Increased Plasma P-Selectin and Decreased Thrombomodulin in Pulmonary Arterial Hypertension Were Improved by Continuous Prostacyclin Therapy

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Background—Thrombosis in situ related to endothelial cell injury may contribute to the development of pulmonary hypertension (PH). P-selectin, a leukocyte adhesion receptor present in endothelial cells and platelets, reflects endothelial injury and platelet activation, and thrombomodulin (TM), a receptor for thrombin and a major anticoagulant proteoglycan on the endothelial membrane, reflects the anticoagulant activity of the endothelium.

Methods and Results—To assess abnormal coagulation due to endothelial injury in patients with PH, plasma levels of soluble P-selectin and TM were measured in 32 patients with primary PH (PPH), 25 with secondary pulmonary arterial hypertension (sPAH), 31 with pulmonary venous hypertension (PVH), and 17 healthy subjects (Control). These measurements were repeated after continuous infusion of prostacyclin in 15 patients with PPH and 3 with sPAH. P-selectin levels in both the sPAH and PPH groups were significantly higher than those in the Control and PVH groups (P < 0.05). Plasma TM level in the PPH group was significantly lower than those in the other groups (P < 0.01). After prostacyclin therapy, the lower TM level was increased and the higher P-selectin level was decreased (P < 0.05).

Conclusions—Decreased TM and increased P-selectin in PPH and sPAH may reflect in situ thrombosis due to endothelial injury. Prostacyclin may act not only as a vasodilator but also as an agent that improves endothelial injury and altered hemostasis in pulmonary arterial injury. (Circulation. 2000;102:2720-2725.)

Key Words: prostaglandins ■ pulmonary heart disease ■ hypertension, pulmonary ■ endothelium ■ thrombosis

Primary pulmonary hypertension (PPH) is characterized by a progressive increase in pulmonary vascular resistance (PVR) that leads to right ventricular failure and death.1 A small number of studies have revealed that patients with PPH or secondary pulmonary arterial hypertension (sPAH) who have been treated with oral anticoagulants appear to have an improved survival.2,3 Histopathological studies in PPH demonstrate thrombosis in situ as well as medial smooth muscle hypertrophy and intimal endothelial proliferation in small pulmonary arteries.4 sPAH due to congenital heart disease, obstructive or restrictive lung disease, collagen vascular disease, or chronic thromboembolic disease also reveals histopathological findings similar to those of PPH.5 Thus, abnormal thrombotic or thrombolytic activity due to endothelial dysfunction has been suggested to be important in the pathogenesis of PAH.

P-selectin, a membrane glycoprotein existing in the Weibel-Palade bodies of endothelial cells and the α-granules of platelets, plays a key role in neutrophil adhesion to the endothelium and platelet activation during the inflammatory process.6 Several reports have demonstrated that a soluble form of P-selectin is present in the normal circulation.7 Previous investigations related to thrombotic, inflammatory, and atherosclerotic disorders have suggested that plasma P-selectin may reflect endothelial cell injury and platelet activation.8–10 There is, however, no report concerning the relationship between PH and P-selectin.

Thrombomodulin (TM) is an endothelial cell surface protein responsible for binding thrombin, with subsequent activation of protein C, which acts as an anticoagulant.11 Plasma soluble TM level is increased in disseminated intravascular coagulation and atheromatous arterial disease.12,13 Two investigations reported that plasma TM levels in patients with PPH or sPAH were lower than those in control subjects.14,15 We speculated that P-selectin and TM in blood from patients with PH may reflect endothelial dysfunction leading to abnormal coagulation and fibrinolytic events. The first purpose of our study was to evaluate the clinical impact of endothelial dysfunction and altered hemostasis in patients with PPH and sPAH compared with those with pulmonary venous hypertension (PVH) by measuring plasma parameters including TM, P-selectin, and other coagulation and fibrinolytic factors.

Recently, continuous infusion of prostacyclin (PGI₂) for patients with PPH has resulted in clinical and hemodynamic
improvement as well as increased survival.\textsuperscript{16} Significant hemodynamic improvement was achieved in patients in whom no significant improvement occurred with acute PGI\textsubscript{2} administration.\textsuperscript{16,17} These data indicate that the beneficial effects of PGI\textsubscript{2} may be caused not only by vasodilation but also by continuous attenuation of hypercoagulation as well as remodeling of the pulmonary vascular bed. The second objective was to evaluate the effects of PGI\textsubscript{2} therapy on these parameters of endothelial cell injury leading to altered hemostasis.

**Methods**

**Patients**

We studied 88 patients with PH. The diagnosis of PH was defined as a mean pulmonary artery pressure at rest of $\geq 25$ mm Hg. Patients were divided into 3 groups according to the type of PH. The first group consisted of 31 patients who were considered to have PVH, in whom pulmonary capillary wedge pressure (PCWP) was increased as a consequence of left-sided cardiac dysfunction. The causes of PVH included 21 cases of mitral valvular disease, 7 of dilated cardiomyopathy, 2 of hypertrophic cardiomyopathy, and 1 of coronary artery disease. Anticoagulant therapy with warfarin was prescribed to 18 patients (58\%) in the PVH group. The second group consisted of 25 patients who were considered to have sPAH, which was defined as an increase in PVR with a normal pulmonary wedge pressure. The causes of sPAH consisted of 16 cases of congenital heart disease with right-to-left shunt and 9 of collagen vascular disease. Anticoagulant therapy with warfarin was prescribed to 14 patients (56\%) in the sPAH group. The third group was 32 patients with PPH, which was diagnosed by the criteria of the National Institutes of Health registry of PPH.\textsuperscript{18} Anticoagulant therapy with warfarin was prescribed to 19 patients (59\%) in the PPH group. Heparin, platelet inhibitors, and thrombolytic agents were not given to any study subjects during the study period.

Seventeen subjects without obvious cardiopulmonary disease (4 men and 13 women, mean age 46 years, range 30 to 76 years) served as controls. No subjects had previously known conditions that might affect the measured values of P-selectin and TM.\textsuperscript{8-10,12,13} All subjects received an explanation of the purpose of the study and gave informed consent.

**Hemodynamic Studies**

Right heart catheterization was performed in the 3 PH groups. Mean pulmonary arterial pressure (MPAP), PCWP, and systemic arterial pressure were obtained at end expiration. Cardiac output (CO) was determined by the Fick method. Total pulmonary resistance (TPR) was calculated by dividing MPAP by CO (Wood units). PVR was calculated by the formula $PVR=(MPAP−PCWP)/CO$ (Wood units). The series of examinations was performed within a 1-week period. Treatment for all patients was not changed during the study period.

**Assay of Plasma Parameters**

Venous blood samples were taken and anticoagulated in plastic tubes containing trisodium citrate at a concentration of 0.01 mol/L. Plasma samples were obtained by centrifugation at 3500 rpm for 10 minutes at 4°C and stored at $-80^\circ$C until the assay.

Plasma P-selectin level was measured as previously described with a sandwich ELISA technique, with 2 distinct murine monoclonal antibodies against P-selectin (PL7-6 and WGA-1: GMP-140-EIA Kit; Takara Biomedical).\textsuperscript{1} Plasma TM level was determined by a 1-step sandwich enzyme immunoassay for soluble TM using 2 monoclonal antibodies for human TM.\textsuperscript{11}

Other hemostatic parameters, including prothrombin time, activated partial thromboplastin time, antithrombin III, thrombin-antithrombin III complex, protein C, fibrinogen degradation products, tissue plasminogen activator, plasminogen activator inhibitor-1, plasminogen, $\alpha_2$-plasmin inhibitor, and von Willebrand factor (vWF), were measured by standard clinical laboratory methods at our institute. All assays for these plasma factors were performed by standard methods, with an established normal range based on the mean±2 SD of $\approx 30$ normal individuals.

**PGI\textsubscript{2} Therapy**

Plasma levels of P-selectin and TM were measured before and during continuous infusion of PGI\textsubscript{2} in 15 patients in the PPH group and 3 in the sPAH group; these 3 patients had PH associated with collagen vascular disease. Warfarin had been prescribed to 9 subjects and dobutamine administered to 5 patients when the PGI\textsubscript{2} therapy was started. PGI\textsubscript{2} was administered via the internal jugular or subclavian vein at an initial rate of 2 ng kg$^{-1}$ min$^{-1}$, with increments of 1 to 2 ng kg$^{-1}$ min$^{-1}$ every week. Right heart catheterization and assays for plasma parameters were repeated during a mean follow-up period of 90 days. Percent changes of P-selectin and TM level from baseline and those of MPAP, TPR, and PVR were also calculated. The percent change of a parameter was calculated by the following formula: $\%\text{ change} = [(value\ after\ PGI_2)−(value\ before\ PGI_2)]\times 100\ (%)/value\ before\ PGI_2$. A significant improvement was defined as a percent change $>20\%$.

**Statistical Analysis**

Data are shown as mean±SD. Significance of differences between the 4 groups was determined by 1-way ANOVA with multiple comparisons. Differences were considered significant when $P<0.05$ by Scheffe’s test. Correlation between plasma P-selectin or TM level and pulmonary hemodynamic variables, MPAP, TPR, and PVR, was determined by simple regression test. The significance of differences in pulmonary hemodynamic data and levels of TM and P-selectin between patients with and without warfarin treatment was determined by Student’s unpaired $t$ test. In patients who were treated by continuous infusion of PGI\textsubscript{2}, the significance of changes in levels of plasma parameters and hemodynamic variables from baseline was assessed by Student’s paired $t$ test.

**Results**

**Hemodynamic Data**

The results of pulmonary hemodynamic studies in the 3 types of PH are shown in Table 1. In the PVH, sPAH, and PPH groups, there was no significant difference in MPAP, TPR, or PVR between patients with warfarin treatment and those without.

**P-Selectin, TM, and Other Plasma Parameters in All Groups**

Plasma levels of P-selectin and TM in the Control, PVH, sPAH, and PPH groups are shown in Figures 1 and 2, respectively. The plasma levels of P-selectin in both the PPH and sPAH groups were significantly higher than that in the Control and PVH groups. In the PVH, sPAH, and PPH groups, there was no significant difference in P-selectin level (ng/mL) between patients who were treated with warfarin (PVH, 142±69; sPAH, 249±143; PPH, 308±154) and those without (PVH, 155±83; sPAH, 237±201; PPH, 227±160). The plasma TM level in the sPAH and PPH groups was significantly lower than that in the Control and PVH groups. In the PPH group, plasma TM level was significantly lower than that in the other 3 groups. In the 3 PH groups, there was no significant difference in TM level (ng/mL) between patients with warfarin treatment (PVH, 3.1±0.9; sPAH, 2.9±1.7; PPH, 1.8±0.5) and those without (PVH, 4.0±1.5; sPAH, 2.7±1.5; PPH, 2.2±0.8). There was a weak correla-
tion between P-selectin and TM levels at baseline in all subjects ($r=0.23; P<0.05$).

For other plasma parameters relating to hemostasis, the plasma level of vWF antigen was significantly higher in the PVH, sPAH, and PPH groups than in the Control group, whereas the ratio of ristocetin cofactor to vWF antigen in the 3 PH groups was lower than that in the Control. However, there was no significant difference among the 4 groups in parameters other than vWF (Table 2).

MPAP, TPR, and PVR were positively correlated with P-selectin level in patients in the PPH group (MPAP, $r=0.40$, $P<0.05$; TPR, $r=0.42$, $P<0.05$; PVR, $r=0.46$, $P<0.01$), although there was no significant correlation between MPAP, TPR, or PVR and P-selectin level in the sPAH and PVH groups. TPR and PVR were negatively correlated with plasma TM level in the PPH group (TPR, $r=0.41$, $P<0.05$; PVR, $r=0.43$, $P<0.05$), although MPAP was not significantly correlated ($r=0.33$, $P=0.07$). However, there was no significant correlation between MPAP, TPR, or PVR and TM level in the sPAH and PVH groups.

Effects of PGI$_2$ Therapy on Plasma P-Selectin and TM

Patient profiles, therapeutic modalities including warfarin and dobutamine, and P-selectin and TM levels at baseline and follow-up measurement are shown in Table 3. There was no significant difference in pulmonary hemodynamic data, presented as MPAP and PVR at baseline between the 9 patients with warfarin treatment (MPAP, $61\pm11$ mm Hg; PVR, $20\pm6$ U) and 9 subjects without (MPAP, $64\pm10$ mm Hg; PVR, $23\pm7$ U). There was no significant difference in plasma P-selectin and TM levels at baseline between patients with warfarin treatment (P-selectin, $303.7\pm177.2$ ng/mL; TM, $1.7\pm0.4$ ng/mL) and those without (P-selectin, $299.2\pm148.1$ ng/mL; TM, $2.0\pm0.5$ ng/mL).

During continuous PGI$_2$ therapy, pulmonary hemodynamic parameters, including MPAP ($62\pm10$ versus $54\pm12$ mm Hg; baseline versus follow-up) and PVR ($22\pm7$ versus $15\pm5$ Wood units), were improved ($P<0.05$; paired $t$ test). During PGI$_1$ therapy, plasma P-selectin level decreased ($307.5\pm187.8$ versus $187.8\pm84.8$ ng/mL, baseline versus follow-up) and plasma TM level increased ($1.9\pm0.5$ versus $2.5\pm0.6$ ng/mL) in all 18 subjects ($P<0.01$; paired $t$ test). Changes in plasma levels of P-selectin and TM before and after PGI$_1$ are shown in Figure 3.

There was no significant difference in percent change of MPAP, TPR, and PVR between subjects who were treated with warfarin and those who were not. Similarly, there was no significant difference in percent change of P-selectin and TM levels between patients with warfarin treatment and those without. A significant improvement, defined as percent change $>20\%$ of the P-selectin level, was attained in 12 of 18 patients (67%), and a significant improvement of the TM level was attained in 11 of 18 subjects (61%). There was no significant difference between percent change of P-selectin and that of TM.

Discussion

We demonstrated that the plasma level of P-selectin is elevated and TM is decreased in patients with PAH. These

![Figure 1](https://circ.ahajournals.org/)

**Figure 1.** Plasma level of soluble P-selectin in 4 groups. Data are presented as mean±SD. Plasma levels (ng/mL) of P-selectin in both PPH (273±159) and sPAH (243±170) groups were significantly higher than those in Control (99±30) and PVH (147±74) groups.

![Figure 2](https://circ.ahajournals.org/)

**Figure 2.** Plasma level of TM in 4 groups. Data are presented as mean±SD. Plasma levels (ng/mL) of TM in both PPH (2.0±0.7) and sPAH (2.8±1.6) groups were significantly lower than those in Control (4.0±0.6) and PVH (3.5±1.2) groups. In PPH group, plasma TM level was even lower than that in sPAH group.
abnormal levels of P-selectin and TM were improved after continuous infusion of PGI₂.

Platelet activation or vessel injury and loss of coagulation regulation may enhance in situ thrombosis, increasing vessel injury and promoting vascular remodeling and perpetuating thrombus formation and PH. Measuring soluble P-selectin, which originates from both platelets and endothelial cells, is proposed as a marker of increased membrane-bound P-selectin expression, attributable to vascular dysfunction and/or platelet activation, and may provide information on in vivo interactions among vascular and circulating cells.⁶,⁷,¹⁰ In vitro experiments revealed that P-selectin induced tissue factor expression in human circulating monocytes and suggest that an increase in plasma P-selectin may have as a consequence the induction of a hypercoagulable state.¹⁹

Plasma TM level appears to be initially increased with acute vascular injury, perhaps through cleavage from the cell surface, and is then decreased with subsequent downregulation of production after chronic vessel injury.¹⁴,²⁰ This loss of TM could result in failure to inactivate locally generated thrombin and diminished protein C activation at the site of vessel injury, resulting in local thrombosis. Such thrombi are much less frequent in PVH, and this might be explained by the differences in plasma TM and P-selectin levels between the PPH or sPAH group and the PVH group.¹⁴

### Table 2. Plasma Levels of Clotting Factors Other Than P-Selectin and TM

<table>
<thead>
<tr>
<th>Control</th>
<th>PVH</th>
<th>sPAH</th>
<th>PPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWF antigen, %</td>
<td>108±35</td>
<td>208±80*</td>
<td>173±76*</td>
</tr>
<tr>
<td>vWF cofactor, %</td>
<td>119±33</td>
<td>165±44</td>
<td>142±71</td>
</tr>
<tr>
<td>vWF activity ratio (cofactor/antigen)</td>
<td>1.26±0.69</td>
<td>0.91±0.42*</td>
<td>0.89±0.22*</td>
</tr>
<tr>
<td>AT-III, %</td>
<td>100±13</td>
<td>92±13</td>
<td>88±13</td>
</tr>
<tr>
<td>TAT, µg/L</td>
<td>2.8±1.4</td>
<td>3.6±3.0</td>
<td>3.5±3.2</td>
</tr>
<tr>
<td>FDP, µg/mL</td>
<td>8.0±4.4</td>
<td>10.3±2.1</td>
<td>10.6±2.9</td>
</tr>
<tr>
<td>IPA, ng/mL</td>
<td>7.4±5.2</td>
<td>8.5±3.2</td>
<td>8.6±4.5</td>
</tr>
<tr>
<td>PAI-1, ng/mL</td>
<td>25±11</td>
<td>50.0±46.8</td>
<td>31.5±30.0</td>
</tr>
<tr>
<td>Plasminogen, %</td>
<td>96±8</td>
<td>90±15</td>
<td>85±18</td>
</tr>
<tr>
<td>α₂-Plasmin inhibitor, %</td>
<td>94±7</td>
<td>93±16</td>
<td>94±18</td>
</tr>
</tbody>
</table>

AT-III indicates antithrombin-III; TAT, thrombin–antithrombin III complex; IPA, tissue plasminogen activator; and PAI-1, plasminogen activator inhibitor-1. Values are mean±SD.

*P<0.05 vs Control.

### Table 3. Profile, Therapeutic Modalities, and Changes of P-Selectin and TM in Patients Treated With PGI₂

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y</th>
<th>Sex</th>
<th>Disease</th>
<th>Warfarin</th>
<th>DOB</th>
<th>P-selectin, ng/mL</th>
<th>TM, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>M</td>
<td>PPH</td>
<td>Yes</td>
<td>No</td>
<td>Baseline 196.3</td>
<td>Follow-Up 62.1 (−68.4)</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>F</td>
<td>PPH</td>
<td>Yes</td>
<td>No</td>
<td>Baseline 570.5</td>
<td>Follow-Up 247 (−56.7)</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>F</td>
<td>PPH</td>
<td>No</td>
<td>No</td>
<td>Baseline 366.2</td>
<td>Follow-Up 267.3 (−27.0)</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>F</td>
<td>PPH</td>
<td>No</td>
<td>No</td>
<td>Baseline 84.8</td>
<td>Follow-Up 64.3 (−24.2)</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>M</td>
<td>PPH</td>
<td>Yes</td>
<td>No</td>
<td>Baseline 561.6</td>
<td>Follow-Up 384 (−31.6)</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>F</td>
<td>PPH</td>
<td>No</td>
<td>No</td>
<td>Baseline 373.9</td>
<td>Follow-Up 165.3 (−55.8)</td>
</tr>
<tr>
<td>7</td>
<td>41</td>
<td>F</td>
<td>PPH</td>
<td>No</td>
<td>No</td>
<td>Baseline 273.3</td>
<td>Follow-Up 145 (−46.9)</td>
</tr>
<tr>
<td>8</td>
<td>27</td>
<td>M</td>
<td>PPH</td>
<td>Yes</td>
<td>Yes</td>
<td>Baseline 344.7</td>
<td>Follow-Up 192 (−44.3)</td>
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<tr>
<td>9</td>
<td>27</td>
<td>M</td>
<td>PPH</td>
<td>Yes</td>
<td>No</td>
<td>Baseline 235.6</td>
<td>Follow-Up 187.3 (−20.5)</td>
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<tr>
<td>10</td>
<td>44</td>
<td>F</td>
<td>PPH</td>
<td>No</td>
<td>No</td>
<td>Baseline 148.9</td>
<td>Follow-Up 128.6 (−13.6)</td>
</tr>
<tr>
<td>11</td>
<td>27</td>
<td>M</td>
<td>PPH</td>
<td>No</td>
<td>Yes</td>
<td>Baseline 565.8</td>
<td>Follow-Up 310 (−45.2)</td>
</tr>
<tr>
<td>12</td>
<td>41</td>
<td>F</td>
<td>PPH</td>
<td>Yes</td>
<td>Yes</td>
<td>Baseline 143.4</td>
<td>Follow-Up 72.6 (−49.4)</td>
</tr>
<tr>
<td>13</td>
<td>48</td>
<td>M</td>
<td>PPH</td>
<td>No</td>
<td>Yes</td>
<td>Baseline 104.5</td>
<td>Follow-Up 114.8 (−9.9)</td>
</tr>
<tr>
<td>14</td>
<td>36</td>
<td>M</td>
<td>PPH</td>
<td>No</td>
<td>No</td>
<td>Baseline 253.3</td>
<td>Follow-Up 213.3 (−15.8)</td>
</tr>
<tr>
<td>15</td>
<td>31</td>
<td>F</td>
<td>PH+SLE</td>
<td>No</td>
<td>Yes</td>
<td>Baseline 284.3</td>
<td>Follow-Up 147.1 (−48.3)</td>
</tr>
<tr>
<td>16</td>
<td>27</td>
<td>F</td>
<td>PH+MCTD</td>
<td>No</td>
<td>No</td>
<td>Baseline 386.7</td>
<td>Follow-Up 239 (−38.2)</td>
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<tr>
<td>17</td>
<td>27</td>
<td>F</td>
<td>PH+SLE</td>
<td>Yes</td>
<td>No</td>
<td>Baseline 119.3</td>
<td>Follow-Up 105.8 (−11.3)</td>
</tr>
<tr>
<td>18</td>
<td>63</td>
<td>F</td>
<td>PPH</td>
<td>Yes</td>
<td>Yes</td>
<td>Baseline 413</td>
<td>Follow-Up 209.6 (−49.2)</td>
</tr>
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</table>

DOB indicates dobutamine; SLE, systemic lupus erythematosus; and MCTD, mixed connective tissue disease.
that a stable analogue of PGI2, beraprost sodium, attenuated endothelial cells. However, we should be cautious not to stress with increased pulmonary arterial pressure also may load or shear stress on both the pulmonary arteries and veins. Secondary vascular endothelial injury due to pressure overload or shear stress on both the pulmonary arteries and veins. Endothelial injury and platelet activation in PPH with PGI2 therapy (Follow-up) in 15 patients with PPH and 3 patients with PH associated with collagen vascular disease. Higher P-selectin level at baseline (307.6±161.0 ng/mL; mean±SD) was decreased during PGI2 therapy (187.8±84.8 ng/mL;  \( P < 0.01 \)). Lower level of TM at baseline (1.9±0.5 ng/mL) increased during PGI2 therapy (2.5±0.6 ng/mL;  \( P < 0.01 \)). Rectangles enclosed with a dotted line denote mean±2 SD of subjects in Control group.

It is difficult to evaluate endothelial injury and microthrombosis in PH by measuring conventional coagulation and fibrinolytic parameters. Indeed, there was no significant difference among the 4 groups in parameters other than vWF (Table 3). The plasma level of vWF antigen in the PVH, sPAH, and PPH groups was higher than that in the Control group. These data relating to vWF are in agreement with a previous study on PH. However, there was no significant difference among the 3 different groups of PH. From our results, increased vWF antigen associated with a decreased ratio of ristocetin cofactor to vWF antigen may reflect secondary vascular endothelial injury due to pressure overload or shear stress on both the pulmonary arteries and veins.

P-selectin and TM levels were correlated with TPR and PVR in patients with PPH. Vascular wall damage due to shear stress with increased pulmonary arterial pressure also may have some effects on TM and P-selectin expression on endothelial cells. However, we should be cautious not to overemphasize this association in PPH, because the correlation was weak, even though it was statistically significant. There was no significant correlation between TPR or PVR and P-selectin or TM level in the sPAH group. In sPAH, increased pulmonary arterial pressure or PVR might be caused not only by pulmonary vascular endothelial injury or microthrombi but also by increased pulmonary blood flow due to intracardiac shunt. Furthermore, abnormal levels of TM and P-selectin in the sPAH group could be affected by systemic inflammation due to collagen vascular disease and other factors.

After PGI2 therapy, the lower TM level and higher P-selectin level showed a tendency to improve. Experimental data from studies of PGI2 in animals with both systemic and pulmonary vascular disease have demonstrated its potential to reverse platelet activation and vascular lesions. The improvement in increased P-selectin may indicate an attenuation of endothelial injury and platelet activation in PPH with PGI2 therapy. The study by Kainoh and coworkers demonstrated that a stable analogue of PGI2, beraprost sodium, attenuated the decreased expression of TM on cultured endothelial cells by stimulation with interleukin-1. The improvement in TM suggests that the reduced anticoagulant activity of endothelial cells in PPH may recover with PGI2 therapy. Friedman and coworkers demonstrated that the increased levels of vWF antigen and ristocetin cofactor and decreased ratio of ristocetin cofactor to vWF antigen, which may reflect biological activity of vWF, were improved with long-term PGI2 therapy. Similarly, they revealed that platelet aggregation abnormalities were also normalized after PGI2 therapy. Our results appear to support the results of these previous studies.

Study Limitations
First, abnormal levels of P-selectin and TM in venous blood may reflect vascular injury and microthrombosis not only in the lungs but also in the systemic circulation, although no subject in this study had any other known cause that has an effect on values of P-selectin and TM. Second, a mean follow-up period of 90 days during PGI2 therapy may be too short to evaluate the long-term effectiveness of PGI2. Further study, however, is needed to truly evaluate the longer-term effectiveness of PGI2 against vascular endothelial damage and altered hemostasis.

Third, the effect of anticoagulant therapy with warfarin on P-selectin and TM levels should be considered. Indeed, there was no significant difference in hemodynamic data or plasma levels of P-selectin and TM at baseline between patients with and without warfarin treatment as well as in percent change of P-selectin and TM levels during PGI2 therapy between subjects with and without warfarin treatment, although patient subsets receiving warfarin in the PPH groups had a tendency to show higher P-selectin and lower TM levels. However, the possible effect of warfarin may be undetectable by this study, because each subset of patients divided by warfarin was too small to conclude the real effect of warfarin on levels of plasma parameters, hemodynamic data, and the effect of PGI2 in these results.

Conclusions
We demonstrated that the plasma level of soluble P-selectin was increased and that of TM was decreased in patients with PAH. The abnormal values of P-selectin and TM were improved by continuous PGI2 infusion. These results suggest that the increased plasma level of P-selectin and decreased level of TM may reflect in situ thrombosis due to endothelial injury, which may contribute to the pathogenesis of PAH. The normalization of these parameters with PGI2 therapy indicates that PGI2 may act not only as a pulmonary vasodilator but also as an agent that attenuates endothelial damage and abnormalities of platelet activation and the coagulation-fibrinolytic systems in PPH.

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References


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Fumio Sakamaki, Shingo Kyotani, Noritoshi Nagaya, Nagato Sato, Hideo Oya, Toru Satoh and Norifumi Nakanishi

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