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Plasma Level Changes of Fibrinopeptide A after Uncomplicated Coronary Angioplasty

ANTONIS S. MANOLIS, M.D., F.A.C.C.,‡ HELEN MELITA-MANOLIS, M.D.,* CHRISTODOULOS STEFANADIS, M.D.,† PAVLOS TOUTOUZAS, M.D.,†

*Biochemistry and †Catheterization Laboratories, Departments of Microbiology and Cardiology, Hippokration Hospital, Athens University Medical School; ‡Catheterization Laboratory, Department of Cardiology, Tzaneio State Hospital, Athens, Greece

Summary: Fibrinopeptide A (FPA) is a small polypeptide cleaved from fibrinogen by thrombin, has a short half-life, and is considered a sensitive biochemical marker of thrombin activity, fibrin generation, and ongoing thrombosis. Increased plasma levels of FPA have been reported in various procoagulable and thrombotic medical and cardiovascular disorders, including acute myocardial infarction, unstable angina, and sudden cardiac death. However, activation of thrombosis by the arterial injury incurred during coronary angioplasty has not been systematically examined with use of plasma FPA measurements.

To detect and monitor activation of thrombosis by coronary angioplasty, plasma levels of FPA were obtained by venipuncture and measured by radioimmunoassay before, immediately after, 24 to 48 h later, and 1 and 3 months after uncomplicated coronary angioplasty. From December 1990 through June 1991, FPA was measured in 30 patients (28 men and 2 women, aged 54 ± 9 years) with coronary artery disease who were undergoing coronary angioplasty. The mean left ventricular ejection fraction was 55 ± 7%. The dilated vessel was the left anterior descending coronary artery in 20 patients (together with a second vessel in 2), the right coronary artery in 9, and the left circumflex in 1. The procedure was successful and free of major complications in all patients. Before angioplasty the FPA levels averaged 6.50 ± 1.18 ng/ml. Shortly after angioplasty they rose to 20.20 ± 7.91 ng/ml ($p = 0.08$) despite intravenous heparin. At 24 to 48 h and after heparin had been discontinued for at least 4 h, the mean FPA levels were significantly higher (32.33 ± 10.86 ng/ml) compared with baseline values ($p = 0.025$). At 1 month after the procedure, the FPA levels measured in 22 patients were lower but still elevated (20.25 ± 9.29 ng/ml), albeit nonsignificantly, compared with baseline values, and at 3 months they had fallen to baseline values (4.84 ± 2.20 ng/ml, $n = 11$). No patient developed restenosis during the study period of 1 to 3 months, during which all patients were receiving aspirin. We conclude that, as reflected by increased FPA levels, angioplasty, most likely due to arterial injury incurred, activates thrombin and generates ongoing coronary thrombosis, which is not suppressed by heparin or aspirin and appears to extend at least through the first month after the procedure.

Key words: fibrinopeptide A, coronary thrombosis, fibrin, coronary angioplasty, radioimmunoassay, restenosis

Introduction

Fibrinopeptide A (FPA) is a product of fibrinogen cleavage by thrombin with a short half-life of about 3 to 5 min and is considered a sensitive marker of fibrin generation and ongoing thrombosis.[1–4] Increased plasma levels of FPA have been measured in various procoagulable and thrombotic states including cardiovascular disorders, such as acute myocardial infarction, vasospastic and unstable angina.[4, 5–10] Coronary arterial injury incurred during coronary angioplasty (PTCA) may variably activate the thrombotic mechanism with serious imme-

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Address for reprints:

Antonis S. Manolis, M.D.
Co-Director
Cardiac Electrophysiology and Pacemaker Laboratory
Box 868
Tufts/New England Medical Center
750 Washington Street
Boston, MA 02111, USA

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diate and late consequences.[11–13] To detect and monitor activation of thrombosis by PTCA, serial measurements of FPA plasma levels were conducted in patients undergoing PTCA.

Patients and Methods

Patients

From December 1990 through June 1991, 38 patients with critical coronary artery stenosis undergoing elective PTCA in two hospitals were considered candidates for this prospective study. Blood was therefore drawn (see below) from these patients prior to PTCA and within the preceding 24 h. However, the final study group (Table I) included 30 patients (28 men and 2 women), aged 54 ± 9 years (range 26–66), who fulfilled the inclusion criteria of a successful and uncomplicated PTCA and absence of conditions known to be associated with elevated FPA levels, such as immunologic, malignant, infectious, or known thromboembolic disease. Also, patients with active ischemic events such as unstable angina or evolving acute myocardial infarction (MI) and patients with technical difficulties in blood sampling were excluded from this study.

All patients participating in the study had symptoms of either stable or unstable angina which, however, had been medically stabilized for > 48 h prior to PTCA with use of nitrates, beta blockers, and/or calcium antagonists. All patients had evidence of exercise-induced ischemia either during a regular treadmill test or on thallium scintigraphy. Eleven patients had a history of previous MI (3 weeks to 6.5 months earlier). Diagnostic coronary angiography had preceded PTCA as a separate procedure several (> 7) days or usually weeks earlier. The mean left ventricular ejection fraction was 55 ± 7% (range 40–63%).

TABLE I Clinical and procedural data in 30 patients undergoing coronary angioplasty and fibrinopeptide A plasma level assay

Men/women	28/2
Mean age (years)	54 ± 9
Angina class	
I–III	22
IV	8
Previous MI	11
Anterior	5
Inferior/posterior	6
LVEF (%)	55 ± 7
Dilated vessel	
LAD	20
RCA	9
LCx	2
RI	1
Initial percent stenosis (%)	87 ± 8
Residual percent stenosis (%)	18 ± 9

Abbreviations: LAD = left anterior descending coronary artery, LCx = left circumflex, LVEF = left ventricular ejection fraction, MI = myocardial infarction, RCA = right coronary artery, RI = ramus interrmedius branch.

Coronary Angioplasty

The procedure was performed via the femoral artery with use of standard techniques and systems (United States Catheters, Inc.-USCI, Advanced Cardiovascular Systems-ACS, Schneider, Medtronic). Intravenous heparin was used as a bolus injection of 10,000 to 15,000 U during the procedure and was then continued for up to 12–24 h as a constant infusion at 750 to 1200 U/h. Multiple balloon inflations with pressures up to 8–10 bar for a maximum of 60–120 s were performed. Antiischemic therapy with nitrates and calcium antagonists and antiplatelet therapy with aspirin were continued periprocedurally. Angioplasty was considered successful when the lesion was crossed and dilated; angiographically, the percent coronary artery stenosis was reduced to < 50% luminal diameter narrowing; and clinically, there occurred no death, myocardial infarction, angina, or new ischemic electrocardiographic changes during the hospitalization period. After hospital discharge the patients were routinely maintained on nifedipine and aspirin, while in some patients, nitrates, small doses of beta blockers, and hypolipidemic agents were also continued.

Single-vessel PTCA was performed in 28 patients (one lesion in 25 patients and two lesions in 3 patients) and double-vessel PTCA in 2 patients. The dilated vessel was the left anterior descending (LAD) coronary artery in 20 patients (together with the left circumflex or a ramus intermedius branch in 2 respective patients), the right coronary artery in 9, and the left circumflex in 1 patient (Table I). Percent critical coronary artery stenosis in 29 patients ranged from 75 to 99% and only 1 patient had total occlusion of the LAD. Residual stenosis after PTCA ranged from 0 to 35%.

Acquisition of Blood Samples and Assay of Fibrinopeptide A

Plasma levels of FPA were determined before PTCA, immediately after (while on heparin), 24–48 h later (while off heparin and > 12 h after vascular sheaths had been removed), and at 1 month after the procedure. In a smaller number of patients FPA was also measured at 3 months after PTCA. Using the same meticulous technique, blood samples for FPA determination were obtained from each patient by two of us (HM-M, ASM) with careful trauma-free venipuncture. The tourniquet was removed after the needle had been inserted into the vein. Free-flowing blood was collected into prepared 5 ml vacutainers containing 0.5 ml of FPA anticoagulant preparation including ethylene diamine-tetra-acetic (EDTA), aprotinin, and a thrombin inhibitor. Blood and anticoagulant were then thoroughly mixed by several gentle inversions. Within 15 min after collection the samples were centrifuged at 1500 rpm for 20 min. Plasma samples were thus obtained and were stored at −70°C until the FPA assay.

Quantitative determination of FPA was performed using the radioimmunoassay method of RIA-mat® FPA by Byk-Sangtec

Diagnostica (Dietzenbach, Germany). The specificity of this method is > 96%; the FPA antibody used in the test kit shows a cross reactivity to fibrinogen of < 4%; the fibrinogen in the plasma sample is completely removed by treatment with bentonite solution. With regard to sensitivity, the lower detection limit of the method is 0.1 ng/ml. Coefficients of variation obtained with different control samples yielded intra-assay variance between 3.5% for high values, and 6.46% for low values, and interassay variance between 4.72% for high values and 10.08% for low values.

Statistical Analysis

The FPA data are presented as mean ± SEM. The Student's paired *t*-test was used for comparison of pre- and postangioplasty FPA plasma level changes. The unpaired *t*-test was used to compare values between study patients and control subjects. A p value of < 0.05 was considered significant. Transformation of (not normally distributed) data into the natural logarithm was also performed and statistical analysis with parametric tests was repeated. Most normal FPA levels are < 2 ng/ml, and less frequently normal levels as high as 5.25 ng/ml have been measured.[1] In our laboratory, the mean normal plasma FPA level, as measured in 10 healthy volunteers, was 2.97 ± 0.76, with all values < 7.6 ng/ml and six values < 2.05 ng/ml (Figs. 1, 2). The higher values were confirmed twice.

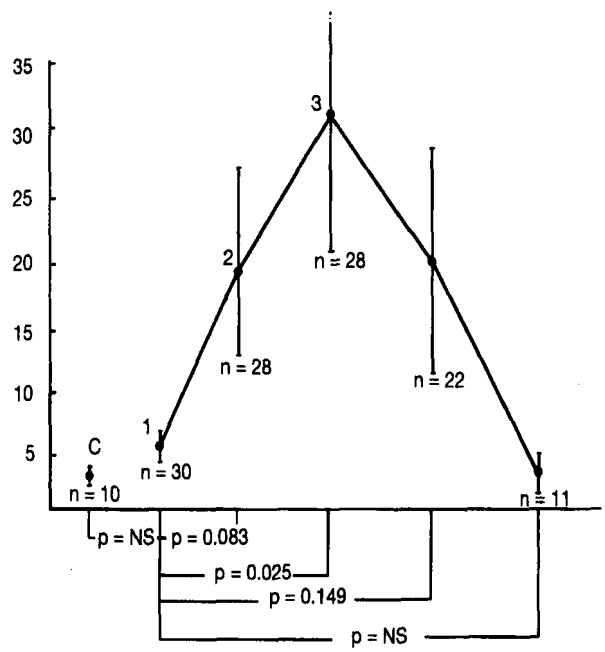

FIG. 1 Serial changes of plasma levels (ng/ml) of fibrinopeptide A after coronary angioplasty. Data are presented as mean ± SE. C = control group; 1 = baseline values before angioplasty; 2 = immediately after angioplasty; 3 = 24 to 48 h after angioplasty; 4 = 1 month after angioplasty; 5 = 3 months after angioplasty. NS = nonsignificant. With regard to statistical significance the results remained unchanged when data were logarithmically transformed for nonnormal distribution.

Results

The baseline mean FPA plasma level for all patients prior to PTCA was 6.50 ± 1.18 ng/ml, which was not significantly different from the value of 2.97 ± 0.76 ng/ml in the control subjects (Fig. 1). Immediately after PTCA, and while the patients had received intravenous heparin, the plasma levels of FPA increased to 20.20 ± 7.91 ng/ml (p = 0.083, or p = 0.122 for log-transformed data). At 24 to 48 h later, after heparin had been discontinued for at least 4 h and the sheaths had been removed for > 12 h, the FPA levels further increased to 32.33 ± 10.86 ng/ml (Figs. 1, 2; p = 0.025, or p = 0.001 for log-transformed data, when compared with baseline values). No abrupt vessel closures occurred during this study. Small inconsequential local dissection was observed in the patient who underwent PTCA of a totally occluded LAD. This patient had sustained an anterior MI 2.5 months earlier with subsequent development of exertional angina and reversible anterior wall ischemia visible by thallium imaging. Coronary arteriography showed total occlusion of the LAD at the mid segment with the distal vessel supplied by collaterals from the right coronary artery. The FPA level reached one of the highest values after PTCA: 198.85 ng/ml immediately after the procedure and 214.45 ng/ml 36 h later (Fig. 2). No major complication occurred post-PTCA. Two patients developed asymptomatic nonspecific T-wave changes.

The FPA plasma levels, measured at 1 month after PTCA in 22 patients, averaged 20.25 ± 9.29 ng/ml (p = 0.149, when compared with baseline values), while at 3 months, as measured in 11 patients, they had dropped to baseline levels (4.84 ± 2.20 ng/ml). After hospital discharge three patients developed recurrent effort angina. One of these three patients underwent repeat catheterization which failed to demonstrate restenosis. Plasma levels of FPA had returned to baseline in all three patients during the last measurement. Throughout the study period no patient developed evidence of thromboembolic disease.

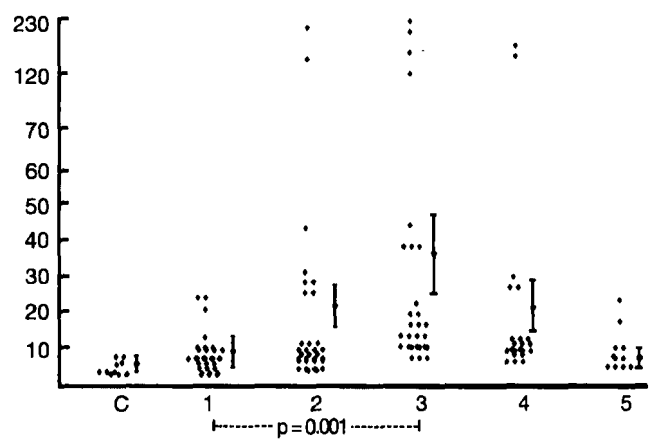

FIG. 2 The individual values of plasma levels (ng/ml) of fibrinopeptide A are plotted in this scatterdiagram during the different periods of measurement (see Fig. 1 and text). This p value was obtained from the analysis of the logarithmically transformed data.

Discussion

Percutaneous transluminal coronary angioplasty (PTCA) is an increasingly and widely applied, highly successful revascularization technique used as an alternative to coronary artery bypass grafting for patients with significant coronary artery disease. Local trauma of the coronary vessel with disruption of the atherosclerotic plaque and intra-arterial tearing of variable degree virtually accompanies all successful PTCA procedures.[14] This angioplasty balloon-induced arterial injury may, however, activate platelets and the coagulation system and generate intracoronary thrombus formation which, in some patients, may lead to abrupt vessel closure and perhaps also contribute to the mechanisms of restenosis.[11, 12]

Methods to detect such activation of thrombosis include coronary angiography or angioscopy to visualize intracoronary thrombus or assessment of biochemical or scintigraphic markers of platelet and thrombin activity.[15–18] Angiographic detection of intracoronary thrombi is quite limited as suggested by angioscopy studies, while angioscopy and scintigraphic methods using indium-111-labeled platelets are not readily available. Among the biochemical markers of platelet, thrombin, and plasmin activity, respective assays of beta thromboglobulin, fibrinopeptide A (FPA), and D-dimer have been employed to monitor the thrombotic mechanism in various conditions including stable, vasospastic, and unstable angina, acute MI and thrombolysis, and sudden cardiac death.[4–9, 19–22]

Fibrinopeptide A is a small polypeptide cleaved from fibrinogen when the latter is converted to fibrin by the action of thrombin. Its half-life is short (3–5 min). It is considered a reliable and sensitive biochemical marker of ongoing thrombosis. To avoid artifactual elevations of FPA levels in blood samples drawn through catheters,[23] particular attention must be paid to the technique of blood drawing. This was accomplished in our study by careful direct venipuncture without using catheters or intravenous tubing. D-dimer is a breakdown product of plasmin-mediated fibrinolysis. Since fibrinolysis occurs only after fibrin has been formed, D-dimer levels are an indirect marker of thrombotic activity (formation and lysis). Although not affected by blood collection techniques, as is the case in FPA, a major limitation of D-dimer assay is the prolonged half-life of this product (4–8 h) that may both overestimate and underestimate thrombotic activity by not reflecting recent or ongoing thrombus formation. Beta thromboglobulin is a specific marker of platelet activation (alpha-granule release) rather than thrombin generation, but may not be as sensitive and reliable a marker of thrombus activity as FPA.[9, 24]

Scarcely, and in a very limited and preliminary way, have these assays been employed to monitor thrombotic activity in response to PTCA.[18, 25] One study reported that thrombotic activity, as detected by increased D-dimer levels, is evident immediately after PTCA despite pretreatment with antithrombotic agents (aspirin, dipyridamole, and heparin).[18] Another report attempted to correlate FPA elevation and angiographically visible dissection occurring after PTCA.[25] However, FPA levels were also elevated in patients without dissection. A major limitation of that study was the absence of baseline pre-PTCA measurements.

The present study confirms and further extends these preliminary observations by providing serial FPA measurements at baseline, immediately (while on heparin), and 24 to 48 h (off heparin) after PTCA, at 1 month, and in a smaller number of patients at 3 months after the procedure. The pattern that became apparent was that of ongoing thrombosis immediately evident at the completion of PTCA despite heparin and aspirin administration, with maximal thrombotic activity detected prior to hospital discharge (24–48 h postangioplasty), but surprisingly with continuing thrombin activity still present at 1 month, and finally returning to baseline values at 3 months.

While interpreting the herein reported results, one should, however, consider potential limitations of the present study. The variability of plasma FPA levels is large; however, this is mostly a reflection of intersubject variability. Particular attention was given to the importance of meticulous sample collection for obtaining reliable data. The possibility that the initial postangioplasty plasma FPA rise could be due to arterial puncture or catheterization alone may be considered, but for the reasons detailed below this could not account for the persistent FPA elevation during the subsequent serial measurements. Finally, the importance of the follow-up data may be somewhat limited by the fewer patients available for FPA assay.

The finding of elevated FPA levels in serial measurements, despite the short half-life of this product, is important and suggests that the increase in thrombin activity was not transient as that caused by the intra-arterial sheath and catheter placement,[23, 25] but a persistent and ongoing process. Presumably due to localized thrombin activity, intravascular sheath and catheter insertion for either angiography or PTCA causes transient plasma FPA elevation which is promptly and fully suppressed by subsequent heparin administration.[25] Plasma FPA levels that are persistently high despite adequate anticoagulation indicate ongoing fibrin generation.[4] Thus, even if one considers the influence of cardiac catheterization per se on the FPA levels, with vascular trauma causing a transient artifactual increase during the period of the second sample received immediately after PTCA (although such an increase should have been prevented by full heparinization if only due to local causes), the significant FPA elevation noted later, prior to hospital discharge (third sampling period) and in the absence of any noncoronary vascular manipulation, provides evidence that the FPA elevation was due to elaboration of thrombin from the coronary vessel which was itself traumatized during the angioplasty procedure. This is further supported by the persistent although statistically nonsignificant FPA elevation still present 1 month later, finally returning to baseline levels at 3 months.

Conclusion

The important implications of this study are those of ongoing thrombosis initiated by PTCA and silently continuing for at least 1 month after the procedure despite conventional antithrombotic measures. Further studies are needed to examine

whether such ongoing thrombotic activity places patients at increased risk for restenosis and to explore other more potent or novel therapeutic interventions.

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