Circulating Platelet Products in Unstable Angina Pectoris

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SUMMARY In 19 patients with unstable angina pectoris at rest, plasma levels of the platelet-derived proteins β-thromboglobulin and platelet factor 4 were significantly elevated in blood samples obtained during or within 4 hours after episodes of angina, but were usually normal during quiescent intervals. Plasma levels of the arachidonic acid metabolite thromboxane B2 were less clearly related to angina, and there was no association of angina with levels of the coagulation product fibrinopeptide A. This demonstration of an association of platelet activation and secretion with unstable angina pectoris by radioimmunoassay of circulating platelet constituents offers a new approach to assessment of therapy in ischemic heart disease and suggests that agents that alter platelet function should be evaluated in patients with unstable angina.

PATIENTS with ischemic heart disease represent a heterogeneous group in whom a variety of pathogenetic factors may be at work to reduce myocardial perfusion. Increasing evidence suggests that classic views of the pathogenesis of angina pectoris and myocardial infarction are oversimplifications. For example, coronary thrombosis is no longer thought to be the inevitable precursor of myocardial infarction.2 Further, angina pectoris cannot always be attributed solely to reduction of blood flow through coronary arteries with fixed stenosis from atherosclerosis. With refined techniques of selective angiography, vascular spasm has been identified as an important mechanism in variant angina3 and also in effort-induced angina4 and in myocardial infarction.4,6

A critical role has been claimed for products of arachidonic acid metabolism in the control of coronary vasomotor tone. According to this hypothesis, vessel diameter is modulated by a balance between the vasoconstricting effects of thromboxane A2 (TxA2) generated by platelets and the vasodilating action of prostacyclin produced by the vessel wall.7,8 Thromboxane A2 also induces platelet aggregation, whereas prostacyclin is the most potent inhibitor of aggregation yet discovered.8 Turbulent flow, ulceration and hemorrhage into atherosclerotic plaques may promote platelet adhesion, TxA2 generation and release of other vasoactive substances.9 Reduced production of prostacyclin by diseased vessels would also favor vasoconstriction and platelet aggregation.

In an effort to clarify the role of platelets and the plasma coagulation system in myocardial ischemia, we measured blood levels of several products secreted by platelets and of an early product of coagulation in such patients.

Beta-thromboglobulin (βTG) and platelet factor 4 (PF4) are platelet-specific proteins stored in platelet α-granules10 and secreted during the activation of platelets. Their physiologic function is unknown, although PF4 is known to have heparin-neutralizing activity and could influence heparin-dependent coagulation reactions. Elevated plasma levels of these proteins have been found in various thromboembolic states,11-14 after myocardial infarction15 and in association with stable coronary artery disease.16-18

Thromboxane B2 (TxB2) is a relatively stable and inert metabolite of TxA2, which is generated during platelet stimulation and aggregation. Elevated plasma levels of TxB2 have been reported during such pronounced types of platelet stimulation as cardiopulmonary bypass.19

During plasma coagulation, thrombin acts on fibrinogen to cleave two pairs of small polypeptides, fibrinopeptide A (FpA) and fibrinopeptide B (FpB). FpA is the first to be released, and its presence is a sensitive indicator of thrombin activity.20 Elevated levels of this peptide have been reported in patients with venous thromboembolism.21

We sought evidence of platelet activation and plasma coagulation in myocardial ischemia by measuring circulating plasma levels of PF4, βTG, TxB2 and FpA in patients hospitalized for unstable angina pectoris.

Materials and Methods

The patients studied were part of an ongoing prospective trial of different methods of prophylaxis against deep vein thrombosis in patients admitted with a diagnosis of unstable angina. The assessment of these prophylactic methods is still in progress and will be reported elsewhere. The project was approved by...
the Committee on Clinical Investigations, New Procedures and New Forms of Therapy of the Beth Israel Hospital, and all patients gave informed consent. During the investigation, every patient admitted to the hospital who met the following criteria was invited to join the study:

**Symptoms:** New or changing angina pectoris abruptly or inexplicably increasing in frequency or severity or changing in character. There must be an interval of less than 2 weeks between the change in angina and admission to the study.

**ECG:** No evidence of myocardial infarction during the first 24 hours in hospital.

**Transaminases, CPK:** Normal or elevation less than 50% of the normal maximum or less than 50% above the patient's baseline.

**Exclusion criteria** included warfarin treatment, known bleeding disorder or thrombocytopenia, and aggravating causes for angina, such as anemia or arrhythmia.

These criteria were established to define a spectrum of intermediate coronary syndromes, including a group with more accelerated symptoms than those with chronic stable angina, while excluding those with presumed or established myocardial infarction. All patients entered the study within 24 hours of their admission to the hospital. Patients were followed throughout their hospital stay unless they required coronary revascularization or full anticoagulation with heparin for deep vein thrombosis. Patients were assigned according to a table of random numbers to one of three groups: (1) treatment with heparin, 5000 U subcutaneously every 12 hours; (2) treatment with intermittent external pneumatic compression of the calves; (3) control group. Blood samples were drawn daily or more frequently depending on the patient's clinical course. Careful daily observations were made especially regarding the following: (1) timing and character of angina pectoris, (2) medications administered, (3) presence and location of intravenous or intraarterial catheters, and (4) special procedures (e.g., cardiac catheterization, exercise tolerance test). If the venipuncture for blood sampling was traumatic, the sample was discarded. For comparison, samples were also drawn from healthy adult volunteer subjects of either sex who had no history of drug ingestion in the previous 2 weeks.

Collection of blood samples was by fresh venipuncture with a 19-gauge scalp vein needle. The first 1–2 ml of blood were discarded, and the sample was then drawn into a dry polypropylene syringe. The blood was mixed (9:1) in polypropylene tubes containing different anticoagulants for FpA and platelet studies.

The FpA anticoagulant contained sodium heparin (Hyson, Westcott and Dunning, 150 U/mg) 100 mg and Trasylol (FBA Mobay Chemical Corp., 10,000 kIU/ml) 1 ml in 9 ml of barbital-buffered saline (pH 7.4). The platelet anticoagulant contained adenosine (Sigma) 0.01 M, theophylline (Sigma) 0.02 M, disodium EDTA (Fisher) 0.027 M, and indomethacin (Sigma) 0.01 M. Samples were kept on ice and centrifuged within 4 hours at 1700 g for 15 minutes. Plasma for FpA determination was stored at −80°C. Plasma for platelet studies was centrifuged once more at 50,000 g for 10 minutes, and the supernatant was stored at −80°C. All samples were assayed at least in duplicate. Normal controls and previously assayed plasmas of known value were included in each assay of patient samples to ensure quality control. Intraassay variation was less than 3%, and interassay variation was less than 10% in all of the assays. FpA levels were determined by radioimmunoassay using reagents from the IMCO Corporation and the methods of Cronlund et al. We were able to detect as little as 100 pg of FpA per sample. The antibody for the TXB2 radioimmunoassay was supplied by J. B. Smith and the methods of Sors et al. were used. The sensitivity of the assay was 10 pg of TXB2. PF4 was measured by the method of Handin et al. Levels of βTG were determined using the assay kit from the Amersham Co. Both of these assays could measure 0-5 ng/ml of plasma.

Statistical analysis was performed on the PROPHET System of the Chemical/Biological Information Handling Program, NIH. Normality of data was tested by Shapiro and Wilk's W statistic. Analysis of differences was by t test for normally distributed data and by the Wilcoxon paired-sample test for data that were not distributed normally.

### Results

**Patient Profile**

Nineteen patients were studied, five females and 14 males, ages 32–83 years (mean 61.2 years). Ten additional patients were excluded from the study because they developed evidence of myocardial infarction within 24 hours after admission or their symptoms were not cardiac in origin. Seven of the 19 patients studied had a history of myocardial infarction. Three had new onset of angina; the rest had had previously stable angina for many years. One patient had taken 600 mg of aspirin the day before admission. All patients received a medical regimen of propranolol, long- and short-acting nitrates, sedation and bedrest. No other platelet-active drugs were taken by the patients before or during the study. All patients survived hospitalization.

Eight patients, including the one who had aspirin the day before admission, responded promptly to the standard program. Many of these had no angina after admission; some had a rare isolated episode. Three patients showed electrocardiographic and enzyme changes diagnostic of myocardial infarction within 2–5 days of admission; thereafter, they had an uncomplicated and angina-free recovery. Two patients developed evidence of myocardial infarction but continued to have a stormy course, with frequent bouts of angina at rest until they eventually stabilized. No patient had a transmural infarction by classic ECG criteria; myocardial infarctions were evidenced by persistent ST-segment and T-wave abnormalities and elevation of CPK and its MB isomer. Six had an unstable course throughout hospitalization, with many episodes of severe angina. Four eventually stabilized
and two underwent direct myocardial revascularization by aortocoronary bypass grafting.

Correlation of $\beta$TG and PF4 Values

Simultaneous measurements of PF4 and $\beta$TG were made in 117 blood samples from the 19 patients. The correlation coefficient for these pairs was $r = 0.433$, suggesting a moderately positive correlation between the two tests. There was no correlation between either of these two measurements and levels of $\text{TxB}_2$ or $\text{FpA}$.

Angina During Sampling

In analyzing the relationship of the blood measurements to the occurrence of angina pectoris, any samples drawn in proximity to some other noteworthy clinical event such as pulmonary embolism or cardiac catheterization were omitted. One hundred seven samples remained for analysis, 25 of which were drawn during or within 4 hours after an episode of angina at rest. The levels of the platelet proteins $\beta$TG and PF4 in samples drawn in association with angina pectoris were significantly higher than in the samples obtained in a quiescent period (table 1). Samples drawn at a time remote from original episodes were not different from values in healthy normal subjects. $\text{TxB}_2$ and $\text{FpA}$ levels were not correlated with anginal symptoms; mean levels of both were above normal values irrespective of the presence or absence of symptoms. To clarify these associations we analyzed the levels measured during specific clinical situations. In 11 patients, the time of sampling made it possible to compare by paired analysis blood drawn in association with angina and blood from the same patient during quiescent periods. In general, these patients had isolated episodes of angina less than 4 hours before blood sampling and were free of ischemic symptoms the previous or following day. Figure 1 illustrates a pattern typical of such patients. The mean values for each patient with and without angina are presented graphically in figure 2. The rise of $\beta$TG and PF4 values in association with angina was highly significant ($p < 0.01$). On the average, $\text{TxB}_2$ levels did not change, although two patients showed elevations to 0.47 and 0.50 ng/ml in association with angina. $\text{FpA}$ levels were unrelated to angina or its absence, being mildly elevated in both circumstances.

Blood was also obtained once from five patients while they were having protracted angina at rest. The levels of $\beta$TG in these patients were higher than normal (38.4 ± 22.1 ng/ml [sd] vs 23.4 ± 13.0 ng/ml, 0.05 < $p$ < 0.1), and higher in samples taken a day earlier or later when the same patients were asymptomatic (16.0 ± 10.9 ng/ml, $p < 0.02$). PF4 levels were likewise higher than normal during angina (21.4 ± 13.3 ng/ml vs 1.8 ± 4.4 ng/ml, $p < 0.001$) and higher than the same patient's own values during quiescent periods (5.9 ± 6.7 ng/ml, 0.05 < $p$ < 0.1). $\text{TxB}_2$ measurements revealed the same trend (angina: 0.473 ± 0.29 ng/ml vs baseline: 0.295 ± 0.083

<table>
<thead>
<tr>
<th>TABLE 1. Blood Tests in Patients with Unstable Angina*</th>
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<tr>
<td>Sampling of blood</td>
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*Includes all blood samples collected, except those in association with cardiac catheterization, pulmonary embolism, deep vein thrombosis, or intraaortic balloon pumping.

All values are in ng/ml (± sd).

‡$p < 0.05$ vs normal.

§$p < 0.01$ vs normal.

$\text{Abbreviations: } \beta$TG = $\beta$-thromboglobulin; PF4 = platelet factor 4; $\text{TxB}_2$ = thromboxane B$_2$; $\text{FpA}$ = fibrinopeptide A.
ng/ml), although neither the elevation nor the change was statistically significant. There was no change at all in measured FpA levels during and after angina (18.0 ± 16.9 ng/ml vs 13.5 ± 17.2 ng/ml).

Patients with a benign course tended to have normal levels of βTG and PF4, except during their rare episodes of angina. Their average values, excluding the anginal episodes, were not significantly different from the normal range (21.1 ± 19.7 ng/ml for βTG, 6.8 ± 6.6 ng/ml for PF4). On the other hand, some patients had such frequent angina that few samples could be obtained when the patient was pain-free. Figure 3 illustrates such a case. Early in his hospital course he had isolated elevations of βTG and PF4 associated with angina at rest (day 3). From the fifth to the eighth hospital day his pattern of angina accelerated, and was matched by a progressive increase in the plasma concentrations of platelet proteins. Like other such patients, he showed elevations of TxB₂ up to 0.5 ng/ml. FpA levels did not change.

Patients with myocardial infarction or more severe anginal symptoms had frequent elevation of plasma levels of platelet proteins and higher and more frequent peaks in their levels of TxB₂, although their mean levels of TxB₂ were not different from those of the other patients. Figure 4 illustrates the course of a patient who suffered a myocardial infarction on his second hospital day.

**Analysis by Treatment Group**

There were no obvious differences in frequency of angina in the three treatment groups. The mean FpA value of patients treated with low-dose heparin was no
different from that of the control group or of patients treated with intermittent compression of the calves. Mean βTG levels were the same in the three treatment groups. PF4 levels were highest in the control group (16.3 ± 13.1 ng/ml), lower in the heparin group (11.27 ± 9.0 ng/ml), and lowest in the calf compression group (7.32 ± 4.9 ng/ml), but these differences were not statistically significant.

Venous Thromboembolism

Two patients suffered venous thromboembolism. One patient sustained pulmonary embolus diagnosed by ventilation-perfusion lung scan. Plasma levels of all four substances measured within 24 hours of the onset of symptoms were abnormally elevated: PF4, 74 ng/ml; βTG, 88 ng/ml; TxB2, 0.45 ng/ml; FpA, 21.4 ng/ml. During heparin therapy, all values decreased toward normal. Another patient developed deep venous thrombosis in the thigh, detected by impedance plethysmography. FpA levels were high (18.8 ng/ml), as were levels of βTG (36 ng/ml) and TxB2 (0.4–0.47 ng/ml). When the patient was treated with heparin, the FpA level decreased to within the normal range, but βTG and TxB2 remained elevated as long as angina persisted.

Discussion

This study has shown a strong association between platelet activation and secretion and myocardial ischemia. Plasma levels of platelet-specific proteins increased and decreased in direct relation to episodes of angina pectoris. These patients had preinfarction or unstable angina, and all their attacks occurred spontaneously while at rest in the hospital. Their elevations of βTG and PF4 must reflect platelet activation, as there are no other known sources of these proteins. In the less severely symptomatic patients, these increases were well correlated with symptoms and contrasted with essentially normal levels during asymptomatic periods. These manifestations of platelet activity demonstrated significant differences between patients whose symptoms subsided quickly and those who had persistent angina at rest. After myocardial infarction we found elevated levels of thromboxane B2, βTG, and PF4 in asymptomatic patients. These results were obtained even though platelet reactivity may have been blunted by propranolol and nitrates, mild inhibitors of platelet function that were administered to all patients.

The fact that FpA levels were unchanged in these clinical situations, with the exception of two patients with venous thromboembolism, suggests that thrombin generation was not responsible for the release of platelet constituents, as 100-fold less thrombin is necessary for FpA generation than for platelet secretion of βTG or PF4.26 Even the small amounts of thrombin generated by an indwelling intravenous catheter will raise the level of FpA above the normal range,27 and the mild persistent elevation of FpA in our patients can probably be attributed to this cause. Nonetheless, the mean FpA levels in 13 of our patients were in a narrow range of 4–9 ng/ml, which is well below the levels reported in venous thrombosis.21

The elevations of TxB2 did not correspond as closely to episodes of angina as did the platelet proteins. There may be several reasons for this. Recently, Maclouf et al.28 suggested that some TxA2 may be covalently bound to albumin, thus escaping assay as free TxB2. Although the concentration of TxA2 could be high in the coronary vessels, dilution in the circulating blood volume may reduce its level to less than the sensitivity of the assay. The half life of TxB1 (10–15 minutes) is probably shorter than those of PF4 and βTG, which are reported to range from 20 minutes to several hours.12,29 Difference in biologic half lives may also account for the relatively weak correlation between levels of PF4 and TxB1, which are thought to be secreted together by platelets.26 Nonetheless, we found elevations of these substances in patients after myocardial infarction and pulmonary embolism and during unstable angina.

None of these patients had variant angina, a group of patients in whom Lewy et al.30 noted elevations of TxB1. Kuzuya and colleagues31 have also shown levels of TxB1 to be higher in the coronary sinus than in the aorta of patients with variant angina as well as in effort-induced angina pectoris.

The experimental model devised by Folts and associates32,33 is perhaps a closer approximation for
most patients with coronary artery disease. Extrinsic narrowing of canine coronary arteries produced periodic decreases in blood flow due to reversible obstruction with platelet aggregates. Epinephrine infusion enhanced this effect; aspirin and ibuprofen blocked it. Not all dogs exhibited the phenomenon, but those that did had platelets more sensitive to ADP. Atheromatous plaques may behave similarly and may also reduce the amount of prostacyclin generated locally by the vessel wall, further upsetting the balance in favor of platelet aggregation and vasoconstriction. A variety of related factors may be operative: simple obstruction of flow by atheromatous plaques, disturbed flow beyond stenoses producing platelet aggregation, excess TxA2 generation unchecked by endothelial prostacyclin, resultant vasospasm, and hyperaggregable platelets in some patients.

Evidence from clinical studies links platelets to ischemic heart disease, although some of the data are conflicting. Mehta and Mehta34 noted an increase in circulating platelet microthrombi after myocardial infarction. Gjesdal36 found a similar trend in patients with severe angina pectoris, although Guyton and Willerson38 did not find increased microaggregate formation in another group with unstable angina. Salby and Dugdale37 observed increased platelet responsiveness to collagen in stable ischemic heart disease. Green et al.38 observed transient elevation of plasma PF4 in association with exercise-induced myocardial ischemia.

In patients with stable coronary artery disease, it has been suggested that platelets are sequestered or destroyed while traversing the atherosclerotic coronary vasculature.39,40 Studies by Steele et al.41 of platelet survival in such patients showed a less striking consumption of platelets. Aspirin, dipyridamole and sulfinpyrazone were found to improve the shortened platelet survival in coronary disease.42,43 Although aspirin in high doses (2.4 g/day) failed to improve exercise tolerance in patients with effort-induced angina.44 Indomethacin failed to improve the symptoms of variant angina.45 It is possible that high doses of these inhibitors of prostaglandin synthesis and thromboxane production blocked prostacyclin formation as well.

Radioimmunoassay of circulating platelet-derived substances offers a promising new method for investigation of the pathogenesis and treatment of ischemic heart disease. Even though no causal relationship was proved, the results suggest that inhibitors of prostaglandin synthesis (such as aspirin) might be worth assessment in patients with unstable angina pectoris, perhaps curtailing a vicious cycle of platelet stimulation, aggregation, embolization and coronary spasm.

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