THROMBOXANE A₂ AS AN ENHANCING FACTOR OF CORONARY VASOSPASTICITY IN VARIANT ANGINA

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To clarify the role of thromboxane A₂ (TXA₂) in evoking coronary spasm, we compared coronary arterial spasticity induced by ergonovine maleate (EM) with coronary sinus thromboxane B₂ (TXB₂): a stable catabolite of TXA₂) in 34 patients with documented variant angina and 11 patients with chest pain syndrome (CPS). We also examined the effect of OKY-1581 (8 mg/kg, i.v.), a TXA₂ synthetase inhibitor, on the coronary arterial spasticity of these patients. When blood samples were taken from coronary sinus just before EM test, all patients with variant angina exhibiting markedly augmented TXB₂ levels (424 ± 138 pg/ml), had positive EM test results, while CPS exhibiting lower TXB₂ levels (223 ± 38 pg/ml), had negative EM test. We found that the amounts of EM needed to induce coronary spasm were inversely correlated with TXB₂ levels in coronary sinus. In 7 out of these 8 patients, OKY-1581 was found to attenuate the increased spasticity with reduction of coronary sinus TXB₂ levels. In 3 patients, an EM rechallenge at symptomatically quiescent stage resulted in negative test with augmented TXB₂ levels being markedly decreased.

These findings indicate that increased TXA₂ in circulating plasma is closely correlated with the hypersensitivity of coronary arteries to EM in patients with variant angina, suggesting a possible role of augmented TXA₂ production in the enhancement of coronary vascular spasticity.

Previously we noted that increased levels of thromboxane B₂ (TXB₂), a stable catabolite of thromboxane A₂ (TXA₂), have been found in both peripheral and coronary sinus blood in patients with angina pectoris.¹² In variant angina a marked increased in TXB₂ levels was seen just before the induction of anginal attack. Others³–⁶ also indicate similar results in the ischemic events of coronary artery disease, indicating that the excessive TXA₂ production is accompanied by coronary vasospasm. In view of the fact that TXA₂ produced by aggregating platelets could serve to increase vascular spasticity, it is of clinical interest to understand the mechanism underlying the excessive TXA₂ production and its relationship with vascular disorders.

Coronary arteries in patients with spontaneous spasm are markedly hypersensitive to ergonovine⁷–¹¹. The spasticity of the vessel could be

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assessed by determining the amount of ergonovine to induce spasm during the catheterization procedures.

The present study reports that the sensitivity of coronary vessels to ergonovine in variant angina is intimately related to the baseline TXB2 levels in coronary sinus, and that the reduction of augmented TXB2 levels by a TXA2 synthetase inhibitor resulted in decreased susceptibility of coronary arteries against ergonovine.

MATERIALS AND METHODS

Patient population

Studies were conducted in 34 patients with variant angina (31 males and 3 females; mean age 54 years, range 31–66 years). These patients had spontaneous episodes of angina pectoris accompanied by S-T segment elevation (≥0.2 mV). About two thirds of these (21 cases) exhibited organic stenosis (≥75%) in any of the major coronary arteries. Eleven cases with atypical chest pain (“chest pain syndrome”) who had no S-T segment change and no coronary vasospasm during ergonovine test (9 males and 2 females; mean age 53 years, range 39–66 years) served as control subjects. An ergonovine test was performed in all of these cases. Patients with variant angina had at least two anginal attacks within the last week before the study. None of these subjects received any long-acting nitrates, calcium antagonists and adrenergic blocking agents for 72 hours, nor had they taken non-steroidal antiinflammatory drugs for at least two weeks. Only sublingual nitrates were allowed for symptoms of chest pain as needed.

Coronary angiography and blood sampling

Selective coronary angiography was performed by Judkin’s technique. #8F right and left coronary catheters were inserted into right and left femoral arteries respectively, and were placed in the ascending aorta. Heparin (100 U/kg) was intravenously administered at the beginning of the procedure and 1000 U was added at every one hour. Both left and right coronary angiograms were obtained before and immediately after an ergonovine-induced anginal attack. Particular care was taken to avoid catheter-induced coronary arterial spasm. Nitroglycerin was not given before the initial administration of ergonovine. A #7F coronary sinus flow catheter (Webster Co.) was positioned in coronary sinus, its location being confirmed by fluoroscopy.

Prior to the ergonovine challenge, blood was sampled from coronary sinus immediately before and 3 minutes after control coronary angiography. Five milliliters of blood were drawn from the coronary sinus into heparinized plastic syringes and transferred into polypropylene tubes containing 1-mM ethylenediamine tetraacetic acid (EDTA) and 0.1 mM indomethacin (in final concentration) for assay of thromboxane B2. Blood samples were stored on ice until the completion of the ergonovine test. Plasma was subsequently separated by centrifugation at 1500g for 15 minutes.

Procedure of ergonovine provocative test

The ergonovine test was performed in the cardiac catheterization laboratory as heart rate and arterial blood pressure were continuously monitored. A standard 12 leads electrocardiogram (ECG) was also recorded using radiolucent carbon fiber electrodes. Nitroglycerin was available for intracoronary administration. Prior to ergonovine tests blood sampling and coronary angiography were performed. Ergonovine was administered intravenously as bolus injections of 0.1 mg every 3 minutes until induction of typical angina or up to a maximal total dose of 0.4 mg. Unless ECG change (S-T segment elevation) and symptom (anginal pain) were positive 3 minutes after the administration, next dose of ergonovine was administered. When either anginal pain or S-T segment elevation of greater than 1 mm compared with control was recognized within 3 minutes after ergonovine, coronary angiography was taken at a dose of ergonovine. If coronary angiography revealed total or subtotal vasospastic obstruction in proximal portions of coronary arteries, the ergonovine test was terminated in positive and an intravenous bolus dose of 0.1–0.2 mg of nitroglycerin was administered as soon as possible. The test was also terminated when maximal dose of ergonovine (0.4 mg) did not cause an ECG change and symptom. A negative test result was defined as a test without S-T segment elevation and vasospastic coronary obstruction at the maximal ergonovine dose of 0.4 mg.

To examine the effect of a thromboxane synthetase inhibitor: (E)-3-[4-(3-pyridylmethyl) phenyl]-2-methyl-2-propenoic acid sodium salt (OKY-1581)12 on susceptibility of coronary vessels to ergonovine, 8 cases exhibiting ergonovine-induced vasospasm were given OKY-1581 (8 mg/kg) one hour after the first ergonovine
Thromboxane A₂ in Variant Angina

Fig. 1. Coronary sinus TXB₂ levels in patients with chest pain syndrome (CPS) and variant angina (VA). Eleven patients with normal coronary arteries with CPS (open circles) served as controls. All of the patients with VA had angiographically documented coronary spasm during ergonovine provocative test; closed triangles and open triangles represent the cases with had without significant stenosis (≤ 75%) in major coronary arteries. Number at the bottom indicate the mean ± SD (pgTXB₂/ml plasma).

challenge. Fifteen minutes after OKY-1581 administration the second ergonovine test was performed under protocol identical to the first challenge. In all of the eight patients, changes in arterial pressure were insignificant after injection of OKY-1581.

In 3 patients whose anginal symptoms became quiescent 6 to 8 months after the first test, a second ergonovine test was also performed.

Reproducibility of ergonovine provocative test

To determine whether a dose of ergonovine could reproduce an anginal episode individually, 14 out of 34 patients with variant angina were rechallenged by ergonovine under the same protocol as described above. The second ergonovine test was performed 75 minutes (3 cases) and approximately 24 hours (11 cases) after the first test. The former 3 cases were rechallenged by ergonovine during catheterization under the same fashion as OKY-1581 study. In the latter 11 cases, ergonovine was injected into the antecubital vein under the monitoring of symptom and electrocardiogram in the cardiac catheterization laboratory.

Radioimmunoassay for TXB₂

Plasma TXB₂ levels were measured by radioimmunoassay as previously described. The sensitivity of the assay was 10 pg per plasma sample (0.25 ml); no cross-reaction was seen with other prostaglandins. In our hand, this method had a coefficient of variation of 10% for duplicate determination.

Statistic analyses

The unpaired Student's t test was employed for statistical analyses in comparing patients with and without coronary artery stenosis of greater than 75%. Because of unequal variances among groups, TXB₂ levels in patients with variant angina and chest pain syndrome were analyzed for intergroup differences with a nonparametric
TABLE 1  EFFECTS OF OKY-1581 ON TXB₂ LEVELS IN CORONARY SINUS AND ERGONOVINE TEST IN PATIENTS WITH VARIANT ANGINA

<table>
<thead>
<tr>
<th>Case</th>
<th>Age &amp; Sex</th>
<th>Spastic Vessel</th>
<th>Dose of Ergonovine</th>
<th>TXB₂ Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>After OKY</td>
</tr>
<tr>
<td>1.</td>
<td>F.S. 50M</td>
<td>LAD</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>2.</td>
<td>Y.S. 49F</td>
<td>LAD</td>
<td>0.1</td>
<td>0.4*</td>
</tr>
<tr>
<td>3.</td>
<td>I.T. 43M</td>
<td>LAD</td>
<td>0.1</td>
<td>0.4*</td>
</tr>
<tr>
<td>4.</td>
<td>Y.D. 52M</td>
<td>LAD</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>5.</td>
<td>S.T. 60M</td>
<td>LCX</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>6.</td>
<td>T.O. 57M</td>
<td>RCA</td>
<td>0.2</td>
<td>0.4*</td>
</tr>
<tr>
<td>7.</td>
<td>K.H. 57M</td>
<td>RCA</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>8.</td>
<td>R.N. 59M</td>
<td>LAD</td>
<td>0.3</td>
<td>0.4*</td>
</tr>
</tbody>
</table>

\[ P \text{ value} \]

\[ N.S.^{**} \]

\[ < 0.05^{**} \]

*: Spasm was not provoked by 0.4 mg ergonovine.

**: Calculated by paired t test comparing control with absolute change in before or after OKY administration.

Abbreviation: LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; RCA = right coronary artery; TXB₂ = thromboxane B₂

Analysis (the Welch procedure). In the dose response protocol, linear regression using the method of least squares was performed on the TXB₂ levels in coronary sinus vs the reciprocal of ergonovine dose. Data were expressed as mean ± standard deviation.

RESULTS

Ergonovine test results

In all 34 patients with variant angina, the intravenous administration of ergonovine doses ranging between 0.1 and 0.4 mg, induced total or subtotal obstruction in the proximal portion of any coronary branches. In 11 cases with atypical chest pain syndrome who served as control subjects, no coronary spasm was provoked by the maximum dose of ergonovine (0.4 mg). When the second ergonovine test was performed on 3 patients with variant angina 75 minutes after the first test, vasoconstrictive attacks were reproduced by the same dose of ergonovine (0.1 mg) as the first test. Also in 11 cases who were rechallenged by ergonovine 24 hours after the first test, the ergonovine threshold at which the attack occurred was almost identical; in 8 cases the second test was positive at the same ergonovine dose as the first test and in the other 3 cases it was positive at an adjacent dose level.

TXB₂ levels in coronary sinus

TXB₂ levels in coronary sinus were measured in 29 patients with variant angina and in 11 patients with chest pain syndrome. Most of the patients with variant angina were associated with markedly increased TXB₂ levels (424 ± 138 pg/ml) in coronary sinus during nonanginal period; this was in sharp contrast to those with the chest pain syndrome whose TXB₂ levels (223 ± 38 pg/ml) were found to be within normal limit. These TXB₂ levels did not alter after the control angiography. The extent of atherosclerosis in variant angina did not appear to affect the TXB₂ levels. There was no virtual difference in TXB₂ levels between the two groups with and without significant stenosis (Fig. 1), whose TXB₂ levels were 447 ± 137 pg/ml and 384 ± 124 pg/ml, respectively.

Since the elevated TXA₂ may serve to precipitate coronary vasospasm, we attempted to relate coronary sinus levels of TXB₂ to the susceptibility of coronary vessels to ergonovine in

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Fig. 3. Representative cine-frames of the left coronary artery in the right anterior oblique projection from 49 year old female patient with variant angina. The control arteriogram (A) demonstrated 50% stenosis in the left anterior descending artery (LAD). After ergonovine (0.1 mg), complete vasospastic obstruction was provoked in LAD (B). After the treatment of OKY-1581 (8 mg/kg i.v.), vasospastic obstruction in LAD could not be provoked at the maximal dose of ergonovine (0.4 mg) (C).

Fig. 4. Left coronary arteriogram (right anterior oblique projection) in 53 year old male patient with variant angina. Left anterior descending artery (LAD) showed a 75% organically stenotic lesion in the proximal portion before the ergonovine test (A). Localized subtotal occlusion occurred in the stenotic site during anginal attack induced by ergonovine (0.1 mg) (B). At the symptomatically quiescent stage 8 months after the first challenge, 0.4 mg ergonovine could not provoke coronary spasm (C).

Patients with variant angina. When the cumulative amounts of ergonovine to induce vasospasm were compared with coronary sinus TXB₂ levels prior to the test, we found that TXB₂ levels are inversely correlated with the cumulative amounts of ergonovine (Fig. 2). Higher TXB₂ levels were associated with lower dose of ergonovine, whereas lower TXB₂ levels were associated with higher dose of ergonovine. When mean TXB₂ levels were plotted against the reciprocal of ergonovine dose, such an inverse relationship was more clearly indicated (r = 0.63, p < 0.001, Fig. 2 inset).

Effects of TXA₂ synthetase inhibitor (OKY-1581) on ergonovine test

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In eight patients with variant angina the ergonovine tests were performed before and after administration of OKY-1581 (Table I). Intravenous infusion of OKY-1581 (8 mg/kg) to these patients resulted in significant reduction of coronary sinus TXB₂ levels. This was accompanied by reduced susceptibility of coronary vessels to ergonovine, in that 7 out of 8 cases required higher dose of ergonovine to induce spasm after administration of OKY-1581. Four cases exhibited no coronary spasm at the maximal ergonovine dose of 0.4 mg. In one case, OKY-1581 significantly reduced TXB₂ levels, but did not alter the susceptibility to ergonovine. Fig. 3 represents a typical case, whose vasospasticity induced by ergonovine was suppressed by the
TABLE II ALTERATION OF PLASMA THROMBOXANE B\(_2\) LEVELS IN CORONARY SINUS AND ERGONOVINE TEST RESULTS ACCORDING TO THE CHANGE OF DISEASE ACTIVITY IN PATIENTS WITH VARIANT ANGINA

<table>
<thead>
<tr>
<th>Case</th>
<th>Age &amp; Sex</th>
<th>Spastic Vessel</th>
<th>Ergonovine Test</th>
<th>Follow-up period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dose of Ergonovine</td>
<td>TXB(_2) Level</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>1. N.S.</td>
<td>57M</td>
<td>RCA</td>
<td>0.1</td>
<td>0.4*</td>
</tr>
<tr>
<td>2. K.K.</td>
<td>53M</td>
<td>LAD</td>
<td>0.1</td>
<td>0.4*</td>
</tr>
<tr>
<td>3. K.I.</td>
<td>53M</td>
<td>LAD</td>
<td>0.1</td>
<td>0.4*</td>
</tr>
</tbody>
</table>

\(p\) value: N.S.**

*: Spasm was not provoked by 0.4 mg ergonovine.
**: Calculated by paired t test comparing 1st ergonovine test with absolute change in 2nd ergonovine test.

Abbreviations: LAD = left anterior descending coronary artery; RCA = right coronary artery; TXB\(_2\) = thromboxane B\(_2\).

administration of OKY-1581. In this patient, with 50% stenosis in left anterior descending coronary artery (LAD) (Fig. 3A), ergonovine challenge (0.1 mg) elicited chest pain with S-T segment elevation (leads V4-6) and vasospastic occlusion of LAD at the stenotic site (Fig. 3B). Chest pain and electrocardiographic abnormalities were abolished promptly by the intravenous nitroglycerin in a dose of 0.1 mg. OKY-1581 was administered sixty minutes after the ergonovine test. Fifteen minutes after OKY-1581, the second ergonovine challenge was performed. Ergonovine at the maximal dose of 0.4 mg failed to evoke chest pain, with no electrocardiographic change, nor was focal coronary spasm demonstrable in LAD (Fig. 3D).

Plasma TXB\(_2\) levels of variant angina in quiescent stage

All of 34 patients with variant angina responded well to the calcium antagonists such as nifedipine and diltiazem. Five of these were asymptomatic during 3 months of therapy; they had no anginal attack after the termination of calcium antagonist. In 2 out of these 5 patients the ergonovine test was repeated during the quiescent stage 6 to 8 months after the prior test. All had normal TXB\(_2\) levels in coronary sinus and exhibited no S-T segment change at the maximal dose of 0.4 mg (Table II). Fig. 4 represents coronary angiography of a typical case (case 2 in Table II) who showed a marked reduction in TXB\(_2\) levels at the quiescent stage. Prior to ergonovine test coronary angiography revealed fixed stenosis (75%: LAD), while left circumflex coronary artery and right coronary artery were almost normal (Fig. 4A). Ergonovine (0.1 mg) induced an episode of anginal pain accompanied by S-T segment elevation in the anterior chest leads with total occlusion of the LAD at the stenotic site (Fig. 4B). At angina-free period taken 8 months after the prior test, coronary spasm was not induced by the maximal dose of ergonovine (0.4 mg) (Fig. 4C).

DISCUSSION

The present study demonstrated that patients with variant angina exhibited markedly augmented TXB\(_2\) levels in coronary sinus. The vasospasticity of coronary arteries, defined by the effectual amount of ergonovine to induce spasm, were inversely correlated with the TXB\(_2\) levels. We also found that the reduction of TXB\(_2\) levels by a thromboxane synthetase inhibitor was effective in attenuating the spasticity of coronary arteries induced by ergonovine.

Waters et al.\(^{11}\) reported that the symptom activity during the active state of variant angina was inversely correlated with the ergonovine dose to provoke coronary spasm. We found that under these conditions augmented TXB\(_2\) levels in the coronary sinus were intimately associated with the increased susceptibility of the coronary arteries to ergonovine. These results are indicative of the possibility that increased TXA\(_2\) production plays an important role in determining symptom activity of variant angina.

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It is important to understand the mechanism relating increased vasosasticity to the augmentation of TXA2 production. The present study demonstrated that these two parameters are strongly interrelated when a TXA2 synthetase inhibitor was applied (Table I) and when long-term natural courses were followed (Table II). These findings suggest that the augmentation of TXA2 production may contribute, to a large extent, to the increase of vascular spasticity. However, these results do not always provide information as to the causal relationship in the occurrence of vasospasm. For instance, it is not clear whether TXA2 serves to trigger vasospasm or it only serves to determine the basic tone of the vessel with other factors functioning to trigger spasm.13–15

Vasospasticity of variant angina was found in this study to be attenuated by the use of a TXA2 synthetase inhibitor, OKY-1581, with doses that are enough to suppress thromboxane formation of platelets.16 In fact, the augmented TXB2 levels in variant angina reduced significantly after OKY-1581 was administered (Table I). The reduction of TXA2 synthesis is capable of suppressing the contractile response of coronary arteries, suggesting the possible role of TXA2 for determining coronary arterial reactivity.

It must be considered that the baseline elevation in TXB2 levels may have been a result of ischemic events because the frequency of spasm in patients with active variant angina is greater than those with anginal pain.17,18 Robertson et al.16 showed that TXB2 levels increased in the coronary sinus after coronary spasm occurred, suggesting the possibility that the rise in TXB2 may represent a result of spasm, rather than a cause. Thus, the elevation in TXB2 levels (Fig. 2) may reflect the frequent ischemic events of patients, even if these levels should underlie the condition where spasm is evoked by ergonovine. In this sense, it is difficult to define exactly the causal relation between TXA2 production and the occurrence of spasm owing to the methodological limitations of these studies. At quiescent stage of variant angina where coronary arteries are less sensitive to ergonovine, TXB2 levels in the coronary sinus were significantly reduced (Table II). These findings may indicate that the fluctuation of coronary artery responsiveness could be assessed by determining the productivity of TXA2 in coronary circulation.

Yui et al.19 reported that the oral administr-

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