Plasma Thromboxane B₂ Concentration in Pulmonary Hypertension Associated With Congenital Heart Disease

Shigeto Fuse, MD; Tetsuro Kamiya, MD

**Background** We investigated the plasma concentration of thromboxane B₂ (TXB₂), a stable metabolite of thromboxane A₂ (TXA₂), 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) ≤10 U/m² (Rp≤10 group) and >10 U/m² (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 TXB₂ levels were measured by radioimmunoassay. Plasma TXB₂ levels in the three groups (control, Rp≤10, and Rp>10) were significantly different (P<.005); the TXB₂ levels in the Rp≤10 group were significantly higher than the others. Among the Rp≤10 patients, the plasma TXB₂ levels were significantly higher in the dyspnea(+) group than in the dyspnea(−) group (P<.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 TXA₂ release in patients with pulmonary hypertension, especially those with dyspnea and Rp≤10. TXA₂ 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 platelelets could be activated had been reduced. (Circulation. 1994;90:2952-2955.)

**Key Words** • thromboxane • radioimmunoassay • catheterization • pediatrics

**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 ≤10 U/m² (Rp≤10 group) and a group in which it was >10 U/m² (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. Oxygen consumption was estimated from the age, sex, and heart rate data according to the method of LaFarge and Miettinnen. The oxygen content of blood was measured with a CO oxymeter.

**Plasma TXB₂ 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 μL of 4x10⁻³
Patient Profile

<table>
<thead>
<tr>
<th>PH (n=78)</th>
<th>Rp≤10 (n=59)</th>
<th>Dyspnea(−) (n=45)</th>
<th>Dyspnea(+) (n=14)</th>
<th>Rp&gt;10 (n=19)</th>
</tr>
</thead>
<tbody>
<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>
</tr>
<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, ×10⁴/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>
</tr>
<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>
</tr>
<tr>
<td>Pp:Pπ</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>2.8±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>
</tr>
<tr>
<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>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocarditis</td>
<td>VSD:17 (po 1)</td>
<td>VSD:6</td>
<td>ASD:6</td>
<td></td>
</tr>
<tr>
<td>(improved):1</td>
<td>VSD-ASD-PS:1</td>
<td>VSD:3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCM:1, SSS:1</td>
<td>VSD-PDA:2</td>
<td>CAVC:2</td>
<td>CAVC:3 (po 2)</td>
<td></td>
</tr>
<tr>
<td>AVF:1, CAVB:1</td>
<td>CAVC:7 (po 3)</td>
<td>DORV:1</td>
<td>TOF-PA po:2</td>
<td></td>
</tr>
<tr>
<td>AS:1, SVAS:1</td>
<td>TOF-PA:4 (po 3)</td>
<td>ECD:1</td>
<td>APW-IAA:1</td>
<td></td>
</tr>
<tr>
<td>PDA small:1</td>
<td>ASD-3, PDA-3</td>
<td>TOF-PA:1</td>
<td>ECD-PDA:1</td>
<td></td>
</tr>
<tr>
<td>Ebstein:1</td>
<td>DORV:3 (po 2)</td>
<td>UVH-TGA po:1</td>
<td>Truncus po:1</td>
<td></td>
</tr>
<tr>
<td>Long QT:1</td>
<td>ECD:1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD. PH indicates pulmonary hypertension; Rp, pulmonary vascular resistance; Ppa, mean pulmonary arterial pressure; Pp:Pπ, 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 myocardio-cerebral vascular disease; HCM, hypertrophic cardiomyopathy; SSS, sick sinus syndrome; AVF, arteriovenous fistula; CAVB, complete atrioventricular block; AS, aortic valve stenosis; SVAS, supravalvar aortic stenosis; PDA, patent ductus arteriosus; Ebstein, Ebstein’s anomaly; Long QT, long QT syndrome; VSD, ventricular septal defect; Po, postoperative; 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.

mol/mL EDTA-2K and 100 μL of 1×10⁻⁵ mol/mL indomethacin. The samples were centrifuged at 3000 rpm for 10 minutes at 4°C, and the serum thus obtained was stored at −30°C until further analysis. A 1-mL aliquot of plasma delipidated with petroleum-ether was mixed with 3 mL of ethylacetate/isopropanol (1:1 vol/vol), followed by the addition of 5 mL of ethylacetate/distilled water (2:3 vol/vol) and further mixing. The upper phase separated by centrifugation at 3000 rpm for 2 minutes was discarded, and the lower phase was evaporated at 55°C under a stream of nitrogen. The dried residue was reconstituted in a benzene/ethylacetate mixture (6:4) and applied to a silicic acid column. The materials adsorbed to the column were eluted sequentially with benzene/ethylacetate/methanol solutions at 60/40/2, 100/ 0/0, 60/40/1, 60/40/5, and 60/40/30. The eluates obtained were evaporated using a centrifugal concentrator, and the residues were reconstituted in phosphate-saline buffer for the TXB2 radioimmunoassay.13 The TXB2 radioimmunoassay was performed in a reaction mixture consisting of 0.8 mL of sample or standard (Ono Pharmaceutical Co.), 0.1 mL of ³H-TXB2 (specific activity: 196.8 Ci mmol, New England Nuclear Co.), and 0.1 mL of anti-TXB2 serum (final dilution, 1/16 000, Ono Pharmaceutical Co.). After the mixture was let stand for 1 hour at 4°C, 0.2 mL of assay buffer containing 10 μL of goat anti-rabbit γ-globulin (Daiichi-RI) and 1 μL of normal rabbit serum was added, and 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);
TXB$_2$ levels in the Rp≤10 group were significantly higher than in the others (Fig 1). No TXB$_2$ level was >300 pg/mL in the Rp>10 group (Fig 1). In addition, plasma TXB$_2$ concentrations were significantly higher in the dyspnea (+) group (957.5±486.8) than in the dyspnea (−) group (145.1±87.5) (P<.0001) (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<.02 and P<.002, respectively). However, there was no relation between the plasma TXB$_2$ concentration and Qp:Qs or Qp/BSA in the Rp≤10 group.

Discussion

Plasma TXA$_2$ values could not be directly measured in this study because this substance is metabolized so rapidly in human biological fluids (t$_1/2$=32 seconds at 37°C). The most common approach to the assessment of TXA$_2$ biosynthesis is measurement of TXB$_2$, its stable and biologically inactive hydration product. Plasma TXB$_2$ values closely reflect but do not exactly represent TXA$_2$ production.

Our study showed that the plasma TXB$_2$ concentration was high in patients with pulmonary hypertension associated with congenital heart disease, especially those with respiratory symptoms (dyspnea) and Rp<10 U/m$^2$.

Rabinovitch et al$^{15}$ 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/m$^2$. If platelets become activated on the damaged or abnormal pulmonary vascular endothelium, chemical substances such as TXA$_2$ will be released in the pulmonary arterioles of patients with grade A or B pulmonary vascular disease. Thus, it appears that the plasma TXB$_2$ 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 TXB$_2$ concentration probably suffered from more platelet activation in the pulmonary arterioles because the number of vessels had not decreased. Thus, the plasma TXB$_2$ 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.

TXA$_2$ is a potent vasoconstrictor and bronchoconstrictor, and it also promotes platelet aggregation.$^9$ 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 TXB$_2$ 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, TXA$_2$ is released, and the resultant vasoconstriction may increase pulmonary artery pressure and decrease pulmonary blood flow. At the same time, TXA$_2$ 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<.02, P<.002). However, there was no relation between the plasma TXB$_2$ levels and Qp:Qs or Qp/BSA in the Rp≤10 group. This suggested that, although TXA$_2$ 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
10. Fick A. Ueber die Messung des Blutquantum in den Herzven-
Plasma thromboxane B2 concentration in pulmonary hypertension associated with congenital heart disease.
S Fuse and T Kamiya

_Circulation_. 1994;90:2952-2955
doi: 10.1161/01.CIR.90.6.2952

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/90/6/2952

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/