Biochemical Markers of Coagulation Activation in Mitral Stenosis, Atrial Fibrillation, and Cardiomyopathy

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Summary: Advances in the understanding of the biochemistry of the mechanism of hemostasis have led to the development of sensitive methods for determining levels of markers which reflect thrombin activity (thrombin-antithrombin III complex, fibrinopeptide A, F1+2 fragment), active fibrinolysis (D-dimer, plasmin- α 2-plasmin inhibitor complex), and platelet activity (platelet factor 4, β -thromboglobulin) in vivo. Measurement of these markers may be useful in identifying patients with various cardiovascular disorders at high risk of thromboembolism.

Key words: embolism, coagulation, fibrinolysis, platelet, mitral stenosis, atrial fibrillation, cardiomyopathy

Introduction

Advances in our knowledge of the biochemistry of coagulation activation in recent years have facilitated the development of sensitive and specific assays capable of detecting platelet activation, the generation of coagulation enzymes, and products of intravascular fibrin formation or dissolution in human circulation.¹ In this review article, we discuss the usefulness of biochemical markers of coagulation activation for assessment of hypercoagulability in various cardiovascular disorders.

Assay for Coagulation Activation

Although the blood coagulation cascade is classically described as functionally independent extrinsic and intrinsic pathways that separately activate factor X and generate thrombin, it seems likely that the extrinsic pathway is the most important in vivo. The extrinsic pathway is initiated when factor VII binds to tissue factor, an integral membrane glycoprotein expressed constitutively by subendothelial components of the vessel wall. Initiation of the intrinsic system is thought to occur with damage to the endothelium and the resulting exposure of subendothelial components such as collagen to the blood. Factor Xa generated by either of the two cascades then converts prothrombin to thrombin by binding to factor Va on activated platelets (Fig. 1).

During thrombin generation, the amino terminus of the prothrombin molecule is released as the inactive F1+2 fragment. Once evolved, thrombin converts fibrinogen into fibrin, thereby releasing fibrinopeptide A and B, or it can be inhibited by the endogenous heparin sulfate-antithrombin III mechanism to form thrombin-antithrombin III complex, a stable enzyme-inhibitor complex. F1+2 fragment, fibrinopeptide A and thrombin-antithrombin III complex can thus be used as indices of thrombin generation in vivo.

The fibrinolytic system consists of a series of activators and inhibitors that regulate the conversion of plasminogen to plasmin. The major activators of plasminogen are tissue type plasminogen activator and urokinase, both of which are inactivated by the circulating inhibitor, plasminogen activator inhibitor. Free circulating plasmin is inactivated by α 2-plasmin inhibitor, forming a plasmin- α 2-plasmin inhibitor complex. On the other hand, fibrin formed from fibrinogen is hydrolyzed by plasmin, releasing D-dimer. Thus, both plasmin- α 2-plasmin inhibitor complex and D-dimer levels reflect fibrinolytic status.

Platelets form a crucial part of both the hemostatic plug and thrombus. Circulating platelets contain protein secretory granules known as α -granules. When platelets are activated by various stimuli such as thrombin, adenosine diphosphate (ADP), epinephrine, or arachidonic acid, platelet factor 4 and β -thromboglobulin are released from α -granules. Thus, plasma levels of these platelet-specific proteins reflect platelet activity.

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FIG. 1 Pathways and biochemical markers of coagulation activation. FPA = fibrinopeptide A, TAT = thrombin-antithrombin III complex, PAI = plasminogen activator inhibitor, TPA = tissue plasminogen activator, $\alpha 2$ PI = $\alpha 2$ -plasmin inhibitor, PIC = plasmin- $\alpha 2$ -plasmin inhibitor complex, PF-4 = platelet factor 4, β -TG = β -thromboglobulin, FDP = fibrin degradation product.

Immunoassays have been developed for the above new molecular markers, and these markers with higher sensitivity for evaluating thrombotic and fibrinolytic status and assessing platelet activity have made it possible to evaluate such activities in patients with valvular heart disease, ischemic heart disease, deep vein thrombosis, pulmonary embolism, and cardiomyopathy.²⁻⁸ Measurement of levels of these markers may be useful in identifying patients at high risk of thromboembolism.

Clinical Applications

Mitral Stenosis

Systemic thromboembolism is a serious complication in patients with valvular heart disease, and its incidence is highest in those with mitral stenosis.⁹⁻¹⁵ The incidence of embolism in large groups of such patients is ~4% per year.¹⁶ The Framingham Study¹⁷ reported an 18-fold increase in the incidence of stroke in patients who had mitral stenosis and atrial fibrillation compared with normal control subjects.

Previously, Asakura *et al.*⁷ demonstrated that patients with mitral stenosis and atrial fibrillation showed a highly hypercoagulable status. Adams *et al.*¹⁸ evaluated 84 patients with mitral stenosis followed up for 20 years and found that patients without anticoagulation had a higher mortality rate than those with anticoagulation. Although there have been no prospective randomized trials to evaluate the efficacy of anticoagulation in the primary prevention of systemic embolism in mitral stenosis, anticoagulation is generally recommended for treating patients with mitral stenosis, particularly those with atrial fibrillation, a large left atrium or heart failure, or patients with a history of systemic embolism.¹⁹ In vivo activation of blood coagulation was more effectively controlled in patients who received warfarin than in those taking as-

pirin; however, the incidence of thromboembolism still cannot be disregarded with those medications.

Thus, we investigated plasma levels of biochemical markers for the status of thrombin generation, fibrinolysis, and platelet activity in blood from the peripheral vein and the right and left atria of 12 consecutive patients with rheumatic mitral stenosis who were undergoing percutaneous mitral valvuloplasty.² Eleven patients had chronic atrial fibrillation, and one had sinus rhythm before valvuloplasty. All 11 patients with atrial fibrillation had received warfarin [international normalized ratio (INR) 2.0-4.0], but no antiplatelet drugs. Levels of fibrinopeptide A and thrombin-antithrombin III complex, indices of thrombin generation, were significantly higher in the left atrium than in the peripheral blood or right atrium of the patients. On the other hand, plasma levels of D-dimer and plasmin- $\alpha 2$ -plasmin inhibitor complex, which reflect active fibrinolysis, and platelet factor 4 and β -thromboglobulin, used to reflect platelet activity, did not differ significantly among the peripheral blood and the right and left atria of the patients.

These results suggest that coagulation activity is significantly increased in the left atrium of patients with mitral stenosis even during anticoagulation therapy with warfarin, which might contribute to the pathogenesis of thromboembolism in this disorder. Therefore, monitoring of peripheral INR levels may not be accurate enough to evaluate the coagulation activity in the cardiac chamber of patients with valvular heart disease. Our observations also suggest that antiplatelet agents such as aspirin are not recommended for the prevention of thromboembolism in patients with mitral stenosis, because platelet activity was not significantly increased in the left atrium of these patients.

Cardiomyopathy

Thromboembolic events are serious complications in patients with idiopathic cardiomyopathy.²⁰⁻²⁶ The incidence of

stroke was reported to be ~3% per patient year in hypertrophic cardiomyopathy.²¹ Systemic or pulmonary embolism is more common in patients with hypertrophic cardiomyopathy and atrial fibrillation.^{20, 21} On the other hand, at least 11% of patients with dilated cardiomyopathy suffer from one or more embolic events during the course of their illness,²⁵ and mural thrombus is more frequently observed in the left ventricle than in the left atrium.²⁷

Controversy continues over the usefulness of anticoagulants in treating patients with idiopathic cardiomyopathy. Kyrle *et al.*²⁴ reported that arterial or pulmonary embolism occurred in 17 of 38 patients with dilated cardiomyopathy before initiation of oral anticoagulant therapy, but no episode of systemic embolism occurred during the period of oral anticoagulant therapy, thus demonstrating the usefulness of anticoagulant therapy in dilated cardiomyopathy. However, no prior study indicated the usefulness of anticoagulant therapy in patients with hypertrophic cardiomyopathy.

We measured levels of hemostatic molecular markers in 13 patients with hypertrophic cardiomyopathy and 17 patients with dilated cardiomyopathy.²⁸ All patients had normal sinus rhythm and no history of systemic emboli, and were receiving no hemorrhologically active agents. Plasma levels of fibrinopeptide A and thrombin-antithrombin III complex in patients with hypertrophic cardiomyopathy and dilated cardiomyopathy significantly exceeded those in normal subjects. On the other hand, plasma levels of platelet factor 4, β -thromboglobulin and plasmin- α 2-plasmin inhibitor complex did not differ significantly between normal subjects and cardiomyopathic patients.

We then investigated the relationship between elevated coagulation activity and left atrial diameter in patients with hypertrophic cardiomyopathy. Plasma levels of fibrinopeptide A and thrombin-antithrombin III complex in these patients correlated significantly with left atrial diameter. On the other hand, in patients with dilated cardiomyopathy, plasma levels of fibrinopeptide A and thrombin-antithrombin III complex correlated positively with left ventricular end-diastolic volume and correlated negatively with fractional shortening of the left ventricle.

These findings indicate that thrombin activity, not platelet activity, is augmented in patients with hypertrophic cardiomyopathy and dilated cardiomyopathy, and may be linked to left atrial dilation in patients with hypertrophic cardiomyopathy, and to left ventricular enlargement and dysfunction in patients with dilated cardiomyopathy. These findings support the usefulness of anticoagulants in patients with hypertrophic cardiomyopathy with left atrial enlargement, and in patients with dilated cardiomyopathy with enlarged left ventricle and dysfunction to prevent the development of intracardiac mural thrombi.

Atrial Fibrillation

Atrial fibrillation is the most common cardiac condition associated with presumed embolic stroke, accounting for almost half of the total incidence of cardiogenic embolic

stroke.^{14, 29, 30} The Framingham Study¹⁷ reported a 6-fold increase in the incidence of stroke in patients with atrial fibrillation compared with age-, gender-, and blood pressure-matched control subjects. Recent clinical trials^{31–33} have shown that low-dose warfarin therapy significantly reduces the incidence of stroke in patients with nonvalvular atrial fibrillation. Thus, prophylactic administration of anticoagulant agents appears to be indicated for selected patients with atrial fibrillation. However, the efficacy of platelet antiaggregating agents such as aspirin in preventing stroke in patients with atrial fibrillation has not been demonstrated convincingly.^{34, 35} In the Copenhagen Atrial Fibrillation, Aspirin, Anticoagulation (AFASAK) study,³⁴ 1,007 patients were assigned randomly to receive warfarin, aspirin (75 mg/day), or placebo. The incidence of stroke, transient ischemic attack, and systemic embolism did not differ significantly between the aspirin and placebo groups at the end of 2 years. However, in the Stroke Prevention in Atrial Fibrillation (SPAF) study,³⁵ in which 1,330 patients were allocated randomly to receive warfarin, aspirin (325 mg/day), or placebo, ischemic stroke and systemic embolism or death were significantly (32%) reduced by the administration of aspirin. The differing results of the AFASAK and SPAF studies may be related to the doses of aspirin used.

Therefore, we measured plasma levels of molecular markers for platelet activity and the status of thrombin generation and fibrinolysis before and after the administration of aspirin (330 mg/day, the same dose as in the SPAF study) for 14 days in patients with nonvalvular atrial fibrillation.³⁶ Basal levels of platelet factor 4 and β -thromboglobulin did not differ significantly between patients with atrial fibrillation and normal subjects. After 14 days of aspirin therapy, plasma levels of these markers in the patients had decreased significantly. On the other hand, basal levels of fibrinopeptide A and thrombin-antithrombin III complex were significantly higher in patients with atrial fibrillation than in normal subjects. However, no significant reductions in plasma levels of these markers in the patients were observed on Day 14 of aspirin treatment.

These findings demonstrate that thrombin activity is augmented in patients with nonvalvular atrial fibrillation. Although aspirin (330 mg/day) did not affect the coagulation system, it significantly suppressed the platelet activity. These findings also suggest that the effectiveness of aspirin in the SPAF study may have been due to the suppression of platelet activity, not to the suppression of thrombin generation, although we detected no increased platelet activity in patients with atrial fibrillation. Alternatively, the effectiveness of aspirin may have been due to the prevention of cerebral thrombosis, but not cardiogenic embolism. These results also support the premise that the administration of an anticoagulant is more appropriate than that of aspirin for preventing systemic embolism in patients with nonvalvular atrial fibrillation.

Conclusion

Advances in our understanding of the biochemistry of the mechanism of hemostasis have led to the development of sen-

sitive methods for measuring levels of peptides, enzyme-inhibitor complexes, or enzymes that are liberated with the activation of the coagulation system in vivo. Studies employing these markers have indicated a biochemical imbalance between procoagulant and anticoagulant mechanisms in the blood of humans prior to the appearance of thrombotic phenomena. Properly designed prospective studies will be required to determine whether these assay techniques will enable us to identify individuals who are entering a clinically relevant hypercoagulable state and intervene with appropriate therapy prior to the onset of overt thrombotic disease.

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