Dynamic Nature of Thrombin Generation, Fibrin Formation, and Platelet Activation in Unstable Angina and Non–Q-Wave Myocardial Infarction

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Abstract. Background: Thrombin and platelets are directly involved in arterial thrombosis, typically occurring at sites of atherosclerotic plaque rupture among patients with acute coronary syndromes. Understanding the dynamic nature of pathologic thrombosis has important clinical implications.

Methods: Fibrinopeptide A (FPA), thrombin-antithrombin complexes (TAT), and prothrombin activation fragment 1.2 (F1.2), plasma markers of fibrin formation (thrombin activity) and thrombin generation, and platelet activation, determined by the recognition of a surface-expressed platelet α-granule protein, P-selectin, using flow cytometry, were measured in 36 consecutive patients with unstable angina and non-Q-wave myocardial infarction participating in the Thrombolysis In Myocardial Ischemia (TIMI) III B trial.

Results: Thrombin generation (TAT 12.1 ± 17.5 ng/ml vs. 3.4 ± 1.0 ng/ml; F1.2 0.19 ± 0.14 nmol/1 vs. 0.12 ± 0.8 nmol/1), fibrin formation (FPA 15.8 ± 23.5 ng/ml vs. 7.5 ± 2.3 ng/ml), and platelet activation (10.6 ± 2.4% vs. 2.5 ± 2.0%) were increased significantly in patients compared with healthy, age-matched controls (p < 0.01). Fibrin formation, represented by plasma FPA levels, did not correlate with the percentage of activated platelets (r = −.10, p = 0.69). Thrombin generation and platelet activation also did not correlate. A statistically insignificant trend between TAT and platelet activation was observed (r = .42, p = 0.07); however, even with TAT levels in excess of 20 ng/ml (nearly sixfold greater than normal healthy controls) platelet activation was increased by only 1.7-fold.

Conclusions: Thrombin generation, fibrin formation, and platelet activation are increased modestly among patients with unstable angina and non-Q-wave myocardial infarction. Despite the involvement of platelets and coagulation proteins in arterial thrombotic processes, their relative contributions may vary, providing a pathophysiologic basis for the dynamic expression of disease and response to treatment observed commonly in clinical practice.

Rupture or fissuring of intimal plaques is known to occur frequently in the evolution of human atherosclerotic coronary artery disease. Plaque ruptures vary considerably in size and depth. While most are microscopic, others are extensive; both occur at the plaques periphery, where the fibrous cap is thinnest and most heavily infiltrated by foam cells (lipid-laden macrophages) [1,2].

Plaque rupture is common among patients with unstable angina and non–Q-wave myocardial infarction. Typically it is accompanied by varying degrees of mural thrombosis, coronary vasospasm, and intracoronary thromboembolism [1,2]. Of particular interest, careful microscopic examination of coronary thrombi has revealed a layered structure, suggesting strongly that repeated mural deposits and episodic growth, over time, cause a progressive decrease in the artery’s luminal dimensions [1]. However, recent studies have shown that atherosclerotic plaques differ markedly in their composition and vulnerability to rupture. Moreover, these differences in makeup translate to important differences in thrombogenicity following rupture and exposure of plaque components to circulating blood [3]. Accordingly, the clinical expression could be as diverse as the underlying pathologic events.

The importance of intracoronary thrombosis in unstable angina and non–Q-wave myocardial infarction

Key Words. acute coronary syndromes, thrombin, platelets

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Biochemical assays

Prothrombin fragment 1 + 2. Prothrombin fragment 1.2 (F1.2) was measured using the Dade F1.2 kit, according to the manufacturer’s instructions (Baxter Diagnostics, Deerfield, IL). This two-site ELISA uses both capture and signal antibodies specific for F1.2 antigenic sites. The coefficient of variation for this assay in our laboratory is 7.2% for a control material with a mean value of 0.4 nM.

Thrombin-antithrombin III complexes. Thrombin antithrombin III complex (TAT) was determined using the Ensaynost TAT micro immunoassay kit (Behringwerke AG, Marburg, Germany). This two-site immunoassay uses antithrombin antibodies as the capture reagent and anti-antithrombin III antibodies coupled to peroxidase as the signal generating reagent. We observed a coefficient of variation in this assay of 14.6%, using the control material supplied with the kit (mean value 9.6 ng/ml).

Fibrinopeptide A. Fibrinopeptide A (FPA) was measured using a RIA-MAT radioimmunoanalytical assay (ByK - Sangtec Diagnostics gmb H and Co, Vietzenbach, Germany). Blood was collected by venipuncture into prepared vacutainers containing 0.5 ml of FPA anticoagulant preparation. The samples were centrifuged at 1500 x g for 20 minutes within 1 hour of collection. Then 1 ml of bentonite solution was added to each sample to remove fibrinogen. To each tube, 100 ml ~125I-fibrinopeptide A solution was added and mixed by gentle vortexing. Then 100 ml fibrinopeptide A antiseraum was then added. The samples were incubated for 60 minutes at room temperature. Then 500 µl of goat-anti-rabbit-γ-gamma globulin in polyethylene glycol solution was added, and each sample was centrifuged at 1500 x g for 20 minutes. FPA concentrations in plasma were determined by a standard curve. The FPA antibody used has a cross reactivity to fibrinogen of <4%. The lower detection limit is 0.1 ng/ml.

Platelet activation using flow cytometry

The method used to detect the expression of P-selectin has been described previously [10]. Briefly, at the start of each day, 1.0 ml of 2.0% paraformaldehyde (Polysciences, Washington, PA) was placed in each of several 12 × 75 mm tubes (one tube for each patient), which were inverted, capped, and stored at 4°C. Fresh Tyrode’s buffer was also prepared, 3.0 ml for each anticipated sample (Tyrode’s buffer: 0.42 mM NaPi, 0.14 mM NaCl, 2.7 mM KCl, 12 mM NaHCO3, 1 mM MgCl₂, 2 mM CaCl₂, adjusted to pH 7.35). Blood samples were obtained by a trained phlebotomist from a peripheral vein using evacuated collection tubes. Care was taken to avoid vessel trauma, prolonged tourniquet time, and vigorous aspiration. Approximately 50 µl of whole blood anticoagulated with EDTA was placed into tubes containing paraformaldehyde and was mixed with a vortex mixer. The capped tubes were stored for 2 hours at room temperature (or overnight at 4°C). The cells were then centrifuged...