

ORIGINAL ARTICLE

Effects of cigarette smoke on methacholine- and AMP-induced air trapping in asthmaticsLuis Prieto, PhD¹, Julio Palop, MD², Ruth Llusar, MD³, Susana Herrera, MD², Carmen Perez-Frances, PhD³, Amparo Lanuza, PhD³, and Daniela Aguilar, MD³¹Department of Medicine, University of Valencia, Medicina, Spain, ²Department of Pulmonary Diseases, and ³Department of Allergology, Doctor Peset University Hospital, Valencia, Spain**Abstract**

Objective: No information is available on the effect of cigarette smoke on bronchoconstrictor-induced air trapping in asthma. The aim of this study was to evaluate the additional influence of smoking on methacholine- and adenosine 5'-monophosphate (AMP)-induced air trapping in subjects with asthma. **Methods:** Airway responsiveness to methacholine and AMP, bronchial (J'aw_{NO}) and alveolar (CA_{NO}) nitric oxide (NO) and exhaled breath condensate pH were measured in 68 adults (23 current smokers with asthma, 23 non-smokers with asthma and 22 current or former smokers with chronic obstructive pulmonary disease; COPD). The degree of air trapping induced by each bronchoconstrictor agent was expressed by the percent fall in forced vital capacity (FVC) at a 20% fall in forced expiratory volume in 1 s relative to FVC after saline inhalation (Δ FVC%). **Results:** The Δ FVC% for AMP was higher in both smokers with asthma and patients with COPD than in non-smokers with asthma ($p < 0.001$). By contrast, Δ FVC% for methacholine was similar in the three groups of subjects ($p = 0.69$). In smokers with asthma, but not in the other two groups, there was a correlation between the residual volume/total lung capacity at baseline and the Δ FVC% induced by each bronchoconstrictor agent. Mean values for J'aw_{NO} were higher in non-smokers with asthma than in the other two groups ($p < 0.05$). **Conclusions:** The results of this study suggest that factors underlying bronchoconstriction induced by indirect agonists are different in smokers and non-smokers with asthma. These observations might be clinically relevant, because triggers that frequently induce bronchial obstruction in the real world act by an indirect mechanism.

Keywords

Adenosine 5'-monophosphate, air trapping, exhaled nitric oxide, methacholine, smoking

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It is now widely accepted that airway hyperresponsiveness (AHR), defined as the tendency of the airway to bronchoconstrict in response to many nonsensitizing stimuli, may occur both in asthma and chronic obstructive pulmonary disease (COPD) [1,2]. Methacholine and histamine are the most frequently used spasmogens for the investigation of AHR; these two agonists cause bronchoconstriction by a direct effect on airway smooth muscle cells. In contrast, inhaled adenosine 5'-monophosphate (AMP) acts indirectly, causing "primed" mast cell degranulation and the release of histamine and other mediators with subsequent smooth muscle contraction [3].

During routine bronchoprovocation procedures, the response is generally expressed as the provocation concentration of agonist that caused a decrease in forced expiratory volume in 1 s (FEV₁) of 20% (PC₂₀ or sensitivity). It has long

been appreciated, however, that during bronchoconstriction induced by acetylcholine or methacholine, residual volume (RV) increases [4,5] and forced vital capacity (FVC) decreases [6]. The conventional explanation for why the FVC decreases during induced bronchoconstriction is that change in the smooth muscle tone causes an increase in air trapping. Assuming that during induced bronchoconstriction, total lung capacity (TLC) remains constant [7], this air trapping could be measured by the dose-dependent decline in FVC. It has been suggested that changes in FVC may reveal information about the underlying asthma pathogenesis that is not apparent from the changes in FEV₁ [6]. In particular, small airway abnormalities lead to peripheral heterogeneity of the ventilation and to premature airway closure and air trapping. Thus, air trapping can be considered a marker of small airway involvement.

On the other hand, exhaled nitric oxide (NO) levels and acidification of exhaled breath condensate (EBC) have been proposed as markers of airway inflammation in asthma [8,9]. Moreover, peripheral airway inflammation can be indirectly examined by measuring NO concentrations in exhaled air at different flow rates [10]. Using this method, it is possible to discriminate between the bronchial and alveolar NO levels

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and, at least theoretically, to evaluate peripheral airway inflammation.

Asthma patients who smoke have greater asthma severity [11] and reduced sensitivity to the beneficial effect of treatment with inhaled corticosteroids [12] compared to asthma patients who do not smoke. The mechanisms accounting for these adverse effects of smoking in asthma are poorly understood but are likely to be due to differences in airway inflammation in smokers compared to nonsmokers with asthma. In line with this suggestion, studies have found increased numbers of neutrophils [13] and mast cells [14] in the lower airways of smokers with asthma compared to never-smokers. Given the relevant role of bronchial mast cells in AMP-induced bronchoconstriction, one may predict that bronchial challenge with inhaled AMP could provide a better tool for detecting inflammatory changes induced by smoking. In addition, several studies demonstrated that, both in patients with COPD and nonasthmatic subjects with allergic rhinitis, cigarette smoke is associated with increased airway sensitivity (lower PC₂₀ value) to methacholine [15,16] and AMP [17,18]. By contrast, smoking has an apparent protective effect on airway responsiveness in subjects with asthma [19,20]. Although these differences in the effect of smoking among different populations may be explained by the well-described self-selection bias in cross-sectional studies, they may also indicate that inflammatory changes in the airways induced by smoking determine the AMP responsiveness in COPD and allergic rhinitis, but not in allergic asthma. Despite the extensive research performed to evaluate the effect of smoking on airway sensitivity to direct and indirect bronchoconstrictor agents, no information is available on the effect that cigarette smoke has on methacholine- and AMP-induced air trapping in subjects with asthma. Given these knowledge gaps in the literature, we hypothesized that smokers with asthma would experience greater air trapping in response to inhaled AMP than non-smokers with asthma. We also hypothesized that AMP-induced air trapping would be similar in smokers with asthma and patients with COPD. Consequently, the primary aim of this study was to evaluate the additional influence of smoking on methacholine- and AMP-induced air trapping, as reflected by changes in FVC, in subjects with asthma. Secondary objectives were to explore the effect of smoking on bronchial NO (J'aw_{NO}), alveolar NO (CA_{NO}) and EBC pH.

Materials and methods

Subjects

A total of 68 subjects (23 current smokers with asthma, 23 non-smokers with asthma and 22 current or former smokers with COPD) were included in the study. Asthma was identified by the presence of asthmatic symptoms (recurrent episodes of wheezing, dyspnea, chest tightness and cough) plus AHR with a methacholine PC₂₀ of less than 8 mg/ml if the FEV₁/FVC was 70% or greater or an improvement of the FEV₁ from predicted of 15% or greater after 200 µg of inhaled salbutamol if the FEV₁/FVC was less than 70%. Non-smokers were individuals who had never smoked; current smokers were those who smoked at least one cigarette a day. The inclusion criteria of patients with COPD were aged between

40 and 70 years, a history of chronic respiratory symptoms (dyspnea, productive cough or both) and a documented absence of reversible airflow obstruction in the past (increase in FEV₁ < 12% and ≤ 200 ml following 400 µg of inhaled salbutamol). All asthmatics and subjects with COPD had baseline FEV₁ ≥ 60% of predicted normal, and no subjects had respiratory tract infections during the four weeks before the study. Subjects with reduced, single-breath CO diffusion capacity were not included. At the time of this study, all patients were in a stable clinical condition. The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from all the participants.

Study design

The study had an open label design. Subjects attended the laboratory on three visits. On the *first visit*, subject characteristics were documented by a general questionnaire and pulmonary function tests were performed. At each of the *next two visits* (7–10 days apart), spirometry, concentration-response studies with either methacholine or AMP, exhaled NO and EBC pH were performed. Exhaled NO and EBC pH measurements were carried out before spirometry and challenge tests. The methacholine and AMP challenges were performed in random order. Before measurements, patients were asked to refrain from smoking for at least 2 h, but no other restrictions were placed on the smoker's smoking pattern. Short-acting inhaled beta₂-agonists were withheld for at least 6 h before the study, long-acting inhaled beta₂-agonists, long-acting muscarinic antagonists and oral antihistamines for at least 72 h, and inhaled and nasal topical corticosteroids for at least four and two weeks, respectively. No other medications for nasal or pulmonary symptoms were used.

Methods

Inhalation challenge tests

Bronchial challenge tests were performed using a modified dosimeter method [21] as previously reported [22]. Methacholine (Provocholine, Diater, Madrid, Spain) and AMP (Sigma Chemical, St Louis, MO) were dissolved freshly in 0.9% saline solution to produce doubling concentration ranges of 0.095–25 mg/ml for methacholine and 1.56–800 mg/ml for AMP. Each solution was administered from a jet nebulizer attached to a breath-activated dosimeter (Mefar MB3; Mefar, Brescia, Italy) at a nebulization time of 1.5 s and an activation pressure of 2.0 bar. With these conditions, the nebulizer showed an output of 18 µl per actuation and, therefore, the subject was exposed to approximately 90 µl of aerosol (18 µl/actuation × 5 breaths) at each concentration. Following baseline spirometry, FEV₁ and FVC were measured 60–90 s after inhalation of isotonic sodium chloride and each concentration of agonist. The FVC manoeuvre was continued until a plateau in the volume–time curve was achieved by visual inspection, and the minimum duration of the FVC manoeuvre was 6 s. The test was interrupted when a 20% decrease in FEV₁ from the post-saline solution administration value was recorded or when the highest concentration was administered.

NO measurements

Exhaled NO was measured with a chemiluminescence technique (NiOx, Aerocrine, Stockholm, Sweden) according to ATS/ERS standards [23], with the exception of the choice of exhalation flow rates, which were 100, 200 and 300 ml/s. To enable the subjects to achieve the target flow rate, dynamic flow resistors were used. The exhalation times varied from 6 to 10 s, depending on the exhalation flow rate, and exhaled NO was always measured during the last 3 s of the exhaled NO maneuver. The average of two values performed at each of the three flow rates was recorded. The technique of Tsoukias and George [10] was used to calculate $J'aw_{NO}$ (pl/s) (y intercept) and steady-state CA_{NO} concentration (ppb) (slope) using a linear regression. CA_{NO} was adjusted for the trumpet shape of the airway tree and axial diffusion from central to peripheral airways (CA_{NOcor}) with the following equation [24]:

$$CA_{NOcor}(\text{ppb}) = CA_{NO}(\text{ppb}) - J'aw_{NO}(\text{pl/s})/860.$$

Lung function

Pulmonary function tests including spirometry, plethysmographic lung volumes and diffusing capacity for carbon monoxide were measured according to standardized guidelines [25].

Measurement of EBC pH

EBC was collected using the RTube collection system (Respiratory Research Inc., Charlottesville, VA) during tidal breathing for 10 min without a nose clip. A 200 µl sample of EBC was then deaerated bubbling argon at 350 ml/min for 8 min, before pH measurement. The pH was measured [26] using a thin and sensitive glass electrode (Metrohm AG, Herisau, Switzerland) and pH meter (model pH 900), with an accuracy of ± 0.01 pH.

Statistical analysis

The primary outcome of the study was the AMP-induced air trapping. Secondary outcomes were the methacholine-induced air trapping, exhaled NO (CA_{NO} and $J'aw_{NO}$) and EBC pH. The sample size requirement of this study was calculated considering a difference of 5% in the AMP-induced drop in FVC as clinically relevant. Based on a calculated SD of 4% for the AMP-induced fall in FVC from previous studies [27], it was estimated that 60 patients (20 in each study group) would be required to provide 90% power at the two-sided 0.05 level.

The provocative concentration of agonist required to produce a 20% fall in FEV_1 (PC_{20}) was calculated using an algebraic formula [28]. Occurrence of air trapping during airway narrowing was determined by the percent fall in FVC at the PC_{20} value relative to FVC after saline inhalation ($\Delta FVC\%$), calculated using log-linear interpolation [6]. With this approach, the percent fall in FVC became the dependent variable (response), and the degree of airway narrowing (20% fall in FEV_1) became the independent variable.

A PC_{20} methacholine value of 25 mg/ml was assigned to seven patients with COPD in whom FEV_1 dropped $<20\%$

even when the highest concentration of methacholine was administered. Furthermore, PC_{20} AMP could not be calculated in seven patients with COPD and two smokers with asthma. On these occasions, the PC_{20} value was censored to the highest concentration of AMP given (800 mg/ml). In these subjects with censored PC_{20} values, $\Delta FVC\%$ was expressed as the % fall in FVC following the inhalation of the highest concentration of each agonist. Methacholine PC_{20} values are not normally distributed because demonstrate a skewed distribution with a long tail into the high range of values. Consequently, all PC_{20} values were log-transformed before analysis and are presented as geometric means with 95% confidence intervals (CIs). Differences in PC_{20} between the groups are expressed in terms of doubling concentrations of methacholine or AMP calculated as $\Delta \log PC_{20} / \log 2$. All other numerical variables are reported as arithmetic means with 95% CIs. Differences among the groups were assessed using one-way analysis of variance and the Student *t* test. Categorical variables were analyzed with the Fisher's exact test. Linear regression analysis was used when appropriate, and the Pearson test was used to investigate correlations. The null hypothesis was rejected at $p < 0.05$. All analyses were performed with GraphPad Prism 5.0 for Windows (GraphPad Software, Inc, La Jolla, CA).

Results

Demographic and baseline characteristics of the patients participating in the study are listed in Table 1. As expected, our patients with asthma were significantly younger ($p < 0.01$) and were more often female ($p < 0.001$) than patients with COPD. Furthermore, subjects with COPD had a higher RV/TLC than non-smokers with asthma ($p < 0.05$). There were no differences between the two groups with asthma in demographic and pulmonary function parameters.

Differences in methacholine- and AMP-induced air trapping

The $\Delta FVC\%$ for AMP was higher in both smokers with asthma and patients with COPD than in non-smokers with asthma (Table 2; Figure 1), the mean difference (95% CI) being 6.0% (2.4–9.7%, $p < 0.001$) for smokers vs non-smokers with asthma and 6.2% (2.5–9.8%, $p < 0.001$) for subjects with COPD vs non-smokers with asthma. Differences in $\Delta FVC\%$ for AMP between smokers with asthma and subjects with COPD were not significant, the mean difference being -0.1% (95% CI: -3.8 to 3.5%). By contrast, $\Delta FVC\%$ for methacholine was similar in the three groups (Table 2; Figure 1), the mean difference (95% CI) being 1.2% (-2.9 to 5.3%) for smokers vs non-smokers with asthma, -0.1% (-4.3 to 4.0%) for smokers with asthma vs COPD and 1.3 (-2.8 to 5.5%) for non-smokers with asthma vs COPD.

Differences in methacholine and AMP PC_{20} values

Methacholine PC_{20} values were higher in subjects with COPD than in either non-smokers or smokers with asthma (Table 2), the mean difference being 1.8 doubling concentrations (95% CI: 0.3–3.3 doubling concentrations, $p < 0.05$) for subjects with COPD vs smokers with asthma and 2.1 doubling

Table 1. Subject characteristics by groups^a.

Characteristics	Asthma non-smokers	Asthma smokers	COPD	<i>p</i>
Subjects	23	23	22	
Age, year	45.3 (39.0–51.7)	39.0 (34.0–43.8)	58.9 (55.6–62.4)	<0.001
Male/female gender	7/16	7/16	20/2	<0.0001
Duration of symptoms, year	10.9 (5.8–16.0)	11.9 (6.2–17.5)	5.2 (2.8–7.5)	0.09
Body mass index, kg/m ²	27.9 (26.1–29.8)	25.5 (23.5–27.5)	28.1 (25.9–30.3)	0.12
Atopy (yes/no)	19/4	20/3	–	0.97
Smoking history (pack-years)	–	37.7 (29.6–45.8)	41.9 (32.9–51.0)	0.36
FEV ₁ , % predicted	87.9 (82.3–93.4)	87.4 (81.5–93.3)	82.0 (74.2–89.7)	0.34
FVC, % predicted	108.4 (102.5–114.3)	104.8 (99.8–109.9)	107.4 (99.8–115.0)	0.68
RV/TLC, % predicted	111.9 (98.7–119.9)	116.2 (101.1–123.1)	129.6 (112.8–139.2)	0.03
DL _{CO} , % predicted	90.3 (85.2–96.4)	88.9 (84.0–94.2)	86.7 (81.5–90.7)	0.23

^aData are presented as no or mean (95% confidence intervals).
FEV₁ = forced expiratory volume in 1 s; FVC = forced vital capacity; RV = residual volume; TLC = total lung capacity; and DL_{CO} = diffusing capacity for carbon monoxide.

Table 2. Methacholine- and AMP-induced air trapping and airway sensitivity in the three groups.

Variables	Asthma non-smokers	Asthma smokers	COPD	<i>p</i>
ΔFVC% ^a				
Methacholine	13.2 (10.9–15.5)	14.4 (11.7–17.1)	14.5 (12.0–17.1)	0.69
AMP	9.3 (7.4–11.1)	15.3 (12.6–18.0)	15.4 (13.5–17.4)	<0.001
PC ₂₀ , mg/ml ^b				
Methacholine	1.7 (0.8–3.6)	2.1 (1.1–4.0)	7.5 (4.5–12.5)	0.003
AMP	46.9 (18.5–118.9)	65.3 (26.2–162.9)	134.3 (61.2–294.4)	0.21

PC₂₀ = concentration of agonist that causes a 20% fall in FEV₁; ΔFVC = fall in FVC at the PC₂₀ value; and AMP = adenosine 5'-monophosphate.

^aData are given as mean (95% confidence intervals).
^bData are given as geometric mean (95% confidence intervals).

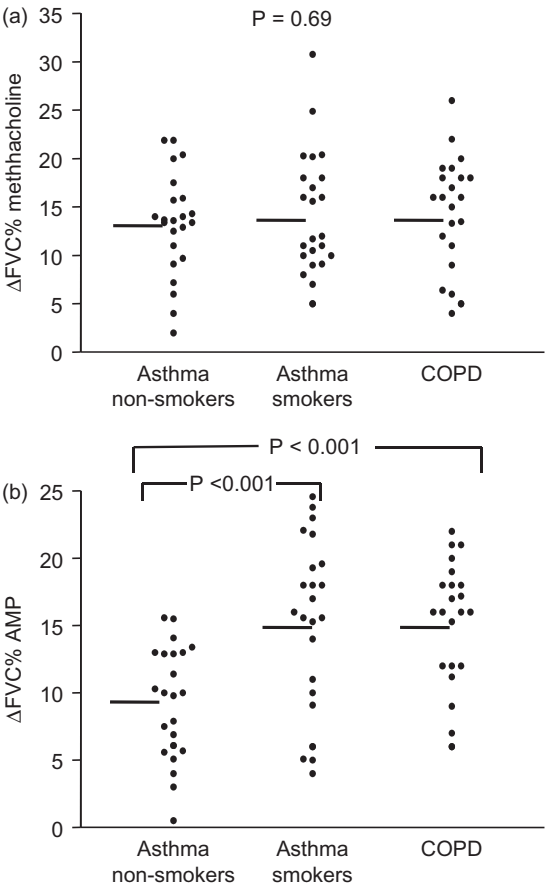


Figure 1. Individual values for the fall in FVC at the PC₂₀ value (ΔFVC) obtained with (a) methacholine and (b) AMP in smokers and non-smokers with asthma and subjects with COPD. Horizontal lines indicate means.

concentrations (0.6–3.7 doubling concentrations, $p < 0.01$) for subjects with COPD vs non-smokers with asthma. Differences in methacholine PC₂₀ values between smokers and non-smokers with asthma were not significant (Table 2), the mean difference (95% CI) being 0.3 doubling concentrations (–1.2 to 1.8 doubling concentrations). AMP PC₂₀ values were similar in the three groups, the mean difference (95% CI) being 0.47 doubling concentrations (–1.6 to 2.6 doubling concentrations) for smokers vs non-smokers with asthma, 1.0 doubling concentrations (–3.1 to 1.1 doubling concentrations) for smokers with asthma vs COPD and 1.5 doubling concentrations (–0.57 to 3.7 doubling concentrations) for non-smokers with asthma vs COPD.

Differences in NO and EBC pH

Mean values for J'aw_{NO} were higher in non-smokers than in smokers with asthma (Table 3; Figure 2), the mean difference being 2143 pl/s (95% CI: 302–3983 pl/s, $p < 0.05$). These values were also higher in non-smokers with asthma than in subjects with COPD, the mean difference being 1763 pl/s (95% CI: 102–3627, $p < 0.05$). Differences in J'aw_{NO} between smokers with asthma and subjects with COPD were not significant, the mean difference being –379 pl/s (95% CI: –2245 to 1485 pl/s).

Values for CA_{NO} were similar in the three groups (Table 3; Figure 2), the mean difference (95% CI) being 0.48 ppb (–0.99 to 1.96 ppb) for smokers vs non-smokers with asthma, 0.15 ppb (–1.34 to 1.64 ppb) for smokers with asthma vs subjects with COPD and –0.32 ppb (–1.82 to 1.17 ppb) for non-smokers with asthma vs subjects with COPD.

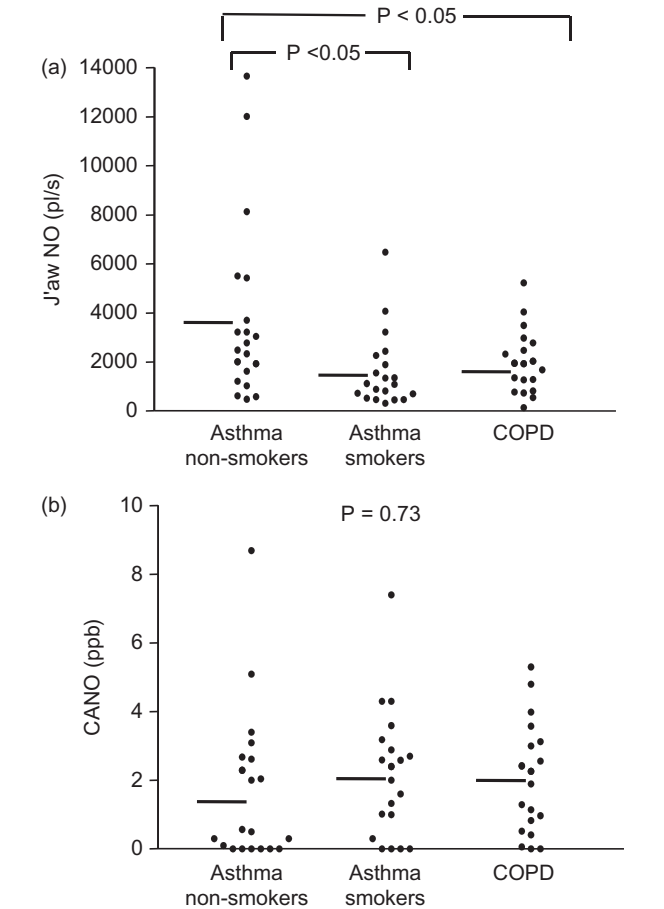


Figure 2. Individual values for (a) J'awNO and (b) CA'NO in the three groups of subjects studied. Horizontal lines are means.

Table 3. J'awNO, CA'NO and EBC pH values in the three groups studied^a.

Variables	Asthma non-smokers	Asthma smokers	COPD	<i>p</i>
J'awNO, pl/s	3757 (2050–5464)	1614 (905–2324)	1994 (1368–2620)	0.02
CA'NO, ppb	1.7 (0.6–2.7)	2.2 (1.3–3.0)	2.0 (1.2–2.8)	0.73
EBC pH	7.08 (6.83–7.34)	6.84 (6.35–7.33)	6.50 (6.02–6.97)	0.13

^aData are given as mean (95% confidence intervals). EBC = exhaled breath condensate; J'awNO = bronchial nitric oxide; and CA'NO = alveolar nitric oxide.

Values for EBC pH were also similar in the three groups (Table 3), the mean difference (95% CI) being –0.24 (–0.91 to 0.43) for smokers vs non-smokers with asthma, 0.34 (–0.35 to 1.03) for smokers with asthma vs subjects with COPD and 0.58 (–0.10 to 1.27) for non-smokers with asthma vs subjects with COPD.

Correlations

In smokers with asthma, there was a correlation between the ΔFVC% induced by methacholine and AMP ($r=0.83$, $p<0.0001$). By contrast, this correlation was not detected in non-smokers with asthma ($r=0.05$, $p=0.81$) and patients with COPD ($r=0.18$, $p=0.43$). Furthermore, in this group of smokers with asthma, but not in the other two groups, a correlation was found between the RV/TLC at baseline and

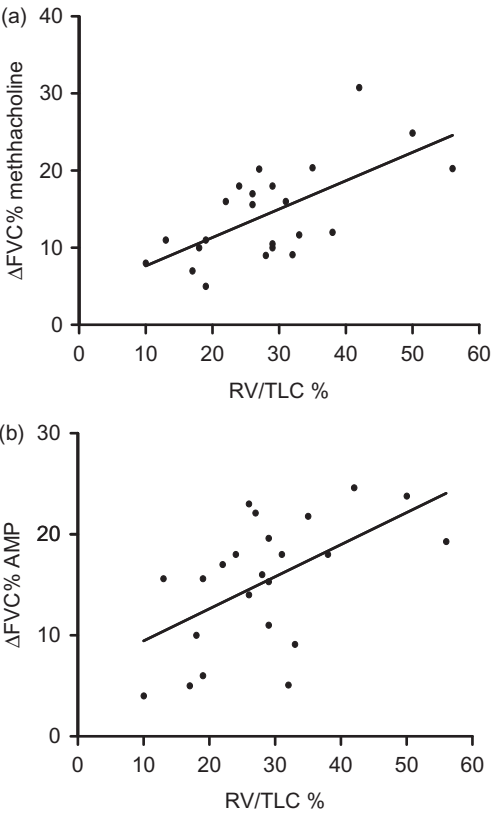


Figure 3. Correlations between RV/TLC at baseline and ΔFVC% induced by (a) methacholine and (b) AMP in smokers with asthma.

the ΔFVC% induced by each agonist ($r=0.65$, $p=0.0008$ for methacholine and $r=0.55$, $p=0.006$, for AMP, Figure 3).

No correlation could be identified between the ΔFVC% in response to each bronchoconstrictor agent and either NO (J'awNO and CA'NO) or EBC pH values.

Discussion

Our data show that, when are stimulated by inhaled AMP, both smokers with asthma and patients with COPD suffer greater air trapping than non-smokers with asthma. Interestingly, we additionally show that the degree of air trapping induced by AMP is similar in the group of smokers with asthma and patients with COPD. By contrast, inhaled methacholine induces a similar degree of air trapping in the three groups of subjects studied. Actually, the relationship between baseline air trapping, expressed as the RV/TLC, and AMP-induced air trapping was significant only in smokers with asthma, suggesting possible different mechanisms of bronchoconstriction in smokers and non-smokers with asthma.

This study fills a gap regarding the effect of smoking on bronchoconstrictor-induced air trapping in subjects with asthma. In our group of subjects with asthma, inhaled AMP caused greater air trapping in smokers than in non-smokers. By contrast, methacholine-induced air trapping was similar in these two groups of subjects with asthma. It has been demonstrated that air trapping reflects small airway closure [6] and, therefore, the results of this study support the findings of Michilis et al. [29] who showed that, in patients with

asthma, inhaled AMP elicits a response in the lung periphery, whereas methacholine induces a response in less peripheral airways. Given that inhaled AMP induces bronchoconstriction causing “primed” mast cell degranulation [3], the greater effect of inhaled AMP on distal airways is in line with previous investigations that reported higher number of mast cells in small vs large airways [30].

Furthermore, in our study, the degree of air trapping identified during the bronchoconstriction induced by AMP was similar in the group of smokers with asthma and patients with COPD. These findings would support the opinion that mechanisms determining airway narrowing during bronchoconstriction induced by indirect agonists are similar in smokers with asthma and subjects with COPD. In addition, these results suggest that pathophysiological mechanisms that generate air trapping and the resulting lung hyperinflation develop earlier in smokers than in non-smokers with asthma. Previous investigations have demonstrated that stimulation of airway smooth muscle is a crucial determinant of bronchoconstriction in subjects with asthma, whereas this is less important for the induction of airway narrowing in patients with COPD [31]. In this latter group of subjects, the increased bronchoconstrictive response may result from structural changes in the lower airways, such as airway wall thickening [32] and reduced loads on airway smooth muscle [6]. Given the similarities in the bronchoconstrictive response generated by AMP in smokers with asthma and patients with COPD, it is tempting to speculate that structural changes in the airways might be a major determinant of bronchoconstriction in smokers with asthma. It has been demonstrated that air trapping in asthma is associated with small airway closure or near closure [33,34], but the mechanisms and the location of airway closure are not well understood. A force that opposes small airway narrowing and closure is airway-parenchymal coupling. Reduction of airway-parenchymal coupling, due either to reduced lung elastic recoil or to an uncoupling of the tethering force from the airway lumen, could be a contributing factor to air trapping in asthma [35,36]. Comparing to non-smoking asthma patients, smokers have more emphysematous changes [13]. Therefore, a significant proportion of the airflow limitation in smokers with asthma could be attributed to reduced lung elastic recoil. In this study, the elastic recoil of the lung could not be determined and, therefore, possible effects of differences in the external elastic load on airway smooth muscle cannot be evaluated. Pulmonary emphysema was, however, unlikely to be present in our subjects, as those with reduced CO diffusion capacity were not selected.

Despite these remarkable differences in AMP-induced changes in FVC between smokers and non-smokers with asthma, change in FEV₁ induced by AMP (PC₂₀ value) was similar in both groups. The significance of these findings is that FVC and FEV₁ may be altered differentially by the same indirect bronchoconstrictor agent. Thus, a significant change in FVC could discern an underlying difference in pathophysiology of acute bronchoconstriction that would not be apparent from the FEV₁.

Similar to findings by Boulet et al. [13], we have found that methacholine-induced bronchoconstriction is associated with a similar degree of air trapping in smokers and non-smokers with asthma. In addition, our finding that inhaled

methacholine induces a similar degree of air trapping in asthmatics and subjects with COPD is apparently discordant with those previously obtained by Milanese et al. [37]. These investigators reported that bronchoconstriction induced by methacholine was associated with greater air trapping in subjects with COPD than in asthma. The reasons for such discrepancies might be related, at least in part, to differences in subject characteristics. Pulmonary function parameters at baseline were significantly lower in the group of subjects with COPD studied by Milanese et al. [37] than in those selected for this study. Mean baseline FEV₁ was normal in our three groups of patients, reflecting the fact that we deliberately selected patients with mild to moderate asthma or COPD. The reason was to obtain similar baseline values for FEV₁ and FVC in the three populations studied. However, the findings of this study may have been different in a more severe asthmatic population. Furthermore, our patients with COPD were significantly older than subjects with asthma. Although the effect of age on AMP responsiveness has not been studied, we cannot exclude that differences in AMP-induced air trapping between asthmatics and subjects with COPD might be explained, at least in part, by differences in age.

Another parameter analyzed in our study was airway inflammation, assessed by bronchial NO, alveolar NO and EBC pH. Although bronchial NO was significantly higher in nonsmokers with asthma than in the other two groups, we think this can be explained simply by considering the negative effect of smoking on exhaled NO [38]. However, it has been reported that activation of neutrophils is associated with decreased exhaled NO values, due to generation of peroxynitrite [39]. Thus, another possible explanation for decreased bronchial NO values in our group of smokers with asthma could be related to the smoking-induced neutrophilic inflammation. The reported absence of differences in alveolar NO between our group of smokers and non-smokers with asthma represents another argument suggesting that the decrease in bronchial NO occurs as a consequence of a selective effect of smoking on bronchial NO production.

On the other hand, EBC pH values were similar in smokers and non-smokers with asthma. This finding appears to contradict those of a previous study performed by Boulet et al. [13] who reported an increased acidity of EBC in smoking asthma patients. There are obvious differences between the study by Boulet et al. [13] and this study that might explain the discrepancies. Asthmatic subjects studied by Boulet et al. [13] had a smoking history significantly lower than those included in this study. In addition, EBC samples in the study by Boulet et al. [13] were obtained using a frozen sampling syringe, whereas we used the RTube. This is important, because it has been reported that EBC pH value is dependent on the collection device used [40]. In addition, in our group of subjects with COPD, EBC pH tended to be more acidic than in non-smokers with asthma, but differences did not reach statistical significance ($p = 0.13$). Previous investigations have reported that EBC acidification is predominantly detected in subjects with severe COPD [41]. Therefore, no inference about the EBC pH values in patients with greater COPD severity should be made on the basis of the results of this study.

Although the clinical implications of differences in air trapping between the three populations remain speculative, our observation of a significant correlation between baseline air trapping (reflected by RV/TLC) and experimentally-induced air trapping (reflected by changes in FVC induced by an indirect bronchoconstrictor, such as AMP) in the group of smokers with asthma would suggest that these different responses may be important in real life because triggers that frequently induce bronchial obstruction in the real world, such as exercise and allergens, act by an indirect mechanism. In addition, it has been reported that air trapping is a frequent finding in subjects with severe asthma [42] and develops early in patients with COPD [4]. Therefore, the determination of air trapping in response to inhaled AMP might be useful to identify those smoking asthmatic patients at risk of develop COPD. This should be confirmed, however, in future prospective studies.

Conclusions/key findings

The results of this study suggest that the factors underlying AHR to indirect bronchoconstrictor agents seem to be different in smokers and nonsmokers with asthma. These differences may be important in real life because triggers that frequently induce bronchial obstruction in the real world, such as exercise and allergens, act by indirect mechanism.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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