PATHOPHYSIOLOGY AND NATURAL HISTORY
CORONARY ARTERY DISEASE

Coagulant activities of platelets in coronary artery disease

A. Koneti Rao, M.D., Paul D. Mintz, M.D., Steven J. Lavine, M.D., Alfred A. Bove, M.D.,
Michael T. McDonough, M.D., James F. Spann, M.D., and Peter N. Walsh, M.D., Ph.D.

ABSTRACT Platelets have been implicated in the pathogenesis of coronary artery disease, and a number of studies have examined platelet function and coagulation parameters in such patients. We have examined platelet coagulant activities, volumes, and aggregate ratios in 23 patients with chest pain, seven of whom had normal coronary angiograms (group I) and 16 of whom had angiographically proven coronary artery disease (group II). There were no significant differences in the mean values for platelet volume or platelet aggregate ratios between the two groups. The platelet coagulant activities concerned with initiation and the early stages of intrinsic coagulation were significantly increased in patients in group II as compared with those in group I. No significant differences were noted between the two groups with respect to prothrombin time, partial thromboplastin time, and plasma levels of fibrinogen and coagulation factors V and VIII. However, the mean activity in plasma of antithrombin III (but not the level of antithrombin III antigen) was significantly lower in patients of group II compared with group I. Overall, our observations provide evidence for an enhanced contribution of platelets to the intrinsic coagulation system in patients with coronary artery disease. The platelet coagulant hyperactivity noted in these patients may reflect a role of platelets in the pathogenesis of coronary artery disease or may be secondary to the underlying arterial disease.


PLATELETS have been implicated in the pathogenesis of coronary artery disease, and a number of studies have been carried out to examine various aspects of platelet function in this disorder. These have included the study of platelet responses such as adhesion, aggregation, and the formation of vasoactive prostaglandin derivatives, the quantitation of platelet aggregate ratio and plasma levels of platelet-specific proteins (β-thromboglobulin and platelet factor 4), and the estimation of the survival of platelets in patients with coronary artery disease.

Previous studies carried out in our laboratory and those of other investigators have shown that human platelets participate in intrinsic coagulation at several stages, including the initiation of intrinsic coagulation by two alternative pathways, the activation of factor X, and the activation of prothrombin. Assays for platelet coagulant activities have been developed in our laboratory and applied to the study of a variety of patients with thrombotic disorders. We have suggested that platelet coagulant activities during the initiation and early stages of intrinsic coagulation are important determinants of normal hemostasis and that they may also play a part in the development of thromboembolic disease. However, this role of platelets has not received attention in studies of platelets in coronary artery disease. Extensive studies have been carried out in patients with coronary artery disease defining the changes in plasma coagulation factors and platelets, but the interaction of platelets and the coagulation system has not been adequately examined. To assess the possible role of platelets in the pathogenesis of coronary artery disease, we have examined platelet coagulant activities, platelet volume, and platelet aggregate ratio in 23 patients who were evaluated for chest pain by coronary angiography; in 16 of these there was angiographic evidence of coronary artery disease. In addition, we have studied plasma coagulation and levels of antithrombin III in these patients. Our studies provide evidence of an enhanced contribu-
tion of platelets to the intrinsic coagulation system in patients with coronary artery disease. In addition, plasma levels of antithrombin III, measured by a functional assay, were found to be decreased in patients with angiographically proven coronary artery disease.

Patients and methods

We have studied 23 patients with a history of chest pain who were evaluated by coronary arteriography and ventriculography at Temple University hospital. Each consecutive patient who gave informed consent for the study was included with the exception of those in whom the following exclusion criteria were present: (1) history of a bleeding disorder, (2) ingestion of aspirin within 14 days of the time of study or ingestion of other medications known to interfere with platelet functions within 72 hr of the time of study, (3) receipt of anticoagulant medication within 14 days of study, (4) myocardial infarction within 6 weeks of study, (5) history of cardiac disease other than coronary artery disease, myocardial ischemia, or myocardial infarction, (6) history of myeloproliferative diseases, paroxysmal nocturnal hemoglobinuria, or congenital antithrombin III deficiency, (7) renal insufficiency (BUN > 25 mg/dl or creatinine > 1.5 mg/dl) or significant liver disease, and (8) diabetes mellitus. A detailed history was elicited from and a complete general physical examination was performed on each patient.

All patients were evaluated in the cardiac catheterization laboratory by selective coronary angiography and studies of left ventricular function, on the basis of which it was determined whether or not patients had significant occlusive disease of the coronary artery and, if so, which coronary arteries were involved. Without knowledge of the results of the coronary angiographic examination, laboratory investigations were carried out simultaneously including a complete blood count, urinalysis, SMA, SMA, and the studies of platelet function and blood coagulation described below. Blood samples for the studies were drawn after a 14 hr period of fasting and before the cardiac catheterization in all patients. Thirty-eight normal subjects (20 men and 18 women), 20 to 48 years old, were also studied to establish normal values.

Preparative procedures. Nine volumes of blood were collected by clean venipuncture directly into one volume of 3.8% trisodium citrate in plastic tubes. Platelet-rich plasma (PRP) and high-susp platelet-poor plasma (PPP) were prepared and platelets were washed and suspensions prepared as previously described. Platelets were counted by phase microscopy and electronically with a Model ZBI Particle Counter (Coulter Electronics, Hialeah, FL) that was used together with a Coulter Channelizer to calculate mean platelet volumes.

Coagulation assays. Determination of one-stage prothrombin times and activated partial thromboplastin times and assays for fibrinogen, factor V, and factor VIII were done as previously described. Determinations of antithrombin III antigen levels were done with a radial immunodiffusion method (M-partigen, Behring-Diagnostics, NJ). The antithrombin III activity was measured with the Thrombo-Screen assay (Pacific Hemostasis, Los Angeles, CA). In this assay plasma is clotted with snake venom (Reptilase) and the serum obtained is incubated with thrombin in the presence of heparin. By addition of exogenous fibrinogen, the activity of uninhibited thrombin is quantitated and the antithrombin III activity obtained from a standard curve.

Estimation of platelet aggregate ratio. The quantitation of platelet aggregate ratio in blood was carried out by the method of Wu and Hoak, which involves comparing platelet counts in PRP obtained from blood collected in EDTA and formalin with those in EDTA alone. The results are expressed as a ratio obtained by dividing the platelet count in EDTA-PRP into the platelet count in EDTA/formalin-PRP. A decrease in the ratio denotes an increase in circulating platelet aggregates.

Platelet coagulant activities. The assays for the platelet coagulant activities reflect the contribution of the platelets to the intrinsic coagulation system and include: (1) contact product–forming activity, the capacity of normal platelets to respond to adenosine diphosphate (ADP) and participate in the activation of factor XII, collagen-induced coagulant activity, the capacity of collagen-stimulated platelets to participate in the initiation of intrinsic coagulation by a alternative mechanism in the apparent absence of factor XII, (3) intrinsic factor X,–forming activity, by which platelet membrane components become available and promote the interactions of factors XI, VIII, and IX to activate factor X in the presence of calcium, and (4) platelet factor 3 activity by which platelet membrane phospholipoproteins become available and promote the interactions of factor X and factor V to activate prothrombin in the presence of calcium. These platelet coagulant activities were assayed by modifications of previously described methods. Briefly, these assays involve incubation of various dilutions of PRP from patients or normal subjects (suspended in PPP from patients or normal subjects) or washed platelets (in calcium-free Tyrode’s buffer) with the activating agents ADP, collagen, or kaolin, followed by determination of clotting times after addition of either calcium chloride (contact product–forming activity) or Russell’s viper venom and calcium chloride (platelet factor 3 activity). For the assay of collagen-induced coagulant and intrinsic factor X,–forming activity, washed platelet suspensions were incubated with partially purified factors XI, IX, X, and thrombin-activated factor VIII, and the factor X, formed was measured by determination of clotting times in factor X–deficient plasma. The results were expressed as percentages of normal platelets by reference to a double logarithmic plot of clotting time and platelet concentration.

Statistical methods. Results are expressed as mean and SEM. The groups were compared by Student’s t test and, for the various assays of the platelet coagulant activities, by the Wilcoxon’s rank-sum test. The results of the analysis by both methods were similar. The level of significance (α error) for the comparison of the two groups was set at .05. The magnitude of type II error for two groups of the size of those in this study was computed for an expected difference of 50% in the coagulant activities. For the assays of collagen-induced coagulant activity, intrinsic factor X,–forming activity and the platelet factor 3, the type II error was between 0.1 and 0.25; it was approximately 0.5 for the assay of the contact product–forming activity. For the assay of antithrombin III activity the type II error was less than 0.25 for an expected difference of 30%, while for the antithrombin III antigen it was between 0.5 and 0.6 for an expected difference of 7 mg/dl. For an expected difference between the groups of 0.2 in the platelet aggregate ratio, the type II error was less than 0.2.

Results

The clinical characteristics of the 23 patients studied are listed in table 1. Seven of these patients presented with chest pain but had normal coronary angiograms (group I), and the remaining 16 (group II) had chest pain or had had a previous myocardial infarction and were found to have coronary artery disease that was documented by coronary angiography. Patients in group II had one or more coronary arteries with a
steno- sis of greater than 50% diameter reduction while group I patients had normal arteries without any obstructive lesions. As shown in table 1, the age and sex distributions of these patients and the frequency of hypertension were similar. Since the groups were similar with respect to these variables, patients in group I constitute appropriate controls for those in group II and it is possible to make valid comparisons of the results obtained in the studies of platelet function and coagulation parameters. Five of 16 patients with documented coronary artery disease had been diagnosed previously as having experienced acute myocardial infarction, whereas none of the patients with normal coronary angiograms had been so diagnosed. The number of coronary vessels demonstrating atherosclerotic lesions in patients with significant coronary artery disease is shown in table 1. Fasting serum cholesterol levels were on average lower in patients with normal coronary angiograms, but the difference was not significant.

Coagulation results (table 2). The results of determinations of prothrombin times, activated partial thromboplastin times, factor V, factor VIII, and fibrinogen concentrations, and antithrombin III levels are listed in table 2. Although prothrombin times and activated partial thromboplastin times were slightly shorter in patients with coronary artery disease than in those with normal coronary angiograms, these differences were not significant. Factor VIII coagulant activity and fibrinogen levels tended to be higher and factor V levels lower in patients with coronary artery disease than in those with normal coronary angiograms, but these differences were not significant.

Determination of antithrombin III levels was carried out by two methods; one measured the amount of the antigen while the other measured the antithrombin III activity in plasma. There was no difference in the mean values obtained in group I and group II patients for antithrombin III antigen levels, whereas in contrast the antithrombin III activity was on average significantly lower (p < .02) in patients with coronary artery disease than in those with normal coronary angiograms (table 2). Within group II the results obtained for the levels and activity of antithrombin III antigen were not significantly different between patients with and without previous myocardial infarction. To determine the impact of age, the mean levels of antithrombin III in the patients of group I and II were compared with those in a group of normal laboratory controls (age range, 20 to 40 years) who were considerably younger (table 2).

No significant differences were noted in the levels of antithrombin III antigen. As compared with the normal laboratory controls, the mean antithrombin III activity was significantly (p < .02) higher in patients of group I; it was not significantly different in group II patients.

Platelet counts, volumes, aggregate ratios, and coagulant activities (table 3, figures 1 and 2). Whole blood platelet counts and mean platelet volumes were not significantly different in patients with coronary artery disease (group II) compared within those with chest pain and normal coronary angiograms (group I). The mean platelet aggregate ratio was slightly lower in patients in group II compared with those in group I, but this difference was not significant (1 > p > .05). In group II the mean values obtained in the five patients with a history of previous myocardial infarction were not different from those in patients without a history of infarction. However, when compared with the results in

| TABLE 1 |
| Patient characteristics |
| Normal angiogram | Coronary artery disease |
| (group I, n = 7) | (group II, n = 16) |
| Age (yr) | 46 (36–54) | 51 (40–66) |
| Male (n) | 6 | 13 |
| Female (n) | 1 | 3 |
| Hypertension (n) | 3 | 9 |
| Cholesterol (150–300 mg/dl) | 205 ± 2 (SEM) | 238 ± 1 (SEM) |
| Previous myocardial infarction (n) | 0 | 5 |
| No. of diseased vessels | 0 | 2.1 ± 0.02 (SEM) |

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All results are mean ± SEM. The figures in parentheses represent the normal ranges.

*Group I vs group II. p < .02; group I vs normal controls p < .02

| TABLE 2 |
| Coagulation tests and antithrombin III levels |
| Normal angiogram | Coronary artery disease |
| (group I, n = 7) | (group II, n = 16) |
| Prothrombin time (10–14 sec) | 12.0 ± 0.2 | 11.5 ± 0.3 |
| Partial thromboplastin time (25–45 sec) | 32.5 ± 2.2 | 31.1 ± 1.1 |
| Factor V (50%–150%) | 133 ± 10 | 116 ± 8 |
| Factor VIII (50%–150%) | 148 ± 17 | 209 ± 27 |
| Fibrinogen (150–300 mg/dl) | 245 ± 32 | 298 ± 38 |
| Antithrombin III Antigen (28.1 ± 0.7 mg/dl, n = 51) | 28.3 ± 2.8 | 29.1 ± 1.7 |
| Activity (102.5 ± 2.5%, n = 41) | 123 ± 12^a | 95 ± 5 |

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TABLE 3
Platelet counts, volumes, and circulating platelet aggregate ratios

<table>
<thead>
<tr>
<th></th>
<th>Normal angiogram (group I, n = 7)</th>
<th>Coronary artery disease (group II, n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>(150-300 × 10⁹/L)</td>
<td>235 ± 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>254 ± 11</td>
</tr>
<tr>
<td>Platelet volume</td>
<td>(7.15 ± 0.08 μm³)</td>
<td>6.08 ± 0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.30 ± 13</td>
</tr>
<tr>
<td>Circulating platelet</td>
<td>(0.94 ± 0.01)</td>
<td>0.79 ± 0.07¹</td>
</tr>
<tr>
<td>aggregate ratio</td>
<td></td>
<td>0.71 ± 0.03²</td>
</tr>
</tbody>
</table>

All results shown are mean ± SEM. The results shown in parentheses are the values for normal controls.

As compared with normal controls¹ p < .001, ²p < .02. The differences between group I and group II were not significant.

251 normal laboratory controls, the mean platelet aggregate ratios were decreased in both groups (group I p < .02, group II p < .001). This normal control group consisted of laboratory personnel who as a group were considerably younger than the patients.

Platelet coagulant activities concerned with the initiation of intrinsic coagulation by two alternative pathways (contact product–forming activity and collagen-induced coagulant activity) and with the early stages (intrinsic factor X₃–forming activity) of intrinsic coagulation were significantly increased in group II patients compared with in group I patients (figure 1). The mean activities of platelet factor 3 in response to collagen and kaolin were not significantly different in the two groups (figure 2). The results of the various assays of the platelet coagulant activities in patients of group II with and without histories of previous myocardial infarction were not significantly different from each other.

Discussion

Our studies demonstrate significant elevations in platelet coagulant activities concerned with the initiation and early stages of intrinsic coagulation in patients with chest pain and angiographically demonstrated coronary artery disease as compared with those with chest pain but normal coronary angiograms. In addition, compared with in patients with normal coronary angiograms, significant decreases in antithrombin III functional activity were observed in patients with documented coronary artery disease without concomitant decreases in antithrombin III antigen levels.

The results of various assays of the platelet coagulant activities reflect the role of platelets at different stages of intrinsic coagulation. The contact product–forming activity reflects the capacity of platelets to respond to ADP and participate in the activation of factor XII, while the collagen-induced coagulant activity represents the ability of collagen-stimulated platelets to promote the proteolytic activation of factor XI in the presence of either factor XII₃ or of kallikrein (in the apparent absence of factor XII). Furthermore, the intrinsic factor X₃–forming activity reflects the role of platelets in the activation of factor X in the presence of factors XI₃, VIII, IX, and calcium. Thus, these assays quantitate the contribution of platelets to the early events in the intrinsic coagulation cascade. In patients with angiographically proven coronary artery disease, the results in all of these three assays were elevated. However, assays of the platelet factor 3, which quantitate the role of platelets at a later stage of the coagulation system, namely, activation of prothrombin, showed elevations in group II as compared with in group I patients, but these were not statistically significant. Overall, our study provides evidence of platelet coagulant hyperactivity in patients with coronary artery disease.

These results should be interpreted in the context of our previous studies. We have demonstrated similar...
abnormalities of the platelet role in intrinsic coagulation in patients who suffer transient cerebral ischemic episodes but do not have hyperlipidemia or hypertension and in patients with diabetes mellitus, particularly those with retinal vascular changes. In addition, platelet coagulant hyperactivity has been demonstrated in a group of younger patients with limited thrombosis, i.e., patients with retinal vascular occlusion. On the basis of these observations we have suggested a role of platelets in the pathogenesis of vascular occlusive diseases. However, the demonstration of platelet coagulant hyperactivity in coronary artery disease does not constitute evidence for a role of platelets in pathogenesis. An issue concerning these patients that still needs resolution, despite the large number of published studies, is whether the platelet abnormalities demonstrated are the cause or the result of the vascular disease. Thus, the platelet coagulant hyperactivity observed in patients with coronary obstructive disease may be a result of in vivo platelet activation secondary to other factors or may represent a primary platelet abnormality that is pathogenetically important in producing the lesions in the vessels and/or the complications of coronary artery disease such as angina pectoris or acute myocardial infarction. Obviously these two possibilities are extremely difficult to differentiate on the basis of studies in humans. Nevertheless, there is compelling evidence from other sources that suggests that platelets and their constituents, such as the platelet-derived growth factors, participate in the pathogenesis of atherosclerosis, the main process leading to the altered coronary arteries. These studies have been extensively reviewed elsewhere.

On the premise that formation of platelet aggregates may contribute to the pathogenesis of acute myocardial ischemia, several studies have quantitated the circulating platelet aggregate ratio in the blood of patients with coronary artery disease. These studies have been summarized elsewhere. Wu and Hoak noted that, compared with control subjects, patients with acute myocardial infarction had a decreased platelet aggregate ratio, but this was not true of patients with stable angina. Similar elevations in circulating microaggregates have been noted by other investigators in patients with acute myocardial infarction.

Furthermore, Neri-Serneri et al. have found a significant decrease in circulating platelet aggregate ratios in all patients with coronary artery disease, regardless of the acuteness of the disease. In contrast, some studies have failed to demonstrate this abnormality even in patients with myocardial infarction. In our studies none of the patients had an acute myocardial infarction at the time of evaluation and there was no difference between the platelet aggregate ratios in patients with normal angiograms and those in patients with coronary artery disease. Interestingly, when compared with normal laboratory controls, the platelet aggregate ratio was significantly decreased in both patient groups. Since the laboratory controls were considerably younger than the patient groups, the observed differences may reflect an age-related phenomenon and provide evidence of enhanced platelet activation with age. Such a hypothesis is supported by the observations of Ludlam and Zahavi et al. that β-thromboglobulin levels in plasma increase with age in apparently healthy individuals. Increased plasma levels of this platelet α-granule protein have generally been considered to be evidence of in vivo platelet activation. However, our inability to demonstrate a difference between the circulating platelet aggregate ratios in the two patient groups may be related to the small sample size.

Nichols et al. have studied the plasma levels of two platelet-specific proteins, platelet factor 4 and β-thromboglobulin, in patients with ischemic heart disease and abnormal coronary arteriograms to obtain evidence of in vivo platelet activation. They found the levels of these proteins to be elevated in patients who had had a myocardial infarction more than 6 months earlier, but not in those without such a history. It was concluded that the enhanced plasma levels of the platelet proteins in patients with ischemic heart disease was related to the interaction of platelets with previously infarcted myocardium rather than to coronary athero-
sclerotic disease. To assess the effect of the previous myocardial infarction on the platelet parameters, we divided the patients in group II into two categories: five patients with a history of previous myocardial infarction and 11 patients without such a history. The acute infarctions in the former group had occurred more than 6 weeks before the laboratory studies. In the assays of the platelet coagulant activities and the circulating platelet aggregates, there were no differences between the two groups in our study, suggesting that the platelet abnormalities were not solely due to the presence of previously infarcted myocardium.

The results of antithrombin III assays by antigenic and functional methods deserve a special comment. They should be interpreted with caution due to the small number of patients studied. The mean antithrombin III activity in group I but not group II patients was significantly higher than that in the normal controls. Since the latter group was considerably younger, this may reflect an age-related phenomenon. Such an age-related rise in plasma levels has been previously reported for some of the proteins of the coagulation system, such as factors V, VII, VIII, and fibrinogen.

Furthermore, patients with documented coronary artery disease had levels of antithrombin III antigen that were similar to those observed in patients with chest pain and normal coronary angiograms. In contrast, the mean antithrombin III activity in patients with coronary artery disease was significantly lower compared with that in patients with normal coronary angiograms. Thus, even though the mean value for antithrombin III activity in group II patients was not different from that in the normal control subjects, it represents a decline from the expected higher value observed in patients of group I. The lack of difference between the groups in the antigen measurements suggests the presence of a molecule that is partially inactive in the functional assays in plasma samples obtained from patients with coronary artery disease. A possible explanation may be the appearance in the plasma of these patients of antithrombin III molecules complexed with activated serine proteases such as thrombin and factor Xa, which are inactivated by antithrombin III. If these complexes persist in plasma and retain antigenic activity they might be measured in assays for antithrombin III antigen but not in assays measuring antithrombin III activity. Another possible explanation for the discrepancy in the antithrombin III measurements made by antigenic and functional assays may involve the recently described second heparin cofactor in plasma, which is different from antithrombin III.41-44 Griffith et al.45 have reported hereditary antithrombin III deficiency in a family in which the plasma levels of antithrombin III activity were always greater than the levels of the antigen. Studies of this family indicate that the measurement of antithrombin III activity in plasma by methods involving thrombin neutralization reflects the combined concentration of antithrombin III and a second substance referred to as heparin cofactor A,46 while the determinations of antigen represent only antithrombin III due to a lack of cross reactivity between the two heparin cofactors. Based on these observations it may be speculated that differences noted between the antithrombin III activity in patients with coronary artery disease and those with normal angiograms may be related to differences in the heparin cofactor A levels since the antigen levels were comparable in the groups. However, such an hypothesis needs to be established with direct measurements of heparin cofactor A when assay methods become available.

Our observation of decreased antithrombin III activity in patients with coronary artery disease is consistent with the findings of Innerfield et al.,47 who noted decreased antithrombin III activity in patients with angiographically proven coronary artery disease. In their study, while most patients with normal angiograms had normal antithrombin III activity, the majority of patients with abnormal angiograms had decreased levels. Stomorken and Eriksen48 compared patients with chest pain and normal control subjects and found antithrombin III activity to be decreased in patients with chest pain and abnormal angiograms but not in those with normal angiographic results. The decreased antithrombin III activity may reflect a greater activation of the coagulation pathways with consumption of antithrombin III in patients with marked coronary atherosclerosis. There have been several other studies49-52 that have examined antithrombin III levels in patients with ischemic heart disease, and the results have often been conflicting. However, in these studies the diagnosis of coronary artery disease was not angiographically documented, making comparisons with our study somewhat difficult.

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