therapeutic efficacy was based on assessment of the severity of a number of nasal symptoms (sneezing, rhinorrhea, pruritus and congestion). A point scale was used at the start of the trial and after 10 days of treatment by the investigator for evaluation of symptoms according to a 0–3 grading (0, absent; 1, mild; 2, moderate; 3, severe). Using the same scale, patients were instructed to keep a diary for the daily evaluation of symptoms.

As a control group normal healthy children (mean age 10.5 ± 0.5 years, range 5-15 years) with a negative history of allergic disease or recurrent rhinitis were enrolled. All children had to be disease free in the last 3 weeks prior recruitment.

Nasal NO concentrations were determined from the air drawn from the nose of all included children. Measurements were performed at baseline and after 10 days of treatment in the affected children. The investigator who performed the measurements of NO (N.M.A.) was blinded regarding the treatment used. At each measurement the children were asked to take a deep inspiration, to close the mouth and to hold the breath for 25 s. A nasal occluding olive was placed into the vestibulum of one nostril during the procedure and air samples were continuously collected by a short sealing Teflon tube connected to the NO analyser. The peak NO levels obtained after 25 s of breath holding were recorded. The contralateral nostril was left open. Two measurements were made on each session and the mean was considered for analysis. We used a chemiluminescence analyser (Orion, Rotork 447, England) sensitive to NO from 1 to 1000 ppb, adapted for on-line recording of NO concentration. The chemiluminescence analyser was calibrated with a certified calibration mixture of NO (Air Liquid, Milano, Italy). In healthy children NO levels were also measured in the air exhaled from the mouth during tidal volume breathing using an open circuit. The study was approved by the medical committee of our University Hospital and all patients gave informed consent.

All mean NO concentrations are reported in parts per billion with the corresponding standard error of the mean (mean ± SEM). As statistical tests Wilcoxon's matched-pair test and the Mann-Whitney rank sum test were used; a P value lower than 0.05 was considered as significant.

**Results**

Twenty-one children with allergic rhinitis and 21 healthy controls were included in the study. Patients' characteristics are presented in Table 1. Of the children with allergic rhinitis, 20 completed the study because one patient of the anti-H group withdrew. Of these, 13 received beclomethasone dipropionate (BDP group) while seven were given levocabastine (anti-H group). The main results are presented in Table 2. Investigator assessments of score symptoms (total nasal symptoms) at the start of the study (7.5 in the BDP group and 7.2 in the levocabastine group) were not different in the two groups (P not significant). Mean baseline measurement of nasal NO in the 21 affected children was 267 ± 18 ppb while in the control group it was 186 ± 15 ppb. The difference is statistically significant (P<0.01). NO baseline values of the BDP group (271 ± 21 ppb) and the anti-H group (261 ± 33 ppb) did not show a significant difference (P not significant) (Table 2).

**Table 1. Patients' characteristics**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Number</th>
<th>Age (years)</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic rhinitis</td>
<td>21</td>
<td>9.6 ± 0.6 (5-17)</td>
<td>17  4</td>
</tr>
<tr>
<td>BDP group</td>
<td>13</td>
<td>10 ± 0.8 (5-17)*</td>
<td>11  2</td>
</tr>
<tr>
<td>Anti-H group</td>
<td>8</td>
<td>8.5 ± 0.7 (6-11)*</td>
<td>7   1</td>
</tr>
<tr>
<td>Controls</td>
<td>21</td>
<td>10.5 ± 0.5 (5-15)</td>
<td>18  3</td>
</tr>
</tbody>
</table>

BDP group: beclomethasone dipropionate 400 μg day⁻¹. Anti-H group: levocabastine 200 μg day⁻¹.

*P=not significant.
cant reduction of nasal NO levels while L-arginine nebulization (precursor of NO synthesis) provokes a restoration of nasal NO concentration (9). Furthermore, the presence of the NOS enzymes has been demonstrated in the epithelium of human nasal mucosa, of both healthy and rhinitic subjects, using immunohistochemistry (6,10).

The results of our study show that NO nasal levels measured in children affected by allergic rhinitis are significantly higher compared with healthy control children (267 ppb vs. 186 ppb). The role of NO in allergic rhinitis is undefined; it can be speculated that high nasal NO levels are determined by the expression of iNOS in the nasal mucosa possibly induced by the cytokines of the allergic reaction, as is described for asthmatic flogosis (3). This hypothesis is supported by the significant correlation found by Furukawa et al. (6) between the extent of nasal inflammation and the cellular expression of NOS in patients with chronic rhinitis and by the preliminary data of Springall et al. (11) showing an upregulated expression of iNOS in nasal biopsies of patients with allergic rhinitis. NO nasal measurement in subjects with allergic rhinitis could therefore similarly represent an index of local allergic inflammation in asthmatic patients. Our findings are consistent with results of one previous study (8) in which exhaled levels of NO were determined in adult patients with seasonal allergic rhinitis during the grass pollen season and compared with non-atopic individuals. These authors (6) found that, in the subjects with seasonal rhinitis, levels of exhaled NO were significantly higher both in nasally exhaled air and in orally exhaled air compared with non-atopic individuals, suggesting generalized inflammation of the airways in patients with rhinitis but no obvious evidence of clinical asthma. Also, these authors hypothesized that the high levels of nasal NO could be consistent with induction of iNOS in association with mucosal mast cell, eosinophil and T-lymphocyte activation.

The second main finding of our study is about the effect of topical therapy on nasal NO production. Up-to-date treatment of allergic rhinitis includes, in addition to removing the specific allergen, the usage of topical or systemic drugs such as corticosteroids, anti-histamine compounds, sodium cromoglycate and anticholinergics (12). The most efficacious drugs on symptoms (nasal itching, sneezing, closing nose) are anti-histamine and corticosteroid drugs. Recently, levocabastine, an H1-receptor antagonist designed for topical application, has been proposed as a valid alternative to oral antihistamines (13).

Rhinitic children given topical glucocorticoids (BDP group) presented a significant reduction of nasal NO levels compared with baseline values after 10 days of treatment. On the contrary, no significant difference in nasal NO has been detected in children treated with levocabastine, a recent H1-receptor antagonist. However, regarding the comparison of inhaled steroids and anti-histamine drugs of the present study, we believe that no definitive conclusion can be reached because of the limited sample size of these subgroups which may have entailed insufficient statistical power. Regarding the changes of nasal NO after BDP treatment it can be speculated that the anti-inflammatory effect of glucocorticoids results in a downregulation of the transcription of iNOS with subsequent reduced values of nasal NO levels. This hypothesis has not yet been demonstrated but it has been shown that corticosteroids inhibit the expression of iNOS probably by blocking the transcription factor nuclear factor κB that is critical for transcription of the iNOS gene (14). The reduction of nasal NO after nasal steroids found in our study is in agreement with preliminary data of Kharitonov et al. in adult patients with allergic rhinitis treated with inhaled steroids (15) and to our knowledge this is the first study in children. Different conclusions were obtained by a Dutch group that recently reported that 2 weeks of therapy with a new topical steroid (fluticasone propionate) was ineffective in changing NO nasal production in subjects with allergic rhinitis (7).

Table 2. Main results: concentrations of nasal NO expressed in ppb in children with allergic rhinitis and controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Baseline</th>
<th>After 10 days of therapy</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic rhinitis</td>
<td>267 ± 18*</td>
<td>212 ± 20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(whole group)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDP group</td>
<td>271 ± 21**</td>
<td>252 ± 31</td>
<td>n.s.</td>
</tr>
<tr>
<td>Anti-H group</td>
<td>261 ± 33</td>
<td>252 ± 31</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>186 ± 15</td>
<td></td>
<td></td>
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</table>

*P<0.01: allergic rhinitis (whole group) vs. control group.
**P=not significant: BDP group vs. anti-H group, baseline values.

Discussion

NO has been identified as an important mediator in numerous physiological and inflammatory processes in the respiratory system and it seems to be involved in the pathophysiology of several airway diseases (1). The exact NO production site in the respiratory airways is not definitely established. There is persuasive evidence that in the nasal mucosa there is a production of NO that is easily measurable in the nasal cavity (5). Nasal nebulation of NOS inhibitors such as L-arginine analogues (L-NAMES), that act as false substrate for the enzyme, causes a significant reduction of nasal NO levels while L-arginine nebulation (precursor of NO synthesis) provokes a restoration of nasal NO concentration (9). Furthermore, the presence of the NOS enzymes has been demonstrated in the epithelium of human nasal mucosa, of both healthy and rhinitic subjects, using immunohistochemistry (6,10).

The results of our study show that NO nasal levels measured in children affected by allergic rhinitis are significantly higher compared with healthy control children (267 ppb vs. 186 ppb). The role of NO in allergic rhinitis is undefined; it can be speculated that high nasal NO levels are determined by the expression of iNOS in the nasal mucosa possibly induced by the cytokines of the allergic reaction, as is described for asthmatic flogosis (3). This
However, these authors did not measure NO concentrations directly in nasal cavities but determined NO metabolites (nitrites and nitrates) in nasal wash material.

In conclusions these data suggest that (1) children with allergic rhinitis have higher levels of nasal NO than non-atopic controls and (2) intranasal steroid therapy significantly reduces nasal NO production in children with allergic rhinitis. Although further studies are required to confirm these preliminary data, we speculate that, in allergic rhinitis, NO nasal levels can be considered a useful marker of allergic immunolglosis and its measurement an easy method for monitoring the response to therapy.

Acknowledgements

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References