Measurement of nitric oxide and 8-isoprostane in exhaled breath of children with atopic eczema

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doi:10.1111/j.1365-2230.2008.03142.x

Summary

Background. Children with atopic eczema (AE) are at risk of developing asthma. Airway inflammation has been shown to be present before the onset of clinical asthma. Increased exhalation (forced expiration; FE) of nitric oxide (FE_{NO}) and 8-isoprostane seems to be a feature of bronchial inflammation in people with asthma.

Aim. To determine whether the exhalation of these two molecules is increased in children with eczema, even in the absence of overt asthma.

Methods. In total, 21 children with AE were recruited and compared with healthy controls. A questionnaire was completed to identify respiratory symptoms compatible with asthma. The severity of AE was graded clinically. Spirometry, FE_{NO} measurements and exhaled breath condensate collection for 8-isoprostane were performed.

Results. The mean level of 8-isoprostane was similar for children with AE (2.33 ± 4.76 pg/mL) and controls (3.37 ± 3.43). FE_{NO} was increased in children with AE (mean 64.97 parts per billion) compared with the normal range, even in the absence of respiratory symptoms and in the presence of normal lung function.

Conclusions. FE_{NO} but not 8-isoprostane levels in exhaled breath condensate are higher in children with AE without asthma. Our finding may indicate a predictive role for FE_{NO} for the development of asthma.

Introduction

Epidemiological studies have shown that children with atopic eczema (AE) are at high risk for development of asthma.[1] Research has focused on the identification of factors that could predict this progression. Bronchial inflammation is present before the clinical onset of asthma and changes in lung function.[2] The measurement of inflammatory airway markers in exhaled breath is a promising tool as it is noninvasive, reproducible and easy to perform. One of these markers, 8-isoprostane, a prostaglandin F_{2a} isomer formed by the free radical-induced peroxidation of arachidonic acid in the cell membrane, is considered a measure of oxidative stress.[3] Compared with other inflammatory markers, 8-isoprostane has several advantages, being chemically stable[4] and specific for lipid peroxidation. High levels of 8-isoprostane have been found in the exhaled breath condensate (EBC) of people with asthma,[5] but 8-isoprostane EBC values have not been measured in children with AE.

Another inflammatory marker is nitric oxide, which is derived from L-arginine by nitric oxide synthase (NOS), of which at least three isoforms exist.[6] Two of these enzymes are constitutively expressed by certain cell types, including neurones (nNOS or NOS1) and endothelial cells (eNOS or NOS3). The third (iNOS or NOS2) is not normally present but it can be induced in a variety of cell types (endothelial, epithelial, fibroblasts,

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Conflict of interest: none declared.

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Accepted for publication 15 August 2008

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smooth muscle cells, neutrophils and macrophages) particularly by inflammatory cytokines, endotoxin and viral infection. High levels of fractional exhaled nitric oxide (FE_{NO}) have been found in children with asthma.[7] It is unclear whether FE_{NO} values are increased in children with AE.[8,9]

This study aimed to investigate whether biomarkers of airway inflammation might be increased in children with eczema. Therefore, we evaluated 8-isoprostane EBC and FE_{NO} in children with AE to investigate whether they are influenced by the coexistence of asthma.

Methods

The study was approved by the local ethics committee. An information sheet was provided to parents of all children, and written consent was obtained from all parents or guardians.

Patients

Children with eczema and normal controls who were able to carry out the measurements were invited to take part to the study. Children with AE were recruited from the dermatology clinic at Great Ormond Street Hospital for Children. All fulfilled the criteria of Hanifin and Rajka.[10] Any child receiving any systemic therapy, including oral corticosteroids or azathioprine, was excluded from the study. Healthy children who were relatives of medical and nursing staff were recruited as controls.

In total, 21 children with AE (9 boys, 12 girls; mean ± SD age 10.92 ± 3.49 years, range 6–18) were recruited. The control group comprised 10 children (2 boys, 8 girls; mean ± SD age 11.3 ± 3.80, range 6–15). There was no significant difference in mean age of the two groups ($P = 0.438$).

A questionnaire on asthma and allergy, which was validated by a clinical examination, was completed. Respiratory symptoms reported by the children with AE are shown in Table 1. Any child with at least four episodes of wheezing per year was considered to have asthma.[11] Using this definitions, asthma was reported by seven children with AE (33%). One child was taking inhaled corticosteroid. There were 12 children (57%) who had experienced wheezing in the previous year, and 14 children reported symptoms of allergic rhinitis in the previous 12 months; 1 of these had symptoms at the time of testing.

AE was graded as mild in seven children, moderate in nine and severe in five.[12]

Table 1 Frequency of respiratory symptoms in eczematous children.

Respiratory symptoms		
Questions	Possible answers	Respondents (*n*)
Ever wheezing	Yes	17
	No	4
Wheezing last year	Yes	12
	No	9
No. of attacks of wheezing last year	1–3	7
	4–12	5
Sleep disturbed by wheezing last year	Ever	8
	< 1/week	4
	> 1/week	1
Wheezing impairing speech last year	Yes	0
	No	13
Ever asthma (at least 4 wheezing attacks/year)	Yes	7
	No	14
Wheezing during exercise	Yes	11
	No	10
Dry cough at night	Yes	6
	No	15
Continue coughing after a cold	< 1 week	13
	1–3 weeks	8
	> 3 weeks	0
Ever allergic rhinitis	Yes	14
	No	7
Allergic rhinitis last year	Yes	14
	No	7
Rhinitis and conjunctivitis	Yes	11
	No	10
Rhinitis season	Winter	4
	Spring	1
	Summer	5
	Autumn	4
Did nose problem interfere with daily activities?	At all	7
	A little	5
	A moderate amount	3
Ever hay-fever?	Yes	12
	No	9

EBC collection for 8-isoprostane measurement was undertaken in both study groups. Spirometry and FE_{NO} measurements were undertaken in nine children with AE. Children were not allowed to take inhaled corticosteroids or β2 agonists on the day of the tests (EBC collection, spirometry, FE_{NO} measurements). Oral antihistamines were discontinued at least 24 h before the tests.

Questionnaire

The International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire[13] was completed by parents, or by the children if they were aged > 14 years

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old. The questionnaire comprised questions relating to upper and lower respiratory tract symptoms and to eczema.

Pulmonary function

Standard lung function indices were measured with a spirometer (Jaeger Vitalograph Ltd, Buckingham, UK) according to American Thorax Society guidelines. The best of three measurements was chosen, expressed as a percentage of the predictive value.[14]

Exhaled nitric oxide measurement

FE_{NO} measurement was performed with a single-breath online measurement (SBOL), according to the American Thoracic Society Guidelines,[15] using a device to measure airway inflammation (NiOX; Aerocrine, Stockholm, Sweden). In order to avoid contamination from the nasopharynx, air was exhaled through the mouth against a constant pressure that ensured palatal closure. The mean FE_{NO} was calculated according to standard methods.[16] The normal range used by our respiratory laboratory is 6.4–26.8 parts per billion (ppb), with a mean of 15.6 ppb).[15]

Exhaled breath condensate collection

EBC was collected by having the child breathe air tidally for 15–20 min through a standardized condenser (Ecoscreen, Jaeger, Germany), using a nonrebreathing valve to separate inspiratory and expiratory air. Approximately 2 mL of EBC was collected from every patient and stored at –80 °C in a 1-mL sterile plastic tube for later analysis, as 8-isoprostane concentrations remain stable for 6 months of storage.[17]

Measurement of 8-isoprostane

The 8-isoprostane levels in EBC samples were measured using a specific enzyme immunoassay kit according to the manufacturer's protocol (Cayman Chemical, London, UK). In brief, the assay is based on competition with 8-isoprostane-acetylcholinesterase (AchE) conjugate for a limited number of 8-isoprostane-specific rabbit antiserum binding sites. Ultimately, the amount of competing AchE is determined spectrophotometrically and is inversely proportional to the amount of free 8-isoprostane in the EBC samples. The lower limit of detection in this assay was 3.9 pg/mL. Intra-assay variation was calculated using a pooled sample with detectable 8-isoprostane levels. This sample was analysed in triplicate on every plate and the variation determined to be 7.5%. Analysis of 8-isoprostane was performed on undiluted and nonconcentrated EBC samples and all samples were assayed in duplicate.

Statistics

Data are expressed as mean ± SD. Differences among groups were analysed using Student's *t*-test and ANOVA was used for comparisons between groups. The χ^2 test with Yates' correction was used to compare ordinal variables. Fisher's exact test was used when the expected number of individuals in the cell was < 5. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated. Correlations were evaluated by Spearman's rank test. Results were considered significant at $P < 0.05$.

Results

8-isoprostane measurement

EBC collection and 8-isoprostane detection were performed in all the children (21 with AE and 10 controls). The levels of 8-isoprostane were below the detection limit in 13 of 21 children with AE and 6 of 10 controls. There was no difference in the mean level of 8-isoprostane between children with AE and healthy controls (children with AE 2.33 ± 4.76 pg/mL, controls 3.37 ± 3.43) (Fig. 1).

Levels of 8-isoprostane were not correlated with AE severity. Children with AE with asthma, previous or current wheezing, or with a diagnosis of hay fever or allergic rhinitis did not have higher 8-isoprostane values than controls.

Children with levels of 8-isoprostane below the detection limit were then excluded from the analysis. In the remaining children, there was again no difference in 8-isoprostane values between children with AE and controls. In these subjects, 8-isoprostane values were not increased in children with asthma, previous or current wheezing, hay fever or allergic rhinitis.

Exhaled nitric oxide measurement

In a subgroup of nine children, FE_{NO} was measured and spirometry was performed. We found levels of FE_{NO} to be much higher than the normal range in all except one of the children with AE; this was the child taking inhaled steroids (Table 2).

Two of the nine children had already received a previous diagnosis of asthma or respiratory symptoms

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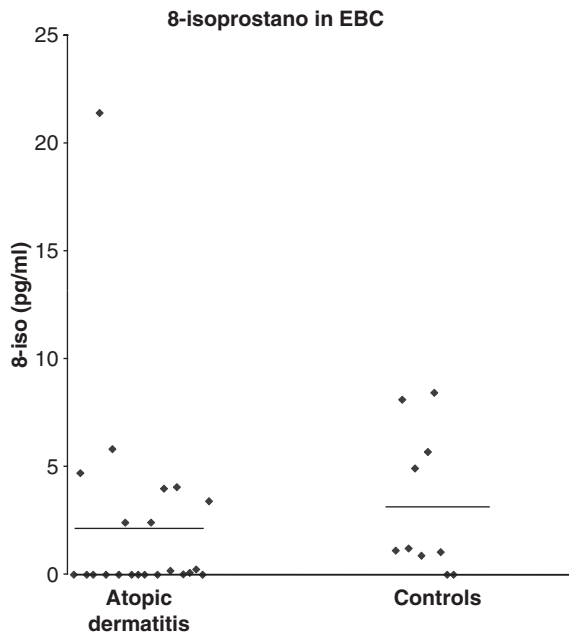


Figure 1 Distribution of 8-isoprostane in children with atopic eczema and controls. Horizontal lines indicate mean concentration of 8-isoprostane (P = not significant).

before the time of the test (Table 2, patients 2 and 7). Seven had had wheezing at some time in the past, five having had it during the past 12 months and during exercise. None of them had coughing during the night.

All of the nine children with AE had normal lung function indices (Table 2). The mean concentration of FE_{NO} was higher in children who had experienced wheezing in the past 12 months (71.2 ± 50.44 ppb) than in those who had not (47.4 ± 14.9 ppb), but this was not significant. There was no difference in FE_{NO} levels between children who reported at least four wheezing attacks/year (n = 5) and those who did not. FE_{NO} was significantly higher in children with severe AE compared with those with less severe AE (mild AE 50.06 ± 34 ppb, moderate AE 56.06 ± 21.5 ppb, severe AE 88.8 ± 73.8 ppb; $P = 0.012$).

No correlation was found between levels of FE_{NO} and 8-isoprostane (P = 0.98) in children with AE in whom both were measured.

Discussion

We found that values of 8-isoprostane in the EBC of children with AE were not different from those of control subjects. In contrast, we found raised levels of exhaled NO in children with AE, even in the absence of respiratory symptoms and in the presence of normal lung function tests.

To our knowledge, levels of 8-isoprostane in the EBC of children with eczema have not been previously reported. In contrast to previous studies,[18,19] we found that 8-isoprostane EBC was not enhanced in children with asthma. Several possibilities could explain such discrepancies. Firstly, low levels of 8-isoprostane EBC may reflect the existence of a subpopulation of children with asthma and AE in which the oxidative stress measured by EBC 8-isoprostane does not play a fundamental pathogenetic role in the airway inflammation. Secondly, we observed that 8-isoprostane values were below the detection limit in many children with AE, whether or not they had asthma. This could reflect a lack of sensitivity of the 8-isoprostane analysis method used.[19,20] Gas chromatography/mass spectrometry[4] may be a preferable method of analysis. Finally, there may be a risk of misclassification error of children with asthma, due to parental recall bias. However, we validated the questionnaire with a medical examination, and therefore we believe that our results are reliable.

Table 2 Peak expiratory flow rate, forced expiratory volume in 1 second, forced vital capacity, forced expiratory flow 75%, mean level of nitric oxide in the exhaled breath and severity of atopic dermatitis.

	Lung function					
Patient	PEFR, %	FEV_1, %	FVC, %	FEF_{75}, %	Mean FE_{NO}, ppb	AE severity
1	117	110	102	101	51.4	Moderate
2	104	103	110	68	9.8	Mild
3	79	104	102	83	88.8	Mild
4	118	109	125	140	66.9	Mild
5	124	105	118	58	79.6	Moderate
6	130	137	124	101	141	Severe
7	101	128	119	110	37.2	Mild
8	107	133	125	104	34.7	Moderate
9	108	112	100	109	36.6	Severe

AE, atopic eczema; FEF_{75}, forced expiratory flow at 75% of vital capacity; FEV_1, forced expiratory volume in 1 second; FE_{NO}, forced expiration of nitrous oxide; FVC, forced vital capacity, PEFR, peak expiratory flow rate; PPB, parts per billion.

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FE_{NO} is a marker of eosinophilic airway inflammation in children with asthma. Increased Fe_{NO} levels have been found in people with asthma[7] but not in children with wheezing.[21] We observed that in children with AE, the FE_{NO} levels were increased irrespective of the coexistence of episodes of wheezing or asthma. Our results are in agreement with the findings of Dinakar[9] in children aged < 3 years who had eczema but no asthma. In contrast, Profita *et al.*[8] showed that FE_{NO} levels were not increased in children with eczema who had a similar age to our population and who did not report asthma exacerbations in the previous 12 months. A possible explanation for our failure to see normal FE_{NO} values may be that we studied children who had more severe eczema than those included in the study by Profita *et al.*,[8] a possibility that is supported by our finding of higher FE_{NO} levels in children with more severe AE.

Mutually nonexclusive hypotheses can be offered to interpret the finding of increased FE_{NO} in children with eczema without asthma. The first is that the NO originated in the lungs, perhaps reflecting a mild degree of lung inflammation that was asymptomatic at the time of testing, and undetectable at that time in standard lung-function tests.[2,7] If this is correct, monitoring NO in children with AE could be a useful tool to predict future development of asthma. High levels of FE_{NO} might even justify pre-emptive treatment. The second possibility is that the NO originated in the skin itself in these children, entering the circulation and being expelled in the lungs. In support of this possibility, it has been found that iNOS is expressed in the skin of people with AE,[22] and that NO causes skin vasodilatation.[23] Furthermore, there are data suggesting reduction of erythema and oedema in AE by intradermal administration of NOS inhibitors in human skin.[24] However, this view is not supported by finding that NO has a very short half-life in plasma, being rapidly oxidized to the more stable NO_3,[25] which is increased in the serum of people with AE.[24]

In summary, the analysis of exhaled breath analysis in children with eczema shows that FE_{NO} but not 8-isoprostane EBC levels may enable identification of a population susceptible to asthma. Therefore, we intend to study the predictability of increased FE_{NO} levels in children with AE without asthma for the development of asthma in a follow-up population study.

Acknowledgement

We thank Dr W. Muller for technical support.

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