The bronchoconstrictive peptide endothelin-1 (ET-1) has been demonstrated in the airway epithelial and endothelial cells. In this study we investigated the pathophysiological significance of endothelin-1 in asthma. We addressed the issue by assessing the concentration of ET-1 in plasma and bronchoalveolar lavage fluid (BALF) in patients with a different intensity of asthma. Twenty one asthmatic patients (11 men, 10 women) and 6 healthy control subjects (C) were included in the study. Eleven asthmatic patients were classified as moderate persistent asthma (SA), all of them were atopic, and another 10 were mild persistent asthmatics (AA). Lung function tests were carried out in all patients investigated. The ET-1 concentration was determined by an ELISA method in plasma and BALF. We found that the SA patients had the highest level of ET-1 (SA - 11.4 ±3.6 fmol/ml; AA - 7.1 ±2.7 fmol/ml; C - 5.6 ±1.8 fmol/ml) in BALF. The same concerned the ET-1 level in plasma (SA - 27.8 ±3.8 fmol/ml; AA - 18.1 ±4.3 fmol/ml; C - 17.3 ±3.0 fmol/ml). A positive correlation between the plasma ET-1 level and lung function indices was observed. We conclude that the higher levels of ET-1 in more severe asthma suggest that endothelins may contribute to the pathophysiology of the disease, its severity, and the regulation of bronchial tone.

Key words: asthma, bronchoalveolar lavage, endothelin-1

INTRODUCTION

Bronchial epithelial cells are known to play an integral role in the airway physiology. Airway epithelium is involved in allergic inflammatory processes. Many recent studies indicate that airway epithelium produces and releases
substances, such as endothelin, cytokines and chemokines, growth factors, and eicosanoids, active in the pathophysiology of airway diseases.

In 1988, Yanagisawa et al (1) isolated from a culture medium of vascular endothelial cells a novel peptide, endothelin. Endothelin-1 (ET-1) is known to be the most potent endothelium-derived vasoconstrictor peptide identified so far. Progress in endothelin research brought us new informations about their properties. Endothelin has a potent effect on smooth muscle contractile properties and has been linked to airway constriction in asthma. Endothelin released from both endothelial and epithelial cells, is binding to the specific endothelin receptors rET-A, rET-B1, rET-B2 (2-4). Subsequently, Uchida et al (5) described ET-1 as one of the most potent contractile agents in airway smooth muscles. ET-1 is implicated in the fibrotic response in the airway remodeling process, occurring in asthma and other pulmonary diseases. ET-1 immunoreactivity was found mainly in alveolar macrophages in patients with sarcoidosis (6). Quantitative autoradiography in human and animal airway smooth muscle has demonstrated high densities of specific binding sites for ET-1, stimulation of which produces a slow, long lasting contraction (7, 8).

The contractile action of ET-1 is presumably mediated in vascular tissue through the voltage dependent calcium channel (9). There are reports that in isolated human bronchus this kind of endothelin action is independent of the influx of extracellular calcium ions (9). Recent studies suggest an essential role of the endothelium in asthmatic airway disorders. ET-1 seems at play in sustained bronchoconstriction, mucus secretion, plasma exudation, and structural remodeling of respiratory mucosa (5, 10-12).

Extrapolating results from animal models to human diseases is still problematic. Therefore, the aim of the present study was to extent the findings from previous animal and laboratory experiments and to evaluate the levels of ET-1 in plasma and bronchoalveolar lavage fluid (BALF) from asthmatic patients who were at various stages of the disease.

MATERIAL AND METHODS

The study was approved by a local institutional Review Board for Human Experiments. All subjects participating in the study gave informed consent to study procedures.

We included 21 stable asthmatic patients into the study, 11 men and 10 women, aged 18-42 years (mean age 24.6 ±3.6 years). According to the GINA guidelines, the patients were divided into two groups. One group consisted of 11 patients (6 men and 5 women) with moderate persistent asthma (SA) (mean age 30.4 ±42 years) and a second group of 10 patients (5 men and 5 women) with mild persistent asthma (AA) (mean age 21.7 ±2.4 years). Thirteen patients were atopic, with a positive skin prick test to one or more common inhalant allergens (mites, alternaria, grass, tree and ragweed pollens, cat and dog dander). All 11 patient classified as moderate persistent asthma were atopic.

Before inclusion in the study, all patients had measured and put in a diary card their peak expiratory flow (PEF) twice daily for a week. On the last day, the patients had spirometry and blood samples were collected. The SA patients presented diurnal PEF variation >20% and FEV1% <80%
of predicted values. These patients were taking long-acting β2 agonists, inhaled corticosteroids, and theophyllin derivates. The AA patients were symptomatic 1-3 times a week and once a week had night symptoms, demonstrated diurnal variation of PEF 20-30% and FEV1% > 80% of predicted values. They received inhaled corticosteroids only, < 500 µg BDP. All of the investigated patients demonstrated reversible airway obstruction, as evidenced by a 15% increase in FEV1 after inhalation of 200 µg of salbutamol. Throughout the study the patients were free of infections, which was confirmed by physical, radiological, and laboratory examinations. The control group (C) consisted of 6 healthy subjects, 1 woman and 5 men, of the mean age 22.3 ± 2.5 years.

**BALF and blood sampling**

Bronchoalveolar lavage was performed according to the guidelines presented by the American Thoracic Society (13). Sterile isotonic saline solution warmed to body temperature was used for lavage. The tip of a flexible bronchoscope (Olympus, Japan) was wedged into the right middle lobe. Five 20 ml portions of the saline were instilled into the segmental lobe, and the fluid was recovered by suction. The fluid was centrifugated at 1000 x g for 10 min at 4°C. The supernatant were pooled and 7.5 mM EDTA and 500 KIU/ml apronitin were added. The samples we stored at -20°C until further analysis. Total protein concentration in BALF was assessed according to the method of Lowry (14).

Blood samples were collected from cubital vein into tubes with 7.5 mM EDTA and apronitin (500 KIU/ml) and were centrifugated at 1000 x g for 10 min at 4°C. Plasma samples were stored at -20°C prior to analysis. The sampling was performed at the same time of day in every patient. In the symptomatic asthmatics, venous blood was collected during an episode of bronchoconstriction.

**Endothelin assay**

Endothelin was extracted from plasma and BALF using Amprep 500mg C2 minicolumns (Amersham International, Amersham, UK). Immunoreactives of ET-1 in plasma and BALF were evaluated in duplicate samples using an Endothelin-1[125I] radioimmunoassay system (Amersham International, Amersham, UK). The method was described in detail by Black et al (16). The sensivity of this method was 1.03 fmol per tube. The inter-assay reproducibility for duplicate determinations was calculated as 4.1%.

**Statistical analysis**

Data are expressed as means ± SD. Results were analyzed by one-way analysis of variance, Spearman's rank test, and an unpaired t-test. A commercial Statistica for Windows packet was used for statistical analylsis. P<0.05 was considered to indicate statistical significance.

**RESULTS**

The baseline mean FEV1 value in the SA patients (moderate persistent asthma) was 72.6 ± 6.3% of predicted values and that in the AA patients (mild persistent asthma) was 92.2 ± 8.1% of predicted values. The difference between the FEV1 in the two groups was significant (P<0.005).

The volume of BALF recovered from asthmatic patients (64.3 ± 8.2 ml) was comparable with that in the control subjects (68.3 ± 7.8 ml). We observed a higher mean level of total protein in BALF in the moderate asthma group than
those in the other groups (SA - 1128.5 ±157.3, AA - 847.3 ±182.8, C - 685.1 ±175.6 mg/ml). The difference between the SA and other groups was significant (P<0.001).

There were detectable levels of ET-1 in both plasma and BALF in all investigated subjects. The ET-1 concentration in BALF, calculated to total protein content, in th SA patients was significantly higher than those in the AA and control subjects (SA - 11.43 ±3.6 fmol/mg/ml, AA - 7.08 ±2.7 fmol/mg/ml, C - 5.58 ±1.8 fmol/mg/ml) (P<0.05) (Fig. 1).

There also were differences among the study groups regarding the plasma ET-1 concentration. Again, the ET-1 concentration was appreciably higher in the moderate asthma patients, but there were no differences in it between the mild asthma and control subjects (SA - 27.77 ±3.8 fmol/ml, AA - 18.14 ±4.3 fmol/ml, C - 17.33 ±2.98 fmol/ml) (Fig. 2).
A significant inverse correlation was noted between the ET-1 plasma concentration and spirometric values of FEV1% (Fig. 3).

DISCUSSION

In vitro studies have revealed that human bronchial smooth muscles, epithelium, and submucosal glands contain endothelin and endothelin receptors (3, 8). ET-1 has emerged as one of the most potent smooth muscle constrictors (1, 15). The study of Black et al (16) has indicated that airway epithelium could produce and release endothelin and supported the opinion that epithelium plays a major role in respiratory pathologies.

The present study demonstrates the increased concentration of ET-1 in BALF from patients with moderate persistent asthma, which points to the bronchial cells, rather than increased vascular leakage, as an important source of ET-1 released into the airways of asthmatic patients. It is interesting that all of the patients with moderate asthma were atopic. This observation is consistent with Campbell's et al (17) who have shown the ET-1 releasing CD-23 positive epithelial cells in allergic asthmatics after incubation with IgE.

Presumably, inflammatory cells and mediators present in bronchial mucosa could upregulate production and release of ET-1. A vascular origin of the peptide also cannot be excluded. The relationship between the ET-1 plasma concentration and FEV1, observed in the present study, could support this hypothesis. Increased plasma ET-1 could be explained in two ways. Firstly, hypoxemia could locally stimulate endothelial cells to release endothelin and secondly, endothelin could be released as a result of a stress reaction associated with respiratory disorders. It is known that stressful, long-lasting exercise, as opposed to short exercise, increases the ET-1 plasma concentration (18). ET-1 induces human lung fibroblast
proliferation through ET(A) receptor-dependent mitogen activated protein kinases (MAPK) and IL-11 release (19).

Sofia et al (20) have demonstrated that ET-1 is detectable in BALF from patients with bronchopulmonary diseases, but is rarely present in subjects with normal airways (20). A study on endobronchial biopsy specimens revealed the substantial presence of ET-1 in the epithelium and vascular endothelium in 11 of the 17 asthmatics, but in only 1 of 11 control subjects (12).

However, Lagente et al (15) have found that neither inhaled nor infused ET-1 induces bronchial hyperreactivity in the guinea pig, a hallmark of bronchial asthma. It is possible that a low concentration of ET-1, augments NO production, but is insufficient to induce bronchoconstriction (21). In a study of Makker et al (22) local exposure of asthmatic airways to an aerosol of hypertonic saline, although it induced bronchospasm, did not increase endothelin release into BALF (22). That could be simply explained by a dilutional phenomenon (hypertonicity-induced passive water flux from the epithelium). Patients with asthma frequently use β2-adrenergic agonists. There are recent observations that fenoterol potentiates constriction of smooth muscle in reaction to ET-1 (23). A possible explanation of this feature is modulation of epithelial ETA and ETB receptors (23). There is evidence that ET-1 contributes to the evolution of allergic inflammation by stimulating eicosanoid production in cultured human nasal mucosa and canine lungs (24, 25). Hay et al (24) have shown that ET-1 elicits release of prostaglandin D2, tromboxane B2, PGF2, PGF2, but not that of leukotrienes and histamine, from human bronchial tissue (24). These results are at variance with those of Uchida et al (5) who have found that ET-1 induces histamine release from mast cells in a receptor-dependent way. Vice versa, some inflammatory mediators (histamine, IL-1) are conducive to endothelin production in bronchial epithelial cells.

Endothelin also can promote fibroblast proliferation leading to structural alteration of airways (6, 26). Increased concentration of ET-1 in alveolar macrophages and BALF could enhance fibrogenesis in pulmonary sarcoidosis (6). ET-1 slows proliferation of human bronchial epithelial cells, which potentially might lead to inhibition of epithelium repair and enhanced airway remodeling (26).

Endothelin should be considered as a potential constrictor factor in the pathophysiology of bronchial asthma. It is likely that a selective ETb receptor antagonist might offer a new therapeutic way for asthma treatment (1). Further comprehensive studies are necessary to better characterize a specific role of ET-1 in respiratory pathophysiology.

REFERENCES


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