SHORT COMMUNICATION

Discrepant nasal and bronchial nitric oxide kinetics during early and late phase allergic reactions

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Received 3 January 2005

Summary Late allergic reactions (LAR) following allergen challenge occur in different compartments. We studied the kinetics of nasal and bronchial nitric oxide (NO) in mild asthmatics after allergen challenge.

Twelve males with intermittent asthma (28 yr, FEV\textsubscript{1} 97% of predicted, PC\textsubscript{20} methacholine <8 mg/ml) and known LAR after bronchial allergen challenge underwent nasal and bronchial allergen provocation using the same allergen separated by a washout of 3 weeks. Nasal and bronchial NO were measured before challenge, during early (EAR) and late phase reactions, and 24 h after allergen.

The mean (SEM) maximum fall of FEV\textsubscript{1} at EAR was 31.9 ± 3.2\% (\(P = 0.001\)), and 17.6 ± 2.2\% (\(P = 0.004\)) during LAR. All patients developed nasal EAR (max. fall in nasal rhinomanometric flow 64.8 ± 7.6\% of baseline) after nasal challenge, and 10 patients demonstrated nasal LAR with a fall in nasal flow of 65.9 ± 6.6\% (both \(P = 0.002\), respectively). During EAR, there was stronger reduction of nasal (−19.2 ± 6.2\%, \(P = 0.039\)) than bronchial NO (−6.9 ± 5.2\% of baseline, \(P = \text{ns}\)). In contrast, bronchial NO also tended to decrease during bronchial LAR (−8.8 ± 6.8\%, \(P = \text{ns}\)), while nasal NO slightly increased non-significantly (+17 ± 10.8\%, \(P = \text{ns}\)). After 24 h, bronchial NO was significantly elevated (+78.1 ± 40.1\%, \(P = 0.039\)), whereas nasal NO was unchanged (+6.1 ± 15.1\%, \(P = \text{ns}\)). The intraindividual difference between bronchial and nasal changes of NO during LAR, but not EAR or after 24 h, was significant (lung vs. nose: −35.6 ± 14.1\% relative difference, \(P = 0.039\)).

Despite similar functional responses in nose and bronchi, nasal NO kinetics following allergen challenge differ from bronchial NO. The concise mechanisms accounting for this discrepancy warrant further investigations.

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KEYWORDS
Nitric oxide; Inflammation; Asthma; Rhinitis; Allergen challenge; Kinetics; Late allergic reactions
Introduction

Allergic rhinitis and asthma share several pathophysiological features, and these similarities have led to the introduction of a unified concept of “united airways”.1,2 Allergic reactions in the lung and nose involve many of the same inflammatory cells and mediators.3 There has been intense research into monitoring of inflammation in asthma and rhinitis. In this regard, nitric oxide (NO) has been studied as a marker of eosinophilic airway inflammation.4–9 Exhaled NO is elevated in the airways of stable asthmatics and has been shown to increase following bronchial allergen challenge.6 Despite the general utility of NO in asthma, various factors influence NO levels in exhaled breath, including airway narrowing,10,11 caffeine12 or hypertonic saline inhalation.6,11,13 Nasal NO is increased in subjects with seasonal14 or perennial15 allergic rhinitis and responds to topical corticosteroid therapy.16 However, the kinetics of nasal NO following allergen challenge have been studied only to a minor extent.17

To the best of our knowledge, there are no studies directly comparing the kinetics of nasal and bronchial NO in the same subject after allergen challenge. This study therefore compares nasal and bronchial NO kinetics during early (EAR) and late phase allergic reactions (LAR) in patients with both asthma and rhinitis.

Materials and methods

Patients

Twelve males (median age 27.5 years) with a history of allergic rhinitis and intermittent asthma (mean FEV1 97% of predicted; PC20 methacholine <8 mg/ml,16,18 only short-acting beta-agonist prn) sensitized (skin test and specific IgE) to grass pollen (n = 9), house dust mite (2) and horse dander (1) were studied. All patients were non-smokers who had demonstrated dual asthmatic reactions following allergen inhalation on a prior occasion. None of the patients used anti-inflammatory drugs. Subjects gave written informed consent and the study was approved by the local hospital ethics committee.

Study design and measurements

The study was performed outside the pollen season. At baseline, a bronchial allergen challenge was performed according to a method described by Taylor et al.16,19 using a five-breath dosimeter protocol (MEFAR nebulizer, Markos, Monza, Italy). A single bolus of allergen (ALK, Hamburg, Germany) was administered at a calculated (historic) dose (PD20) that had resulted in a dual response on a previous occasion using an incremental allergen challenge.16,19 Serial spirometry was performed using the Masterscope system (Jaeger; Würzburg, Germany) according to ATS.20 Exhaled NO was measured prior to challenge, at the peak of EAR and LAR and 24 h after allergen.

After a washout of 3 weeks, nasal allergen challenge was performed with the same allergen according to the EAACI guidelines.28 Responses were evaluated every 15 min after allergen administration and considered positive when symptoms occurred.29 Nasal EAR was defined as a flow reduction >40% (150 Pa, within 30 min after allergen). Nasal LAR was defined as delayed (3–8 h after allergen) occurrence of symptoms accompanied by at least 30% decrease of nasal flow. Nasal flow and resistance were measured by active anterior rhinomanometry (Jaeger; Würzburg, Germany).21 Nasal NO was measured prior to and during EAR, LAR, and 24 h after nasal allergen.

Exhaled bronchial and nasal NO

Exhaled NO was measured by a chemiluminescence analyzer (ECOmedics CLD88sp) according to European Respiratory Society (ERS) and ATS guidelines for the measurement of exhaled NO.11 Nasal NO was measured according to ATS guidelines.11 The patient exhaled against expiratory resistance while targeting a mouth pressure of 10 cm H2O to close the velum, with a nasal olive introduced into one nostril and air aspirated from the nasal cavity at constant flow (50 ml/s) via the olive by a suction pump. The mean of three measurements was recorded, respectively.

Statistical analysis

Unless otherwise stated, data are presented as arithmetic mean values with standard error of mean (SEM). Due to technical problems, rhinomanometry could not be performed in one patient. Changes of NO levels between lung and nose were compared intraindividually using pairwise comparisons based on nonparametric sign tests with P values <0.05 indicating significance. Individual changes of nasal or bronchial NO compared to baseline values were analyzed by paired signed test. Correlations between bronchial and nasal NO
were estimated by Spearman’s nonparametric correlation ($\rho$).

**Results**

A summary of baseline characteristics of subjects is presented in Table 1. All subjects developed bronchial and nasal EAR with a mean maximum fall in FEV$_1$ of 31.9 $\pm$ 3.2\% ($P = 0.001$) and nasal flow reduction of 64.8 $\pm$ 7.6\% ($P = 0.002$) of baseline. The mean bronchial LAR was a fall in FEV$_1$ of 17.6 $\pm$ 2.2\% ($P = 0.004$) of baseline. Ten patients also developed nasal LAR with a maximum flow reduction 65.9 $\pm$ 6.6\% ($P = 0.002$) of baseline.

Bronchial NO decreased non-significantly during EAR from 71 $\pm$ 21 to 61 $\pm$ 17 ppb, ($P = \text{ns}$), equalling a mean NO reduction of $-6.92 \pm 5.22$\% from baseline. There was a further decrease in bronchial NO during LAR to 59 $\pm$ 16 ppb ($P = 0.146$ vs. baseline, mean decline of $-8.80 \pm 6.77$\%). In contrast, exhaled bronchial NO increased significantly 24 h after bronchial allergen challenge to 95 $\pm$ 23 ppb ($P = 0.039$ vs. baseline, mean rise of 78.1 $\pm$ 40.1\%).

Nasal challenge with allergen caused a decrease in nasal NO during EAR from 85 $\pm$ 7 to 68 $\pm$ 9 ppb ($P = 0.039$, mean reduction of $19.2 \pm 6.2$\% from baseline). In contrast, nasal NO slightly increased non-significantly during LAR to 95 $\pm$ 7 ppb ($P = 0.74$, mean increase 17 $\pm$ 10.8\%), while levels returned to baseline after 24 h (85 $\pm$ 12 ppb; $P = 0.63$, mean change $6.1 \pm 15.1$\%) (Fig. 1).

On an individual basis, there was no significant difference between bronchial and nasal NO changes during EAR (lung–nose: $5.7 \pm 7$\%, $P = \text{ns}$). In contrast, significant discrepancies were observed during LAR (lung–nose: $-35.6 \pm 14.1$\%, $P = 0.039$).

![Figure 1: Bronchial and nasal NO kinetics after allergen challenge. Plots represent mean (+SEM) values for individual changes (% of baseline) of bronchial (bold square) and nasal (open circle) NO during early (EAR), and late phase reaction (LAR), and 24 h after allergen challenge. *$P = 0.039$ nose vs. lung, **$P < 0.05$ vs. corresponding baseline value.](image)

After 24 h, there was a trend towards increased bronchial compared to nasal NO levels (lung–nose: 40.6 $\pm$ 31.3\%, $P = \text{ns}$).

At baseline, exhaled bronchial NO was strongly correlated with nasal NO ($\rho = 0.71$, $P = 0.02$). However, there was no further correlation of bronchial or nasal NO with either corresponding changes in lung or nose, respectively, or changes in bronchial or nasal airflow during EAR or LAR (all correlations $P > 0.05$).

**Discussion**

To the best of our knowledge, individual comparisons of bronchial and nasal NO kinetics have not previously been reported and ours is the first study that systematically investigates whether or not nasal and bronchial NO kinetics after local allergen challenge are similar. In the present study, we describe dissimilarities between nasal and bronchial NO kinetics after allergen challenge in atopic patients with both asthma and rhinitis. Levels of both bronchial and nasal NO decreased during EAR. With regard to bronchial NO, this observation is consistent with a previous study by Dweik et al.\textsuperscript{23} Adding to this, a decrease of nasal NO during EAR has also been described by Silkoff et al.\textsuperscript{17} This reduction of exhaled NO may be a consequence of the allergen-induced reduction in airflow as previously demonstrated by others.\textsuperscript{24–26} Nasal NO

<table>
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<tr>
<th>Table 1</th>
<th>Summary: patient baseline characteristics.</th>
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<tr>
<td>Age (years)</td>
<td>Median (interquartile range) 28 (26–34)</td>
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<tr>
<td>FEV$_1$ (% of predicted)</td>
<td>Mean (SD) 97 (5)</td>
</tr>
<tr>
<td>Allergen (n)</td>
<td>Grass 9</td>
</tr>
<tr>
<td></td>
<td>House dust mite 2</td>
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<tr>
<td></td>
<td>Horse dander 1</td>
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<tr>
<td>RAST class</td>
<td>Median (range) 3 (2–6)</td>
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output is also considered to be flow-dependent. However, there was no correlation between nasal or bronchial NO and nasal or bronchial flow reduction during EAR, hence the observed reduction of NO cannot be fully explained by airway obstruction. Decreased expired NO during EAR may also reflect rapid consumption of NO to form stable nitrogen intermediates, e.g. peroxynitrite or nitrate. It has been demonstrated that early antigen challenge results in an immediate release of airway superoxide anion, thus promoting the formation of peroxynitrite through NO and O₂⁻.

During bronchial LAR, exhaled NO levels also tended to decrease or remain stable in all patients. At first glance, this finding was somewhat surprising, since late phase allergic reactions are usually associated with airway eosinophilia and increased NO levels, despite airway obstruction. However, the majority of our patients displayed LAR at an relatively early timepoint (between 3 and 5 h post challenge), therefore it can be speculated that an early influx of neutrophils during the initial stages of LAR might have partially contributed to the failure to find increased bronchial NO during LAR, since NO levels can be decreased by superoxide generated from neutrophils. In contrast, nasal NO increased during nasal LAR, and the individual rise of nasal NO was significant compared to bronchial NO. The reasons for this discrepancy are not fully clear, in particular since there was a marked reduction in nasal flow during LAR. Hence, the observed NO levels are rather an underestimation of “true” nasal NO, as demonstrated by Djupesland et al. On the other hand, it might be argued that the magnitude of eosinophilic nasal inflammation and NO release overcomes NO reduction by decreased nasal flow, thus resulting in a net increase of NO. Pastorello et al. demonstrated that large numbers of eosinophils appear in nasal lavage fluid rapidly after allergen challenge, peak at 8 h and tend to normalize after 24 h.

Bronchial NO significantly increased 24 h after allergen, which is consistent with earlier reports. We therefore hypothesize that the discrepant nasal and bronchial NO levels are probably at least in part due to the fact that eosinophilic inflammation after allergen challenge in the lung is fully established at a later timepoint compared to the nasal compartment.

In conclusion, our study demonstrates similar nasal and bronchial NO kinetics during early, but not late, allergic responses in subjects with both rhinitis and asthma. Further studies need to clarify the relation of nasal and bronchial NO to inflammatory cells and mediators.

Acknowledgements

The authors wish to thank PD Dr. Frank Krummenauer, Department of Medical Biometry, Epidemiology and Informatics, University of Mainz, for statistical advice.

References


