

Dissimilarity between Seasonal Changes in Airway Responsiveness to Adenosine-5'-Monophosphate and Methacholine in Patients with Grass Pollen Allergic Rhinitis: Relation to Induced Sputum

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Key Words

Airway hyperresponsiveness · Allergic rhinitis ·
Grass pollen · Sputum · Methacholine ·
Adenosine-5'-monophosphate

Abstract

Background: In patients with allergic rhinitis, bronchial hyperresponsiveness (BHR) and airway inflammation may increase during pollen exposure. BHR can be assessed by adenosine-5'-monophosphate (AMP) or methacholine challenge. It has been suggested that BHR to AMP is more closely related to airway inflammation than BHR to methacholine. Seasonal allergic rhinitis offers a dynamic model to study changes in BHR and airway inflammation during natural allergen exposure. **Meth-**
ods: We measured BHR [provocative concentration causing a 20% fall (PC_{20}) in forced expiratory volume in 1 s (FEV_1)] to AMP and methacholine, and induced sputum cells in 16 rhinitis patients before and during the 2001 grass pollen season. **Results:** There was a decrease in PC_{20} methacholine during pollen exposure (geometric mean PC_{20} from 3.22 to 1.73 mg/ml, $p = 0.0023$), whereas no reduction was observed for PC_{20} AMP ($p = 0.11$). There was no increase in sputum eosinophils [pre: 0.69% (95% confidence interval 0.22–2.07); during: 1.85 (0.55–

5.6), $p = 0.31$]. Although the correlation of log PC_{20} methacholine and log PC_{20} AMP at baseline was good ($r = 0.76$, $p = 0.001$), individual seasonal changes (doubling concentrations) in PC_{20} methacholine were not correlated with changes in PC_{20} AMP ($\rho = 0.21$, $p = 0.44$). There was no correlation between baseline log PC_{20} methacholine or seasonal changes in PC_{20} methacholine and sputum eosinophils ($p > 0.12$, all correlations). In contrast, there was a significant correlation between seasonal changes in PC_{20} AMP and changes in sputum eosinophils ($\rho = -0.59$, $p = 0.025$). **Conclusions:** These data show dissimilarity between seasonal changes in PC_{20} AMP and methacholine in patients with seasonal allergic rhinitis. Moreover, PC_{20} AMP seems to be more closely related to sputum eosinophils than PC_{20} methacholine. The clinical significance of this discrepancy is unclear.

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Introduction

Bronchial asthma is predominantly characterized by eosinophilic inflammation of the lower respiratory tract [1]. Atopic sensitization to aeroallergens remains the commonest single risk factor for the development of asthma,

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and affected patients frequently suffer from coincident allergic rhinitis. However, allergic rhinitis itself may predispose to incident asthma, since epidemiologic data suggest that as many as 30–80% of patients with allergic rhinitis occasionally develop asthma [2]. Increased bronchial hyperresponsiveness (BHR) and eosinophilic airway inflammation can be found even in patients with seasonal allergic rhinitis (SAR) only upon pollen exposure [3, 4]. Hence, SAR offers a model of natural exposure to study changes in BHR in correlation with inflammatory indices. BHR can be assessed by direct, e.g. methacholine, or indirect stimulation, e.g. adenosine-5'-monophosphate (AMP) with a bronchoconstrictor. While methacholine directly contracts airway smooth muscle, the precise mechanisms by which AMP elicits bronchoconstriction are not completely understood. AMP challenge stimulates mast cells mainly via activation of adenosine A2a receptors, leading to mast cell degranulation and mediator release [5]. A growing body of evidence suggests that BHR to AMP may reflect airway inflammation more accurately than BHR to methacholine [6, 7]. It has therefore been proposed that BHR to AMP should be preferred as a clinical outcome parameter in trials with anti-inflammatory drugs (e.g. corticosteroids).

Polosa et al. [8], have studied the correlation between BHR to AMP and induced sputum and exhaled nitric oxide in SAR patients during pollen exposure and observed a close correlation between PC₂₀ AMP, but not PC₂₀ methacholine with sputum eosinophils. However, this study was designed as a cross-sectional analysis, and did not include natural allergen exposure during the pollen season. In the present study, we have therefore evaluated dynamic changes in BHR to AMP and methacholine, and induced sputum and plasma inflammatory markers in SAR patients before and during pollen exposure. We report broad discrepancies between changes in PC₂₀ methacholine and PC₂₀ AMP in correlation with induced sputum inflammatory cells.

Methods

Patients

The study population consisted of 16 patients (46% males, mean age 39 years, range 24–53) with a history of SAR due to grass pollen allergy, confirmed by positive skin prick tests and specific IgE of CAP-FEIA-RAST (Pharmacia, Freiburg, Germany) class ≥ 2 against grass allergen. All patients were nonsmokers, had a negative history of any asthmatic symptoms, e.g. wheezing or cough during pollen seasons, and a history of prior use of β -agonists, and were free of other active allergic or respiratory diseases, such as acute or chronic infectious rhinitis, respiratory infection or atopic dermatitis at the

time of the investigation. None of the patients had active perennial allergic rhinitis during the study. A small proportion of patients (4/16) also had positive skin tests to perennial allergens, in particular house dust mite. The median total IgE was 143 U/ml (range 7–453), and the median RAST class for grass pollen was 3 (range 2–6). For symptom relief, all patients used short- or medium-acting antihistamines (e.g. loratadine or fexofenadine) during the pollen season. Patients were asked to withhold their antihistamines for at least 12 h prior to airway challenge procedures. None of the patients were using nasal, inhaled or systemic corticosteroids throughout the pollen season.

The investigational design complied with local regulatory authorities; all patients were informed about the purpose of the challenge procedures and sputum induction and gave written informed consent.

Overall Investigational Design

Bronchial challenges with AMP and methacholine were performed on two consecutive days at baseline (at least 2 weeks before the expected start of the 2001 grass pollen season) and during the pollen season. Sputum induction was performed after each methacholine challenge, or whenever possible, on a third day. This procedure was adopted in order to avoid any influence of challenge procedures on sputum cells as much as possible, since AMP [9], but not methacholine challenge [10], can alter sputum cells, at least within 1 h. Airborne pollen counts were followed via information from the German Pollen Information Service, and all challenges during the pollen season were performed at the time of high ambient pollen counts for at least 6 weeks after the start of the pollen season in May 2001.

Methacholine and AMP Challenge

Methacholine and AMP challenge were performed according to ATS guidelines [11] and a previously published method [12] using a five-breath dosimeter protocol (Mefar nebulizer, Italy). Doubling concentrations of methacholine or AMP were inhaled, and the procedure was stopped when a 20% fall in forced expiratory volume in 1 s (FEV₁) was observed, or the final concentration was reached (32 mg/ml methacholine and 800 mg/ml AMP, respectively). Purified methacholine (Provocholine®) was obtained from Metapharm, Canada, and purified AMP was purchased from Sigma Chemicals, Heidelberg, Germany.

Sputum Induction and Processing

Sputum induction was performed according to a previously described method [13]. 400 nonsquamous cells were then independently counted by two blinded observers. The relative proportion of cell types was counted and expressed as a percentage of all nonsquamous cells.

Statistical Analysis

Statistical analysis was performed using STATA 5.0 (Stata Corp., Texas, USA) for personal computers. Unless otherwise stated, data are given as geometric means with 95% geometric confidence intervals (CI) for 16 patients. Due to an inadequate sample quality in 2 patients, paired sputum values were only available in 14 patients. Seasonal changes in PC₂₀ AMP and PC₂₀ methacholine are reported as doubling concentrations [11]. In patients who did not demonstrate a 20% fall in FEV₁ after the final concentration of AMP, an arbitrary PC₂₀ of 800 mg/ml AMP was used for the calculations. Individual

Fig. 1. Individual and geometric mean (thick bars) seasonal changes in airway responsiveness (PC_{20}) to methacholine (**a**) and AMP (**b**). * $p = 0.0023$.

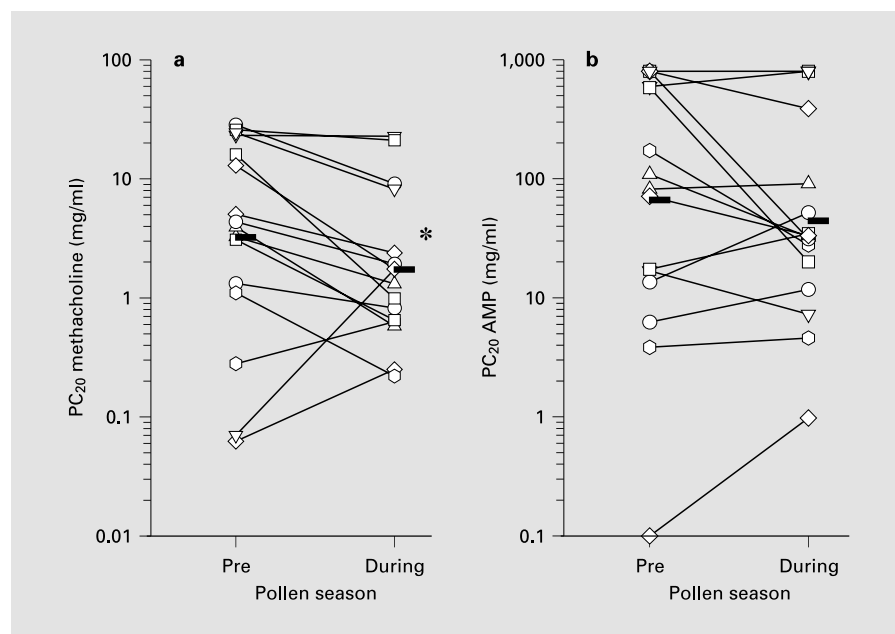


Table 1. Lung function ($n = 16$) and sputum parameters ($n = 14$) before and during the grass pollen season

	Before the season	During the season	p value
FEV ₁ , % predicted	85 ± 2	84 ± 1	0.21
FEF _{25–75} , % predicted	91 ± 4	88 ± 4	0.25
Total cells, × 10 ⁶ /ml	1.82 (1.15–2.88)	1.75 (1.07–2.81)	0.47
Neutrophils, %	19.6 (13.5–28.2)	15.5 (9.8–24)	0.36
Macrophages, %	66 (55–77.6)	66 (56–77.6)	0.49
Eosinophils, %	0.69 (0.22–2.07)	1.85 (0.55–5.6)	0.31
Lymphocytes, %	4.03 (2.75–5.88)	5.2 (4.36–6.6)	0.06

Lung function data (FEV₁ and FEF_{25–75}) are presented as arithmetic mean ± standard error. Sputum data are presented as geometric means with 95% geometric confidence intervals.

pre-post values were compared by paired nonparametric Mann-Whitney analysis. Correlations between data were calculated by Pearson's correlation after log transformation of PC_{20} methacholine, PC_{20} AMP and sputum eosinophils. Nonparametric correlations between seasonal changes in PC_{20} AMP, PC_{20} methacholine and sputum cells were calculated by Spearman's rank correlation (ρ). A p value of less than 0.05 was considered statistically significant. Based on prior observations in similar patients [14], and assuming an intra-class correlation coefficient of at least 0.8 for the methacholine protocol used, the study had at least 80% power to detect a change of one doubling concentration of PC_{20} methacholine with a one-sided α -level of 0.05 (Sample Power 2.0, SPSS).

Results

Spirometry and BHR to Methacholine and AMP

There was no change in baseline pulmonary function before and during the pollen season (table 1). The mean preseasonal PC_{20} methacholine and PC_{20} AMP were 3.22 mg/ml (95% CI 1.11–9.33) and 66.6 mg/ml (17–257), respectively. There was a significant decrease in PC_{20} methacholine (1.73, 0.81–3.6 mg/ml, $p = 0.0023$), but not in PC_{20} AMP (44.5, 16–125.9 mg/ml, $p = 0.36$) during the grass pollen season (fig. 1a, b). There was a sig-

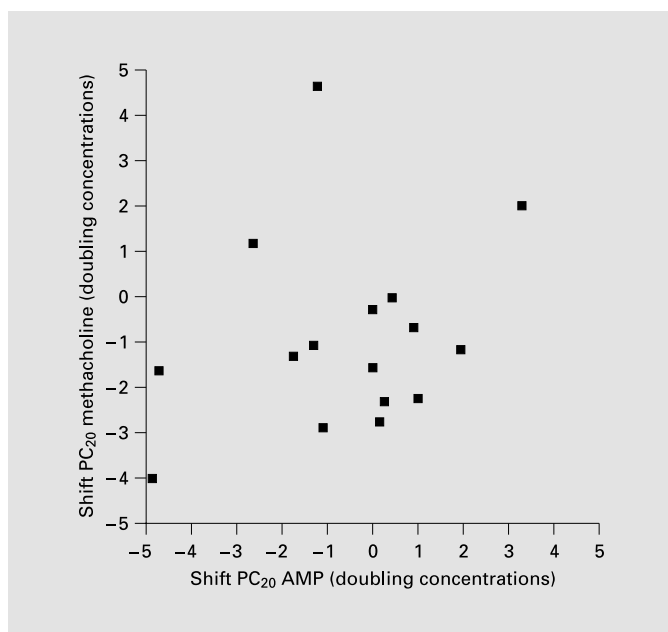


Fig. 2. Absence of correlation between seasonal changes (doubling concentrations) in airway responsiveness to methacholine and AMP. $\rho = 0.21$, $p = 0.44$.

nificant correlation of log PC₂₀ methacholine and log PC₂₀ AMP before ($r = 0.76$, $p = 0.001$), and during the pollen season ($r = 0.79$, $p < 0.001$), respectively. However, there was no correlation between the seasonal changes in PC₂₀ methacholine and PC₂₀ AMP, expressed by doubling concentrations ($\rho = 0.21$, $p = 0.44$) (fig. 2). Moreover, baseline PC₂₀ methacholine was not correlated with changes in PC₂₀ AMP during the grass pollen season ($p = 0.23$). Finally, seasonal changes in PC₂₀ methacholine were not correlated with changes in FEV₁ ($p = 0.42$).

Sputum Cell Counts and Differentials

During the grass pollen season, there was no significant overall change in sputum total cells or any sputum cell population (table 1).

Correlation of BHR to Methacholine or AMP with Sputum Inflammatory Cells

There was no correlation of log PC₂₀ methacholine before or during the grass pollen season with corresponding log sputum eosinophils ($p > 0.12$, both comparisons).

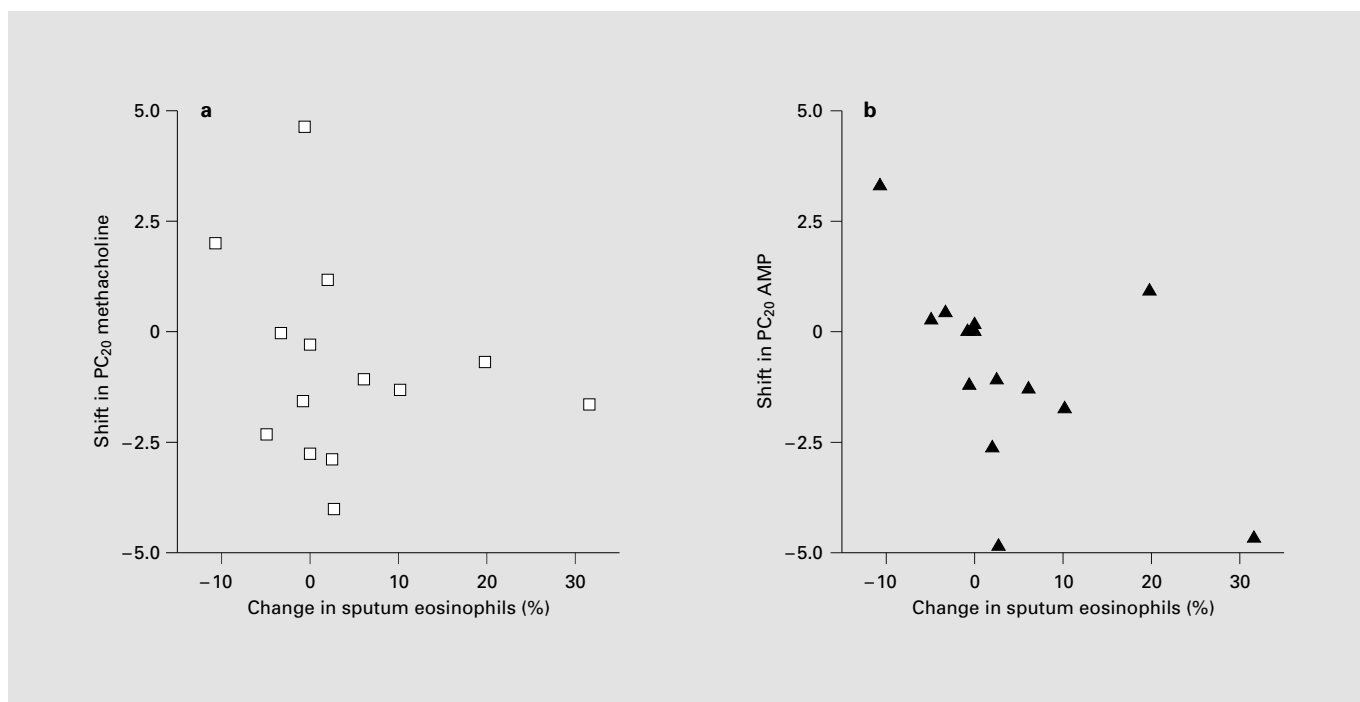


Fig. 3. Correlation of changes (doubling concentrations) in PC₂₀ methacholine (**a**) ($\rho = -0.32$, $p = 0.27$) and PC₂₀ AMP (**b**) ($\rho = -0.59$, $p = 0.02$) with changes in percentage sputum eosinophils (difference between pre/during season visits).

In contrast, there was a borderline statistical correlation between baseline ($r = -0.47$, $p = 0.08$) and a significant correlation between seasonal ($r = -0.58$, $p = 0.028$) log PC₂₀ AMP and the corresponding log sputum eosinophils. During pollen exposure, there was no correlation between changes in PC₂₀ methacholine and changes in sputum eosinophils (fig. 3a). In contrast, a significant correlation between doubling concentrations in PC₂₀ AMP before and during the grass pollen season and seasonal changes in sputum eosinophils was observed (fig. 3b). Neither changes in PC₂₀ methacholine nor in PC₂₀ AMP were correlated with any other sputum cell type ($p > 0.43$, all correlations).

Discussion

Patients with SAR may demonstrate increased BHR, and airway inflammation during pollen exposure, even in the absence of clinical asthma. In the present study, we demonstrate dissimilarity of dynamic changes in BHR to methacholine and AMP in SAR patients during the pollen season. The results of our observation are remarkable in several aspects:

Most importantly, PC₂₀ AMP and seasonal changes in AMP BHR were closely correlated with airway inflammatory cells, i.e. eosinophils. This was true despite the fact that there was no overall change in PC₂₀ AMP and sputum eosinophils during pollen exposure. However, on an individual base, there were changes in both parameters in either direction, and these changes were shown to be correlated. A potential limitation of our study is the relatively small number of subjects studied. Therefore, it might have lacked power to detect small changes, e.g. in sputum eosinophils or PC₂₀ AMP, and weak correlations, e.g. of sputum eosinophils with PC₂₀ methacholine. Crimi et al. [15] failed to show a correlation between sputum eosinophils and PC₂₀ methacholine in a cross-sectional analysis using data from 28 subjects. In contrast, following allergen challenge, positive correlations between influx of eosinophils and changes in PC₂₀ methacholine have been demonstrated in as few as 8 subjects [16]. Nevertheless, our study had at least 80% power to detect a change of one doubling dose in PC₂₀ methacholine during the pollen season, and comparable studies, e.g. by Prieto et al. [17], showed a significant increase in PC₂₀ AMP after pollen exposure in only 14 subjects. Therefore, we anticipated that our study design would at least allow to detect major changes in these parameters.

The observation of a correlation between PC₂₀ AMP and eosinophils adds to a growing body of evidence supporting AMP challenge as a more accurate surrogate marker of airway inflammation than BHR to methacholine [7, 18]. Polosa et al. [8] have previously shown that sputum eosinophils of rhinitis patients during pollen exposure were more closely associated with BHR to AMP than BHR to methacholine. Moreover, in a large trial with inhaled corticosteroids, improvement in airway inflammation due to corticosteroid therapy was closely associated with improvements in BHR to AMP, but not BHR to methacholine [19]. AMP challenge effects bronchoconstriction mainly via mast cell activation, and eosinophil-derived mediators may directly or indirectly promote mast cell migration and maturation [20, 21], thus providing a possible explanation for the close relationship between airway eosinophils and BHR to AMP.

In our study, there was a good baseline correlation of PC₂₀ methacholine and PC₂₀ AMP. This is, at least to a certain extent, in contrast to a recent cross-sectional study by Prieto et al. [22], who have compared airway responses to methacholine and AMP in patients with allergic rhinitis out of season. In their study, some patients with BHR to AMP did not demonstrate BHR to methacholine and vice versa, which led to the conclusion that BHR to methacholine and AMP did not reflect the same airway abnormalities in rhinitis patients. However, in full compliance with our results, the same authors have also recently demonstrated that postseasonal changes in PC₂₀ methacholine and PC₂₀ AMP were not correlated [17].

In our patients, only PC₂₀ methacholine decreased during the pollen season, even without a concomitant increase in eosinophilic inflammation; the reason for this exclusive decrease is not fully clear. It has been suggested by other authors that PC₂₀ methacholine may be related to baseline airway caliber, since BHR to methacholine was closely correlated with baseline FEV₁ in a large group of asthmatics [23]. Bearing in mind that we have studied patients with rhinitis, the former hypothesis is nevertheless not supported by the data from our study, since there was no association between changes in PC₂₀ methacholine and airway caliber. An alternative explanation may be the relationship between upper and lower airways through nasobronchial reflexes, since nasal irritants increase bronchial hyperresponsiveness to methacholine via vagal mechanisms [24, 25]. Nasal allergic inflammation could therefore contribute to increased BHR to methacholine in our patients, without affecting BHR to AMP due to the absence of bronchial eosinophilic inflammation in the majority of the subjects surveyed.

In summary, this study reports dynamic changes in BHR to methacholine or AMP correlating with induced sputum inflammatory cells of SAR patients before and during pollen exposure. The data of our investigation show a marked dissimilarity between seasonal changes in

BHR to methacholine and AMP, and the stronger association of PC₂₀ AMP with sputum eosinophils further supports the hypothesis that AMP challenge may be a more accurate marker of eosinophilic airway inflammation than methacholine.

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