Plasma Thromboxane B$_2$ Concentration in Pulmonary Hypertension Associated With Congenital Heart Disease

Shigeto Fuse, MD; Tetsuro Kamiya, MD

Background We investigated the plasma concentration of thromboxane B$_2$ (TXB$_2$), a stable metabolite of thromboxane A$_2$ (TXA$_2$), to assess platelet activation in 78 patients who had pulmonary hypertension associated with congenital heart disease (PH group) and 16 patients with almost normal hemodynamics (control group).

Methods and Results The PH group was divided into two subgroups: pulmonary vascular resistance (Rp) $\leq$ 10 U/m$^2$ (Rp≤10 group) and $>$ 10 U/m$^2$ (Rp>10 group). In addition, the Rp≤10 group was divided on the basis of clinical symptoms into groups with dyspnea (dyspnea$^+$ group) and without dyspnea (dyspnea$^-$ group). Plasma TXB$_2$ levels were measured by radioimmunoassay. Plasma TXB$_2$ levels in the three groups (control, Rp≤10, and Rp>10) were significantly different ($P<.005$); the TXB$_2$ levels in the Rp≤10 group were significantly higher than the others. Among the Rp≤10 patients, the plasma TXB$_2$ levels were significantly higher in the dyspnea$^+$ group than in the dyspnea$^-$ group ($P<0.0001$).

In addition, the pulmonary-to-systemic flow ratio and pulmonary blood flow divided by body surface area were significantly higher in the dyspnea$^+$ group than in the dyspnea$^-$ group ($P<.02$ and $P<.002$, respectively).

Conclusions These findings suggest that platelet activation led to increased TXA$_2$ release in patients with pulmonary hypertension, especially those with dyspnea and Rp≤10. TXA$_2$ release from platelets probably caused constriction of the pulmonary arterioles and the bronchi, thus worsening pulmonary hypertension and dyspnea in these patients. In the patients with high Rp values, it was considered that the number of pulmonary arterioles where platelets could be activated had been reduced. (Circulation. 1994;90:2952-2955.)

Key Words • thromboxane • radioimmunoassay • catheterization • pediatrics

In patients who have pulmonary hypertension associated with congenital heart disease, ultrastructural abnormalities are observed in the endothelial cells of the pulmonary arterioles.1-3 In these patients, there is an increase in von Willebrand factor antigenic activity and a loss of large von Willebrand factor multimeric forms,4 or plasma endothelin levels are increased.5 These studies suggest that the endothelial cells have abnormal or heightened metabolic activity. If endothelial function is abnormal, an increase of platelet adhesion or activation may also occur. In some patients who have pulmonary hypertension associated with congenital heart disease, platelets have been observed in close apposition to the endothelial cells by scanning electron microscopy.1

Thromboxane B$_2$ (TXB$_2$) is a metabolic product of arachidonic acid that is rapidly formed from thromboxane A$_2$ (TXA$_2$) and has no biological activity. TXA$_2$ is released from platelets by many stimuli and causes marked vasoconstriction, platelet aggregation, and bronchoconstriction.6 It has also been suggested that plasma TXA$_2$ levels are correlated with pulmonary artery pressure6,9 and respiratory symptoms. In the present study, we measured the plasma TXB$_2$ concentration to evaluate platelet activation in patients with pulmonary hypertension and congenital heart disease.

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From the National Cardiovascular Center, Osaka, Japan.
Correspondence to Shigeto Fuse, MD, Department of Pediatrics, Kushiro City General Hospital, 1-12, Shunkodai, Kushiro, Hokkaidou, 085, Japan.
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Methods

Subjects

We studied 94 patients (55 male and 39 female) who were not under treatment with any anticoagulants. Pulmonary hypertension (mean pulmonary artery pressure ≥20 mm Hg) was present in 78 patients aged 2 months to 37 years (PH group). Another 16 patients aged 6 months to 22 years had almost normal hemodynamics despite the presence of acquired heart disease and arrhythmias (control group). The PH group was divided into two subgroups on the basis of pulmonary vascular resistance (Rp), ie, a group in which Rp was $\leq$ 10 U/m$^2$ (Rp≤10 group) and a group in which it was $>$ 10 U/m$^2$ (Rp>10 group). Furthermore, the Rp≤10 group was divided by the presence of dyspnea into a dyspnea$^+$ group and a dyspnea$^-$ group. The clinical profiles of these groups are given in the Table.

Informed consent to the study was obtained from the patients or their parents, and the study protocol conformed to the guidelines of the ethics committee of our institution.

Cardiac Catheterization

Cardiac catheterization was performed in all patients in the control and the PH groups. Pulmonary and systemic blood flow volumes were determined according to the Fick principle.10 Oxygen consumption was estimated from the age, sex, and heart rate data according to the method of LaFarge and Miettinen.11 The oxygen content of blood was measured with a CO oxymeter.12

Plasma TXB$_2$ Assay

Blood samples (5 mL) were taken from a peripheral vein (antecubital, femoral, or external jugular); collection was completed within 10 seconds, and each sample was withdrawn into an ice-chilled plastic tube containing 50 $\mu$L of 4×10$^{-3}$


diluted A2

in...
Patient Profile

<table>
<thead>
<tr>
<th>PH (n=78)</th>
<th>RP≤10 (n=59)</th>
<th>Dyspnea(+) (n=14)</th>
<th>Dyspnea(−) (n=45)</th>
<th>Male:female</th>
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</thead>
<tbody>
<tr>
<td>Control (n=16)</td>
<td>25:20</td>
<td>11:3</td>
<td>12:7</td>
<td>7:9</td>
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<tr>
<td>Age, y</td>
<td>8.6±6.2</td>
<td>3.7±6.4</td>
<td>0.9±0.8</td>
<td>9.5±8.3</td>
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<tr>
<td>Hematocrit, %</td>
<td>38.7±4.2</td>
<td>39.7±4.2</td>
<td>39.5±5.4</td>
<td>48.0±9.2</td>
</tr>
<tr>
<td>Platelets, ×10^3/μL</td>
<td>31.5±12.8</td>
<td>27.6±10.1</td>
<td>30.8±12.9</td>
<td>18.8±8.8</td>
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<tr>
<td>Ppa, mm Hg</td>
<td>11.8±2.7</td>
<td>48.0±12.4</td>
<td>52.6±13.0</td>
<td>67.9±12.7</td>
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<tr>
<td>Pp:Ps</td>
<td>0.20±0.04</td>
<td>0.75±0.23</td>
<td>0.80±0.15</td>
<td>1.00±0.20</td>
</tr>
<tr>
<td>Qp:Qs</td>
<td>1.0±0.1</td>
<td>2.6±1.5</td>
<td>4.2±3.9</td>
<td>1.1±0.3</td>
</tr>
<tr>
<td>Qp/BSA, L · min⁻¹ · m⁻²</td>
<td>4.3±1.2</td>
<td>9.1±4.1</td>
<td>16.0±13.0</td>
<td>3.2±1.1</td>
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<td>Rp, U/m²</td>
<td>1.6±0.6</td>
<td>4.9±2.0</td>
<td>4.5±3.1</td>
<td>23.2±11.7</td>
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<table>
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<th>Diagnosis</th>
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<th>VSD:17 (po 1)</th>
<th>VSD:6</th>
<th>ASD:6</th>
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<td>VSD-ASD-PS:1</td>
<td>VSD:3</td>
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<td>(improved):1</td>
<td>VSD-CoA:2</td>
<td>VSD-PDA:1</td>
<td>VSD-PDA:2</td>
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<tr>
<td>HCM:1, SSS:1</td>
<td>VSD-PDA:2</td>
<td>CAVC:2</td>
<td>CAVC:3 (po 2)</td>
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<td>AVF:1, CAVB:1</td>
<td>CAVC:7 (po 3)</td>
<td>DORV:1</td>
<td>TOF-PA po:2</td>
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<td>AS:1, SVAS:1</td>
<td>TOF-PA:4 (po 3)</td>
<td>ECD:1</td>
<td>APW-IAA:1</td>
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<td>PDA small:1</td>
<td>ASD:3, PDA:3</td>
<td>TOF-PA:1</td>
<td>ECD-PDA:1</td>
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<td>Ebstein:1</td>
<td>DORV:3 (po 2)</td>
<td>UVH-TGA po:1</td>
<td>Truncus po:1</td>
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<td>Long QT:1</td>
<td>ECD:1</td>
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Values are mean±SD. PH indicates pulmonary hypertension; Rp, pulmonary vascular resistance; Ppa, mean pulmonary arterial pressure; Pp:Ps, systolic pulmonary-to-systemic pressure ratio; Qp:Qs, pulmonary-to-systemic flow ratio; Qp/BSA, pulmonary blood flow divided by body surface area; Hx.MCLS, history of mucocutaneous lymph node syndrome; HCM, hypertrophic cardiomyopathy; SSS, sick sinus syndrome; AVF, arteriovenous fistula; CAVB, complete atrioventricular block; AS, aortic valve stenosis; SVAS, supravalvular aortic stenosis; PDA, patent ductus arteriosus; Ebstein, Ebstein's anomaly; Long QT, long QT syndrome; VSD, ventricular septal defect; po, postoperatively; ASD, atrial septal defect; CoA, coarctation of aorta; CAVC, common atrioventricular canal; TOF, tetralogy of Fallot; PA, pulmonary atresia; DORV, double-outlet right ventricle; ECD, endocardial cushion defect; PS, pulmonary stenosis; UVH, univentricular heart; TGA, transposition of the great arteries; APW, aortopulmonary window; IAA, interrupted aortic arch; and Truncus, truncus arteriosus.

Further incubation was performed for 24 hours at 4°C. After centrifugation at 3000 rpm for 10 minutes, the radioactivity in the supernatant was counted using a Packard TriCarb liquid scintillation spectrometer. A standard curve was constructed using B/Bo versus the TXB₂ standard. The amount of TXB₂ in the plasma samples from the patients was determined from the standard curve linearized by logit-log transformation, and the recovery was calculated by adding [³H]-TXB₂ to each plasma sample during the extraction procedure.

Statistical Analysis

Comparisons among the three groups were analyzed using one-way ANOVA. Comparisons of mean values between two groups were performed with the Mann-Whitney U test. Comparison of the mean values of Qp:Qs or Qp/BSA (body surface area) in the Rp≤10 group was performed with the unpaired t test. Probability values <.05 were considered to indicate statistical significance.

Results

Plasma TXB₂ concentrations were determined for individual patients of the control group (70.7±35.4 pg/mL, mean±SD), the Rp≤10 group (337.9±424.8), and the Rp>10 group (101.4±76.1). The TXB₂ levels in the three groups were significantly different (P<.005);
TXB₂ levels in the Rp≤10 group were significantly higher than in the others (Fig 1). No TXB₂ level was >300 pg/mL in the Rp>10 group (Fig 1). In addition, plasma TXB₂ concentrations were significantly higher in the dyspnea(+) group (957.5±486.8) than in the dyspnea(−) group (145.1±87.5) (P<0.001) (see Fig 2). The dyspnea(+) group had significantly higher Qp:Qs and Qp/BSA values (4.2±3.9 and 16.0±13.0 L·min⁻¹·m⁻², respectively) than the dyspnea(−) group (2.8±1.5 and 9.1±4.1) (P<0.02 and P<0.002, respectively). However, there was no relation between the plasma TXB₂ concentration and Qp:Qs or Qp/BSA in the Rp≤10 group.

Discussion

Plasma TXA₂ values could not be directly measured in this study because this substance is metabolized so rapidly in human biological fluids (t₁/₂=32 seconds at 37°C).¹⁴ The most common approach to the assessment of TXA₂ biosynthesis is measurement of TXB₂, its stable and biologically inactive hydration product. Plasma TXB₂ values closely reflect but do not exactly represent TXA₂ production.

Our study showed that the plasma TXB₂ concentration was high in patients with pulmonary hypertension associated with congenital heart disease, especially those with respiratory symptoms (dyspnea) and Rp<10 U/m².

Rabinovitch et al⁵ have reported three grades (grades A through C) of pulmonary vascular disease in patients with congenital heart disease that were correlated with hemodynamic data. The patients with grade C disease and a reduction in the number of small pulmonary arteries had Rp>3.5 U/m².¹² If platelets become activated on the damaged or abnormal pulmonary vascular endothelium, chemical substances such as TXA₂ will be released in the pulmonary arterioles of patients with grade A or B pulmonary vascular disease. Thus, it appears that the plasma TXB₂ concentration was lower in our patients with a high Rp because of a reduction in the number of pulmonary arterioles where platelets could be activated. In contrast, the patients with a high plasma TXB₂ concentration probably suffered from more platelet activation in the pulmonary arterioles because the number of vessels had not decreased. Thus, the plasma TXB₂ concentration may reflect the grade of pulmonary vascular disease and may be lower in patients with progressive pulmonary vascular occlusive disease and a high Rp.

TXA₂ is a potent vasoconstrictor and bronchoconstrictor, and it also promotes platelet aggregation.⁶ The lungs are the major site where circulating platelet aggregates are trapped.¹⁶ Thus, if platelets were activated and became aggregated in the systemic arterioles of the patients with high Rp values (including the 14 patients with right-to-left shunts), many of these aggregates would become trapped in the lungs as well as the systemic arterioles. Because the patients with high Rp values did not have high plasma TXB₂ concentrations (Fig 1), we concluded that there was little systemic platelet activation and trapping of aggregates and that platelets were mostly activated in the pulmonary arterioles and trapped in the lungs. When platelet aggregates form in the pulmonary arterioles, TXA₂ is released, and the resultant vasoconstriction may increase pulmonary artery pressure and decrease pulmonary blood flow. At the same time, TXA₂ reaches the bronchi through the pulmonary arterioles and may cause bronchoconstriction with dyspnea.

Because it is well known that the respiratory symptoms of patients who have pulmonary hypertension associated with congenital heart disease are reduced or abolished by successful surgical operation, the respiratory symptoms of such patients may be due to pulmonary hypertension and a large pulmonary blood flow associated with congenital heart disease. Thus, this dyspnea differs from that in patients with cyanotic congenital disease, which is often due to an exercise-induced increase in right-to-left shunt that leads to a sudden change in the systemic arterial blood gas composition and pH, stimulating the respiratory center and causing hyperventilation. The present study showed that Qp:Qs and Qp/BSA were significantly higher in the dyspnea(+) group (4.2±3.9 and 16.0±13.0) than in the dyspnea(−) group (2.8±1.5 and 9.1±4.1) (P<0.02, P<0.002). However, there was no relation between the plasma TXB₂ levels and Qp:Qs or Qp/BSA in the Rp≤10 group. This suggested that, although TXA₂ is
closely related to pulmonary hypertension associated with congenital heart disease, especially influencing pulmonary vascular resistance and pulmonary blood flow, some other factors or chemical mediators must also influence pulmonary hypertension.

Accordingly, TXA₂ synthetase inhibitors such as oza-grel hydrochloride¹⁷ may have a beneficial effect on pulmonary hypertension in patients with a high plasma TXB₂ concentration and may decrease pulmonary vascular resistance and improve respiratory symptoms by reducing constriction of the pulmonary arterioles and bronchi.

References


Plasma thromboxane B2 concentration in pulmonary hypertension associated with congenital heart disease.
S Fuse and T Kamiya

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