Augmented Platelet Reactivity and Thromboxane A₂ Production
Possible Aggravating Factors in Unstable Angina

MICHIHIKO TADA, M.D., SHIRO HOSHIDA, M.D.
AND TSUNEHIKO KUZUYA, M.D.

We examined platelet aggregation and plasma levels of thromboxane B₂, a stable metabolite of thromboxane A₂, in patients with unstable angina and correlated these platelet indices with the response to antianginal conventional therapy such as isosorbide dinitrate and calcium channel blocker. Eight of 36 patients exhibited anginal attacks more than 5 times/week in spite of the therapy, designated refractory unstable angina, associated with augmented platelet aggregation induced by arachidonate (0.3 mM, 71 ± 3%, mean ± SEM) and collagen (2 µg/ml, 72 ± 5%), and elevated plasma levels of thromboxane B₂ (350 ± 19 pg/ml). In the remainder of the patients whose anginal attacks were effectively subsided by the therapy, platelet aggregation was much lower (arachidonate: 34 ± 9%, collagen: 31 ± 8%, p < 0.01) and plasma levels of thromboxane B₂ were also lower (295 ± 12 pg/ml, p < 0.05).

To evaluate the effect of selective thromboxane A₂ blockade on clinical findings and platelet reactivity in refractory unstable angina, OKY-046 (600 mg/day, p.o.) was administered to another 14 patients with refractory unstable angina in addition to the conventional therapy. We found that platelet aggregation induced by arachidonate (71 ± 4%) and collagen (65 ± 8%) was markedly reduced (44 ± 7% and 24 ± 3%, respectively, p < 0.01) and plasma levels of thromboxane B₂ (358 ± 31 pg/ml) and thromboxane B₂ production in serum (29 ± 5 ng/ml) were also significantly reduced after OKY-046 treatment (262 ± 21 pg/ml, p < 0.05, and 1.4 ± 0.2 ng/ml, p < 0.001). In accordance with these findings, frequency of anginal attacks (8.4 ± 1.1 times/week) was markedly decreased to less than one half (3.6 ± 1.1 times/week, p < 0.01). Under these conditions, during the first month of admission occurrence of myocardial infarction in refractory unstable angina treated with OKY-046 was effectively decreased (7%) compared with that in controls (50%, p < 0.05). These results suggest that augmented platelet reactivity and thromboxane A₂ production by platelets play a key role in the pathogenesis of refractory unstable angina.

Key Words:
Unstable angina
Platelet aggregation
Thromboxane A₂
Selective thromboxane A₂ blockade

EXPERIMENTAL studies have shown that platelet aggregates may form in a partially constricted coronary artery, leading to acute luminal occlusion and myocardial ischemia.1–3 The pathophysiological importance of platelet dysfunction in patients with unstable angina was

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also suggested in a large, randomised, clinical trial of an antiplatelet agent. In fact, there are some patients with unstable angina whose anginal attacks can not be stabilized by treatment with coronary vasodilating agents and agents that decrease myocardial oxygen demand. These findings support the concept that coronary circulatory disorders resulting from the formation of platelet aggregates due to augmented platelet reactivity might play an important role as an aggravating factor in the pathogenesis of unstable angina.

The intervention between platelets and coronary vascular endothelium is thought to depend on the balance between the production of proaggregatory thromboxane A2 by platelets and antiaggregatory prostacyclin by endothelium. Since nonsteroidal anti-inflammatory drugs block prostaglandin synthesis nonselectively, resulting in blockage of both thromboxane A2 and prostacyclin syntheses, it is unclear whether these agents truly exert a beneficial influence in coronary circulatory disorders such as unstable angina.

In the present study, we examined the correlation between platelet function and the response to antianginal conventional therapy such as isosorbide dinitrate and calcium channel blockers in patients with unstable angina. Attempts were also made to assess the effects of selective thromboxane A2 blockade, OKY-046, on platelet function and other clinical data in patients with refractory unstable angina.

### MATERIALS AND METHODS

**Patients and Treatment Protocol**

Fifty patients (mean age 60.1 ± 1.3 years; 32 men and 18 women) admitted to our hospital with the clinical diagnosis of unstable angina participated in this study. All patients exhibited transient ST segment or T wave changes during an episode of chest pain. Isosorbide dinitrate and calcium channel blockers (nifedipine or diltiazem) were prescribed in doses as tolerated. In 7 patients, β-adrenergic receptor blockers which had been used before admission were continued. Frequency of anginal attacks was monitored for 1 or 2 weeks under this conventional therapy and patients who continued to experience attacks more than 5 times/week in spite of the therapy were judged to have refractory unstable angina. To define the characteristics of clinical findings and platelet reactivity in these refractory patients, 36 patients (mean age 59.1 ± 1.5 years; 24 men and 12 women) with unstable angina were entered into our first study. To assess the effect of selective thromboxane A2 blockade treatment in unstable angina, (E)-3-[4-(1-imidazolylmethyl) phenyl]-2-propenoic acid hydrochloride (OKY-046, 600 mg/day, p.o.) was applied to another consecutive 14 patients (mean age 62.6 ± 2.4 years, 8 men and 6 women) with unstable angina of refractory type in addition to the conventional therapy used in our second study. We focused on the differences in clinical findings and platelet reactivity before

### TABLE 1 CLINICAL CHARACTERISTICS OF REFRACTORINESS FOR CONVENTIONAL THERAPY IN UNSTABLE ANGINA

<table>
<thead>
<tr>
<th></th>
<th>Effective (n = 28)</th>
<th>Refractory (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years, mean ± SEM)</strong></td>
<td>58.6 ± 1.8</td>
<td>60.8 ± 2.8</td>
</tr>
<tr>
<td><strong>Male/female</strong></td>
<td>20/8</td>
<td>4/4</td>
</tr>
<tr>
<td><strong>Disease history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior myocardial infarction*</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Angina on effort*</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td><strong>Clinical type of unstable angina</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New onset</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Worsening</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td><strong>Coronary arteriography findings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of diseased vessels showing ≥ 75% narrowing (mean ± SEM/{cases})</td>
<td>1.0 ± 0.2{23}</td>
<td>2.2 ± 0.3{8}</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01

Patients (n = 28) who experienced anginal attacks less than 5 times/week by the conventional therapy were designated as the "effective" type, while the patients (n = 8) whose anginal attacks occurred more than 5 times/week regardless of the conventional therapy were designated as the "refractory" type.
TABLE II  DIFFERENCES IN PLATELET REACTIVITY BETWEEN PATIENTS OF THE EFFECTIVE TYPE AND THE REFRACTORY TYPE

<table>
<thead>
<tr>
<th></th>
<th>Effective (n = 28)</th>
<th>Refractory (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet aggregation (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arachidonate (0.3 mM)*</td>
<td>34 ± 9</td>
<td>71 ± 3</td>
</tr>
<tr>
<td>Collagen (2 μg/ml)*</td>
<td>31 ± 8</td>
<td>72 ± 5</td>
</tr>
<tr>
<td>Plasma thromboxane B₂ level (pg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-anginal period**</td>
<td>295 ± 12</td>
<td>350 ± 19</td>
</tr>
<tr>
<td>After attack**</td>
<td>310 ± 35</td>
<td>414 ± 34</td>
</tr>
<tr>
<td>Thromboxane B₂ production in serum (ng/ml)</td>
<td>33 ± 4</td>
<td>28 ± 5</td>
</tr>
</tbody>
</table>

\[ \text{mean} \pm \text{SEM} \]
\[ * p < 0.01, \quad ** p < 0.05 \]

and after thromboxane A₂ blockade treatment in patients with refractory unstable angina. Patients of refractory type in the first study served as controls.

Excluded from the study were those patients who had received anti-platelet therapy such as aspirin and sulfinpyrazone for at least 2 weeks prior to the study.

Platelet Aggregation Studies

Peripheral blood was collected into one tenth volume of 3.8% sodium citrate. Platelet rich plasma (PRP) was prepared by centrifugation at 600 rpm for 10 min at room temperature. The upper turbid layer of PRP was transferred to polypropylene tube and residual blood was centrifuged at 3000 rpm for 15 min to obtain platelet poor plasma (PPP). The platelet count in the PRP was determined using a Coulter Counter and the PRP was diluted to a count of 2.5 × 10⁵/µl using autologous PPP and was used within 2 hours of preparation. Platelet aggregation in PRP was monitored in a model RAM Multi-channel Platelet Aggregometer (Rikadenki) with stirring at 1,100 rpm using the method of Born. Platelet aggregation was determined at 5 min after the addition of aggregating agents, arachidonate and collagen (0.3 mM and 2 μg/ml in final concentrations, respectively). Arachidonate (sodium salt) was obtained from the Sigma Chemical Company and collagen was purchased from Hormon-Chemie.

Determination of Plasma Thromboxane B₂ Levels

Five ml peripheral venous blood was gently drawn to avoid trauma to platelets or blood vessels into a heparinized plastic syringe and transferred into polypropylene tubes containing 1 mM ethylendiamine tetracetic acid and 0.1 mM indomethacin (in final concentrations). Blood was withdrawn during non-anginal period, more than 4 hours after subsistence of the last anginal attack, and immediately after anginal attack, within 1 hour of the appearance of chest symptoms. Plasma was separated by centrifugation at 3000 rpm for 20 minutes, kept frozen at −20°C, and subjected to determination of thromboxane B₂ by radioimmunoassay, employing the previously described method!! Plasma thromboxane B₂ levels were expressed as pg/ml of plasma.

Determination of Serum Thromboxane B₂ Production

Five ml peripheral venous blood was drawn during non-anginal period into a glass syringe and transferred into glass tubes. Thromboxane B₂ production in serum was determined by leaving the serum at room temperature for 1 hour and was measured by radioimmunoassay. Serum thromboxane B₂ production was expressed as ng/ml of serum.

Coronary Arteriographic Findings

In the convalescent phase, most of the patients (44 cases) underwent selective coronary arteriography using the Judkins technique to examine the number of diseased coronary arteries. A stenosis reducing the luminal diameter by 75% or more was considered significant.

Analysis of Findings

Chi-square and unpaired t-tests were used in the statistical analysis of the data. Data are expressed as mean ± SEM.

RESULTS

Clinical Characteristics of Refractoriness to the Conventional Therapy in Unstable Angina

Among 36 patients with unstable angina, 8 patients exhibited anginal attacks more than 5 times/week in spite of the conventional therapy. There was no difference in the administered doses of isosorbide dinitrate and calcium channel blockers between these 8 patients (refractory
type) and the other 28 patients (effective type). Two patients in the former and 3 patients in the latter group were given β-adrenergic receptor blockers. Table I shows the differences in the clinical profiles between these two groups. The incidence of prior myocardial infarction and angina on effort in their disease history was more frequent in the patients of refractory type (p<0.05). In these patients arteriographic examination at their convalescent phase showed severe coronary stenotic lesions (p<0.01). On the other hand, there were no significant differences in their clinical subtype of unstable angina (new onset type and worsening type) and in their electrocardiographic changes during anginal attacks (ST-elevation and ST-depression).

Platelet reactivity, as measured by the aggregation in PRP and plasma thromboxane B₂ levels, was found to demarcate the pathological profile of these patient groups (Table II). Platelet aggregation induced by arachidonate (0.3 mM) and collagen (2 µg/ml) in the patients of refractory type was markedly higher, 71 ± 3% and 72 ± 5%, than that in the patients of effective type, 34 ± 9% and 31 ± 8%, respectively (p<0.01). The former patients also exhibited elevated plasma levels of thromboxane B₂ not only during non-anginal period (350 ± 19 pg/ml) but also immediately after anginal attacks (414 ± 34 pg/ml) compared with those in the latter group (295 ± 12 pg/ml and 310 ± 35 pg/ml, respectively, p<0.05).

Effect of Selective Thromboxane A₂ Blockade in Refractory Unstable Angina

Since the patients with unstable angina of refractory type exhibited markedly augmented platelet reactivity, another consecutive 14 patients with unstable angina of refractory type were administered with OKY-046 (600 mg/day, p.o.) in addition to the conventional therapy. Figure I shows a representative case whose anginal attacks were effectively treated and stabilized by the additive OKY-046. In this case, the administration of isosorbide dinitrate (80 mg/day) and nifedipine (40 mg/day) did not attenuate the frequency of anginal attacks, accompanied by marked elevation of thromboxane B₂ levels. The oral administration of OKY-046 produced a precipitous decrease in frequency of attacks with a marked reduction of plasma thromboxane B₂. Table III summarizes the therapeutic effects of OKY-046 on 14 patients with unstable angina of refractory type. Platelet aggregation induced by arachidonate (71 ± 4%) and collagen (65 ± 8%) was markedly
TABLE III  EFFECT OF OKY-046 ON PLATELET FUNCTION AND CLINICAL FINDINGS IN PATIENTS WITH UNSTABLE ANGINA OF REFRACTORY TYPE

<table>
<thead>
<tr>
<th></th>
<th>OKY-treatment</th>
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<tr>
<td></td>
<td>Before</td>
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<tr>
<td>Platelet aggregation (%)</td>
<td></td>
</tr>
<tr>
<td>Arachidonate (0.3 mM)*</td>
<td>71 ± 4</td>
</tr>
<tr>
<td>Collagen (2 μg/ml)*</td>
<td>65 ± 8</td>
</tr>
<tr>
<td>Plasma thromboxane B2 level** (during non-anginal period, pg/ml)</td>
<td>358 ± 31</td>
</tr>
<tr>
<td>Thromboxane B2 production in serum*** (ng/ml)</td>
<td>29 ± 5</td>
</tr>
<tr>
<td>Frequency of anginal attacks* (times/week)</td>
<td>8.4 ± 1.1</td>
</tr>
</tbody>
</table>

*mean ± SEM
*p < 0.01, **p < 0.05, ***p < 0.001

reduced after the administration of OKY-046 (44 ± 7% and 24 ± 3%, respectively, p < 0.01). Plasma levels of thromboxane B2 (358 ± 31 pg/ml) and thromboxane B2 production in serum (29 ± 5 ng/ml) were also significantly reduced after the treatment (262 ± 21 pg/ml, p < 0.05, and 1.4 ± 0.2 ng/ml, p < 0.001, respectively). Under these conditions, frequency of anginal attacks (8.4 ± 1.1 times/week) was effectively decreased to less than one half (3.6 ± 1.1 times/week, p < 0.01). The treated and control patients with refractory unstable angina were not different with respect to age, sex, disease history, coronary arteriographic findings in the convalescent phase and administered doses of isosorbide dinitrate and calcium channel blockers. During the first month follow-up for these refractory patients, myocardial infarction occurred in 5 patients, 4 in the control patients (50%) and 1 in the OKY-046 treated patients (7%, p < 0.05).

DISCUSSION

The present study demonstrated that marked augmentation of platelet aggregation and significant elevation of plasma thromboxane B2 levels were seen in peripheral circulation in patients with refractory unstable angina, and that the reduction of augmented platelet aggregation and elevated thromboxane B2 levels, brought about by the selective thromboxane A2 block-

ad, was associated with a decrease in frequency of anginal attacks and occurrence of myocardial infarction. Of 36 patients with unstable angina, 8 were diagnosed as being refractory type, based on their weak response to isosorbide dinitrate and calcium channel blockers. These patients were associated with augmented platelet aggregation induced by arachidonate and collagen, and elevated plasma thromboxane B2 levels. In the remainder of the patients whose anginal attacks were effectively reduced by the therapy, platelet aggregation was much lower and plasma thromboxane B2 levels were also lower. When another 14 patients with refractory unstable angina were treated with selective thromboxane A2 blockade, OKY-046, platelet aggregation and thromboxane A2 production were markedly reduced, associated with a decrease in frequency of anginal attacks. In accordance with these findings, occurrence of myocardial infarction in OKY-046-treated patients was significantly decreased compared with that in controls. These results suggest that augmented platelet reactivity and thromboxane A2 production by platelets play a key role in the pathogenesis of refractory unstable angina.

Several studies have suggested an important role for platelets and metabolites of arachidonate in the pathogenesis and manifestations of coronary artery disease. Thromboxane A2 generated by platelets is a potent vasoconstrictor and proaggregant prostanoïd, and counteracts the effect of prostacyclin generated by vascular endothelium which is a potent vasodilator and antiaggregant prostanoïd. Thromboxane A2 formation is found to increase in patients with coronary artery disease while atheromatous vessels exhibit a decreased ability to synthesize prostacyclin. The interaction between platelets and vascular endothelium in coronary circulatory disorders is thought to depend on the balance between the production of thromboxane A2 and prostacyclin. Therefore, the observed augmentation of platelet aggregation and thromboxane A2 production could contribute to microthrombus formation at the atheromatous coronary vessel in refractory unstable angina. Recently, receptors for prostacyclin in platelets are found to decrease transiently during the active phase of coronary artery disease. These findings may underlie the augmented platelet reactivity of anginal patients. Selective thromboxane A2 blockade is thought to exert not only inhibition of throm-
boxane A₂ synthesis by platelets but also augmentation of endogenous prostacyclin synthesis by vascular endothelium. Such a mechanism is termed the "prostaglandin H₂ steal" in which prostaglandin H₂ accumulated in platelets is utilized by vascular enzyme to produce prostacyclin. The dual effects of thromboxane A₂ blockade, found in experimental models, may favour the use of this drug in coronary artery disorders. It remains to be examined whether the "prostaglandin H₂ steal" mechanism is demonstrable in the treatment of angina pectoris.

It is not known whether the excessive production of thromboxane A₂ accompanying augmented platelet reactivity could contribute to the occurrence of thrombotic event in acute myocardial infarction. In experimental animals, administration of thromboxane A₂ into the coronary artery caused formation of platelet aggregates in coronary circulation, resulting in the onset of acute myocardial infarction indicating a possible role of platelet dysfunction in the initiation of myocardial infarction. In fact, the present study suggested that selective thromboxane A₂ blockade could effectively suppress the occurrence of myocardial infarction. These findings may stress the role of platelet dysfunction in triggering acute myocardial infarction. Considering the interaction between platelets and vascular endothelium, it should be also noted that acute vascular endothelial damage may be essential in precipitating of acute myocardial infarction.

In our selective thromboxane A₂ blockade treatment of refractory unstable angina, we prescribed OKY-046 to 14 consecutive refractory patients in a single blind fashion in association with conventional therapy because of the high occurrence of myocardial infarction in these patients. A large, double blind, clinical trial of unstable angina would be needed to define the possible clinical importance of this drug in the stabilization of disease activity and occurrence of myocardial infarction.

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REFERENCES

5. HAMBERG J, SVENSSON J, SAMUELSSON B: Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci USA 72: 2994, 1975
15. TADA M, HOSHIDA S, KUZUYA T, INOUE M, MINAMINO T, ABE H: Augmented thromboxane

Japanese Circulation Journal Vol. 50, February 1986
A2 generation and efficacy of its blockade in acute myocardial infarction. Int J Cardiol 8: 301, 1985
16. GRYGLEWSKI RJ, BUNTING S, MONCADA S, FLOWER RJ, VANE JR: Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. Prostaglandins 12: 685, 1976