Hirudin Reduces Tissue Factor Expression in Neointima After Balloon Injury in Rabbit Femoral and Porcine Coronary Arteries

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Background—Tissue factor (TF) is a transmembrane glycoprotein that, after binding to factor VII/VIIa, initiates the extrinsic coagulation pathway, resulting in thrombin generation and its sequelae. Thrombin has been shown to induce TF mRNA in endothelium, monocytes, and smooth muscle cells, further perpetuating the thrombogenic cycle. This study was designed to determine the effect of specific inhibition of thrombin by recombinant hirudin (r-hirudin) on TF distribution after balloon angioplasty in the cholesterol-fed rabbit femoral artery and porcine coronary artery models.

Methods and Results—Thirty-five femoral arteries from 32 cholesterol-fed New Zealand White rabbits and 84 coronary arteries from 55 Yorkshire-Albino swine were studied by use of a recently developed in situ method of TF localization based on digoxigenin labeling of recombinant factor VIIa (Dig-VIIa), with correlative studies of TF immunoreactivity by use of anti-rabbit (AP-1) or anti-human (sTF) antibodies. At sites of balloon angioplasty in rabbit femoral or pig coronary arteries (double or single injury), TF-antibody and Dig-VIIa staining were noted in association with endothelial cells, smooth muscle cells, and foam cells and within the fibrous tissue matrix primarily of the adventitia and neointima. Staining was significantly greater after balloon angioplasty than in vessels that had not undergone angioplasty but was similar after single and double balloon injury. Animals treated with r-hirudin (rabbits, 1 mg/kg bolus plus 2-hour infusion; pigs, 1 mg/kg bolus plus 0.7 mg·kg⁻¹·d⁻¹ infusion for 14 days with implantable pump) had diminished TF-antibody and Dig-VIIa staining 28 days after balloon angioplasty compared with controls (bolus heparin only). This effect was more prominent on the neointima and was more striking in the porcine than the rabbit model.

Conclusions—TF expression, persistent 1 month after balloon angioplasty in rabbit femoral arteries and porcine coronary arteries, is attenuated by specific thrombin inhibition with hirudin. These results suggest that thrombin inhibition, in addition to its effect on acute thrombus formation and its effect on luminal narrowing by plaque in experimental animals, may result in a prolonged reduction in thrombogenicity of the restenotic plaque through this effect on TF expression. (Circulation. 1998;98:580-587.)

Key Words: hirudin ■ tissue factor ■ restenosis ■ balloon ■ plaque

Tissue factor is a low-molecular-weight transmembrane glycoprotein that initiates the extrinsic coagulation cascade. The binding of factor VII to exposed TF results in an enzymatic complex that cleaves factor IX to IXa and factor X to Xa, ultimately resulting in the generation of thrombin, followed by fibrin formation, platelet activation, and thrombus generation. TF mRNA and antigen are prominent in the adventitia of normal, uninjured vessels, and very little TF immunoreactivity is present in the endothelial or smooth muscle cells of uninjured vessels. TF mRNA is inducible in endothelial and monocyte cultures by a variety of agents and perturbations. TF mRNA also has been shown to be rapidly induced (within 10 to 60 minutes) in smooth muscle cells in vivo after endothelial denudation and in vitro by numerous agents such as calf serum, platelet-derived growth factor, angiotensin II, epidermal growth factor, calcium ionophores, and thrombin.

We have shown previously that specific thrombin inhibition at the time of angioplasty resulted in reduced luminal
narrowing by angiography and cross-sectional area narrowing by neointima by histomorphometry 28 days after the procedure in the double-injury, cholesterol-fed rabbit model. It has also been shown with this same model that administration of inactivated factor VIIa (DEGR-VIIa), which blocks the binding of factor VIIa to TF, and treatment with recombinant TF pathway inhibitor, which binds factor Xa and inhibits the complex of TF factor VIIa and factor Xa, reduce angiographic restenosis and decrease luminal narrowing by neointima. Administration of TF pathway inhibitor has also been shown to markedly reduce neointimal formation after severe balloon injury in the carotid arteries of hypercholesterolemic minipigs. The present study was designed to determine the effect of specific inhibition of thrombin by r-hirudin on the distribution of TF after balloon angioplasty in the cholesterol-fed rabbit femoral artery and porcine coronary artery models.

Methods

Study Design

This study was performed on 35 femoral arterial segments from 32 adult male New Zealand White rabbits (weight, 3 to 4 kg) fed a high-cholesterol diet and 106 coronary arterial segments from 55 Yorkshire-Albino pigs (weight, 27 to 32 kg) fed a normal diet (Figure 1). Animals were housed according to Laboratory Animal Welfare Act specifications, and all surgical procedures conformed to the guidelines detailed in the “Position of the American Heart Association on Research Animal Use.” Femoral arterial segments of the present study were from rabbits that formed part of a previous study of predictors of inactivated factor VIIa (DEGR-VIIa), which blocks the high-affinity binding of TF to factor VIIa. This method, which involves digoxigenin labeling of recombinant factor VIIa, localized TF in arteries that had, versus those that had not, undergone angioplasty. The 13 hirudin-treated rabbits, although originally part of that study, were not included in the final report. Coronary artery segments of the present study (28-day survival only) were from pigs that formed part of a recent study of the effect of thrombin inhibition by neointima by histomorphometry 28 days after the procedure in the double-injury, cholesterol-fed rabbit model. It has also been shown with this same model that administration of inactivated factor VIIa (DEGR-VIIa), which blocks the binding of factor VIIa to TF, and treatment with recombinant TF pathway inhibitor, which binds factor Xa and inhibits the complex of TF factor VIIa and factor Xa, reduce angiographic restenosis and decrease luminal narrowing by neointima. Administration of TF pathway inhibitor has also been shown to markedly reduce neointimal formation after severe balloon injury in the carotid arteries of hypercholesterolemic minipigs. The present study was designed to determine the effect of specific inhibition of thrombin by r-hirudin on the distribution of TF after balloon angioplasty in the cholesterol-fed rabbit femoral artery and porcine coronary artery models.

Localization of TF was achieved by demonstration of TF-antibody immunoreactivity and by a recently developed, highly sensitive in situ method of TF localization that takes advantage of the specific high-affinity binding of TF to factor VIIa. This method, which involves digoxigenin labeling of factor VIIa, localized TF in the atherosclerotic plaques of several species, including rat, rabbit, pig, and human. The rabbit model was used to compare the distribution of TF in arteries that had, versus those that had not, undergone angioplasty, in single- versus double-injured vessels, and in r-hirudin–treated animals versus control animals that underwent angioplasty (bolus heparin only) (Figure 1). The porcine model was used primarily to substantiate the attenuating effect of hirudin on TF expression after balloon injury in a second species in coronary arteries.

Induction of Lesions in Rabbits

Rabbits were anesthetized by intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). Lesions were induced in 1- to 2-cm segments of the femoral arteries by a combination of nitrogen gas desiccation of the endothelium and a 2% cholesterol, 6% peanut oil diet for 1 month. Pigs were fed a normal diet, and the coronary arteries were not subjected to endothelial injury before balloon angioplasty.

Balloon Angioplasty

Rabbit

A 5F Berman angiographic catheter (Arrow International) was inserted into the right common carotid artery and advanced to 2 vertebral segments above the iliac bifurcation. Animals scheduled for angioplasty (n = 28) (Figure 1) received either single-bolus heparin (150 U/kg, heparin sodium injection [source, porcine intestinal mucosa], 1000 USP U/mL; Solopak Laboratories) or r-hirudin (recombinant desulfatohirudin, CGP39393, Ciba-Geigy Ltd; intravenous bolus of 1 mg/kg followed by infusion of 1 mg/kg for 1 hour and 0.5 mg/kg for a second hour; prolongation of aPTT for control and hirudin-treated rabbits to greater than twice baseline at the time of angioplasty and after 2 hours). In animals subjected to double injury (Figure 1), a baseline angiogram was performed to identify the site of maximum stenosis. The angiographic catheter was replaced by a 2.5-mm balloon dilation catheter (Advanced Cardiovascular Systems, Inc), which was advanced across the femoral stenosis under fluoroscopic guidance. For all animals that underwent angioplasty, three 60-second, 10-atm inflations were performed at 60-second intervals by use of a hand inflator, as described previously. After removal of the catheter system, the carotid arteries were ligated, the wound was sutured, and the animals were returned to their cages. Rabbits undergoing angioplasty by single injury (without prior deendothelialization) and those which were deendothelialized
without undergoing angioplasty (Figure 1) received bolus heparin at the time of intervention.

**Pig**

Pigs were anesthetized with ketamine (15 mg/kg IM) followed by sodium pentobarbital (25 mg/kg IV), intubation, and ventilation with room air. Animals received either bolus heparin (100 U/kg IV) or r-hirudin (Figure 1: 1 mg/kg bolus plus infusion of 0.7 mg·kg⁻¹·h⁻¹ for 14 days via an implantable infusion pump) (prolongation of aPTT for heparin and hirudin-treated animals to greater than twice baseline at the time of angioplasty and at the end of the 14-day infusion). In the porcine model, hirudin was administered for 14 days in view of a previous study that showed that the thrombogenicity of injured arteries is high even 11 days after angioplasty.²³

**Implantation of Infusion Pump**

After a midline neck incision was made, the right common carotid artery and internal jugular vein were cannulated with 0.8-mm-diameter Teflon tubing. The tubes were then tunneled subcutaneously to exit on the dorsal side of the neck. The venous cannula was connected to a Synchromed 8615 miniature infusion pump containing an 18-mL internal reservoir (Medtronic Inc). The pump was secured to the animals with a specially designed vest. On the 14th day after balloon angioplasty, the pump was removed and the cannulas were sealed.

After implantation of the infusion pump and administration of bolus heparin or r-hirudin, an 8F catheter was inserted via the right carotid artery and advanced under fluoroscopic guidance to the right, left anterior descending, or circumflex coronary artery. Angioplasty was performed by use of a 4.0×2.0-mm-diameter balloon catheter (Cordis) that was inflated 3 times to 10 atm (15 seconds, 30-second intervals). After angioplasty, animals were returned to their pens and maintained on a normal diet.

**Pressure Perfusion and Specimen Preparation**

A catheter was inserted via the left common carotid artery in rabbits and positioned above the aortoiliac bifurcation. After administration of an overdose of sodium pentobarbital (Nembutal), the distal arterial tree was pressure perfused with 4% buffered formaldehyde (100 mL, 100 mm Hg, at room temperature for 15 minutes). In pigs, after a midsternal thoracotomy, a cannula was inserted retrograde into the ascending aorta. After a prewash with 500 mL of PBS (with heparin and papaverine), the coronary arteries were pressure perfused with 4% buffered paraformaldehyde (500 mL, 100 mm Hg, 4°C). The heart was then excised and immersion fixed overnight at room temperature. Segments of rabbit femoral arteries and porcine coronary arteries were excised, cut in cross section at 1- to 2-mm intervals, dehydrated in increasing concentrations of ethanol and xylene, and embedded in paraffin. Sections for histomorphometry (5 μm) were stained by the Movat pentachrome technique (rabbits)²⁴ or the combined Masson elastin method (pigs). Adjacent sections were processed for immunohistochemical localization of TF antigen, in situ identification of TF–Dig-VIIa binding, and localization of macrophages and smooth muscle cells.

**Immunohistochemistry**

In rabbits, TF antigen was localized in formalin-fixed, paraffin-embedded sections with the use of a mouse monoclonal antibody to rabbit TF (AP-1)²⁵ (a kind gift by William Konigsberg, Yale University, New Haven, Conn.). Sections were deparaffinized at 55°C to 60°C (2 hours), rinsed in xylene (10 minutes), and rehydrated in decreasing concentrations of ethanol and deionized water. Sections were then blocked with horse anti-rabbit IgG (10 minutes) and 1% hydrogen peroxide (15 minutes), washed in PBS, and incubated with monoclonal antibody to rabbit TF (0.5 μg/mL; 1:5 dilution; 2 hours at 37°C). After washing in PBS, the primary antibody was detected with the use of a biotin-streptavidin amplification detection system (SuperSensitive Kit, Biogenex) (20 minutes at room temperature). The slides were washed again in PBS, reacted with horseradish peroxidase–conjugated streptavidin (20 minutes at room temperature), and developed with DAB (Biogenex) (10 minutes at room temperature in the dark). After washing in tap water, all sections were then counterstained with Harris hematoxylin and put under coverslips. Porcine sections were stained with the use of the polyclonal rabbit anti-human TF antibody raised to the extracellular domain of recombinant TF, residues 1 to 128 (sTF), as reported previously.²

**In Situ Staining for Dig-VIIa**

Deparafilesed sections from all rabbit femoral and pig coronary arteries were rehydrated, washed with TBS and CaCl₂ (pH 7.5, three times), and incubated with human recombinant factor VIIa (a gift from Novo Nordisk A/S, Gentofte, Denmark; 100 nmol/L) bound to digoxigenin-3-α-methylcarbonyl-e-aminocaproic acid-N-hydroxy-succinimide prepared in accordance with the method of Thiruvikraman et al.²⁶ (2 hours at 37°C). After a second wash in TBS/ CaCl₂, the sections were treated with 4% paraformaldehyde (5 minutes at 37°C), rinsed again as before, and incubated with sheep Fab anti-digoxigenin antibody conjugated to horseradish peroxidase (1:400 dilution, 37°C for 1 hour). After another washing in PBS, the sections were developed with DAB (10 minutes) and put under coverslips.

For correlative histochemical comparisons, macrophages were identified in rabbit arteries by use of RAM-11 (mouse anti-rabbit macrophage, Dako). Smooth muscle cells in both species were labeled with a mouse anti-human α-actin antibody (1A4; Dako; 1:1000 dilution).

Control sections were prepared by omission of the primary antibody and substitution with nonimmune serum from the same species (in the case of TF antigen, RAM-11 and α-actin) or by substitution of unlabeled factor VIIa for the Dig-VIIa.

The distribution of TF immunoreactivity and Dig-VIIa binding activity was assessed, by direct light microscopic inspection, for the different wall components of all experimental groups, and the overall intensity of stain of the intimia and media of each section was compared with that of its adventitia. The overall intensity of the stain in the intimia or media was reported as being less than, equal to, or greater than that of its adventitia. Sections were studied by 2 observers blinded to treatment group. This analysis did not include assessment of the intensity of the stain in the endothelium because some groups had endothelial injury as part of the experimental design and because of marked inconsistencies in the intensity of staining of this element that were seemingly unrelated to treatment.

Because the localization of TF-antibody staining in rabbits and pigs virtually paralleled that of Dig-VIIa staining, the semiquantitative estimation of the effect of hirudin versus control (bolus heparin) on porcine arteries was assessed by use of Dig-VIIa staining only (see Table).

**Statistical Analysis**

Data are reported as the number of femoral arteries in each treatment group and expressed as mean±SD. Comparison of categorical data was made by coded χ² analysis. The Statview 512+ statistical package (Brain Power, Inc) was used for these calculations.

**Results**

**Baseline Histopathological Features**

The nature and extent of arterial injury after balloon angioplasty in the cholesterol-fed rabbit model and the pig coronary artery model have been reported in detail previously.¹⁴,¹⁵,¹⁶,²² Relevant correlative points are summarized as follows: Neointimal formation after angioplasty, whether in the rabbit femoral or the pig coronary artery model, consisted primarily of fibrocellular hyperplasia. Noninjured arteries had no neointima even if animals (rabbits) were fed cholesterol. In double-injured rabbit femoral arteries (endothelial injury, cholesterol feeding for 28 days followed by angioplasty) excised 7 or 28 days after balloon angioplasty (at 35 or 56

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days), foam cells occupied on the average ≈10% to 15% of the plaque area (mostly near the internal elastic lamina), with the remainder of the plaque area being occupied by smooth muscle cells and fibrous tissue matrix, as reported previously.22 The striking feature of the rabbit angioplasty model is the abundant infiltration of foam cells into the media, which ranged from ≈25% to a situation in which the majority of the media often appeared to be replaced by foam cells.19,22 Medial foam cells were intermingled with fibrous tissue and smooth muscle cells and were contiguous with foam cells of the adventitia and, through the defect in the internal elastic lamina, with foam cells of the intima. Foam cells were not found in the intima or media of vessels of animals fed cholesterol without prior endothelial injury or subsequent angioplasty. Foam cells present in the intima of the normcholesterolemic, porcine coronary arteries were much more sparse than those of the cholesterol-fed rabbit model, being scattered throughout the intima and adventitia with virtually none within the media. Thrombi were present in 5 of the 35 rabbit arteries, 1 in a deendothelialized artery that had not undergone angioplasty and 4 in double-injured arteries excised 28 days after balloon angioplasty (at 35 days) (2 control and 2 hirudin-treated animals). Thrombi were found at the site of angioplasty in all 39 porcine coronary arteries excised between 1 hour and 7 days after the procedure. By 28 days, organizing thrombus and submedial hematoma appeared to contribute on the average to 10% and 5%, respectively, of intimal thickening in control porcine coronary arteries. In hirudin-treated pigs, the percentage of thrombus declined to ≈5%, with very little change from control in what appeared to be remnants of submedial hematoma.23

TF Localization

Rabbit Femoral Artery

TF antibody and Dig-VIIa staining were noted at sites of balloon angioplasty (double or single injury) not only within the adventitia but also in the media and intima. TF staining was present in association with endothelial cells, smooth muscle cells, and foam cells and within the fibrous tissue matrix primarily of the adventitia and neointima (Figure 2). Dig-VIIa staining essentially paralleled that of the distribution of TF-antibody staining in this model; however, it appeared somewhat more intense in the area of foam cells relative to smooth muscle cells and its immediate extracellular environs (Figure 2).

In rabbits, no significant differences were detected in overall stain intensities between arteries with double injury excised 28 days after balloon angioplasty, arteries with double injury excised 7 days after balloon angioplasty, and arteries with single injury (no prior deendothelialization) excised 28 days after balloon angioplasty. TF immunoreactivity and Dig-VIIa staining were detected in the medial smooth muscle cells of rabbits without angioplasty or endothelial injury that were fed cholesterol. However, in general, the overall intensity of both stains was greater in the intima of deendothelialized arteries that did, versus those that did not, undergo angioplasty. This increase in intensity of both stains was most apparent in the smooth muscle cells of the media and intima, with the intensity of the staining of foam cells appearing somewhat less variable across angioplasty and nonangioplasty groups.

Pig Coronary Artery

Very little TF-antibody or Dig-VIIa staining was detected in the media and intima of normal pig coronary arteries that had not undergone angioplasty. After angioplasty, there appeared to be a biphasic increase in staining early and late after angioplasty. One hour after angioplasty, staining was clearly visible in the media adjacent to the site of injury and in the mural thrombus. Prominent staining in the thrombus and media persisted at 6 and 24 hours. However, staining was markedly less at 3 and 7 days except in the area of the macrophages, which were most numerous at these 2 time points. Staining was again prominent in the media at 14, 21, and 28 days, at which times substantial staining was noted in the hypercellular neointima (Figure 3). TF-antibody and Dig-VIIa staining were markedly positive in the adventitia at all time points, even in arteries that did not undergo angioplasty.

Effect of Hirudin

The overall intensity of TF-antibody and Dig-VIIa staining in rabbits euthanized 7 days after angioplasty (double-injury model; see Figure 1) did not appear to be different between hirudin-treated and control rabbits. However, by 28 days, the
The intensity of TF-antibody and Dig-VIIa staining in rabbits treated with hirudin at the time of angioplasty was noticeably less than in those that received bolus heparin (Figure 2). This reduction in stain intensity appeared more obvious in the smooth muscle cells and extracellular portions of neointima, with little or no effect on the media, possibly because of the large number of foam cells present in the media of these arteries. Hirudin did not appear to affect the intensity of staining of foam cells in this model. By semiquantitative analysis of adjacent Dig-VIIa–stained sections of the femoral arteries of rabbits euthanized 28 days after angioplasty, the number of individual arteries in which the overall intensity of intimal staining was less than (rather than equal to or greater than) the intensity of staining in the adventitia was greater in hirudin than control animals (Table), but contingency analysis reached only borderline statistical significance ($P = 0.07$). For this reason, additional studies were conducted to test the reproducibility of this phenomenon.

The effect of hirudin was much more striking in the porcine coronary artery model. The overall intensity of TF staining in coronary arteries excised 28 days after angioplasty was markedly less in the neointima (and, to a lesser extent, the media) of swine receiving hirudin (bolus plus 14-day infusion) than of swine receiving bolus heparin (Figure 3). By semiquantitative analysis, the number of porcine coronary arteries in which the intensity of intimal staining was less than that of adventitial staining was significantly greater in hirudin versus control animals ($P = 0.009$), further substantiating the effect suggested from the rabbit model (Table).

**Discussion**

We have shown marked TF immunoreactivity and in situ staining of TF binding of Dig-VIIa after balloon angioplasty in the neointima of femoral arteries of cholesterol-fed rabbits (double and single injury) and the coronary arteries of pigs fed a normal diet. This is consistent with previous studies in rats and human atherectomy specimens. TF immunoreactivity and Dig-VIIa staining persisted 1 month after balloon angioplasty in both animal models of the current study and were markedly attenuated by specific thrombin inhibition with r-hirudin. The effect of hirudin was substantiated by overall qualitative inspection and by semiquantitative assessment of the intensity of Dig-VIIa staining in the intima or media of each animal with respect to the staining of its adventitia.

The in situ Dig-VIIa staining method is based on the high affinity that TF has for factor VIIa. It is generally accepted that TF–factor VIIa binding is the principal event that initiates the extrinsic coagulation cascade in vivo. These 2 factors do not exhibit substantial procoagulant activity by themselves. Only after binding is the enzymatic activity of factor VIIa enhanced toward its substrates, factors IX and X. Previous studies have shown that digoxigenin labeling of factor VIIa can be performed without interfering with its binding to TF. The primary structure of TF varies between species, but the tertiary structure of this protein appears to be substantially conserved, resulting in the ability, as previously reported, to use labeled human recombinant factor VIIa to stain rabbit and pig tissues. On the other hand, because of lack of cross-reactivity, it was necessary to use different antibodies for demonstration of TF immunoreactivity.
The overall intensities of both stains were greater in the media and intima of arteries that underwent angioplasty versus those that did not, which were either less severely injured arteries (endothelial cell injury only) in the rabbit or noninjured arteries in both animal models. The finding of increased TF immunoreactivity and Dig-VIIa staining after angioplasty is consistent with the studies of Speidel et al., who showed a marked attenuation of “functional presence of tissue factor” as evidenced by reduced fibrinopeptide A in the effluent of ex vivo segments of balloon-injured rabbit abdominal aortas preincubated with a monoclonal antibody to TF. These findings are also consistent both with the results of studies showing the rapid induction of TF mRNA in monocytes and smooth muscle cells in vitro in response to a variety of insults and with the results of Taubman et al., who found very low levels of TF mRNA in vivo in the normal rabbit aorta but markedly upregulated levels 30 to 60 minutes after balloon withdrawal injury. When considered together, the results of these studies emphasize that the increased thrombogenic potential of arteries subjected to balloon angioplasty is not limited to the consequences of exposure of thrombogenic elements of the subendothelial tissues of the vascular wall, but it also includes induction of TF expression, which may be facilitated by a wide variety of growth factors released or induced at the site of injury.

The presence of TF immunoreactivity and Dig-VIIa staining in the media of arteries that did not undergo angioplasty but that underwent initial endothelial injury is not surprising.

Figure 3. Porcine left anterior descending coronary arteries 28 days after balloon angioplasty (normal diet). Left, control; right, hirudin treatment. Top, combined Masson elastic stain; bottom, TF-antibody stain. Note the marked overall reduction in TF staining (brown) in the intima and fragmented media of hirudin-treated versus control animals despite significant TF staining in the adventitia (A). Arrows indicate disrupted internal elastic lamina. Top, magnification ×20; bottom, ×200.
in view of previous studies showing upregulation of TF mRNA staining in the media after deendothelialization by balloon withdrawal injury.12,13 It is intriguing that some TF immunoreactivity and Dig-VIIa staining was found not only in the adventitia but also in the media of femoral arteries of noninjured rabbits that did not undergo angioplasty, but little or none was found in uninjured pig arteries. This appears to be in partial contradiction to the reports of Fleck et al and Wilcox et al in human arteries and the in vivo studies of Taubman et al in rabbits in which the presence of TF in presumably normal vessels was limited primarily to the adventitia, with little or no TF in the media of these vessels. However, Drake et al found a variable amount of TF reactivity, ranging from weak to moderate intensity, in the media of the human aorta and other elastic arteries, and Thiruvikraman et al found variable amounts of TF–factor VII/VIIa staining in smooth muscle cells of the media in human and other mammalian arteries and, routinely, in venous medial smooth muscle cells. Additional studies comparing injured and noninjured arteries in cholesterol-fed and non–cholesterol-fed animals of both species may provide additional information concerning this issue. It would also be of interest to know whether the increased TF present in the media of noninjured arteries of the rabbit model versus porcine arteries might be related to the tendency, thus far considered unique, for hypercholesterolemic rabbits to have abundant foam cell accumulation in the media after injury.22 This would require extensive additional comparative analysis across species and various vascular beds.

The overall intensity of the Dig-VIIa staining of foam cells, whether in the media or intima, appeared somewhat greater than that of the smooth muscle cells. It is therefore tempting to question whether Dig-VIIa staining, which reflects TF–factor VII/VIIa binding,7 may indicate increased functionally active TF in foam cells compared with smooth muscle cells. This differential staining was not apparent with TF-antibody staining. A variety of stimuli have been shown to induce TF in monocytes within the arterial wall and, under certain circumstances, in peripheral blood, such as in patients with evidence of heightened inflammatory processes and in unstable angina pectoris.2,4,8,9,28,29 It has been suggested that this TF, which belongs to the family of immediate-early genes,13 is responsible for the marked procoagulant activity of monocyte/macrophages in such situations.

The finding of Thiruvikraman et al11 that α-thrombin is itself an agonist for TF induction by smooth muscle cells pointed to the important role these cells play in the perpetuation of the thrombogenic state of the intima and raised the question of whether specific inhibition of thrombin might also attenuate thrombogenicity after balloon angioplasty by this effect on TF expression.

It is uncertain why hirudin has a prominent effect on the neointima rather than the media or adventitia in the rabbit model and on the neointima and, to a lesser extent, the media rather than the adventitia in the porcine model. One possibility is an effect of hirudin on injury-induced TF production rather than on the prominent and already present preinjury baseline levels of TF in the adventitia and, to a lesser extent, the media. This may be one explanation for why hirudin appeared to have less of an effect on the intensity of TF staining in foam cells and why this regimen appeared to affect TF-antigen or Dig-VIIa staining 28 days after angioplasty but not in animals killed just 7 days after angioplasty. High levels of preinjury TF may still be present 7 days after angioplasty, with the effect of hirudin on new TF expression being seen only in arteries excised later. Hirudin also did not appear to have a significant effect on luminal narrowing 7 days after angioplasty, as measured by I/M area ratio (control, 1.1±0.4 versus hirudin, 0.7±0.9) or by %CSAN (control, 31±9% versus hirudin, 24±27%). However, the effect of hirudin on luminal narrowing was significant by both parameters 28 days after angioplasty in rabbits (I/M ratio: control, 2.5±1.2 versus hirudin, 1.3±0.6, P<0.01; %CSAN: control, 66±9% versus 45±18%, P<0.05 by 1-way ANOVA with Fisher protected least significant difference as post hoc test) and in pigs (I/M ratio: control, 2.6±1.1 versus hirudin, 1.8±0.8, P<0.05; %CSAN: control, 58±12% versus 35±10%, P<0.005), as reported previously.20

In conclusion, we have presented evidence that intimal TF expression, induced by balloon angioplasty in femoral arteries of cholesterol-fed rabbits and coronary arteries of pigs fed a normal diet, persistent 1 month after balloon angioplasty, is attenuated by specific thrombin inhibition with hirudin. These results provide support for the suggestion that thrombin inhibition, in addition to its effect on acute thrombus formation and its effect on luminal narrowing by plaque,14–16,20 may also result in prolonged reduction of the thrombogenicity of restenotic plaque after angioplasty through an effect on TF expression. These findings also point to what seems to be a strong, though not yet completely understood, relationship between foam cells and TF expression in plaque and media after angioplasty in the rabbit model. Effort should continue to identify factors regulating the infiltration of monocytes and their conversion to foam cells, which remains to be an important component of the pathogenesis of primary and restenotic lesions in experimental animals and humans.

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