Plasma Endothelin Concentrations in Patients With Pulmonary Hypertension Associated With Congenital Heart Defects

Evidence for Increased Production of Endothelin in Pulmonary Circulation

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Background. To elucidate the pathophysiological significance of endothelin in pulmonary hypertension associated with congenital heart defects, we measured plasma endothelin-like immunoreactivity (ET-LI) concentrations by using radioimmunoassay in 18 patients with pulmonary hypertension (PH group; age, 6 months to 12 years) in comparison with 27 patients without pulmonary hypertension (non-PH group; age, 6 months to 12 years).

Methods and Results. Blood samples were obtained from the vena cava, right atrium, right ventricle, left or right pulmonary artery, and pulmonary vein or the pulmonary arterial wedge position (pulmonary venous blood) during cardiac catheterization. Plasma ET-LI concentrations in the PH group were significantly higher than those in the non-PH group at all sampling sites. In the PH group, plasma ET-LI concentration showed a significant increase between the right ventricle and pulmonary artery and between the pulmonary artery and pulmonary vein. The increment of plasma ET-LI concentrations from the right ventricle to the pulmonary vein was significantly larger in the PH group than in the non-PH group and was significantly correlated with pulmonary artery pressure.

Conclusions. Plasma ET-LI concentrations were elevated in patients with pulmonary hypertension; the elevation was due to the increased production of ET-LI in pulmonary circulation, indicating the possible involvement of endothelin in the pathophysiology of pulmonary hypertension. (Circulation 1991;84:2280-2285)

Patients with congenital heart defects and left-to-right shunts often reveal pulmonary hypertension associated with progressive pulmonary vascular changes,1-4 the severity of which is one determinant of their prognosis. The mechanism for these changes is still poorly defined.

It has been reported that in patients with pulmonary hypertension related to congenital heart defects, ultrastructural abnormalities are observed in pulmonary artery endothelial cells from the early stage of vascular changes.5,6 Endothelin, a newly isolated peptide from vascular endothelial cells,7 has vasoconstricting activity8,9 and induces vascular smooth muscle cell proliferation.10 These observations raise the possibility that endothelin plays an important role in the pathophysiology of pulmonary hypertension and the development of pulmonary vascular changes.

In the present study, to elucidate the pathophysiological significance of endothelin in pulmonary hypertension, we measured plasma endothelin-like immunoreactivity (ET-LI) concentrations in blood samples obtained from various sites during cardiac catheterization in patients with congenital heart defects and pulmonary hypertension and compared them with those in patients without pulmonary hypertension by using a radioimmunoassay that we recently developed.11-13
Methods

Subjects
We studied 45 patients (29 males and 16 females) with congenital heart defects. Their ages ranged from 6 months to 12 years (mean±SEM, 3.2±0.3 years). Their diagnoses were ventricular septal defect in 29 patients (postoperative, six), tetralogy of Fallot in seven (postoperative, four), transposition of the great arteries in three, aortic stenosis in three, pulmonary stenosis in two, and atrial septal defect in one. We also studied 30 healthy children (18 males and 12 females; age, mean±SEM, 3.4±0.6 years) who visited Kyoto University Hospital because of heart murmur, slight electrocardiographic abnormalities, or chest pain and were proved to have no cardiovascular or other organic lesions.

Informed consent was obtained from the parents of each child for the blood sampling. This study protocol was in agreement with the guidelines of the ethical committee of our institutions.

Cardiac Catheterization
Cardiac catheterization was performed under heparinization (70 units/kg) and sedation with meperidine hydrochloride (1.0 mg/kg) and promethazine hydrochloride (0.5 mg/kg). Pressure measurement was done using a manometer-tipped catheter (Millar Instrument Inc.). Pulmonary and systemic blood flow volumes were determined according to the Fick principle. Oxygen consumption was estimated based on age, sex, and heart rate according to the method of LaFarge et al. Oxygen content in blood was measured by a CO oxymeter. Pulmonary (Rp) and systemic (Rs) vascular resistances were calculated according to the following equations:

\[
Rp (\text{dyne} \cdot \text{sec} \cdot \text{cm}^{-5} \cdot \text{m}^2) = \frac{\text{mean pulmonary artery pressure (mm Hg)}}{\text{mean left atrial or pulmonary arterial wedge pressure (mm Hg)}} \times 80
\]

\[
Rs (\text{dyne} \cdot \text{sec} \cdot \text{cm}^{-5} \cdot \text{m}^2) = \frac{\text{mean aortic pressure (mm Hg)}}{\text{mean right atrial pressure (mm Hg)}} \times 80
\]

Blood Sampling
Blood samples of 1 or 2 ml were obtained from the patients at the following sites during cardiac catheterization: superior or inferior vena cava (VC), right atrium (RA), right ventricle (RV), left or right pulmonary artery (PA), and pulmonary vein or the pulmonary arterial wedge position (pulmonary venous blood; PV). In patients with transposition of the great arteries, blood was sampled only at VC, PA, and PV. The blood sampling in each patient was performed in a random order and was finished within 5 minutes. In healthy children, blood was obtained from the antecubital vein in the supine position at rest after more than a 5-hour fast.

Blood samples were transferred to chilled, siliconized disposable glass tubes containing aprotonin (1,000 kallikrein inactivator units/ml) and ethylenediaminetetraacetic acid (1 mg/ml), immediately placed on ice, and promptly centrifuged at 4°C. An aliquot of plasma was immediately frozen at −20°C and thawed only once at the time of extraction.

Measurement of Plasma ET-LI Concentration
Plasma ET-LI concentration was measured by the radioimmunoassay (RIA) with the monoclonal antibody (KY-ET-1-IV), as previously reported. Figure 1 shows the standard curve of endothelin-1 (ET-1) and cross-reactivities with other related peptides in the RIA with this antibody. This antibody has a high affinity for ET-1 (association constant, 4.8×10^13 M^-1). The value of 50% inhibitory concentration of the RIA with this antibody is 2 pg/tube. Cross-reactivity with ET-2, ET-3, and human big ET-1 is 80%, 20%, and 80%, respectively.

Standard ET-1 was purchased from the Peptide Institute Inc. (Minoh, Japan). The intra- and interassay variations of the RIA with KY-ET-1-IV were 4.0% (n=10) and 6.4% (n=10), respectively. The extraction of endothelin from plasma was performed with polystyrene beads coated with the purified monoclonal antibody (KY-ET-1-I), as previously reported. The volume of plasma used for the extraction was 0.5 ml.

Statistical Analysis
Comparison of mean values between PH and non-PH groups was performed with the unpaired t
TABLE 1. Patient Profile

<table>
<thead>
<tr>
<th></th>
<th>PH group</th>
<th>Non-PH group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>27</td>
</tr>
<tr>
<td>Age (years)</td>
<td>2.8±0.5</td>
<td>3.5±0.5</td>
</tr>
<tr>
<td>Sex (male:female)</td>
<td>12:6</td>
<td>17:10</td>
</tr>
<tr>
<td>Pp systole (mm Hg)</td>
<td>62.4±3.6</td>
<td>25.5±1.6</td>
</tr>
<tr>
<td>Pp diastole (mm Hg)</td>
<td>24.7±2.4</td>
<td>10.9±0.7</td>
</tr>
<tr>
<td>Pp mean (mm Hg)</td>
<td>38.1±2.7</td>
<td>16.1±1.0</td>
</tr>
<tr>
<td>Ps systole (mm Hg)</td>
<td>91.0±2.2</td>
<td>95.4±1.9</td>
</tr>
<tr>
<td>Ps diastole (mm Hg)</td>
<td>56.1±1.9</td>
<td>60.1±1.4</td>
</tr>
<tr>
<td>Ps mean (mm Hg)</td>
<td>70.6±2.6</td>
<td>74.7±1.6</td>
</tr>
<tr>
<td>Qp (/min/m²)</td>
<td>9.4±1.2</td>
<td>5.8±0.7</td>
</tr>
<tr>
<td>Qs (/min/m²)</td>
<td>4.7±0.3</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Rp (dyne · sec · cm⁻³ · m²)</td>
<td>273±37</td>
<td>141±12</td>
</tr>
<tr>
<td>Rs (dyne · sec · cm⁻³ · m²)</td>
<td>1,187±66</td>
<td>1,318±62</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>117.0±4.7</td>
<td>104.2±3.0</td>
</tr>
<tr>
<td>CRE (mg/dl)</td>
<td>0.26±0.02</td>
<td>0.25±0.02</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>13.7±0.7</td>
<td>12.0±0.8</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37.7±0.9</td>
<td>40.0±1.1</td>
</tr>
<tr>
<td>Pao₂ (mm Hg)</td>
<td>80.7±3.3</td>
<td>83.0±4.1</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>VSD, 10; ASD, 1; TGA, 2; po VSD, 5</td>
<td>VSD, 13; TOF, 3; AS, 3; PS, 2; TGA, 1; po VSD, 1; po TOF, 4</td>
</tr>
</tbody>
</table>

Values are shown as mean±SEM. Pp, pulmonary arterial pressure; Ps, aortic pressure; Qp, pulmonary blood flow; Qs, systemic blood flow; Rp, pulmonary vascular resistance; Rs, systemic vascular resistance; CRE, serum creatinine level; BUN, blood urea nitrogen level; Pao₂, oxygen partial pressure in the aorta; VSD, ventricular septal defect; po, postoperation; ASD, atrial septal defect; TGA, transposition of the great arteries; TOF, tetralogy of Fallot; AS, aortic stenosis; FS, pulmonary stenosis.

test. To compare mean values among the PH group, the non-PH group, and the normal subjects, one-way analysis of variance with multiple comparisons (Bonferroni method) was used. The gender distribution among the two groups and the normal subjects was compared using χ² analysis. To analyze the differences in plasma ET-LI concentrations between adjacent sites in each group, the paired t test with Bonferroni correction was used. Linear regression analysis was used to determine correlations between results. Probability values of less than 0.05 were considered statistically significant.

Results

Patients were classified into two groups according to the ratio of pulmonary arterial to aortic systolic pressure (Pp/Ps). In 18 patients, Pp/Ps was equal to or greater than 0.5 (PH group), and in the other 27 patients, Pp/Ps was less than 0.5 (non-PH group). Table 1 shows the patient profiles of each group. There were no significant differences between the two groups in parameters except those related to the pulmonary circulation and heart rate. There were neither significant differences in age nor in gender distribution among the two groups and the normal subjects.

Figure 2 shows plasma ET-LI concentrations at various sampling sites in the PH and non-PH groups and those in the peripheral vein of the normal subjects. Plasma ET-LI concentrations in the PH group were significantly higher than those in the non-PH group at all sampling sites (1.2 to 1.3 times higher at VC, RA, and RV and about 1.5 times higher at PA and PV). In the PH group, a significant increase in plasma ET-LI concentration was observed between RV and PA and between PA and PV, although there were no significant changes between VC and RA or between RA and RV. On the other hand, in the non-PH group, no significant change was observed in plasma ET-LI concentration between any one site and the next. Plasma ET-LI concentrations (pg/ml; mean±SEM) at sampling sites were as follows: VC, 16.2±1.1; RA, 15.3±0.7; RV, 16.0±1.0; PA, 19.2±1.6; and PV, 20.7±1.8 in the PH group, and VC, 12.9±0.4; RA, 12.8±0.4; RV, 12.9±0.4; PA, 13.1±0.5; and PV, 13.5±0.4 in the non-PH group.

Plasma ET-LI concentration (mean±SEM) in peripheral venous blood of normal subjects was 13.3±0.3 pg/ml. In the PH group, plasma ET-LI concentrations were significantly higher than the normal value at all sampling sites, whereas in the non-PH group, there was no significant difference from this value at any site.

Figure 3 shows the mean±SEM value of the increments of plasma ET-LI concentrations from RV to PV in both groups. The increment of plasma ET-LI concentrations from RV to PV in the PH group was about 30% and was significantly higher than that in the non-PH group.

There was a significant (p<0.01) correlation between plasma ET-LI concentration at PV and pulmonary artery pressure (systole, r=0.67; diastole, r=0.47;
mean, $r=0.58$). The increment of plasma ET-LI concentrations from RV to PV was also significantly ($p<0.01$) correlated with pulmonary artery pressure (systole, $r=0.66$; diastole, $r=0.43$; mean, $r=0.57$).

**Discussion**

In the present study, we demonstrated that plasma ET-LI concentrations at VC, RA, RV, PA, and PV were significantly elevated in patients with congenital heart defects and pulmonary hypertension in comparison with those in patients without pulmonary hypertension. We also indicated that this elevation was mainly due to the increased production of ET-LI in pulmonary circulation. These observations suggest the possible involvement of endothelin in the pathophysiology of pulmonary hypertension associated with congenital heart defects.

Measurements of plasma ET-LI concentrations at multiple sites during cardiac catheterization enabled us to speculate on the source of elevated plasma ET-LI concentration in patients with pulmonary hypertension. We observed a significant increase in plasma ET-LI concentration between RV and PA and between PA and PV in patients with pulmonary hypertension. These observations clearly indicate that ET-LI is substantially produced in pulmonary circulation including the pulmonary artery, capillaries, and pulmonary vein. It should be noted that left-to-right shunts caused the elevation of plasma ET-LI concentration at PA. No significant increase in plasma ET-LI concentration between RV and PV in patients without pulmonary hypertension suggests that the production of ET-LI in pulmonary circulation is not substantial in normal children.

The mechanism for elevated plasma ET-LI concentration in patients with pulmonary hypertension is not clear at present. Recently, hemodynamic shear stress has been reported to stimulate the production of endothelin by cultured endothelial cells. In patients with pulmonary hypertension, abnormal hemodynamic forces such as high pressure or increased blood flow on pulmonary arterial walls may stimulate endothelin production by pulmonary artery endothelial cells. It has been observed in patients with congenital heart defects and pulmonary hypertension that there are ultrastructural changes suggesting heightened metabolic activity in pulmonary artery endothelial cells. These changes may have relevance to the increased production of ET-LI during pulmonary circulation.

Endothelin has been reported to have contractile and proliferative effects on vascular smooth muscle cells and to produce pulmonary vasoconstriction. The increased muscularization of the pulmonary artery observed in patients with pulmonary hypertension may be related to this increased plasma ET-LI concentration in pulmonary circulation. On the other hand, low doses of endothelin have been reported to cause pulmonary vasodilation. Therefore, it may also be speculated that the increased plasma ET-LI concentration plays a role in relieving the hypertensive state and modulates the pulmonary vascular tone in patients with pulmonary hypertension. Further studies are necessary to elucidate the actual role of this increase in plasma ET-LI concentrations in pulmonary hypertension.

Recently, we measured plasma ET-LI concentrations in the peripheral vein of healthy children and adults by using the same RIA as in the present study.
study.\(^{21}\) In that report, we showed that the normal value of plasma ET-LI concentrations in children varies with age. In infants younger than 3 months, it is higher than that in older children; after 3 months of age, it is nearly constant and similar to that in adults. Therefore, we studied patients and normal subjects ranging from 6 months to 12 years in age in the present study.

There are some limitations in the interpretation of the results in the present study. The normal value of the plasma ET-LI concentration in adults measured with this RIA\(^{11-13,21}\) is higher than that reported by other investigators.\(^{22-24}\) The difference is due mainly to the specificity of the antibody used in each laboratory. We have reported recently that ET-LI in normal plasma consists of ET-1, big ET-1, and another precursor form of endothelin (6K daltons) and that the ratio of ET-1 to total ET-LI is about 1:5, using gel permeation chromatography.\(^{11}\) The monoclonal antibody used in our study recognizes ET-1, big ET-1, and another precursor form of endothelin (6K),\(^{11}\) but the other antibodies do not recognize big ET-1 or the other components of ET-LI significantly. Recently, plasma big ET-1 concentration was reported to be around 5 to 6 pg/ml.\(^{25}\) This finding is consistent with our results.

In the present study, it remains to be clarified which components (ET-1, big ET-1, or 6K) are preferentially increased in patients with pulmonary hypertension. Because ET-1 and big ET-1 in plasma are almost equally elevated in patients with acute myocardial infarction,\(^{26}\) it is possible that patients with pulmonary hypertension show parallel elevation of ET-1 and big ET-1. In a clinical study of children, we did not obtain blood samples of high enough volume to perform gel permeation chromatography.

Most patients in the PH group had been administered with digitalis and some patients with diuretics, whereas no patients in the non-PH group had been taking medications. It has not been clarified at present whether these drugs have any effect on the ET-LI production in pulmonary circulation.

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