

Exhaled and nasal nitric oxide is increased in laboratory animal allergy

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Summary

Background Allergens from rats, mice, guinea pigs or rabbits cause up to 30% of exposed persons to develop specific immunoglobulin E (IgE) responses. Laboratory animal allergy (LAA) is among the highest occupational risks for asthma in the UK. Elevated levels of nitric oxide (NO) are found in exhaled breath in asthma. In LAA symptoms may progress from conjunctivitis, rhinitis to asthma. Health surveillance aims to detect early sensitization.

Objective To assess whether an association exists between LAA and exhaled NO.

Methods A cross-sectional study was performed in 39 laboratory workers undergoing LAA health surveillance. Volunteers completed two health questionnaires, had skin-prick tests, spirometry, total IgE and RAST tests. Exhaled and nasal NO was measured by chemiluminescence analyser (LR2000, Logan Research, Rochester, UK).

Results There were 23 asymptomatic subjects (mean age 29.53 years) and 16 symptomatic subjects (29.63 years, $P = 0.95$); 9 early LAA, seven LAA asthma. Exhaled NO was raised in those with LAA symptoms $17.97 \text{ ppb} \pm 1.24$ (mean $\pm$ SEM) compared with asymptomatics $6.08 \text{ ppb} \pm 1.15$, $P < 0.05$. A trend of increased NO by allergic status was observed; asymptomatic, to early LAA, to asthma. One-way analysis of variance compared differences between groups (F ratio 13.93, $P < 0.001$). Symptomatic subjects also had raised nasal NO, vs asymptomatic subjects (mean difference 378 ppb, $P < 0.05$). A trend was again observed by allergic status (F ratio 5.28, $P = 0.01$).

Conclusion Raised NO levels in LAA increasing with symptom severity suggest NO may prove a useful additional tool in monitoring for LAA, and possibly the response to exposure reduction or allergy due to other respiratory sensitizers.

Keywords: nitric oxide, animal allergy, asthma, occupational disease

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Introduction

Levels of nitric oxide (NO) in exhaled air are increased in patients with asthma [1–5], and during the late response to inhaled allergen, there is a further increase in NO [6]. Workplace exposure to allergens affords the opportunity to learn more about the process of sensitization. Asthma due

to occupational exposure has been estimated to have a prevalence from 2 to 15% in the United States [7]. In the UK asthma due to laboratory animal allergy (LAA) forms a significant proportion of reported cases of occupational asthma, and is amongst the leading causes [8]. There may be a progression from conjunctivitis, rhinitis and contact urticaria to asthma.

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Study design

A cross-sectional study was performed in a group of laboratory workers undergoing health surveillance for

animal allergy. The study was performed to establish whether NO is raised in the expired air of subjects with occupational asthma induced by laboratory animals, and to examine the relationship between laboratory animal allergy and exhaled NO.

Study population

Subjects were recruited from a group of 175 workers undergoing health surveillance for laboratory animal allergy. The subjects were either research workers or animal technicians exposed to rats, mice, guinea-pigs or rabbits. Consent to participate in this study was sought from 80 consecutive subjects, when they attended for health surveillance screening. Sixty-six subjects were recruited. New employees were included if they had previously been working with laboratory animals for at least 6 weeks. There were no payments or inducements made to the volunteers, travelling expenses were reimbursed.

Workers with known constitutional asthma and no evidence of sensitization to laboratory animals did not have nitric oxide levels measured. This was because constitutional asthma has previously been demonstrated to be associated with raised exhaled air nitric oxide [1,3,4]. Subjects who had occupational asthma but were taking inhaled or systemic corticosteroid therapy were also excluded since steroids are known to reduce exhaled air NO [1,9]. Measurements of exhaled air NO were not made for at least three weeks in anyone giving a history of upper respiratory tract infection [10]. It was also decided not to include any women who declared that they were pregnant, due to the unknown effect of hormone alterations during pregnancy on exhaled air NO. Workers with less than 6 weeks exposure to laboratory animals were not included since although LAA may occur this early it is uncommon [11].

Methods

All participants completed two questionnaires. One, asking about work related symptoms, was self completed and based on the format recommended by the UK Health and Safety Executive. The other questionnaire was the Medical Research Council 'Respiratory Symptoms (1986)' questionnaire. All subjects had skin-prick tests for reactions to extracts of common allergens (three tree pollens mix, six grass pollens mix, *Aspergillus fumigatus*, cat, house-dust mite), to a range of laboratory animal allergens (rat, mouse, guinea-pig, rabbit, dog), a negative control and histamine as a positive control (Allerayde reagents, Slough UK). Weals were measured in two diameters at right angles, the mean was taken as the result. A positive skin-prick reaction was considered as 3 mm greater than the negative control. A

blood sample was tested for total immunoglobulin E (IgE) and according to a local policy (St. Thomas' hospital, immunology laboratory) only if raised above 40 ku/l, a radioallergosorbent test (RAST) was done (Cardiff protein reference laboratory) for the animal species used. The forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) were recorded by spirometry. Methacholine challenge testing using the method described by Yan [12] and serial peak expiratory flow measurements (PEF) using a peak flow gauge (Wright mini-meter) were undertaken in patients with a history suggestive of asthma. Measurements of skin-prick reactivity were made on the day of recruitment to the study, blood was taken for IgE/RAST at this time. An appointment was made to attend for spirometry with/without methacholine challenge, and nitric oxide measurement within 3 weeks. These tests were performed on a Friday between 09.00 h and 15.00 h away from the usual place of work. All subjects were currently working with laboratory animals and had been exposed within the preceding 7 days.

Study definition of LAA

For the purpose of the study individuals with a positive skin-prick or RAST to the relevant laboratory animal, who had indicated the presence of work related lower respiratory symptoms and a positive response to any of the MRC questions for 'wheezing' were regarded as having occupational asthma. Subjects who had evidence of sensitization and other work-related symptoms (of nose, eyes or skin) were classified as 'early LAA'.

Nitric oxide measurement

Exhaled nitric oxide (NO) was measured using a modified chemiluminescence analyser (Model LR2000, Logan Research, Rochester, UK), sensitive to NO from 1 to 5000 parts per billion (ppb, by volume), and with a resolution 1 ppb, which was designed for on-line recording of exhaled NO concentration. This feature obviates the need for collection in a reservoir and gives greater sensitivity (with a response time <0.5 s) with high reproducibility. In addition to NO, the analyser also measured CO₂ (resolution 0.1% CO₂, response time 200 ms), and sample pressure and volume in real-time. The sampling rate of the analyser was 250 mL/min for all measurements. The analyser was calibrated daily using certified NO mixtures (90 ppb and 500 ppb) in nitrogen (BOC Special gases, Guildford UK). Ambient air NO was recorded before and after each subject was studied.

Measurements of exhaled NO were made by slow exhalation (6 L/min) from total lung capacity for 30 s via a wide bore Teflon tube by-passing the analyser and thus

with minimal resistance to flow; NO (end-exhaled) was sampled from a side arm. The final NO result was taken as the value corresponding to the plateau of the end-exhaled CO₂ reading (5% CO₂) and at a point of the NO recording where the level was found to stabilize at end exhalation [13]. In these measurements subjects wore a nose-clip and the pressure during expiration was kept constant (3 ± 0.4 mmHg) by using a visual display of expiratory flow measured by the pressure and volume sensors in the analyser.

Nasal NO was measured with a Teflon tube inserted into one of the nares, whilst the subject held their breath, i.e. with no active exhalation. This allowed analysis of the local NO concentration with free flow of ambient air (250 mL/min) from one nostril to the other and subsequent direction into the analyser. The NO level was measured when a plateau level was achieved and in the absence of a CO₂ signal, indicating satisfactory breath holding [13]. Three readings each of exhaled and nasal air were taken, with the results being expressed as the mean value.

Exclusions

Three persons were excluded, one with LAA asthma because of current inhaled steroid use, another with asthma since there were no work-related symptoms and there was no evidence of occupational sensitization from skin prick, RAST and peak flow monitoring, and a third without LAA symptoms who had bacterial sinusitis.

Statistical methods

Nasal NO levels conformed to a normal distribution so were analysed untransformed, whereas exhaled NO levels followed a log-normal distribution, so were transformed to logarithms for analysis; the results for this measure are presented converted back into the original units. Both measures were summarized using (geometric) means with 95% confidence limits. Group differences were

investigated by one-way analysis of variance with an appropriate multiple comparison test.

Results

Complete data were obtained on 39 subjects. For these subjects studied (Table 1), 23 had no symptoms of LAA, nine had symptoms of early LAA without asthma, seven had occupational asthma due to LAA. There was a high prevalence of atopy as defined by skin prick response to one or more common allergens and serum Ig E levels were higher amongst those with symptoms (Table 1). Where methacholine challenge tests had been performed there were more positive responses in those with LAA symptoms (Table 1). Twelve subjects in the group with LAA symptoms recorded serial peak flow measurements. Amongst this group four of the peak flow recordings were considered consistent with occupational asthma. In the group without LAA symptoms three subjects who had reported positive responses to the MRC questions on wheezing also performed serial peak flow recordings. None of these recordings were considered consistent with asthma.

Geometric mean exhaled NO levels (95% confidence limits) were 6.08 ppb (4.58–8.07) for the asymptomatic group, 12.53 ppb (6.50–24.14) for the early LAA group, and 28.58 ppb (15.66–52.17) for the asthma group. The differences between all three groups were significant [$F(2,36) = 13.93$, $P < 0.001$], indicating a trend of increase in NO from asymptomatic to early LAA to asthma (Fig. 1).

Mean nasal NO levels were 920 ppb (744–1097), 1220 ppb (990–1451), and 1398 ppb (1100–1696) for the asymptomatic, early LAA and asthma groups, respectively (Fig. 2). Again a significant overall trend was observed across the groups according to allergic status [$F(2,36) = 5.28$, $P = 0.01$], although the difference between the early LAA and asthma groups did not achieve statistical significance. The exhaled and nasal NO levels for the asymptomatic group and the entire group with LAA symptoms are presented in Table 2.

Table 1. Characteristics of the asymptomatic and LAA symptoms groups

		Gender		Age Mean years (range)	Smokers (n)	Atopy n (%)	Serum Ig E (GM) kU/L $\pm$ SEM	FEV ₁ (% Pred) L $\pm$ SEM	Methacholine PD ₂₀ responders
		female	male						
Asymptomatic	(n = 23)	12	11	29.53 (20–40)	2	5 (22)	15.1 $\pm$ 1.5	104 $\pm$ 3	1 of 9
LAA symptoms	(n = 16)	7	9	29.63 (22–39)	2	12 (75)	108.2 $\pm$ 1.3	107 $\pm$ 4	6 of 11
$P = 0.95$							$P = 0.001$		

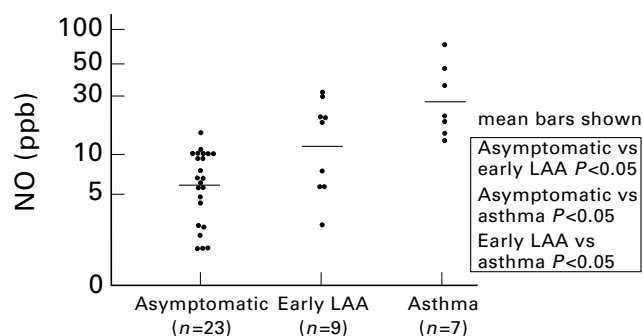


Fig. 1. Geometric mean values are shown by the horizontal bars. Significant differences were present for the overall trend ($P < 0.001$) and between the groups as shown.

Discussion

The results of this study show that exhaled breath NO is raised in early LAA and asthma due to LAA compared with levels in asymptomatic laboratory workers. Nasal NO levels are similarly but not as markedly raised.

That the nasal NO levels did not show such a large difference between and across the groups as exhaled NO, is consistent with the clinical progression of the allergy and the pathophysiology of NO in the respiratory tract. Nasal symptoms are the most frequently reported manifestation of LAA and a significant proportion of affected individuals progress to asthmatic symptoms [11,14,15]. Also, those with asthma have an increased prevalence of nasal sensitization [11,14,15], actually almost invariable. Hence, a large difference in nasal NO would not be expected between the early LAA and asthma groups. NO originating from the nasal sinuses may contribute to the exhaled air measurement [16,17]. However, we have recently demonstrated that the increased exhaled NO in asthma is derived from the lower respiratory tract [13].

The results for exhaled NO comparing the asymptomatic group and those with asthma are similar to the findings in

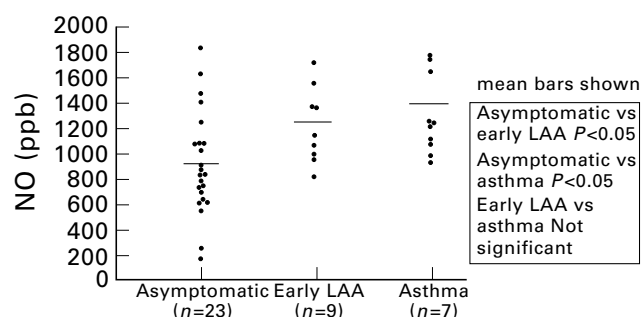


Fig. 2. Mean values are shown by the horizontal bars. A significant overall trend was present ($P = 0.01$).

Table 2. Nitric oxide results by presence of LAA symptoms

	Exhaled no mean ppb ($\pm$ SEM)	Nasal no mean ppb ($\pm$ SEM)
Asymptomatic ($n = 23$)	6.08 (± 1.15)	920 (± 85)
LAA symptoms ($n = 16$)	17.97 (± 1.24)	1298 (± 78)
	$P < 0.05$	$P < 0.05$

asthma of nonoccupational origin. The trend across the three groups probably reflects the extent of lower airways inflammation. The more obvious trend seen for exhaled NO is likely to be the effect of the larger surface area of the lungs as compared with the nasal passages. The highest exhaled NO levels being found in asthmatics probably highlight the predominant site of inflammation in these subjects. It has been shown that nasal NO levels are raised in allergic rhinitis and can be suppressed by steroids suggesting that the elevated levels arise from inducible NO synthase [18].

Exhaled air NO measurement currently requires specialized and expensive equipment. It is however, noninvasive, rapid and there is no associated discomfort. In respiratory allergy the result recorded relates both to the target organ(s) of interest and to the inflammatory process. It is not yet known whether asymptomatic individuals with already high NO levels are more likely to develop symptoms. Neither is it at present possible to predict from NO levels which individuals with symptoms of 'early LAA' may progress to asthma. Further longitudinal studies using NO measurement may however, give useful information towards answering these questions. It may also be possible to monitor the effect of reducing the occupational exposure to allergen on inflammatory activity as indicated by NO levels. It seems likely that similar results would be found with other occupational or environmental allergens of high molecular weight and probably also of low molecular weight.

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