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Pathophysiology of airway hyperresponsiveness in patients with nasal polyposis

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Nasal polyposis;
Reactivity;
Sensitivity

Summary
Background: It has been hypothesized that airway hyperresponsiveness (AHR) is characterized by sensitivity (strength of stimulus) and reactivity (responsiveness to stimulus); the latter could be the intrinsic characteristic of AHR. The underlying mechanisms leading to AHR could be 1) airway inflammation, 2) reduction of forces opposing bronchoconstriction, and 3) structural airway changes/geometric factors.
Objective: Our main objective was to assess the relationships between reactivity in patients with nasal polyposis and these three mechanisms using measurements of 1) bronchial and bronchiolar/alveolar NO, 2) bronchomotor response to deep inspiration, and 3) forced expiratory flows and an index of airway to lung size, i.e. $FEF_{25-75\%}$/FVC.
Methods: Patients underwent spirometry, multiple flow measurement of exhaled NO (corrected for axial diffusion), assessment of bronchomotor response to deep inspiration by forced oscillation technique and methacholine challenge allowing the calculation of reactivity (slope of the dose–response curve) and sensitivity (PD_{10}).
Results: One hundred and thirty-two patients were prospectively enrolled of whom 71 exhibited AHR. Airway reactivity was correlated with alveolar NO concentration (rho = 0.35; $p = 0.017$), with airflow limitation ($FEF_{25-75\%}$: rho = −0.40; $p = 0.003$) and with an index of airway size to lung size ($FEF_{25-75\%}$/FVC: rho = −0.38; $p = 0.005$), of which only alveolar NO remained the only independent factor in a stepwise multiple regression analysis (variance 25%). Airway sensitivity was not correlated with any pulmonary function or exhaled NO parameter.

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Conclusion: In patients with nasal polyposis, alveolar NO is associated with airway reactivity, suggesting that bronchiolar/alveolar lung inflammation may constitute one intrinsic characteristic of increased responsiveness.

Introduction

Airway hyperresponsiveness (AHR), the tendency of the airways to narrow too much and too easily in response to various stimuli, is a universal feature of asthma, although it is not exclusive to this disease. Airway inflammation and structural airway changes can lead to this heightened airway response. Accordingly with these statements made by Boulet,[1] inflammation and structural changes might be associated with a change in smooth muscle mechanical properties and/or a reduction of forces opposing bronchoconstriction, such as reduced airway-parenchymal interdependence. Other factors, such as "geometric factors" (eg, airway caliber related to lung size), can also modulate the degree of AHR.[2] Nevertheless, the mechanisms by which all these potential factors modify airway function are still unclear. The two major functional components of AHR are airway reactivity and sensitivity, which can be obtained from a dose—response curve to methacholine. Accordingly to Sterk and Bel, reactivity could constitute the intrinsic characteristic of AHR (responsiveness of the airways to stimulus), while sensitivity could be related to extrinsic factors as allergic exposure (strength of triggering stimulus).[3] Consequently, one may hypothesize that the relationships between reactivity/sensitivity and the potential modifiers of AHR deserve to be studied.

Although the increase in exhaled NO commonly observed in atopic subjects with or without symptoms has classically been attributed to eosinophilic inflammation (one dimension of asthma), the relationships between fractional exhaled NO (FE_{NO}) and both AHR[4] and bronchodilator response[5] suggest a specific link between NO and another dimension of asthma, namely an increased airway tone. Consequently, one may also hypothesize that a link between exhaled NO and airway reactivity would be evidenced in patients with AHR. For this demonstration, partitioning of exhaled NO in its bronchial and bronchiolar/alveolar origins is mandatory since a single expiratory flow measurement at 50 mL/s mainly reflects the bronchial contribution to FE_{NO},[6] which is mainly linked to bronchial inflammation,[7] while alveolar NO should better represent airway responsiveness.[8,9]

The aim of our cross-sectional physiological study was to assess the relationships between airway reactivity/sensitivity and 1) bronchial/alveolar NO origins (using multiple flow exhaled NO measurement), 2) reduction of forces opposing bronchoconstriction (using bronchomotor response to deep inspiration [DI]), 3) structural airway changes/geometric factors (using forced expiratory flows and a crude assessment of airway to lung size, i.e. $FEF_{25-75\%}$/FVC).[2,10] These three pathophysiological factors were chosen because they may constitute three dimensions of airway hyperresponsiveness accordingly to Boulet,[1] even if there is some links between them.

Patients with nasal polyposis were enrolled because the prevalence of non atopic asthma is elevated, which may favour the discrimination of the role of NO that is not associated with allergic inflammation.

Patients and methods

Design

All consecutive patients suffering from nasal polyposis (diagnosis based on endoscopic examination and on computed tomography, as previously described [10]) referred for baseline pulmonary function testing were eligible with the exception of those suffering from another respiratory disease than asthma or from a severe cardiac disease. Patients were divided according to the presence of AHR, and further divided in symptomatic (asthmatic) and asymptomatic subjects. Our patient database has been declared to our regulatory agency for computer data collection (Commission Nationale Informatique et Libertés, *n°* 1391593v0), and approval from our local Ethics Committee was obtained. All patients were informed of the prospective recording of clinical and physiological data.

Diagnosis of confirmed asthma

The diagnosis of confirmed asthma was based on the fact that symptoms of recurrent episodes of airflow obstruction and AHR (based on PD_{20}) were both present, and alternative diagnoses were excluded, as recommended by GINA guidelines.

Pulmonary function tests

The tests were conducted in the following order and are summarized in Fig. 1.

Exhaled nitric oxide measurements

Exhaled NO was measured using a chemiluminescent nitric oxide analyser (ENDONO 8000, Seres, Aix en Provence, France) before performing spirometry (Fig. 1). Maximum

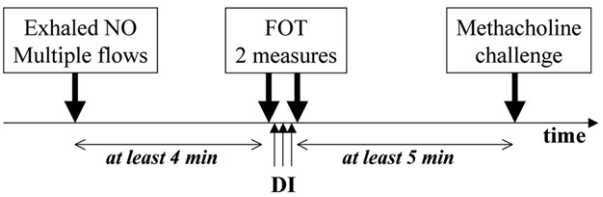

Figure 1 Sequence of investigations. The bronchomotor effect of deep inspiration (DI) is of short duration (median 65 s [range, 35—120 s]) as previously demonstrated.[8]

conducting airway flux of NO (J'_{awNO}) and bronchiolar/alveolar NO concentration (C_{alvNO}) were calculated after obtaining several exhaled NO measurements at different expiratory flow rates, using the previously described linear approach.[11] We used a validity criterion of this linear approach.[11] Then, NO exchange parameters were corrected for axial NO back diffusion according to the method of Condorelli and colleagues.[12]

Forced oscillation technique measurement of bronchomotor response to deep inhalation

Respiratory impedance was determined using the standard forced oscillation technique (Oscilink; Datalink-MSR, Rungis, France) according to recommendations.[13] The real component of respiratory impedance [resistance of the respiratory system (Rrs)] was subjected to linear regression analysis to obtain the intercept (R0, resistance extrapolated to 0 Hz). The effect of three deep inspirations was calculated as 100*(R0 after inspiration - R0 before inspiration)/R0 before inspiration, as previously described.[10] A positive result indicates a bronchoconstrictor effect of deep inhalation.

Spirometry and methacholine inhalation challenge

These tests (using MasterScreen Body, Jaeger, CareFusion) were conducted according to international recommendations.[14,15] Anti-asthma drugs were withheld according to recommendations.[14] Non-specific airway responsiveness to methacholine was measured using successive doses from 0.05 to 2.4 mg (0.05, 0.20, 0.40, 0.80, 1.6 and 2.4 mg), delivered using a nebulization dosimeter (Aero Doseur Atomisor, Diffusion Technique Française, Saint-Etienne, France) as previously described.[10] The test was stopped if there was a $\geq$20% decline in forced expiratory volume at 1 s (FEV_1) from the control value or if the maximum cumulative dose had been reached. The change in FEV_1 as a percentage of the reference value has been plotted on the ordinate against the log concentration on the abscissa. In patients with AHR, PD_{20} and PD_{10} (sensitivity) were calculated using interpolation (after log-linear regression), and the slope of the dose–response curve defined reactivity. Reference values for spirometry were those of Stanojevic and colleagues.[16]

Statistical analysis

Values are expressed as mean $\pm$ SD or median [interquartile range] as appropriate. Between groups comparisons used Student-t test, except when stated. Correlations were evaluated using Spearman's rank correlation coefficient. The association between the different explanatory variables and the dependent variable was examined in a multiple linear regression model using the procedure for general linear models (with log-transformed values for non normally distributed variables). The multivariate analysis was performed with a backward selection method and variables with P values of less than 0.10 were retained in the FE_{NO} model. We further performed a multivariable logistic regression analysis to evaluate AHR pathophysiology. Since our main aim was restricted to patients with AHR we decided to include at least 120 patients with nasal polyposis in order to have a group with AHR $>$ 50 (based on the prevalence of AHR in this setting [10]), allowing multivariate analyses including a maximum of four independent variables (10–15 patients/variable). Statistical significance was defined as $P < 0.05$. Data were analysed using Statview 5.0 (SAS Institute, Berkeley, CA, USA).

Results

The characteristics of the 132 patients enrolled are described in Table 1. Seventy-one patients exhibited AHR. These patients, as compared to those exhibiting no AHR, had an increased alveolar NO concentration, increased constrictor response to DI and a lower lung function.

Relationships between airway reactivity/sensitivity and potential dimensions of AHR

Airway reactivity was correlated with alveolar NO concentration (Fig. 2), with airflow limitation ($FEF_{25-75\%}$: rho = -0.40; $p = 0.003$), with an index of airway size to lung size ($FEF_{25-75\%}$/FVC: rho = -0.38; $p = 0.005$), and was not correlated with response to DI ($p = 0.23$).

Airway sensitivity was not correlated with any pulmonary function or exhaled NO parameter.

PD_{20} was not significantly correlated with $FENO_{0.05}$, alveolar NO and bronchial NO.

We then assessed whether the third potential dimension of AHR (response to DI) was linked to another dimension of AHR. The bronchomotor effect of DI was correlated with structural airway changes/geometric factor index (airflow limitation $FEF_{25-75\%}$: rho = -0.30; $p = 0.020$ and airway to lung size $FEF_{25-75\%}$/FVC: rho = -0.26; $p = 0.043$).

Comparison of symptomatic and asymptomatic subjects with AHR

The Table 2 describes the patients with AHR according to the presence of asthmatic symptoms. Asthmatic patients had similar levels of exhaled NO, but exhibited a lower lung function and higher degrees of airway reactivity and sensitivity.

We further evaluated in the whole population the pathophysiology of AHR using a logistic regression model with AHR as dependent variable and log of alveolar NO, $FEF_{25-75\%}$, $FEF_{25-75\%}$/FVC and symptoms (asthma) as independent variables that demonstrated that both alveolar NO ($p = 0.048$) and $FEF_{25-75\%}$ ($p = 0.004$) remained independently associated with AHR (r^2 of the model = 0.18) while $FEF_{25-75\%}$/FVC and symptoms were not independent predictors of AHR ($p = 0.072$ and $p = 0.066$, respectively).

Discussion

The main result of this cross-sectional study is the suggestion that airway reactivity is linked to some extent to bronchiolar/alveolar NO (corrected for back diffusion) in patients with nasal polyposis. The statistical significance of the relationship is weak ($\sim$25% of variance of the model), implying that other factors contribute to airway reactivity

Table 1 Characteristics of the patients with nasal polyposis according to the presence of AHR.

Characteristics	Whole population $N = 132$	With AHR $N = 71$ (54%)	Without AHR $N = 61$ (46%)	*P* value
Female (%)	81 (61)	39 (55)	42 (69)	NS
Age, yrs	48 ± 14	50 ± 14	46 ± 13	0.08
BMI, kg m^{-2}	24.5 ± 3.9	24.1 ± 3.7	24.9 ± 4.0	0.27
Never-smokers, *n* (%)	78	42	36	NS
Ex-smokers, *n* (%)	35	19	16	NS
Current-smokers, *n* (%)	19	10	9	NS
Tobacco, Pack-year $	16 ± 12	19 ± 15	14 ± 9	0.14
Asthma, *n* (%)	16 (12)	15 (28)	1	
age at asthma onset, years	17 [9–45]	18 [9–45]	12	
Exhaled NO				
linearity[a], *n*	122	64	58	NS
$FENO_{0.05}$, ppb	25.4 [14.2–34.6]	26.4 [14.8–44.5]	21.5 [14.1–31.4]	0.098
C_{alvNO}, ppb	4.6 [2.2–8.3]	5.4 [2.8–10.7]	3.8 [1.4–6.9]	0.018
J'_{awNO}, pL.s-1	1374 [652–2437]	1587 [538–3017]	1275 [793–1983]	0.59
PFT				
FEV_1, % predicted	100 ± 19	94 ± 21	106 ± 14	0.0001
sVC, % predicted	102 ± 17	100 ± 18	109 ± 13	0.004
FEV_1/sVC	0.76 ± 0.09	0.73 ± 0.11	0.79 ± 0.06	0.0006
FEF_{25-75}, % predicted	79 ± 30	69 ± 31	91 ± 26	<0.0001
$FEF_{25-75\%}$/FVC	0.73 ± 0.28	0.67 ± 0.32	0.79 ± 0.21	0.013
R0 rs, cmH2O	3.27 ± 1.66	3.57 ± 1.87	2.91 ± 1.29	0.024
R0 rs, % predicted	129 ± 63	139 ± 72	118 ± 47	0.060
Response to DI, % increase	+6 [−4 − +19]	+13 [+1 − +24]	+4 [−8 − +13]	0.019
Methacholine challenge test				
Reactivity (slope), %/μg		0.053 [0.015–0.117]		
Sensitivity (PD_{10}), μg		128 [48–317]		
PD_{20}, μg		372 [174–1325]		

The *P* values are related to the comparisons of patients with and without AHR.
A dose of methacholine of 2.4 mg or less causing a 20% fall in FEV_1 (PD_{20}) was used to identify AHR.
$FENO_{0.05}$ (ppb) denotes fractional exhaled nitric oxide obtained at a constant expiratory flow rate of 0.05 L/s.
[a] $FENO_{0.05}$ has been obtained in the whole population, but the linearity of relationship between expiratory flow rate and NO output was not observed for the whole population (122/132, 92%), accordingly to our previous results.[11]

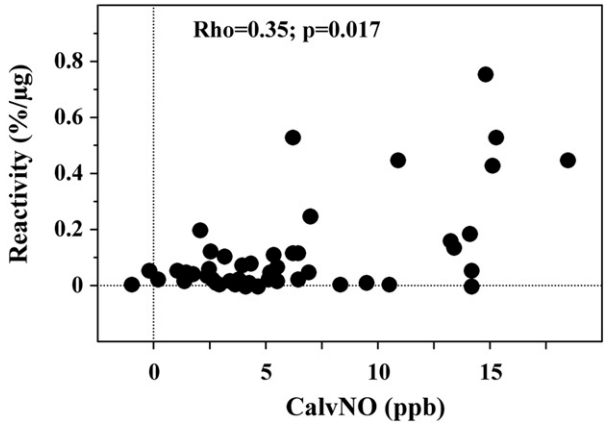

Figure 2 Relationship between alveolar NO and airway reactivity in patients with airway hyperresponsiveness. The X axis is alveolar NO concentration (Calv,NO ppb, corrected for axial diffusion), while the Y axis is airway reactivity (slope of the dose–response curve of methacholine challenge test).

obviously. We further suggest that occurrence of symptoms is related to both a higher degree of airway reactivity/sensitivity and decreased airway to lung size ratio and/or airway remodeling.

Airway reactivity and sensitivity are the two major underlying mechanisms of AHR. In 1989, Sterk and Bel reviewed the mechanisms of airway sensitivity and airway reactivity.[3] They proposed that airway sensitivity is determined by the strength of the stimulus that triggers the airways to narrow. Determinants of airway sensitivity include epithelial damage and malfunction, neural control, inflammatory cell number/activity, interactions among these factors, and altered metabolism or absorption of inflammatory mediators. Accordingly, several studies support the view of Sterk and Bel that the degree of cellular inflammation, as measured by the sputum eosinophil count, is related to airway sensitivity[17] but conflict with the results of others.[18] Since eosinophilic airway inflammation and exhaled NO are usually linked,[7] a correlation between exhaled NO and sensitivity should be expected. Nevertheless, the former relationship is weak, and the relationship between FENO and AHR has inconsistently been demonstrated.[19–21] In our series, FENO measured at 0.05 L/s was not significantly correlated with PD_{20} in the patients with

Table 2 Characteristics of the 71 patients with AHR according to the presence of asthmatic symptoms.

Characteristics	With asthma $N = 15$ (21%)	Without asthma $N = 56$ (79%)	P value[a]
Female (%)	10 (67)	22 (39)	0.081
Age, yrs	42 ± 13	46 ± 13	0.018
BMI, kg m^{-2}	24.3 ± 4.3	24.1 ± 3.6	0.79
Exhaled NO			
$FENO_{0.05}$, ppb	27.5 [19.7–44.1]	25.7 [12.4–51.9]	0.68
C_{alvNO}, ppb	5.8 [4.3–14.8]	5.3 [2.6–9.5]	0.25
J'_{awNO}, pL s^{-1}	1558 [623–3173]	1587 [453–3003]	0.83
PFT			
FEV_1, % predicted	81 ± 16	98 ± 21	0.001
sVC, % predicted	97 ± 18	100 ± 18	0.56
FEV_1/sVC	0.68 ± 0.08	0.75 ± 0.11	0.012
$FEF_{25-75\%}$, % predicted	46 ± 18	75 ± 31	0.0005
$FEF_{25-75\%}$/FVC	0.51 ± 0.16	0.71 ± 0.34	0.013
Response to DI#, % increase	+19 [+1 − +32]	+12 [0 − +21]	0.31
Methacholine challenge test			
Reactivity (slope), %/μg	0.186 [0.081–0.473]	0.035 [0.010–0.083]	0.001
Sensitivity (PD_{10}), μg	48 [21–58]	171 [64–369]	0.004
PD_{20}, μg	103 [43–174]	619 [202–1388]	0.0003

A dose of methacholine of 2.4 mg or less causing a 20% fall in FEV_1 (PD_{20}) was used to identify AHR.
$FENO_{0.05}$ (ppb) denotes fractional exhaled nitric oxide obtained at a constant expiratory flow rate of 0.05 L/s.
[a] using Mann Whitney U test due to the restricted size of the group of asthmatic patients. The P values are related to the comparisons of patients with and without asthma.

AHR. At this expiratory flow rate, FENO mainly originates from its bronchial source, which may explain this lack of significance since no significant correlation was evidenced between maximum NO flux from airways and airway sensitivity. Furthermore, nasal polyposis constitutes a specific setting in which the prevalence of non allergic asthma is elevated.

As for airway reactivity, Sterk and Bel regarded this variable to represent the responsiveness of the airways to the stimulus given.[3] Determinants of airway reactivity include airway smooth muscle contractility, viscous and elastic loads on muscle shortening, swelling of the airway wall, and intralumenal exudate and secretions. To our best knowledge this is the first study that shows a significant relationship between bronchiolar/alveolar NO and airway reactivity, which is relevant from a pathophysiological point of view. Airway reactivity may constitute an intrinsic characteristic of asthma disease, being linked to smooth muscle cell physiology. We previously showed that NO inhibition decreased the airway tone of ex vivo human bronchi in COPD[22] suggesting that NO may surprisingly be involved in bronchoconstriction, which is further supported by the recent demonstration of a link between bronchiolar/alveolar NO and bronchodilator response in asthma.[9] An increase in bronchiolar/alveolar NO may be present in up to 25% of asthmatic patients.[23] We also evidenced a relationship between bronchiolar/alveolar NO and remodeling ($FEF_{25-75\%}$),[7,24] suggesting that its increase may represent a marker of severity rather than control. Accordingly, the degree of AHR is usually in proportion to the severity of the underlying asthma.[25] Computational modeling of the tracheobronchial tree has suggested that increased airway wall thickness has the potential to cause increased reactivity and maximal narrowing without changing sensitivity,[26] which may further explain the observed relationship between reactivity and remodeling in our study. Recent studies have emphasized to potential clinical relevance of reactivity/sensitivity determination. For instance, in patients with stable asthma, bronchial reactivity (but not sensitivity) has been associated with health-related quality of life, and it has also been shown that the adolescents with asthma remission may show a significant decrease of reactivity, whereas sensitivity was not changed.[27,28]

The relationship between an index of airway to lung size as $FEF_{25-75\%}$/FVC and the degree of AHR is well-established in the general population.[2] Obviously, this index is a crude assessment of airway to lung size ratio,[29] which is also sensitive to remodeling. Our results further suggest that this relationship is also valid in the subgroup of patients with nasal polyposis and AHR, and that this pathophysiological link is associated with reactivity rather than sensitivity to methacholine. We further show in the whole population that AHR pathophysiology relies on both alveolar NO and an index of airway size ($FEF_{25-75\%}$), while logically the presence of symptoms (asthma) was not an independent factor of AHR, logically.

Deep inspirations are known to exert strong beneficial effects on the airways of healthy humans. These effects appear to be of dual nature: bronchoprotective and bronchodilatory. The bronchoprotective effect of deep inspiration is lost in asthma.[30] It is also lost in individuals with rhinitis and AHR, but no asthma.[31] Therefore, it has been postulated that the loss of bronchoprotection could mainly be related to AHR.[31] No relationship is evidenced in our study between the bronchomotor effect of DI and both exhaled NO (alveolar, bronchial) and AHR (reactivity,

sensitivity) parameters, but this bronchomotor effect was related to an index of airway to lung size, thereby potentially indirectly contributing to AHR.

Clinical perspectives

It has been shown that distal NO production is insensitive to inhaled corticosteroid in asthma,[32] which may explain the non significant effect of steroid on AHR.[14] Since the association of smaller airways and higher degree of AHR may explain the occurrence of asthmatic symptoms (see Table 2), accordingly with the statement of Boulet,[1] specific therapies targeting bronchiolar/alveolar spaces could be warranted.

Study limitations

First, the degree of significance of the observed relationships are weak (rho values 0.35 to 0.40), which underlines that other factors are involved, obviously. These relationships do not imply causality, which may be inferred from experimental studies.[22] Tobacco smoking may have biased our results to some extent, even if smoking history do not greatly impact alveolar NO fraction.[33] We only evaluated the bronchomotor response to DI, and not the bronchoprotective role of DI, which could give different results. Given the small number of patients with asthma ($n = 15$), our study was underpowered to confidently assess the potential confounding role of asthmatic patients on the relationship between AHR and exhaled NO parameters. Finally, our results have been obtained in a specific context, nasal polyposis, and remain to be confirmed in a broader set of patients with AHR. Nevertheless, our demonstration is important since the presence of AHR constitutes a severity factor in patients with nasal polyposis.[34]

In conclusion, in patients with nasal polyposis and airway hyperresponsiveness, increased airway reactivity is associated with increased alveolar NO, suggesting that bronchiolar/alveolar lung inflammation may constitute one intrinsic characteristic of asthma.

Authorship

BM, LP and CD2 acquired the data. BM, PB, and CD2 conceived this study. BCB and DM performed the statistical analyses. All authors have drafted the submitted article or revised it critically for important intellectual content and have provided final approval of the version to be published.

Conflict of interest

All the authors declare no competing interest. The authors alone are responsible for the content and writing of the paper.

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