Involvement of vascular endothelial growth factor in exercise induced bronchoconstriction in asthmatic patients

H Kanazawa, K Hirata, J Yoshikawa

BACKGROUND: There is evidence that the bronchial microcirculation has the potential to contribute to the pathophysiological mechanisms of exercise induced bronchoconstriction (EIB) in asthmatic subjects. Vascular endothelial growth factor (VEGF), which is highly expressed in asthmatic airways, increases vascular permeability. The relationship between VEGF levels in induced sputum and the severity of EIB in asthmatic subjects was studied.

METHODS: The concentration of VEGF in induced sputum was examined in 23 asthmatic subjects and 11 normal controls. The asthmatic subjects performed an exercise test and the % maximal fall in forced expiratory volume in 1 second (FEV₁) was measured. Beclomethasone dipropionate (BDP) 400 µg twice daily was administered to the asthmatic subjects for 8 weeks and the exercise test and sputum induction were repeated.

RESULTS: The concentration of VEGF in induced sputum was significantly higher in asthmatic subjects than in normal controls. There was a significant correlation between the concentration of VEGF and the % maximal fall in FEV₁ (r=0.826, p=0.0001) and between the concentration of VEGF and airway vascular permeability index (r=0.621, p=0.0037). After treatment with inhaled BDP there was a significant decrease in the concentration of VEGF in the asthmatic subjects (before treatment: 7051 (2361) pg/ml, after treatment: 4498 (2135) pg/ml, p<0.0001). The change in the concentration of VEGF was significantly correlated with the change in the % maximal fall in FEV₁ (r=0.463, p=0.031).

CONCLUSIONS: Excessive production of VEGF in asthmatic airways may contribute to the pathogenesis of EIB via increased airway vascular permeability.

EXERCISE INDUCED BRONCHOCONSTRICTION (EIB) is used to describe the increase in airway resistance that follows exercise in asthmatic patients. Two major hypotheses have been put forward to explain the mechanism of EIB. One suggests that evaporative water loss associated with exercise causes a transient increase in osmolarity of the fluid interface of the mucosal surface in the airways, resulting in mast cell degranulation. The second hypothesis proposes that EIB is a mechanical event in which the airways are rapidly rewarmed by reactive hyperaemia of the bronchial circulation with subsequent oedema of the airway wall. However, since mast cell derived mediators such as histamine and leukotrienes may cause airway oedema, it is possible that both of these hypotheses explain EIB in asthmatic subjects. There is no doubt that the bronchial microcirculation has the potential to contribute to the pathophysiological mechanisms of EIB in asthma.

Vascular endothelial growth factor (VEGF) is one of the most potent inducers of endothelial cell growth. It also increases vascular permeability, allowing plasma proteins to leak into the extravascular space leading to mucosal oedema and thereby narrowing of the airway diameter which could amplify the effects of airway smooth muscle contraction. VEGF is widely expressed in many highly vascularised organs including the lung. Hoshino et al recently reported that VEGF positive cells are significantly increased in the airway mucosa of patients with bronchial asthma compared with healthy control subjects. However, there have been no reports of the possible role of VEGF in EIB in asthma. We therefore examined the relationship between VEGF levels in induced sputum and the severity of EIB in patients with asthma.

METHODS

Subjects

Twenty three non-smoking asthmatic patients of mean (SD) age 34.1 (7.7) years, forced expiratory volume in 1 second (FEV₁) 106.6 (5.7)% with no history of lung disease formed the control group.

Methacholine inhalation challenge testing was performed in the patients with asthma. All challenge tests were performed at 13.00 hours to eliminate the effect of diurnal variation. Following baseline spirometric tests and inhalation of diluent to establish the stability of FEV₁, the subjects were instructed to take slow inspirations in each set of inhalations. All the asthmatic patients had bronchial hyperreactivity to methacholine. Their regular medication consisted of β₂ agonists and theophylline, and none were receiving oral or inhaled corticosteroids. Medications were not changed during the month before the study and were withdrawn for at least 12 hours before the methacholine challenge and exercise test. All patients were clinically stable and none had a history of respiratory infection for at least 4 weeks before the study.

All subjects gave their written informed consent for participation in this study which was approved by the ethics committee of Osaka City University, Japan.

Sputum induction and processing

Spirometric tests were performed before inhalation of 200 µg salbutamol via a metered dose inhaler. All subjects were instructed to wash their mouth thoroughly with water. They then inhaled 3% saline at room temperature, nebulised by an ultrasonic nebuliser (NE-U12; Omron Co, Tokyo, Japan) at...
maximum output. They were then asked to cough deeply at 3 minute intervals. After sputum induction the spirometric tests were repeated. If the FEV₁ fell, the subjects were required to wait until it returned to baseline value. The sputum samples were kept at 4°C for no longer than 2 hours before further processing. A portion of the sample was diluted with phosphate buffer solution (PBS) containing 10 mmol/l dithiothreitol (DTT) (Sigma Chemical Co, St Louis, MO, USA) and gently vortexed at room temperature. It was then centrifuged at 4000 g for 10 minutes and the cell pellet was resuspended. Total cell counts were performed with a haemocytometer and slides were made using a cytospin (Cytospin 3; Shandon, Tokyo, Japan) and stained with May-Grunwald-Giemsa stain for differential cell counts. The mean differential cell counts of at least two chest physicians on separate occasions in a blind manner were used.

The supernatant was stored at −70°C for subsequent assay for albumin, VEGF, and eosinophil cationic protein (ECP). The concentration of VEGF was measured using an enzyme linked immunosorbent assay kit (R&D System Inc, Minneapolis, MN, USA), ECP measurement was carried out using a radioimmunoassay kit (Pharmacia Diagnostics, Uppsala, Sweden), and albumin was measured by laser nephelometry. We thus could calculate the airway vascular permeability index (ratio of albumin concentrations in induced sputum and serum).

All subjects produced an adequate specimen of sputum of at least 2 ml with differential cell counts of <10% squamous cells.

Exercise challenge testing

Three days after sputum induction the exercise test was performed on an electrically driven treadmill (O25xt, Series 90; Quinton Instrument Co, Seattle, WA, USA) for 6 minutes with a fixed workload adjusted to increase the cardiac frequency to 90% of the maximum predicted for the age of the patient. All subjects breathed unconditioned room air (temperature 22–25°C) and were trained to overcome hyperventilation during testing. A single lead electrocardiogram (ECG) and pulse oximeter (502-US; CSI, Tokyo, Japan) were monitored continuously. The criteria for exclusion were the presence of coronary artery disease or cardiac arrhythmias. A spirometer (Chestac–25F; Chest Co, Tokyo, Japan) was used to obtain spirometric measurements before and after exercise challenge. The higher of two measurements of FEV₁ obtained before exercise challenge was taken as the baseline value. Single measurements of FEV₁ were obtained at 1, 3, 5, 10, 15, 20, 25 and 30 minutes after completion of the exercise challenge. The response to exercise challenge was taken to be the percentage fall in FEV₁ after exercise:

\[ \text{% fall in FEV}_1 = \left( \text{FEV}_1 \text{ (baseline)} - \text{FEV}_1 \text{ (after exercise)} \right) / \text{FEV}_1 \text{ (baseline)} \times 100 \]

For 8 weeks after the first exercise test beclomethasone dipropionate (BDP) 400 µg twice daily was administered to the asthmatic patients. During this 8 week period all subjects continued their previous treatment of β₂ agonists and theophylline. All of the above tests were repeated following treatment with BDP.

Statistical analysis

All data are expressed as mean (SD) values. The Mann-Whitney U test was used for intergroup comparisons and Wilcoxon's signed rank test was used to compare paired values. The significance of correlation was evaluated using Spearman's rank correlation coefficients. A p value of <0.05 was considered significant.

RESULTS

The clinical characteristics of the 23 asthmatic subjects and 11 age matched normal controls are shown in table 1. The percentage of eosinophils and the concentration of ECP in induced sputum were significantly higher in asthmatic subjects than in normal controls. The concentration of VEGF in induced sputum was also significantly higher in patients with asthma than in normal controls. There was a significant correlation between the concentration of VEGF and the% maximal fall in FEV₁ (r=0.826, p=0.0001; fig 1) and between the concentration of VEGF and the airway vascular permeability index (r=0.621, p=0.0037; fig 2).

After treatment with inhaled BDP there was a significant decrease in the percentage of eosinophils (from 17.0 (6.9)% before treatment to 8.1 (1.1)% after treatment; p<0.0001), the concentration of ECP (from 705 (288) ng/ml to 117 (100) ng/ml; p<0.0001), and in VEGF (from 7051 (2361) pg/ml to 4498

### Table 1 Clinical characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Normal controls</th>
<th>Asthmatic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F</td>
<td>11 (6/5)</td>
<td>23 (13/10)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>34.1 (7.7)</td>
<td>34.8 (7.8)</td>
</tr>
<tr>
<td>FEV₁ (% predicted)</td>
<td>106.6 (5.7)</td>
<td>90.1 (5.4)</td>
</tr>
<tr>
<td>PC&lt;sub&gt;20&lt;/sub&gt; methacholine (µg/ml)*</td>
<td>ND</td>
<td>3.34 (0.31)</td>
</tr>
<tr>
<td>4% Maximal fall in FEV₁, after exercise (%)</td>
<td>ND</td>
<td>24.0 (13.3)</td>
</tr>
<tr>
<td>Sputum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Eosinophils (%)</td>
<td>1.0 (0.7)</td>
<td>17.0 (6.9)</td>
</tr>
<tr>
<td>ECP (µg/ml)</td>
<td>113 (66)</td>
<td>705 (288)</td>
</tr>
<tr>
<td>VEGF (µg/ml)</td>
<td>1345 (1304)</td>
<td>7051 (2361)</td>
</tr>
</tbody>
</table>

All values are mean (SD).

*Geometric mean.

†p<0.01 compared with normal controls.

Figure 1 Correlation between VEGF levels in induced sputum and % maximal fall in FEV₁ in asthmatic patients.

Figure 2 Correlation between VEGF levels in induced sputum and airway vascular permeability index in asthmatic patients.
(2135) pg/ml; \( p < 0.0001 \) in patients with asthma. The severity of EIB was also significantly decreased after treatment with inhaled BDP (% maximal fall in FEV \(_2\) 24.0 (13.3)% before treatment \( r = 14.7 \) (9.4)% after treatment, \( p < 0.0001 \)). The change in the concentration of VEGF was significantly correlated with the change in the % maximal fall in FEV \(_2\) \( r = 0.463, p = 0.031 \); fig 3). In contrast, neither the change in percentage geometry—vascular engorgement, capillary leakage, and bronchial circulation could exert an important influence on airway bed is hypertrophied and hyperplastic. Because of its location, this work was supported by grant-in-aid for Scientific Research (1360611) from the Ministry of Education, Science and Culture, Japan.

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