SHORT SURVEY

Endothelin-1 in exhaled breath condensate of stable and unstable asthma patients

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Summary
Endothelins are proinflammatory, profibrotic, broncho- and vasoconstrictive peptides, which play an important role in the development of airway inflammation and remodeling in asthma. The study was undertaken to evaluate the endothelin-1 (ET-1) levels in exhaled breath condensate (EBC) of asthmatics with different degree in asthma severity.

EBC was collected from 31 patients with allergic asthma (11 with steroid-naive mild asthma, 10 with ICS-treated, stable mild-to-moderate asthma, 10 with ICS-treated unstable, severe asthma) and 7 healthy volunteers.

In the three groups of asthmatics, ET-1 concentrations in EBC were significantly higher than in healthy volunteers. ET-1 levels were significantly higher in patients with unstable asthma than in the two groups with stable disease. There was a significant correlation between ET-1 levels and FENO in the three groups of asthmatics and between ET-1 and blood eosinophil counts in the group of patients with unstable asthma.

Measurements of ET-1 in EBC may provide another useful diagnostic tool for detecting and monitoring inflammation in patients with asthma.

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Introduction

Endothelins are a family of peptide mediators that have a number of biological properties, including proinflammatory, profibrotic, broncho- and vasoconstrictive influence in human airways.1 Endothelin-1 (ET-1) has been demonstrated in the airway epithelial and endothelial cells and is involved in the pathogenesis of bronchial asthma.2,3 It has also been suggested that ET-1 influences asthmatic inflammation, provoking the concentration and proliferation of bronchial smooth muscle cells as well as subepithelial fibrosis. This leads to airway remodeling and severe bronchial hyperreactivity.4

Exhaled breath condensate (EBC), collecting by cooling exhaled air, is non-invasive, easily performed, a rapid and effort independent method for obtaining samples from the lower respiratory tract. There has been increasing interest in measuring EBC—a very useful method, especially in the assessment of inflammatory mediators related to the bronchial epithelium, in the pathophysiology and evaluation of new strategies for the treatment of asthma.5

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The aim of the study was to assess ET-1 concentrations in the EBC of asthmatics with different degrees of asthmatic severity, and to establish the possible correlation of these measurements with the parameters of airway inflammation.

Material and methods

Patients

The study was conducted upon groups of 11 steroid-naive mild allergic asthma patients, 10 patients treated with inhaled corticosteroids (ICS) with stable mild-to-moderate allergic asthma, and 10 ICS-treated patients with severe, unstable allergic asthma. Asthma was diagnosed according to the criteria recommended by the GINA 2002.6 The steroid-naive asthmatics have not been treated with ICS and were free from acute exacerbations and respiratory tract infections during the 3 months prior to the study. Patients with stable, mild-to-moderate asthma had been treated with low to medium doses of ICS at a constant dose for at least 3 months. Stable asthma was defined as a minimal need for rescue medications (short-acting $\beta_2$-agonists), no exacerbations, and no use of systemic steroids in the previous 12 months. The patients with severe, unstable asthma had required one or more hospitalizations for asthma and more than three oral steroid bursts in the last year. They had been taking high-doses of ICS and long-acting $\beta_2$-agonists for at least 6 months. Patients who had respiratory tract infections in the last month before the study were excluded from this study. All the patients were atopic and sensitized to common inhaled allergens, as evaluated by skin prick tests.

Seven healthy volunteers were used as a negative control group. They were free of respiratory tract infection within 3 months prior to the study and from other significant illnesses known to affect exhaled nitric oxide ($F_{ENO}$) measurements. Asthma patients and healthy volunteers were non-smokers and during the last year have not been passive smokers.

All of the patients and healthy volunteers were examined by a physician, then underwent EBC collection, $F_{ENO}$ measurement, and spirometry. Blood samples were collected to determine serum total IgE and blood eosinophil count.

The study protocol was approved by the Ethics of Research Committee of the Medical University of Bialystok, number of agreement: R-I-003/80/2006. Informed consent was obtained from every patient entered into the study.

Measurements

Exhaled nitric oxide ($F_{ENO}$) was measured by the chemiluminescence technique using a Sievers 280i NO Analyzer (Boulder, CO, USA). The measurements were performed at an expiratory flow of 50 ml/s according to ATS recommendations for on-line measurement of $F_{ENO}$ in adults.7

The spirometry ($FEV_1$) was performed using a MasterScreen Pneumo PC spirometer (Jaeger, Hoechberg, Germany), according to ATS standards.8

EBC was collected by using a commercially available condenser (EcoScreen; Erich Jaeger GmbH) according to the current ATS/ERS guidelines, as previously described.5,9,10 Serum total IgE concentrations were measured using ImmunoCAP™ Technology (Pharmacia Diagnostics, Uppsala, Sweden). Blood eosinophil count was measured using a hematologic analyzer (Coulter Electronics GmbH, Miami, FL, USA). The concentrations of ET-1 in EBC were determined using enzyme immunoassay kits for quantitative determination (ET-1-Biomedica Gruppe, Vienna, Austria). Detection limit (0 fmol/ml±3 S.D.):0.02 fmol/ml.

Analysis

Statistical analyses were completed using the Student’s $t$-test. All values were expressed as means±S.D.; $p$ values <0.05 were considered significant. Correlations were evaluated by Pearson’s linear correlation test.

Results

Characteristics of patients and healthy volunteers are presented in Table 1.

In the three groups of asthmatics, EBC concentrations of ET-1 were significantly higher than those detected in healthy volunteers (steroid-naive stable asthma: $0.88±0.24$ fmol/ml, $p=0.017$; ICS-treated stable asthma: $0.99±0.23$ fmol/ml, $p=0.0018$; unstable asthma: $2.27±0.82$ fmol/ml, $p=0.0001$; healthy volunteers: $0.59±0.19$ fmol/ml) (Figure 1). ET-1 levels were significantly elevated in patients with unstable asthma compared with ICS-treated stable asthma patients ($p=0.00018$) and steroid-naive asthma patients ($p=0.0004$). There was no significant difference ($p=0.29$) in ET-1 concentrations between stable ICS-treated and steroid-naive asthma patients.

We revealed statistically significant correlations between concentrations of ET-1 in EBC and $F_{ENO}$ in the three studied groups of asthmatics; the degree of correlation increased as asthma severity intensified. There were no correlations between ET-1 in EBC and $F_{ENO}$ in the group of healthy volunteers. We discovered a significantly positive correlation between ET-1 in EBC and blood eosinophil count in the groups of asthmatics with severe, unstable asthma. There was no statistically significant correlation between ET-1 in EBC and other studied parameters in any of the studied groups of asthmatics and healthy volunteers (Table 2).

Discussion

There have been numerous studies investigating whether differences exist between asthmatics and non-asthmatic volunteers, especially with regard to the expression of ET-1 by cultured airway epithelial cells, as well as the levels of ET-1 in saliva, sputum, plasma, BAL fluid, and bronchial biopsy specimens.11–13 Furthermore, the relationship between levels of ET-1 and markers of airway inflammation and bronchial reactivity has also been the object of several studies.6,14,15 The influence of anti-inflammatory therapy on levels of ET-1 has been investigated as well.16–18 In human airways, immunoreactive ET-1 is located principally in the bronchial epithelium. Expression at this site increases with asthma and is correlated with the severity of the disease.16 Except for human bronchial
epithelial cells, ET-1 is produced by vascular endothelial cells, as well as inflammatory cells such as macrophages and mast cells. Many interactions between ET-1 and other cytokines essential in asthma have been described. Tumor necrosis factor- \( \text{(TNF-} \text{)} \) induces secretion of ET-1 from cultured

### Table 1 Characteristics of study subjects and healthy volunteers.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Healthy volunteers</th>
<th>Stable asthma, steroid naïve</th>
<th>Stable asthma, ICS-treated</th>
<th>Unstable asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>7</td>
<td>11</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>4/3</td>
<td>6/5</td>
<td>6/4</td>
<td>7/3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>28.00 ± 4.93</td>
<td>27.36 ± 7.50</td>
<td>44.70 ± 8.11</td>
<td>45.90 ± 6.04</td>
</tr>
<tr>
<td>Duration of symptoms (years)</td>
<td>2.54 ± 1.21( ^{1,1} )</td>
<td>8.60 ± 3.86( ^{1,2} )</td>
<td>16.9 ± 7.04( ^{1,1} )</td>
<td>56.70 ± 12.46( ^{1,1} )</td>
</tr>
<tr>
<td>Baseline FEV(_1) (% predicted)</td>
<td>106.85 ± 9.7( ^{1,2} )</td>
<td>95.63 ± 18.54( ^{1} )</td>
<td>83.60 ± 6.61( ^{1} )</td>
<td>315.0 ± 123.5</td>
</tr>
<tr>
<td>Serum total IgE concentration (kU/l)</td>
<td>65.42 ± 31.65( ^{1,2} )</td>
<td>327.9 ± 265.6</td>
<td>275.0 ± 88.60</td>
<td>315.0 ± 123.5</td>
</tr>
<tr>
<td>Blood eosinophil count (cells/mm(^3))</td>
<td>51 ± 26( ^{1,1} )</td>
<td>239 ± 138</td>
<td>271 ± 70</td>
<td>302 ± 104</td>
</tr>
<tr>
<td>( F_{\text{ENO}} ) (ppb)</td>
<td>18.00 ± 5.59( ^{1,1} )</td>
<td>76.00 ± 33.66( ^{1} )</td>
<td>38.70 ± 10.26( ^{1,1} )</td>
<td>74.80 ± 28.64( ^{1} )</td>
</tr>
<tr>
<td>ET-1 (fmol/ml)</td>
<td>0.59 ± 0.19( ^{1,1} )</td>
<td>0.88 ± 0.24( ^{1} )</td>
<td>0.99 ± 0.23( ^{1} )</td>
<td>2.27 ± 0.82( ^{1} )</td>
</tr>
</tbody>
</table>

Data are presented as medians (ranges). FEV\(_1\): forced expiratory volume in 1 s.

\( ^{1} \)Values significantly different from patients with stable, steroid-naïve asthma, \( p<0.05 \).

\( ^{2} \)Values significantly different from patients with stable, ICS-treated asthma, \( p<0.05 \).

\( ^{1,1} \)Values significantly different from patients with unstable asthma, \( p<0.05 \).

### Figure 1 Concentrations of ET-1 in EBC in studied groups of asthma patients and healthy volunteers.

### Table 2 Correlations between concentrations ET-1 in EBC and other studied parameters in the groups of asthma patients and healthy volunteers.

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>( F_{\text{ENO}} )</th>
<th>Blood eosinophil count</th>
<th>Serum total IgE</th>
<th>Baseline FEV(_1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers</td>
<td>( r = 0.38 )</td>
<td>( r = 0.48 )</td>
<td>( r = 0.22 )</td>
<td>( r = 0.53 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.39 )</td>
<td>( p = 0.11 )</td>
<td>( p = 0.62 )</td>
<td>( p = 0.22 )</td>
</tr>
<tr>
<td>Stable asthma steroid-naïve</td>
<td>( r = 0.73 )</td>
<td>( r = 0.26 )</td>
<td>( r = -0.17 )</td>
<td>( r = -0.24 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.01 )</td>
<td>( p = 0.42 )</td>
<td>( p = 0.61 )</td>
<td>( p = 0.46 )</td>
</tr>
<tr>
<td>Stable asthma ICS-treated</td>
<td>( r = 0.94 )</td>
<td>( r = 0.47 )</td>
<td>( r = -0.48 )</td>
<td>( r = 0.12 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.00005 )</td>
<td>( p = 0.16 )</td>
<td>( p = 0.15 )</td>
<td>( p = 0.73 )</td>
</tr>
<tr>
<td>Unstable asthma</td>
<td>( r = 0.94 )</td>
<td>( r = 0.67 )</td>
<td>( r = 0.43 )</td>
<td>( r = -0.47 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.00005 )</td>
<td>( p = 0.03 )</td>
<td>( p = 0.07 )</td>
<td>( p = 0.16 )</td>
</tr>
</tbody>
</table>
ET-1 in EBC of stable and unstable asthma

ET-1 may contribute significantly to the remodeling of the airway by slowing epithelial cell migration while increasing the proliferation of airway fibroblasts and smooth muscle cells. The damage of asthmatic airways by environmental agents and allergens may be additionally increased by slower repair mechanisms, in which ET-1 may be involved.25

EBC examination being simple and non-invasive could be exploited to detect specific levels of biomarkers and monitor disease severity in response to appropriate prescribed therapy.24 There are two reports concerning elevated ET-1 levels in EBC in patients with fibrosing lung disease and lung cancer.26,27 However, there are no reports about using EBC for the assessment of ET-1 levels in asthma patients.

Our study shows that ET-1 levels in EBC are higher in asthmatic patients than in healthy controls. The concentrations of ET-1 increase as the severity of asthma is augmented. In patients with unstable asthma, levels of ET-1 were significantly higher than in steroid-naive and ICS-treated patients with stable asthma. The level of ET-1, depending on the severity of the asthma, significantly correlated with other inflammatory markers. We have uncovered no significant difference in ET-1 levels between steroid-naive mild asthmatics and ICS-treated mild-to-moderate asthmatics. This could suggest the beneficial effect of ICS-treatment in downregulation of ET-1 in the airways. However, more studies are needed to assess the influence of ICS on ET-1 levels in EBC.

What is the possible reason of the elevated ET-1 levels in unstable asthma? A number of studies have reported, that in severe asthma, the several proinflammatory cytokines including TNF-α and TGF-β can induce the inflammatory cascade reaction, which may lead to airway hyperresponsiveness and structural changes characteristic for severe asthma. The mechanism of increase in ET-1 level could be not only the consequence of promoting the interaction between cytokines, but also of higher expression of this cytokine on inflammatory modified bronchial epithelium cells and stimulating ET-1 secretion, resulting in its elevated levels in the airways. The increased levels of ET-1 in patients with unstable asthma can be also associated with the rise in the number of inflammatory cells involved in ET-1 production (e.g. macrophages, mast cells).28,29

He et al. revealed that ET-1 expression in bronchial mucosa was still high in patients with severe asthma who had a poor response to ICS. It is indicated that the airway hyperresponsiveness in patients with severe asthma might be associated with stronger expression of ET-1.30

Measurements of ET-1 in EBC of asthma patients may provide another useful diagnostic tool for detecting and monitoring inflammation, disease severity, and response to the treatment.

Competing interests

The authors declare that they have no competing interests in the publication of the manuscript. This work was supported by research grant no. 3-35523P from the Medical University of Bialystok, Poland.

References