Endothelin-1 in the lungs of patients with pulmonary hypertension

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Abstract

Background: Pulmonary hypertension (PH) is characterized by an increase in vascular tone and an abnormal proliferation of muscle cells in the walls of pulmonary arteries. Recent studies have found high plasma endothelin-1 (ET-1) concentrations in patients with PH. This study was conducted to assess whether elevated circulating ET-1 levels in PH really reflect excessive local pulmonary production.

Methods: We prospectively studied ET-1 concentration in lung specimens from 6 control subjects and 13 patients with severe PH referred for lung or heart-lung transplantation (6 patients had primary PH and 7 PH secondary to congenital heart defect). Endothelin-like immunoreactivity (ET-LI) was measured in plasma and lung tissue, using a radioimmunoassay, after ET-1 extraction. Reverse-phase high-performance liquid chromatography was also performed.

Results: Peripheral venous plasma ET-LI concentrations in patients with PH, whatever the cause, were greater than those in controls (10.7 ± 0.8 vs 5.3 ± 0.7 pg/ml; P < 0.0005). Pulmonary ET-LI was significantly higher in patients with PH, irrespective of its cause, than in controls (25.2 ± 5.1 vs 8.1 ± 1.1 pg/mg, P < 0.03). ET-LI pulmonary concentrations were slightly higher in Eisenmenger than in primary PH, but this was not significant (27.1 ± 8.6 vs 22.8 ± 5.4 pg/mg). Linear regression analysis indicated a small but significant correlation between ET-LI pulmonary concentrations and pulmonary vascular resistance in the patients with PH (r = 0.38; P = 0.047). In each case, HPLC separation of ET indicated that most of the immuno-reactivity was detected in the same fraction as ET-1.

Conclusions: The striking increase in ET-1 pulmonary concentration provides new evidence that excessive local pulmonary ET-1 production may contribute to the vascular abnormalities of pulmonary hypertension.

Keywords: Pulmonary hypertension; Eisenmenger’s syndrome; Endothelin-1; Human, lung

1. Introduction

Pulmonary hypertension (PH) is often a progressive condition, characterized by an increase in pulmonary vascular resistance that ultimately leads to right-heart failure and death. PH is histologically characterized by endothelial injury and the proliferation of pulmonary arterial smooth-muscle cells. A role for vasoconstriction in the pathophysiology of PH is supported by the possibility of spontaneous reversal at early stages and the higher-than-expected incidence of Raynaud’s phenomenon in primary PH patients [1]. What is far from obvious is the mechanism(s) of the initiation of vasoconstriction and of the perpetuation or progression leading to obstruction. Recently, it has been recognized that the vascular endothelium plays a crucial part in local regulation of the function of smooth-muscle cells. Under physiologic conditions, the endothelium produces a variety of vasodilator mediators, which maintain an appropriate level of vascular tone and prevent the proliferation of smooth-muscle cells [2]. Under pathologic conditions, the endothelium can secrete factors that induce profound vasoconstriction. Endothelin-1 (ET-1) is a peptide recently isolated from vascular endothelial cells that has the most potent vasoconstricting activity and which is
also a mitogen for smooth-muscle cells in vitro [3–5]. These observations raise the possibility that ET-1 plays an important role in the increased vascular tone and medial hypertrophy of small arteries seen in PH. We and others have previously reported that patients with PH have significantly higher plasma ET-1 concentrations than healthy controls [6,7]. Together, these results strongly suggest that the pulmonary production of ET-1 may contribute to the vascular abnormalities associated with PH. Only one study suggests that elevated circulating ET-1 levels in PH really reflect an excessive local pulmonary production and then can contribute to the vascular abnormalities of PH [8].

In the present study, to determine the role of local ET-1 pulmonary production, we measured pulmonary ET-1 concentrations in patients with PH and compared them with those found in normal subjects.

2. Methods

2.1. Patients

We prospectively studied ET-1 concentration in the lungs of 13 consecutive patients referred for lung or heart-lung transplantation after diagnosis of severe PH had been established at cardiac catheterization. Six patients had primary PH based on the criteria established by the National Heart, Lung and Blood Institute Primary Pulmonary Hypertension Registry [9], and 7 patients had PH secondary to congenital heart defect (Eisenmenger’s syndrome). In addition, lung biopsies from 6 men (40 to 67 years old, mean 54) with lung cancer and without PH by echocardiography were also analyzed as a control group. No patient had known causes of elevation of ET-1 such as diabetes mellitus, systemic hypertension, sepsis, renal failure or cardiogenic shock. None of the patients received NO or prostacyclin for the treatment of their PH.

2.2. Endothelin assay

Peripheral venous blood samples were obtained, in patients and controls, from an antecubital vein after at least 15 min seated rest. Venous blood samples were collected in glass tubes containing ethylenediamine tetraacetic acid, centrifuged at 2000 × g for 15 min and plasma was frozen and stored at −80°C until assay. Plasma endothelin-like immunoreactivity (ET-LI) was measured by radioimmunoassay using polyclonal antibody (obtained from rabbit after human ET-1 immunization; intra- and inter-assay reproducibility 13.3 and 7.5%, respectively; sensitivity 0.2 pg/tube) after plasma extraction on a C2 ethyl microcolumn (Amersham, UK). Antiserum cross-reactivity was ET-1 100%, ET-2 130%, and ET-3 0.1%; “big” ET < 5%, but the antibody exhibited no cross-reactivity with unrelated peptides (i.e., atrial natriuretic factor, vasopressin, angiotensin II).

ET-LI lung concentrations were measured after ET-1 extraction. We obtained lung tissue specimen of 100–150 g from patients and controls. Tissues were homogenized in 10 vol (vol/wt) HCl (0.1 mol/L) and heated at 95°C for 10 min, then centrifuged at 20,000 × g for 30 min. The supernatant was adjusted to pH 7.5 with 1 mol/L Tris and the cloudy precipitate was eliminated by a second precipitation at 20,000 × g for 30 min. The final clear supernatant was lyophilized and the dry residue was stored at −80°C. ET-LI was measured by the same radio-immunoassay using polyclonal antibody. ET-LI pulmonary concentration was expressed in pg/mg of dry pulmonary tissue. ET-1 was stable at 95°C. We determined an extraction efficiency of ET-1 by the ratio of ET-1-125I added to lung tissue specimen/ET-1-125I measures in the final extract. The extraction efficiency of ET-1 with our procedures was 59.8 ± 6.2%.

Reverse-phase high-performance liquid chromatography was also performed. The lung tissue was solubilized with water and injected (0.250 µl) into a lichrosorb RP 18
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<th>Variables in patients with primary pulmonary hypertension (n = 6) and Eisenmenger's syndrome (n = 7)</th>
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<td><strong>Age, Sex (years-M/F)</strong></td>
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<td>Primary pulmonary hypertension (PPH)</td>
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Pao² = arterial oxygen tension; MABP = mean systemic arterial blood pressure; mPAP = mean pulmonary artery pressure; PCWP = pulmonary capillary wedge pressure; PVR = pulmonary vascular resistance; ET-LI = endothelin-like immunoreactivity; NA = not available.
column pre-equilibrated with 0.001 mol/l ammonium acetate, pH 5.8. Elution was achieved at room temperature with a linear gradient of methanol (0–75% in 75 min) in the same buffer at a flow rate of 1 ml/min. Elution fractions were lyophilized before ET-1 radio-immunoassay.

2.3. Statistical analysis

All values are expressed as mean ± s.e.m. Comparison of mean values between the groups was performed with the unpaired two-tailed t-test or the two-tailed Mann-Whitney test. Pulmonary vascular resistance was calculated in Wood units from the formula (mean pulmonary artery pressure – pulmonary wedge pressure)/cardiac output. To identify relevant relations, regression analysis of hemodynamic values with pulmonary ET-LI was performed including mean systemic arterial pressure, mean pulmonary artery pressure, pulmonary wedge pressure, cardiac index and pulmonary vascular resistance. Probability values of less than 0.05 were considered statistically significant.

3. Results

The characteristics of patients are shown in Table 1. Peripheral venous plasma ET-LI concentrations in patients with PH, whatever the cause, were greater than those in controls (10.7 ± 0.8 vs 5.3 ± 0.7 pg/ml; P < 0.0005). Pulmonary ET-LI was significantly higher in patients with PH, irrespective of its cause, than in controls (25.2 ± 5.1 vs 8.1 ± 1.1 pg/mg, P < 0.03) (Fig. 1). Linear regression analysis indicated a weak but significant correlation between ET-LI pulmonary concentrations and pulmonary vascular resistance in the patients with PH (r = 0.38, P = 0.047; Fig. 2). There was no correlation between pulmonary ET-LI and all other hemodynamic variables. There was no correlation between plasma and pulmonary ET-LI levels. Compared to patients with primary PH, patients with Eisenmenger’s syndrome were younger (29 ± 5 vs 41 ± 2 years; P = 0.04) and had a more severe PH with a higher mean pulmonary artery pressure (80 ± 7 vs 58 ± 7 mmHg; P = 0.04) and hemoglobin level (18.3 ± 0.9 vs 13.9 ± 1.1 g/dl; P = 0.01), and a lower arterial oxygen tension (50 ± 2 vs 59 ± 2 mmHg; P = 0.01). ET-LI pulmonary concentrations were slightly higher in Eisenmenger than in primary PH, but this difference was not significant (27.1 ± 8.6 vs 22.8 ± 5.4 pg/mg). In each case, high-performance liquid-chromatography separation of ET indicated that most of the immuno-reactivity was detected in the same fraction as ET-1.

4. Discussion

In the present study, we found that pulmonary ET-1 concentrations are significantly elevated in patients with PH. These results provide new evidence of increased local production of ET-1 in PH and may explain earlier observations of increased circulating ET-1 levels in patients with this disorder.

The pathophysiology of PH is still unclear, but a generalized angiopathic process has been suggested. At an early stage, elevated pulmonary vascular resistance has been shown to be amenable to vasodilators [10], indicating a significant component of vasoconstriction. Migration of myofibroblasts into the intima of pulmonary arteries has been reported early in the course of PH [11]. ET-1 could promote all these processes by virtue of its potent vasoconstrictor effects on pulmonary vasculature [3,12] and vascular smooth-muscle cells proliferative actions [5]. Numerous studies have found elevated circulating ET-1 levels in PH, but it remains unclear if such elevations were associated with biological activity. We have previously reported [6] that venous plasma ET-LI concentrations were significantly elevated in patients with primary or secondary PH. Stewart et al. [7] have found that the arterio-venous ratio of ET-LI was significantly greater than unity in PH patients, suggesting pulmonary production of ET-1. In children with congenital heart defects, plasma ET-LI concentrations were
found to be increased in those with PH [13,14]. In these children, there was a significant increase of plasma ET-LI concentrations between right ventricle and pulmonary vein, which indicates increased pulmonary production. Moreover, after successful surgical repair of the congenital abnormalities, ET-1 plasma levels decreased significantly [14]. It has been argued that elevated levels of ET-1 found in the plasma of patients with PH could not play a major role in pulmonary vascular resistance modifications because (1) such levels of plasma ET-1 are not sufficient to induce significant vasoconstriction and (2) ET-1 should act in a paracrine fashion rather than as a circulating hormone. However, others have demonstrated that, in dogs, an only two-fold increase in plasma ET-1 was sufficient to increase renal and systemic vascular resistance [15]. Recent studies using an endothelin receptor antagonist in two animal models of renal and cerebral vasoconstriction reinforce the pathological role of ET-1 [16].

It seems important to know whether elevated circulating ET-1 levels in PH really reflect excessive local pulmonary production and then whether they contribute to the vascular abnormalities of PH. Stelzner et al. [17] have reported increased production of ET-1 in the lung in rats with idiopathic PH. There is little expression of ET-1 in the normal adult lung or in the pulmonary vasculature of patients with pulmonary diseases devoid of PH [18]. Giaid et al. [8] described an increased expression of ET-1 mRNA in vascular endothelial cells of patients with PH, whereas no staining was found in systemic vessels. These findings are in agreement with ours of an excessive local pulmonary production of ET-1 in PH without correlation between plasma and pulmonary ET-1 levels. The ET-1 level measured in pulmonary samples represents an average ET-1 level of the parenchyma and the level in the vessel might be considerably higher. Therefore, the tissue levels may underestimate the true vascular levels. We found a slight correlation between the increase in pulmonary resistance and the degree of ET-LI in lung samples, but these results must be analyzed cautiously because of the small size of the sample.

In the present study, we found that excessive pulmonary production of endothelin in PH consisted of ET-1, as demonstrated by high performance liquid chromatography separation. High-performance liquid chromatography separation showed that the high level of ET-1 in lung tissue from patients with PH could not be related to an abnormal conversion of 'big ET'-ET or to an abnormal rate of synthesis affecting all the endothelins.

Although the stimulus of excessive ET-1 production in the lungs of patients with PH is still unknown, our finding of a striking increase in ET-1 pulmonary concentration provides new evidence in support of the view that excessive local pulmonary ET-1 production may contribute to the vascular abnormalities of PH. However, the demonstration of increased pulmonary production of ET-LI does not prove a cause-and-effect relationship. Further studies with endothelin receptor blockers are required to confirm this hypothesis.

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References