Activation of Clotting Factors XI and IX in Patients With Acute Myocardial Infarction


Abstract—In acute coronary events, plaque rupture and the subsequent formation of the catalytic tissue factor–factor VIIa complex is considered to initiate coagulation. It is unknown whether clotting factors XI and IX are activated in acute coronary events. Therefore, we prospectively investigated the activation of clotting factors XI and IX as well as activation of the contact system and the common pathway in 50 patients with acute myocardial infarction (AMI), in 50 patients with unstable angina pectoris (UAP), and in 50 patients with stable angina pectoris (SAP). Factor XIa–C1 inhibitor complexes, which reflect acute activation of factor XI, were detected in 24% of the patients with AMI, 8% of the patients with UAP, and 4% of the patients with SAP (P<0.05), whereas factor Xla–α1-antitrypsin complexes, which reflect chronic activation, were observed equally in all 3 study groups. Factor IX peptide levels were significantly higher in the patients with AMI and UAP compared with the patients with SAP (P<0.01). No differences regarding markers of the common pathway were demonstrated. Fibrinopeptide A levels were elevated in patients with AMI compared with patients with UAP and those with SAP (P<0.01). Factor XIIa– or kallikrein–C1 inhibitor complexes were not increased. In conclusion, this is the first demonstration of the activation of clotting factors XI and IX in patients with acute coronary syndromes. Because these clotting factors are considered to be important for continuous thrombin generation and clot stability, their activation might have clinical and therapeutic consequences. (Arterioscler Thromb Vasc Biol. 2000;20:2489-2493.)

Key Words: coagulation ■ thrombosis ■ myocardial infarction ■ cardiovascular disease

Thrombus formation on a fissured coronary atherosclerotic plaque plays a fundamental role in the onset of acute coronary events, such as unstable angina pectoris (UAP) and acute myocardial infarction (AMI).1 In addition to activated platelets, fibrin contributes significantly to thrombus formation, and reperfusion with fibrinolytic agents reduces the mortality associated with myocardial infarction.2 Fibrin formation is thought to be triggered by the formation of tissue factor (TF)–factor VIIa complexes. TF is found in the atherosclerotic plaque itself, and expression of TF after plaque rupture facilitates the formation of the catalytic TF–factor VIIa complex.3–5 On exposure to blood, this complex activates clotting factors X and IX, leading to the generation of thrombin and formation of fibrin.6

The role of the contact system is less defined in this process. Although activation of the contact system, ie, factor XII and prekallikrein, has been suggested to occur in acute coronary syndromes, direct evidence (such as obtained by specific activation markers) is lacking.7–9

In 1991, it was demonstrated that thrombin is capable of activating factor XI in vitro,10,11 and this would imply a factor XII–independent activation of factor XI. Thrombin-dependent activation of factor XI constitutes an amplification pathway, because activation of factor XI leads, via the activation of factors IX and X, to the formation of additional thrombin.12 This additional thrombin may be important for the activation of a carboxypeptidase B, called thrombin-activatable fibrinolysis inhibitor (TAFI), resulting in the attenuation of fibrinolysis.13–15 Hence, thrombin-dependent activation of factor XI may be a critical element in the onset of acute coronary syndromes.

To study the role of factor XI in coronary vascular disease, we used sensitive assays for detecting the activation of factor XI, factor IX, and the contact system in patients with AMI or UAP compared with patients with stable angina pectoris (SAP).

Methods

Patients

Consecutive patients with characteristic chest pain within the last 12 hours before presentation at the emergency room were eligible for the present study.
UAP was defined by the presence of at least 1 episode of ischemic pain during rest (Braunwald IIIB classification, “primary” UAP), accompanied by diagnostic ST-segment shift or T-wave changes, but without enzymatic evidence of AMI (creatine kinase-MB levels less than twice the upper limit of normal). AMI was defined as the combination of characteristic chest pain, ECG changes, and creatine kinase-MB levels twice the upper limit of normal. Patients with chronic SAP from the outpatient clinic, characterized by exercise-induced ischemic chest pain or proven coronary artery disease on angiogram, without changes in symptoms in the previous 3 months, served as a control group.

Exclusion criteria were the use of heparin or fibrinolytic therapy before blood sampling, thrombotic diseases, or surgery in the past 3 months or oral anticoagulant medication. Use of aspirin was not an exclusion criterion.

The study was approved by the institutional review board of the Academic Medical Center, and written informed consent was obtained from all patients.

**Blood Sampling and Assays**

On admission, venous blood samples for the determination of prothrombin fragment 1+2 (F1+2) were collected with minimal stasis in sodium citrate (0.105 mol/L) Vacutainer tubes (Becton Dickinson), and those for fibrinopeptide A (FPA) were collected in siliconized Vacutainer tubes (Becton Dickinson) containing the provided anticoagulant mixtures provided in the kits. Plasmas for the factor Xla, factor XIIa, and kallikrein complex assays were collected in siliconized Vacutainer tubes containing EDTA 0.34 mol/L (Becton Dickinson), to which a solution of Polybrene (0.05% [wt/vol] final concentration, Janssen Chimica) and benzamidine (100 mol/L final concentration, Acros) was added. The factor IX and factor X activation peptides were assessed from blood collected in siliconized Vacutainer tubes containing the following anticoagulant: 38 mmol/L citric acid, 75 mmol/L sodium citrate, 136 mmol/L dextrose, 6 mmol/L EDTA, 6 mmol/L adenosine, and 25 U/mL heparin. Platelet-poor plasma was obtained by centrifuging at 1600g for 30 minutes at room temperature. Plasma samples were immediately frozen on dry ice and stored at −70°C until assayed.

Activation of factor XI in the samples was measured by assessing levels of factor Xla–C1 inhibitor and factor Xla–α1-antitrypsin complexes as described. In these sandwich-type ELISAs, microtiter plates are coated with monoclonal antibody (mAb) XIa-5, directed against the heavy chain of factor XI. Complexes between factor Xla and C1 inhibitor or α1-antitrypsin, present in the plasma samples to be tested, are detected with mAbs against C1 inhibitor or α1-antitrypsin, respectively. In the plasma of normal volunteers, levels of these complexes are below the detection limit of 10 pmol/L for these assays. Complexes between factor XIIa and kallikrein with C1 inhibitor were measured with ELISAs, which were modified from radioimmunoassays.

In short, microtiter plates were coated with a mAb against factor XIIa, present in the plasma samples to detect 0.02% activation of the plasma concentration of the respectivezymogens. As an in-house standard, dextran sulfate–activated EDTA, 75 mmol/L sodium citrate, 136 mmol/L dextrose, was used. Activation of factor XI in the samples was measured by assessing levels of factor Xla–C1 inhibitor and factor Xla–α1-antitrypsin complexes as described. In these sandwich-type ELISAs, microtiter plates are coated with monoclonal antibody (mAb) XIa-5, directed against the heavy chain of factor XI. Complexes between factor Xla and C1 inhibitor or α1-antitrypsin, present in the plasma samples to be tested, are detected with mAbs against C1 inhibitor or α1-antitrypsin, respectively. In the plasma of normal volunteers, levels of these complexes are below the detection limit of 10 pmol/L for these assays.

Complexes between factor XIIa and kallikrein with C1 inhibitor were measured with ELISAs, which were modified from radioimmunoassays. In short, microtiter plates were coated with a mAb against complexed C1 inhibitor and incubated with the samples to be tested. Bound complexes were detected with biotinylated mAbs against factor XII or kallikrein. These assays are specific and able to detect 0.02% activation of the plasma concentration of the respectivezymogens. As an in-house standard, dextran sulfate–activated EDTA plasma, which was calibrated against purified factor XII– or kallikrein–C1 inhibitor complexes, was used. Normal values are &lt;60 pmol/L for factor XIIa–C1 inhibitor complexes and &lt;350 pmol/L for kallikrein–C1 inhibitor complexes.

The factor IX and factor X peptide were assayed with double-antibody radioimmunoassays as described. These assays measure the fragment that is liberated from factor IX and factor X during activation. F1+2 fragment was measured by use of a commercially available kit according to the manufacturer’s instructions (Enzymost F1+2, Behringwerke). FPA levels were measured with a double-antibody radioimmunoassay (Byk-Santec).

**Statistical Analysis**

Patient characteristics are given as mean±SD; differences between groups were analyzed by χ² tests. Results of the measured coagulation parameters are presented as median (range), and differences between the individual study groups were analyzed by the Mann-Whitney U test. Factor IX and X peptides are also presented as median (range) but were analyzed, after logarithmic transformation, by the Student t test. Correlations between observations were analyzed by the Spearman rank correlation test and are presented as correlation coefficients (r values). A 2-sided value of P&lt;0.05 was considered statistically significant.

**Results**

**Patients**

In total, 50 patients with AMI and 50 patients with UAP were included in the study. The control group consisted of 50 patients with SAP. Table 1 shows the clinical characteristics of the patients. The groups were similar in age, sex, and risk factors for atherosclerosis, such as family history, cholesterol levels, diabetes, and hypertension. Smoking was more common in the SAP and AMI groups compared with the UAP group (P&lt;0.05). As can be expected, antianginal medication on admission was different between the groups, as was prior cardiology history, with a higher frequency of previous myocardial infarction or coronary bypass surgery in patients with SAP than in patients with UAP or AMI. The use of aspirin was not an exclusion criteria because a direct effect of aspirin on the levels of coagulation markers has not been demonstrated (H. ten Cate, K.A. Bauer, R.D. Rosenberg, unpublished data, 1989). By use of the Spearman rank correlation test, no correlation between factor Xla–C1 inhibitor levels and aspirin use was found (r=0.06, P=NS).

There was no difference in time between the first symptoms of chest pain and time of admission (ie, collection of blood samples) between the AMI and UAP group (295±233 minutes and 287±210 minutes, respectively; P=NS).

**Activation of Factor XI**

There were significantly more patients with elevated factor Xla–C1 inhibitor complexes in the AMI group than in the
Factor XIa (FXIa)–C1 inhibitor (C1inh) and FXIa–α1-antitrypsin (α1AT) complexes in patients with AMI, UAP, or SAP. In 50 patients with AMI, who presented at the emergency room, blood samples were collected before any therapy was given. Fifty patients with SAP served as a control group. FXIa-inhibitor complexes were measured by ELISA. Normal value of both assays is $\leq 10$ pmol/L.

UAP ($P=0.02$) and SAP ($P=0.006$) groups (Figure). Twelve patients in the AMI group (24%) had levels above the normal value of 10 pmol/L for this assay. Five patients in the UAP (10%) group and 2 patients in the SAP group also had elevated levels of factor XIa–C1 inhibitor complexes ($P=NS$).

The levels of factor XIIa–α1-antitrypsin complexes did not differ between the study groups. Nine patients with AMI (18%) had levels above the normal value of 10 pmol/L for this assay. Five patients in the UAP (10%) group and 2 patients in the SAP group also had elevated levels of factor XIIa–C1 inhibitor complexes ($P=NS$).

Activation of Factor IX

The median levels of the factor IX peptide were 354.4 pmol/L in the AMI group, 316.5 pmol/L in the UAP group, and 259.2 pmol/L in the SAP group (Table 2). Although the differences in the AMI and UAP groups were not statistically different, both groups had significantly higher levels of the activation peptide factor IX compared with the levels in the SAP group ($P<0.01$). No significant correlations with factor XIa–C1 inhibitor or factor XIa–α1-antitrypsin complexes were observed.

Activation of the Contact System

In 3 patients with AMI and in 2 patients with UAP, factor XIIa–C1 inhibitor complexes were above the normal value of 60 pmol/L. Factor XIIa–C1 inhibitor complex levels in these patients were 61, 94, and 372 pmol/L for the 3 AMI patients and 81 and 100 pmol/L for the 2 UAP patients. None of the patients with SAP had detectable factor XIIa–C1 inhibitor complexes ($P=NS$, results not shown).

Kallikrein–C1 inhibitor complexes were detectable in 2 patients with AMI (64 and 85 pmol/L), in 3 patients with UAP (60, 70, and 90 pmol/L), and in 1 patient with SAP (68 pmol/L; $P=NS$, results not shown). Because the normal value of these complexes is $<350$ pmol/L, none of the patients had elevated kallikrein–C1 inhibitor complexes.

Activation of the Common Pathway

The concentration of the factor X peptide was not different between the study groups; furthermore, no significant difference was observed in the levels of F1+2 between the groups (Table 2). In contrast, FPA levels differed significantly between patients with AMI and UAP ($P=0.002$) and between patients with AMI and SAP ($ P<0.001$). The FPA
levels between patients with UAP and SAP were also significantly different ($P=0.002$).

There was no significant correlation between the factor XIa–C1 inhibitor or factor XIa–α1-antitrypsin complexes and F1+2, factor X peptide, or FPA levels. Factor X peptide and F1+2 correlated with the factor IX peptide levels in all groups, with $r=0.63$ ($P<0.001$) and $r=0.35$ ($P<0.001$), respectively.

**Discussion**

The development of coronary thrombosis in response to the rupture of atherosclerotic plaques is the primary determinant of the evolution of stable atherosclerotic disease to UAP and AMI. The exposure of TF and subsequent activation of the intrinsic pathway of coagulation leads to the generation of thrombin and formation of fibrin. However, the extrinsic pathway is rapidly inhibited by TF pathway inhibitor, and continuation of thrombin formation is dependent on the feedback activation by thrombin of the clotting factors V, VIII, and (probably also) factor XI. We investigated whether activation of factors XI and IX would be detectable in acute coronary events.

Activated factor XI was measured as a complex with the C1 inhibitor as its major inhibitor and was indeed detected in 24% of the patients with AMI, which was significantly greater than the percentage found in patients with UAP or SAP. Thus, the formation of a coronary thrombus may be reflected by relatively high levels of factor XI activation in some patients, apparent as factor XIa–C1 inhibitor complexes. However, factor XIa–C1 inhibitor complexes were not more elevated in patients with UAP compared with the control group. This likely reflects the well-known clinical variability of this syndrome, with (despite strict inclusion criteria) difficulty in assessing the beginning of the “instability.” No difference in factor XIa–α1-antitrypsin complexes was demonstrated among the study groups. Factor XIa–α1-antitrypsin complexes are cleared at a much slower rate than are factor XIa–C1 inhibitor complexes; thus, they are more easily detectable. Complexes between factor XIa and its dominant inhibitor (the C1 inhibitor) can only be measured within hours after the initiation of clotting activation.

Indeed, factor XIa–α1-antitrypsin complexes have been demonstrated in patients 7 to 10 days after a myocardial infarction and in patients with coronary artery disease. The lack of difference in levels of factor XIa–α1-antitrypsin complexes between the study groups might be caused by the relationship between the extent of coronary atherosclerosis and clotting activity.

The present study clearly demonstrates, for the first time, the involvement of clotting factor IX in acute coronary syndromes. Although a correlation between factor XI and factor IX activation was not observed, it is likely that factor XI generation contributed to factor IX conversion, as shown in a study in primates. Moreover, discordant plasma half-life times between factor XIa–C1 inhibitor complexes and the factor IX peptide and the activation of factor IX directly via the TF–factor VIIa complex influence the plasma levels of the factor IX peptide. Also, under experimental conditions, such as the controlled administration of tumor necrosis factor to normal subjects, temporal dissociation in the activation of coagulation markers has been demonstrated.

In the patients with SAP, the IX peptide levels were slightly higher (geometric mean 267.7 pmol/L) compared with levels found in another study in 654 men (geometric mean 204.4 pmol/L) without a history of unstable angina pectoris or myocardial infarction but with a high risk of fatal coronary heart disease.

In contrast to the enhanced factor XI and factor IX activation in patients with acute coronary events, activation markers of the common pathway of coagulation, ie, the factor X peptide and F1+2, were in the same range in all 3 study groups. FPA, a marker of thrombin activity, was significantly higher in AMI and UAP patients than in the control group. This confirms the results of other studies, indicating that FPA is a sensitive marker of acute coronary thrombosis. The lack of difference in markers of factor Xa and thrombin generation may reflect a difference in assay sensitivity.

In the revised model of coagulation, factor XI is activated by thrombin, whereas factor XII is believed to have a profibrinolytic function rather than initiating coagulation via the intrinsic pathway. This is illustrated by several case reports, linking a deficiency of factor XII with myocardial infarction and depression of factor XII–dependent fibrinolytic activity with risk of reinfarction. We could not detect any significant activation of factor XII or prekallikrein in either study group, suggesting no role for factor XII in the activation of factor XI in patients with coronary artery disease. Using another, very sensitive, assay for factor XIIa, Coppola et al also did not detect enhanced activation of factor XII in 58 patients with myocardial infarction and 28 patients with UAP compared with an age-matched control group. Our results seem to be in contrast with those of Hoffmeister et al, who demonstrated activation of the contact system in patients with UAP. However, patients in that study were included after recurrent symptoms of angina pectoris at rest, and 98% of the patients were already being treated with drugs, indicating that these patients were more likely to be in a postacute than in an acute phase of their disease.

In conclusion, the present study demonstrates increased activation of factor XI and factor IX in a subset of patients with AMI and UAP. This activation can have important implications, inasmuch as activation of factors XI and IX may have a function in the consolidation of clot formation. Continuous generation of thrombin not only leads to fibrin formation but also contributes to enhanced stability of the fibrin clot via activation of TAFI. Recently, Klement et al have demonstrated that inhibition of TAFI activity is capable of significantly potentiating tissue plasminogen activator–induced lysis of an arterial thrombus in a rabbit model, an important finding with possible clinical implications in the future.

Furthermore, inasmuch as clot-bound thrombin contributes to the activation of factor XI, these results underline the importance of therapeutic strategies that inhibit the activity of clot-bound thrombin in the management of patients with acute coronary events.

**Acknowledgments**

M.C.M. is supported by a grant from the Netherlands Heart Foundation. H.T.C. is an established investigator of the Netherlands Heart Foundation.
References


Activation of Clotting Factors XI and IX in Patients With Acute Myocardial Infarction
M. C. Minnema, R. J. G. Peters, R. de Winter, Y. P. T. Lubbers, S. Barzegar, K. A. Bauer, R. D. Rosenberg, C. E. Hack and H. ten Cate

Arterioscler Thromb Vasc Biol. 2000;20:2489-2493
doi: 10.1161/01.ATV.20.11.2489

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/20/11/2489

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/