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 ± 0.11 nmol/l vs. 0.12 ± 0.8 nmol/l), fibrin formation (FPA 15.8 ± 23.5 ng/ml vs. 7.5 ± 2.3 ng/ml, 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). Thrombin formation, represented by plasma FPA levels, did not correlate with the percentage of activated platelets (r = -0.10, p = 0.69). Thrombin generation and platelet activation also did not correlate. A statistically insignificant trend between TAT and platelet activation was observed (r = 0.17, 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.

Key Words. acute coronary syndromes, thrombin, platelets

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

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is underscored by the effectiveness of treatment strategies inhibiting either thrombin [4] or platelet [5–7] activity. Although it has been assumed that thrombin and platelet activity operate equally and are directly and intimately linked [8], limited information on the relative contributions of each to the thrombotic process in acute coronary syndromes is available. Clearly, while thrombin and platelet activity are associated, should they act independently, at least in a relative sense within certain settings, diagnostic and treatment strategies would have to be tailored according to the predominant mechanistic driving force.

As a means to explore the dynamic nature of thrombin generation, fibrin formation, and platelet activation among patients with unstable angina and non-Q-wave myocardial infarction, a prospectively designed Thrombolysis in Myocardial Ischemia (TIMI III) ancillary study was undertaken.

Methods

Patient population

The specific aim of the TIMI III trial was to study the comprehensive management of patients with unstable angina and non-Q-wave myocardial infarction. All patients were required to have experienced an episode of rest pain presumed to be ischemic in origin and lasting 5 minutes or more within the prior 24 hours. In addition, patients were required to have evidence of coronary artery disease defined as (1) new or presumably new electrocardiographic evidence of myocardial ischemia, including ST-segment or T-wave shifts; (2) prior myocardial infarction; or (3) ≥70% luminal diameter narrowing of a major epicardial coronary artery on a prior angiogram. Patients with myocardial infarction within 21 days of hospital presentation were excluded, as were patients with previous coronary artery bypass grafting (at anytime) or coronary angioplasty within the prior 180 days. The study was approved by the Committee to Protect Human Subjects Participating in Clinical Research at the University of Massachusetts Medical School.

Biochemical assays

Blood was collected prior to treatment in evacuated collection tubes containing 50 μM D-Phe-Pro-Arg chloroethyl ketone (PPACK), 200 KIU/ml aprotinin, and 4.5 mM ethylenediamine tetraacetic acid (EDTA) (Haematologic Technologies, Essex Junction, VT) or EDTA alone. Platelet-poor plasma was prepared and used for the assays listed later. The samples were centrifuged for 1500 × g for 20 minutes within 1 hour of collection. The tubes used in TIMI III have been shown to inhibit effectively thrombin activity [9]. Plasma was stored at −70°C until the time of analysis.

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 Ensysgen 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 radioimmunological 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 × 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 antiserum was then added. The samples were incubated for 60 minutes at room temperature. Then 500 μl of goat-anti-rabbit-y-gamma globulin in polyethylene glycol solution was added, and each sample was centrifuged at 1500 × 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