

Nasal and Bronchial Inflammation After Nasal Allergen Challenge: Assessment Using Noninvasive Methods

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■ Abstract

Background: Links between the upper and lower airways have been demonstrated in recent years. However, few studies have evaluated inflammation using noninvasive methods.

Methods: A nasal allergen challenge was performed with pollen outside the pollen season in 30 patients with allergic rhinitis due to pollen but no asthma. Clinical and inflammatory nasal and bronchial responses to nasal allergen challenge were evaluated using the nasal symptoms score (NSS), visual analog scale (VAS), nasal geometry (volume between 2 and 5 cm [Vol₂₋₅]) by acoustic rhinometry, lung function by spirometry, nasal nitric oxide (nNO), and exhaled nitric oxide (eNO). Values were recorded at baseline, 15 minutes, and 2 and 24 hours after challenge. Nasal lavage and exhaled breath condensate (EBC) samples were collected at 2 and 24 hours to assess 8-isoprostane, cys-leukotrienes, eosinophil cationic protein (ECP), tryptase, granulocyte-macrophage colony-stimulating factor, and interleukin (IL) 5.

Results: NSS and VAS increased significantly at 15 minutes and 2 and 24 hours after challenge. Vol₂₋₅ decreased significantly at 15 minutes and 2 hours, while nNO decreased at 15 minutes. All inflammatory mediators except ECP increased significantly at 2 hours in nasal lavage samples, while ECP, 8-isoprostane, and cys-leukotrienes increased at 24 hours ($P < .01$). In EBC, 8-isoprostane and cys-leukotrienes increased at 2 and 24 hours ($P < .01$). No significant changes were found at any time in lung function or eNO.

Conclusion: Nasal allergen challenge induces clinical and inflammatory responses in the nose and bronchi that can be assessed using noninvasive methods such as nasal lavage, EBC, and nNO.

Key words: Nasal and bronchial inflammation. Nasal allergen challenge. Noninvasive methods.

■ Resumen

Introducción: La interrelación inmuno-inflamatoria entre nariz-bronquios ha sido demostrada recientemente. Pocos estudios la han evaluado a través de métodos no invasivos.

Métodos: Se incluyeron 30 pacientes con alergia a polen. Se les realizó provocación nasal con alérgeno fuera de la época de polinización. Se evaluaron: puntuación de síntomas (NSS), escala visual analógica (VAS), volumen nasal entre los 2 y 5 cm (Vol₂₋₅) mediante rinometría acústica, función pulmonar mediante espirometría (FEV₁), óxido nítrico nasal (ONn) y exhalado (ONe) mediante quimioluminiscencia, a los 15 minutos, 2 y 24 horas tras la provocación. Se recogieron lavados nasales y condensado de aire exhalado antes y a las 2 y 24 horas de la provocación, en los que se determinaron niveles de: 8-isoprostano (8-iso), cisteinil leucotrienos, proteína catiónica de los eosinófilos (ECP), triptasa, factor estimulante de colonias de granulocitos/macrófagos, interleucina 5.

Resultados: La NSS y la VAS aumentaron a los 15 minutos y a las 2 y 24 horas. El Vol₂₋₅ se redujo a los 15 minutos y a las 2 horas. El ONn disminuyó a los 15 minutos. Todos los mediadores de inflamación, excepto la ECP, aumentaron a las dos horas en los lavados nasales, y esta misma, el 8-iso y los cisteinil leucotrienos lo hicieron a las 24 horas ($p < 0,01$). Estos dos últimos aumentaron en el aire exhalado condensado tanto a las dos como a las 24 horas ($p < 0,01$). No hubo cambios en la función pulmonar ni en el ONe.

Conclusión: La provocación nasal con alérgeno induce inflamación nasal y bronquial medible a través de métodos no invasivos como lavado nasal, aire exhalado condensado y ONn.

Palabras clave: Inflamación nasal y bronquial. Provocación nasal con alérgeno. Métodos no invasivos.

Introduction

Asthma and allergic rhinitis (AR) are inflammatory airway diseases in which inflammatory cell infiltration and production of inflammatory mediators are prominent. The epidemiological, immunological, and clinical features of both diseases are similar, as is their management [1,2]. Braunsthal et al [3-5] demonstrated the presence of bronchial inflammation after nasal allergen challenge (NAC) and nasal inflammation after segmental bronchial provocation. Their findings confirmed the concept that allergic inflammation induced at specific points on the mucosal surface tends to spread systemically. However, ethical issues surrounding the use of invasive methods such as bronchoscopy with bronchial biopsy make it very difficult to reproduce these results in studies.

Several noninvasive techniques have been used to evaluate nasal and bronchial inflammation in patients with AR [6,7]. Furthermore, immediate and late asthmatic responses (reduction in forced expiratory volume in the first second [FEV₁]) after NAC have been described in patients with AR and asthma [8].

The aim of the present study was to assess the presence of nasal and bronchial inflammation using noninvasive methods, such as nasal nitric oxide (nNO), exhaled nitric oxide (eNO), nasal lavage, and exhaled breath condensate (EBC) after NAC in patients with pollen-induced AR outside the pollen season.

Methods

Sample

The study sample comprised 30 patients (70% women) with a mean (SD) age of 27.7 (10.1) years and intermittent or persistent AR (ARIA classification [9]) due to pollen, with 2 or more years from onset and without asthma. Twenty-one patients (70%) were challenged with grass pollen, 3 (10%) with *Parietaria judaica*, 3 (10%) with *Olea europaea*, and 3 (10%) with *Platanus species* (Table 1).

Nasal Allergen Challenge

The challenge test was performed using solutions containing extracts of the relevant clinical allergens (Diater). Based on the positive skin prick test concentrations, 100 µL of the

trigger pollen extract was applied at progressive concentrations (1:100, 1:10, and 1:1). Nasal volume between 2 and 5 cm (Vol_{2.5}) was measured using acoustic rhinometry (SER 2000, RhinoMetrics) following a standardized technique [10-12]. NAC was considered positive when a reduction $\geq 30\%$ was reached. The allergen concentration needed to induce a positive response was recorded.

One week later, a second NAC was performed with the previously established allergen concentration. Vol_{2.5} was assessed at baseline, 15 minutes (with normal saline in each nostril to exclude nasal hyperreactivity), and at 2 and 24 hours.

Inflammatory Markers

nNO and eNO were assessed by chemoluminescence (N6008, SIR) [13,14], at baseline, 15 minutes, and 2 and 24 hours. The remaining inflammatory markers were assessed in nasal lavage samples (5 mL of saline in each nostril using a modified bladder catheter) and EBC (ANACON, Biostec) collected while breathing at a tidal volume of 200 L/min [15]. Cys-leukotrienes, tryptase, eosinophil cationic protein (ECP), granulocyte-monocyte colony stimulating factor (GM-CSF), 8-isoprostane (8-iso), and interleukin (IL) 5 were assessed by means of enzyme-linked immunosorbent assay (ELISA) kits following the manufacturer's instructions. The kits used were Human IL-5 ELISA (eBioscience), Amersham Leukotriene C4/D4/E4 Biotrak Enzymeimmunoassay (EIA) System (GE Healthcare), Amersham High Sensitivity Granulocyte-Macrophage Colony Stimulating Factor [(h)GM-CSF] human, Biotrak ELISA System (GE Healthcare), and the 8-Isoprostane EIA Kit (Cayman Chemical). Nasal lavage and EBC samples (1 mL of each) were freeze-dried (FTS Systems) and concentrated to determine levels of cys-leukotrienes, GM-CSF, 8-iso, and IL-5. In the case of cys-leukotrienes, 1 mL of sample was resuspended in 100 µL of buffer from the commercial kit. One milliliter from each sample was resuspended in 500 µL of buffer from the commercial kit in order to determine levels of GM-CSF, 8-iso, and IL-5.

Clinical Evaluation

The presence of nasal symptoms was assessed using a visual analog scale (VAS) and the nasal symptoms score (NSS), which analyzes itching, rhinorrhea, sneezing, and obstruction [9].

Table 1. Distribution by Type of Pollen and Concentration Needed for a Positive Nasal Challenge^a

Concentration for positive challenge		1:100	1:10	1:1
Pollen type (N=30)	Grass pollen (n=21)	9	9	3
	<i>Parietaria judaica</i> (n=3)	3	0	0
	<i>Olea europaea</i> (n=3)	1	1	1
	<i>Platanus</i> (n=3)	0	1	2

^aCarried out by applying 100 µL of the trigger pollen extract at progressive concentrations based on the positive skin prick test concentration.

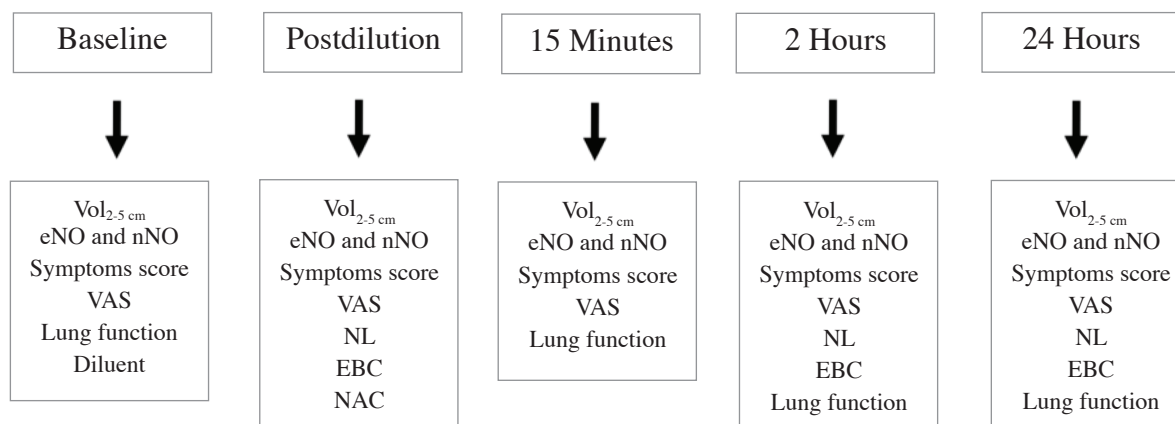


Figure. Summary of the study protocol. Vol_{2.5} indicates nasal volume between the second and fifth centimeters of the nasal cavity; eNO, exhaled nitric oxide; nNO, nasal nitric oxide; VAS, visual analog scale; NL, nasal lavage; EBC, exhaled breath condensate; NAC, nasal allergen challenge.

The protocol (Figure) was evaluated and approved by the Institutional Review Board at Hospital Clínic (Barcelona, Spain), and patients gave their written informed consent.

Statistical Analysis

For quantitative variables, the mean (SD), median, and range were calculated. Qualitative variables were expressed as relative frequencies. Descriptive analyses were used to summarize demographic data, baseline characteristics, and postbaseline measurements. The results for the efficacy variables were analyzed using the Wilcoxon signed rank test. Analysis of covariance was used to compare the mean change from baseline. All statistical tests were performed using SAS version 8.2 for Windows (SAS Institute Inc). *P* values less than .05 were considered statistically significant.

Results

NSS and VAS increased significantly at 15 minutes and 2 and 24 hours after challenge. Vol_{2.5} decreased significantly at 15 minutes and at 2 hours while nNO decreased at 15 minutes. There were no significant changes in eNO or lung function (Table 2).

All the inflammatory markers except ECP showed a significant increase at 2 hours in nasal lavage samples, while cys-leukotrienes and ECP increased at 24 hours. Levels of 8-iso and cys-leukotrienes in EBC increased significantly at 2 and at 24 hours (*P*<.01). Additional inflammatory markers were not detected in EBC (Table 3).

Table 2. Clinical Values^a

Parameter	Baseline	Postdilution	15 Minutes	2 Hours	24 Hours
VAS	30.3 (47.7)	22.5 (31.2)	178.3 (89.9) ^b	46.1(39.4) ^b	45.7 (73.3) ^b
NSS	1.5 (1.7)	1.3 (1.6)	9.1 (2.5) ^b	2.9 (2.1) ^b	2.7 (2.9) ^b
Vol _{2.5} , cm ³	5.6 (2.2)	5.6 (2.4)	3.1 (1.2) ^b	5.0 (2.3) ^c	5.3 (2.0)
nNO, ppb	1497 (765)	1579 (754)	754 (692) ^d	1261 (800)	1466 (1045)
eNO, ppb	15.8 (20.18)	16.0 (20.17)	15.5 (19.7)	15.6 (19.9)	15.7 (17.7)
FEV ₁ , L/min	95 (7.1)	ND	97 (5.9)	91 (11.4)	89 (12.7)
FVC, L	85 (8.6)	ND	91 (6.4)	88 (11.9)	85 (10.7)
Tiffenau index	102 (8.0)	ND	104 (5.9)	103 (7.9)	101 (9.8)

Abbreviations: eNO, exhaled nitric oxide; FEV₁, forced expiratory flow in the first second of expiration; FVC, forced vital capacity; ND, not determined; nNO, nasal nitric oxide; NSS, nasal symptoms score; VAS, visual analog scale.

^aValues are expressed as mean (SD)

^b*P*<.01

^c*P*<.03

^d*P*=.056

Table 3. Collected Volume and Inflammatory Mediators After Nasal Challenge

		Baseline	2 Hours	24 Hours
Nasal Lavage	Volume, mL	6.1 (0.9)	6.0 (0.9)	6.0 (0.9)
	Cys-leukotrienes	1.46 (0.3)	2.98 (0.4) ^a	2.3 (0.3) ^a
	8 Isoprostane	7.0 (1.7)	13.0 (2.7) ^a	14.8 (2.7) ^a
	GM-CSF	2.82 (0.5)	4.11 (0.6) ^a	3.97 (0.8)
	Interleukin 5	7.3 (2.6)	9.35 (3.8) ^a	8.58 (2.2)
	Tryptase	0.10 (0.07)	0.89 (0.35) ^a	0.19 (0.09)
	ECP	11.21 (2.4)	15.9 (5.0)	35.6 (10.7) ^a
Exhaled Breath Condensate	Volume, mL	2.7 (1.4)	2.6 (0.3)	2.6 (0.5)
	Cys-leukotrienes	0.46 (0.3)	2.41 (0.4) ^a	2.03 (0.4) ^a
	8 Isoprostane	0.95 (0.6)	4.45 (1.0) ^a	8.15 (3.6) ^a
	GM-CSF	ND	ND	ND
	Interleukin 5	ND	ND	ND
	Tryptase	ND	ND	ND
	ECP	ND	ND	ND

Abbreviations: ECP, eosinophilic cationic protein; GM-CSF, granulocyte-monocyte colony-stimulating factor; ND, not detected.

^a $P < .01$

Discussion

The present study demonstrates that NAC produces a clinical response in the nose that was evidenced by a significant reduction in nasal volume ($Vol_{2.5}$) and inflammatory nasal and bronchial responses measured using noninvasive methods (nasal lavage and EBC).

Several studies have used noninvasive methods to evaluate the presence of bronchial inflammation in AR patients [16-20], and a consensus report describing the different techniques and their usefulness has already been published [21]. However, few authors have used multiple evaluation methods in the same study [6,7]. Gratzou et al [6] used eNO and the presence of inflammatory markers in the EBC of patients with seasonal AR.

Pedroletti et al [20] found that natural exposure to seasonal allergens in patients with AR increased eNO levels, which were assessed as the only inflammatory marker. By contrast, Bergmann-Hug et al [18] reported that allergen challenge outside the pollen season did not induce this effect in adults or children, a finding that is consistent with our results, both for the challenge technique and for eNO. It seems that production of NO in the bronchial tree needs sustained exposure to the culprit allergen; however, no evidence exists to justify this hypothesis.

The only study to evaluate the effect of NAC on nNO is that of Boot et al [22], who observed a significant reduction in nNO levels at 20 minutes and a progressive increase that became statistically significant at 24 hours [22]. In the present study, we also found a significant reduction in nNO at 15 minutes after NAC, but not at 24 hours. The acute reduction after NAC could be explained by the obstruction of the ostiomeatal complex caused by edema and nasal secretions blocking the passage of NO from the paranasal sinuses (where it is produced) to the nasal cavity [23].

Inflammatory marker levels in induced sputum are increased after a single nasal challenge [19]. Inal et al [24] evaluated the presence of ECP and cys-leukotrienes in the induced sputum of children monosensitized to house dust mite who were analyzed according to the clinical expression of their allergic disease (rhinitis, rhinitis and asthma, asymptomatic atopsics, and nonallergic healthy controls) before and 24 hours after NAC. They found that levels of these mediators were significantly higher at 24 hours in the 2 groups with diagnosed disease and in the asymptomatic atopsics than in the healthy controls, suggesting that allergic inflammation is present before the onset of symptoms. In the present study, we did not include patients with different patterns of allergic disease or a control group. However, using different methods (sputum and EBC), both studies found that ECP levels were increased at 24 hours after NAC. Since we did not find any increase in ECP at 2 hours, our results suggest that this mediator, which can be detected in various fluid samples, should not be measured early after NAC.

Analysis of inflammatory markers in EBC has been proposed as an innovative diagnostic technique, not only in asthma research, but also in other respiratory diseases [25-29], although few studies have assessed bronchial inflammation using this method. Cap et al [16] analyzed the presence of leukotrienes B_4 and E_4 in EBC from patients with seasonal AR during the pollen season and 5 months after, finding significant differences with respect to controls. The same research group had reported similar results in a previous study, in which they also measured leukotrienes C_4 and D_4 , although they found no differences between seasonal and postseasonal levels [17]. Gratzou et al [6] evaluated the presence of 8-iso, leukotriene B_4 , and nitrate/nitrite in the EBC of patients with seasonal AR with or without asthma during and after the pollen season and found a significant increase in 8-iso and

leukotriene B₄ during the pollen season compared to controls, with normalization of values out of season. These 3 studies used natural exposure, while our study used a single NAC out of season in asymptomatic AR patients. Initially, no cyste leukotrienes (C₄, D₄, and E₄) or 8-iso were found in nasal lavage or EBC. Lyophilization and subsequent sample concentration were necessary to assess the different concentrations.

Finally, although the presence of an asthmatic response (reduction in FEV₁) after NAC in patients with AR and uncontrolled asthma has been reported [8], our patients did not develop early or late changes in lung function after nasal NAC. However, the increased bronchial inflammatory marker levels found after NAC were consistent with the absence of a correlation between lung function and inflammation, indicating that these assessments should complement each other.

The methods used in the present study and other methods such as induced sputum could serve to determine inflammatory mediators in the upper and lower airways. However, applicability in daily clinical practice remains low, because the techniques demand time and logistical preparation. Furthermore, the cost of the kits used to detect mediators is high. nNO and eNO are probably the most practical methods, and the cost of kits could fall in the future if they are more widely used. As for the mediators measured in nasal lavage samples and EBC, cyste leukotrienes and metabolites of oxidative stress (8-iso) seem to be detectable in both early and late phase reactions, suggesting that their increase in nasal lavage samples reflects their presence in the lower airways. However, these results should be reproduced in further studies.

In conclusion, using noninvasive methods such as nasal lavage, EBC, nNO, and eNO, we observed that NAC induced a clinical nasal response and both inflammatory nasal and bronchial responses (at 2 and 24 hours) in patients with AR due to pollen. We show that these methods could be useful in the study of nasal and bronchial responses after exposure to an allergen.

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