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Increased Serum Levels of Endostatin in Patients With Idiopathic Pulmonary Fibrosis

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Endostatin is an angiogenesis inhibitor that is an endogenously produced proteolytic fragment of type XVIII collagen. Serum levels of endostatin have been studied extensively in patients with malignant diseases. Recently, elevated serum endostatin levels were observed in patients with systemic sclerosis accompanying pulmonary fibrosis. To determine whether elevated serum endostatin can be observed in patients with idiopathic pulmonary fibrosis (IPF), we measured serum levels of endostatin in 69 patients with benign respiratory disease using an ELISA kit. The median of the serum endostatin levels in these patients was 50.8 pg/mL. Seven of 11 patients (63.6%) with collagen disease-associated pulmonary fibrosis (CDPF), and 19 of 24 patients (79.2%) with IPF had higher serum endostatin levels than the median level of the 69 patients. There was no statistical difference in serum endostatin levels between the patients with IPF and those with CDPF (P=0.7898). Serum endostatin levels in 24 patients with IPF were significantly higher than those in 34 patients with respiratory diseases other than IPF and CDPF (P=0.0001). Elevated serum levels of endostatin were observed in patients with IPF. Although the mechanisms are unclear, elevated serum levels of endostatin may be related to the fibrosing process in the lung. J. Clin. Lab. Anal. 19:146–149, 2005. © 2005 Wiley-Liss, Inc.

Key words: idiopathic pulmonary fibrosis; serum; endostatin; collagen disease; chronic obstructive pulmonary disease

INTRODUCTION

Endostatin has been identified as a C-terminal fragment of collagen XVIII. The proteolytic release of endostatin can occur via several pathways, which may lead to a switch from a matrix-associated form to a more soluble endocrine form.

Elevated serum levels of endostatin have been observed in patients with cancers originating from the kidney, head and neck, and lung (1–3). Suzuki et al. (3) reported that serum endostatin levels were higher in patients with both lung cancer and interstitial pneumonia compared to those without interstitial pneumonia. Very recently, two study groups reported the serum levels of endostatin in patients with systemic sclerosis (4,5). One of these study groups demonstrated elevated serum levels of endostatin in such patients, especially those who presented with pulmonary fibrosis, and they speculated that the endostatin detected was released by tissue-activated fibroblasts (4). If elevated serum endostatin is derived from activated fibroblasts in the lung, elevated serum endostatin may be observed in patients with fibrosing lung diseases, such as collagen disease-associated pulmonary fibrosis (CDPF) and idiopathic pulmonary fibrosis (IPF). To test this hypothesis, we evaluated serum levels of endostatin in patients with benign respiratory disease using an ELISA kit, which is designed to measure the amount of ''free'' forms of endostatin.

MATERIALS AND METHODS

Patients

A total of 241 patients were admitted to the Division of Respiratory Medicine, University of Tsukuba Hospital, in April 1998–April 2000, and diagnosed with benign respiratory diseases. These patients were

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followed up for 3 years, and 227 of them did not develop malignant disease at any site. Of the 227 patients, 69 were involved in this study. These patients were chosen at random. Fifteen nondiseased control subjects were also enrolled in this study.

A diagnosis of IPF was established by a combination of medical history, physical examination, laboratory tests, chest radiograph, pulmonary function tests, arterial blood analyses, and lung biopsy according to previously described criteria (6–11). The criteria used included a history of exertional dyspnea and cough, fine crackles on physical examination, compatible findings on the chest radiograph, and physiological abnormalities of restrictive lung defects, including decreased diffusing capacity and abnormal arterial oxygen tension. Histological confirmation was obtained in all cases by video-assisted thoracoscopic biopsy or transbronchial lung biopsy. The patients with CDPF were clinically classified as having rheumatoid arthritis, polymyositis and dermatomyositis, Sjogren's syndrome, systemic sclerosis, and mixed connective tissue disease according to the criteria for each disease (12–16). A diagnosis of chronic obstructive pulmonary disease (COPD) was based on medical history and pulmonary function tests that confirmed the presence of severe irreversible bronchial obstruction (forced expiratory volume/FEV $1.0 < 1.5$ L, FEV $1.0\% < 70\%$ predicted value), hyperinflation (total lung capacity/TLC $> 100\%$ predicted value), and reduced diffusing capacity of lung for carbon monoxide (DLCO) ($< 70\%$ predicted value).

Circulating Endostatin Assay

Serum samples were obtained from the patients with benign respiratory disease before they received any clinical treatment, and were stored at –20°C until they were analyzed. Concentrations of circulating endostatin were measured in duplicate by means of a commercial enzyme immunoassay kit (CytElisa Human Endostatin; CytImmune Sciences, College Park, MD). This kit is designed to measure the amount of "free" forms of endostatin in tissue cell culture supernatants and biological fluid samples, such as serum and pleural effusion. The typical sensitivity of the immunoassay kit used in this study was < 15.6 pg/mL, which is sufficient to detect minimal physiologic concentrations.

Statistical Analysis

The data are shown as box plots with median and upper and lower quartiles if not otherwise indicated. We used the Kruskal-Wallis test to analyze differences between more than two groups, and the Mann-Whitney test to analyze differences between two specific groups. $P < 0.05$ was considered of statistical significance.

RESULTS

The general characteristics of the 69 patients studied are outlined in Table 1. The average age was 65 years (range = 23–84 years), and 43 were male. The distribution of age did not differ between 69 patients with benign respiratory disease and 15 nondiseased control subjects ($P = 0.7700$). There also was no statistical difference in age between the control subjects and patients with CDPF ($P = 0.8152$), and those with IPF ($P = 0.6337$).

We analyzed the distribution of serum endostatin levels in 69 patients with begin respiratory diseases (Fig. 1, Table 2). The median and interquartile ranges of the serum endostatin levels in the 69 patients with benign respiratory disease were 50.8 pg/mL and 29.3–172.3 pg/mL, respectively. Seven of 11 patients with CDPF (63.6%; median = 139.3 pg/mL, interquartile range = 39.7–243.3 pg/mL) had higher serum endostatin levels than the median level of the 69 patients tested. Interestingly, 19 of 24 patients with IPF (79.2%) also had higher serum endostatin levels (median = 107.9 pg/mL, interquartile range = 52.3–279.1 pg/mL) than the median level of the 69 patients. On the other hand, 10% and 0% of patients with COPD and bronchial asthma had higher endostatin levels than the median level, respectively (Table 2). Serum levels of endostatin were significantly higher in patients with CDPF ($P = 0.0052$) and IPF ($P = 0.0006$) than in the control subjects. There was no significance difference between the control subjects and patients with COPD ($P = 0.6151$), bronchial asthma ($P = 0.6112$), and patients with respiratory diseases other than IPF and CDPF ($P = 0.7283$).

Serum endostatin levels in 11 patients with CDPF were significantly higher than those in 34 patients with respiratory diseases other than IPF and CDPF (median = 29.0 pg/mL; interquartile range = 19.6–50.8 pg/mL; $P = 0.0001$) (Fig. 1). Serum levels in 24 patients with

TABLE 1. Patient characteristics

No. of patients	69
Gender, M : F	43 : 26
Age, median, range, year	65, 23–84
Diagnosis, patients	
IPF	24
CDPF	11
COPD	10
Bronchiectasis/diffuse panbronchiolitis	8
Bronchial asthma	4
Pneumonia	4
Others[a]	8

[a]Others include two patients with pneumoconiosis, two patients with proteinosis, two patients with thromboembolism, one patient with sacroidosis, one patient with tuberculosis.

IPF, idiopathic pulmonary fibrosis; CDPF; collagen disease-associated pulmonary fibrosis, COPD, chronic obstructive pulmonary disease.

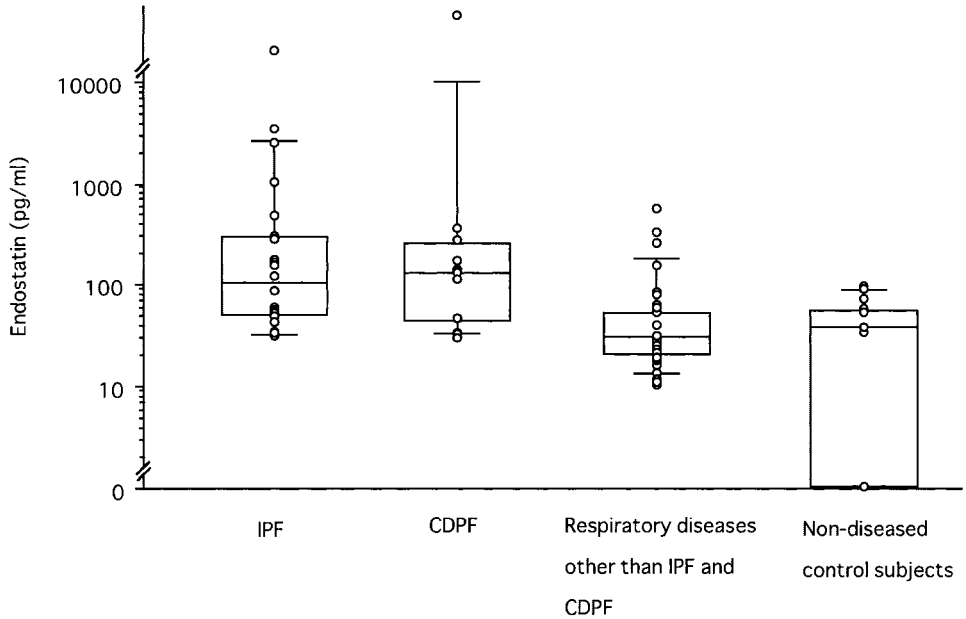

Fig. 1. Serum endostatin levels in patients with benign respiratory diseases. Each box indicates the 25th and 75th percentiles, with the median values indicated by the lines within the boxes. The bars extending above and below the box indicate the 90th and 10th percentiles, respectively. Plots indicate all individual data points for each subject.

TABLE 2. Serum endostatin levels in patients with benign respiratory diseases

	Endostatin (pg/ml)	
Diagnosis	Median	Interquartile range
IPF	107.9	52.3–279.1
CDPF	139.3	39.7–243.3
COPD	24.0	16.9–32.0
Bronchial asthma	21.6	16.8–29.7
Bronchiectasis/diffuse panbronchiolits	51.7	38.1–113.1
Pneumonia	28.2	21.8–34.0
Others[a]	23.3	19.1–166.9
Non-diseased controls	37.7	0–59.9

[a]Others include two patients with pneumoconiosis, two patients with proteinosis, two patients with thromboembolism, one patient with sarcoidosis, one patient with tuberculosis.
IPF, idiopathic pulmonary fibrosis; CDPF, collagen disease-associated pulmonary fibrosis, COPD, chronic obstructive pulmonary disease.

IPF were significantly higher than those in 34 patients with respiratory diseases other than IPF and CDPF ($P = 0.0001$; Fig. 1), whereas, there was no statistical difference in serum endostatin levels between the patients with CDPF and those with IPF ($P = 0.7898$; Fig. 1).

DISCUSSION

Serum levels of endostatin have been extensively investigated and found to correlate with disease extent or prognosis in patients with malignant diseases, and elevated serum endostatin levels have been observed in patients with cancers originating from several organs, including the lung (1–3). Suzuki et al. (3) revealed that serum endostatin levels were higher in patients with both lung cancer and interstitial pneumonia compared to those without interstitial pneumonia. Endostatin is an angiogenesis inhibitor that is an endogenously produced, 20-kD proteolytic fragment of type XVIII collagen (17,18). Type XVIII collagen is a basement membrane heparan sulfate proteoglycan, and is found in abundance along blood vessels in the lungs (19). Recently, Hebbar et al. (4) showed that patients with systemic sclerosis (especially those with pulmonary fibrosis, which was detectable on chest radiograms) had higher concentrations of endostatin. Hebbar et al. (4) suggested that elevated serum levels in patients with systemic sclerosis may result from the activation of tissue fibroblasts, leading to collagen deposition. However, Distler et al. (5) showed that serum levels of endostatin were not increased in systemic sclerosis patients compared to healthy controls. The reasons for these differences are not clear. In both study groups, enzyme immunoassays for the determination of endostatin were purchased from the same manufacturer, and serum samples were processed in similar ways. Distler et al. (5) suggested that a possible explanation included clinical differences in the study populations. At least two different types of kits are used to measure human endostatin. One of the kits, Accucyte Human Endostatin, is a competitive ELISA designed to measure

endostatin in biological samples. The dynamic range in this assay ranges from 2.0 ng/mL to 500 ng/mL. A number of studies have used this kit (1,3,4). The CytElisa is the more sensitive of the two kits, and measures endostatin in the dynamic range of 15.6–4,000 pg/mL. In the present study, we measured serum levels of endostatin using the CytElisa kit, which is designed to measure the amount of "free" forms of endostatin. Serum endostatin levels were evaluated in CDPF patients, including two patients with systemic sclerosis whose serum endostatin levels were 139.3 pg/mL and 30.1 pg/mL, respectively. The former patient had more extensive pulmonary fibrotic change than the latter patient. However, on the basis of these results we could not conclude whether elevated levels of endostatin are specific for certain subgroups of systemic sclerosis. Large-scale studies with precise analyses will be required to clarify this issue.

In the present study, we found that serum levels of endostatin were significantly higher in patients with CDPF or IPF compared to patients with benign respiratory diseases other than these two fibrotic lung diseases. The mechanisms behind the elevated serum endostatin levels in such patients may be quite different from those in patients with malignant disease. Kanazawa et al. (20) recently reported that decreased levels of vascular endothelial growth factor were associated with alveolar destruction in patients with pulmonary emphysema. In this study, we also observed lower serum endostatin levels in patients with COPD. These results suggest that decreased levels of angiogenic growth factors and their inhibitors may play a role in the pathogenesis of benign respiratory diseases, such as COPD, or have little relationship with the development of COPD. However, we have no definitive information about this issue.

In summary, elevated serum levels of endostatin were observed in patients with CDPF and patients with IPF. Although the precise mechanism and function of increased endostatin levels in patients with CDPF or IPF are unclear at present, we speculate that elevated serum levels of endostatin in such patients may represent the activation of tissue fibroblasts in the lung, leading to collagen deposition, and endostatin may participate in the fibrosing process in the lung.

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