Fibrinopeptide A, Platelet Factor 4, and β-Thromboglobulin Levels in Coronary Heart Disease


In vivo platelet alpha-granule release and fibrin I formation were measured in 82 patients with ischemic heart disease by radioimmunoassay of platelet factor 4, β-thromboglobulin, and fibrinopeptide A. The presence and extent of coronary artery disease were determined by coronary arteriography, and the extent of left ventricular regional dysfunction was assessed by contrast left ventriculography. In patients with abnormal coronary arteriograms without previous myocardial infarction, mean levels of platelet factor 4, β-thromboglobulin, and fibrinopeptide A were not elevated. In patients in whom myocardial infarction had occurred more than 6 mo previously, platelet factor 4 (8.3 ng/ml; p < 0.01) and β-thromboglobulin (33.2 ng/ml; p < 0.001) levels were significantly elevated, but fibrinopeptide A levels were normal. Levels of platelet factor 4 and β-thromboglobulin were unrelated to the extent of coronary artery disease. In the patients with prior infarction, β-thromboglobulin correlated directly with extent of left ventricular regional dysfunction (r = 0.53; p < 0.01) and inversely with ejection fraction (r = −0.56; p < 0.05). In a small group of patients with left ventricular aneurysm, mean fibrinopeptide A levels were also elevated. We interpret these findings as indicating that platelet release in patients with ischemic heart disease results from platelet reaction with previously infarcted myocardium rather than with the atherosclerotic coronary arteries.

Recent clinical and experimental evidence indicates that platelet activation and release may play an important role in the pathogenesis of coronary atherosclerosis and in the complications of ischemic heart disease. Other clinical and autopsy evidence implicates fibrin formation in the development of coronary atheroma. Nevertheless, direct evidence for in vivo platelet activation and fibrin formation has been difficult to obtain in patients with coronary artery disease, largely because sensitive techniques for detecting in vivo platelet activation and fibrin formation have not been available.

In the present study, platelet release and fibrin I formation were investigated in patients with coronary artery disease by radioimmunoassay of specific products of these reactions in vivo. Platelet factor 4 (PF4) and β-thromboglobulin (βTG) are specific markers of platelet alpha-granule release. Fibrinopeptide A (FPA) is the first peptide released from fibrinogen by thrombin to produce fibrin I. Fibrinopeptide A levels were normal. Levels of platelet factor 4 and β-thromboglobulin were unrelated to the extent of coronary artery disease. In the patients with prior infarction, β-thromboglobulin correlated directly with extent of left ventricular regional dysfunction (r = 0.53; p < 0.01) and inversely with ejection fraction (r = −0.56; p < 0.05). In a small group of patients with left ventricular aneurysm, mean fibrinopeptide A levels were also elevated. We interpret these findings as indicating that platelet release in patients with ischemic heart disease results from platelet reaction with previously infarcted myocardium rather than with the atherosclerotic coronary arteries.

MATERIALS AND METHODS

Patients undergoing coronary arteriography for evaluation of chest pain were studied if the following conditions were absent: myocardial infarction within 6 mo, valvular or congenital heart disease, idiopathic congestive cardiomyopathy, pulmonary embolism, or thrombophlebitis. Other indications for exclusion were heparin or coumadin therapy, implanted prosthetic devices, including cardiac pacemakers, and indwelling venous or arterial cannulas. Eighty-two patients provided informed written consent for participation in the study, according to a protocol approved by the Committee on Human Investigation, Health Sciences, Columbia University. Sixty-eight men and 14 women, ranging in age from 32 to 75 yr (mean 57 yr) were studied.

The following clinical data were recorded for each patient: coronary risk factors, including systemic hypertension, cigarette smoking, and diabetes mellitus; a history of unstable angina (defined as recurrent precordial pain at rest despite medication); and all medications received during the 2-wk period prior to study. The criteria for previous myocardial infarction were abnormal Q waves on electrocardiography or a typical rise in cardiac enzymes and segmental left ventricular asynergy documented by contrast left ventriculography.

Left ventricular catheterization and coronary arteriography were performed percutaneously from the femoral artery. Coronary arteriograms were considered normal if no focal narrowings were visualized. Coronary artery disease was considered present if a focal narrowing of luminal diameter greater than 70% was present in any coronary artery. The extent of disease was determined from the number of coronary arteries with greater than 70% obstructions. Left ventriculograms were scored for the extent of segmental ventricular dysfunction using a scoring system previously described. A summary score of left ventricular asynergy, normalized for the number of segments scored, was calculated for each patient. Left ventricular ejection fraction was measured from left ventricular volumes determined angiographically.

Left ventricular aneurysm was defined as a discrete sacular protuberance in the left ventricular silhouette, which had either akinetic or dyskinetic regional wall motion. A mural thrombus of...
the left ventricle was diagnosed if the contrast left ventriculogram disclosed a persistent filling defect in the left ventricular cavity during both systole and diastole.20

**Blood Collection and Processing**

Blood samples for FPA, PF4, and βTG measurements were collected within the 24-hr period prior to cardiac catheterization. None of the patients experienced angina during blood collection or during the 30-min period prior to venipuncture. Each sample was drawn from the antecubital vein with a 21-gauge scalp vein needle (Abbott Laboratories) through a Luer-lock adapter into a siliconized 10-mI vacutainer tube containing 1 ml 0.15 M saline with 1400 U heparin, 1000 U of aprotinin (Trasylol from FBA Pharmaceuticals, Inc., New York), 0.01 M adenosine, and 0.02 M theophylline. Samples were placed on ice immediately and centrifuged within 1 hr at 3000 g at 4°C for 20 min. For PF4 and βTG measurements, the supernatant was recentrifuged at 48,000 g for 20 min at 4°C, and the platelet-poor plasma frozen at −60°C. PF4 and βTG levels were measured by radioimmunoassay.20,21

FPA levels were measured by radioimmunoassay on plasma from which fibrinogen had been adsorbed with bentonite.22 Platelets were counted by a model S plus Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.). Creatinine and blood urea nitrogen (BUN) values were determined for each patient.

In 56 patients, fasting blood samples were drawn for measurement of serum cholesterol,23 triglyceride,24 and high density lipoprotein (HDL) cholesterol concentrations, by standardized procedures of the Lipid Research Clinics Program.25

**Statistical Analysis**

FPA, PF4, and βTG levels for each patient group are expressed as geometric means with standard errors of the logarithmically transformed data. The geometric means are the inverse of the means of the log transforms of these variables. Since the measured levels of these factors show a marked skewness toward high values, logarithmic transformation was required to obtain normally distributed variables. The significance of differences among groups for the measured variables was tested by analysis of variance.

**RESULTS**

Patients were separated into three groups: group 1 consisted of 14 patients with normal coronary arteriograms and normal left ventriculograms; group 2 was comprised of 32 patients with coronary artery disease without prior myocardial infarction; and group 3 was comprised of 36 patients with coronary artery disease and previous myocardial infarction. The patient groups did not differ significantly with regard to the

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**Table 1. Mean Values (± SD) for Patient Age, Use of Nitrates, Propranolol and Aspirin, and Serum Creatinine Levels**

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Age</th>
<th>Nitrates Use</th>
<th>Propranolol Use</th>
<th>Aspirin Use</th>
<th>Serum Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>49 ± 9</td>
<td>6/14 (43%)</td>
<td>7/14 (50%)</td>
<td>1/14 (7%)</td>
<td>0.99 ± 0.20</td>
</tr>
<tr>
<td>2</td>
<td>60 ± 8</td>
<td>26/32 (81%)</td>
<td>23/32 (72%)</td>
<td>6/32 (19%)</td>
<td>1.07 ± 0.26</td>
</tr>
<tr>
<td>3</td>
<td>57 ± 9</td>
<td>26/36 (72%)</td>
<td>17/36 (47%)</td>
<td>7/36 (19%)</td>
<td>1.20 ± 0.27†</td>
</tr>
</tbody>
</table>

*Significantly different from groups 2 and 3 (p < 0.05).
†Significantly different from group 1 (p < 0.05).
patients with coronary disease with prior infarction (group 3) compared to group 2. When patients within groups 2 and 3 with comparable degrees of multivessel coronary artery disease were compared, mean PF4 and βTG levels were significantly higher in the group 3 patients. Within the group 3 patients, βTG levels correlated directly with summary scores of the extent of left ventricular regional dysfunction ($r = 0.53$, $p < 0.05$) and inversely with left ventricular ejection fractions ($r = -0.56; p < 0.05$).

Eight of the patients in group 3 had left ventricular aneurysms demonstrated angiographically. Mean βTG levels (45.9 ng/ml) for these patients were significantly higher ($p < 0.05$) than βTG levels in group 3 patients without aneurysms (30.3 ng/ml). Mural thrombi were identified angiographically in three of the patients with aneurysms. FPA levels (mean 3.5 pmole/ml) were elevated for these 3 patients.

In 12 patients in group 2 with a history of angina at rest, mean FPA (0.83 pmole/ml), PF4 (6.1 ng/ml), and βTG (22.9 ng/ml) levels were not different from FPA (0.79), PF4 (5.6), and βTG (20.8) levels in the other 20 patients in group 2 without this symptom.

**DISCUSSION**

In the present study, fibrinopeptide A, platelet factor 4, and β-thromboglobulin levels were not elevated in patients with angiographically documented coronary artery disease without electrocardiographic or ventriculographic evidence of previous myocardial infarction. Peripheral blood levels of FPA, PF4, and βTG did not differ significantly from levels in patients with normal coronary arteriograms. Furthermore, levels of FPA, PF4, and βTG were unrelated to the severity of coronary artery disease assessed by the number of arteries with significant stenotic lesions. These observations indicate that a sustained state of increased platelet activation or fibrin formation is not detectable in the peripheral venous blood of patients with coronary artery disease.

However, mean PF4 and βTG levels were elevated in patients with coronary artery disease who had previously experienced myocardial infarction. The association observed between elevated platelet proteins and remote myocardial infarction was unrelated to the greater severity of coronary disease in these patients. Platelet protein levels were significantly elevated in patients with multivessel coronary artery disease with previous infarction compared to patients with comparable multivessel coronary artery disease without previous infarction. None of the patients in the present study experienced angina immediately before or during blood sample collection, and none had experienced...
acuted myocardial infarction within the previous 6 mo. Therefore, elevated platelet protein levels in the patients with prior infarction could not be attributed to acute myocardial ischemia.

Other investigators have reported that mean PF4 levels are elevated in patients with coronary artery disease. In these studies, however, patients with prior infarction were not analyzed separately from those with coronary disease without infarction. Elevated mean levels of PF4 may have resulted from inclusion of patients with previous infarction. The present data suggest that elevated platelet proteins in patients with coronary artery disease are related to the presence of previous myocardial infarction rather than the presence of coronary atherosclerotic lesions.

The mechanism for the association observed between platelet protein levels and prior infarction can only be inferred. However, two types of evidence suggest the possibility that increased βTG and PF4 levels reflect platelet reaction with the infarcted ventricular wall. First, in the 36 patients of group 3, βTG levels correlated with the extent of prior infarction as assessed by ejection fraction and abnormalities of segmental left ventricular contraction. Secondly, βTG levels were highest in the small group of patients with left ventricular aneurysms.

In 3 of the 8 patients with left ventricular aneurysms, mural thrombi were detected by angiography. In these 3 patients, FPA levels were significantly elevated, implying thrombin action. These hemostatic alterations are analogous to findings in patients with prosthetic heart valves. This observation raises the possibility that measurement of FPA levels may be useful for monitoring the activity and response to therapy of cardiac mural thrombus.

For each patient group in the present study, mean ratios of βTG to PF4 concentrations were approximately 4:1. Since platelets contain and release these two proteins in equivalent amounts in vitro, the high ratio of βTG to PF4 found in peripheral venous blood has been attributed to rapid in vivo clearance. Elevated peripheral venous levels of these proteins with this relative ratio have been interpreted to indicate increased platelet release in vivo and to exclude in vitro release. The presence of normal FPA levels indicates that platelet release was not due to thrombin action, since the FPA level is more sensitive than the platelet protein level to the action of this enzyme.

Several of the platelet alpha-granule constituents released have important biologic activities. Platelet-derived growth factor, which is released in parallel with PF4 and βTG, stimulates the proliferation of arterial smooth muscle cells; PF4 neutralizes the anticoagulant effect of heparin; and βTG has been claimed to inhibit PGI₂ production by these cells, although this latter observation has not been confirmed. Chronic release of these substances, particularly if localized to the left ventricle, might be expected to favor both atherogenesis and thrombosis in the aorta and coronary arteries by a self-perpetuating mechanism. Additional studies are required to determine whether patients with elevated platelet protein levels, such as those in group 3, have a higher incidence of reinfarction and whether therapy can suppress platelet release and reduce the incidence of reinfarction.

The findings of this study indicate that the reaction of blood with injured cardiac wall must be considered...
in the interpretation of tests of hemostatic function in patients with atherosclerosis or myocardial infarction. Such a mechanism may explain the abnormal results of platelet survival reported in some patients with coronary artery disease.35,34

REFERENCES


ACKNOWLEDGMENT

We would like to thank Carol Wilkins and Betty Grossman for excellent technical assistance and Leonard L. Norbert for typing the manuscript.


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