Prolonged Activation of Prothrombin on the Vascular Wall After Arterial Injury

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Abstract—This study was designed to characterize the relative roles of bound Xa/Va and thrombin activity in vascular wall procoagulant activity after balloon-induced injury and the extent to which intravenous aspirin and heparin attenuate procoagulant activity associated with the vascular wall. Abdominal aortic injury was induced in rabbits by overinflation and multiple passages of a 4F embolectomy catheter. Rabbits were killed 15 minutes or 4, 8, 24, 48, 72, 96, or 120 hours after injury. Aortic segments were incubated ex vivo to define bound procoagulant activity. Thrombin activity bound to the aorta was detected by 4 hours after injury and was most marked over the first 24 hours, as estimated by increases in concentration of fibrinopeptide A during incubation of segments with recalcified barium-adsorbed plasma or activity against the thrombin-synthetic substrate S-2238. Based on comparison with purified human thrombin incubated under the same conditions, a maximum of 0.04 to 0.1 nmol/L per square centimeter of thrombin activity was associated with the vascular wall during the first 24 hours and remained detectable for 72 hours. In contrast, bound Xa/Va complex activity to injured segments was detected within 15 minutes and induced activation of prothrombin added to recalcified barium-adsorbed plasma incubated with injured segments for 96 hours. Aspirin (15 mg/kg) administered 30 minutes before injury attenuated 111In-platelet deposition at 4 hours by 67%, with an associated decrease in bound Xa/Va and thrombin activity at 15 minutes and 4 hours. However, intravenous heparin did not attenuate bound Xa/Va activity at 15 minutes or thrombin activity at 15 minutes and 4 hours. Platelet-dependent bound Xa/Va activity occurs rapidly after arterial injury and may promote thrombin elaboration for up to 96 hours. Bound thrombin activity and de novo thrombin elaboration on the vascular wall may play an important role in the progression of thrombosis and vascular wall remodeling. (Arterioscler Thromb Vasc Biol. 1998;18:250-257.)

Key Words: thrombin • factor Xa • thrombosis • platelets • tissue factor

The initial response to arterial injury involves platelet adhesion and activation, as well as activation of the coagulation system.1-3 The subsequent development of a thrombus is dependent on platelet and fibrin recruitment to the injured vascular wall, which is mediated primarily by local thrombin elaboration. We and others have shown that local thrombin elaboration is dependent on tissue factor–mediated activation of coagulation.4-6 Tissue factor, a membrane-associated glycoprotein, serves as a cofactor for factor VIIa–mediated activation of factor X.3 Accumulation of platelets and fibrin at the site of arterial injury, even when thrombus is not grossly apparent, also mediates the progression of thrombosis by binding activated coagulation factors.5-8 We have shown that arterial thrombi induce marked activation of the coagulation system attributable to platelet-associated Xa/Va activity and thrombin bound to fibrin.5 However, the extent to which procoagulant factors bind to the injured vessel wall, particularly in the absence of significant mural thrombus, and the duration of procoagulant activity associated with the vascular wall after arterial injury are not well defined.

In a previous study, we demonstrated that tissue factor exposed by balloon-induced deep injury of the aorta in rabbits promoted activation of the coagulation system for up to 24 hours after injury.5 We also found evidence for binding of enzymatically active thrombin to the vascular wall after injury that contributed to the procoagulant activity in the first 24 hours. However, the contribution of other coagulation factors was not specifically assessed. We hypothesized that the Xa/Va complex expressed by platelets that bind to the vascular wall after injury may be an important determinant of thrombin elaboration.6,8,10,11 Accordingly, the objectives of this study were to characterize the functional activity of Xa/Va and thrombin associated with the vascular wall after balloon-induced injury to the rabbit aorta and the extent to which the activity of these factors contributes to procoagulant activity over time. In addition, we characterized the extent to which aspirin and heparin attenuate vascular wall-associated procoagulant activity after injury.

Methods

Preparation of Coagulation Factors and Plasma Samples

Purified coagulation factors were prepared in our laboratory in collaboration with Dr Joseph Miletich. Human prothrombin and
factor X were isolated from pooled citrated plasma and were purified by affinity chromatography against the other vitamin K–dependent proteins, as previously described. Prothrombin and factor X preparations were incubated with 5 mmol/L d-Phe-d-Pro-L-Arg chloromethylketone (PPACK; Calbiochem) to inhibit trace amounts of contaminating active proteases. These preparations were then extensively dialyzed against 0.02 mol/L HEPES, 0.15 mol/L NaCl, pH 7.4 (HEPES saline), to remove free d-Phe-d-Pro-L-Arg chloromethylketone. Thrombin was prepared by activation of prothrombin with Taipan snake venom, followed by purification with monos-S chromato-
graphy. Activated factor X (factor Xa) was prepared by incubation of factor X with purified factor X coagulant protein from Russell’s viper venom at a 1:1 ratio by weight in HEPES saline containing 0.002 mol/L CaCl2, pH 7.4, at 37°C for 60 minutes. Tick anticoag-
ulant peptide (TAP) was a generous gift from Dr George Vlasuk (Corvas, San Diego, Calif). Recombiant desulfathirudin was from Ciba-Geigy (Basel, Switzerland).

Pooled, citrated human plasma was purchased from the American Red Cross (St Louis, Mo). Plasma was depleted of vitamin K–dependent factors by addition of 0.1 mol/L BaCl2 at 4°C for 60 minutes (barium-adsorbed plasma). Plasma was centrifuged to separate the precipitate, the supernatant was recovered, and additional BaCl2 precipitate was allowed to form at 4°C. The centrifugation step was repeated and the supernatant was then exhaustively dialyzed against buffer containing 0.15 mol/L NaCl, 0.012 mol/L sodium citrate, pH 6.0, and stored as 10-mL aliquots at −70°C. All plasma samples were quickly thawed at 37°C immediately before use.

Aortic Injury in the Rabbit

Animal studies were approved by the Animal Studies Committee at Washington University and conform with the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health publication 93–23, revised 1985). New Zealand White rabbits (3 to 4 kg), fed a normal diet, were anesthetized with intramuscular ketamine (65 mg/kg) and xylazine (0.75 mg/kg). The animals were prepared for surgery as previously described. Briefly, deep arterial injury was induced with a 4F Fogarty embolectomy catheter (Baxter Pharmaceuticals) inserted through a left femoral arteriotomy and advanced into the abdominal aorta proximal to the renal branches. The balloon was overinflated with saline with use of a tuberculin syringe to obtain a wedge position, and the balloon volume was then decreased by approximately 0.05 mL to permit withdrawal of the catheter to the level of the distal abdominal aorta. The procedure was repeated twice. Control sham-operated rabbits were subjected to the same surgical procedure, with the exception that the balloon was not inserted through the exposed femoral artery.

The rabbits were killed at 15 minutes or 4, 8, 24, 48, 72, 96, or 120 hours after balloon overinflation–induced injury of the aorta. Initially, a midline incision was made under anesthesia, and the abdominal aorta was exposed and collateral branches were ligated. A catheter was then introduced into the ascending thoracic aorta, which was flushed with 500 mL of saline buffer at a pressure of 100 mm Hg before the aorta was excised. These washing conditions were shown in preliminary studies to be extremely stringent and resulted in no visible adherent clot. The aorta was maintained in phosphate-buffered saline until luminal procoagulant activity was assayed, as described below.

Some animals were randomly assigned to receive intravenous infusions of either heparin (heparin sodium, LyphoMed) administered as a bolus (150 U/kg) followed by a continuous infusion (50 U/kg per hour) (n = 6) or aspirin (Maggioni-Winthrop) administered as a bolus (15 mg/kg) followed by an infusion (15 mg/kg) for 30 minutes (n = 6).

In animals given heparin, the activated partial thromboplastin time (aPTT) was measured with a Coag-A-Mate XC automated coagula-
tion timer (Organon Technica). In animals given aspirin, platelet aggregation in response to addition of collagen (5 μg/mL) to platelet-rich plasma was measured with a ChronoLog aggregometer (ChronoLog Corporation).

Characterization of Extent of Arterial Injury

In each animal, a 1.5-cm segment of the injured abdominal aorta was collected to assess the extent of arterial damage. Segments were fixed in 4% paraformaldehyde and embedded in paraffin; serial sections (5 μm) were collected at 100-μm intervals and stained with Verhoeff-van Gieson’s stain for elastic tissue. At least four serial sections from each dilated arterial segment were examined by two of the investiga-
tors (Drs Abendschein and Eisenberg), who were blinded to the treatment assignment. The extent of injury was defined as deep (ie, involving the media) if one or more areas of the internal elastic lamina were ruptured in each section examined.

Characterization of Procoagulant Activity on the Luminal Surface of Aortic Segments

The aorta was cut into two or three segments as quickly as possible after perfusion from the rabbit. Each aortic segment was cannulated with silicone elastomer tubing at either end (0.062-in internal diameter, 0.125-in external diameter; Medical Division, TPI) such that approximately 1.5 cm of the luminal surface of the segment was exposed between the ends of the tubing. The area of exposed lumen was directly measured at the end of each experiment by transsection-

ing only the segment between the cannulated ends. Segments were then cut longitudinally, traced, and digitized, and the luminal area was quantified with the use of an image analysis program on a Macintosh PC (Image 1.5, NIH). The cannulated segment was placed in a water bath at 37°C, and the tissue was flushed with 5 mL of phosphate-

buffered saline. The segment was then sequentially incubated with one or more of the plasma preparations or chromogenic substrates de-

scribed below.

We have previously shown that rabbit thrombin induces proteolysis of human fibrinogen and that compared with human tissue factor, rabbit tissue factor–induced coagulation of human plasma induces similar degrees of thrombin elaboration. In addition, we have shown that rabbit Xa/Va activates human prothrombin and that activation is completely abolished with TAP (data not shown).

To characterize thrombin activity associated with the injured vessel wall, segments were incubated for 30 minutes with either recalcified (25 mmol/L CaCl2, final concentration) barium-adsorbed plasma, after which the concentration of fibrinopeptide A (FPA) was measured to define fibrin formation induced by bound thrombin, or with 333 μmol/L (final concentration) S-2238 (H-D-Phe-Pip-Arg-p-ni-

trouamine; Chromogenics) in 200 μL of 0.05 mol/L Tris–HCl, 0.175 mol/L NaCl, and 0.002 mol/L CaCl2, pH 7.8, after which bound thrombin activity was assayed. Proteolysis of S-2238 was measured by the increase in absorbance at 405 nm in an automated microplate reader at 37°C (ThermoMax Molecular Devices). The activity of thrombin bound to vessels against the chromogenic substrate was compared with activity of purified human α-thrombin against the same substrate and under the same conditions. To confirm the specificity of the assay for vascular wall–bound thrombin activity, selected segments were incubated with either barium-adsorbed plasma or S-2238 in buffer in the presence of recombinant desulfathirudin.

To define Xa/Va activity bound to the vessel wall, aortic segments were incubated with recalcified (25 mmol/L CaCl2, final concentration) barium-adsorbed plasma repleted with 0.9 μmol/L human prothrombin, and concentrations of FPA were measured after a 30-minute incubation. Under these conditions, increases in FPA were attributable to both the activity of preformed thrombin associated with the segment (which was determined by incubation without added prothrombin) and thrombin elaborated by activation of the added prothrombin by bound Xa/Va. Accordingly, increases in FPA attrib-
utable to Xa/Va activity alone were determined by subtracting the increases in FPA observed after incubation with barium-adsorbed plasma alone from increases observed in the presence of added prothrombin. Bound factor Xa/Va–mediated activation of prothrom-
bin was also assessed in a purified system by incubation of injured segments with 0.9 μmol/L prothrombin in 200 μL of 0.05 mol/L Tris–HCl, 0.175 mol/L NaCl, and 0.002 mol/L CaCl2, pH 7.8. Aliquots of the incubation mixture were removed and assayed for thrombin activity by incubation with 333 μmol/L S-2238 in the same buffer, as described above. To confirm the specificity of the assay for vascular wall–bound Xa/Va activity, selected segments were incubated with barium-adsorbed plasma containing prothrombin in the presence of TAP, a specific Xa inhibitor.
Selected injured aortic segments were incubated in 200 μL of 0.05 mol/L Tris-HCl, 0.175 mol/L NaCl, and 0.002 mol/L CaCl₂, pH 7.8, containing 50 mmol/L purified human factor X to determine whether procoagulants capable of activating factor X are bound to the injured vessel wall (ie, IXa/VIIa or VIIa/tissue factor). Factor Xa activity was determined after a 30-minute incubation by adding S-2222 (N-benzoyl-l-Ilc-Glu-Gly-Arg-p-nitroanilide, Chromogenics) to a final concentration of 450 μmol/L and measuring the change in absorbance at 405 nm.

**11In-Platelet Deposition After Arterial Injury**

To characterize platelet deposition after arterial injury, platelets from separate donor rabbits were washed and labeled with 11In, as previously described. The radiolabeled platelets were injected into the rabbits 1 to 2 minutes before the aortic injury, and the aorta was perfused in situ and excised after 4 hours as described above. Radioactivity associated with vessel segments was counted with the use of a gamma counter. The luminal area was directly measured as described above. The number of platelets per square centimeter adhering to the aortic segment was calculated from the radioactivity and platelet count of the injected platelet suspension, corrected for the luminal area of the injured aortic segment. 11In-labeled platelets were also injected into control, sham-operated animals to determine the binding of platelets on the uninjured aorta.

**Radioimmunoassay of FPA**

Thrombin activity in barium-adsorbed plasma, with or without added prothrombin, was characterized by elaboration of fibrin in plasma determined by measuring changes in the concentration of FPA, a peptide released from fibrinogen by thrombin. Plasma was adsorbed with bentonite before assay of FPA. Concentrations of FPA were measured with a polyclonal antisemur-based commercial radioimmunoassay (Byk Sangtec), and results were compared with a standard curve prepared by use of purified human FPA as previously described.

**Statistical Analysis**

Data are reported as the mean ± SE. FPA data were log transformed before statistical analysis to normalize the distribution of the data. Comparisons between all conditions were analyzed by ANOVA (Statview 4, Abacus Concepts) on a Macintosh PC with the use of Scheffe’s test to compare differences between individual time points; a level of P < .05 was considered indicative of a significant difference.

**Results**

**Procoagulant Activity Bound to the Vessel Wall Immediately After Arterial Injury**

Injury resulting in multiple disruptions of the internal elastic lamina was documented in all animals. FPA was not increased when uninjured segments from sham-operated rabbits were incubated with barium-adsorbed plasma. Thrombin activity associated with the injured vessel wall was not detected in aortic segments acquired 15 minutes after balloon hyperinflation, judging by the lack of significant increase in concentrations of FPA in barium-adsorbed plasma incubated with injured aortic segments compared with concentrations in uninjured segments obtained from control, sham-operated rabbits (Fig 1). The lack of significant bound thrombin activity was also confirmed by a negligible proteolysis of the chromogenic substrate for thrombin when incubated with the injured segments (Table 1). However, within the first 15 minutes after injury, significant Xa/Va activity was associated with the same injured segments, as indicated by marked elaboration of FPA in barium-adsorbed plasma containing 0.9 μmol/L human prothrombin (P < .0001; see Fig 2) and by activation of prothrombin in the buffer system (Table 2). There were no increases in FPA when recalcified barium-adsorbed plasma containing prothrombin was incubated with uninjured segments. Furthermore, there was no activation of the same preparation of prothrombin incubated with uninjured segments in the buffer system containing calcium. These results indicate that activation of prothrombin required incubation with injured aortic segments and was not attributable to the presence of active coagulation proteases in either the prothrombin preparation or the barium-adsorbed plasma.

**Bound Thrombin Activity Over 120 Hours After Injury**

Significant increases in thrombin activity associated with the vascular wall were detected by 4 hours after aortic injury and persisted until 24 hours (Fig 1 and Table 1). Increases in FPA were identical in a subset of segments acquired 4 hours (n = 3) after incubation with nonrecalcified barium-adsorbed plasma (290 ± 298 ng/mL) compared with incubations in the same plasma in the presence of calcium (247 ± 66 ng/mL, P = NS).

**TABLE 1. Thrombin Activity in Uninjured or Injured Aortic Segments, as Characterized by Fibrin Formation (Fibrinopeptide A) or Amidolytic Activity Against S-2238**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Fibrin Formation, nmol · L⁻¹ · cm²</th>
<th>Amidolytic Activity, nmol · L⁻¹ · cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninjured</td>
<td>ND (n = 17)</td>
<td>0.03 ± 0.001 (n = 3)</td>
</tr>
<tr>
<td>15 min after injury</td>
<td>ND (n = 65)</td>
<td>0.04 ± 0.01 (n = 4)</td>
</tr>
<tr>
<td>4 h after injury</td>
<td>0.04 ± 0.01 (n = 11) *</td>
<td>0.1 ± 0.02 (n = 6) *</td>
</tr>
<tr>
<td>8 h after injury</td>
<td>0.05 ± 0.03 (n = 8) *</td>
<td></td>
</tr>
<tr>
<td>24 h after injury</td>
<td>0.05 ± 0.02 (n = 10)</td>
<td></td>
</tr>
</tbody>
</table>

ND indicates not detectable.

*P < .01 vs uninjured segments and 15 minutes after injury.
also a modest but significant increase in concentrations of FPA attributable to bound thrombin activity on segments acquired at 72 hours (Fig 1). By comparison with increases in FPA induced in the same plasma incubated under similar conditions with purified human thrombin, the activity of thrombin associated with the vessel wall was estimated to be 0.04±0.01 nmol/L per square centimeter at 4 hours, 0.05±0.03 nmol/L per square centimeter at 8 hours, and 0.05±0.02 nmol/L per square centimeter at 24 hours (Table 1). The concentrations of thrombin associated with injured vessels 4 hours after injury were somewhat higher when determined by amidolytic assay and compared with activity of purified human thrombin (0.1±0.02 nmol/L per square centimeter; see Table 1). Proteolysis of S-2238 in the amidolytic assay induced by thrombin associated with the vascular wall in segments acquired 4 hours after injury was also abolished by preincubation with hirudin.

**Bound Xa/Va Activity Over 120 Hours After Injury**

Activity of the Xa/Va complex associated with the same injured aortic segments in which bound thrombin activity was characterized was detected at 15 minutes and persisted for up to 96 hours after injury, judging from the activation of prothrombin in recalcified barium-adsorbed plasma or the buffered system (Fig 2 and Table 2). Although concentrations of FPA attributable to activation of prothrombin by bound Xa/Va were increased at 8 hours after injury, they were not significantly higher than those induced by incubation with uninjured segments (P=.054). Because we could not directly quantify the concentrations of bound Xa/Va, we compared Xa/Va activity at each time point by quantifying thrombin elaboration. During the 30-minute incubation with prothrombin in recalcified barium-adsorbed plasma, bound Xa/Va activity induced elaboration of approximately 0.06±0.02 and 0.04±0.02 nmol/L thrombin in segments recovered at 4 and 72 hours, respectively, judging from the increases in concentrations of FPA compared with those induced by purified thrombin in the same plasma (Table 2). The activity of Xa/Va associated with the vascular wall was also assayed by incubating segments acquired at 4 hours with prothrombin in the buffer system. Bound Xa/Va activity in segments obtained at 4 hours induced activation of prothrombin in buffer, resulting in elaboration of 0.16±0.02 nmol/L thrombin (Table 2).

To confirm that the results of these assays reflect activation of prothrombin by Xa/Va associated with the injured vascular aortic segments, recalcified barium-adsorbed plasma with added prothrombin was incubated with segments obtained 15 minutes and 4 hours after injury in the presence of the factor Xa inhibitor TAP. Concentrations of FPA attributable to Xa/Va activity were reduced in the presence of TAP from 244±25 to 18±1 ng/mL at 15 minutes, and from 300±36 to 26±7 ng/mL at 4 hours (P<.01 for both comparisons). Furthermore, when injured aortic segments were incubated with prothrombin in buffer with 0.1 μmol/L TAP, activation of prothrombin was abolished, 0.18±0.02 nmol/L thrombin elaborated in the absence of TAP compared with 0.003±0.003 nmol/L thrombin with TAP (P<.001, n=4).

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**Figure 2.** Increases in fibrinopeptide A (FPA) attributable to de novo thrombin elaboration induced by activation of prothrombin added to recalcified barium-adsorbed plasma by Xa/Va associated with the injured vascular wall (concentrations of FPA shown reflect additional increases in FPA after subtraction from results due to activity of bound thrombin shown in Fig 1). Incubation of uninjured segments with barium-adsorbed plasma containing prothrombin did not induce any significant increase in concentrations of FPA, indicating that there was no bound Xa/Va activity in uninjured segments. In contrast, incubation of segments acquired 15 minutes through 72 hours after injury (with the exception of 8 hours) with barium-adsorbed plasma containing prothrombin induced marked elevations in FPA attributable to de novo thrombin elaboration. *P<.01; **P<.05.

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**TABLE 2. Factor Xa/Va Activity in Aortic Segments, Characterized by the Thrombin Activity Induced by Incubation of Segments with Prothrombin in Barium-Adsorbed Plasma and Measurement of Fibrin Formation (Fibrinopeptide A) or Amidolytic Activity Against S-2238 of Thrombin Elaborated During Incubations With Purified Prothrombin in Buffer**

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</tr>
<tr>
<td>4 h after injury</td>
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<td>0.16±0.02 (n=6)*</td>
</tr>
<tr>
<td>8 h after injury</td>
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<td></td>
</tr>
<tr>
<td>24 h after injury</td>
<td>0.04±0.01 (n=10)*</td>
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</table>

ND indicates not detectable.

*P<.01 vs no injury.
Activation of Factor X by Procoagulants Bound to the Injured Vessel Wall

To determine whether complexes (i.e., IXa/VIIIa and tissue factor/VIIa) that induce activation of factor X were associated with vessel segments after injury, we incubated segments acquired from rabbits 4 hours after injury (n=4) with 50 nmol/L factor X in the buffer system. Minimal, inconsistent activation of factor X was detected as activity against the substrate S-2222 that was too low to permit further characterization.

Dependence of Bound Xa/Va Activity on Platelets and Inhibition by Aspirin

To characterize the dependence of the Xa/Va activity associated with the vascular wall on the binding of platelets, rabbits were given intravenous aspirin 30 minutes before injury. This dose of aspirin decreased collagen-stimulated platelet aggregation in platelet-rich plasma obtained 20 minutes after bolus administration by 80% to 100%. Aortas were acquired 15 minutes and 4 hours after the injury (n=6 for each condition), and bound thrombin and Xa/Va activity were characterized as noted above. In addition, 111In-platelet deposition on the injured segment was measured at 4 hours after injury in separate groups of rabbits injured in the same manner with (n=5) or without (n=9) administration of intravenous aspirin. Treatment with aspirin markedly attenuated 111In-platelet deposition at 4 hours compared with that in untreated animals (Fig 3). Aspirin also did not significantly attenuate bound Xa/Va activity at 15 minutes, but Xa/Va activity was significantly decreased by 4 hours compared with that in injured aortic segments from animals not given aspirin (P<.01; see Fig 5). Heparin did not significantly attenuate 111In-platelet deposition at 4 hours (n=4; see Fig 3).

Discussion

The principal findings of this study were that Xa/Va complex activity associated with the vascular wall is detected immedi-
ately after arterial injury and that bound Xa/Va potentially promotes thrombin elaboration for up to 96 hours after injury (Fig 2). The activity of Xa/Va associated with the vascular wall appears to be dependent on the deposition of platelets on the injured aorta, judging from the marked attenuation that occurs in rabbits given intravenous aspirin (Figs 3 and 5). We also confirmed previous findings from our group and others indicating that thrombin activity is associated with the subendothelium after endothelial injury, most likely because of specific binding to matrix glycoproteins or fibrin. However, thrombin activity is not immediately detectable on the injured vascular wall, and bound thrombin activity diminishes markedly after 24 hours, although it remains elevated to a modest extent up to 72 hours after injury (Fig 1). We also demonstrated that Xa/Va activity detected early after injury on the vascular wall and bound thrombin activity measured 4 hours after injury are resistant to heparin–antithrombin III–mediated inhibition. These results are consistent with previous data indicating that the Xa in the Xa/Va complex on phospholipid membranes and in association with arterial thrombi is resistant to heparin–antithrombin III–mediated inhibition. We were surprised to find a modest but significant increase in thrombin activity associated with the vessel wall early after injury when heparin was administered, but this finding is consistent with a potentiation by heparin of the binding of thrombin to subendothelial matrix and the resistance of matrix- or fibrin-bound thrombin to antithrombin III–mediated inhibition.

Role of Platelet-Dependent Xa/Va Activity in Thrombin Elaboration After Arterial Injury

The importance of platelet- and thrombin-dependent mechanisms of thrombosis in response to arterial injury has been extensively characterized. Platelet adhesion to the arterial wall is one of the earliest events after endothelial injury and is regulated, at least in part, by receptor-mediated binding to collagen in the subendothelial matrix. Activated platelet membranes are thought to be the primary site for assembly of the Xa/Va complex because platelets bind Xa and, when activated, express factor V on their surface. In addition, several investigators have suggested that monocytes may also play a role in supporting the procoagulant activity of the Xa/Va complex. Inhibition of bound Xa/Va activity by aspirin in our study suggests that platelets are the primary site for assembly of the Xa/Va complex early after arterial injury in this preparation. Although aspirin may have effects other than inhibition of platelet function, the correlation we observed between decreased platelet accumulation at the site of injury, decreased bound Xa/Va activity, and local thrombin elaboration suggests that inhibition of platelet adhesion accounts for these observations.

The results of this study are consistent with our previous observations showing that local Xa/Va activity is directly related to the platelet content of arterial thrombi. Reverter et al have also shown that the murine/human chimeric monoclonal antibody 7E3, which inhibits both platelet glycoprotein Ib/IIa and the α,β integrin, attenuates tissue factor-stimulated prothrombin activation. These results are consistent with the hypothesis that the initiation and progression of arterial thrombosis is dependent on a dynamic balance between platelet adhesion/activation, expression of the prothrombinase complex, elaboration of thrombin, and platelet aggregation.

Role of the Tissue Factor Pathway After Arterial Injury

We have previously shown that tissue factor is primarily responsible for activating coagulation in the first 24 hours after balloon-induced injury to the rabbit aorta. Tissue factor–mediated procoagulant activity was identified by preincubation of aortic segments with a monoclonal antibody that inhibits tissue factor. This assay would not be sufficiently sensitive to detect less potent procoagulant activity, such as that expressed by the Xa/Va complex, because recalcified citrated plasma clots after 15 to 20 minutes, even when it is not exposed to the injured aorta. With the assay used in this study, Xa/Va activity associated with the vessel wall did not induce sufficient activation of the added prothrombin to result in clotting during the 30-minute incubation. These results suggest that the extent of Xa/Va activity after injury in this preparation is substantially less than that we have previously observed associated with arterial thrombi formed in vivo; the thrombi induce clotting in less than 15 minutes under similar conditions. However, the importance of bound Xa/Va activity should not be underestimated, because the thrombin elaborated may markedly potentiate thrombosis by activating the coagulation cofactors V and VIII and by activating platelets.

In our preparation of balloon-induced aortic injury, mural thrombus was not observed, but platelet and fibrin deposition occurred. Our previous findings document the observation that conditions which induce more extensive mural vascular injury and platelet-rich thrombosis (ie, high shear, electrical injury) cause considerably more Xa/Va activity to be associated with the thrombus, leading to marked local generation of thrombin.

Persistence of Bound Xa/Va Activity After Arterial Injury

Data from previous experimental and clinical studies are consistent with our observation that Xa/Va activity persists for long periods of time after initial arterial injury. For example, using assays similar to those we used, Barry et al have shown that a 2-hour infusion of hirudin attenuates arterial wall–associated thrombin activity at 24 hours but that bound thrombin activity increases by 48 hours. Although these investigators did not characterize bound Xa/Va activity, our findings that Xa/Va mediates prothrombin activation between 24 and 96 hours (Fig 3) likely account for the late increase in bound thrombin activity they observed. Hatton et al also observed that the injured aortic wall elaborated low levels of thrombin for as long as 10 days, presumably secondary to bound procoagulant complexes that induced activation of prothrombin. Recently, Hatton and Ross-Ouette reported that thrombin elaboration by the injured vascular wall and binding of radiolabeled hirudin decreased substantially after 24 hours and returned to baseline 7 to 10 days after injury. However, none of these studies have characterized the specific mechanisms involved in thrombin elaboration. In our experimental model, in which activation of prothrombin induced by the injured arterial wall was characterized, we found that
bound Xa/Va may induce considerably more thrombin elaboration than has previously been appreciated. This may also occur in patients with acute coronary syndromes, judging from clinical evidence of increased prothrombin activation for up to 6 months after ischemic events or infarction.30

Significance of Procoagulant Activity Bound to the Arterial Wall After Injury

The persistence of Xa/Va and thrombin activity on the vascular wall for up to 4 days after initial arterial injury provides a mechanism for prolonged thrombin elaboration that may play a role in mediating vascular wall remodeling. Thrombin has been shown to induce vascular smooth muscle cell proliferation in culture. In experimental models, vascular smooth muscle cell proliferation is maximal in the first 72 hours after arterial injury, which is the interval during which we observed that thrombin was elaborated and bound to the vascular wall. Sarembock et al31 found that even a 2-hour infusion of hirudin attenuated neointimal hyperplasia after balloon injury of atherosclerotic femoral lesions in the rabbit, but this did not appear to be attributable to decreased smooth muscle cell proliferation.32 However, because direct thrombin inhibition induces only transient attenuation of bound-thrombin activity,19 inhibition of the tissue factor pathway or the Xa/Va complex may be a more effective strategy for inhibiting neointimal hyperplasia after arterial injury.41–44

Our findings are consistent with clinical observations of persistent prothrombin activation in patients with thrombotic complications of coronary artery disease that is not inhibited by heparin or direct thrombin inhibitors.36–41 The persistence of Xa/Va activity after arterial injury also likely contributes to the increases in thrombin activity that occur after discontinuation of thrombin inhibitors or heparin.37 This phenomenon, recently referred to as “thrombin rebound” in one study, did not occur when patients were concurrently treated with aspirin.48 It is possible that the efficacy of aspirin in attenuating or preventing rebound thrombin activity reflects attenuation of platelet-bound Xa/Va activity.

In summary, the results of this study indicate that the progression of arterial thrombosis is dependent on a dynamic balance between adhesion and activation of platelets to the injured vascular wall, tissue factor/VIIa activation of factor X, and the prolonged expression of platelet-dependent Xa/Va activity on the injured vascular wall. Our data suggest that strategies that attenuate platelet adhesion may have antithrombotic efficacy after arterial injury because they decrease thrombin elaboration. Alternatively, inhibition of the activation of factor X or factor Xa activity would appear to be an attractive means of attenuating local thrombin elaboration. Persistence of Xa/Va-mediated thrombin elaboration and binding of thrombin to the vascular wall may also play an important role in vascular wall remodeling.

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References


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