Thrombogenic potential of human coronary atherosclerotic plaques

Diego Ardissino, Piera Angelica Merlini, Kenneth A. Bauer, Ezio Bramucci, Maurizio Ferrario, Raffaella Coppola, Raffaella Fetiveau, Stefano Lucreziotti, Robert D. Rosenberg, and Pier Mannuccio Mannucci

Higher levels of tissue factor (the initiator of blood coagulation) have been found in coronary atherosclerotic plaques of patients with unstable coronary artery disease, but it is not established whether they are associated with a different thrombotic response to in vivo plaque rupture. In 40 patients undergoing directional coronary atherectomy, prothrombin fragment 1 + 2, a marker of thrombin generation, was measured in intracoronary blood samples obtained proximally and distally to the coronary atherosclerotic plaque before and after the procedure. Before the procedure, plasma prothrombin fragment 1 + 2 levels were significantly increased across the lesion in patients with unstable, but not in those with stable, coronary disease (unstable, median increase, 0.37 nM; range, -0.35-1.16 nM) (stable, median increase, -0.065 nM; range, -0.58-1.06 nM) (P = .0021). After plaque removal, an increase in prothrombin fragment 1 + 2 across the lesion was observed only in patients with unstable coronary disease (unstable, median increase, 0.25 nM; range, -1.04-4.9 nM) (stable, 0.01 nM; range, -0.48-3.59 nM) (P = .036). There was a correlation between the tissue factor content of the plaque and the increase in thrombin generation across the lesion (r = 0.33; P = .038). The higher tissue factor content found in plaques obtained from patients with unstable coronary disease was associated with a local increase in thrombin generation, thus suggesting a link with the in vivo thrombogenicity of the plaque. (Blood. 2001;98:2726-2729)

© 2001 by The American Society of Hematology

Introduction

The rupture or fissuring of coronary atherosclerotic plaque and subsequent thrombosis are considered the key events in the pathogenesis of unstable angina or acute myocardial infarction.1,2 Plaque disruption frequently occurs during the course of atherosclerosis, but only some ruptured plaques develop thrombosis.3,4 Tissue factor (the primary initiator of blood coagulation) is contained in human coronary atherosclerotic plaques and is functionally active in vitro.5-7 There are larger amounts of functionally active tissue factor in the plaque of patients with unstable coronary artery disease (unstable angina and myocardial infarction) than in those with stable angina,7 roughly corresponding to the different tendency of stable and unstable disease to be associated with coronary thrombosis. Hence, the different thrombotic response to plaque rupture has been attributed to variations in tissue factor content. However, there is no in vivo evidence of any quantitative association between tissue factor content and thrombin generation in response to plaque disruption.

By cutting the atherosclerotic plaque, directional coronary atherectomy exposes the plaque content to flowing blood, thus mimicking spontaneous plaque rupture. This procedure also provides a unique opportunity to obtain fresh samples of plaque tissue that can be biochemically analyzed and related to the degree of coagulation activation in coronary blood. This study was undertaken to evaluate whether a different amount of tissue factor in atherosclerotic plaques is associated with a different degree of thrombin generation in the coronary blood of patients with coronary artery disease.

Materials and methods

Study population

The study population consisted of 40 consecutive patients with coronary artery disease who were undergoing directional coronary atherectomy at the Division of Cardiology, IRCCS Policlinico San Matteo, Pavia, and the Division of Cardiology, Ospedale Maggiore di Parma, Italy. Patients were prospectively assigned to the diagnostic subgroups by their attending physicians. Stable angina (n = 22) was defined as a history of chest pain induced by exercise or normal daily activity or at least 1-mm ST-segment depression on the exercise test electrocardiogram. Unstable angina (n = 12) was defined as resting chest pain accompanied by transient ischemic electrocardiographic changes and serum creatine kinase MB-fraction concentrations of less than twice the upper limit of normal. Acute myocardial infarction (n = 6) was defined as persistent chest pain at rest with ischemic electrocardiographic changes that evolved into pathologic Q waves or T wave inversions and an increase in the creatine kinase MB fraction of more than twice the upper limit of normal. Patients with unstable angina or myocardial infarction were considered to have unstable coronary artery disease (n = 18). All patients gave their written, informed consent, and the study was approved by the Institutional Review Board of Ospedale Policlinico San Matteo.

From the Division of Cardiology, Ospedale Maggiore and University of Parma, Italy; Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS, Maggiore Hospital, University of Milan, Italy; Division of Cardiology, Ospedale Niguarda, Milan; Division of Cardiology, IRCCS, Policlinico San Matteo, Pavia, Italy; Division of Cardiology, Ospedale Legnano, Italy; Charles A. Dana Research Institute, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School; and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

Submitted January 3, 2001; accepted June 20, 2001.

Reprints: Diego Ardissino, Divisione di Cardiologia, Ospedale Maggiore, Universita’ degli Studi di Parma, Via Gramsci 14, 43100 Parma, Italy; e-mail: ardis001@planet.it.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 U.S.C. section 1734.

© 2001 by The American Society of Hematology
Intracoronary blood sampling

Thrombin generation was quantitated by measuring the plasma levels of prothrombin fragment 1 + 2, a 31-kd polypeptide released from the amino terminal end of prothrombin during its conversion to thrombin, which is considered to be an indirect quantitative index of the thrombin generated by the prothrombinase complex. Although previous studies have usually measured prothrombin fragment levels in peripheral venous blood, our goal was to measure it in coronary artery blood collected by means of a specially designed sampling catheter that allows a blood flow of 80 mL/min. A potential advantage of this technique over peripheral venous sampling is that it avoids dilution of the fragment in the systemic circulation; a potential disadvantage is that blood withdrawal through a catheter may itself artifically increase the levels of the fragment. To evaluate potential artifactual increases, the fragment was measured in venous blood withdrawn directly from a clean antecubital venipuncture made by means of a 19-gauge needle at the same time blood was withdrawn through the sampling catheter in 24 patients who had received 325 mg aspirin and 70 U/kg heparin. There was virtually no difference in plasma prothrombin fragment 1 + 2 levels between the blood samples obtained directly from the vein and those obtained from the sampling catheter (0.84 nM/L ± 0.08 vs 0.86 nM/L ± 0.05, respectively). To assess the variability over time of prothrombin fragment 1 + 2 levels in the samples drawn directly from the coronary artery by means of the sampling catheter, 2 sets of blood samples were drawn from the same 24 patients with an interval of 25 minutes (the average time of the directional coronary atherectomy procedure). An increase was defined as abnormal when fragment levels were above the 95th percentile of the distribution of the changes between the 2 samples (0.11 nM).

Blood processing and measurement of thrombin generation

Samples were collected through the sampling catheter directly into refrigerated plastic tubes containing an anticoagulant mixture consisting of a thrombin inhibitor, EDTA, and aprotonin. The anticoagulant blood ratio used was 1:9 (v/v/v). Blood samples were immediately centrifuged at 2500g for 25 minutes at 4°C; the plasma was frozen on dry ice and stored at −80° C until use. All samples were analyzed by laboratory personnel who had no knowledge of the clinical data. Plasma levels of prothrombin fragment 1 + 2 were measured by means of a previously described double-antibody radioimmunoassay, which has a coefficient of variation of 8%.

Coronary angiography and directional coronary atherectomy

Selective coronary arteriography was performed in multiple views using the Judkins technique. Greater than 70% narrowing in the diameter of the coronary arteries was considered significant coronary artery stenosis. Patients were classified as having 1-, 2-, or 3-vessel disease according to the number of vessels with significant coronary stenoses. Angiographic morphology of the lesions was prospectively assessed and was considered complex if the lesions had irregular borders, overhanging edges, ulcerations, or thromboses.

Before directional coronary atherectomy, patients were given 160 to 325 mg aspirin and heparin U/kg 70 adjusted as needed to maintain an activated clotting time of more than 250 seconds. Directional coronary atherectomy was performed using standard procedures. Briefly, the atherectomy device was directed over a previously inserted 300-cm-long guide wire and positioned at the level of the stenosis, and the support balloon was then inflated up to 1 atm. The cutter was then retracted, and the inflation pressure of the balloon was increased to a maximum of 3 atm. The driving motor was started, and the rotating cutter slowly was advanced to cut and collect the protruding plaque in the collection chamber located at the tip of the catheter. After each pass, the balloon was deflated and either removed or repositioned.

Plaque processing and measurement of tissue factor procoagulant activity

After thawing, the lipid-bound proteins in the plaques were solubilized using 1% Triton X-100 in phosphate-buffered saline for 2 hours at room temperature and then were centrifuged at 50 000g for 1 hour at 20°C. Tissue factor activity was analyzed in the supernatant using a previously published chromogenic factor Xa generation assay.

Statistical analysis

The descriptive statistics include mean values and standard deviations, or median values and ranges, as appropriate. Between-group differences were assessed by using the Student unpaired t test or the Mann Whitney U test. Prevalences were compared by means of the χ² test. Correlation between tissue factor activity and prothrombin fragment 1 + 2 was calculated as Spearman rank correlation (ρ). All tests were 2-tailed, and P < .05 was regarded as statistically significant.

Results

Clinical and angiographic characteristics of the study population are reported in Table 1. There was a higher prevalence of lesions with complex morphology in the patients with unstable coronary artery disease than in those with stable disease (P < .0001).

Before directional coronary atherectomy, there was no difference in the plasma prothrombin fragment 1 + 2 levels measured proximally to the lesion between the patients with stable or unstable coronary artery disease, whereas higher fragment levels were found distally to the lesion in unstable patients (P = .0045). No significant increase in fragment 1 + 2 levels was observed across the lesion in stable patients, whereas a significant increase was observed in unstable patients (Table 2) (P = .002). The median change in prothrombin fragment levels across the lesion was significantly greater in patients with unstable coronary artery disease (P = .0021); there was a median increase of 0.37 nM (range, −0.35–1.16 nM) in unstable patients and a median decrease of −0.065 nM (range, −0.58–1.06) in stable patients. An abnormal increase in the fragment was observed in 71% of the unstable patients but in only 28% of the stable patients (χ² = 7.24; P = .007).

After plaque removal, there was no difference in the fragment levels measured proximally to the lesion between the patients with stable and unstable coronary artery disease, whereas significantly higher levels were found distally to the lesion in patients with unstable disease (P = .027). No significant increase in fragment 1 + 2 was observed proximally to the lesion in unstable patients (P = .027). No significant increase in fragment 1 + 2 was observed distally to the lesion in patients with stable disease (P = .014). No significant increase in fragment 1 + 2 was observed distally to the lesion in stable patients (P = .014).

<table>
<thead>
<tr>
<th>Lesion morphology*</th>
<th>Complex (%)</th>
<th>4 (18)</th>
<th>15 (83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. cuts</td>
<td>5.2 (2-9)</td>
<td>5.6 (2-9)</td>
<td>5.0 (2-7)</td>
</tr>
</tbody>
</table>

Continuous variables are expressed as means ± SD or medians and ranges as appropriate. Means were compared using the Student unpaired t test; medians were compared using the Mann Whitney U test. Frequencies were compared using the χ² test.

*P < .0001.
across the excised lesion in stable patients, whereas a significant increase was observed in unstable patients (Table 2) \( (P = .043) \). The change in fragment levels across the lesion was significantly greater in the patients with unstable coronary disease \( (P = .036) \), with a median increase of 0.25 nM (range, \(-1.04\) to 4.91) compared with 0.01 (range, \(-0.48\) to 3.59) in stable patients. The median changes in prothrombin fragment 1 + 2 across the lesion before and after directional coronary atherectomy were not significantly different in either stable or unstable patients. An abnormal increase in the fragment was observed in 65% of the unstable patients and in 27% of the stable patients \( (\chi^2 = 5.4; P = .01) \). Percentages of patients with abnormal increases before and after directional coronary atherectomy were not significantly different between the stable and the unstable patients. However, although the greater changes in the fragment were observed after directional coronary atherectomy in stable and unstable patients, these were more pronounced in the unstable patients.

Median weights were as follows: extracted plaque, 5.7 mg (range, 0.15-15.1 mg); coronary plaque of patients with stable disease, 4.5 mg (range, 0.15-9.7 mg); coronary plaque of patients with unstable disease, 7.2 mg (range, 0.8-15.1 mg). Tissue factor activity per milligram plaque weight ranged from 0 to 5.3 mU/mg. It was greater in the plaques extracted from patients with unstable disease than from patients with stable disease (0.258 mU/mg [range, 0.08-5.30 mU/mg] vs 0.156 mU/mg [range, 0-1.94 mU/mg]; \( P = .011 \)). There was a statistically significant positive correlation between the tissue factor activity of the removed plaque and the increase in thrombin generation across the lesion after the procedure \( (r = 0.33; P = .038) \).

### Discussion

Tissue factor has been detected within the necrotic cores of endo-atherectomy specimens from patients with carotid atherosclerosis and in atherectomy specimens from patients with coronary artery disease. Furthermore, the tissue factor found in atherosclerotic plaques has been shown to be functionally active in vitro because it is capable of generating activated factor X. In an ex vivo model, Toschi et al and Badimon et al used a perfusion chamber to evaluate the platelet and fibrinogen deposition on aortic atherosclerotic plaques exposed to flowing blood, and they found a positive correlation between the amount of tissue factor detected by means of in situ binding assays and both platelet and fibrinogen deposition. Tissue factor staining of the lipid-rich core was more intense than that of all of the other plaque components, and there was also a higher level of plaque and fibrinogen deposition.

Finally, a recent study has shown that a thrombus could be histologically detected only in coronary atherectomy specimens characterized by immunohistochemically detectable tissue factor. All these findings strongly support the view that the presence and amount of functional tissue factor in coronary atherosclerotic plaques may be critical for thrombus formation in acute coronary events. However, it is still unknown whether higher levels of tissue factor in coronary plaques in vivo is associated with increased thrombus formation after plaque rupture.

This study confirms that tissue factor activity is greater in the coronary atherosclerotic plaques extracted from patients with unstable angina or myocardial infarction than in those extracted from patients with stable angina. The new finding is that the local (intracoronary) measurement of thrombin generation makes it possible to demonstrate that greater tissue factor activity in atherosclerotic plaque is associated with a greater increase in thrombin generation across the lesion, before and after plaque disruption induced by directional coronary atherectomy. Of the patients with unstable coronary artery disease, 71% had abnormal increases in thrombin generation before the procedure and 65% had them after the procedure; of those with stable disease, 27% had abnormal increases in thrombin generation across the lesion before the procedure and 23% had them after the procedure. Perhaps the greater increase in thrombin generation observed before the procedure in patients with unstable coronary artery disease occurred because most of them already had a ruptured plaque that exposed tissue factor. Patients with stable angina have smooth, fibrous, nonulcerated plaques with little exposure of tissue factor. We surmise that in patients with unstable plaque, the thrombogenic material is already exposed and the lesions induced by atherectomy do not further increase such a thrombogenic stimulus. On the other hand, in patients who have stable plaque at baseline, the lesions induced by atherectomy theoretically expose a new surface to prothrombin activation, but the local thrombotic stimulus is too weak and inadequate to reach the level of prothrombin activation seen in unstable patients. The highest values of thrombin generation across the lesion were observed in patients with unstable coronary artery disease after atherectomy. We hypothesize that this strong thrombogenic stimulus may lead to the thrombotic complications occasionally observed during percutaneous coronary interventions despite intensive antithrombotic therapy.

On the whole, these findings further support the notion that plaque rupture and exposure of the atheromatous core is not the only precipitating event in determining coronary thrombosis. The nature of the plaque components exposed to flowing blood, together with the local rheologic and systemic blood factors in unstable patients, are also critical in determining the extent of the prothrombic response. In this respect, tissue factor appears to be a critical plaque component.

### References


---

**Table 2. Prothrombin fragment 1 + 2 levels before and after directional coronary atherectomy**

<table>
<thead>
<tr>
<th></th>
<th>Stable (n = 22)</th>
<th>Unstable (n = 18)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before directional coronary atherectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( F_1 ) + 2 proximal nM</td>
<td>0.95 (0.44-2.08)</td>
<td>1.16 (0.74-3.22)</td>
<td>NS</td>
</tr>
<tr>
<td>( F_1 ) + 2 distal nM</td>
<td>1.00 (0.44-2.64)</td>
<td>1.5 (0.51-3.96)</td>
<td>.0045</td>
</tr>
<tr>
<td>( P )</td>
<td>NS</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>Change nM</td>
<td>-0.065 (-0.58-1.06)</td>
<td>0.37 (-0.35-1.16)</td>
<td>.0021</td>
</tr>
<tr>
<td>After directional coronary atherectomy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( F_1 ) + 2 proximal nM</td>
<td>1.06 (0.42-2.61)</td>
<td>1.03 (0.68-3.49)</td>
<td>NS</td>
</tr>
<tr>
<td>( F_1 ) + 2 distal nM</td>
<td>0.97 (0.49-5.71)</td>
<td>1.31 (0.69-6.17)</td>
<td>.027</td>
</tr>
<tr>
<td>( P )</td>
<td>NS</td>
<td>.043</td>
<td></td>
</tr>
<tr>
<td>Change nM</td>
<td>0.01 (-0.48-3.59)</td>
<td>0.25 (-1.04-4.91)</td>
<td>.036</td>
</tr>
</tbody>
</table>

Data are expressed as medians and ranges. NS indicates not significant.
Thrombogenic potential of human coronary atherosclerotic plaques

Diego Ardissino, Piera Angelica Merlini, Kenneth A. Bauer, Ezio Bramucci, Maurizio Ferrario, Raffaella Coppola, Raffaella Fetiveau, Stefano Lucreziotti, Robert D. Rosenberg and Pier Mannuccio Mannucci