Serum ECP and MPO, but not urinary LTE₄, are associated with bronchial hyper-responsiveness

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A random population-based sample of 131 subjects was used to assess the value of serum eosinophil cationic protein (ECP), serum myeloperoxidase (MPO), and urinary leukotriene E₄ (LTE₄) in predicting bronchial hyper-responsiveness measured by methacholine challenge. Special interest was focused on the history of aspirin intolerance and on smoking as contributing factors.

The mean serum ECP and MPO were higher in hyper-reactive [provocational dose causing a 20% fall in forced expiratory volume in 1 sec. (PD₂₀) ≤ 6900 µg] than in non-hyper-reactive subjects (22.3 vs. 13.2 ng l⁻¹, P < 0.001 and 377 vs. 278 µg l⁻¹, P = 0.001, respectively). This was also seen in current smokers vs. never smokers (17.2 vs. 12.9 µg l⁻¹, P = 0.03 and 372 vs. 286 µg l⁻¹, P = 0.04, respectively). There were no differences in baseline urinary excretion of LTE₄ between hyper-reactive and non-hyper-reactive subjects. During the 2 h after methacholine challenge, urinary LTE₄ excretion increased from 53.8 and 69.0 ng mmol⁻¹ creatinine in non-hyper-reactive subjects, but there was no change in hyper-reactive subjects (non-hyper-reactive vs. hyper-reactive, P = 0.06). The increase was greatest in subjects with aspirin intolerance causing urticaria or angioedema but not aggravation of asthma (from 58.5 to 87.2 ng mmol⁻¹ creatinine), probably due to extrapulmonary leukotriene production.

Our results indicate that serum ECP and MPO, but not urinary LTE₄ (even in subjects with a history of aspirin intolerance), predict bronchial hyper-responsiveness to methacholine. The subject’s smoking history must be taken into account when these parameters are considered.

Introduction

Bronchial hyper-responsiveness to methacholine or histamine is closely associated with asthma but is also found in several other airway disorders, as well as in healthy individuals (1-4). Asthma is characterized by chronic inflammatory changes in the airway mucosa, even in its mildest form (5). The infiltration of inflammatory cells in the lamina propria of the airways of asthmatic patients has been shown to be inversely related to provocational concentration causing a 20% fall in forced expiratory volume in 1 sec (PC₂₀) for methacholine (6), but in atopic subjects with mild to moderate asthma no correlation has been found between the degree of airway responsiveness and the numbers of inflammatory cells in sputum or bronchoalveolar lavage or bronchial biopsy (7). To monitor airway inflammation other markers have also been studied. Myeloperoxidase (MPO), as a parameter of neutrophil activity, and eosinophil cationic protein (ECP), as a parameter of eosinophil activity, are both elevated in induced sputum in patients with asthma and chronic obstructive pulmonary disease (COPD) (8,9). In COPD, the changes in MPO seem to be more prominent (8). Serum ECP and MPO are elevated in children with persistent asthma symptoms (10,11). Further, serum ECP, but not serum MPO, is influenced by atopy and eczema states (10,11).

In asthmatic subjects, plasma leukotriene E₄ (LTE₄) levels have been shown to be higher than in controls and are related to disease activity (12). In contrast, baseline values for urinary LTE₄, which is an index of whole-body production of cysteinyl-leukotrienes, do not differ between atopic asthmatics and non-asthmatics (13). Elevated basal urinary LTE₄ excretion has been reported in aspirin-intolerant asthmatics (13,14). LTE₄ excretion has been increased after aspirin challenge in aspirin-intolerant asthmatics, but methacholine challenge producing comparable bronchial obstruction did not alter eicosanoid excretion (15).

The aim of the present study was to use random population-based material to assess the value of serum MPO, ECP and urinary LTE₄ in indicating bronchial...
hyper-responsiveness as measured by methacholine challenge. Special interest was focussed on the history of aspirin intolerance and on smoking as contributing factors.

Methods

POPULATION SAMPLES

The original study population comprised a population-based random sample (4300) of adult women and men, aged 18-65 years served by the Päijät-Häme Central Hospital (16). Of these subjects, 3102 returned a postal questionnaire, yielding a response rate of 73%. Special interest was focused on markers of intrinsic asthma (16). The study groups of the present study were as follows.

Group 1: Subjects with a History of Aspirin Intolerance Causing Shortness of Breath or Worsening of Asthma

The population-based sample (4300) included a total of 35 subjects with symptoms consistent with the group definition. A trained nurse interviewed 32 of these subjects and 29 of them were confirmed to have aspirin intolerance. Twenty-two of these participated in the study proper. Ten subjects (46%) had doctor-diagnosed asthma and seven had been on inhaled steroid treatment.

Group 2: Subjects with a History of Aspirin Intolerance Causing Urticaria or Angioedema but not Respiratory Symptoms

The population-based sample included 50 subjects fulfilling these criteria and 39 of these were interviewed. Thirty-two has confirmed positive history and 24 took part in the study.

Group 3: Subjects with Doctor-Diagnosed Asthma and Without a History of Aspirin Intolerance

The population-based sample included 93 subjects in this category. A random sample of 39 subjects participated in the study. Twenty-two of them had been on inhaled steroid treatment.

Group 4: Subjects with a History of Symptoms of Asthma or Attacks of Shortness of Breath in the Past 12 Months Without Asthma or Other Respiratory Diagnosis, no Medication and no History of Aspirin Intolerance

The population-based sample included 197 such subjects. A random sample of 27 participated in the study.

Group 5: Subjects Without a History of Respiratory Symptoms and Without Aspirin Intolerance

The population-based sample included 1510 such subjects. A random sample of 19 participated in the study.

Twenty-six per cent of all subjects were regular smokers, 43% were irregular smokers or had stopped smoking and 31% were never-smokers.

The study was approved by Ethics Committee of Päijät-Häme Central Hospital.

METHACHOLINE CHALLENGES AND SPIROMETRY

A rapid dosimetric methacholine challenge test, performed with a pocket turbine spirometer (Micro Spirometer®; Micro Medical Instruments Ltd, Rochester, U.K.), was employed, its volume calibration being checked with calibration pumps as previously described (17). An automatic, inhalation-synchronized dosimeter jet nebuliser, the Spira Elektro 2 (Respiratory Care Center, Hämeenlinna, Finland), was used for methacholine delivery. Patients had to have a forced expiratory volume in 1 sec (FEV1) of at least 65% of predicted before the challenge and were excluded if they had experienced any respiratory infection during the previous 4 weeks. Methacholine chloride was delivered in four cumulative doses of 80, 400, 1700 and 6900 μg. The fall in FEV1 was plotted against the methacholine dose on a log scale, and the provocational dose causing a 20% fall in FEV1 (PD20) was estimated by interpolation from the dose-response cure. The dose-response ratio (DRR) was also calculated as the percentage fall in FEV1 by the last dose, divided by total dose administered (18).

All subjects underwent spirometry and a bell spirometer with a water seal (Could 2400°, SencorMedics Corporation, Yorba Linda, CA, U.S.A.) was used for lung function studies as previously described (19). Measurements of volumes and ventilatory flows were corrected to BTPS (body temperature, pressure, saturated with water vapour).

ECP AND MPO CONCENTRATIONS IN SERUM

Venous blood was collected prior to methacholine provocation in glass Vacutainer tubes without anticoagulants, and allowed to clot at 22°C for 60 min. Serum was separated by centrifugation (1200 g for 10 min at 22°C) and kept at −70°C until analysed. ECP and MPO were measured by radioimmunoassay using reagents from Pharmacia & Upjohn AB (Uppsala, Sweden).

LTE4 CONCENTRATIONS IN URINE

Urine samples were collected prior to and for the 2 h following the methacholine provocation and kept at −70°C until analysed. LTE4 concentrations were measured in serial dilutions by enzyme immunoassay (EIA) using
reagents from Cayman Chemicals as described by Kumlin et al. (13). The results are expressed as ng of LTE4 per mmol of creatinine in the urine sample.

SKIN-PRICK TESTS AND BLOOD EOSINOPHILS COUNTS

Skin-prick tests were carried out on all subjects with a panel of 12 common allergen extracts (Soluprick®, ALK A/S, Copenhagen, Denmark) with a negative (solvent) and a positive (histamine dihydrochloride, 10 mg ml−1) control. The allergens used were birch, timothy, meadow fescue and mugwort pollen; horse, dog, cat and cow dander; the mites Dermatophagoides farinae and Dermatophagoides pteronyssinus; and spores of the moulds Alternaria alternata and Cladosporium herbarum. A subject was considered atopic if any allergen caused a weal size ≥ 3 mm, while control solutions gave expected results. Venous blood was collected and the blood eosinophil counts measured.

STATISTICAL METHODS

The study population consisted of five separate groups for statistical analysis, groups 1 and 3 were combined to form the group of subjects with asthma. This combined group was compared to group 4 (asthmatic symptoms without respiratory diagnosis) and to group 5 (controls). In addition, group 1 (subjects with aspirin intolerance causing shortness of breath or worsening of asthma) was compared to group 2 (subjects with aspirin intolerance causing urticaria or angioedema); and group 4 (asthmatic symptoms without respiratory diagnosis) was compared to group 5 (controls). In pair-wise comparisons, Fisher’s least significant difference method was used and in the case of nominal data the chi-squared test was applied. Logarithmic transformations were carried out for skewed distributions.

With respect to quantitative inflammatory parameters, a t-test for independent samples was used to compare hyper-reactive with non-hyper-reactive subjects and smokers with non-smokers. Nominal data were analysed using the chi-squared test. Spearman’s rank correlation coefficient was used to investigate the correlation between change in urinary LTE4 excretion and methacholine dose.

Sensitivity, specificity and positive and negative predictive values at different cut-off points were calculated to compare serum ECP, MPO and urinary LTE4 as indicators of hyper-reactivity. Receiver operator characteristic ROC curves for bronchial hyper-responsiveness were constructed by plotting sensitivity against 1 – specificity.

Results

COMPARISONS BETWEEN THE GROUPS

Anthropometric data on the subjects are given in Table 1. There were no statistically significant differences between the groups in this context.

Data for the methacholine challenges and FEV1 measurements are similarly given in Table 1. There were no hyper-reactive subjects (PD20 ≤ 6900 µg) in groups 2 or 5 (subjects without a history of asthmatic symptoms). In subjects with asthma (groups 1 and 3 together), hyper-reactivity (49%) was observed significantly more often than in group 4 (asthmatic symptoms without diagnosis, 26%) and group 5 (controls, 0%) (P = 0.05 and P = 0.0001, respectively). Also, the difference between groups 4 and 5 was significant (P = 0.02). Among aspirin-intolerant subjects, hyper-reactivity was significantly more common in group 1 (respiratory symptoms) than in group 2 (urticaria or angioedema) 38% vs. 0%, P = 0.001).

In subjects with asthma, the FEV1 % of predicted value (FEV1 pred.) was significantly lower (mean 85%) than in groups 4 and 5 (P = 0.04 and P = 0.001, respectively). The difference between groups 4 and 5 was not significant (P = 0.16). Among aspirin-intolerant subjects between groups 1 and 2, the difference was not significant (P = 0.67).

TABLE 1. Anthropometric data, metacholine challenges, and FEV1 data for the subjects (n = 131)

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (µ)</td>
<td>22</td>
<td>24</td>
<td>39</td>
<td>27</td>
<td>19</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7/15</td>
<td>9/15</td>
<td>14/25</td>
<td>7/20</td>
<td>4/15</td>
</tr>
<tr>
<td>Age (years), mean (range)</td>
<td>46 (24-65)</td>
<td>45 (21-66)</td>
<td>50 (20-67)</td>
<td>52 (22-67)</td>
<td>43 (25-61)</td>
</tr>
<tr>
<td>PD20, min-max (µg methacholine) in hyper-reactive subjects</td>
<td>32-6900</td>
<td>45 (21-66)</td>
<td>50 (20-67)</td>
<td>52 (22-67)</td>
<td>43 (25-61)</td>
</tr>
<tr>
<td>Hyper-reactive subjects*, n (%)</td>
<td>8/21 (38)</td>
<td>0/23 (0)</td>
<td>17/30 (57)</td>
<td>7/27 (26)</td>
<td>0/19 (0)</td>
</tr>
<tr>
<td>DRR (% mmol−1 methacholine) median (range)</td>
<td>0-0.51</td>
<td>0-0.23</td>
<td>0-0.64</td>
<td>0-0.40</td>
<td>0-0.14</td>
</tr>
<tr>
<td>FEV1 (l), mean (range)</td>
<td>3.09 (1.58-5.38)</td>
<td>3.33 (1.87-5.41)</td>
<td>2.73 (0.64-5.2)</td>
<td>2.92 (1.66-4.36)</td>
<td>3.40 (1.73-4.87)</td>
</tr>
<tr>
<td>FEV1 % pred.†, mean (range)</td>
<td>92 (62-127)</td>
<td>94 (62-113)</td>
<td>82 (27-119)</td>
<td>94 (67-123)</td>
<td>101 (81-115)</td>
</tr>
</tbody>
</table>

*PD20≤6900 µg.
†See reference (20).
Table 2. Parameters of airways inflammation and skin-prick tests

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophil count (×10^9/L), median (range)</td>
<td>0.19 (0.06-1.0)</td>
<td>0.15 (0.03-0.59)</td>
<td>0.23 (0.05-1.09)</td>
<td>0.19 (0.10-0.74)</td>
<td>0.16 (0.01-0.44)</td>
</tr>
<tr>
<td>Eosinophil count (n&gt;0.4×10^9/L), n (%)</td>
<td>4/22 (18)</td>
<td>2/23 (9)</td>
<td>10/39 (26)</td>
<td>3/27 (11)</td>
<td>1/19 (5)</td>
</tr>
<tr>
<td>Serum ECP (µg/L), median (range)</td>
<td>13.6 (1.8-71.3)</td>
<td>9.2 (2.1-32.3)</td>
<td>16.9 (4.9-39.8)</td>
<td>13.8 (3.3-34.6)</td>
<td>12.0 (1.7-25)</td>
</tr>
<tr>
<td>Serum ECP, (n≥15 µg/L), n (%)</td>
<td>9/22 (41)</td>
<td>6/24 (25)</td>
<td>24/39 (62)</td>
<td>12/27 (44)</td>
<td>8/19 (42)</td>
</tr>
<tr>
<td>Urine LTE4 (ng/mmol creatinine), median (range)</td>
<td>40.8 (11.9-184.1)</td>
<td>57.8 (13.3-130.6)</td>
<td>47.6 (8.4-307.5)</td>
<td>49.9 (14.3-94.6)</td>
<td>47.5 (20.9-171.6)</td>
</tr>
<tr>
<td>Urine LTE4 (n≥50ng/mmol creatinine), n (%)</td>
<td>8/22 (36)</td>
<td>15/24 (63)</td>
<td>18/38 (47)</td>
<td>14/27 (52)</td>
<td>9/19 (47)</td>
</tr>
<tr>
<td>Serum MPO (µg/L), median (range)</td>
<td>262 (120-788)</td>
<td>201 (84-540)</td>
<td>338 (133-764)</td>
<td>311 (137-900)</td>
<td>254 (131-480)</td>
</tr>
<tr>
<td>Serum MPO (n&gt;300) (µg/L), n (%)</td>
<td>9/22 (41)</td>
<td>7/24 (29)</td>
<td>22/39 (56)</td>
<td>14/27 (52)</td>
<td>6/19 (32)</td>
</tr>
<tr>
<td>Skin prick, [n≥1 positive (≥3mm)], n (%)</td>
<td>9/22 (41)</td>
<td>8/24 (33)</td>
<td>16/39 (41)</td>
<td>9/27 (33)</td>
<td>7/19 (37)</td>
</tr>
</tbody>
</table>

Airway inflammation parameters and skin-prick tests are given in Table 2. The peripheral eosinophil count was higher in asthmatics than in controls (0.29 vs. 0.18×10^9/L; P=0.01) as was serum ECP (18.6 vs. 12.0 µg/L; P=0.03) and serum MPO (337 vs. 266 µg/L; P=0.06). Serum ECP in the non-atopic control group (no positive prick tests) was 12.0 µg/L (range from 5.8 to 25.0 µg/L). The other pair-wise comparisons were not significant. With respect to urinary LTE4 and atopic status (skin-prick test), there were no differences between the groups.

SERUM ECP, MPO AND URINARY LTE4 AS INDICATORS OF BRONCHIAL HYPER-RESPONSIVENESS

ROC curves for bronchial hyper-responsiveness using different cut-off points are given in Fig. 1. The cut-off points of 15 µg/L for ECP, 300 µg/L for MPO and 50 mg/mmol creatinine for LTE4 gave the best sensitivity and specificity. The mean values of serum ECP, MPO, and urinary LTE4 in hyper-reactive and non-hyper-reactive subjects are given in Table 3.

ASSOCIATION BETWEEN SERUM ECP AND BRONCHIAL HYPERRESPONSIVENESS

Mean serum ECP was higher in subjects with a positive response to methacholine challenge than in those with a negative response (Table 3). Mean serum ECP was also increased in current or ex-smokers (mean values 17.2 (SD 9.7) µg/L 18.7 (12.1) µg/L, respectively, as compared to never-smokers 12.9 (9.0) µg/L; P=0.03 never-smokers vs. Current smokers; Fig. 2).

Seventy-two per cent of hyper-reactive subjects had serum ECP levels ≥15 µg/L as compared to 35% of non-hyper-reactive (P=0.0004; sensitivity 72%, specificity 65%, positive predictive value 43% and negative predictive value 86%). Serum ECP correlated with the blood eosinophil count (r=0.67, P<0.0001). Twenty per cent of those with elevated blood eosinophil count (≥ 0.4×10^9
TABLE 3. The mean values of serum ECP (µg l⁻¹), MPO (µg l⁻¹) and urinary LTE₄ (ng mmol⁻¹ creatinine) in hyper responsive (PD₂₀≤6900 µg) and non-hyper-responsive (PD₂₀>6900 µg) subjects

<table>
<thead>
<tr>
<th></th>
<th>Hyper-reactive subjects (n = 32)</th>
<th>Non-hyper-reactive subjects (n = 88)</th>
<th>t-test:</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ECP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>22.3</td>
<td>13.2</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>12.8</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum MPO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>377</td>
<td>278</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>181</td>
<td>131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Urinary LTE₄</td>
<td></td>
<td></td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>57.7</td>
<td>53.8</td>
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</table>

Fig. 2. Serum MPO (□) and ECP (■) concentrations in the study subjects divided according to smoking history (mean ± SEM).

SERUM MPO AND BRONCHIAL HYPER-RESPONSIVENESS

The mean serum MPO was higher in subjects positive to methacholine challenge than in those who were negative (Table 3). Mean serum MPO was also higher in current or ex-smokers [mean values 372 (so 166) µg l⁻¹ and 304 (134) µg l⁻¹], than in never-smokers [286 (166) µg l⁻¹]; P = 0.04, never-smokers vs. current smokers; Fig. 2).

URINARY LTE₄ AND BRONCHIAL HYPER-RESPONSIVENESS

There were no statistically significant differences between the study groups in baseline urinary LTE₄ excretion (Table 2). During the 2 h following methacholine challenge, urinary LTE₄ excretion increased from 53.8 to 69.0 ng mmol⁻¹ creatinine in non-hyper-reactive subjects, whereas there was no change in LTE₄ excretion in hyper-reactive subjects (non-hyper-reactive vs. hyper-reactive, P = 0.06). There was no correlation between the increase in LTE₄ excretion and methacholine dose (r = 0.11, P = 0.23).

Aspirin intolerance was also associated with the change in LTE₄ excretion after methacholine challenge. In aspirin-intolerant subjects, the urinary LTE₄ increased from 55.4 to 73.9 ng mmol⁻¹ creatinine, while in subjects with no history of aspirin intolerance the change was from 54.5 to 62.5 ng mmol⁻¹ creatinine (aspirin intolerance subjects vs. others, P = 0.09). No interaction was detected between hyper-reactivity and aspirin intolerance (ANOVA, interaction P = 0.99). The greatest change in LTE₄ was seen in subjects with aspirin intolerance causing urticaria or angiodema without respiratory side-effects (from 58.5 to 87.2 ng mmol⁻¹ creatinine, Fig. 3). The mean increase in these subjects was 28.7 ng mmol⁻¹ creatinine vs. 7.8 ng mmol⁻¹ creatinine in other subjects (P = 0.02). The
lowest increase, if any, was seen in patients with asthma (groups 1 and 3, Fig. 3).

Discussion

In this study a random population-based sample group was used to assess the value of serum MPO, ECP and urinary LTE4 as measures of bronchial hyper-responsiveness. Special interest focused on subjects with a history of aspirin intolerance, which is associated with the most aggressive form of asthma (21); new leukotriene modifiers have been shown to be effective in blocking adverse reactions to aspirin and other non-steroidal anti-inflammatory drugs (NSAID) in susceptible asthmatic patients (22).

The main purposes in developing inflammatory indices for asthma are to help early detection and differential diagnosis of the disease and, further, to make possible better-targeted treatment as well as follow-up of patients on the basis of objective measurements. The need for simple parameters to detect and monitor airway inflammation is obvious, especially in general practice. The infiltration of inflammatory cells into the lamina propria of the airways of asthmatic patients has been shown to be inversely related to PC_{20} for methacholine but not clearly to symptoms or changes in lung function tests, including peak expiratory flow (PEF) variability (6). This finding emphasizes the importance of the methacholine test in the follow-up of asthmatic patients; however, the test procedures are technically challenging and time-consuming and cannot easily be expanded to use in general practice. On the other hand, some studies have failed to reveal any correlation between methacholine responsiveness and the number of inflammatory cells evaluated by any method (7). Hence, in the treatment of asthma, the assessment of all three components, airway obstruction, airway hyperresponsiveness and airway inflammation, has recently been stressed (23).

In this present study, serum ECP and MPO levels were higher in hyper-reactive than in non-hyper-reactive subjects, which confirms findings in previous reports (10,11). Serum ECP has also been shown to correlate with the percentage of eosinophils in bronchoalveolar fluid and bronchial biopsy specimens and reflects the intensity of eosinophil airway inflammation as well as disease activity (24). Serum ECP and MPO can thus be used to monitor anti-inflammatory treatment in asthmatic patients (11,25,26). They are, however, not changed in smoking asthmatics even with high-dose budesonide (25) or in COPD patients using inhaled steroids (26), which would imply that smoking asthmatics and COPD patients might not benefit from these drugs (25,26). In the present study serum ECP and MPO levels were higher in current smokers than in never-smokers, suggesting that smoking increases airway inflammation characterized by eosinophil and neutrophil activation. This is also in concordance with the results of Jensen et al. (27), who have shown raised serum levels of ECP and lactoferrin, another neutrophil marker, in smokers.

The role of increased neutrophil number in asthmatic airways is not clear. In a study by Nordman et al. (28) no significant differences in serum MPO values between normoreactive and hyper-reactive subjects in methacholine challenge were found. It has been suggested that in both allergic and non-allergic asthma, airway recruitment and activation of neutrophils occur parallel to eosinophil migration. Airway neutrophils might not, however, contribute to epithelial cell injury or to airway hyper-responsiveness in the steady state (29). On the other hand, neutrophils may induce tissue damage and participate in the shedding of the epithelium in status asthmaticus (30). Neutrophil recruitment, together with mast cells, may also contribute to the bronchoconstriction observed in occupational asthma induced by grain dust (31).

No differences were found between the study groups in baseline urine LTE4 excretion. Some previous studies have reported higher urinary LTE4 levels in aspirin-sensitive asthmatics as compared to aspirin-tolerant asthmatics and healthy controls (14,15). One possible reason for the discrepancy is that in the present study patients were considered aspirin-sensitive according to their history and no provocations were performed. The measurement of urinary LTE4 in a single sample did not predict bronchial hyper-responsiveness, which confirms previous results obtained by Smith et al. (14) Bronchial provocation with specific allergen in atopic asthmatics induces a prompt increase in cysteinyl-leukotriene release which is reflected in increased urinary LTE4 excretion during the first few hours after the challenge (32). Enhanced urinary LTE4 excretion has also been reported after aspirin challenge in aspirin-intolerant asthmatics, but methacholine challenge producing comparable bronchial obstruction has not been shown to alter eicosanoid excretion (15), as has provocation with histamine (32). In the present case, LTE4 excretion was not altered during the 2 h following methacholine challenge in hyper-reactive subjects but was elevated in non-hyper-reactive subjects. The largest increase was found in subjects with aspirin intolerance causing urticaria or angioedema, whereas the least (if any) increase was found in patients with asthma (groups 1 and 3). The reason for these results is not clear. The data in the present study suggest that higher doses of methacholine (6900 μg) provoke extrapulmonary cysteinyl-leukotriene production in non-hyper-reactive subjects (possibly in the skin, especially in aspirin-intolerant patients).

The present data which suggest a negligible role of leukotrienes in bronchial hyper-responsiveness, are complicated by the findings of a beneficial clinical action of S-lipoxygenase inhibitor zileuton (33) and a cysteinyl-leukotriene receptor antagonist pranlukast (34). A single oral dose of zileuton (400 mg) is found to increase PC_{20} to histamine by 2-1 doubling doses and the PL_{20} to ultrasonically nebulized distilled water 1-3 doubling doses (33). These results, need, however, to be confirmed with a larger number of subjects with treatment given both acutely and long-term (35). With the leukotriene receptor antagonist pranlukast, a small but significant reduction (from 0.30 to 0.48 mg ml^{-1}) in methacholine responsiveness was observed after 1-week of treatment in asthmatic patients.
Our present data suggest that increased cysteinyl-leukotriene synthesis is not associated with bronchial hyper-responsiveness. Therefore other mechanisms, e.g. increased reactivity to leukotrienes rather than increased leukotriene synthesis, might explain the mediator role of cysteinyl-leukotrienes in bronchial hyper-responsiveness, as would indeed be implied by therapeutic interventions with 5-lipoxygenase inhibitors and leukotriene receptor antagonists.

In conclusion, the present results indicate that serum ECP and MPO, but not urinary LTE4, predict bronchial hyper-responsiveness to methacholine. The smoking history has to be taken into account when these inflammation parameters are used in clinical practice.

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