Endothelin Production in Pulmonary Circulation of Patients With Mitral Stenosis

Keiji Yamamoto, MD; Uichi Ikeda, MD; Hideaki Mito, MD; Hideyuki Fujikawa, MD; Hiromichi Sekiguchi, MD; Kazuyuki Shimada, MD

**Background** Although plasma endothelin concentrations are elevated in patients with pulmonary hypertension, the precise sites of endothelin production have not been defined. We investigated the endothelin production in the pulmonary circulation of patients with mitral stenosis and its effects on pulmonary vascular tone.

**Methods and Results** We measured plasma concentrations of endothelin-1, angiotensin II, and thrombomodulin in blood samples obtained from the right and left atria of 10 consecutive patients with rheumatic mitral stenosis (mean age, 55 years; range, 39 to 68) who were undergoing percutaneous mitral valvuloplasty. Plasma levels of endothelin-1 were significantly higher in the left atrium than in the right atrium (3.25±0.45 vs. 2.53±0.36 pg/mL, mean±SE, P<.001). The increased plasma endothelin-1 level in the left atrium, which reflected endothelin-1 production in the pulmonary circulation, was correlated with mean pulmonary artery pressure (r=.65, P=.04), mean pulmonary arterial wedge pressure (r=.67, P=.03), total pulmonary resistance (r=.68, P=.03), and 1/mitral valve area (r=.85, P=.002) but not with pulmonary vascular resistance (r=.04, P=.91). There were no significant differences in plasma levels of angiotensin II and thrombomodulin between the right and left atria (angiotensin II, 16.40±3.08 versus 15.50±4.85 pg/mL; thrombomodulin, 2.96±0.34 versus 2.85±0.37 ng/mL).

**Conclusions** Endothelin-1 production is increased in the pulmonary circulation of patients with mitral stenosis in response to increased pulmonary artery pressure but is not directly related to increased pulmonary vascular tone in this disorder. (*Circulation. 1994;89:2093-2098.)*

**Key Words** • angiotensin II • thrombomodulin • atrium • valvuloplasty

Endothelin, an endothelium-derived peptide, has contractile and proliferative effects on vascular smooth muscle cells.1-4 Endothelin consists of 21 amino acid residues with two sets of intrachain disulfide linkages and is classified by its deduced amino acid sequences as three distinct isopeptides: endothelin-1, endothelin-2, and endothelin-3.5 Increased plasma endothelin concentrations have been observed in various cardiovascular disorders such as coronary spasm,6 acute myocardial infarction,7 essential hypertension,8-10 mitral stenosis,11 cardiogenic shock,12 and subarachnoid hemorrhage.13

Pulmonary hypertension refers to the abnormal hemodynamic effects of various conditions that lead to a chronic increase in pulmonary artery pressure and pulmonary vascular resistance. Chemical and hormonal regulation of pulmonary vascular resistance is a complex process. Catecholamine, acetylcholine, prostaglandin, histamine, bradykinin, serotonin, and angiotensin II have been found to play roles in the regulation of pulmonary vascular resistance.14 Recent studies have shown that plasma endothelin concentrations are elevated in patients with pulmonary hypertension,14-21 suggesting that endothelin is involved in the pathophysiology of pulmonary hypertension. Lippton et al22 reported that endothelin-1 induced contractions in isolated pulmonary vessels and increased pulmonary vascular resistance. Although evidence suggests increased endothelin production by pulmonary endothelial cells in pulmonary hypertension, the precise sites of endothelin production have not been defined.

Mitral stenosis is associated with increases in pulmonary artery pressure and resistance. We measured plasma levels of endothelin-1, angiotensin II, and thrombomodulin in right and left atrial blood samples obtained from 10 patients with rheumatic mitral stenosis who were undergoing percutaneous mitral valvuloplasty to determine whether endothelin-1 production is increased in the pulmonary circulation of patients with mitral stenosis.

**Methods**

**Subjects** We studied 10 patients with severe rheumatic mitral stenosis (9 with chronic atrial fibrillation and 1 with sinus rhythm) who underwent percutaneous mitral valvuloplasty using the anterograde transeptal approach with an Inoue balloon23 between March 1992 and April 1993 (Table 1). None of the subjects had significant mitral regurgitation, aortic stenosis, aortic regurgitation, a history of systemic embolism, a left atrial clot (as detected by two-dimensional transesophageal echocardiography), diabetes mellitus, or renal or hepatic insufficiency. Hemodynamic studies were performed the morning after an overnight fast. Vasodilators were withheld for at least 24 hours before evaluation. Chronic, stable doses of digoxin and diuretics were continued but were administered on an evening schedule. All patients signed informed consent forms.

M-mode, two-dimensional, and Doppler echocardiography studies were performed the day before patients underwent percutaneous mitral valvuloplasty (Table 1). No intracardiac thrombi were detected by transesophageal echocardiography.
TABLE 1. Clinical Characteristics of 10 Patients Who Underwent Percutaneous Mitral Valvuloplasty

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men:women</td>
<td>2:8</td>
</tr>
<tr>
<td>Mean age, y</td>
<td>55±2*</td>
</tr>
<tr>
<td>History of rheumatic fever</td>
<td>4</td>
</tr>
<tr>
<td>Previous surgical commissurotomy</td>
<td>1</td>
</tr>
<tr>
<td>Previous PMV</td>
<td>1</td>
</tr>
<tr>
<td>NYHA class</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>7</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
</tr>
<tr>
<td>Rhythm</td>
<td></td>
</tr>
<tr>
<td>Sinus</td>
<td>1</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>9</td>
</tr>
<tr>
<td>Mitral calcification (fluoroscopy)</td>
<td>8</td>
</tr>
<tr>
<td>Mean left atrial diameter, mm</td>
<td>57±2*</td>
</tr>
<tr>
<td>Echocardiographic score &gt;8</td>
<td>6</td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5</td>
</tr>
<tr>
<td>1/4</td>
<td>3</td>
</tr>
<tr>
<td>2/4</td>
<td>2</td>
</tr>
</tbody>
</table>

PMV indicates percutaneous mitral valvuloplasty; NYHA, New York Heart Association. *Values are mean±SE.

The morphological characteristics of the mitral valve and subvalvular structures were classified using an echocardiographic scoring system.24

Cardiac Catheterization and Valvuloplasty

Right and left heart studies, including measurement of cardiac output, biplane left ventriculography and right angiography, and coronary angiography, were performed using a percutaneous catheter inserted via the left groin immediately before percutaneous mitral valvuloplasty. Percutaneous mitral valvuloplasty was performed with an Inoue single-balloon catheter (Toray Industries, Inc), as previously described.23 Briefly, an Inoue single-balloon catheter was inserted transseptally into the left atrium by the Brockenbrough technique. The distal half of the balloon was inflated at the left ventricle, and the balloon was pulled back to the mitral orifice position. The balloon then was fully inflated until the indentation disappeared. The balloon then was deflated and pulled back to the left atrium. The diameters of the inflated balloon were 24 mm in four patients, 26 mm in five patients, and 28 mm in one patient. These procedures were performed under fluoroscopy at the same predetermined projections as used for left ventriculography. Effective balloon dilating areas were determined by geometric analysis and normalized for body surface area. Severity of mitral regurgitation was determined by the method of Sellers. Hemodynamic variables, including pressure in the right atrium, right ventricle, pulmonary artery, pulmonary arterial wedge position, left atrium and ascending aorta, as well as the cardiac index, were measured before valve dilatation. Simultaneous left atrial and left ventricular pressures were recorded to determine the mean transmural gradient. Cardiac output was determined by the thermodilution method in nine patients with mild or no tricuspid regurgitation. In one patient with moderate tricuspid regurgitation, cardiac output was determined by the direct Fick method. The following variables were determined: systemic vascular resistance, total pulmonary resistance, and pulmonary vascular resistance.15 Mitral valve area was calculated by the Gorlin formula.

Blood Sampling and Plasma Assay

Immediately before the valvuloplasty balloon was inflated, blood samples were obtained from the right and left atria through a thermodilution catheter (7F) and an Inoue balloon catheter (12F), respectively. A 6-mL sample of whole blood was drawn into a plastic tube containing 9 mg Na3-EDTA and 3000 KIU (kallidinogenase inactivator units) aprotinin for use in assays. All samples were placed on ice. Plasma was separated by prompt centrifugation of blood samples at 1800g at 4°C for 20 minutes, immediately frozen, and stored at −80°C until needed for assays.

Plasma endothelin-1 was measured using a modification of a previously described radioimmunoassay.12 Samples were extracted using SepPak C18 cartridges (Waters) that had been activated with methanol, 8 mol/L urea, and water. Elution of endothelin-1 using 100% methanol yielded a recovery rate of 75±3.3%. Samples and standards (endothelin-1, Peptide Institute, Osaka, Japan) were reconstituted in the assay buffer and incubated for 24 hours with rabbit anti-endothelin-1 serum (Peninsula Laboratories, Belmont, Calif) at 4°C. This antibody primarily recognizes and has 100% cross-reactivity with endothelin-1. It also has 17% cross-reactivity with human big endothelin and 7% cross-reactivity with endothelin-2 and endothelin-3. It has no cross-reactivity with human atrial natriuretic peptide, brain natriuretic peptide, vasopressin, the angiotensins, vasactive intestinal peptide, or adrenocorticotropic hormone. After approximately 8000 cpm of [125I] endothelin-1 (Amersham) was added to the mixture, the preparation was incubated for 24 hours at 4°C. Immune complexes were precipitated with goat anti-rabbit serum, and the radioactivity in precipitates was measured. B/Bo, percent of maximum [125I]endothelin-1 bound, was calculated for each standard and plotted against the log of endothelin-1 on semilog graph paper, and the best-fit standard curve was drawn. Radioactivity in the sample concentrations was determined by comparing values with this standard curve.

Angiotensin II was extracted by adsorption onto an elution from florisor and was measured by an antibody with sufficient specificity, as previously described.25 The extraction rate was 84%, and the minimum detectable value was 0.75 pg per tube. Intra-assay and interassay reproducibilities were 11.7% and 12.9%, respectively.

Plasma levels of thrombomodulin were determined using the one-step sandwich enzyme immunoassay kit (TM Panacea, Fuji Chemical Industries Ltd).26

Statistical Analysis

Data are expressed as mean±SE of multiple experiments. Differences were analyzed using the Student’s t test. Simple linear regression analysis was used to compare measurements obtained by catheterization and levels of molecular markers. Values of P<.05 were considered statistically significant.

Results

Hemodynamic Characteristics of Mitral Stenosis

As shown in Table 2, the following values represent the hemodynamic characteristics of 10 patients with mitral stenosis: mean pulmonary artery pressure, 31.3±2.5 mm Hg; mean pulmonary arterial wedge pressure, 22.3±2.2 mm Hg; mean right atrial pressure, 6.9±0.8 mm Hg; mean left atrial pressure, 21.0±2.7 mm Hg; cardiac index, 2.35±0.14 L·min⁻¹·m⁻²; systemic vascular resistance, 2918±171 dyne·s·cm⁻⁵·m²; total pulmonary resistance, 1022±72 dyne·s·cm⁻⁵·m²; pulmonary vascular resistance, 295±36 dyne·s·cm⁻⁵·m²; mean transmi-
TABLE 2. Hemodynamic Characteristics of Patients With Mitral Stenosis

<table>
<thead>
<tr>
<th>Heart rate, beats per minute</th>
<th>82±5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>81.4±3.9</td>
</tr>
<tr>
<td>Mean pulmonary artery pressure, mm Hg</td>
<td>31.3±2.5</td>
</tr>
<tr>
<td>Mean pulmonary arterial wedge pressure, mm Hg</td>
<td>22.3±2.2</td>
</tr>
<tr>
<td>Mean right atrial pressure, mm Hg</td>
<td>6.9±0.8</td>
</tr>
<tr>
<td>Mean left atrial pressure, mm Hg</td>
<td>21.0±2.7</td>
</tr>
<tr>
<td>Cardiac index, L · min⁻¹ · m⁻²</td>
<td>2.35±0.14</td>
</tr>
<tr>
<td>Systemic vascular resistance, dyne · s · cm⁻³ · m⁻²</td>
<td>2918±171</td>
</tr>
<tr>
<td>Total pulmonary resistance, dyne · s · cm⁻³ · m⁻²</td>
<td>1022±72</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, dyne · s · cm⁻³ · m⁻²</td>
<td>295±36</td>
</tr>
<tr>
<td>Mean transmitral gradient, mm Hg</td>
<td>14.0±1.9</td>
</tr>
<tr>
<td>Mitral valve area, cm²</td>
<td>0.77±0.06</td>
</tr>
<tr>
<td>$\text{Pao}_2$, mm Hg</td>
<td>87.5±2.1</td>
</tr>
</tbody>
</table>

Values are mean±SE.

...tral gradient, 14.0±1.9 mm Hg; and mitral valve area, 0.77±0.06 cm².

Plasma Endothelin-1 Levels

Plasma endothelin-1 levels in peripheral vein blood samples obtained from normal subjects (n=10) were not significantly different from plasma levels in right atrial blood samples obtained from patients with mitral stenosis (2.14±0.10 versus 2.53±0.36 pg/mL). Endothelin-1 levels were significantly higher in left atrial blood samples (3.25±0.45 pg/mL) than in right atrial blood samples in patients with mitral stenosis (Fig 1).

Fig 1. Scatterplot showing plasma levels of endothelin-1 in blood from the right atrium (RA) and the left atrium (LA) in patients with mitral stenosis. Plasma levels of endothelin-1 in the LA were significantly higher than those in the RA. Bars show mean±SE of 10 samples.

Fig 2. Scatterplot showing relation between the increase in plasma endothelin-1 levels from the right atrium (RA) to the left atrium (LA) and mean pulmonary artery pressure in patients with mitral stenosis. Regression analysis revealed a highly significant relation between the increase in endothelin-1 levels and mean pulmonary artery pressure. $y=12.82x+22.04$; $r=.65$, $P=.04$.

Correlation Between Hemodynamic Variables and Endothelin-1 Levels

The increase in plasma endothelin-1 levels between the left and right atria, reflecting endothelin-1 production in the pulmonary circulation, was significantly correlated with mean pulmonary artery pressure ($r=.65$, $P=.04$) (Fig 2), mean pulmonary arterial wedge pressure ($r=.67$, $P=.03$), total pulmonary resistance ($r=.68$, $P=.03$), mean transmitral gradient ($r=.63$, $P=.04$), and 1/mitral valve area ($r=.85$, $P=.002$) (Table 3). However, the increase in endothelin-1 levels was not significantly correlated with pulmonary vascular resistance ($r=.04$, $P=.91$) (Fig 3), cardiac index, or oxygen partial pressure in the aorta ($\text{Pao}_2$) (Table 3).

Plasma Angiotensin II and Thrombomodulin Levels

The plasma angiotensin II levels in peripheral vein blood samples obtained from normal subjects (14.2±2.2 pg/mL, n=10) were not significantly different from right (16.40±3.08 pg/mL) and left atrial plasma levels (15.50±4.85 pg/mL) in patients with mitral stenosis. There was no significant difference in plasma angiotensin II levels between the right and left atria (Fig 4). Plasma thrombomodulin levels in peripheral vein blood samples obtained from normal subjects (3.20±0.30 ng/mL, $P=.65$).

TABLE 3. Correlations Between Increase in Plasma Endothelin-1 Levels and Hemodynamic Variables

<table>
<thead>
<tr>
<th>$r$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pulmonary artery pressure, mm Hg</td>
<td>.65</td>
</tr>
<tr>
<td>Mean pulmonary arterial wedge pressure, mm Hg</td>
<td>.67</td>
</tr>
<tr>
<td>Cardiac index, L · min⁻¹ · m⁻²</td>
<td>.10</td>
</tr>
<tr>
<td>Total pulmonary resistance, dyne · s · cm⁻³ · m⁻²</td>
<td>.68</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, dyne · s · cm⁻³ · m⁻²</td>
<td>.04</td>
</tr>
<tr>
<td>Mean transmitral gradient, mm Hg</td>
<td>.63</td>
</tr>
<tr>
<td>1/Mitrval valve area, cm²</td>
<td>.85</td>
</tr>
<tr>
<td>$\text{Pao}_2$, mm Hg</td>
<td>.16</td>
</tr>
</tbody>
</table>
n=10) were not significantly different from those in the right (2.96±0.34 ng/mL) and left atria (2.85±0.37 ng/mL) in patients with mitral stenosis. There was no significant difference in plasma thrombomodulin levels between the right and left atria (Fig 5).

Discussion

Plasma levels of endothelin-1 were significantly higher in the left atrium than in the right atrium in patients with mitral stenosis, and the increase in endothelin-1 levels in the left atrium was significantly correlated with mean pulmonary artery pressure but not with pulmonary vascular resistance, suggesting that endothelin-1 production is increased in the pulmonary circulation of patients with mitral stenosis in response to increased pulmonary artery pressure but is not directly related to increased pulmonary vascular tone in this disorder.

In vitro and in vivo studies have demonstrated that endothelin is a potent constrictor of pulmonary arteries and veins but that it exerts a greater effect on pulmonary arteries. Endothelin has been found to increase pulmonary artery pressure in a time-dependent manner. Yoshibayashi et al reported that plasma endothelin concentrations were significantly higher in subjects with congenital heart disease who had pulmonary hypertension than in subjects without pulmonary hypertension. Stewart et al reported that plasma endothelin-1 levels were significantly higher in arterial plasma samples compared with venous plasma samples obtained from patients with primary pulmonary hypertension. Chang et al reported that plasma levels of endothelin-1 but not endothelin-3 were increased in patients with pulmonary hypertension. They speculated that both endothelin-1 and endothelin-3 were cleared mainly through the pulmonary circulation and that the constant level of endothelin-3 in plasma indicated that the clearance rate of endothelin in the lung was not impaired in patients with pulmonary hypertension.

Whether the elevated endothelin-1 in pulmonary hypertension contributes directly to increased pulmonary tone is controversial. Cody et al reported that elevated endothelin-1 concentrations in arterial plasma were correlated with hemodynamic variables such as pulmonary artery pressure and pulmonary vascular resistance in patients with congestive heart failure. On the other hand, Stewart et al described that there was no correlation between endothelin-1 levels in venous or arterial plasma and pulmonary vascular resistance. In the present study, the increased levels of endothelin-1 in the pulmonary circulation of patients with mitral stenosis were not significantly correlated with pulmonary vascular resistance, suggesting that endothelin-1 production is not directly related to increased pulmonary vascular tone in this disorder.
The increase in plasma endothelin-1 levels also may be related to proliferation of the cells of the vessel wall in pulmonary hypertension. The morphology of the lungs in pulmonary venous hypertension associated with mitral valve disease is distinctive, and the veins, arteries, and parenchyma are affected. Medial hypertrophy and proliferation of smooth muscle cells are present in veins and arteries. Komuro et al reported that endothelin-1 caused proliferation of smooth muscle cells. Thus, increased secretion of endothelin-1 from endothelial cells may stimulate and further aggravate pathological changes in the underlying intima and media of the pulmonary vasculature in mitral stenosis.

The mechanism for increased endothelin-1 production in the pulmonary circulation of patients with mitral stenosis has not been clarified. Endothelin synthesis in endothelial cells is stimulated by epinephrine,1 thrombin,29 angiotensin II,30 endotoxin,31,32 shear stress,33 and hypoxia.34-36 Shirakami et al34 showed that exposure to alveolar hypoxia increased endothelin levels in the plasma and lung in conscious unrestrained rats and that the increase of plasma and lung endothelin levels were paralleled to the severity of hypoxia. Elton et al35 reported that plasma endothelin-1 levels in rats were increased significantly during 48 hours of hypoxia (10% O2) compared with air controls and that endothelin-1 mRNA levels were increased twofold in the lung and right atrium after 48 hours of hypoxia. However, in our study, plasma levels of endothelin-1 in the right and left atria and the increase in endothelin-1 levels from the right atrium to the left atrium were not related to PaCO2 levels.

On the other hand, Vincent et al18 found that endothelin levels were increased in patients with congenital heart disease associated with left-to-right shunts and that the increase was related to increased pulmonary blood flow and was independent of pulmonary artery pressure. In our study, the increase in endothelin-1 concentrations from the right atrium to the left atrium was not correlated with cardiac index, suggesting that the increased endothelin-1 production is not related to pulmonary blood flow. Recently, Yoshizumi et al33 reported that hemodynamic low shear stress stimulated the expression of endothelin mRNA in endothelial cells with an increased release of immunoreactive endothelin into the culture medium. In the present study, the increased levels of endothelin-1 in the pulmonary circulation of patients with mitral stenosis were significantly correlated with mean pulmonary artery pressure, supporting the premise that abnormal hemodynamic forces such as high pressure on pulmonary artery walls may stimulate endothelin-1 production by pulmonary artery endothelial cells.

Angiotensin II has vasoconstrictive effects similar to those of endothelin and stimulates the growth of vascular smooth muscle cells and endothelin-1 synthesis in endothelial cells.30 Angiotensin II has been implicated in hypoxia-induced pulmonary hypertension.37-39 However, Cassis et al39 reported that angiotensin II did not contribute to pulmonary hypertension in rats treated with the pyrrolizidine alkaloid monocrotaline. We observed no significant increase in plasma levels of angiotensin II in the right atrium or the left atrium, suggesting that angiotensin II did not contribute to increased vascular resistance and endothelin-1 production in mitral stenosis.

Thrombomodulin is a high-affinity thrombin receptor present on vascular endothelial cells, placental syncytiotrophoblasts, and platelets.40,41 It is a cofactor for thrombin-catalyzed activation of the anticoagulant protease zymogen protein C. Thrombomodulin has been detected in the circulating blood after endothelial injury.42 Plasma thrombomodulin levels are elevated in various conditions such as diabetes mellitus,43 hemodialysis,44 systemic lupus erythematosus, thrombotic thrombocytopenic purpura, and disseminated intravascular coagulation.45 Karmochkin et al40 reported that plasma thrombomodulin levels were decreased in patients with pulmonary hypertension compared with control subjects. We observed a tendency toward decreased plasma thrombomodulin levels in both atria compared with peripheral vein of normal subjects, although there was no significant difference between the right and left atria. This observation suggests that increased plasma levels of endothelin-1 may not result from leakage of the peptide from injured endothelial cells but are likely to be related to increased biosynthesis in pulmonary endothelial cells.

Our results show that endothelin-1 production is significantly increased in the pulmonary circulation of patients with mitral stenosis in response to increased pulmonary artery pressure. However, there was no evidence to implicate circulating endothelin-1 in the pathogenesis of pulmonary hypertension in this disorder. Further studies are needed to clarify the pathophysiological role of endothelin-1 in pulmonary hemodynamics in vivo.

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References


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