

Thrombin Generation in Human Coronary Arteries After Percutaneous Transluminal Balloon Angioplasty

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Objectives. The aim of this study was to investigate the relation between coronary atherosclerotic plaque injury and activation of the coagulation cascade.

Background. Thrombus formation after atherosclerotic plaque disruption has been implicated in the pathogenesis of atherosclerosis, unstable angina and myocardial infarction.

Methods. Biochemical markers of thrombin generation (prothrombin fragment F_{1+2}) and thrombin activity (fibrinopeptide A) were measured in coronary blood before, during and immediately after percutaneous transluminal coronary angioplasty. After demonstrating that blood withdrawn through an angioplasty catheter does not artificially elevate the plasma levels of these markers in patients after heparinization, coronary artery samples were collected proximal and distal to the lesion before and distal to the lesion after balloon inflation in 26 patients.

Results. Plasma levels of F_{1+2} measured proximal to the lesion before angioplasty (median 0.47 nmol/liter, 95% confidence interval [CI] 0.40 to 0.50) were significantly elevated after angioplasty (median 0.55 nmol/liter, 95% CI 0.46 to 0.72, $p = 0.001$). In

contrast, plasma fibrinopeptide A levels measured proximal to the lesion before angioplasty (median 2.0 ng/ml, 95% CI 1.3 to 2.2) were similar to those measured after angioplasty (median 1.8 ng/ml, 95% CI 1.3 to 3.0, $p = NS$). After we defined a normal range of interassay variability on the basis of values obtained from samples drawn proximal and distal to the lesion before angioplasty, seven patients (27%) had a significant increase in F_{1+2} plasma levels. A significant increase in plasma fibrinopeptide A occurred in five of these seven patients. Lesions with dissection, filling defects or haziness on postangioplasty angiography were associated with more thrombin generation than lesions without these features.

Conclusions. Markers of thrombin generation and activity can be collected safely and assayed accurately in heparinized blood withdrawn through an angioplasty catheter. Balloon dilation of coronary stenoses increases thrombin generation and activity within the coronary artery in a substantial subgroup of patients undergoing angioplasty.

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Percutaneous transluminal coronary angioplasty is commonly used for the treatment of symptomatic coronary artery disease (1) but may be complicated by the generation of a thrombus at the site of balloon dilation. Mural thrombus formation may result in abrupt closure (2) and may participate in the development of intimal hyperplasia (3,4), the pathologic hallmark of restenosis after angioplasty.

The presence of thrombus as an early event after coronary angioplasty has been demonstrated by a variety of techniques, including angiography (5) and angiography (6). In addition, pathologic examination of atherectomy specimens derived

from restenotic lesions has demonstrated organized thrombus in ~25% of lesions (7). These examples of thrombus detection suggest that activation of the clotting cascade occurs in association with angioplasty. However, the aforementioned techniques provide a static image of thrombosis and are therefore insufficient to detect whether blood clotting was initiated before, during or after angioplasty.

By measuring peptides that are released at each step of the coagulation cascade, it is possible to assess the dynamic biochemical events that are associated with active clot formation (8). These peptides include fibrinopeptide A (9,10) and prothrombin fragment F_{1+2} (11,12). Fibrinopeptide A is released from the alpha-chain of fibrinogen when thrombin converts fibrinogen to fibrin and is therefore an indicator of thrombin activity. Prothrombin fragment F_{1+2} is released from the amino-terminal portion of prothrombin when it is converted to thrombin by activated factor X (Factor Xa) and is therefore an indicator of thrombin generation. The plasma levels of these peptides are elevated during active thrombosis (8).

We hypothesized that these peptides may serve as biochemical markers of thrombosis in the setting of coronary balloon

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angioplasty. In this study, peptide levels were therefore measured before and after coronary angioplasty in blood withdrawn directly from the coronary artery. The results demonstrate that measurement of coagulation peptides from blood withdrawn through an angioplasty catheter is feasible and safe and suggest that angioplasty results in increased thrombin generation and activity in a subset of patients and that this subset is associated with a higher incidence of lesion complexity on postangioplasty angiography.

Methods

Pilot study. Although plasma activation peptide levels have generally been assayed in peripheral venous blood, our goal was to measure them in coronary artery blood collected through a coronary angioplasty catheter positioned near the site of balloon dilation. A potential advantage of this technique over peripheral venous sampling is that dilution of peptide levels with mixed venous blood is avoided. A potential disadvantage is that blood withdrawal through a long, narrow angioplasty catheter may itself elevate plasma levels of fibrinopeptide A and F_{1+2} . To determine the feasibility of blood withdrawal through an angioplasty catheter, a pilot study was therefore performed.

The pilot study included six patients treated with coronary angioplasty for stable angina. All patients received a daily dose of 325 mg of oral aspirin. Peptide levels were compared in each patient by means of four sets of samples. The first set (set 1) was withdrawn by venipuncture from the right antecubital fossa and the second set (set 2) by aspiration from the femoral artery sheath (USCI) (8F, 2.7-mm internal diameter, 11.5-cm length), both before heparinization. After systemic intravenous heparinization (10,000-U bolus, followed by infusion of 1,000 U/h), an angioplasty catheter was advanced to the ostium of the left main or right coronary artery, and a third set of samples (set 3) was aspirated through the angioplasty catheter (Baxter Scientific) (0.020-in. [0.5 mm] internal diameter, 135-cm length). A coronary guide wire was not used during advancement of the angioplasty catheter to the ostium of the vessel, and therefore set 3 was collected through the balloon catheter lumen with no guide wire present. A final set (set 4) was then collected from the left antecubital fossa by venipuncture. Set 4 was withdrawn immediately after set 3. The preheparin arterial samples (set 2) were not withdrawn through the angioplasty catheter because of the risk of blood clotting on the external surface of the balloon or within the catheter lumen.

Coronary angioplasty study. After a feasibility determination, patients treated for stable and unstable angina with coronary angioplasty at the Mount Sinai Medical Center Cardiac Catheterization Laboratory (New York) were considered for enrollment in the study. Exclusion criteria included acute myocardial infarction, use of a fixed wire balloon or coronary atherectomy catheter, hemodynamic instability, renal failure, hematocrit <34%, presence of an aortic aneurysm, enrollment in a thrombolytic trial for unstable angina and

failure to obtain informed consent. The protocol was approved by the Institutional Review Board of the Mount Sinai Medical Center, and all patients gave written informed consent.

In contrast to the pilot study, blood in the coronary angioplasty study was collected at three separate times. After baseline coronary angiography and before balloon insertion, the first set of samples (proximal to lesion before angioplasty) was withdrawn from the guiding catheter positioned in the ostium of the left main or right coronary artery. The second set of blood samples (distal to lesion before angioplasty) was withdrawn through the angioplasty catheter after the lesion had been crossed, before the balloon was inflated, to determine baseline fluctuations of plasma levels of fibrinopeptide A and F_{1+2} across the lesion. The coronary guide wire was removed before withdrawal of this set and then reinserted. If a patient experienced chest pain or hemodynamic instability after the lesion was crossed with the balloon catheter, the second set of samples was not collected. After successful dilation, the wire was again removed, and the third and complete set of samples (after angioplasty) was withdrawn through the angioplasty catheter positioned just distal to the site of the lesion. Angioplasty was performed using standard procedures as previously described (5).

As in the pilot study, all patients received 325 mg of oral aspirin and intravenous heparin (10,000-U bolus, followed by infusion of 1,000 U/h) before coronary angioplasty. Before blood samples were drawn, the activated clotting time was measured in the catheterization laboratory using the Hemochron (International Technidyne Corporation). Throughout the procedure, the rate of intravenous heparin was adjusted to maintain the activated clotting time >300 s.

Collection and processing of blood samples. All blood samples were obtained by two investigators (J.D.M., P.A.M.) using precooled plastic syringes preloaded with anticoagulant provided by the manufacturer of the kit used for radioimmunochemical determination of plasma fibrinopeptide A (Byk-Sangtec, Dietzenbach, Germany). No heparin-bonded catheters or sheaths were used in this study. Venous samples were collected by means of meticulous venipuncture using a 19-gauge butterfly without a tourniquet. The first 4 ml of venous blood was discarded. Arterial samples were collected from the femoral sheath and coronary angioplasty catheter after discarding 4 and 1 ml of blood, respectively. A total of 2.7 ml of blood was then withdrawn to determine the levels of fibrinopeptide A and F_{1+2} (blood/anticoagulant ratio 0.9:1 v/v). After collection of blood samples, plasma fractions were obtained by centrifugation at 4°C for 20 min at 2,500 rpm (1,600 g) and stored at -80°C.

Determination of plasma activation peptide levels. Fibrinopeptide A was measured by enzyme immunoassay in plasma extracted twice with bentonite to remove fibrinogen (Byk-Sangtec). This method has an interassay coefficient of variation of 5.4%. The plasma concentrations of F_{1+2} were determined by double-antibody radioimmunoassay, as previously described (12). The interassay coefficient of variation for this technique is

8%. Samples were analyzed without knowledge of the clinical data.

Angiographic analysis. Atherosclerotic lesions to be treated with coronary angioplasty were visualized angiographically in two orthogonal views before and after the procedure. Angiograms were interpreted by two experienced angiographers without knowledge of the results of the peptide assays. Lesions were characterized before angioplasty as complex or simple according to Ambrose et al. (13). Lesions were characterized after angioplasty by determining the presence or absence of angiographic filling defects (definite thrombus), translucency (possible thrombus) and dissection (a linear radiolucency adjacent to the site of dilatation) (5). A nonideal lesion morphology after angioplasty was defined as a lesion associated with dissection, translucency or filling defects.

Statistical analysis. Coefficients of skewness and kurtosis were calculated to test for deviations from a normal distribution. Because the coagulation peptide levels were found to be nonnormally distributed, nonparametric statistics were used. Paired data were analyzed by means of the Wilcoxon signed-rank test. Descriptive statistics include mean value $\pm$ SD or median and 95% confidence interval (95% CI). The normal range of values for plasma fibrinogen A and F_{1+2} in serial samples withdrawn from an angioplasty catheter in patients after heparinization is not known. To determine which patients exhibited a significant increase or decrease in plasma levels between two samples, we therefore defined the upper normal limit of change as the 95th percentile of the distribution of the changes between samples collected proximal versus distal to the lesion before coronary angioplasty (17 patients), which was 1.73 ng/ml for fibrinogen A and 0.11 nmol/liter for F_{1+2} . To calculate changes in peptide levels before and after angioplasty (ΔF_{1+2} , ΔFPA), the peptide plasma value in the samples drawn proximal to the lesion before angioplasty were subtracted from the plasma value of the corresponding peptide measured in the postangioplasty set of samples. All tests presented in this report were two-tailed. Plasma peptide concentrations for a group of patients were deemed significantly different from a reference group when both the median was outside the 95% CI of the reference group and $p < 0.05$ (14).

Results

Pilot study. The plasma levels of the activation peptides from the six patients enrolled in the pilot study are shown in Figure 1, in which each line connects data derived from an individual patient. Sets 1, 3 and 4 include peptide levels derived from all six patients. Set 2 includes data from four of the six patients. Compared with venous blood collected by venipuncture (set 1), preheparin fibrinogen A levels were significantly elevated in arterial blood withdrawn through the femoral sheath (set 2) (median 5.1 ng/ml [95% CI 2.2 to 13.7] vs. 32.8 ng/ml [95% CI 19.3 to 45.2], respectively, $p < 0.05$). This artifactual elevation in plasma fibrinogen A concentration was completely inhibited by the administration of

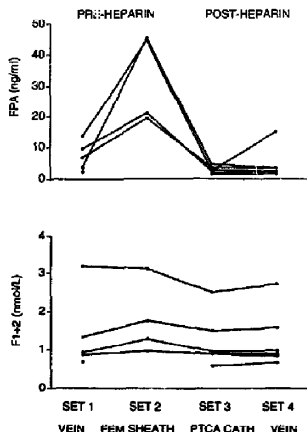


Figure 1. Pilot study. Serial plasma fibrinogen A (FPA) and prothrombin fragment F_{1+2} levels before (sets 1 [$n = 6$] and 2 [$n = 4$] and after (sets 3 and 4 [$n = 6$ for both]) heparin administration. Each line connects data points derived from the same patient. Blood was collected by venipuncture (VEIN), aspiration through a femoral artery sheath (FEM SHEATH) and by aspiration through an angioplasty catheter (PTCA CATH).

intravenous heparin. The fibrinogen A levels in samples withdrawn either from the catheter or from a peripheral vein after the patients received full anticoagulation with heparin were similar to those of venous samples withdrawn before catheterization and heparin administration (set 3, median 2.5 ng/ml [95% CI 1.5 to 4.6]; set 4, median 2.8 ng/ml [95% CI 1.5 to 4.9]; set 3 and set 4 vs. set 1, $p = NS$).

The plasma levels of F_{1+2} varied minimally as a function of the sampling conditions. Blood withdrawal through the arterial sheath before heparin (set 2) resulted in a small, statistically insignificant artifactual elevation of F_{1+2} levels (set 1, median 0.93 nmol/liter [95% CI 0.69 to 3.18] vs. set 2, median 1.54 nmol/liter [95% CI 0.96 to 3.10], $p = NS$). This elevation was eliminated by the immediate systemic administration of heparin. The concentration of F_{1+2} in postheparin angioplasty catheter blood (set 3) was similar to the venipuncture samples before (set 1) and after (set 4) heparinization (set 3, median 0.92 nmol/liter [95% CI 0.58 to 2.49]; set 4, median 0.94 nmol/liter [95% CI 0.67 to 2.71], $p = NS$).

After heparinization no significant difference in either fibrinogen A or F_{1+2} levels was observed between samples obtained by venipuncture and samples obtained through the coronary angioplasty catheter (set 3 vs. 4). These observations support the feasibility of accurately measuring the levels of

Table 1. Plasma Fibrinopeptide A and Prothrombin Fragment F_{1+2} Levels (median and 95% confidence interval) in 17 Patients With Samples Collected Both Proximal and Distal to the Lesion Before Coronary Angioplasty

Sample	FPA (ng/ml)	F_{1+2} (nmol/liter)
Before PTCA		
Proximal to lesion	1.6 (1.0-2.5)	0.39 (0.40-0.65)
Distal to lesion	1.5 (1.1-2.3)	0.45 (0.39-0.56)
After PTCA	1.8 (1.2-3.0)	0.57 (0.46-0.78)*

* $p < 0.05$ versus preangioplasty (distal to lesion). FPA = fibrinopeptide A; PTCA = coronary angioplasty.

coagulation peptides in coronary blood withdrawn through an angioplasty catheter in patients after heparinization.

Coronary angioplasty study. *Patient characteristics.* Between January 7 and March 9, 1992, 101 patients underwent percutaneous coronary revascularization at the Mount Sinai Medical Center in New York. Seventy-two patients were excluded from the study for the following reasons: participation in a thrombolytic trial (5) ($n = 26$), use of a fixed wire device ($n = 15$), atherectomy ($n = 10$), unavailability of technical staff ($n = 7$), systemic disease ($n = 5$), age > 80 years ($n = 4$), failure to obtain informed consent ($n = 3$), inability to cross the lesion with a balloon ($n = 1$) and presence of a thoracoabdominal aneurysm ($n = 1$). The remaining 29 patients were enrolled in the study. In three patients the levels of fibrinopeptide A after coronary angioplasty were > 20 ng/ml. These patients were excluded from the analysis because such levels are likely to represent artifacts and to interfere with the accurate measurement of F_{1+2} (unpublished data). Thus, the study included 26 patients (mean $\pm$ SD) age 57 ± 12 years; 19 men, 7 women).

Twenty of the 26 patients had a diagnosis of unstable angina. Of these 20, 9 had crescendo exertional angina (Canadian Cardiovascular Society class III), 9 had experienced rest

pain (class IV), and 2 had rest pain after a myocardial infarction within the previous month. All patients were treated with oral aspirin (minimal dose 325 mg on the day of coronary angioplasty). Patients with rest pain were treated with intravenous heparin that was stopped ~ 2 to 3 h before angioplasty.

Coagulation peptide levels. In all 26 patients, blood samples were collected proximal to the lesion before angioplasty and distal to the lesion after angioplasty. In 17 of these patients, samples were also collected before balloon inflation with the angioplasty catheter positioned distal to the lesion. Results from these 17 patients are summarized in Table 1. Before angioplasty, the plasma levels of fibrinopeptide A and F_{1+2} measured proximal to the stenosis were statistically indistinguishable from those measured distal to the stenosis. Compared with samples collected distal to the lesion before angioplasty, samples collected after angioplasty demonstrated a significant increase in F_{1+2} levels (median 0.45 nmol/liter [95% CI 0.39 to 0.56] vs. median 0.57 nmol/liter [95% CI 0.46 to 0.78], respectively, $p < 0.05$).

For all 26 study patients, the individual changes in F_{1+2} and fibrinopeptide A plasma levels during coronary angioplasty are shown in Figure 2. Compared with the levels seen before angioplasty (proximal to lesion), plasma levels of F_{1+2} after angioplasty were significantly elevated (median 0.47 nmol/liter [95% CI 0.40 to 0.50] vs. median 0.55 nmol/liter [95% CI 0.46 to 0.72], respectively, $p = 0.001$). In contrast, the plasma fibrinopeptide A levels before angioplasty (proximal to lesion) were similar to those found after the procedure (median 2.0 ng/ml [95% CI 1.3 to 2.2] vs. median 1.8 ng/ml [95% CI 1.3 to 3.0], $p = \text{NS}$).

Figure 2 demonstrates that although the overall changes in plasma F_{1+2} and fibrinopeptide A are minimal, there are a number of individual patients in whom a marked elevation in peptide levels is apparent. Because the normal range of values for our patients is unknown, we used data from the angioplasty

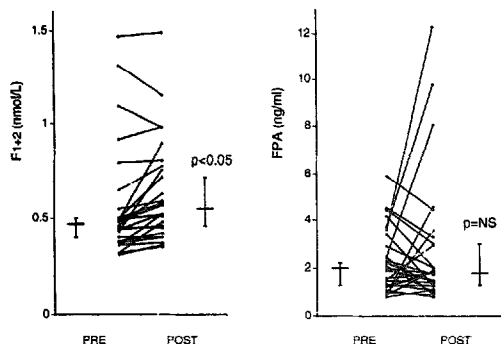


Figure 2. Coronary angioplasty study. Plasma levels of fibrinopeptide A (FPA) and prothrombin fragment F_{1+2} in coronary blood before (PRE) and after (POST) balloon angioplasty. Horizontal bars represent medians; error bars span the 95% confidence intervals.

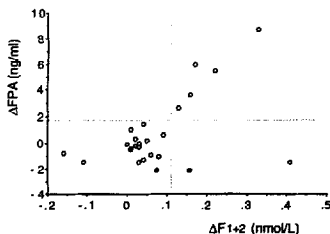


Figure 3. Coronary angioplasty study. Correlation of changes in plasma fibrinopeptide A (Δ FPA) levels with those in plasma prothrombin fragment F_{1+2} (Δ F $_{1+2}$) levels before versus after angioplasty. Stippled lines indicate the upper normal limits of change for repeated sampling. The two patients with a significant decrease in plasma fibrinopeptide A are indicated by solid circles. Each point is derived from a single patient ($n = 26$, $r = 0.5$, $p < 0.01$).

study to define as significant (for an individual patient) an increase in $F_{1+2} > 0.11$ nmol/liter and fibrinopeptide A > 1.73 ng/ml (see Statistical analysis). Of the 26 study patients, seven (27%) and five (19%) had a significant increase in plasma F_{1+2} and fibrinopeptide A levels, respectively (Fig. 3). Of the seven patients with an elevation in plasma F_{1+2} , five had a concomitant increase in plasma fibrinopeptide A. In two patients, an isolated increase in plasma F_{1+2} occurred without an increase in plasma fibrinopeptide A. No elevations in fibrinopeptide A occurred in the absence of an increase in plasma F_{1+2} . Peptide levels in patients who had increased plasma levels of fibrinopeptide A or F_{1+2} , or neither, are shown in Table 2.

For the 26 study patients, a significant correlation was found between the changes in plasma fibrinopeptide A and F_{1+2} levels before and after coronary angioplasty ($r = 0.5$, $p < 0.01$) (Fig. 3).

As shown in Figure 2, 15 patients had a decrease in plasma fibrinopeptide A after angioplasty. A significant decrease in plasma fibrinopeptide A (1.73 ng/ml) (see Statistical analysis) occurred in only two of these patients (Fig. 3).

Adequacy of heparinization. The degree of anticoagulation by heparin was assessed by measuring the activated clotting time (see Methods). In all patients an activated clotting time > 300 s was documented before blood was drawn for plasma peptide analysis. For all patients in the coronary angioplasty study, the mean activated clotting time was 398 ± 67 s (range 303 to 610). Activated clotting time in the 7 patients with a significant increase in plasma F_{1+2} was not different from that in the remaining 19 patients (418 ± 63 vs. 392 ± 69 s, $p = \text{NS}$). Similarly, activated clotting time in the 5 patients with a significant increase in plasma fibrinopeptide A did not differ from that in the remaining 21 patients (405 ± 59 vs. 397 ± 71 s, $p = \text{NS}$).

No correlation was found between activated clotting time and change in fibrinopeptide A or between activated clotting time and change in F_{1+2} ($r = 0.05$ and $r = 0.001$, respectively, $p = \text{NS}$).

Angiographic results and relation to coagulation peptide levels. Successful coronary angioplasty, as defined by a residual stenosis $< 50\%$, was achieved in all patients. Postangioplasty angiography revealed that 16 patients had a nonideal lesion morphology (dissection in 7, translucent lesion appearance in 7, filling defect in 2). These characteristics were absent in the remaining 10 patients. A greater increase in F_{1+2} levels after angioplasty occurred in patients with a nonideal lesion morphology (median increase 0.07 nmol/liter [95% CI 0.03 to 0.16]) than in those with a more desirable lesion morphology (median increase 0.02 nmol/liter [95% CI 0.00 to 0.07], $p < 0.05$) on postangioplasty angiography. The median increase in fibrinopeptide A was not significantly different between the two groups (-0.3 ng/ml [95% CI -1.5 to 2.6] vs. -0.2 ng/ml [95% CI -0.4 to 0.4], respectively).

Of the 16 patients with a nonideal lesion morphology on postangioplasty angiography, a significant increase in plasma F_{1+2} and fibrinopeptide A was detected in 6 and 4 patients, respectively. Activated clotting time showed no difference in extent of anticoagulation (397 ± 62 s vs. 401 ± 79 s, respectively, $p = \text{NS}$).

A nonideal lesion morphology on postangioplasty angiography was seen in 6 of the 7 patients with a significant increase in plasma F_{1+2} (vs. 10 of the 19 with no increase in plasma F_{1+2}) and in 4 of the 5 patients with a significant increase in

Table 2. Peptide levels (median and 95% confidence interval) in Patients Who Had Increased Plasma Fibrinopeptide A or Prothrombin Fragment F_{1+2} or Neither

Pt Group	No. of Pts	FPA (ng/ml)		F_{1+2} (nmol/liter)	
		Before PTCA	After PTCA	Before PTCA	After PTCA
Increased FPA	5	2.5* (1.0-3.7)	8.1† (3.6-12.3)	0.46‡ (0.32-0.65)	0.72§ (0.48-0.78)
Increased F_{1+2}	7	3.4 (1.0-5.9)	4.6 (1.3-12.3)	0.46‡ (0.32-0.65)	0.72§ (0.48-0.90)
No increase in FPA or F_{1+2}	19	1.6 (1.3-2.4)	1.5 (1.1-2.0)	0.48 (0.38-0.80)	0.52 (0.40-0.81)

* $p < 0.05$ versus plasma fibrinopeptide A (FPA) levels after angioplasty. † $p < 0.05$ versus plasma fibrinopeptide A levels before angioplasty. ‡ $p < 0.05$ versus F_{1+2} levels after angioplasty. § $p < 0.05$ versus F_{1+2} levels before angioplasty. Includes the five patients with increased plasma fibrinopeptide A levels. PTCA = coronary angioplasty; Pts = patient(s).

plasma fibrinopeptide A (vs. 12 of the 21 with no increase in plasma fibrinopeptide A, $p = NS$ for both groups).

Relation of coagulation peptide levels to clinical syndromes and preangioplasty angiographic lesion morphology. Neither the presenting clinical syndrome (i.e., unstable vs. stable angina, rest vs. exertional pain) nor the preangioplasty angiographic lesion morphology (i.e., complex vs. simple) was found to be associated with significantly greater or lesser baseline plasma levels or changes in plasma levels of F_{1+2} or fibrinopeptide A. Baseline levels and changes in plasma F_{1+2} levels were similar in the 11 patients with and the 15 patients without rest pain (rest pain present, median baseline F_{1+2} 0.46 nmol/liter [95% CI 0.36 to 1.09], median change in F_{1+2} 0.04 nmol/liter [95% CI 0.00 to 0.17]; rest pain absent, median baseline F_{1+2} 0.49 nmol/liter [95% CI 0.37 to 0.65], median change in F_{1+2} 0.24 nmol/liter [95% CI 0.01 to 0.16], $p = NS$). There was a slightly greater increase in plasma fibrinopeptide A levels in patients with than without rest pain (rest pain present, median change in fibrinopeptide A 0.2 ng/ml [95% CI -1.4 to 5.6]; rest pain absent, median change in FPA -0.3 ng/ml [95% CI -0.9 to 1.4], $p = NS$).

A complex lesion morphology on preangioplasty angiography was present in seven patients, five (71%) of whom had rest angina. Baseline plasma levels and changes in plasma levels of F_{1+2} and fibrinopeptide A were not significantly different in patients with versus those without a complex lesion morphology (complex morphology present, median baseline F_{1+2} 0.46 nmol/liter [95% CI 0.31 to 0.65], median change in F_{1+2} 0.08 nmol/liter [95% CI 0.04 to 0.41], median baseline fibrinopeptide A 3.7 ng/ml [95% CI 1.0 to 5.9], median change in fibrinopeptide A -1.1 ng/ml [95% CI -2.2 to 6.0]; simple morphology present, median baseline F_{1+2} 0.48 nmol/liter [95% CI 0.37 to 0.80], median change in F_{1+2} 0.03 nmol/liter [95% CI 0.01 to 0.09], median baseline fibrinopeptide A 1.5 ng/ml [95% CI 1.1 to 2.5], median change in fibrinopeptide A -0.1 ng/ml [95% CI -0.8 to 1.0], $p = NS$).

Discussion

The pathologic event that underlies the clinical syndromes of unstable angina and myocardial infarction is the disruption of an atherosclerotic plaque in association with a superimposed thrombus (15). Balloon angioplasty results in plaque fracturing and dissection (16) and therefore provides a model to study the events associated with coronary plaque rupture.

In its chronic, stable state the atherosclerotic plaque is covered with a nonthrombogenic endothelialized fibrous cap. Disruption of this cap permits circulating blood to come into contact with procoagulant elements within the plaque, such as collagen and tissue factor (17). This sequence of events has been presumed to be in part responsible for the development of thrombosis in atherosclerotic vessels (18).

The changes in coronary plasma F_{1+2} reported in this study demonstrate that the generation of thrombin within the coronary artery is detectable in a substantial portion of patients undergoing coronary angioplasty. Because an increase in

plasma F_{1+2} was seen only in samples collected after balloon inflation and not in samples collected after crossing the lesion but before balloon inflation, the generation of thrombin was most likely caused by balloon-mediated plaque injury. Additional evidence that the increase in plasma F_{1+2} was related to plaque injury is provided by the observation that the changes in F_{1+2} levels were greater in arteries that demonstrated dissection, translucency or filling defects on postangioplasty angiography. These angiographic morphologies have been associated with plaque disruption and thrombosis in both human and experimental models of vessel injury (4,5,15,19). The association of greater increases in plasma F_{1+2} with these morphologies suggests that plasma F_{1+2} may provide a marker for intracoronary thrombosis during angioplasty.

The generation of thrombin may have important implications in the vascular response to injury, independent of its activity on fibrinogen. Thrombin is a potent stimulant for platelets to aggregate and degranulate (20-22). In cultured vascular smooth muscle, thrombin acts as a growth factor, activating many of the growth-related genes and inducing cellular hypertrophy (23,24). Thrombin may therefore participate in the development of intimal hyperplasia and restenosis by its ability to stimulate smooth muscle proliferation, both directly through its effects on gene expression and indirectly through its effects on platelet accumulation. In the absence of endothelium, thrombin acts as a vasoconstrictor (25) and may therefore play a role in coronary vasospasm after angioplasty.

Although plasma fibrinopeptide A levels increased significantly in a number of individual patients, no significant change occurred in the overall group. This may reflect an inadequate sample size or the known ability of heparin to inhibit the generation of fibrinopeptide A in the presence of thrombin (10). The two patients in whom plasma fibrinopeptide A did not increase despite an increase in plasma F_{1+2} may represent an example of the greater inhibition that heparin exerts on thrombin activity than it does on Factor Xa activity (26). Alternatively, the amount of thrombin generated after plaque disruption in certain cases may be insufficient to result in fibrin formation, even in the absence of heparin. In every patient in whom an increase in plasma fibrinopeptide A did occur, a concomitant increase in F_{1+2} was documented. This suggests that the increase in plasma fibrinopeptide A levels seen in individual patients was not an artifact but represented new thrombin generation.

Heparin inhibits thrombin activity and has been shown to reduce the generation of fibrinopeptide A when added to human blood (10). Results of the pilot study demonstrate that heparin inhibits the increase in fibrinopeptide A that occurs during blood withdrawal through an arterial catheter (see Results). Patients in whom an increase in plasma fibrinopeptide A levels was observed after angioplasty could therefore represent a subgroup in which an inadequate dose of heparin was administered. To ensure that the coronary angioplasty study cohort had adequate anticoagulation, all patients were treated with a dose of heparin sufficient to maintain the activated clotting time >300 s, which is a level that has been

associated with a low incidence of both thrombotic closure and major in-hospital complications (27,28). Compared with patients with no significant increase in plasma peptide levels, patients with a significant increase in plasma levels of F_{1+2} or fibrinopeptide A had a similar prolongation of activated clotting time. Furthermore, there was no correlation between the changes in plasma peptide levels and activated clotting time. These findings suggest that the changes in F_{1+2} or fibrinopeptide A plasma levels after angioplasty were not caused by variations in the degree of heparinization.

The inhibitory effects of heparin on thrombin activity may in part explain why a decrease in fibrinopeptide A was seen in a number of patients (Fig. 2). In patients with thrombophlebitis and elevated plasma fibrinopeptide A, intravenous heparin has been shown to markedly suppress plasma fibrinopeptide A levels within 15 min of initiation of infusion (9). The reduction in plasma fibrinopeptide A seen in some patients in the angioplasty study may therefore represent a case in which the propensity for arterial balloon dilation to generate thrombin was overshadowed by the systemic inhibitory effect of heparin on thrombin activity and fibrinopeptide A generation.

Study limitations. Previous clinical (29) and experimental (30) studies suggest that thrombosis after vessel injury is a time-dependent phenomenon. In a study of 459 patients with unstable angina (5), only 4.5% were found to have a definite filling defect at the site of coronary angioplasty consistent with thrombus at angiography 1 min after angioplasty. On repeat coronary angiography performed 15 min later, 15.9% of patients had evidence of thrombus formation. Because in the present study samples were withdrawn early after successful balloon dilation, a later undetected increase in plasma fibrinopeptide A may have occurred.

Activation of the coagulation cascade is a surface-catalyzed event (31) that in the setting of angioplasty occurs on a damaged vessel wall. Coagulation peptides cleaved from their parent zymogens are likely to be found at a high concentration close to the site of injury and to be rapidly diluted by upstream arterial blood. Although we sampled coronary blood with the coronary angioplasty catheter in close proximity to the site of dilation, the catheter is a coaxial device with a tip that is designed to remain in the center of the arterial lumen. It is possible that more highly significant changes in F_{1+2} and fibrinopeptide A levels did occur, but only in the immediate vicinity of the dilated plaque, in areas close to the vessel wall that were inaccessible to the angioplasty catheter tip.

In contrast to the pilot study, in which blood was withdrawn almost immediately after introduction of the coronary angioplasty catheter in the patient, the angioplasty study involved blood withdrawal through a catheter that had resided in the patient for the duration of the procedure. Because activation of the clotting cascade is a time-dependent process (29), the increases in peptide levels observed in the angioplasty study may have resulted from artifactual activation of the clotting cascade within the catheter lumen or on the coronary guide wire. To minimize this possibility, the guide wire was removed, and the first milliliter of blood withdrawn through the angio-

plasty catheter was discarded before the samples were collected (see Methods). The angioplasty catheter material did not appear to possess potent thrombogenic properties because an increase in fibrinopeptide A levels was not seen in all cases, and no macroscopic clots were detected on inspection of the guide wire or during blood withdrawal.

Implications. This study demonstrates that balloon dilation of coronary artery stenoses induces thrombin generation in a subgroup of patients treated with coronary angioplasty. The extent of this induction appears to be related to the extent of vascular injury as assessed by angiography. In a certain proportion of cases, there is an associated generation of fibrin, suggesting that more potent inhibitors of thrombin may have a role in preventing angioplasty-induced activation of the hemostatic system. Further studies will be needed to determine the short- and long-term implications of thrombin generation and activity during angioplasty.

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